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DESIGN AND *IN-VITRO* EVALUATION STUDIES OF TELMISARTAN LIPOSOMAL FORMULATIONS

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ABSTRACT
The aim of the present study was to develop a liposomal gel formulation for antihypertensive drug telmisatran. Liposomal carriers are well known for their topical drug delivery system with an advantage to overcome serious gastrointestinal complications for steroidal or non steroidal drugs given in oral route. Liposomes with various concentrations of cholesterol were prepared using thin film hydration technique (vacuum rotatory evaporator). The liposomal formulation was incorporated in gel (carbopol) and characterized. The SEM analysis showed surface morphology of liposomal formulation was achieved. The FTIR analysis showed there is no specific interaction between drug and excipients. The in-vitro studies revealed that liposomal gel formulation exhibits increased permeation showing sustain. The future studies are warranted to develop commercial liposomal gel formulation for the treatment of hypertension.

KEYWORDS: Liposomes, Telmisatran, FTIR & in-vitro studies.

INTRODUCTION
Telmisartan is a nonpeptide angiotensin-II receptor (type AT1) antagonist. It blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin-II by selectively blocking its binding to the AT1 receptor in adrenal gland and smooth muscles of vasculature.[1,2] In the past few decades, considerable attention has been focused on the development of new drug delivery system (NDDS). The NDDS should ideally fulfill two prerequisites. Firstly, it should deliver the drug at a rate directed by the needs of the body, over the period of treatment. Secondly, it should channel the active entity to the site of action. Conventional dosage forms including prolonged release dosage forms are unable to meet none of these. At present, no available drug delivery system behaves ideally, but sincere attempts have been made to achieve them through various novel approaches in drug delivery. In recent years, vesicles have become the vehicle of choice in drug delivery. Lipid vesicles were found to be of value in immunology, membrane biology, diagnostic techniques, and most recently, genetic engineering. Vesicles can play a major role in modelling biological membranes, and in the transport and targeting of active agents. Vesicular drug delivery reduces the cost of therapy by improved bioavailability of medication, especially in case of poorly soluble drugs. They can incorporate both hydrophilic and lipophilic drugs. These systems delay drug elimination of rapidly metabolizable drugs and function as sustained release systems and solve the problems of drug insolubility, instability and rapid degradation. Consequently, a number of vesicular delivery systems such as liposomes, proliposomes, transferosomes, pharmacosomes, niosomes or proniosomes etc, were developed.[3] Most commonly used materials for the formation of vesicles are phospholipids cholesterol and non-ionic surfactants. Vesicular system offers number of advantages in drug delivery through the skin such as biocompatibility, non-toxicity, incorporated both hydrophilic and lipophilic drugs, controlled drug delivery rate and extent, act as a depot formation for sustained release of drug, increased permeation of drugs through the skin and penetration enhancer because of their unique composition etc.[4,5] Liposome can be defined as "a colloidal, vesicular structures composed of one or more lipid bilayers surrounding a number of aqueous compartments".[6] Liposomes can be composed of naturally-derived phospholipids with mixed lipid chain like egg phosphatidylethonalimine or of pure components like DOPE (dioleolylphosphatidyl ethanolamine) and cholesterol.[7] A number of evidences demonstrated the ability of liposomes to enhance the efficiency of drug delivery via several routes of administration.[8] Liposome as a vesicular system offers a number of advantages,

such as biocompatibility, nontoxicity and flexibility, protection from the inactivating effect of external conditions, unique ability to deliver the pharmaceutical agents into cells or even inside individual cellular compartments. Apart from the given advantages, liposomes are associated with the some limitations such as high production cost, prone to leakage and short half life.[9,10]

MATERIAL AND METHODS

Materials: Telmisatran was obtained from Dr. Reddy's Laboratories, Hyderabad. Cholestrol and phospholipids were purchased from S. D. Fine Chemicals, Mumbai. Chloroform and methanol were procured from Merck Laboratories, Mumbai. Carbopol 934 and triethanolamine were obtained from Yarrow chemicals, Mumbai, India.

Methods

Pre formulation studies

Analytical Method Development

Procedure: For the preparation of calibration curve stock solution was prepared by dissolving 100 mg of accurately weighed drug in 100ml of methanol (1mg/ml). Further 1ml of the stock solution was pipette out into a 100 ml volumetric flask and volume was made up with phosphate buffer (7.4 pH). From this stock solution concentrations were prepared and subjected for UV scanning in the range of 200-400nm using double beam UV spectrophotometer. The absorption maximum was obtained at 296nm with a characteristic peak. The results are shown in table 1 & in fig 1.

