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Research Article

Preparation and characterization of raloxifene proniosomes using sorbitan esters

Bhama S.*, Sambathkumar R., Shanmuga Sundaram R.

J.K.K. Nattraja College of Pharmacy, Kumarapalayam, Namakkal-638183, Tamil Nadu, India

ABSTRACT

A 2nd-generation selective oestrogen receptor modulator, raloxifene HCl (RLX-HCl) is a non- steroidal benzothiophene that binds to oestrogen receptors. While administer orally due to its extensive first pass metabolism in gut, it possess only 2% absolute bioavailability. In this present work, RLX-HCL was formulated as proniosomes by slurry method, where different ratios of surfactant and cholesterol were used for preparation and evaluating study. The shape of prepared RLX-HCl loaded proniosomes were spherical with an average particle size of 690nm with a high encapsulation efficiency of 83.64%. The study revealed that in 24h, formulation RS606 had the highest cumulative drug release (99.42%). From the stability study, 5°C was identified as the suitable temperature for the optimized proniosomes. In conclusion, an improved bioavailability and prolonged drug release profile of the proniosome formulation seems to be a promising delivery system for RLX-HCl.

Keywords: Lipids; Drug release; Stability study; Selective oestrogen receptor modulator.

INTRODUCTION

A non-steroidal benzothiophene, Raloxifene hydrochloride (RLX- HCl), is a 2nd-generation selective oestrogen receptor modulator, and has mixed oestrogen receptor mediated pharmacological action. RLX- HCl is an oestrogen agonist on bone and the cardiovascular system, and exhibits oestrogen antagonist on endomerital and breast tissues (Cranney et al., 2005). It is used in the treatment of breast cancer, prostate cancer and benign hypertrophy as a result of its anti-estrogenic and anti-androgenic effects (Garg et al., 2009). According to BCS classification RLX-HCL comes under class II category which has poor solubility and their absorption is dissolution rate limited (Elsheikh et al., 2012). RLX-HCl has only 2% oral bioavailability due to its extensive first pass metabolism (Hochner-Celnikier, 1999). Hence to enhance its oral bioavailability, it is a must to improve the solubility, dissolution rate and to lower the hepatic first-pass metabolism. Any appropriate novel drug delivery system could be used to avoid these types of problems.

Many researchers had dealt with improved the bioavailability by enhancing the solubility of RLX- HCl (Bikaris et al., 2009; Wempe et al., 2008; Tran et al., 2013). Recently, it has been reported that self-emulsifying drug delivery systems and solid lipid nanoparticles, which belong to lipid based systems, enhance bioavailability (Thakkar 19et al., 2011; Madhu et al., 2013; Ravi et al., 2014).

In comparison to conventional dosage forms, colloidal particulate drug delivery systems such as liposomes (Betageri et al., 1994) or niosomes (Schreier et al., 1994) are found to possess more merits, because these carriers might modify the particle composition and surface and also act as a drug containing reservoir. Due to these characters it achieve targeting site rapidly by adjusting the release rate. As such liposomes, solutes could be entrapped by non-ionic surfactant vesicles i.e., niosomes. They also increase the stability of entrapped drugs due to its osmotic active nature and its own stable capacity (Baillie et al., 1985; Rogerson et al., 1988). There are no special precautions to handle and storage of surfactants. Niosomes could entrap drug with different solubilities because of its infrastructure which consists both hydrophilic and hydrophobic moieties together (Biju et al., 2006). Though chemically stable and cheap, niosomes have been reported to exhibit the following physical stability problems viz. 1) aggregation 2) sedimentation 3) fusion, and 4) leakage during storage. Specialized equipments necessary for the traditional methods are also time consuming ones and most of such methods allow only for a predetermined lot size, often increasing the material wastage, when smaller quantities are required for particular dose application (Blazek-Welsh et al., 2001).

