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PREPARATION AND EVALUATION OF PRONIOSOMAL ROSUVASTATIN GEL FOR TRANSDERMAL DELIVERY

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ABSTRACT

The main aim of the current study was to formulate and evaluate a stable proniosomal gel using rosuvastatin to avoid problems associated with conventional delivery system such as limited permeation, low dissolution and bioavailability and also to improve bioavailability and hypocholesteromic effect in blood vessels.The preformulation studies were performed to know the physico-chemical and mechanical properties of rosuvastatin encapsulated in proniosomes. The drug-excipient compatability studies were conducted to characterize the drug rosuvastatin present in proniosomes. Eight formulations were taken as trial formulations for optimizing the final formulation. Finally, optimized PGR1 formulation has been selected as the final formulation containing Span 80 with Soya. In-vitro release studies, drug content and drug entrapment studies were performed to evaluate the optimized formulation. FTIR spectra of rosuvastatin indicates there was no chemical alteration of the drug characteristics. The calibration range of rosuvastatin was found to be 5-35µg/ml at λmax of 248 nm. The percentage drug content of optimized formulation was found to be 96.7%, shown higher % drug entrapment i.e.92.8%. In vitro drug release of PGR1 was found to be 96.3% for 24hr that reveals sustainability of drug release to improve bioavailability of rosuvastatin in systemic circulation. Finally, it was concluded that the Proniosomal rosuvastatin gel was found to be a stable alternative transdermal delivery approach to enhance the bioavailability with hypocholestrolemic effect.

KEYWORDS: Novel Drug Delivery System, Transdermal drug delivery, vesicular drug delivery, Proniosomes, Rosuvastatin.

INTRODUCTION

In the past few decades, considerable attention has been focused on the development of a new drug delivery system (NDDS). The NDDS can deliver the drug at a rate directed by the needs of the body over the period of treatment and also effectively releases the active entity to the site of action. In recent times, the transdermal route vied with the oral route as the most successful innovative research area in drug delivery[1].Transdermal drug delivery through skin to the systemic circulation provides a convenient root of administration for variety of clinical indications that include minimization of drug degradation and loss, preventing harmful side-effects and increases bioavailability. In addition, Transdermal route offers several potential advantages over conventional routes like avoidance of first pass metabolism, utility of short half-life drugs, avoiding the fluctuation in drug levels, improving physiological and pharmacological response, and most importantly, it provides patient convince. But one of the major problems in transdermal drug delivery is the low penetration rate through the outermost layer of skin.Vesicular drug release systems have the potential of overcoming the skin barrier problems, as these are made up of bilayered lipid vesicles, consisting primarily of phospholipids and cholesterols[2].Vesicular drug delivery is an approach which increases bioavailability of encapsulated drug. e.g. Liposomes, niosomes, proniosomes and proliposomes.The proniosomal approach is comparably better than niosomes, which reduces the problems of physical stability associated with niosomes during the storage and sterilization of dosage forms[3-8].

Rosuvastatinis chemically known as (3R,5S,6E)-7-[4-(4-fluorophenyl)-2-(N-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid, prevents the biosynthesis of cholesterol there by reduces the risk of cardiovascular diseases. Rosuvastatin is classified as 'non-CYP3A4'-metabolised statins, acts as an inhibitors on HMG-CoA reductase catalyticreations[9]. In addition, it induces the arrest of developmental renal toxicity[10].James W Blasetto et al. reveals that a consistent efficacy of rosuvastatin has been observed against other diseases likeatherosclerosis, obesity, hypertension and type 2 diabetes[11]. Limited studies were reported to prepare proniosome gels using statins.Dalia S Shaker et al. formulated proniosome gel as a transdermal drug delivery using simvastatin to improve the bioavailability and hypocholesteromic effect in blood vessels[12]. In the past,

there was no research was carried out on proniosomal gels using rosuvastatin drug administered through transdermal drug delivery system. Thus, we made an attempt to formulate and evaluate Proniosomal rosuvastatin gel for transdermal drug delivery.