Drug-excipient interaction studies by FTIR: The compatibility between pure drug and cholesterol were detected by FTIR spectra. The potassium bromide pellets were prepared on KBR press. To prepare the pellets, the solid powder sample was grounded together in a mortar with 100 times quantity of KBR. The finely grounded powder introduced into a stainless steel die. The powder was pressed in the die between polished steel anvils at a pressure of about 10t/in2. The spectra's were recorded over the wave number of 4000 to 600 cm-1.

Preparation of liposome formulations

Preparation of Telmisatran loaded liposome formulations: The liposomal formulations containing telmisatran was prepared by film deposition on carrier method using rotary evaporator. The optimization of telmisatran liposomes was done by preparing the different formulations by varying the concentration of cholesterol placed in 100ml round bottom flask which was held at 60-70^{0}C temperature and the flask was rotated at 80-90rpm for 30min under vacuum. After complete drying the temperature of water bath was lowered to 20-30^{0}C.

Telmisatran (40mg), cholesterol and phospholipid were dissolved in mixture of organic solvents (chloroform: methanol, 6:4, v/v) and 5ml of aliquot of organic solution was slowly introduced into the flask via the solvent inlet tube. After complete drying second aliquot (5ml) was introduced. After complete dying, the vacuum was released and liposome vials were placed in a desiccator. The collected liposomes were transferred into glass bottles and stored at the freeze temperature.

Table. 2: Formulation table.

Excipient	F1	F2	F3	F4	F5	F6	F7	F8	F9
Telmisatran (mg)	40	40	40	40	40	40	40	40	40
Cholesterol (mg)	150	100	50	100	100	150	50	50	150
Phospholipid (Soya lecithin)	80	80	80	80	80	80	80	80	80
Chloroform (ml)	6	6	6	6	6	6	6	6	6
Methanol (ml)	4	4	4	4	4	4	4	4	4

Preparation of carbopol gel base: 1gm of carbopol 934 was weighed and dispersed in distilled water. Then, propylene glycol was added and the mixture was neutralized by drop wise addition of 1% triethanolamine. Mixing was continued until the transparent gel was obtained and allowed to swell for 24 hours. Similarly 2% and 3% carbopol gels were prepared.

Preparation of liposomal gels: Liposomes containing telmisatran entrapped drug was mixed into the 1% carbopol gel by using mortar and pestle, the concentration of liposomal gel being 1%. All optimized formulations were incorporated into different carbopol gels (1%, 2% and 3%).

Characterization of liposome formulations: Liposome formulations were characterized by following parameters

Surface morphology: The surface morphology of liposomal gel formulation was examined by scanning electron microscopy (SEM).

Drug content: Telmisatran content in liposomal formulation was assayed by an UV-visible spectrophotometer. Liposomal gel (100mg) was dissolved in 10ml methanol by shaking the mixture for 5mins. One ml of the resultant solution was taken and diluted to 10ml with methanol. Then, aliquots were withdrawn and absorbance was recorded at 294nm.

Entrapment efficiency: Separation of unentrapped drug from the liposomal formulation was done by centrifugation method. The entrapment efficiency of liposomal formulation was determined after hydration with distilled water. 10ml of phosphate buffer (pH 7.4) was added to liposomal formulations and then subjected

to sonication for 10 min using ultra sonicator. The liposomal suspension was subjected to centrifugation on a cooling centrifuge at 15000rpm for 30mins for the separation of unentrapped drug. The clear supernatant (1ml) was taken and diluted to 10ml with buffer and absorbance was recorded at 294 nm using UV-visible spectrophotometer. The percentage drug in the each formulation was then calculated.

Entrapment efficiency (%) = $\frac{Ct-Cf}{Ct} \times 100$ where, Ct – concentration of total drug, Cf – concentration of free drug.

Yield of liposomal formulations: The yield of liposomal formulations was calculated using the formula Percentage yield = (Total weight of proliposomes) / (total weight of drug + weight of added materials) × 100.

