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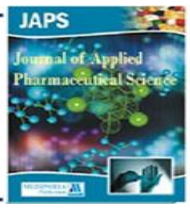

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Formulation and Evaluation of Lisinopril Dihydrate Transdermal Proniosomal Gels

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ABSTRACT

In the present study transdermal Lisinopril proniosomal gels was formulated by using Lecithin, Cholesterol as encapsulating agents, Surfactant, Span and permeation enhancers. The study methodology encompasses compatibility studies using FTIR spectra, evaluation of proniosomal gels for pH determination, Viscosity, Vesicle size analysis, rate of spontaneity, encapsulation efficiency, *in vitro* skin permeation studies and stability studies. The preliminary compatibility studies conducted revealed that there no interaction between Lisinopril and excipients which was as evident from FTIR spectral studies. The physical characterization of proniosomal gels was found to be within the acceptable limits. It was observed that the gel formulations showed good spreadability and viscosity. Determination of vesicle size was found to be 20.10-26.23µm. The proniosomes showed spherical and homogenous structure in optical microscopy. All formulations showed zero order drug release by diffusion mechanism. The stability studies showed that proniosomal gels were stable at 4 to 8^0C and 25 ± 2^0C. The above results indicated that the proniosomal gels of could be formulated for controlled release of Lisinopril. The proniosomal gels are suitable for Lisinopril once a day controlled release formulation.

Key words: Lisinopril, Pronoisomes, Lecithin, Cholesterol, Transdermal gel.

INTRODUCTION

Systemic arterial hypertension (Agarwal et al., 1994) is a chronic medical condition in which the blood pressure is elevated. Increased blood pressure indicated by signs and symptoms like headache, somnolence, and hypertensive encephalopathy. In children it causes fatigue, blurred vision, epistaxis, bell palsy. Most patients with hypertension shows elevated blood pressure when compared to normal as indicated in table 1. Characteristically hypertensive headache occur in the morning and is localized at occipital region was commonly diagnoized symptom. In the treatment of hypertension Angiotensin converting enzyme (ACE) inhibitors (www.circ.ahajournals.org. 2010) were used, they produce vasodilation by inhibiting the formation of angiotensin II. ACE inhibitors are effective in the treatment of primary hypertension and hypertension of renal artery stenosis. Reducing angiotensin II formation lead to arterial and venous dilation inturn reduces arterial and venous pressure. By reducing the effects of angiotensin II on the kidney, ACE inhibitors causes natriuresis and diuresis, which decreases blood volume and cardiac output, thereby lowering arterial pressure. The ideal drug candidates employed were Lisinopril, Benazepril. Captopril, Enalapril and Fosinopril.

Table 1. The normal and hypertensive systolic and diastolic blood pressure values.

Classification	Systolic pressure		Diastolic pressure	
	mmHg	kPa	mmHg	kPa
Normal	90–119	12–15.9	60–79	8.0–10.5
Pre hypertension	120–139	16.0–18.5	80–89	10.7–11.9
Stage 1	140–159	18.7–21.2	90–99	12.0–13.2
Stage 2	≥160	≥21.3	≥100	≥13.3
Isolated systolic hypertension	≥140	≥18.7	<90	<12.0