MATERIALS AND METHODS

Materials: Rosuvastatin was procured from Chandra Labs Hyderabad, India. Soya Lecithin was purchased from Bright Laboratories,Hyderabad, India. Surfactants such as Span 60 and 80, tween 60 and 80were taken from Merck specialties pvt.limited (Mumbai). Volatile solvents like Ethanol and Chloroform were purchased from S.D.Fine Chemicals, (Mumbai). Carbopol-940 as a gelling agent was obtained from Research lab fine chem. Industries(Mumbai).

Instruments: FT-IR spectrophotometer (Thermo Nicolet, ALPHA-T-1020), UV-Visible spectrophotometer (Lab India, UV 3200), Hot air oven (Universal, Q-5247), Electronic balance (Shimadzu, AX-200), Centrifuge (Remi, TROI), Probe sonicator (Heldolph, VCX750), PH meter (Labindia, SAB 5000), Magnetic stirrer (Remi, 5MLH), Weighing balance (Shimadzu, ATX224), Homogenizer (Remi, RQT-124A).

Pre-Formulation Studies

The physico-chemical and mechanical properties of drug substances are characterized by preformulation studies.

FT-IR studies for drug and excipients compatibilities[13] FTIR spectra were recorded with a Thermo Nicolet. Japan in the range 400–4000 cm−1 using a resolution of 4 cm−1 and 16 scans. KBrpowder was mixed with sample to obtain self-supporting disks. In addition, liquid samples formulations were analyzed to form a thin liquid film between two KBr disks.

Calibration curve of Rosuvastatin: 100 mg of rosuvastatin was dissolved in 100 ml Phosphate buffer pH 6.8 to give a stock concentration of 1000 µg/ml and then, the stock solution was diluted to obtain working standards in the concentrationrangeof 5-35µg/ml of Pure sample of rosuvastatin.The absorbance values of all the samples were found at 248 nm wavelength using UV spectrophotometer. Finally, calibration curve was plotted to obtain the concentrationofrosuvastatin presentin the proniosomal gel.

Formulation Procedure[14]

Proniosomes were prepared by phase separation coacervation technique.Different formulae of proniosomal gel (PGR) were prepared by considering different types of Span and tween surfactants and these were coded as PGR1 to PGR8. All the formulation trials are shown in table1.

Precisely weighed amount of drug, soya lecithin, surfactant, and cholesterol in a specified ratio were taken in a dry, clean, wide mouth small test tube. A measured amount of ethanol (absolute alcohol) was added to test tube to dissolve the ingredients. A lid was placed on the test tube to prevent loss of solvent from it warmed over water bath at 67±3^{0}C for about 5 minute until the surfactant mixture was dissolved completely and then, the aqueous phase phosphate buffer saline (pH 6.8) was added and warmed on a water bath till a clear solution was formed and then,the mixture was allowed to cool down to room temperature till the dispersion was converted to proniosomal gel.

Table 1 Composition of different Proniosomal formulations

Ingredients(mg)	PGR1	PGR2	PGR3	PGR4	PGR5	PGR6	PGR7	PGR8
Rosuvastatin	20	20	20	20	20	20	20	20
Soya lecithin	450	450	450	450	225	225	225	225
Span 80	450	--	--	--	450	--	--	--
Span 60	--	--	--	450	--	--	--	450
Tween 60	--	450	--	--	--	450	--	--
Tween 80	--	--	450	--	--	--	450	--
Cholesterol	50	50	50	50	100	100	100	100
Ethanol (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Preparation of topical Proniosomal gel: As a vehicle for incorporation of Proniosomes for topical delivery, carbopol gels were prepared. Proniosomal aqueous dispersion was utilized for the formulation of topical gel. Gel polymer such as carbopol 940 was utilized to prepare proniosomal gel. 1.5g of carbopol- 940 powder was dispersed into vigorously stirred (stirred by magnetic stirrerRemi 5MLH) distilled water (taking care to avoid the formation of in dispersible lumps) and allowed to hydrate for 24 hrs. The dispersion was neutralized with tri ethanolamine to adjust the pH [6.8] by using pH meter (Lab India Sab 5000).Appropriate amount of proniosomes containing rosuvastatinwere then incorporated into gel-base with continuous stirring until homogenous formulation was achieved.

