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Research Article

PRONIOSOMES, AS A DRUG CARRIER FOR TRANSDERMAL DELIVERY of HYDROCHLOROTHIAZIDE AND LISINOPRIL COMBINATION

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ABSTRACT

The objective of the study was to develop a proniosomal carrier system for Lisinopril and Hydrochlorothiazide (HCTZ) drug combination for prophylaxis and treatment of hypertension that is capable of efficiently delivering entrapped drug over an extended period of time. The potential of proniosomes as a transdermal drug delivery system for Lisinopril and hydrochlorothiazide was investigated by encapsulating the drug in various formulations of proniosomal gel composed of various ratios of sorbitan fatty acid esters, cholesterol, drug determined by factorial design and prepared by coacervation-phase separation method. The formulated systems were characterized in vitro for size, drug entrapment, drug release profiles and vesicular stability at different storage conditions. The optimized formulations of HCTZ and lisinopril were obtained by employing factorial design at two phases. Stability studies for proniosomal gel were carried out for 4 weeks. The mean vesicle size of optimized niosome formed upon hydration of gel, determined by compound microscope was found to be 115.52 and 104.16 µm for HCTZ and Lisinopril respectively. Spanontaneity determines the rate of niosome formation upon hydration; data obtained exhibit that Span 40, 60 produce niosome more instantly. The method of proniosome loading resulted in an encapsulation yield of 37.01± 1.26% and 59.94± 1.56% for HCTZ and lisinopril respectively. In vitro studies of HCTZ and lisinopril proniosome was carried by drug diffusion through cellophane membrane and Ex-vivo skin permeation studies, the percent drug released after 24 hrs was found ranging in 79-66 % and 48-61% of total drug entrapped for lisinopril and HCTZ respectively. It is evident with the result of this study that proniosomes are a promising prolonged delivery system for HCTZ and Lisinopril and have reasonable satisfactory stability characteristics.

Keywords: Proniosome, Transdermal drug delivery, Factorial design, Hypertension.

INTRODUCTION

Conventional transdermal systems provides various advantages over oral and parentral routes but, suffer with various shortcomings viz. poor release kinetics, less penetration of large size molecules to overcome these problems vesicular carriers were developed.

Vesicular carriers are colloidal particles in which a concentric bilayer made up of amphiphilic molecules surrounds an aqueous compartment. These amphiphilic molecules viz. phospholipids, surfactant (Non ionic, ionic in combination) are either present separately or in combination along with cholesterol as fluidity buffer. Vesicular carriers show a very promising role in permeability improvement[1] and solubility enhancement[2] and therefore they can also improve the bioavailability of drug by enhancing stability, absorption, targeting to site of action which is actually the result of improved solubility, stability and permeability. The stability of peptide drugs have been reported viz. insulin loaded niosomes[3] showed enhancement in their absorption as they become more resistant to proteolytic enzymes and gastric pH due to protective sheath of non ionic surfactants. Vesicular carrier viz. liposome, niosomes provides alternative route apart from conventional routes, for drug delivery by enhancing permeability and increasing occlusion time on skin. Some drugs Hydralazine[4], methotrexate[5], gallic acid[6], levonorgestrol[7], estradiol[1], flurbiprofen[8], tenoxicam[9], captopril[10], ketorolac[11], carvediol[12], minoxidil[13], ellagic acid[14] have been evaluated for transdermal application in niosomal or proniosomal carriers. Niosomes has been also tested in ophthalmic administration of acetazolamide[15]. Vesicular carriers are further exploited in vaccination as adjuvant for enhancing the presentation of immunogens[16]. These carriers, not only provide alternative routes, they also provide a sustained action due to prolonged release. All mentioned studies showed that vesicular carriers are very promising and effective for drug delivery. However, on other hand these carriers also suffer from some shortcomings viz. liposomes have poor shelf life, less purity of ingredients, high cost, poor yield, restricted storage condition, difficulty in sterilization; niosomes also show aggregation, fusion, leaking, sedimentation of vesicles, difficulty in sterilization. So a new approach of provesicular carriers has been introduced. Proniosomes are semisolid liquid crystal (gel) products of non ionic surfactants[7], prepared by techniques such as coacervation phase separation, slurry method and spray drying, upon subsequent hydration by means of incorporation in hydrophilic gel or by absorbing moisture from site of administration turn to niosome. Proniosomes are more stable and convenient than niosomes and also provide following advantages viz. ease in transportation, distribution, storage, dosing and sterilization.