To minimize all these problems, an ideal drug delivery system, proniosomes, may be used. The proniosomes are more stable, dry, free flowing powder which can

* Corresponding Author
Email: bhamashaswath2006@gmail.com
Contact: +91-9443836674, 9715250375
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transfer and distribute easily and also be stable while sterilization and during storage. Proniosomes, surfactant-coated water soluble carrier particles, can be hydrated easily to form niosomes immediately before use on brief agitation with hot aqueous media (Blazek-Welsh et al., 2001; Hu and Rhodes, 1999; Blazek-Welsh et al., 2001). In this study, the bioavailability enhancement of RLX-HCl was achieved using proniosomes, due to their greater advantages.

MATERIALS AND METHODS

Raloxifene was received from Cipla Ltd,Mumbai, India (as a gift sample). Maltodextrin was purchased from Himedia, Mumbai, India. Cholesterol and span60 were purchased from S.D.Fine Chemicals Limited, Mumbai, India. All other chemical utilized were of analytical grade.

Preparation of proniosomes

Slurry method was used for the preparation of proniosomes (Solanki et al., 2007). Table 1 represents the composition of different proniosomal formulations. Accurately weighed amounts of lipid mixture comprising of span 60 and cholesterol at various molar ratios and drug (60 mg) were dissolved in ethanol. Then maltodextrin was added to the resultant solution to form slurry and was then transferred into a round bottom flask. The organic solvent was then evaporated under reduced pressure, at a temperature of 45±2°C, attached to a rotary flash and thereafter to ensure complete removal of solvent. To obtain dry, free-flowing product the resultant powders were further dried overnight in a vacuum oven at room temperature, stored in a tightly-closed container at 5°C for further evaluation.

Physico-chemical characterization of proniosome powders

Optical microscopy

Optical microscopy was employed to evaluate the formation and morphology of the niosomes. By using vortex mixer for 2 minutes, the niosomes dispersion were obtained by hydrating the proniosome powder with phosphate buffer (pH 7.4) at 80°C. Placed over a glass slide, the vesicles formed in the niosome dispersions were observed through an optical microscope.

Measurement of angle of repose (AoR)

The flow property of powder play an important role during handling and processing operations. The flow properties were studied by measuring the AoR determined by fixed funnel method (Staniforth, 1988).

Number of vesicles per cubic mm

One of the vital parameter to assess the proniosome powder is the no. of vesicles formed from proniosome powder by hydration with Phosphate buffer pH 7.4 was used as a hydration medium. The number of vesicles formed from proniosomes were counted in 80 small squares, utilizing haemocytometer and optical microscope (Subheet et al., 2003). The following formula was used for calculation

$$No.of\ niosomes\ per\ mm^3 = \frac{Total\ No.of\ niosomes\ counted \times dilution\ factor}{Total\ number\ of\ small\ squares\ counted} \times 4000$$

Determination of vesicle size, zeta potential and entrapment efficiency

The resultant dispersion was used for the determination of size, zeta potential and entrapment efficiency by hydrating the proniosomal powders with phosphate buffer (pH 7.4) and subjecting it to bath sonication for 3 min. The vesicle size and zeta potential of niosomes were then determined using Zetasizer. With phosphate buffer of pH 7.4, each sample was diluted to a suitable concentration. Size analysis was performed at 25° C, with an angle of detection of 90 C.

At first, dialysis method was employed to separate the unentrapped drug from proniosome derived niosome. The remained drug present in niosomes was estimated by complete vesicle disruption using TritonX-100. It was then diluted with phosphate buffer (pH 7.4) and filtered through Whatman filter paper. The percentage of drug encapsulation (%EE) was calculated using the following equation

$$\%EE = \left[\left(\frac{A_t - A_r}{A_t}\right)\right] \times 100\%$$

Where, A_t is the concentration of total drug and A_r, the concentration of free drug

In vitro Dissolution study

This study was carried out using open end cylinder method. One end of the tube was tightly covered with a Himedia dialysis membrane. The proniosome powder was placed over the membrane in the donar chamber which was then lowered to the vessels of the glass beaker containing 100 ml of phosphate buffer (pH 7.4), which act as a receptor compartment where in the dissolution medium outside and the vesicles preparation inside were adjusted at the same level. The release study was carried out at 37±0.5ºC, and the stirring shafts were rotated at a speed of 50 rpm. Five millilitre samples were withdrawn periodically at predetermined time intervals. To maintain the sink condition, every withdrawal was followed by replacement with fresh medium and then the samples were analysed spectrophotometrically.