Characterization of gel

Physical appearance: All prepared gel formulations have been observed for their visual appearance, such as transparency, colour, texture, grittiness, greasiness, stickiness, smoothness, stiffness and tackiness. The prepared gels were also evaluated for the presence of any particles. Smears of gels were prepared on glass slide and observed under the microscope for the presence of any particle or grittiness.

pH of formulation: pH measurement of the gel was measured by using a digital pH meter. The glass electrode completely dipped into the gel system. The observed pH values were recorded for all formulations (F1-F9) in triplicates.

Rheological properties: The rheological properties of prepared gels were estimated using a Brookfield viscometer pro D II apparatus, equipped with standard spindle LV1 with 61 marking. Sample holder of the Brookfield viscometer was filled with the gel sample, and then spindle was inserted into sample holder. The spindle was rotated at 100rpm. All the rheological studies were carried out at room temperature.

Homogeneity: The homogeneity of telmisartan liposomal gels was checked by visual inspection. In this regard the gels were filled into narrow transparent glass tubes and were checked in light for the presence of any particulate or lump.

Drug Content: For determination of drug content, accurately weighed quantity (1gm) of gel equivalent to 40mg of Telmisatran was dissolved in phosphate buffer (pH 7.4) and analyzed by UV-Vis Spectrophotometer at 230nm.

***In vitro* studies**

Percent amount of drug release from semi permeable membrane: Franz diffusion cell was used for the *in vitro* drug release studies. Semi permeable membrane was placed between donor and receptor chamber of diffusion cell. Receptor chamber was filled with freshly prepared 7.4 pH phosphate buffer. Liposomal gel equivalent to 1gm was placed on semi permeable membrane. The Franz diffusion cell was placed over magnetic stirrer with 100rpm and temperature was maintained at 37 ± 1^0C. 1ml of samples were withdrawn periodically and replaced with fresh buffer. The withdrawn samples were periodically diluted and analyzed for drug content using UV visible spectrophotometer at 294nm.

***In-Vitro* Release Kinetics:** There are several linear and non-linear kinetic models to describe release mechanisms and to compare test and Reference dissolution profiles are as follows:

Zero-order model: Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equation

$Q_0 - Q_t = K_0t$

Rearrangement of equation yields

$Q_t = Q_0 + K_0t$

Where, Qt is the amount of drug dissolved in time t, Q_0 is the initial amount of drug in the solution (most times, $Q_0 = 0$) K_0 is the zero order release constant expressed in units of concentration/time.

To study the release kinetics, data obtained from in vitro drug release studies were plotted as cumulative amount of drug released versus time.

First order model: This model has also been used to describe absorption and/or elimination of some drugs, although it is difficult to conceptualize this mechanism on a theoretical basis. The release of the drug which followed first order kinetics can be expressed by the equation: Dc/dt=-kc

Where, K is first order rate constant expressed in units of $time^{-1}$.

Above Equation can be expressed as: $\log C = \log C_0 - Kt / 2.303$

where, C_0 is the initial concentration of drug, k is the first order rate constant, and t is the time.

The data obtained are plotted as log cumulative percentage of drug remaining vs. time which would yield a straight line with a slope of -K/2.303.

Higuchi model: The first example of a mathematical model aimed to describe drug release from a matrix system was proposed by Higuchi in 1961.

In a general way it is possible to simplify the Higuchi model (generally known as the simplified Higuchi model): $f\ t = Q = K_Ht^{1/2}$ where, K_H is the Higuchi dissolution constant.

The data obtained were plotted as cumulative percentage drug release versus square root of time.

Korsmeyer-Peppas model: Korsmeyer et al. (1983) derived a simple relationship which described drug release from a polymeric system equation To find out the mechanism of drug release, first 60% drug release data were fitted in Korsmeyer-Peppas model $Mt / M\infty = Kt^n$ Where, $Mt / M\infty$ is a fraction of drug released at time t, k is the release rate constant and n is the release exponent. In this model, the value of n characterizes the release mechanism of drug as described below. To study the release kinetics, data obtained from *in vitro* drug release studies were plotted as log cumulative percentage drug release versus log time.

Hixson-Crowell model: Hixson and Crowell (1931) recognized that the particles regular area is proportional to the cube root of its volume.

They derived the equation
$W_0^{1/3} - W_t^{1/3} = \kappa t$ Where, W_0 is the initial amount of drug in the pharmaceutical dosage form, W_t is the remaining amount of drug in the pharmaceutical dosage form at time and κ (kappa) is a constant incorporating the surface-volume relation.