Hypertension[17] is defined conventionally as a sustained increase in blood pressure 140/90 mm Hg, a criterion that characterizes a group of patients whose risk of hypertension-related cardiovascular disease is high enough to merit medical attention. Actually, the risk of both fatal and nonfatal cardiovascular disease in adults is lowest with systolic blood pressures of less than 120 mm Hg and diastolic BP less than 80 mm Hg; these risks increase progressively with higher systolic and diastolic blood pressures. At very high blood pressures (systolic 210 and/or diastolic 120 mm Hg), a subset of patients develop fulminant arteriopathy characterized by endothelial injury and a marked proliferation of cells in the intima, leading to intimal thickening and ultimately to arteriolar occlusion. This is the pathological basis of the syndrome of immediately life-threatening hypertension, which is associated with rapidly progressive micro vascular occlusive disease in the kidney (with renal failure), brain (hypertensive encephalopathy), congestive heart failure, and pulmonary edema.

The report of National health and Nutrition Examination survey shows that blood pressure is controlled only in 27% case by mono therapy, so treatment with two different pharmacologic classes is necessary to achieve adequate blood pressure reduction[18]. For fixed dose combination AB/CD rule or Cambridge rule which was later modified as A/CD rule[19], was studied for combination of drugs. ACE inhibitor and diuretic combination provide good means of controlling hypertension by showing a synergistic effect. Thiazide diuretics control hypertension by depleting volume of plasma and sodium which in turn activate renin-angiotensin system which suppresses the antihypertensive effect of thiazide diuretics but in combination with ACE inhibitor angiotensin-II conversion does not takes place thus providing a synergistic effect[19]. The effect is more prominent in black patients. This combination is well tolerated according to a clinical study[20]. As low dose of these agents are enough, when administered in combination so it helps to keep the incidence of side effects minimal and facilitates the patient's

compliance with long-term treatment[21]. Lisinopril is absorbed slowly, incompletely, variably and its bioavailability is approximately 30% and it provides a long duration of action >24 hour while hydrochlorothiazide reaches its T_{max} in 1 to 1.5 hour providing prompt relief[22].

MATERIALS AND METHODS

HCTZ and Lisinopril were provided by Abbott India Ltd. DMF (HPLC grade) was purchased from jai appliances, Sagar. Ascorbic acid (AR grade) was procured from Department of biotechnology (GGV). DSC was performed at Indian Pharmacopoeia Commission, Ghaziabad. All other chemicals used throughout this investigation were of analytical grade and no additional purification was carried out. Distilled water was used throughout the study.

Reagents Preparation

Saline pH 7.4, Phosphate-buffer

Dissolve 2.38 g of disodium hydrogen phosphate, 0.19 g of potassium di hydrogen phosphate and 8.0 g of sodium chloride in sufficient water to produce 1000 ml. pH was adjusted accordingly.

Preparation of HCTZ standard curve

Hydrochlorothiazide (10mg) was accurately weighed and dissolved in 100ml of Phosphate buffer pH 7.4 and stock solution of (100µg/ml) was produced. From the standard stock solution,.0.2,0.4, 0.6, 0.8, 1.0,1.2 and 1.4 ml was diluted up to 10 ml and to get 2, 4, 6, 8, 10,12, 14 µg/ml. Further, calibration curves for hydrochlorothiazide were plotted in the concentration range of 2-14 µg/ml .The absorbance values were determined for the drug at the wavelength of 272.0 nm.