Drug release kinetic data analysis

The results of *in vitro* drug release study of niosomes were fitted with various kinetic equation like zero-order (cumulative % release vs. time), first-order (log % drug remaining vs. time), Higuchi's model (cumulative % drug release vs. square root of time), in order to understand the kinetic and mechanism of drug release.

Table 1: Composition of raloxifene loaded maltodextrin based proniosome powders

Sl.No	Formulation Code	Raloxifene	Span60 in mg	Cholesterol in mg	Molar ratio	
					Span60 in mM	Cholesterol in mM
1	RS601	60 mg	346.46	0	1.000	0.000
2	RS602	60 mg	311.814	38.665	0.900	0.100
3	RS603	60 mg	277.168	77.33	0.800	0.200
4	RS604	60 mg	242.522	115.995	0.700	0.300
5	RS605	60 mg	207.876	154.66	0.600	0.400
6	RS606	60 mg	173.23	193.325	0.500	0.500
7	RS607	60 mg	138.584	231.99	0.400	0.600
8	RS608	60 mg	103.938	270.655	0.300	0.700
9	RS609	60 mg	69.292	309.32	0.200	0.800
10	RS610	60 mg	34.646	347.985	0.100	0.900

Table 2: Physico-chemical characterization of various proniosome formulations

Formulation	*Angle of Repose in Θ ± STD	Vesicle size in nm	Zeta potential in *mv*	*Entrapment Efficiency in % ± STD	No of vesicle/ mm^3X10^5
RS601	31°19'± 0.55	240	+14	57.52 ± 0.54	1.4
RS602	30°72'± 0.30	360	+14	63.16 ± 0.58	1.6
RS603	31°54'± 0.45	440	+16	68.78 ± 0.85	1.9
RS604	31°01'± 0.89	580	+19	72.98 ± 0.44	2.6
RS605	30°39'± 0.46	640	+20	77.74 ± 0.45	2.8
RS606	29°22'± 0.17	690	+22	82.44 ± 0.44	3.2
RS607	30°32'± 0.82	660	+21	74.96 ± 0.77	3
RS608	31°38'± 0.20	640	+20	70.74 ± 0.59	2.7
RS609	31°18'± 0.46	640	+20	64.96 ± 0.40	2.8
RS610	31°22'± 0.97	610	+19	61.76 ± 0.47	2.4

#Maltodextrin angle of repose 30°06'±0.14; *n=3

Table 3: Characteristic absorption peaks in FTIR spectrum

Wave Number		Indications
Pure drug Raloxifene	Raloxifene Formulation	
3643.10 cm^{-1}	3557.10 cm^{-1}	O-H
1234.67 cm^{-1}	1232.87 cm^{-1}	C-O PHENOLIC
1519.82 cm^{-1}	1517.78 cm^{-1}	C=C AROMATIC
1730.36 cm^{-1}	1730.80 cm^{-1}	C=O KETO
1051.39 cm^{-1}	1050.69 cm^{-1}	C-O ETHER
1234.67 cm^{-1}	1232.87 cm^{-1}	C-N
3643.10 cm^{-1}	3604.77 cm^{-1}	O-H

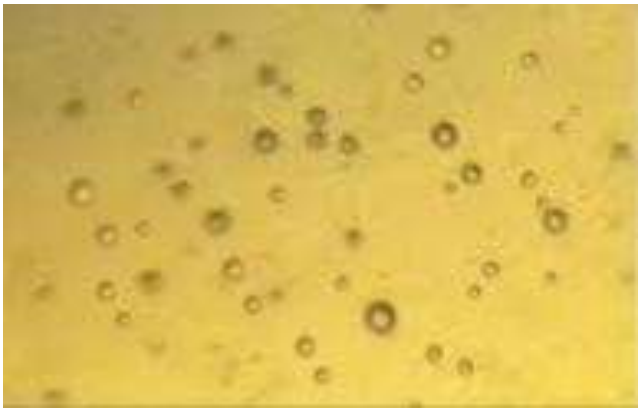

Figure 1: Photomicrograph of Raloxifene Formulation (RS606)

