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Research Article

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Formulation and Evaluation of Darifenacin Hydrobromide Nano-Liposomes

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ABSTRACT

The main objective of this study was to formulate and evaluate darifenacin hydrobromide loaded nano-liposomes for prolonged drug release. Drug and excipient compatibility study was performed by FTIR and the study revealed that there was no interaction between drug and excipients. Various formulations were prepared by conventional thin film hydration method using different phospholipids such as phospholipon 80H, phospholipon 90H and soyalecithin. The ratio of phospholipid to cholesterol was optimized as 3:1 and used for phospholipid further studies. All formulations were evaluated for entrapment efficiency and in vitro drug release studies. The optimized formulation with soyalecithin DLSL6 showed highest entrapment efficiency (78.5%) and highest drug release in 24 hrs (82%). The optimized formulation was evaluated for FTIR, SEM, and particle size analysis and zeta potential studies. The FTIR study revealed that there was no interaction between drug and excipients. The vesicles were smooth surfaced with uniform distribution which is evident from surface morphology analysis from SEM. The optimized formulation was found to be stable with zeta potential value of -51.6 mV and the average particle size of 24.1 nm with uniform distribution. The regression coefficient (R2 value) 0.981 indicating release as zero order, where "n" value 0.455 states the mechanism as fickian diffusion. The optimized formulation was subjected to stability studies at room temperature and 4ºC for two months and liposomes were found to be stable with no significant change in the entrapment efficiency.

Keywords: Chemical derivatization; Drug delivery; Metabolizable drug; Phospholipid

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INTRODUCTION

In the past few decades, an attention has been focused on the development of new drug delivery system (NDDS). The NDDS should fulfill two requisites. First it should deliver the drug at a rate required by the body over the period of treatment. Second, it should channel the active entity to the site of action. None of the dosage forms includes prolonged release are unable to meet the requirement. Novel drug delivery attempts to either sustain the drug action at a predetermined rate or by maintaining constant effective drug level in the body with concomitant minimization of undesirable side effects. Even it can localize the drug action by targeting drug release systems to the diseased tissue/ organ by using carriers or chemical derivatization. There are different types of pharmaceutical carriers like particulate, polymeric, macromolecular and cellular carrier. Particulate type carrier also known as a colloidal carrier system, includes lipid particles (low and high density lipoprotein LDL and HDL respectively), microspheres, nanoparticles, polymeric micelle and vesicular systems like liposomes, niosomes, pharmacosomes, virosomes etc. [1-4]. In recent years, vesicles have become the carriers of choice in drug delivery. Lipid vesicles were found to be of value in immunology, membrane biology, diagnostic techniques and most recently genetic engineering [5-7]. Vesicles can play a major role in modeling biological membranes and in the transport of active agents for targeting. Encapsulation of a drug in vesicular structures can be predicted to prolong the existence of the drug in systemic circulation. If it is being observed with selective uptake, reduces the toxicity [8].

The phagocytic uptake of drug loaded vesicular systems provides an efficient method for delivery of drug directly to the site of infection, leads to reduction of drug toxicity with no adverse effects. Especially vesicular drug delivery is useful in case of poorly soluble drugs due to the reduction in the cost of treatment by improved bioavailability of medication. 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, transferosomes, pharmacosomes, niosomes etc. were developed. Liposomes are concentric bilayered microscopic vesicles with diameter between 20 nm to 20 µm, in which an aqueous volume is entirely enclosed by a membranous lipid bilayer which is mainly composed of phospholipids. There are number of components present in liposomes with phospholipid and cholesterol being the main ingredients. The type of phospholipids includes phosphoglycerides, sphingolipids and together with their hydrolysis products [9]. When phospholipids are dispersed in an aqueous phase, as a result of the interaction of water and the phospholipid molecules which are amphiphilic i.e. they possess hydrophilic and hydrophobic regions. This allows a wide range of materials to be incorporated since hydrophilic drugs are entrapped in the aqueous regions and hydrophobic materials are located in the hydrocarbon region forms a heterogeneous mixture of structures generally referred as vesicles, most of which contain multiple lipid bilayers forming concentric spherical shells are known as liposomes [10].