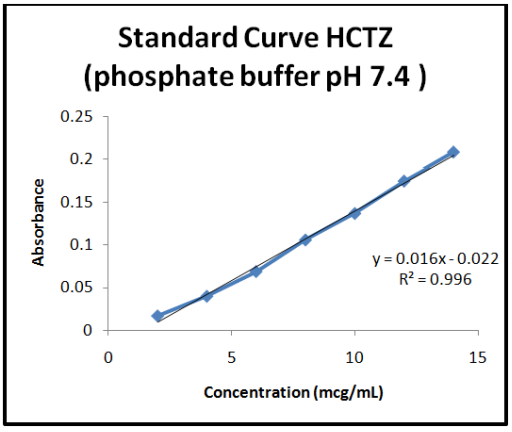

Fig. 1: Standard curve HCTZ

Preparation of Lisinopril standard curve

Stock solution was prepared by weighing 25 mg lisinopril, drug was transferred into a 25 ml volumetric flask and the volume was made up to 25 ml by 0.9/0.1 DMF/water ratio. Further, amount of stock solutions required for aliquots from 5- 50 µg/ml were transferred into 5 ml volumetric flask, then to each tube, 2 ml of ascorbic acid solution (0.2%) was added and volumes were maintained to 5 ml with DMF. The solutions were heated on a water bath at 100 ±1°C for 15 minutes. The solutions were cooled at room temperature. The contents of each tube were transferred to 10 ml volumetric flasks and volume was made up to 10 ml with DMF. The absorbance were measured at 530 nm against the reagent blank prepared simultaneously within the stability period of 3h.

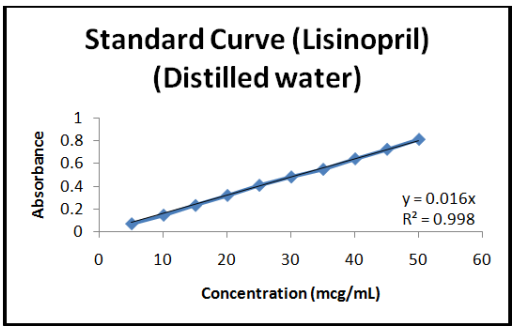

Fig. 2: Standard curve Lisinopril

Preparation of proniosome gel

Proniosomal gel was prepared by the method[23] reported by parrett et al. (1991) with slight modification. Precisely weighed amount of surfactant, cholesterol and drug in a specified ratio were taken in a dry, clean, weighing bottle. A measured amount of ethanol (absolute alcohol) was added to weighing bottle to dissolve the ingredients. The bottle was closed well to prevent loss of solvent from it and warmed over water bath at 65±3°C for about 10 minutes until the surfactant mixture was dissolved completely, then the aqueous phase was added and warmed on water bath till get clear solution was obtained. This clear solution formed was cooled to room temperature to convert to proniosomal gel. The gel obtained was preserved in the same weighing bottle in a dark at refrigerated condition for characterization.

Optimization of formulation

Simultaneous optimization technique was employed to optimize various process variables, which could affect preparation and properties of the Proniosomal gel formulation. These were identified and optimized. Following process variables of proniosome gel formulation were selected for optimization of formulation.

-Type of surfactant

-Ratio of surfactant, cholesterol

-Ratio of drug, cholesterol

In order to optimize the formulation initially a preliminary screening was performed with selected ingredients to use in factorial design rest were kept uniform. A 3^2 incomplete factorial design was employed to ascertain interrelationship between ingredients in first phase then in next phase rest parameters were optimized.

Table 1: Composition and Appearance of Primary Formulations

Surfactant/Cholesterol (in mmol)	Span-20	Span-40	Span-60	Span-80
1	Transparent liquid	White creamy gel	White creamy gel	Transparent liquid
.9/.1	Transparent Liquid	White creamy gel	White creamy gel	Two phase liquid
.8/.2	Transparent gel	White creamy gel	White creamy gel	Two phase liquid
.7/.3	Transparent gel	White creamy gel	White creamy gel	----

Table 2: Fractional Factorial Design

Cholesterol Levels	Cholesterol/Surfactant	Surfactant Levels		
		1=0.9 mmol	0=0.8 mmol	-1=0.7 mmol
	-1=.1 mmol	-1/1	-1/0	-1/-1
	0=.2 mmol	0/-1	0/0	0/-1
	1 =.3 mmol	1/-1	1/0	1/-1

Table 3: Full Factorial design (For HCTZ)

Formula Code	Independent Variable Level Coded form	
	Cholesterol	Drug
FFH1	1	0
FFH2	1	1
FFH3(Replaced with FH1)	0	1
FFH4	0	0

Where: Cholesterol: 0=0.1 mmol, 1=0.2 mmol Drug: 0=35 mg, 1=50 mg

Table 4: Full Factorial design (For Lisinopril)

Formula Code	Independent Variable Level Coded form	
	Cholesterol	Drug
FFL1	1	0
FFL2	1	1
FFL3(Replaced with FL1)	0	1
FFL4	0	0

Where: Cholesterol: 0=0.1 mmol, 1=0.2 mmol Drug: 0=35 mg, 1=50 mg

Characterization of proniosomal gel

Light microscopy

A thin layer of proniosome derived niosomes, obtained by hydration were spread under microscope and size was observed using ocular micrometer. Further, photomicrographs were taken at suitable magnification.