The Proniosomal Gels are becoming more popular due to ease of application and better percutaneous absorption, than other semi solid preparations. Gels can resist the physiological stress caused by skin flexion, mucociliary movement, adopting to the shape of the applied area and for controlling drug release. To achieve optimal drug action, functional molecules should be transported by a carrier to the site of action and released to perform their task. Non ionic surfactant vesicles known as niosomes are microscope lamellar structures formed on admixture of a non ionic surfactant, cholesterol and diacetylphosphate with subsequent hydration in aqueous media. The proniosomes offer versatile drug delivery via transdermal route. Proniosomes provide additional convenience in stability, transportation, storage and dosing with minimized aggregation, fusion and leaking. The reported transdermal niosomal drug formulations include flurbiprofen, Piroxicam and Estradiol. Lisinopril dihydrate, an orally active inhibitor of ACE is considered for anti hypertensive therapy which has 50-60% bioavailability. The oxidation rate of lisinopril in dermal homogenate is significantly lower than the intestinal homogenate because of the oxidative product of Lisinopril, a lisinopril disulfide show poor absorption from intestine. Lisinopril when administered initially cause hypotension, which can prove to be harmful in diuretic treated and congestive heart failure patients. Therefore, the use of transdermal Proniosomal gel could reduce the side effects associated with oral route. Lisinopril competitively inhibits angiotensin converting enzyme resulting to decreased level of angiotensin. Lisinopril is available in tablet dosage form with 2.5 mg, 5 mg, 10 mg, and 20 mg strength. In the literature studied for the present work revealed that (Chandra et al., 2008) prepared piroxicam Proniosomal transdermal reservoir gels and diffusion studies done on Keshery chein diffusion cell. They concluded that span 60 based formulation produced vesicles of smallest and higher entrapment efficiency and there was considerable improvement in flux over the control gel formulation. Anti-inflammatory studies showed significant reduction in rat paw oedema. (Ibrahim et al., 2008) formulated flurbiprofen proniosomal transdermal carrier systems using various spans with cholesterol. Drug release was tested by diffusion through cellophane membrane and rabbit skin. Their microscopic observations showed that either proniosomal solutions or gel formulations immediately converted to niosomal dispersions upon hydration. Due to the skin permeation barrier, rabbit skin showed lower drug diffusion rates compared to cellophane membrane.

Later (Gupta et al., 2007) designed proniosomal Transdermal captopril System using various ratios of sorbitan fatty acid esters, cholesterol and lecithin by coacervation phase separation method. The Proniosomes showed 66.7 – 78.7% encapsulation yield. In vitro studies showed prolonged release of entrapped captopril. Higher drug retention was reported at refrigerated conditions. With the above rationale the present study was planned to formulate proniosomal gels of lisinopril. The drug and excipients compatibility studies was planned to carry out by FTIR spectra, Formulation of proniosomal transdermal gels of Lisinopril dihydrate by using lecithin, cholesterol and surfactants (figure 1), Evaluation by determination of pH, Viscosity, Vesicle size analysis, Rate of spontaneity, Encapsulation efficiency, *In vitro* skin permeation studies and Stability studies.

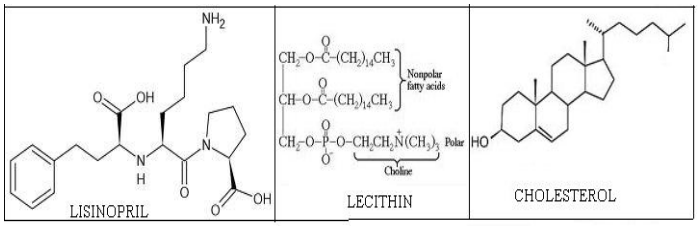

Fig 1. Chemical structures of Lisinopril, Lecithin and Cholesterol.

MATERIAL AND METHODS

Lisinopril dihydrate was procured from Aurobindo Pharma Pvt Ltd., Hyderabad, A.P. India. Lecithin was obtained from Cipla Limited, Maharastra, India. Cholesterol was purchased from Rolex Laboratory, Mumbai. Glycerin (Sigma Laboratory, Mumbai), Span (Rolex Laboratory, Mumbai.), Sodium chloride (Rasayana Chemicals, Hyderabad), Sodium hydroxide (Thomus Baker Chemicals, Mumbai), Potassium dihydrogen ortho phosphate (Finar Chemicals Ltd., Ahamedabad). All other Polymers, chemicals and solvent ethanol used in the investigation were of LR grade.

Reagents Preparation (Indian pharmacopoeia., 2007)
Phosphate buffer: Place 250 ml of 0.2M KH_2 PO_4 in 1000 ml volume metric flask. Add 195.5 ml of 0.2 ml NaoH and make up the volume. *0.2M Potasium di hydrogen phosphate:* 27.218 g of KH_2 PO_4 in water and dilute with water to 1000 ml. 0.2M NaoH: 8g of NaoH in 1000 ml. *Normal Saline:* Dissolve 0.9 % w/v NaCl in 100 ml purified water.

Compatibility Studies

The compatibility between drug and excipients is determined by using FTIR spectrophotometer.

Preparation of calibration curve

Lisinopril stock solution 1mg/ml was prepared and diluted suitably and 10 µg/ml was subjected to wave length scan over 200 to 400 nm, the drug was later quantified by measuring the absorbance at 207 nm in phosphate pH 7.4.