MATERIALS AND METHODS

Materials

Darifenacin hydrobromide obtained as gift sample from Microlabs, Benguluru. Soya phosphotidylcholine 70 purchased from Sonic-Biochem Extractions, Phospholipon 80 H Phospholipon 90 H purchased from Lipoid, Cholesterol, Methanol, Chloroform purchased from S.D. Fine Chemicals Limited.

Drug-Excipient Compatability Studies

Fourier Transforms Infrared Spectroscopy: This study was performed to ensure the compatability between excipient and drug. Fourier transform infrared (FT-IR 8400s, Shimadzu, Japan) spectra were obtained for pure drug darifenacin hydrobromide and liquid FT-IR studies were carried out to the prepared formulations with different excipients and their compatability was checked. Spectrum of drug was obtained using the potassium bromide disc method. The pellet was prepared with the dry samples by applying 10 tons/inch^2 pressure for 10 min.

Preparation and Optimization of Liposomes with Different Phospholipids

Liposomes were prepared using different phospholipids i.e., phospholipon 80H, phospholipon 90H, soyalecithin and cholesterol incorporation. All the formulations were prepared by conventional thin film hydration method. Cholesterol to phospholipid was optimized and this ratio was used for optimization of liposomes with maximum entrapment efficiency and extended drug release in 24 hrs.

Procedure

Liposomes are prepared by the conventional thin film method as given in Table 1. Lipid mixture along with drug is dissolved in methanol: chloroform (1:2, v/v). This mixture is dried to a thin film by slowly reducing the pressure from 500 to 1 mbar at 40ºC and 80 RPM using rotary flash evaporator. The film is kept under vacuum (1 mbar) for overnight at room temperature and subsequently flushed with nitrogen. Then, the film deposited is hydrated with PBS (pH 7.4) by rotating for 2 hrs at room temperature. The obtained vesicles are sonicated for size reduction using probe sonicator for 3 min.

Evaluation of Prepared Liposomes

The prepared liposomes were evaluated for different properties i.e., entrapment efficiency study, vesicle size analysis, surface morphology, *in vitro* diffusion study and stability study.

Entrapment Efficiency Study

1ml of liposome formulation was transferred into an ephendroff tube and was centrifuged at 15,000 rpm at 4ºC for 20 mins in 5 cycles to separate darifenacin hydrobromide containing liposomes from unentrapped drug. The clear fraction was used for the determination of free drug at 284.5 nm spectrophotometrically. The percentage of drug entrapment or entrapment efficiency (EE) was calculated as

$$\frac{C_t - C_f}{C_t} \times 100$$

Table 1: Liposomal formulations using different phospholipids

Formulations	Drug (mg)	Lipid (mg)	Cholesterol (mg)	Chloroform: Methanol	Probe sonication(min)	SPB pH 7.4 (ml)
DL80H1	15	75	15	2:1	3	10
DL80H2	15	75	25	2:1	3	10
DL80H3	15	75	75	2:1	3	10
DL90H1	15	75	15	2:1	3	10
DL90H2	15	75	25	2:1	3	10
DL90H3	15	75	75	2:1	3	10
DLSL1	15	75	15	2:1	3	10
DLSL2	15	75	25	2:1	3	10
DLSL3	15	75	75	2:1	3	10
DL80H4	15	150	50	2:1	3	10
DL80H5	15	300	100	2:1	3	10
DL80H6	15	450	150	2:1	3	10
DL80H7	15	600	200	2:1	3	10
DL90H4	15	150	50	2:1	3	10
DL90H5	15	300	100	2:1	3	10
DL90H6	15	450	150	2:1	3	10
DL90H7	15	600	200	2:1	3	10
DLSL4	15	150	50	2:1	3	10
DLSL5	15	300	100	2:1	3	10
DLSL6	15	450	150	2:1	3	10
DLSL7	15	600	200	2:1	3	10

Note: Values are expressed as Mean± SD, n=3 D=Darifenacin L=Liposomes 80H=Phospholipon 80H 90H=Phospholipon 90H SL=Soyalecithin

Where, Ct is the total concentration of darifenacin hydrobromide and Cf is the concentration of free darifenacin hydrobromide.