The equation describes the release from systems where there is a change in surface area and diameter of particles or tablets. To study the release kinetics, data obtained from in vitro drug release studies were plotted as cube root of drug percentage remaining in matrix versus time.

RESULTS AND DISCUSSION

Pre-Formulation studies: Construction of calibration curve using phosphate buffer (pH7.4)

Table. 1: Concentration and absorbance of drug.

S. No	Concentration	Absorbance
1	0.5	0.2251
2	1	0.4195
3	1.5	0.6407
4	2	0.8013
5	2.5	0.9971

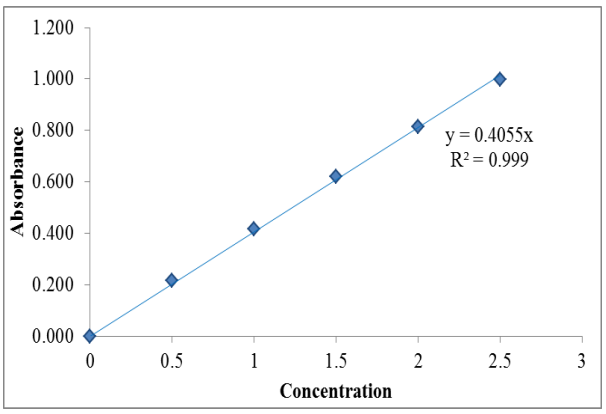

Fig. 1: Standard curve of Telmisatran in phosphate buffer (pH 7.4).

Drug- excipients compatibility studies using FTIR

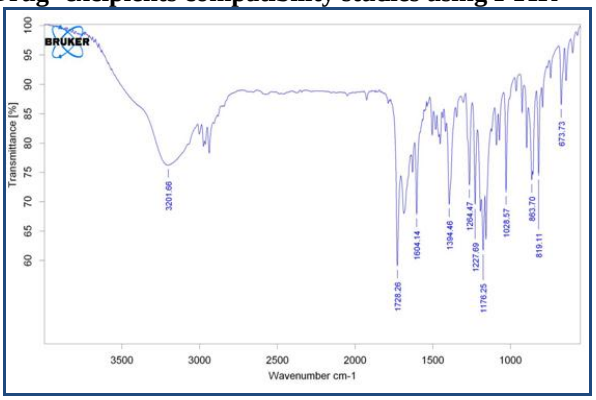

Fig. 2: FTIR spectra of Telmisatran pure drug.

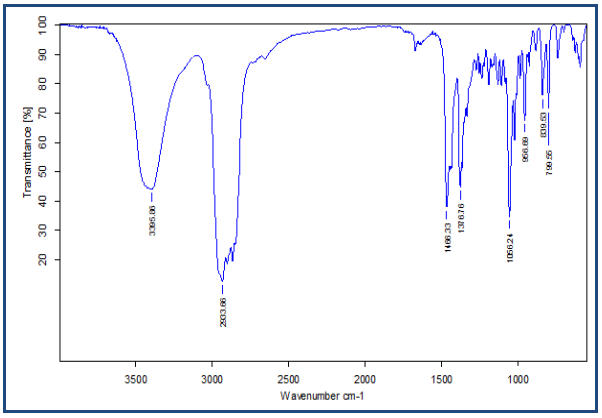

Fig. 3: FTIR spectra of cholesterol.

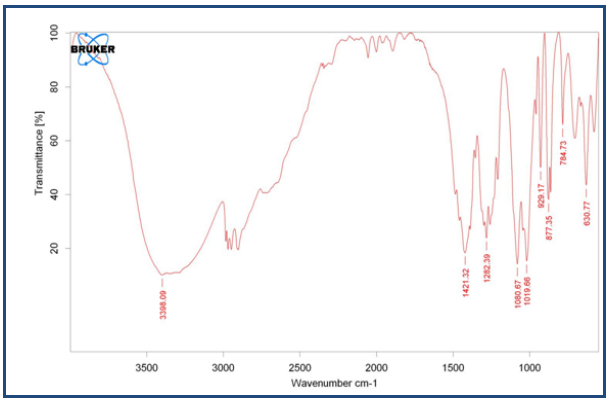

Fig. 4: FTIR spectra of phospholipid.