Preparation Of Transdermal Proniosomal Gel

Proniosomal gel is prepared by a coacervation phase separation method. Precisely weighed amounts of surfactant, lecithin, cholesterol and drug are taken in a wide mouthed glass vial of 0.5 ml capacity and alcohol (0.5 ml) is added to it. After warming, all the ingredients are mixed well with a glass rod, the open end of glass bottle is covered with a lid to prevent the loss of solvent form it and warmed on water bath at 60-70°C for about 5 min until the surfactant mixture is dissolved completely. Then the aqueous phase (0.1% glycerol solution) is added and warmed in water bath till a clear solution is formed which is converted into Proniosomal Gel on cooling. The gel so obtained is preserved in the same glass bottle in dark condition for characterization. Various formulations was developed as indicated in table 2 (Kumar, et al., 2003).

Table 2. Composition of the Lisinopril Proniosomal Gel formulations.

Formulation Code	Lisinopril (mg)	Surfactant Span (S)	Surfactant Ratio (mg)	Lecithin (mg)	Cholesterol (mg)
GF_1	10	S20:S40	500:500	100	100
GF_2	10	S20:S60	500:500	100	100
GF_3	10	S20:S80	500:500	100	100
GF_4	10	S40:S60	500:500	100	100
GF_5	10	S40:S80	500:500	100	100
GF_6	10	S60:S80	500:500	100	100

Evaluation of Proniosomal Gel pH and Viscosity

The pH of the gel is measured using pH meter before and after incorporation of the drug. Viscosity of the gel is determined using a Brook field viscometer (Gopala Krishna Murthy et al., 2008).

Vesicle size analysis

Hydration of Proniosomal gel (100 mg) is done by adding saline solution (0.9% NaCl solution) in a small glass vial with occupation shaking for 10 mins. The dispersion is observed under optical microscope 100X magnification. The size of 100 vesicles was measured using stage micrometer.

Rate of Spontaneity

The number of niosomes formed after hydration of proniosomes for 15-20 min. Approximately 10 or 20 mg of Proniosomal gel is transferred to glass bottle and spread uniformly around walls. 2ml of saline s added along the walls and left aside for 20 min. then a drop was withdrawn and place on Neubauers chamber to count the number of vesicles. The number of noisomes eluted from Proniosomes was counted.

Encapsulation efficiency

The proniosomal gel 100 mg is dispersed in distilled water and warmed a little for the formation of niosomes. Then the dispersion is centrifuged at 18000 rpm for 40 minutes 5°C. The clear fraction is used for the determination of free drug at 207 nm spectrophotometrically. The percentage encapsulation efficiency is calculated from the equation.

% encapsulation efficiency = { 1 - (unencapsulated drug / total drug)} × 100

***In vitro* diffusion studies**

In vitro diffusion studies of proniosomal gel was performed in Franz diffusion cell that has receptor compartment with an effective volume approximately 60 ml and an effective surface area of permeation of 3.14 sq cms. The egg membrane or cellulose membrane was mounted between the donor and receptor compartment. A weighed amount of Proniosomal gel is placed on one side of the skin the receptor medium was phosphate saline buffer pH 7.4. The receptor compartment surrounded by a water jacket to maintain the temperature at 37± 0.5°C. Heat is provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirrer by a Teflon coated magnetic bead fitted to a magnetic stirrer. At each sampling interval, sample is withdrawn and is replaced by equal volume of fresh receptor fluid on each occasion. Samples withdrawn were analyzed spectrophotometrically at 207 nm (Jain et al., 2003).

Stability studies

The stability studies were conducted according to ICH guidelines. The stability of vesicles to retain the drug was assessed by keeping the proniosomal gel at three different temperature conditions like refrigeration temperature (4-8^{0}C), room temperature (25±2^{0}C) in aluminum foil sealed glass vials. The samples were withdrawn at different time intervals over a period of one month and they were observed visually and under optical microscope for the change in consistency and appearance of drug crystals upon storage point and drug leakage from the formulations by analyzing drug content.

Drug Release kinetics

The mechanism of drug release from the gels was studied by subjecting *in vitro* diffusion studies in to different kinetic equations as follows **(**Higuchi T., 1963).

Zero order kinetics

$$At = A_0 - K_{0t}$$

Where, At is drug release at time‘t’. Ao is initial drug concentration. K_0 is Zero order rate constant (hr^{-1}) when the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys zero- order equal to K_0.