Vesicle Size Analysis

The vesicle size was measured by DelsaTM Nano. The polydispersity index (PI) was used as a parameter of the size distribution. The formulation diluted with deionized water before the size measurements. The particle size was measured at 25°C.

Surface Morphology

The surface morphology (roundness, smoothness and formation of aggregates) of liposomes was studied by Scanning Electron Microscopy (SEM).

***In vitro* Drug Release**

In vitro release of drug from liposomes was performed using the dialysis method. The dialysis bags were soaked before use in distilled water at room temperature for 12 hrs to remove the preservative, followed by rinsing thoroughly in distilled water. *In vitro* release of darifenacin hydrobromide from liposomes was conducted by dialysis in a dialysis sac. 2 ml of liposome formulation was placed in dialysis bags with two ends of sac were tightly bound with threads. The sac was hanged inside the beaker containing 200 ml of phosphate-buffered saline (PBS pH 7.4) as medium, with the help of a glass rod so that the portion of the dialysis bag containing the formulation could dip into the buffer solution. The beaker was kept on a magnetic stirrer; temperature was maintained at 37°C with a thermostatic control at speed of 100 rpm with the help of a magnetic bead. Aliquots of the samples (5 ml) were withdrawn at each time point and the same volume of fresh medium was added to the beaker to maintain the constant volume. The samples were analyzed using spectrophotometer at 284.5 nm. The concentration was calculated from the standard curve.

Model Dependent Methods

Regression coefficients (r^2) were calculated for all the formulations. Release component "n" was calculated from Korsemeyer Peppas equation. The release kinetic calculations were carried out using MS – OFFICE EXCEL. Based on 'n' value the release mechanism was characterized.

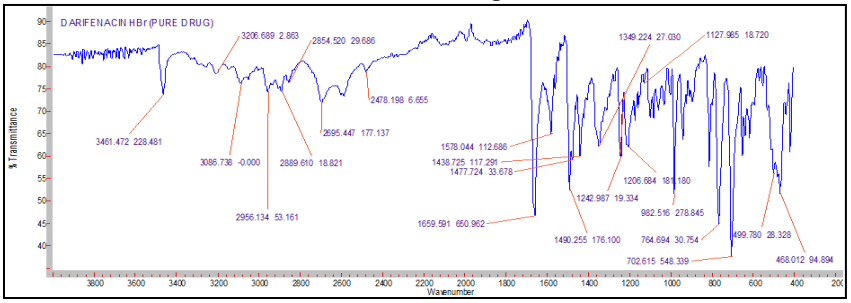
Stability Studies

The optimized formulation was evaluated for physical stability by investigating the leaching of drug from the vesicles. The liposome samples were sealed in 10 ml glass vials and stored at refrigeration temperature (4-8ºC) and at 30ºC for two months. The entrapment efficiency of all the samples was determined for every month in the same manner as prescribed previously.

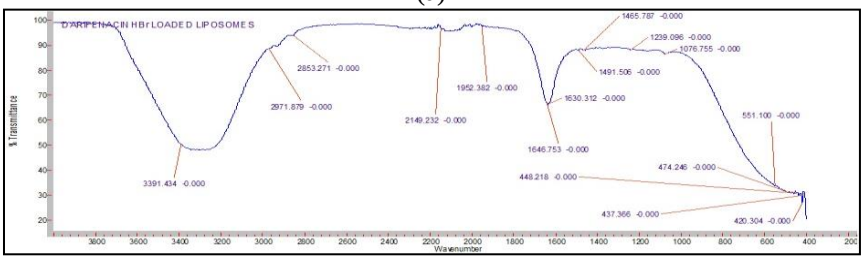
RESULTS AND DISCUSSION

Drug-Excipient Compatibility Study by FTIR

Darifenacin hydrobromide compatibility with excipient was studied by FTIR. The principal peaks of darifenacin hydrobromide were observed at 1213.68, 1351.19, 1440.89, 1665.6, 2958.93, 3466.23 cm^{-1} indicating the presence of C-O, C-N, C=C, C=O, -CH3, O-H groups as shown in Figure 1. It was observed that there was no change in the characteristic peaks of drug in the FTIR spectra of optimized formulation DLSL6. Suggesting, there were no physical or chemical interactions and functional alteration of drug.