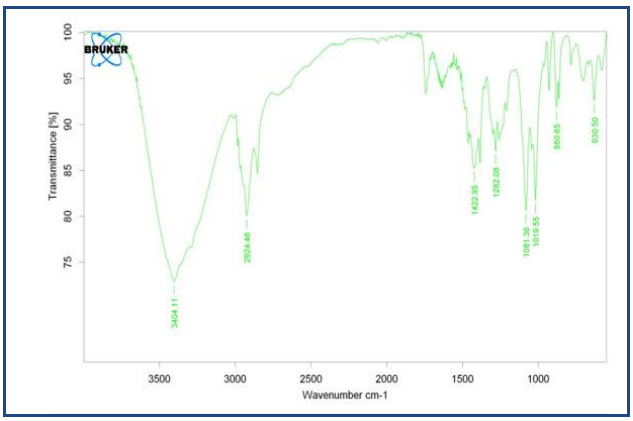

Fig. 5: FTIR spectra of optimized formulation (F4).

Table. 3: FTIR interpretation analysis for pure drug Characteristic peaks.

S. No	Wave number in formulation (cm^{-1})		Characteristic Wave number range (cm^{-1})	Bond nature and bond attributed
	Pure drug	Optimized formulation		
1	3201	3404	3300-2500	O-H stretching Carboxylic acids
2	1720	1753	1760-1690	C=O stretching Carboxylic acids
3	1600	1422	1600-1400	C-C stretch in ring aromatics
4	1264	1282	1320-1000	C-O stretch Esters
5	883	880	900-675	C-H oop aromatics
6	673	680	1000-650	=C-H bend Alkenes

On comparison of IR spectra of liposomes, pure Telmisatran drug, cholesterol and phospholipid it was clear that, there was no significant interaction of encapsulated drug with the phospholipid.

Characterization liposomes and liposomal gels

Determination of entrapment efficiency

Table. 4: Entrapment efficiency of liposome formulations.

S. No	Formulation	Entrapment efficiency ± SD
1	F1	94.9±0.244
2	F2	85.12±1.48
3	F3	91.02±0.613
4	F4	96.5±0.205
5	F5	92.7±0.249
6	F6	94.1±0.509
7	F7	88.1±2.19
8	F8	89.2±0.817
9	F9	86.02±2.90

A positive correlation was observed for both variables phospholipid and cholesterol. Results show that with increase in the concentration of phospholipid and cholesterol entrapment efficiency found to be increased. In the present study, the observed entrapment efficiency for all batches of telmisatran liposome formulation was in the range of 72 to 90%.

Drug content estimation

Table. 5: Drug content of liposomal formulations.

S. No	Formulation	% Drug content ± SD
1	F1	95.03±0.543
2	F2	86.4±0.734
3	F3	93.7±0.664
4	F4	96.8±0.249
5	F5	94.7±0.984
6	F6	94.8±0.860
7	F7	92.4±1.70
8	F8	90.6±0.748
9	F9	87.5±0.953

The telmisatran content in the liposomes were observed in the range of 86.4% to 96.8% at various drug to phospholipid ratios. From the above result F4 formulation showed maximum drug content when compared to other formulations.

Percentage yield of liposomes

Table. 6: Percentage yield of liposomal formulations.

S. No	Formulation	Percentage yield
1	F1	93.4±0.324
2	F2	90.7±0.534
3	F3	89.5±0.654
4	F4	95.4±0.123
5	F5	94.3±0.221
6	F6	94.8±0.212
7	F7	88.7±0.321
8	F8	89.3±0.187
9	F9	86.5±0.265

The results of % yield of various formulations were found to be in the range of 86.5 ± 0.265 to 95.4 ± 0.221 % as the drug to phospholipid ratio in liposomes was changed.

***In-vitro* studies**

Table. 7a: Cumulative percentage drug release of liposomal formulations.

Time (hr)	F1	F2	F3	F4	F5
0	0.00	0.00	0.00	0.00	0.00
1	25.85±0.010	24.07±0.031	21.38±0.159	22.92±0.030	20.11±0.047
2	34.35±0.156	28.70±0.021	30.74±0.026	38.19±0.021	39.32±0.035
3	40.17±0.089	31.95±0.070	35.01±0.023	45.35±0.046	45.70±0.067
4	47.27±0.050	39.88±0.160	43.16±0.021	52.67±0.035	50.42±0.057
5	54.08±0.061	42.77±0.091	48.96±0.025	60.10±0.047	58.89±0015
6	60.40±0.055	49.43±0.046	52.81±0.061	68.00±0.070	64.83±0.072
7	76.05±0.046	55.47±0.051	55.61±0.075	80.42±0.046	69.73±0.046

Table. 7b: Cumulative percentage drug release of liposomal formulations.