Thermal Analysis

Differential scanning calorimetry thermograms of proniosomes were recorded at Indian Pharmacopoeial Commission, India. The DSC runs were performed over a temperature range 30-210^{0}C for lisinopril proniosomes and 30 to 320^o C for HCTZ at a heating rate of 10^{0}C per minute.

Entrapment Studies

To proniosomal gel stored in weighing bottles, 10 ml of distilled water or pH 7.4 phosphate buffer at 55^o C was added, and weighing bottles were heated at 60^o C for 10 min. with shaking. Thus the preformed niosomes dispersion was subjected for centrifugation at 15000 rpm for 45 min by using cooling centrifuge (Remi Instruments, Mumbai). The supernatant was recovered and assayed spectrophotometrically by Shimadzu UV spectrophotometer, applying suitable analytical technique[24, 25] for untrapped drug. The encapsulation percentage of drug (EP) was calculated by the following equation:

$EP= [(C_t-C_r)/C_t]*100$,

Where: C_t, amount of total drug, C_r amount of free drug.

Assessment of Physical stability

Aggregation or fusion of the vesicle as a function of time was determined as the change in entrapment efficiency after storage at refrigerated condition. The vesicles were stored in weighing bottle at refrigerated condition for one month. Stability for each formulation was defined in terms of retaining its initial entrapment efficiency for one month duration. Stable formulations were defined as those showing high entrapment retention (>90%).

Drug retained in proniosomes= (Entrapped Drug after storage/Entrapped Drug before storage)*100

In vitro release studies

In vitro release studies on proniosomal gel were performed using fabricated diffusion cell. The capacity of receptor compartment was kept 100 ml. The area of donor compartment exposed to receptor compartment was 3.95cm^2. The dialysis cellophane membrane was mounted between the donor and receptor compartment. A weighed amount of proniosomal gel was placed on one side of the dialysis membrane. The receptor medium was phosphate saline buffer pH 7.4. The receptor compartment was maintained at temperature 37±0.5^{o}C with the help of thermostatic magnetic stirrer and receptor fluid kept circulating by using Teflon coated magnetic bead. After each sampling interval, samples were withdrawn and were replaced by equal volumes of fresh receptor fluid. Samples withdrawn were analyzed spectrophotometrically (Shimadzu-1800) at 272 nm and 530 nm, employing suitable sample treatment.

Ex-vivo skin permeation study[24]

Ex-vivo skin permeation study was carried out, using hairless mice skin. Before using the skin, it was hydrated overnight then mounted on hollow glass tube, receptor compartment was maintained at 37±2^o c by keeping it on magnetic stirrer, gentamycin was used as a preservative. Sample was withdrawn at scheduled interval and volume of donor compartment was maintained with fresh media further samples were suitably treated and assayed by UV spectrophotometer (Shimadzu 1800).

Data Analysis

The cumulative amount of HCTZ and Lisinopril permeated through cellophane membrane and excised mice abdomen skin was plotted as a function of time. The slope of the linear portion of the plot was derived by regression. The flux (permeation rate) was calculated from slope.

Flux =Slope of cumulative drug release v/s time

Effective permeation area

The release profiles were fitted to the Zero order model (Eq. 1), First order (Eq. 2) and Higuchi square root model (Eq.3).

1. $Q_t = Q_0 - k_0t$ .....Eq. 1

2. $Q_t = Q_0 - e^{-k1t}$ .....Eq. 2

3. $Q_t = k_H \sqrt{t}$ .....Eq. 3

4. $Q_t/Q_\infty = K_kt$.....Eq.4

5. $Q_0^{1/3}-Q_t^{1/3} = K_{HC}\, t^n$.....Eq.5

Q_t is the total amounts of drug release after time t, Q_0 the initial amount of drug, and k_0, k_1, k_H, K_k, K_{HC} are release rate constant for the above mentioned kinetic models, respectively.

Statistical Analysis

The statistical significance of the difference in particle size and percentage entrapment between the two formulation groups prepared with different ratio of surfactant, cholesterol and drug were tested by one way analysis of variance. Differences were considered to be statistically significant at a level of $p \leq 0.05$.