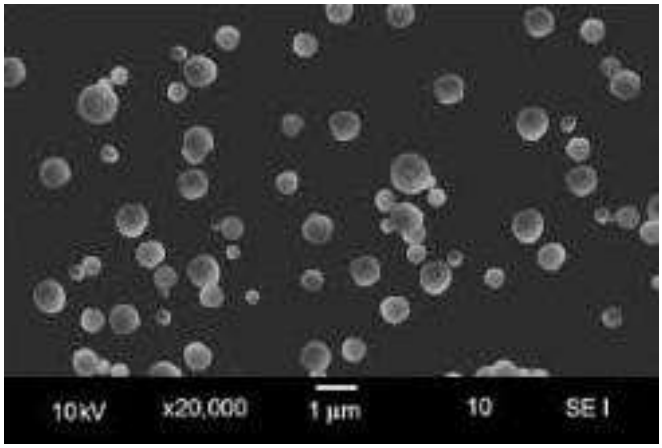

Figure 2: Scanning Electron Microscope of Proniosome formulation (RS606)

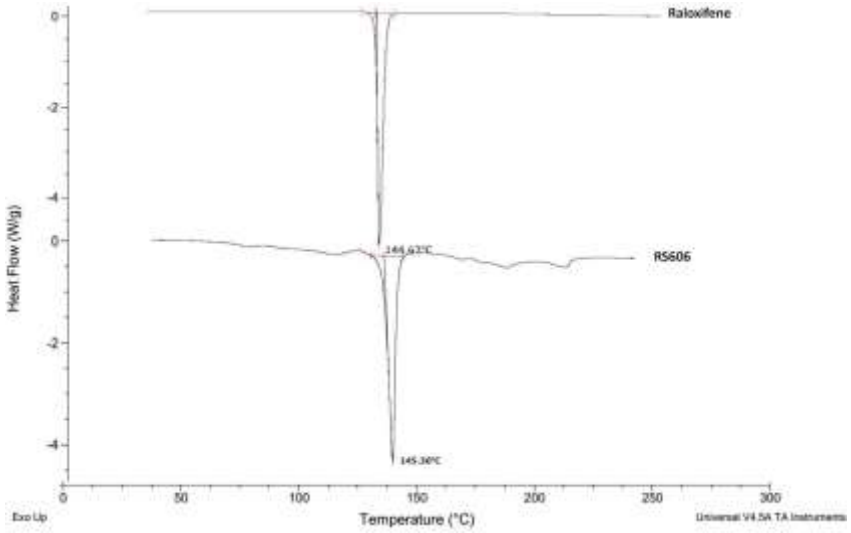

Figure 3: Differential Scanning Calorimetry of Raloxifene and formulation(RS606)