Evaluation of Topical Gel[15]

Formulated gel was evaluated for their physico-chemical properties, *in-vitro* release studies and drug content and drug entrapment studies.

Clarity: All formulations were observed for their clarity by visual inspection under black and white background and it was graded as follows; turbid: +, clear: ++, very clear (glassy): +++.

pH Determination: The pH of rosuvastatin gel formulation was determined by using digtal pH meterstandardized before with pH 4.0 and 7.0 using standard buffers. 1gram of gel was dissolved in 100ml of distilled water and then, average values of triplicate pH measurements were calculated.

Homogeneity: The homogeneity of all formulations was determined by visual inspection after the gels have been stored in the container for their appearance and presence of any aggregate.

Rheological Characterization: The rheological studies of samples were carried out with Brookfield Digital viscometer (LV DV-E model) using S-18 spindle number. All developed formulations were poured into the small sample adaptor rotated with increased angular velocity upto 100 rpm.

Drug content: Proniosomes equivalent to 20 mg were taken into a standard volumetric flask. They were lysed with 25 ml of methanol by shaking for 15 min.Then 10 ml of this solution was diluted to 100 ml with phosphate buffer 6.8. Aliquots were withdrawn and the absorbance was measured at 248 nm and drug content was calculated from the calibration curve.

The drug content was determined by using following equation:
Drug content = (concentration× volume taken) × conversion factor

Entrapment Efficiency[16]

accurately weighed 0.5 g of proniosomal gel was dissolved in 10 ml of phosphate buffer pH 6.8; the aqueous suspension was then sonicated. Niosomes containing Rosuvastatin were separated from un entrapped drug by centrifugation at 9000 rpm for 45 min at 4^{o}C. The supernatant was recovered and assayed spectrophotometrically using Shimadzu UV

spectrophotometer (Japan) at 248 nm. The encapsulation efficiency was calculated by the following equation.

% Encapsulation efficiency = {Total drug - (unencapsulated drug / total drug)} ×100

In vitro diffusion studies[17]: The in vitro diffusion study of prepared gel was carried out in Franz diffusion cell using through an egg membrane. Accurately weighed 1 GM ofrosuvastatin gel was spread uniformly on the membrane and then, The donor compartment was kept in contact with a receptor compartment and the temperature was maintained at 37±0.5^{0}C. The solution on the receptor side were stirred by externally driven Teflon coated magnetic bars at predetermined time intervals, pipette out 2 ml of solution from the receptor compartment at specified time intervals for up to 24hrs and immediately replaced with the fresh 2 ml phosphate buffer. The cumulative % release of drug was calculated against time.

KINETIC STUDIES [18]

An in-vitro release profile of all formulations were plotted as follows:

1. Cumulative percent drug release V/s. Time (Zero-order).
2. Cumulative percent drug release V/s. Square root of Time (Higuchi Model).
3. Log Cumulative percent drug retained V/s. Time (First-order).
4. Log Cumulative percent drug release in V/s. log Time (Korsmeyer-Peppas Model).

Zero order kinetics

Drug dissolution of pharmaceutical dosage forms that do not disaggregate and release the drug slowly, assuming that the area does not change and no equilibrium conditions are obtained can be represented by the following equation.

$$Qt = Qo + Ko\ t$$

Where Qt = amount of drug dissolved in time t, Qo = initial amount of drug in the solution and Ko = zero order release constant.

First order kinetics

The data of first order release kinetics was studied by following equation

$$Log\ Qt = log\ Qo + Kt / 2.303$$

In equation, Qt is the amount of drug released in time t, Qo is the initial amount of drug in the solution and K1 is the first order release constant.

Higuchi model

Higuchi developed several theoretical models to study the release of water-soluble and low soluble drugs incorporated in semisolids and or solid matrices. The drug particles dispersed in diffusion media was studied by the following equation:

$$Q\,t = KH.\ t\ 1/2$$

Where Q t = Amount of drug released in time t, K H = Higuchi dissolution constant.

Korsmeyer and Peppas release model

The release rate data was studied by the following equation

$$Mt / M = K.tn$$

Where Mt / M is the fraction of drug release, K is the release constant, t is the release time and n is the Diffusion exponent for the drug release.