Time (hr)	F6	F7	F8	F9
0	0.00	0.00	0.00	0.00
1	20.76±0.060	4.15±0.035	5.33±0.071	14.19±0.044
2	43.59±0.067	29.33±0.030	31.63±0.097	39.07±0.015
3	51.10±0.071	33.07±0.047	40.21±0.046	46.89±0.032
4	55.76±0.025	39.54±0.05	46.21±0.053	51.54±0.038
5	65.00±0.040	46.19±0.025	55.01±0.060	58.43±0.042
6	69.07±0.026	53.36±0.061	58.84±0.038	61.55±0.093
7	74.06±0.042	60.03±0.062	58.52±0.068	64.42±0.081

From the above results F4 formulation was selected as optimized one which is used for further study.

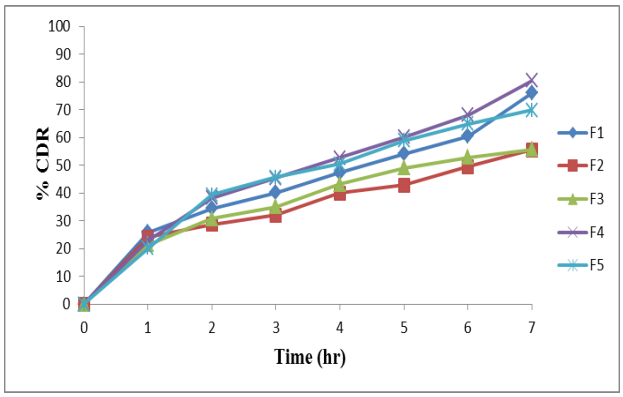

Fig. 6a: *In vitro* diffusion data of (F1-F5).

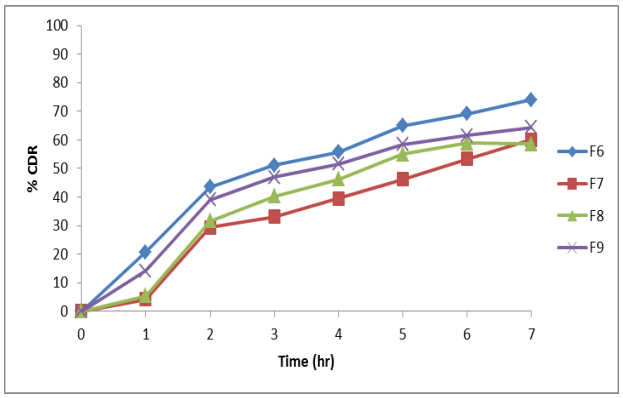

Fig. 6b: *In vitro* diffusion data of (F6-F9).

Surface Morphology: The surface morphology of liposome granules, pure drug was examined by scanning electron microscopy (SEM) and the images were photographed at 100 resolution.

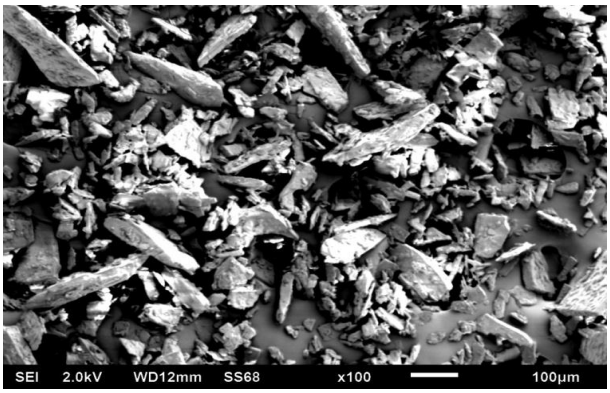

Fig. 7: SEM image of Phospholipid (Soya Lecithin).