RESULT and DISCUSSION

Proniosomal gel was formed by first forming the sol phase at high temperature (65^{0}c). The Excipients were solubilized in small amount

of ethanol with heating further addition of small amount of water forms a w/o emulsion, indicating the arrangement of surfactant at interfacial layers. As temperature decreases surfactants may start converting into gel phase from sol, in which possibly there is a tail to tail arrangement to form bilayer. Due to, less amount of solvents, they remain in gel state at low temperature, on subsequent hydration ethanol starts towards continuous phase, as it has good aqueous solubility this movement may induces a thermodynamic gradient to minimize the energy. Bilayers start folding and form vesicles, to hasten this process we use hot media.

Size and shape Analysis

Size and shape was determined using calibrated stage micrometer by compound microscope. Formulations with higher cholesterol ratio shows lesser size compared lesser cholesterol ratio this may be attributed to some membrane stabilizing property of cholesterol.

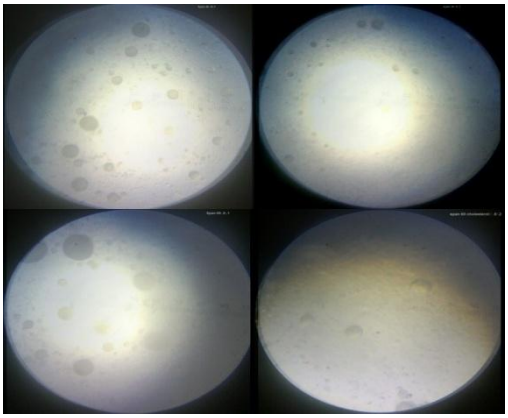

Fig. 3: Photomicrograph of proniosome derived niosomes

Entrapment Studies

Entrapment studies were performed by centrifugation method. Lisinopril has more entrapment than HCTZ, the possible difference may be due to hydration media phosphate buffer pH 7.4 for HCTZ and distilled water for lisinopril. Probably ionization of HCTZ at pH 7.4 may be the reason. Span 60 shows higher entrapment for both drugs which may be due to higher phase transition temperature while span 40 has lower phase transition temperature leading to more chances of leakage and also decreased order in bilayer with increase in temperature. There is less entrapment in proniosomes solely made up of span due to less ordered bilayer. Further, presence of cholesterol enhances membrane stability up to a limit then decrease in entrapment shows presence of disordered bilayer. This means as optimum concentration of cholesterol favors higher entrapment.

Table 5: Table for entrapment efficiency and size analysis

Formulation code	Percent entrapment efficiency	Diameter (µm)
FH1	37.01±1.02	115.52±3.34
FH2	33.85±1.31	70.24±1.31
FH3	26.4±0.98	33.76±1.22
FH4	33.44±1.23	83.68±2.38
FH5	25.4±1.04	40.96±1.41
FH6	20.57±0.94	16.32±0.39
FL1	59.94±1.78	104.16±2.34
FL2	52.4±1.86	73.92±1.83
FL3	50.2±1.12	33.28±0.84
FL4	56.94±1.98	85.12±2.11
FL5	50.42±1.94	41.74±1.12
FL6	47.72±1.45	15.52±0.36
PHASE 2		
FFH1	34.1±1.23	126.4±3.31
FFH2	21.6±1.43	125.3±2.29
FFH3	37±1.02	115.52±3.34
FFH4	22.4±0.91	116.92±3.08
FFL1	53.6±1.04	108.97±2.16
FFL2	41.4±1.28	109.42±2.37
FFL3	59.94±1.78	104.16±2.34
FFL4	45.7±1.04	101.24±2.54