A plot of log drug release verses log time will be linear with slope of n and intercept gives the value of log k

- n = 0.5 indicating pure fickian diffusion.
- n = 0.5-1 or 0.45-0.89 indicating non fickian diffusion ie. Drug release and the rate of solvent penetration are in the same range.
- n = 0.89 or 1 indicate zero order release which can be achieved when drug diffusion is rapid compared to the constant rate of solvent induced relaxation.(Grassi.M.,2005).

RESULTS

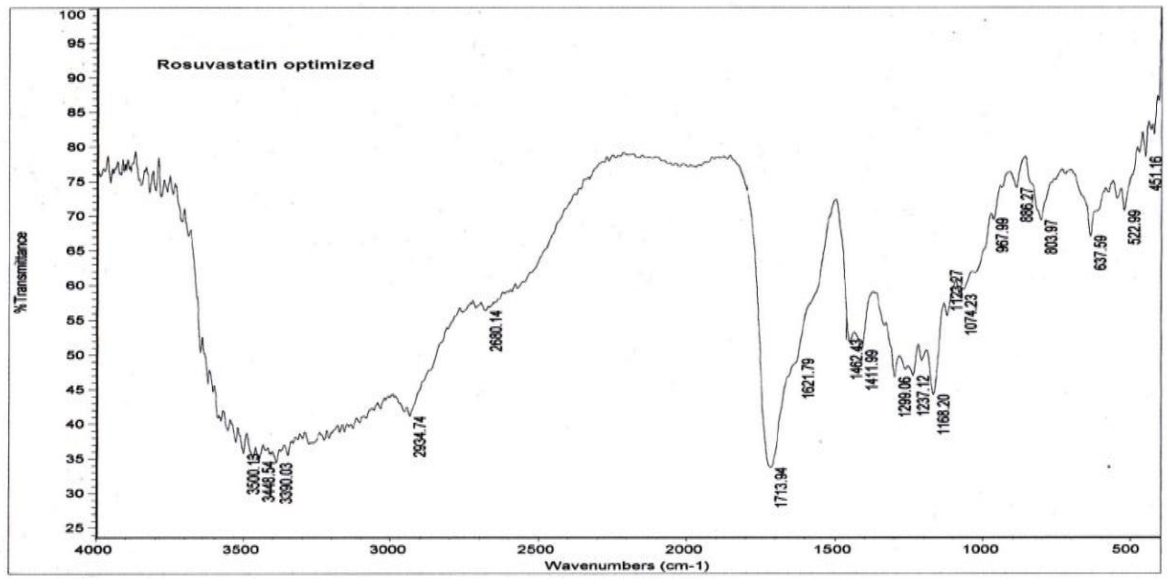

Fig. 1:FT-IR spectra of rosuvastatin physical mixture

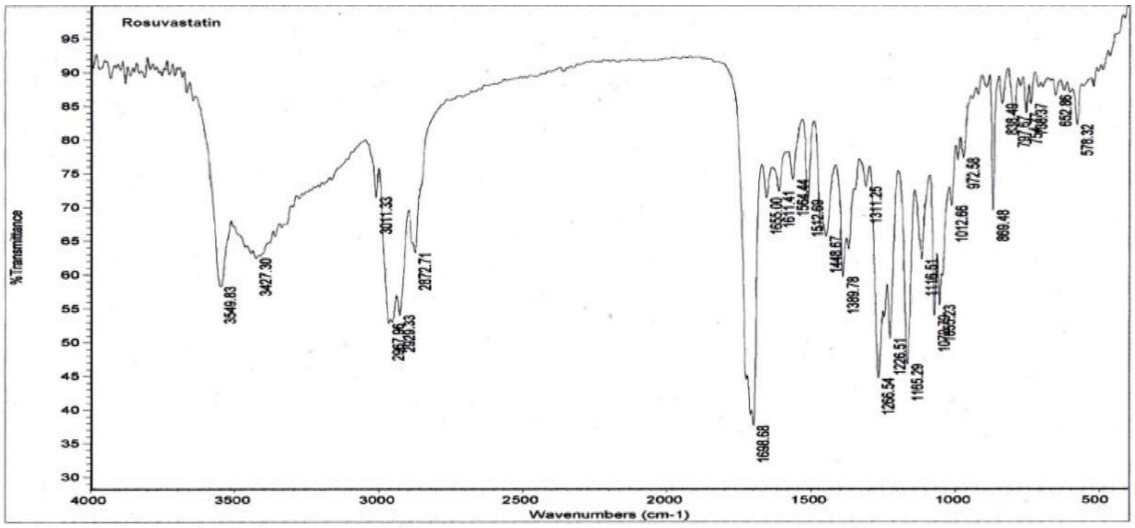

Fig. 2:FTIR spectra data for pure drug

Table 2:Concentration and absorbance of rosuvastatin

S.No	Concentration (µg /ml)	Absorbance at 248nm
1	5	0.071
2	10	0.134
3	15	0.201
4	20	0.261
5	25	0.322
6	30	0.382
7	35	0.443

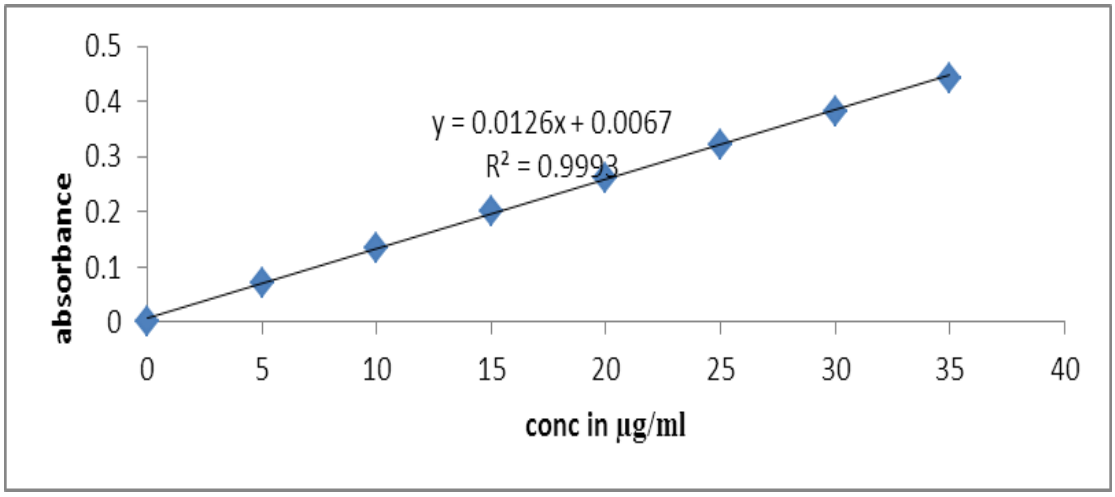

Fig. 3:Calibration curve of rosuvastatin