(a)



(b)

Figure 1: FTIR graph of a) pure drug b) optimized formulation DLSL6

Characterization of Prepared Liposomes

All liposome formulations were evaluated for different properties i.e.; entrapment efficiency, vesicle size analysis and *in vitro* release profile.

Entrapment Efficiency of Liposomal Formulations

The results showed that the phospholipid to cholesterol ratio was found to have significant impact on the formation of liposomes as shown in the Table 2 and Figure 2.

Table 2: Entrapment efficiency of liposomes for PL to Chol ratio optimization

Formulation code	Phospholipid : Cholesterol	Entrapment efficiency(%)
DL80H1	5 : 1	22.5 ± 0.24
DL80H2	3 : 1	31.7 ± 0.21
DL80H3	1 : 1	23.5 ± 0.21
DL90H1	5 : 1	18.1 ± 0.14
DL90H2	3 : 1	26.1 ± 0.14
DL90H3	1 : 1	20.2 ± 0.32
DLSL1	5 : 1	32.5 ± 0.15
DLSL2	3 : 1	45.3 ± 0.17
DLSL3	1 : 1	31.9 ± 0.16

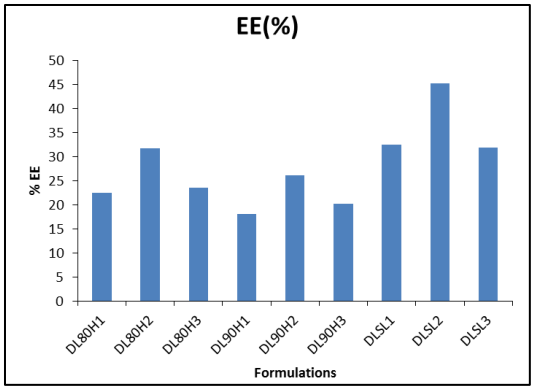


Figure 2: Entrapment efficiency for cholesterol formulations

Entrapment efficiency improved to a certain extent with increase in the cholesterol concentration in the liposomes (3:1). The maximum entrapment efficiency was showed with 3:1 PL and Chol ratio. This could be attributed to the assembling of cholesterol within the phospholipid molecules to provide rigidity to the resultant vesicular structure11. However with an increase in the concentration of cholesterol beyond 3:1 leads to decrease in EE, this might be the result of increase competence of cholesterol with drug to incorporate within the vesicles and leaching of drug molecules owing to disruption of membrane vesicles [11]. Therefore the ratio of phospholipid to cholesterol was selected as 3:1 for further optimization.

The formulations were prepared for PL optimization with optimized PL to Chol ratio (3:1). The results shown that with increase in phospholipid concentration the entrapment efficiency also increased up to 1:30 drug and PL ratios as shown in Table 3 and Figure 3. Further increase in PL resulted in decrease in EE. The formulations with 1:30 (drug:PL) showed highest EE with all types of phospholipids [12].

Table 3: Entrapment efficiency of liposomes for phospholipid optimization

Formulation code	Drug : phospholipid	Entrapment efficiency (%)
DL80H4	1 : 10	40.3 ± 0.16
DL80H5	1 : 20	48.9 ± 0.31
DL80H6	1 : 30	60.0 ± 0.24
DL80H7	1 : 40	51.2 ± 0.14
DL90H4	1 : 10	34.4 ± 0.24
DL90H5	1 : 20	41.2 ± 0.16
DL90H6	1 : 30	52.5 ± 0.17
DL90H7	1 : 40	43.7 ± 0.28
DLSL4	1 : 10	58.0 ± 0.16
DLSL5	1 : 20	67.2 ± 0.25
DLSL6	1 : 30	78.5 ± 0.18
DLSL7	1 : 40	70.1 ± 0.26

Note: Values are expressed as Mean±SD, n=3 D=Darifenacin hydrobromide L=Liposomes 80H=Phospholipon 80H 90H=Phospholipin 90H SL=Soyalecithin