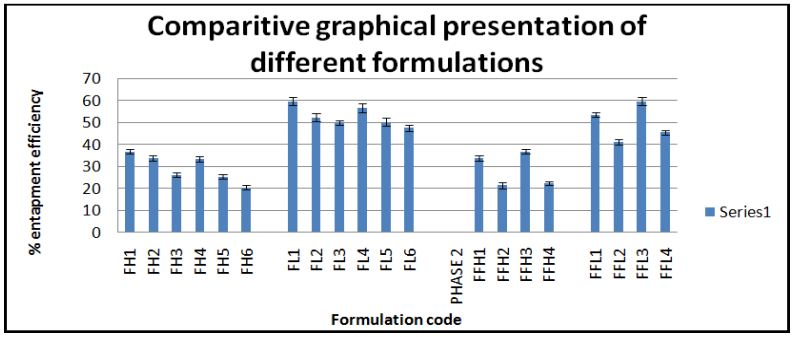

Fig. 4: Comparative graphical presentation of different formulations

In Vitro Release Study

In vitro permeation study was performed using semi permeable membrane of molecular weight cut off 12000-14000. Throughout the experiment period proniosomal gel of span40 showed release rate higher than span 60. This may be due to less phase transition temperature, span 40 has phase transition temperature of 42 ^{0}C while span 60 has phase transition temperature 53 ^{0}C. Proniosome of lisinopril shows higher release rate in comparison to HCTZ. This may be justified on the basis of thermograms of the proniosomes of HCTZ and Lisinopril. According to thermograms HCTZ, lisinopril proniosomes indicates phase transition temperature at 52.10 ^{0}c and 51.68 ^{0}c respectively. For both drugs when level of cholesterol was enhanced beyond a point there is increase in release rate which may be due to increased disorder in bilayer due to cholesterol. Thus the optimized formulation has surfactant cholesterol ratio .9/.1 mmol for both drugs.

Table 6: Data of Cumulative amount release from proniosomal gel (HCTZ) through cellophane membrane at various time point of sample collection expressed as mean value (n=3)

Time(h)	FH1	FH2	FH3	FH4	FH5	FH6	FFH1	FFH2	FFH4
1.1	5.31	7.02	7.84	5.53	6.95	8.11	6.21	6.11	5.12
2	8.52	9.49	10.06	9.47	9.89	10.29	8.41	7.93	8.37
3	12.29	13.58	12.89	15.72	14.89	13.65	12.36	11.37	12.11
4	16.88	16.91	15.43	19.89	17.68	16.91	15.99	14.81	16.41
5	19.7	18.98	18.58	24.92	21.47	20.34	20.02	19.86	19.32
6	23.74	24.12	22.46	28.65	25.74	26.81	23.89	24.89	23.41
7	27.45	28.78	26.93	31.78	29.12	30.47	28.62	28.51	27.12
8	31.34	33.61	31.82	34.67	33.99	33.85	32.14	32.12	31.09
12	36.13	39.73	36.47	38.81	47.87	43.61	36.87	35.89	35.98
24	48.61	57.02	59.51	49.97	59.83	61.28	51.9	50.61	48.93

Table 7: Correlation coefficient and release rate constant of various kinetic equation (through cellophane membrane)

Formulation code	Zero order R^2	First order R^2	Higuchi matrix R^2	Korsmeyer peppas		Hixson crowell R^2	Best fit model
				R^2	n		
FH1	0.874	0.64	0.972	0.907	0.246	0.913	Higuchi
FH2	0.927	0.728	0.983	0.828	0.206	0.96	Higuchi
FH3	0.966	0.797	0.982	0.77	0.185	0.984	Higuchi
FH4	0.806	0.561	0.94	0.94	0.246	0.856	Higuchi
FH5	0.906	0.712	0.974	0.852	0.214	0.942	Higuchi
FH6	0.934	0.749	0.984	0.78	0.19	0.97	Higuchi
FFH1	0.896	0.679	0.977	0.845	0.224	0.934	Higuchi
FFH2	0.878	0.665	0.966	0.82	0.228	0.918	Higuchi
FFH4	0.882	0.645	0.975	0.911	0.253	0.919	Higuchi

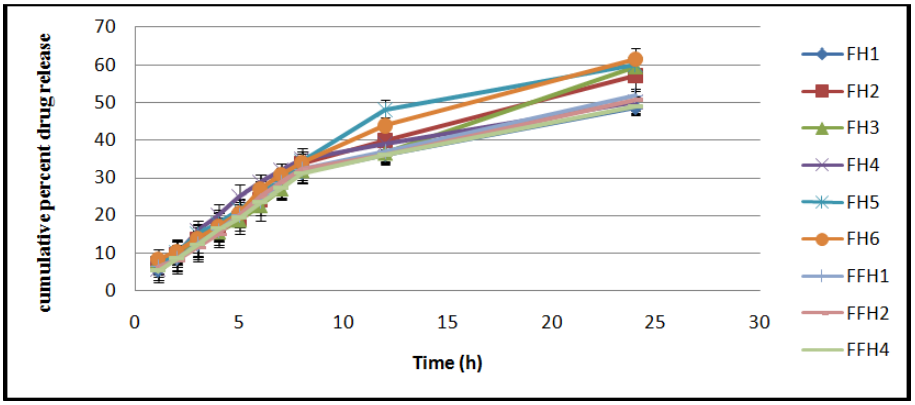

Fig. 5: Percent cumulative drug (HCTZ) release Vs time

Table 8: Data of Cumulative amount released from proniosomal gel (lisinopril) through cellophane membrane at various time point of sample collection expressed as mean value (n=3)