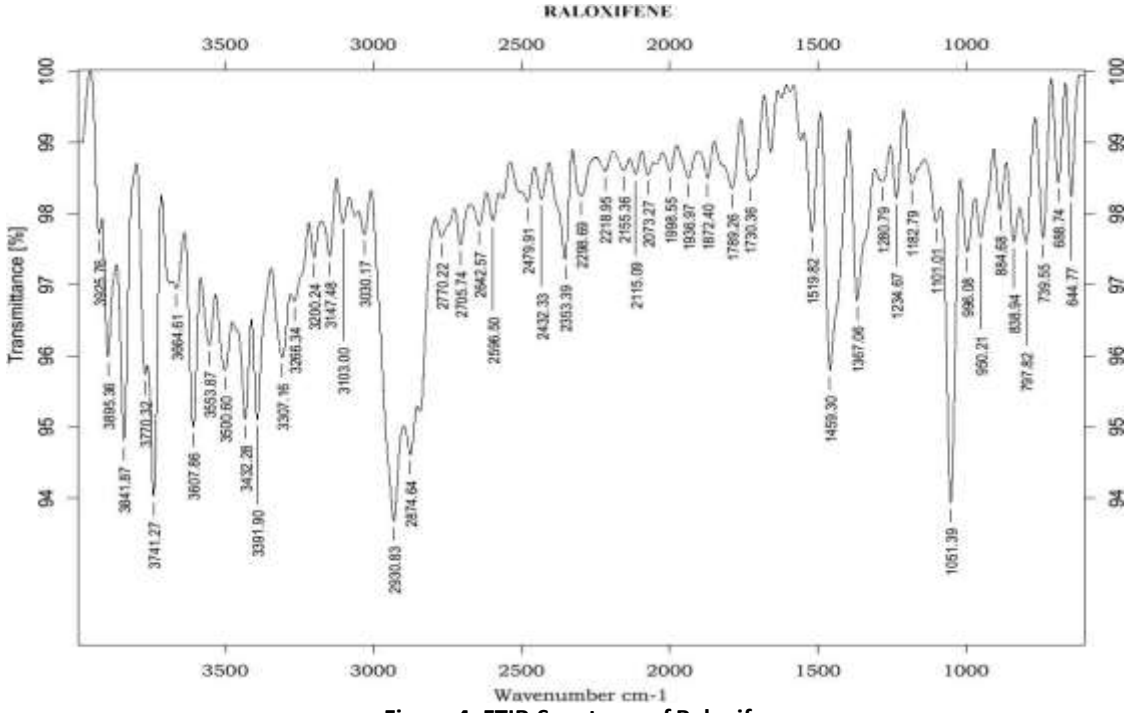

Figure 4: FTIR Spectrum of Raloxifene

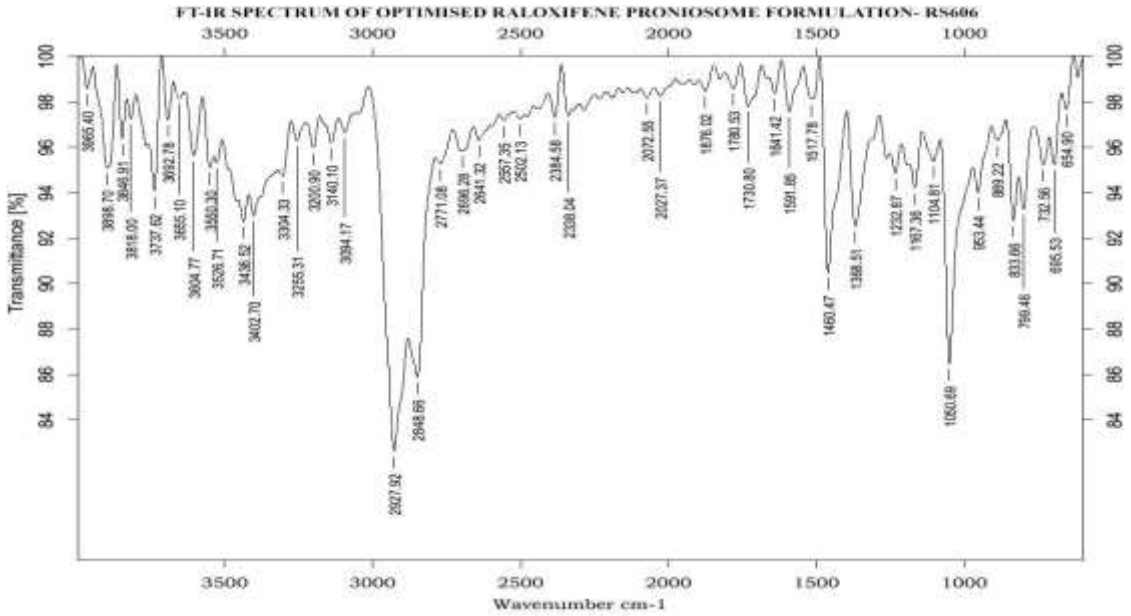

Figure 5: FTIR spectrum of Optimized Raloxifene Proniosome Formulation RS606

Table 4: Kinetic analysis data of Raloxifene Proniosome Formulations

Formula Code	Zero order		First Order		Higuchis model		Koresmayer & Peppas	
	K_0	R^2	K_1	R^2	K_H	R^2	*n*	R^2
RS601	7.0906	0.9988	0.3266	-0.8457	28.0726	0.9813	0.6130	0.9850
RS602	6.3610	0.9910	0.2749	-0.7991	25.1534	0.9713	0.6071	0.9816
RS603	5.0866	0.9935	0.2053	-0.7876	22.8817	0.9713	0.6034	0.9849
RS604	4.6844	0.9933	0.1824	-0.7942	22.0630	0.9693	0.6507	0.9904
RS605	4.3222	0.9966	0.1558	-0.8254	21.4565	0.9727	0.6665	0.9922
RS606	3.8677	0.9983	0.1440	-0.8427	21.0283	0.9783	0.7648	0.9983
RS607	4.4135	0.9953	0.1522	-0.8604	22.0840	0.9607	0.8926	0.9953