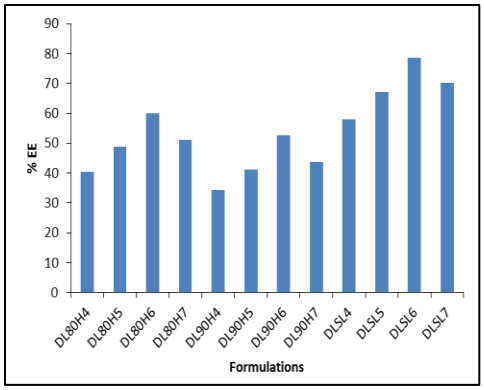


Figure 3: Entrapment efficiency for phospholipid formulations

Vesicle Size Analysis
DARIFENACIN HBr
Measurement Results
Date: Wednesday September 11, 2013
Measurement Type: Particle size
Sample Name: Darifenacin HBr
Scattering Angle: 173
Temperature of the holder : 25.0°C
T% before meas.: 44
Viscosity of the dispersion medium: 0.895 mPa·s
Form of Distribution: Standard
Representation of result: Scattering Light Intensity
Count rate: 19 kCPS

The particle size of the optimized formulation (DLSL6) vesicles was obtained as 24.1 nm as shown in Figure 4 and the formulation was a homogenous suspension. The size and size distribution of liposomes depend on the method of size reduction, by which large lipid vesicles are reduced to small vesicles of nanometer scale. Ultra-sonication helps to reduce the size of the liposomes. In this case, nano-size liposomes may be formed as an effect of ultra-sonication [13]. The liposomes had an average size of 24.1 nm in a narrow size range and with a uniform distribution pattern. Since they had nano-dimensions, longer residence time in systemic circulation could help them reaching the target tissues [14].

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	24.1 nm	32.3 nm	8.6 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	24.1 nm	32.3 nm	8.6 nm

Histogram Operations

% Cumulative (2) : 10.0 (%) - 5.6 (nm)
% Cumulative (6) : 50.0 (%) - 12.9 (nm)
% Cumulative (10) : 90.0 (%) - 53.2 (nm)

Figure 4: Vesicle size analysis

Surface Morphology of Optimized Formulation
Surface morphology and the three-dimensional nature of the liposomes studied through SEM as shown in Figure 5, confirmed the preparation as smooth-surfaced Nano-carriers possessing vesicular characteristics.

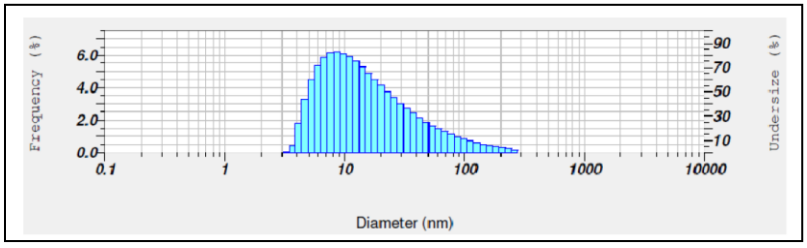


Figure 5: Frequency vs. undersize

Zeta Potential of Optimized Formulation
Measurement Results
Darifenacin HBr
Measurement Results
Date: Wednesday September 11, 2013
Measurement Type: Zeta Potential
Sample Name: Darifenacin HBr
Temperature of the holder: 25.2°C
Viscosity of the dispersion medium: 0.891mPa·s
Conductivity: 6.738 mS/cm
Electrode Voltage: 4.3 V

The zeta potential of optimized formulation (DLSL6) which was selected based on entrapment efficiency is shown in Figure 6. The value was -51.6 mV which indicates that the surface of liposomes was dominated by the anions and proved that prepared liposomes have sufficient charge to avoid aggregation of vesicles. Zeta potentials more positive than +30 mV and more negative than -30 mV are normally considered stable for colloidal dispersion. The optimized liposomal formulation zeta potential value was more than -50 mV, which suggest that the formulation was stable suspension and had nano-dimensions and thus it would be easier for parenteral administration [13].