time (h)	FL1	FL2	FL3	FL4	FL5	FL6	FFL1	FFL2	FFL4
1.1	13.66	12.04	14.02	13.81	14.35	15.64	14.31	13.57	12.94
2	16.21	17.37	18.09	17.19	18.61	19.26	17.45	18.11	16.43
3	24.82	26.89	35.04	25.31	26.7	27.31	25.31	25.78	23.98
4	30.57	32.6	35.7	29.67	33.43	35.81	31.43	31.46	29.87
5	36.79	37.08	40.81	35.81	38.68	42.31	36.98	36.81	35.99
6	40.77	42.56	46.61	41.07	43.77	47.71	42.06	41.91	40.21
7	44.01	47.45	51.05	45.87	49.02	53.06	46.67	46.54	44.45
8	47.04	52.94	55.45	50.43	54.13	59.47	51.64	50.49	47.05
12	52.56	58.78	60.36	57.14	59.89	64.86	56.64	57.02	51.21
24	67.87	73.61	75.04	70.39	75.93	79.63	72.14	71.87	66.17

Table 9: Correlation coefficient and release rate constant of various kinetic equation (Via cellophane membrane) of lisinopril formulation

Formulation Code	Zero order R^2	First order R^2	Higuchi matrix R^2	Korsmeyer peppas R^2	Korsmeyer peppas n	Hixson crowell R^2	Best fit model
FL1	0.825	0.631	0.949	0.829	0.14	0.899	Higuchi
FL2	0.815	0.607	0.946	0.897	0.16	0.902	Higuchi
FL3	0.784	0.586	0.926	0.871	0.146	0.88	Higuchi
FL4	0.823	0.645	0.645	0.883	0.141	0.897	Higuchi
FL5	0.828	0.64	0.64	0.852	0.141	0.914	Higuchi
FL6	0.796	0.793	0.793	1		0.895	Korsmayer
FFL1	0.827	0.645	0.645	0.827	0.139	0.905	Higuchi
FFL2	0.831	0.641	0.641	0.864	0.143	0.909	Higuchi
FFL4	0.809	0.446	0.446	0.852	0.145	0.881	Higuchi

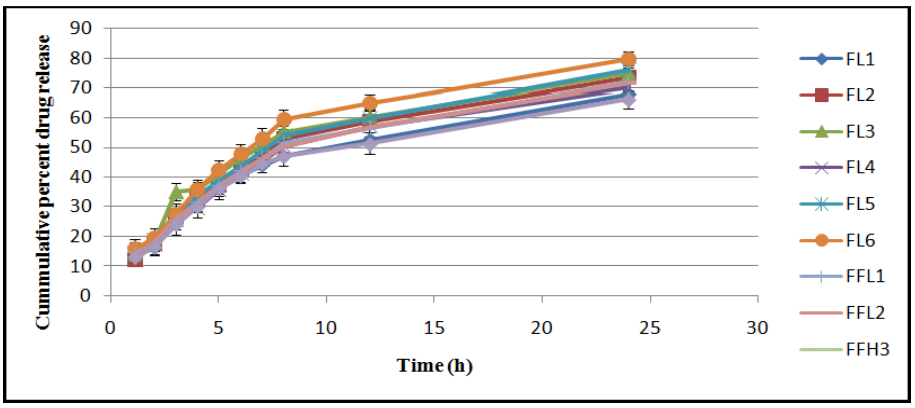

Fig. 6: Percent cumulative drug release Vs time of lisinopril formulations

Ex-vivo release study

Ex-vivo release study was performed using mice skin. Carbopol gel (1%) shows a fast release in comparison to proniosomes entrapped drug. The reason may be, that the low molecular weight drug from Carbopol gel starts permeating via hydrated skin from donor compartment to receiver compartment due to concentration gradient mean while from proniosome gel sustained release was observed, this happened due to controlled release pattern from niosome. Initially, release is more than that of release observed at subsequent stages; reason may be untrapped drug in crevices of proniosomal gel, decreasing concentration of drug in system which results into low concentration gradient either. Therefore, it is observed that proniosome gel system provides a sustained release in comparison to carbopol gel.