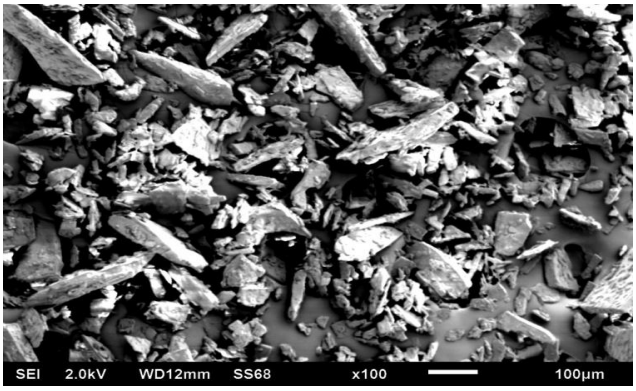

Fig. 8: SEM image of Telmisatran.

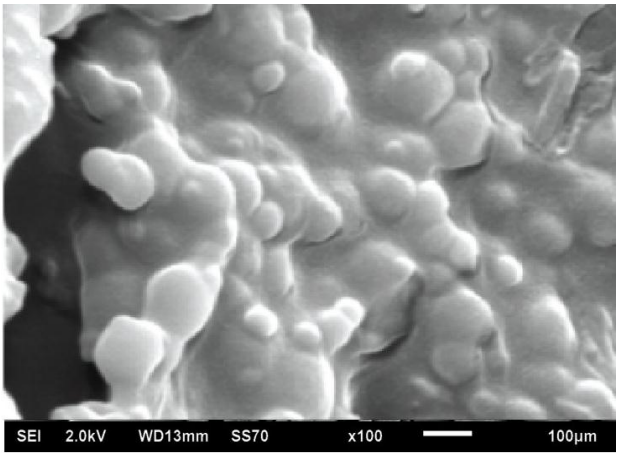

Fig. 9: SEM image of Telmisatran liposomal gel.

The surface morphology of liposomes was examined by scanning electron microscopy. The surface morphology of phospholipid and telmisartan drug showed crystalinity and the liposomal gel exhibited smooth surface.

Viscosity Measurement: Rheological studies revealed that 1% carbopol gel showing better rheological properties when compared to 2% and 3% carbopol gels. So 1% carbopol was used for preparation of liposomal gel. Viscosity of the gel was measured by Brookfield viscometer (LVDV II pro+). Viscosity of liposomal gel showed 1156cps at 100rpm.

pH Measurement: The pH of the developed formulation was in accordance with human skin pH rendering them more acceptable. The formulated liposomal gel was suitable for topical application. The pH values of liposomal gels were within the limits of 5.5 to 5.8.

Release Kinetics: Various mathematical models were used to evaluate kinetics and mechanism of drug release from liposomal gel formulation. Best model was selected for release data which showed high correlation coefficient (r) value. *In-vitro* drug release via semi permeable membrane was performed and release kinetics was calculated.

Table 8: Correlation coefficients (R^2) values of different kinetic models and Release Exponent (n)

S. No	Formulation	Zero order R^2	First order R^2	Higuchi model R^2	Peppa's model R^2	Release Exponent (n)
1	F1	0.9699	0.9889	0.9771	0.9802	0.257
2	F2	0.9972	0.9845	0.9583	0.9715	0.384
3	F3	0.9663	0.9899	0.9908	0.9916	0.418
4	F4	0.9905	0.9857	0.9889	0.9927	0.161
5	F5	0.9783	0.9949	0.9925	0.9959	0.325
6	F6	0.9875	0.994	0.989	0.9965	0.293
7	F7	0.9783	0.9819	0.9828	0.9859	0.502
8	F8	0.9926	0.9922	0.9812	0.9917	0.528
9	F9	0.982	0.9979	0.994	0.9948	0.405

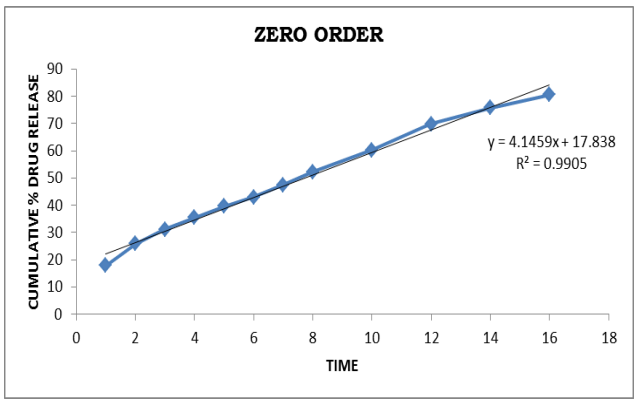

Fig. 10: Zero order plot of optimized liposomal formulation (F4).