No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation
1	0.34	0.000	0.000	22	4.40	1.816	2.292	43	57.09	1.655	90.968	64	740.89	0.000	100.000
2	0.38	0.000	0.000	23	4.97	3.279	5.571	44	64.50	1.448	92.416	65	837.07	0.000	100.000
3	0.43	0.000	0.000	24	5.61	4.477	10.048	45	72.87	1.263	93.680	66	945.74	0.000	100.000
4	0.49	0.000	0.000	25	6.34	5.330	15.379	46	82.33	1.098	94.778	67	1068.52	0.000	100.000
5	0.55	0.000	0.000	26	7.17	5.864	21.243	47	93.02	0.952	95.730	68	1207.24	0.000	100.000
6	0.62	0.000	0.000	27	8.10	6.134	27.377	48	105.10	0.823	96.552	69	1363.97	0.000	100.000
7	0.70	0.000	0.000	28	9.15	6.197	33.574	49	118.74	0.709	97.261	70	1541.04	0.000	100.000
8	0.80	0.000	0.000	29	10.34	6.104	39.678	50	134.16	0.609	97.870	71	1741.10	0.000	100.000
9	0.90	0.000	0.000	30	11.68	5.896	45.574	51	151.57	0.522	98.391	72	1967.14	0.000	100.000
10	1.02	0.000	0.000	31	13.20	5.609	51.183	52	171.25	0.446	98.837	73	2222.51	0.000	100.000
11	1.15	0.000	0.000	32	14.91	5.270	56.452	53	193.48	0.380	99.217	74	2511.05	0.000	100.000
12	1.30	0.000	0.000	33	16.84	4.899	61.352	54	218.60	0.324	99.541	75	2837.04	0.000	100.000
13	1.47	0.000	0.000	34	19.03	4.515	65.866	55	246.98	0.277	99.818	76	3205.35	0.000	100.000
14	1.66	0.000	0.000	35	21.50	4.128	69.995	56	279.04	0.182	100.000	77	3621.48	0.000	100.000
15	1.87	0.000	0.000	36	24.29	3.749	73.744	57	315.27	0.000	100.000	78	4091.63	0.000	100.000
16	2.11	0.000	0.000	37	27.45	3.384	77.128	58	356.20	0.000	100.000	79	4622.81	0.000	100.000
17	2.39	0.000	0.000	38	31.01	3.038	80.166	59	402.44	0.000	100.000	80	5222.96	0.000	100.000
18	2.70	0.000	0.000	39	35.03	2.714	82.880	60	454.69	0.000	100.000	81	5901.02	0.000	100.000
19	3.05	0.000	0.000	40	39.58	2.413	85.293	61	513.71	0.000	100.000	82	6667.10	0.000	100.000
20	3.45	0.026	0.026	41	44.72	2.136	87.430	62	580.41	0.000	100.000	83	7532.65	0.000	100.000
21	3.89	0.449	0.476	42	50.53	1.884	89.313	63	655.76	0.000	100.000	84	8510.56	0.000	100.000

Figure 6: Vesicle size analysis of optimized DLSL6 formulation

In vitro Drug Release Studies

In vitro drug release study of liposome formulations was performed using the dialysis method. Formulation equivalent to 3 mg of darifenacin hydrobromide was taken in the dialysis sac and 200 ml of PBS was taken in the beaker.

The *in vitro* drug release study results of formulations for cholesterol optimization with phospholipon 80H (DL80H1, DL80H2), phospholipon 90H (DL90H1, DL90H2), soyalecithin (DLSL1, DLSL2) showed that with increase in Chol ratio the drug release over 24 hr increased due to the increase in entrapment efficiency. Further increase in cholesterol ratio (DL80H3, DL90H3, DLSL3) resulted in decreased drug release due to the decrease in the entrapment efficiency and also due to the hydrophobic nature of the cholesterol [15]. The results of formulations for PL optimization (DL804 to DL80H6, DL90H4 to DL90H6 and DLSL4 to DLSL6) showed with increase in phospholipid the drug release over 24 hrs increased due to increase in EE and also due to the hydrophilic nature of the phospholipid [15]. Further increase in PL (DL80H7, DL90H7 and DLSL7) resulted in decreased drug release due to decreased EE as shown in Figures 7-9. The formulation DLSL6 with highest EE (78.5%) showed highest drug release 82.4% over 24 hrs.