Table 3:Values of evaluation parameters of developed gel

Formulation code	Clarity	pH	Homogeneity	Viscosity (cps)	% Drug Content	% drug entrapped
PGR1	+++	6.4	Good	1560	96.7	92.8
PGR2	+++	6.5	Good	1625	95.3	83.3
PGR3	+++	6.4	Good	1763	96.0	87.3
PGR4	++	6.5	Good	1656	96.8	89.1
PGR5	+++	6.2	Good	1577	96.3	82.4
PGR6	++	6.7	Good	1770	97.4	84.6
PGR7	+++	6.6	Good	1654	97.1	81.2
PGR8	++	6.5	Good	1653	97.1	80.1

Table 4:In-Vitro drug release of Proniosomal gel formulations

Time(hr)	PGR1	PGR2	PGR3	PGR4	PGR5	PGR6	PGR7	PGR8
0	0	0	0	0	0	0	0	0
1	2.9	4.5	2.5	3.4	4.1	6.1	3.2	4.7
2	4.4	6.2	4.6	5.2	5.8	11.67	5.8	7.3
3	6.1	8.1	8.6	7.9	7.8	16.23	7.5	8.6
4	12.4	16.3	15.0	12.6	15.3	21.48	12.7	12.1
5	25.2	21.8	29.8	23.8	22.1	29.64	24.3	16.4
6	47.6	33.5	46.4	53.6	35.6	34.79	45.1	27.1
8	65.9	46.7	64.1	61.2	45.4	47.16	63.6	48.6
12	87.6	64.78	72.9	76.3	66.5	53.65	73.5	59.3
24	96.3	73.10	84.1	85.6	75.5	66.10	81.4	79.4