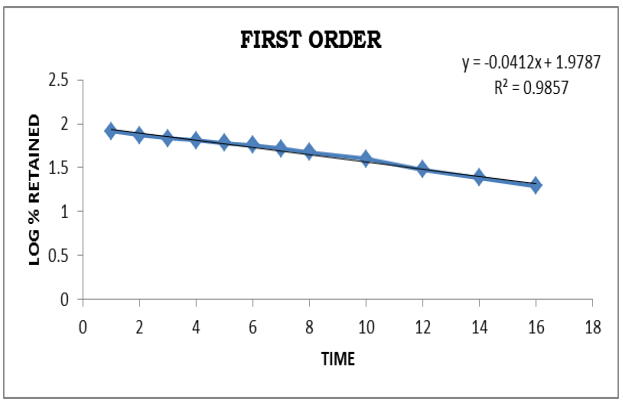

Fig. 11: First order plot of optimized liposomal formulation (F4).

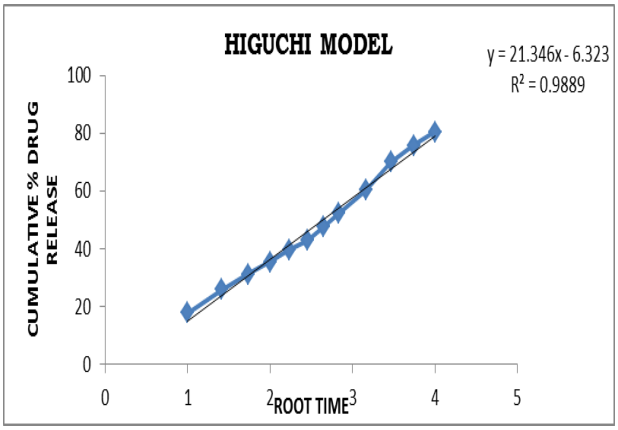

Fig. 12: Higuchi plot of optimized liposomal formulation (F4).

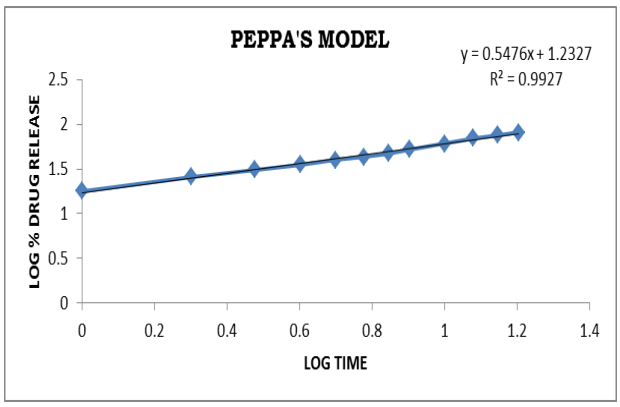

Fig. 13: Peppa's plot of optimized liposomal formulation (F4).

The results clearly show that the liposomal formulations were independent of the initial concentration following zero order kinetics which is ideal for topical drug delivery system. The release data showed regression value near to 1 and best fitted in Peppa's model indicating the drug delivery followed was Fickian diffusion.

CONCLUSION

In conclusion, a sustained delivery of telmisatran can be achieved by liposomal drug delivery system. Variables such as phospholipid and cholesterol have a profound effect on vesicle size and entrapment efficiency. Rheological studies of all liposomal gels gave clear idea of concentration of carbopol (1%) is require for preparation of stable gel formulation. *In-vitro* studies of liposomal gels encapsulating anti-hypertensive drugs were found to increase the skin permeation and deposition showing a sustain effect.

Phospholipids, being the major component of liposomal system, can easily get integrated with the skin lipids and maintain the desired hydration conditions to improve drug permeation. Fusion of lipid vesicles with skin contributed to the permeation enhancement effect. The phospholipid was found to have a significant influence on the lipid matrix of the stratum corneum, suggesting a disruption of the intercellular lipid lamellar structure and act as penetration enhancer. Hence as the phospholipid concentration was increased, it would increase the permeation of drug in to the skin. The properties of the liposomal gel will be beneficial in formulating the liposomes as a transdermal dosage form.

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