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Preparation, optimization and evaluation of liposomes encapsulating diclofenac sodium and charge inducers to enhance stability using lipid hydration method.

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ABSTRACT

Aims

The aim of the study is to encapsulate, optimize and characterize the liposomal preparations of various formulations of Diclofenac sodium (DS) along with phosphatidylcholine, cholesterol, stearylamine and dicetylphosphate.

Settings and design

Rotary evaporator is set at a temperature of 40^0C with constant rotation speed. Instruments used during the study includes Whirl mixer, Hellos software, zetasizer, Ultracentrifugation, Dialysis tube and UV Spectrophotometer.

Methods and Material

Diclofenac Sodium, Phosphatidylcholine, Cholesterol, Dicetylphosphate, Stearylamine, Phosphate buffer and solvents. Liposomes were prepared by Lipid-Hydration technique using rotary evaporator (RE-300). The prepared liposomes were analyzed for size, zeta potential, percentage of drug encapsulated, in-*vitro* drug release and stability studies.

Results

Particle size of the drug loaded liposome was decreased when compared to that of the drug free. Encapsulation efficiency of the drug loaded liposomes with PC shows increase in the percentage of drug encapsulated to that of the lower concentrated vesicles and positive charge inducer have revealed elevated encapsulation efficiency. Liposomes composed of PC: CHOL: SA observed to be released at high rate and stability studies confirms that PC: CHOL: SA is supreme stable at varied temperatures.

Conclusions

Phosphatidylcholine, cholesterol and stearylamine based preparations posses the suitable % drug encapsulated and release rate. The composition PC: CHOL: SA at a concentration of 16:8:4 µmoles proved as a stable suspension. From the study it can be concluded that cholesterol and stearylamine based phosphatidylcholine liposomes are most suitable to encapsulate the Diclofenac sodium.

Keywords: Liposomes, Diclofenac Sodium (DS), Phosphatidylcholine (PC), Entrapment, Lipid, Cholesterol (CHOL), Stearylamine (SA), Dicetylphosphate (DCP).

INTRODUCTION

Since 1974 Diclofenac sodium (DS) has been used as a potent non-steroidal anti inflammatory drug (NSAID) with its antipyretic and analgesic effects. Similar to other NSAID, DS is also associated with serious gastro intestinal (GI) side effects like ulceration when administered orally and cutaneous lesion by intramuscular injection[1]. DS also undergoes first pass metabolism when administered orally. As the amphiphilic drugs have the tendency to form aggregates with celluloses[18], other different techniques have been followed to reduce the side effects. Incorporation of drug with organized structures is of great interest on drug absorption and targeting. Encapsulation by Phospholipids has been suggested one of the ways to improve the condition and the drug causes the structural modification of phospholipids forming the surface active monomers[2]. Liposome has been known as drug carrier for wide variety of compounds, to improve therapeutic effects and to reduce the side effects. The mucus which covers the surface epithelium of the GI tissue poses an adsorbed layer of phospholipids, provides a hydrophobic layer between epithelium and luminal contents[14]. There are a number of lipid species which reminds the surface active phospholipids among which phosphatidyl choline (PC) is suitable to incorporate DS. It has been suggested that presence of ionic surfactants DS shield the changes which are pH-dependant and the complex remains lipophilic.

In the present work, drug is encapsulated into the liposomes along with the positive and negative surfactants and the effect of drug encapsulation on the size and zeta potential is studied. The samples were being stores at three different temperatures and the size and zeta potential were measured at an interval of 7 days. Size, zeta potential, percentage of drug release and encapsulation efficiency was observed.

Since 1970 liposomes have been widely used as drug carriers for improving the drug delivery to specific sites in the body. Lipid-based formulations were developed and the current lipid-based pharmaceuticals are a result of study of lipid-drug interactions and liposome disposition mechanisms. Formulation of drug in the liposome enhances the therapeutic index by altering bio distribution[4].Liposomes are the liquid crystals which are biocompatible and biodegradable. In nature, liposomes are made up of bilayer phospholipids. The hydrophilic head faces the surface and the lipophilic tail away from the surface[5].

Cholesterol plays an important role in stabilising the liposomes, it increases the rigidity, thus improves encapsulation efficiency and increase in concentration decreases the release rate.

Stearylamine and dicetylphosphate induce the charge, which creates an electrostatic repulsion between the adjacent vesicles[4].

AIM AND OBJECTIVES

- Prepare Diclofenac sodium encapsulated liposome.
- Determine of Liposomal characteristics (size and zeta potential).
- Calculate the percentage of drug encapsulated.
- Conduct the drug release in-vitro.
- Conduct the stability studies, by storing the liposomes at 0^0C, 25^0C and 40^0C and measuring its size and zeta potential at an interval of 7 days.
- Compare the results of drug free and drug loaded liposomes.