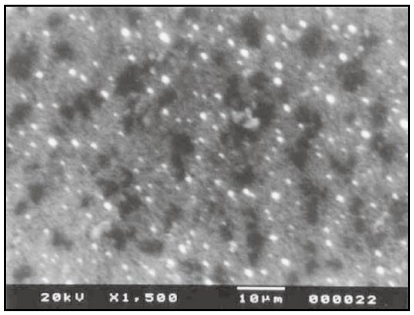


Figure 7: SEM photograph of optimized DLSL6 formulation

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-51.6 mV	-0.000267 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean) : -51.6 mV

Electrophoretic Mobility mean : -0.000267 cm^2/Vs

Figure 8: Measurement results

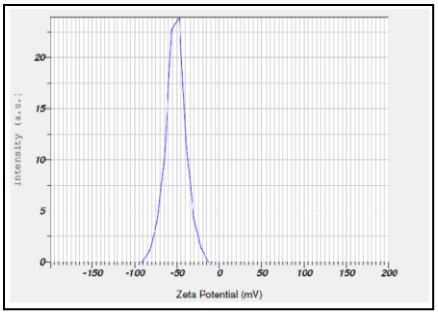


Figure 9: Zeta-potential of optimized DLSL6 formulation

Model Dependent Methods

Release kinetics for all the twenty one formulations were calculated using Microsoft Office Excel 2007 version as shown in Table 4. The release data was analysed by fitting the drug release profiles of all the formulations into zero order release model, first order release model, Higuchi model and Korsmeyer-Peppas model. Regression coefficients (r^2) were calculated for all the formulations. The model that best fits the release data is selected based on the correlation coefficient (r^2) value in various models. The model that gives high r^2 value is considered as the best fit of the release data. Zero order release constant K0 was calculated for zero order release model, first order release constant K1 was calculated for first order release model, Higuchi constant KH was calculated for Higuchi model and release exponent 'n' was calculated for Korsmeyer-Peppas model.

Table 4: Model dependent kinetic study for all formulations diffusion profiles

Formulation	Zero order Release	First order Release	Higuchi release	Korsmeyer-Peppas release	
Parameter	r^2	r^2	r^2	r^2	N
DL80H1	0.841	0.655	0.964	0.954	0.454
DL80H2	0.848	0.683	0.967	0.963	0.461
DL80H3	0.838	0.671	0.963	0.957	0.449
DL80H4	0.929	0.798	0.987	0.988	0.372
DL80H5	0.923	0.797	0.988	0.988	0.342
DL80H6	0.934	0.810	0.986	0.989	0.467
DL80H7	0.938	0.804	0.989	0.989	0.382
DL90H1	0.806	0.645	0.943	0.948	0.435
DL90H2	0.877	0.701	0.975	0.974	0.416
DL90H3	0.833	0.668	0.958	0.959	0.391
DL90H4	0.877	0.711	0.972	0.970	0.319
DL90H5	0.845	0.666	0.966	0.959	0.480
DL90H6	0.917	0.749	0.989	0.987	0.494
DL90H7	0.887	0.716	0.975	0.976	0.447
DLSL1	0.808	0.652	0.950	0.954	0.464
DLSL2	0.808	0.647	0.950	0.951	0.458
DLSL3	0.768	0.638	0.929	0.946	0.324
DLSL4	0.776	0.597	0.937	0.931	0.483
DLSL5	0.830	0.682	0.957	0.964	0.468
DLSL6	0.981	0.735	0.892	0.980	0.455
DLSL7	0.857	0.687	0.966	0.968	0.420

Regression coefficients were reported for all the formulations. DLSL6 was considered as optimized formulation on account of its highest entrapment efficiency and highest drug release over 24 hrs. The optimized formulation by kinetics (based on highest r^2 value) followed zero order release. The correlation coefficient (r^2) was 0.981 for optimized formulation (DLSL6). The release component "n" was calculated from the Korsmeyer-Peppas kinetics equation (0.455) which revealed that the optimized formulation followed fickian diffusion mechanism in drug release.

Stability Studies

The stability profile of darifenacin hydrobromide loaded liposomal formulations evaluated for substantial loss of drug at various temperatures such as 4ºC and room temperature (RT) for two months.