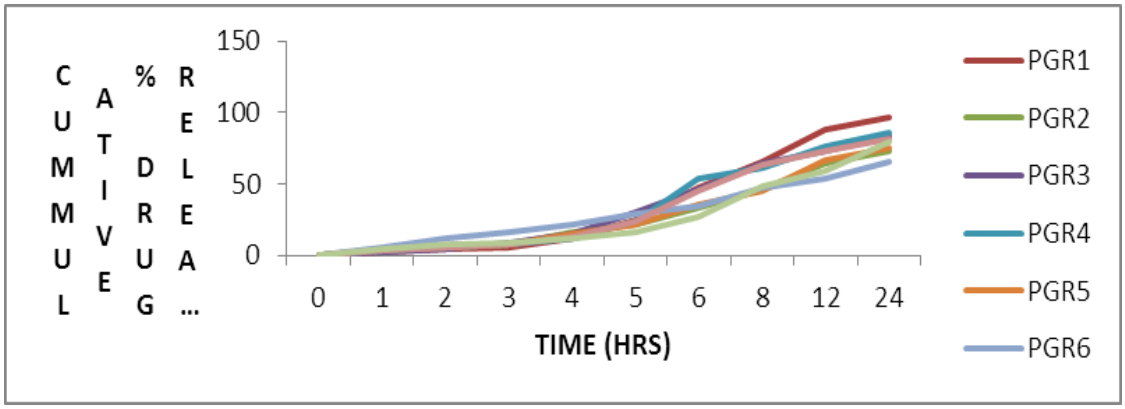

Fig. 4:Showing in vitro drug release for proniosomal formulations

Table 5 Release kinetics for optimized PGR1 formulation

	ZERO	FIRST	HIGUCHI	PEPPAS
Name	**% CDR Vs T**	**Log % Remain Vs T**	**%CDR Vs √T**	**Log C Vs Log T**
Slope	4.676685083	-0.06633457	24.9592609	1.47576491
Intercept	4.441546961	2.098220458	-20.1286047	0.262964082
Correlation	0.89804441	-0.97818203	0.91514192	0.953581567
R^2	0.806483762	0.9568401	0.837484733	0.909317805