First order Kinetics

Predicted by equation;

$$Log\ C = log\ C_o - Kt / 2.30.$$

Where, C is amount of drug remained at time‘t’, C_o is Initial amount of drug and K is First order rate constant (hr^{-1}). When the data is plotted as log cumulative percent drug remaining versus time yields a straight line indicated that the release follow first order kinetics. The constant ‘k’ can be obtained by multiplying 2.303 with the slope values.

Higuchi model

Drug release from the matrix device by diffusion has been described by diffusion equation;

$$Q = [D€ /\tau(2A-€Cs)Cs\ t\ ]^{1/2}$$

Where, Q is amount of drug released at time 't', D is diffusion coefficient of the drug in the matrix, A is total amount of in unit volume of matrix, Cs is the solubility of drug in the matrix, € is porosity of the matrix, τ is Tortuosity, t is time (hrs) at which 'q' amount of drug is released. Above equation may be simplified if one assume that 'D', 'Cs' and 'A' are constant. Then equation become $Q=Kt^{1/2}$ When the data is plotted according to equation i.e. cumulative drug release versus square root of time yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to 'K'.

Korsemeyer peppa's equation model

To the study the mechanism of drug release from the formulation, the released data were fitted to exponential equation which is often used to describe the drug release behavior from polymeric system.

$$Mt/M_{\alpha}=Kt^{n}$$

Where, Mt/M_{α} is the fraction of drug released at a time 't', K is the constant term incorporating the structural and geometrical characteristics of the drug/ polymer system, n is diffusion exponent related to the mechanism of the release. Above equation can be simplified by applying log on both side**;**

$$Log\ Mt/M_{\alpha} = Log\ K + n\ Log\ t.$$

When the data is plotted as log of drug released versus log time, yield a straight line with a slope equal to 'n' and the 'K' can be obtained from y intercept **(**Korsmeyer et al., 1983).

RESULTS AND DISCUSSION

Preformulation studies showed the absorption maxima for Lisinopril at 207 nm and the developed Spectrophotometric method obeyed beer's law with linearity range of 2-20 µg/ml figure 2. Later the drug along with the ingredients showed no change in any characteristic peak in preliminary compatibility studies revealed that there no interaction between Lisinopril and excipients which was as evident from FTIR spectral studies.

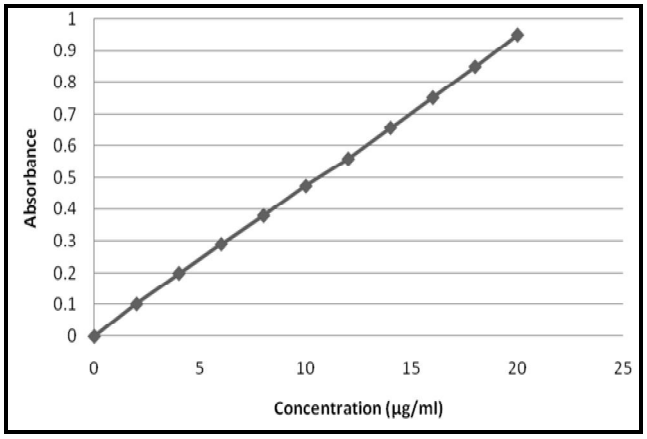

Fig 2. Calibration curve of Lisinopril dehydrate.

Proniosomal gels were prepared by coacervation phase separation method. The formulations were studied for physical characteristics like determination of pH and determination of viscosity and are found to be within the acceptable limits as indicated in table 3.

Table 3. Physicochemical characterization studies of Lisinopril proniosomal gels.

S. No	Formulation Code	pH	Viscosity (CPS)	Vesicle Size in µm	Encapsulation Efficiency
1	GF1	7.31	1625.70	26.23	78.21
2	GF2	7.15	1590.66	24.16	77.89
3	GF3	7.05	1375.15	21.75	74.35
4	GF4	7.14	1611.22	23.17	76.27
5	GF5	7.13	1495.26	22.55	73.58
6	GF6	7.22	1578.02	25.33	66.69

It was observed that the gel formulation showed good spreadability, and viscosity. Determination of vesicle size was found to be 20.10-26.23µm. Size was reduced when the dispersion was agitated. For spontaneity studies, the formulation was treated with ethanol, propanol, butanol and Isopropanol. It was found that niosomes containing isopropanol and butanol were formed more spontaneity than niosomes containing propanol and ethanol due to faster phase separation of isopropanol and butanol due to their lower solubility in water. The morphology of proniosomal gel was studied using optical microscopy revealed that niosomes were spherical and homogenous as shown in figure 3.