The percentage of drug retained in the vesicles after a period of two months at 4ºC and room temperature were found to be 76.43 and 30.21 respectively for DLSL6 formulation as shown in Figure 10. The results indicate that more than 90% of the drug was retained in the liposomal formulation for a period of two months at 4ºC. The entrapment efficiency was decreased may be due to fusion or aggregation of vesicles. From this it can be concluded that liposomal formulations were stable under refrigeration temperature with least leakage.

The stability profile suggested the storage of the liposomal formulations at refrigerated temperature (4 ± 2ºC), as compared to elevated temperatures, the greater drug loss from the system was observed (Figures 10-13), at RT that might be described to the effect of temperature on the gel-to-lipid transition of lipid bilayers together with possible chemical degradation of the phospholipids, leading to defects in the membrane packing.

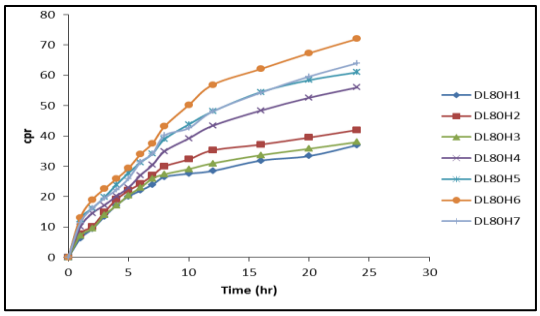


Figure 10: *In vitro* release profile of formulations with phospholipon 80H

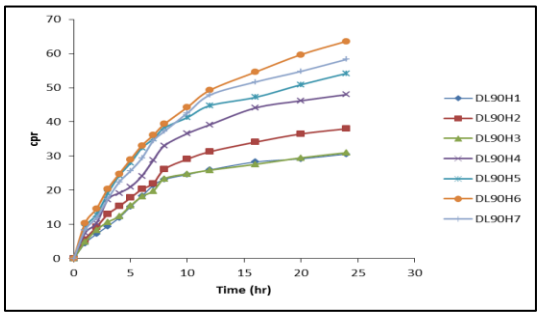


Figure 11: *In vitro* release profile of formulations with phospholipon 90H

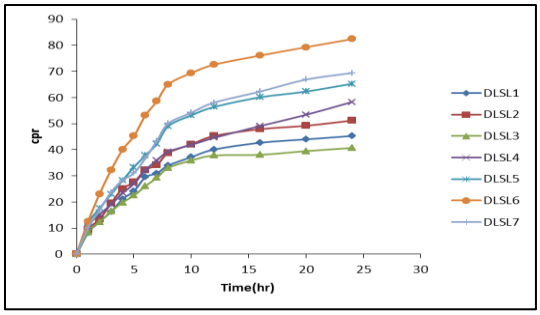


Figure 12: *In vitro* release profile of formulations with soya lecithin

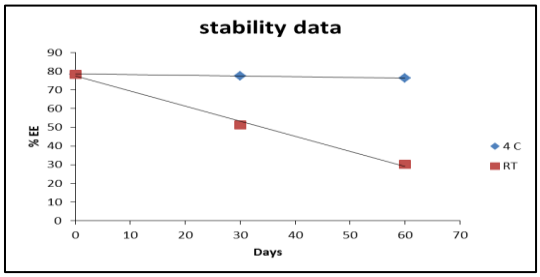


Figure 13: Stability study data for DLSL6 formulation

CONCLUSION

Darifenacin hydrobromide liposomes were prepared and optimized using thin film hydration method with different phospholipids. Promising results were obtained with DLSL6 formulation containing soyalecithin phospholipid because of highest entrapment efficiency and the sustained drug release. The optimized formulation has an average size of 24.1 nm in a narrow size range and with a uniform distribution pattern and it was stable with negative zeta potential value of -51.6 mV. Since they had nano-dimensions, longer residence time in systemic circulation could help them reaching the target tissues. Over active bladder syndrome requires to be controlled for longer duration instead delivery the drug all at once and it was achieved by formulating darifenacin hydrobromide into nano-liposomes.

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