RS608	4.8278	0.9956	0.1710	-0.7913	22.4491	0.9517	1.0088	0.9966
RS609	5.4969	0.9922	0.2121	-0.7476	23.3715	0.9337	1.1424	0.9981
RS610	5.4504	0.9935	0.1721	-0.7976	23.2822	0.9387	1.2196	0.9980

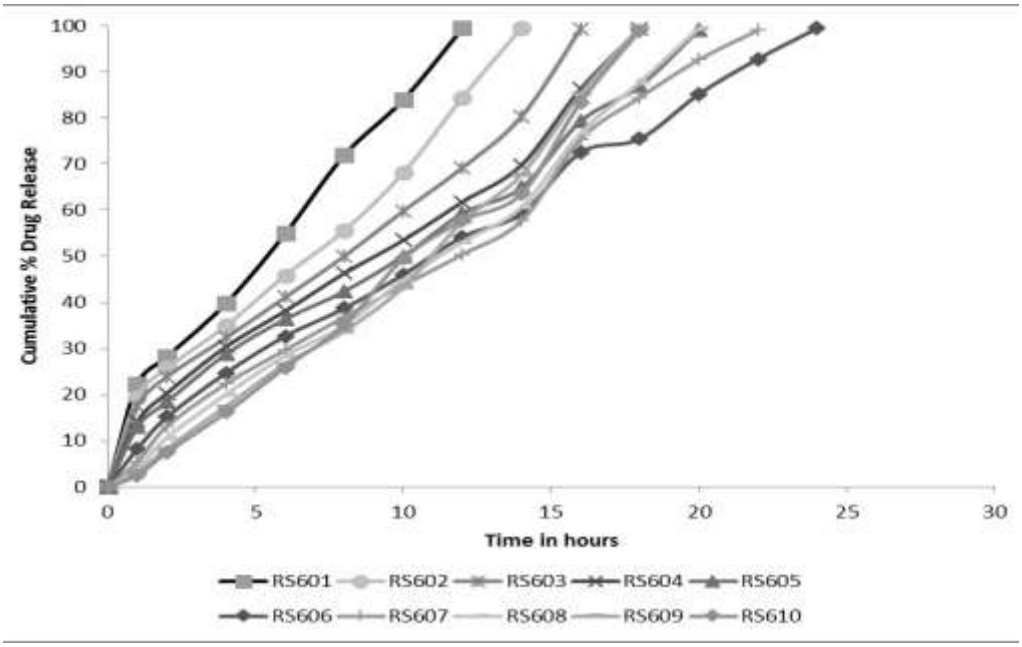

Figure 6: In vitro drug release of Raloxifene Proniosome Formulations

Table 5: Stability Study of Optimized Raloxifene Formulation

Temperature	Values obtained at zero time			Values obtained at 90th day		
	Vesicle size in nm	Drug re-main in %	*In vitro* drug release in %	Vesicle size in nm	Drug re-main in %	*In vitro* drug release%
5±3˚C	690	82.44	99.42	700	81.36	98.35
25±2˚C	690	82.44	99.42	710	79.82	96.54
40±2˚C	690	82.44	99.42	720	72.58	93.92

The $R^2$and k values were then calculated from the li ne-ar curve obtained by regression analysis of the above plots.