In vitro diffusion studies of Proniosomal Transdermal Gel studies showed the cumulative percentage permeation 85.54 %, 83.68 %, 80.42 %, 74.70 %, 75.86 %, 71.81 % in 24 hrs from the formulations GF_1, GF_2, GF_3, GF_4, GF_5, GF_6 respectively as showed in table 4. The stability studies showed that proniosomal gels were stable at 4 to 8^0C and 25 ± 2^0C and no leakage of drug from proniosomes and was found stable at these temperatures. All formulations from GF_1 to GF_6 showed zero order drug permeation kinetics by drug release mechanism though diffusion of drug from proniosomal gels. Graphical representation was given in figure 4. The above results indicated that the proniosomal gels of could be formulate for controlled release of Lisinopril and the formulations would be used for controlled release once a day formulation.

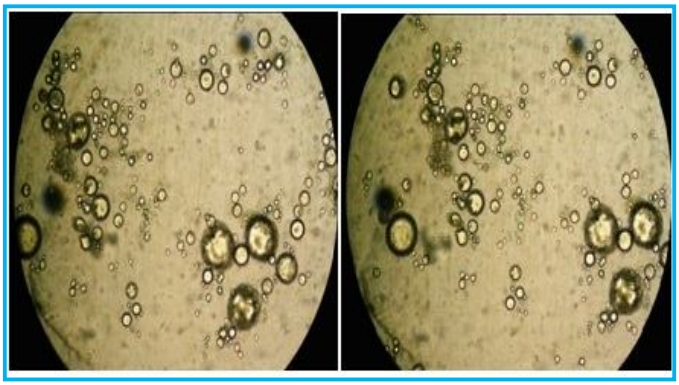

Fig 3: Proniosomes of Lisinopril under optical microscope with uniform size range.

Table 3. Percent drug permeated and *in vitro* kinetics of Lisinopril proniosomal gels.

Formulation	% Dug permeated	Regression			Korsemeyer	
		Zero order	First order	Higuchi	Slope	Regression
GF1	85.54	0.9961	0.9594	0.9618	0.3539	0.6852
GF2	83.68	0.9955	0.9691	0.967	0.3346	0.9439
GF3	80.42	0.9913	0.9862	0.9691	0.9921	0.9418
GF4	74.70	0.9929	0.9824	0.9638	0.9814	0.9407
GF5	75.86	0.9907	0.9710	0.9917	0.6549	0.7264
GF6	71.81	0.9599	0.9808	0.9897	0.6366	0.7154

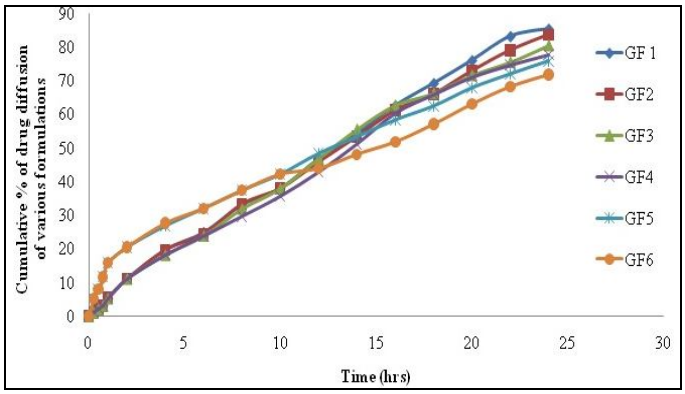

Fig 4: *In vitro* drug diffusion of Lisinopril from various proniosomal gel formulations.

CONCLUSION

The Transdermal Proniosomal Gels showed controlled drug release properties. The results of the present study indicated that Lisinopril dihydrate proniosomal gel containing lecithin, cholesterol and in combination of surfactants like span 20, 40, 60, 80 sustained release of drug over a period of 24 hrs for the management of hypertension. The proniosomal gel system has shown potential for delivery of anti hypertensive drug candidate Lisinopril dihydrate. The proniosomal gel could be an effective alternative vehicle for delivering the drug through transdermal route to avoid side effects associate with oral route.

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