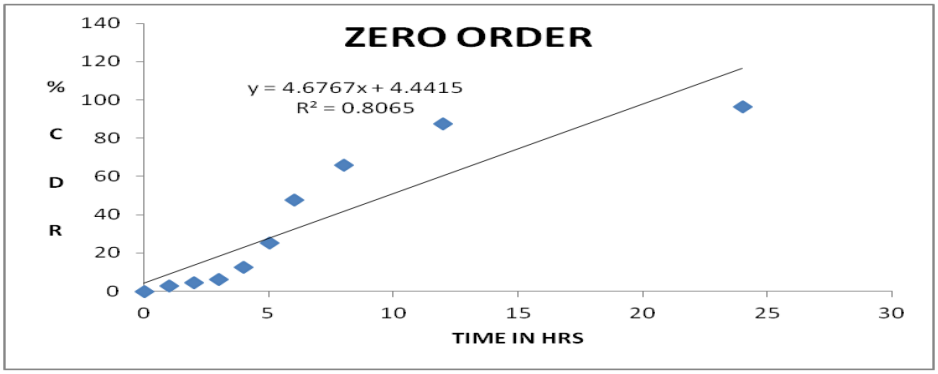

Fig. 5: Zero order graph of optimized formulation

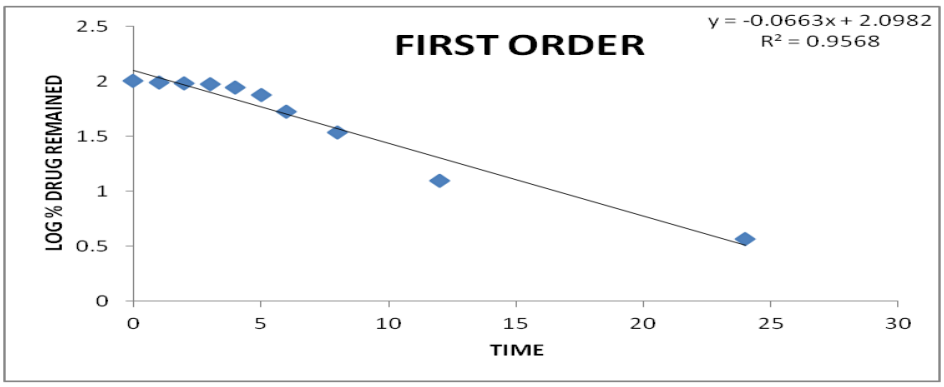

Fig. 6:First order graph of optimized formulation

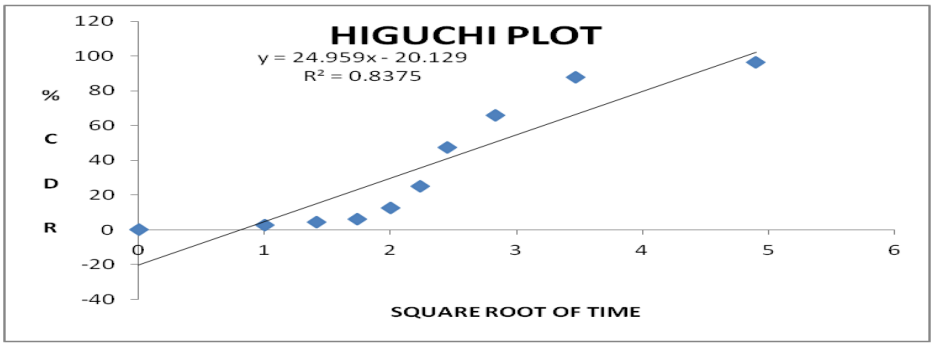

Fig.7: Higuchi graph of optimized formulation

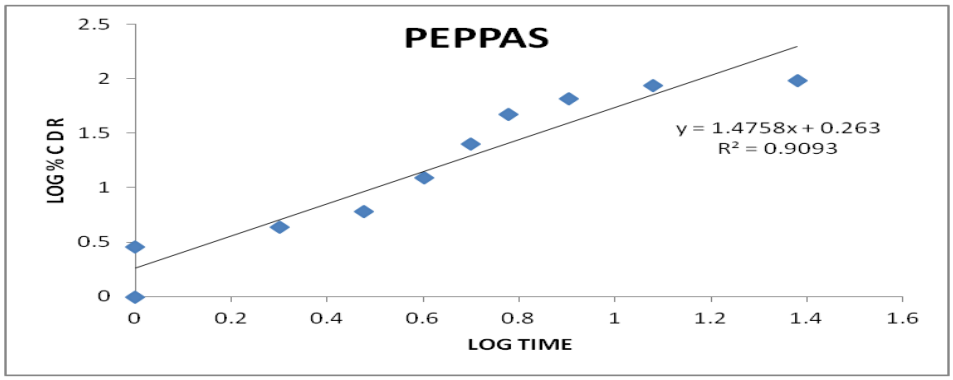

Fig. 8:Peppas graph of optimized formulation

DISCUSSION

Preformulation Studies

Compatibility studies: The drug and polymers were characterized by FTIR spectral analysis for any physical as well as chemical alteration of the drug characteristics. From the results, it was observed that there was no interference in the functional groups as the principle peaks of the rosuvastatin (Fig.2)were found to be unaltered in the spectra of the drug-polymer mixture (Fig.1).

Calibration Curve: λMax of rosuvastatin was found to be 248 nm, it obey beers law in the concentration range 5-35µg/ml. A plot of linear relationship between the concentration (x-axis) and the peak area (y-axis) was observed for rosuvastatin as shown in Fig.3 and Table 2.