Solid state characterization

Scanning electron microscopy (SEM)

Proniosome powders were affixed to double-sided carbon tape which was positioned on an aluminium (Al) stub to remove the excess powder and stored un-der vacuum overnight and the samples were sputter-coated with gold and the electron micrographs were obtained using SEM.Using SEM, the surface morpholo-gy (roundness, smoothness, and formation of aggre-gates of proniosomes were studied.

Differential Scanning Calorimetry (DSC)

By performing DSC analysis of pure drug, maltodextrin, and proniosome powder, the molecular state of the drug in optimized proniosome formulation (RS606) were evaluated and the DSC curves of samples were obtained by a DSC. Each sample was then placed in an Al pan, crimped with an Al cover followed by heating and cooling at the rates of 10° C/min and 250° C/min, respectively. All measurements were performed over 40-450° C under a nitrogen purge at 50 mL/min.

Fourier Transform Infrared (FT-IR) Spectroscopy:

Using FT-IR spectrophotometer, the infrared spectra of raloxifene, maltodextrin, non-ionic surfactant and op-timized proniosome powder formulation (RS606) were obtained with a scanning range of 4000–500 cm^{-1} and a resolution of 4 cm^{-1}.

Stability studies

The optimized batch was stored in airtight sealed vials at different temperatures i.e., 5±3 ˚C, 25±2 ˚C and 40±2˚C to determine the stability of proniosomes, Since the instability of the formulation would reflec t in drug leakage and a decrease in the % drug retained, the surface characteristics were selected as parame-ters for evaluation of the stability. After being hydrated to form niosomes, the proniosomes, sampled at regu-lar intervals of time (0, 30, 60, and 90 days), were ob-served for colour change and surface characteristics, and then subjected to testing for the % drug retained..

RESULTS AND DISCUSSIONS

Maltodextrin based proniosomes could be used for efficient oral delivery of lipophilic or amphiphilic drugs. Further, during formulation, the amount of carrier could be easily adjusted with the surfactant enabling the preparation of proniosomes with high surfac-tant:carrier mass ratio, as a result of high surface area and porous structure of maltodextrin. Raloxifene load-ed maltodextrin based proniosomes with non-ionic surfactant i.e., span 60, were prepared by slurry meth-od. While formulating proniosomes, stability issues raised by aqueous niosomes could be resolved. For achieving maximum therapeutic benefit fr om the proniosome formulations, the stability of the vesicles formed after hydration with gastric fluids may also equally be important for achieving maximum thera-peutic benefit from the proniosome formulations. Therefore, several methods were employed to improve the stability of vesicular systems.

The stability and morphology of the niosomes would greatly vary depending on the non-ionic surfactant concentration and cholesterol (Touitou et al., 1994) leading to disruption of vesicles, leading to leakage of free drug before drug diffusion and fusion of vesicle with gastrointestinal membrane following any alter-ation in their composition (Ogiso et al., 1996). Highest entrapment could be observed with an increase in phase transition temperature of span which has same polar head groups with varied alkyl chain. The phase transition temperatures for span-20, 40 and 60 are 16, 42 and 53°C, respectively whereas span-80 having the lowest phase transition temperature (12°C). Span-60 was selected as a surfactant because of its high phase transition temperature and to facilitate stable vesicle formation and to improve the oral delivery of raloxi-fene from proniosomes (Varshosaz, 2005).

Niosomes were spherical while observed by optical microscope and given in figure 1.The powder flow properties were assessed from AoR. The AoR obtained for prepared proniosomes are shown in table 2, were within the range of 29°22'±0.17 to 31°54'±0.45. A small AoR (<30) is an indicative of good flow properties of proniosome powder formulations.