Table 9: Table for cumulative drug release at various time point of sample collection expressed as mean value (n=3) (Ex-vivo study)

Time (h)	% cumulative drug release (HCTZ) from carbopol gel	% cumulative drug release (Lisinopril) from carbopol gel	% cumulative drug release (Lisinopril) from FL1	% cumulative drug release (HCTZ) from FH1
1.1	67.85	65.67	17.57	14.37
2	77.85	75.12	20.28	18.35
3	78.42	79.12	23.85	20.5
4	81.71	80.05	26.28	22.62
5	83.01	84.67	29.1	24.25
6	84.42	86.21	34.42	26.12
24	95.71	94.21	51.57	39.25

Effect of surfactants

In reference to above study it was observed that phase transition temperature of surfactant may have some role in release profile. It was observed that release rate for span 60 proniosome is less than that of formed by span 40 as phase transition temperature values of span 60 and span 40 was 53-57, 43-48 ^{0}C respectively. Thus it may be possible due to low phase transition temperature span 40 formed proniosomes are found to be more leaky.

Effect of cholesterol

Vesicles formed in absence of cholesterol have lower entrapment than that of formulation having a certain concentration. Thus, by this study it may be concluded that cholesterol being a lipophilic molecule creates some changes in bilayer which results into less permeability of bilayer and above a certain concentration these changes were reversed.

Effect of drug candidates

Drug candidates certainly have some interactions with carrier system; such interaction was clearly observed in case of above drugs by observing DSC results where peak position of same formulations containing both drugs have different phase transition temperature that is 52.10 and 51.68 ^{0}C for HCTZ and Lisinopril respectively. So, change in phase transition temperature may because of the drug candidate, which ultimately results into change into entrapment efficiency and overall release profile.

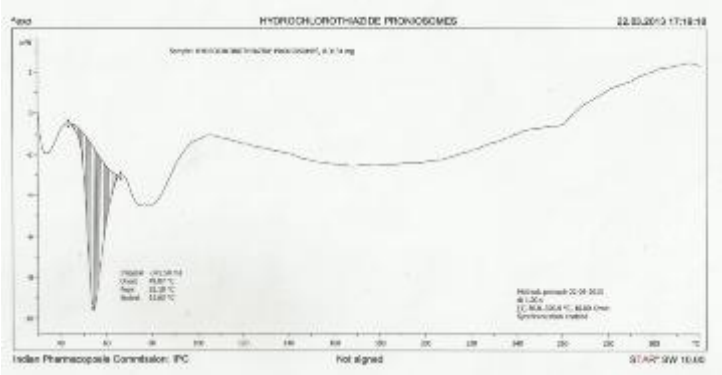

Fig. 7: DSC of HCTZ Proniosomes

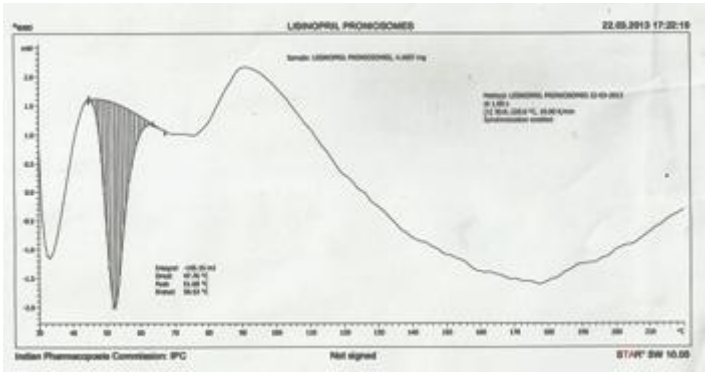

Fig. 8: DSC of Lisinopril Proniosomes

CONCLUSIONS

Concluding the above work, span 60 type surfactants used in proniosomes preparation shows better entrapment and release profile. The type of surfactant, hydration media, transition temperature, cholesterol concentration, drug candidates affect the various characters of proniosome gel. The entrapment and release rate of proniosomes are affected by formulation variables; such as surfactant/cholesterol ratio, drug amount. Ex-vivo study showed that permeation of entrapped drug through mice skin is lesser than cellophane membrane in 24 hour. Study indicates the potential of HCTZ and lisinopril proniosomes in sustained delivery of drugs.

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