Evaluation of Gels

Clarity: Proniosomes containing gels were found to be transparent and white viscous. In the present study, PGR1, PGR2, PGR3, PGR5 and PGR7 have shown higher clarity that all above formulations were free from presence of particles.

pH: The pH value of all developed formulations of gels were in the range of 6.2– 6.7.

Homogeneity: All developed formulations were much clear and transparent aslo showed good homogeneity with absence of lumps.

Viscosity measurement: The viscosity of various formulated rosuvastatin gels was measured using a Brookfield viscometer. The rheological behavior and its consistency of all formulated gels dependson the ratio of solid to liquid fractions. In the present study, Viscosity of various formulated gels was found in range of 1560 to 1770 centipoises.The results of evaluation parameters of all developed formulations were given in table 3.

Drug content: The percentage drug content of all prepared gel formulations were found to be in the range of 96.0 – 97.4 %. The drug content percentage of formulations was found satisfactory. Thus, methods adopted for gels formulations were found suitable.

Entrapment Efficiency: Once the presence of vesicles was confirmed in the niosomal system, the ability of vesicles for entrapment of drug was investigated by ultra-centrifugation. This method was used to separate the niosomal vesicles containing drug and un-entrapped or free drug and also to find out the entrapment efficiency.The maximum entrapment efficiency

of Niosomal vesicles was found to be 92.8% due to presence of Span 80 (PGR1).

***In vitro* drug diffusion studies:** In vitro drug release studies were carried out on dissolution test apparatus Franz diffusion cell. The initial rapid drug release could be attributed to desorption of rosuvastatinfrom the surface of niosomes. The release profiles of encapsulated drug was found to be 96.3 % with over 24 hours. The biphasic release pattern would be beneficial due to saturation of skin epidermis with drug at the initial fast release phase; this will help to achieve high concentration gradient of drug across skin, required for successful transdermal drug delivery to the blood. The results of in vitro drug release profile of all proniosomal formulations were shown in Fig. 4 and Table 4.

Kinetics of drug release profile

In vitro drug release data of Optimized proniosomal formulation PGR1 was utilized for determination of kinetic models such as zero order, first order, Higuchi andKorsmeyer–Peppasmodels. The best fitted line and the highest correlation were observed in order to know the mechanism of drug release. The highest correlation coefficient ($\mathbf{R^2}$=0.956) was resulted with first order kinetic model which indicates that drug release mainly depends on its concentration. The release kinetics data of the selected proniosomal formulawas given in table 5.

CONCLUSION

Proniosomal system is an alternate strategy for transdermal drug delivery because it reduces the toxicity and enhances penetration effect of surfactants. Also shows a significant advance over the conventional vesicular systems. A wide variety of active agents of different therapeutic actions can also be given by proniosomal drug delivery system in the form of tablets, beads or capsules. Based on the investigations proniosomal system appears to be an efficient drug carrier for the future with physical and chemical stability and potentially scalable for commercial viability.

In the present study an attempt was made to formulate and evaluate niosomal system of Rosuvastatin. The preformulation studies include identification, melting point, pH calibration and drug excipient compatibility studies were carried out. Estimation of Rosuvastatin was done by spectrophotometrically using Phosphate buffer 6.8 at 248nm.

All the gels were evaluated for their appearance, drug content, pH, rheological properties,

drug entarppment study and in-vitro release (Franz diffusion cell using through an egg membrane). Visually gels were sparkling and transparent.

The following conclusions are drawn from Promising results were obtained with PGR1 formulation containing Span 80 with Soya Lecithin because of the highest entrapment efficiency and high localization in the stratum corneum than the Tweens with Soya Lecithin.

However Niosomes prepared by phase separation coacervation method were more uniform and small in size which is essential for skin penetration. The proniosomes on hydration with phosphate buffer produced niosomal dispersions. The invitro drug release revealed the formulations followed by slow sustained release of the drug for 24 h.

These findings are very encouraging and confirm that proniosomes are a very promising carrier for the topical administration due to the enhanced delivery of drugs through the skin thus prompting various opportunities for the development of suitable therapeutic strategies through the topical route. The formulation is easy to scale up as the procedure is simple and do not involve lengthy procedure and unnecessary use of pharmaceutically unacceptable additives.

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