The formation of abundant numbers of vesicles formed after hydration in the GI tract may be speculated to derive maximum benefit from the proni osome formu-lations. The proniosome formulation containing span 60 and cholesterol at a ratio of 0.5:0.5(RS606) had dis-played good number of vesicles, which is in correlation with the size and entrapment efficiency results, amongst all the formulations,

An important parameter for the vesicular systems are the vesicle size. The mean size of the vesicles was in the range of 240-690 nm. The zeta potential and entrapment efficiency of the formulations was found to be between+14 to +22mv and 57.52 to 82.44 % res pectively (Table 2). The size and surface charge of the vesicles seems to be dependent on the concentration of cholesterol. Further, the results also substantiated that the entrapment efficiency of raloxifene was dependent on the proniosomes composition. As the concentration of cholesterol increased, the entrapment efficiency has been increased (RS601 to RS606) upto a certain ratio of surfactant &cholesterol, with equimolar ratio, higher entrapment and less leakage attained.

Using SEM, DSC, and FT-IR spectroscopy, molecular interactions between drug and carrier were studied. The absence of native crystalline structures of raloxifene in the proniosome powder and surfactant coating over carrier is attained 33and the mean particle size in the range of 690 ±0. 38nm was revealed by the SEM images (Figure 2).

The thermotropic behaviour and the physical state of the drug in proniosome powder were ascertained from the DSC thermograms of drug, maltodextrin and proniosome formulation (RS606). It is evident from figur e 3, that the drug possessed crystalline behaviour as it showed sharp intense peak at 144.67 to 145.36 corresponding to its melting point. On the other hand, the absence of conspicuous peak over the range of 143 to 147 4°C in proniosome powder is an indicative of the transformation of the native crystalline form of the drug to amorphous or molecular state. Figure 4 and 5 illustrates the FT-IR spectrum of raloxifene pure drug and raloxifene proniosome formulation (RDS606) respectively and table 3 explained the characteristics peaks of raloxifene and raloxifene proniosome formulation. When compared all the peaks of proniosome formulation with pure drug, there is not much variation in the peaks which indicated no chemical interaction between raloxifene, maltodextrin, cholesterol and span60.

Figure 6 shown the release study conducted for all the formulations. Most of the formulations were found to have a linear release and the formulations were found to provide more than 90% release within a period of 24 h. Cholesterol, has been found to abolish the gel to liquid transition of niosomes and found to prevent the leakage of drug from the niosomal formulation. The fact that multimellar vesicles consist of several concentric sphere of bilayer separated by aqueous compartment could be attributed to slower release of drug from multilamellar vesicles. The formulation RS606 was found to be best and to give a cumulative release of 99.42% over a period of 24h. Interpretation of drug release kinetic mechanism was found using *in vitro* release data and applying it to various kinetic models.The release constant was calculated from the slope of appropriate plots, and the regression R^2 values are shown in table 4 also calculated from various plots.

The *in vitro* drug release of noisome was best explained by zero-order kinetics for best formulation RS606, as the plots showed highest linearity and korsemayer peppas plot showed that the drug release followed a non-fickian diffusion mechanism.

Stability studies of optimized proniosomes (RS606) were performed by storing at 5±3˚C, 25±2˚C and 40±2˚C for a period of 90 days. The vesicle size, drug remain, *in vitro* drug release were monitored. The results shown in table 5 indicated that there was no appreciable change in the formulation after 90 days particularly at 5±3˚C. Hence, it may be concluded from the data obtained that the optimum storage condition for niosomes was 5°.

CONCLUSION

Using slurry method, Raloxifene was successfully encapsulated into proniosomes. The nature of non-ionic surfactant and the cholesterol concentration was found to influence the retention of drug in the proniosomal formulation during *in vitro* drug release study. A maximum entrapment efficiency of 82.44% and *in vitro* drug release of 99.42% were attained after 24 hrs. The vesicles were quite stable at 5° over a period of 90 days. Hence, it may be concluded that proniosome formulation proved as efficient carrier for raloxifene oral delivery.

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