METHODOLOGY

Method of preparation

Stock solutions were prepared by using 9:1 ratio of chloroform and methanol and stored at -20^0C

Drug (DS): 1mg/ml

Phosphatidyl choline (PC): 100mg/ml

Cholesterol (CHOL): 15mg/ml

Stearyl amine (SA): 5mg/ml

Dicetyl phosphate (DCP): 5mg/ml

Phosphate buffer saline (PBS): 0.01M of PBS was prepared by dissolving one tablet into 200ml of distilled water. Lipid hydration technique was used in the preparation of drug loaded and drug free liposomes. The above stock solutions were used at different compositions. Appropriate volumes were transferred by using a micro pipette into a dry 100ml round bottom flask. The rotary evaporator (RE-300)[1] was set to temperature of 40^oC, rotation speed was kept constant for all the preparation and the whole system. Round bottom flask was detached from the system after the solvent completely evaporated by releasing the vacuum. The film was hydrated by using PBS solution by vortexing (Whirl mixer) until the lipid film dissolved. The resultant milky suspension

was transferred into a fresh bijou and tested for it size and zeta. The method was followed for all the other preparations.

RESULTS AND DISCUSSION

Standard curve

To prepare standard curve 10mg of the drug (DS) was dissolved in 10ml of phosphate buffer saline (pH–7.4), which gives a concentration 1mg/ml. This was treated as the stock solution and the serial dilutions 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 30µg/ml and 40µg/ml were prepared and their absorbance was measured by UV spectroscopy at 275nm wavelength. The calibration points were estimated by UV method.

Table1: Concentrations of the serial dilutions were prepared and the absorbance of the dilutions was measured by UV spectroscopy.

Concentration	*Absorbance*
5	0.181
10	0.343
15	0.505
20	0.664
30	0.974
40	1.236

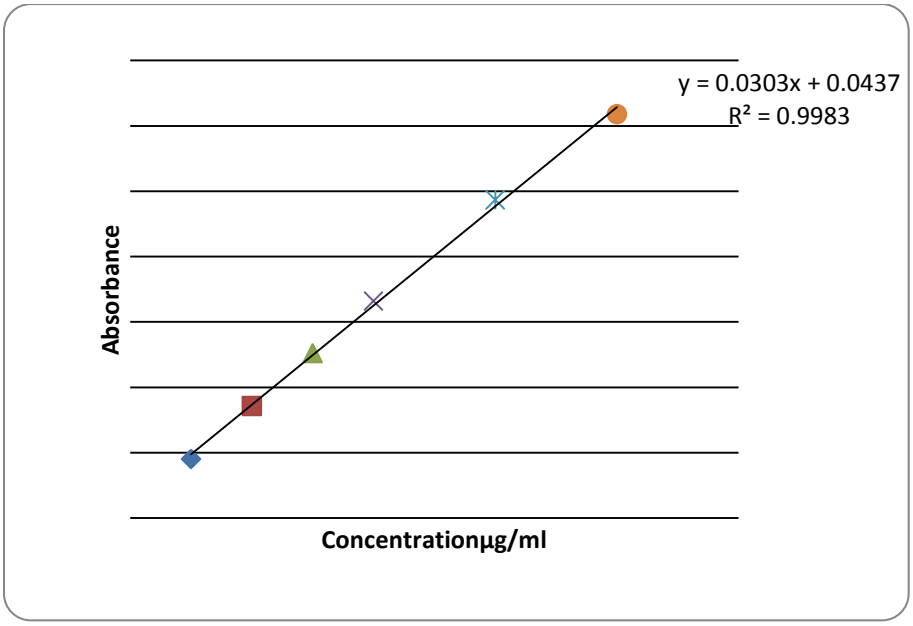

Figure 1: Calibration curve; standard curve was drawn by taking concentration on X-axis and absorbance on Y-axis, the absorbance was measured by UV spectroscopy

Particle Size determination

The size of the drug-loaded liposomes is compared with that of the drug free liposome, and significant difference can be seen between drug free and drug loaded liposome. The interaction of DS with PC: CHOL shows the decrease in the diameter

Table 2: Particle size of the liposomes

Composition	Drug free (µm)	Drug-loaded (µm)
PC (8µ moles)	8.74±1.17	8.5±1.2
PC (16µ moles)	9.09±0.34	7.7±0.3
PC:CHOL (16:8µ moles)	11.26±1.42	6.4±0.3
PC:CHOL (16:16µ moles)	10.52±2.6	7.7±0.8
PC:CHOL:SA (16:8:4µ moles)	6.78±0.34	5.8±0.1
PC:CHOL:SA (16:8:8µ moles)	29.07±10.82	20.7±14
PC:CHOL:DCP (16:8:4µ moles)	38.35±5.49	5.8±0.6
PC:CHOL:DCP (16:8:8µ moles)	42.69±13	22.3±3.8

Zeta potential

Zeta potential of the drug free and drug loaded liposomes did not prove much variation (table 4), but the formulations with stearylamine and dicetylphosphate shows an increase charge and else than the other formulations, zetapotential of the charge induced liposomes have demonstrated increased zetapotential ranging from 55 to 60 which indicates enhanced stable suspension compared to the other formulations.

Table 3: zeta potential of drug free liposomes

	Zeta potential	
Composition	**Drug free**	**Drug –loaded**
PC (8µ moles)	-2.59±0.74	-4.1±0.6
PC (16µ moles)	-2.00±1.1	-2.7±0.7
PC:CHOL (16:8µ moles)	-1.00±0.92	-1.5±0.1
PC:CHOL (16:16µ moles)	-0.77±0.9	-18.7±1.3
PC:CHOL:SA (16:8:4µ moles)	54.83±3.26	59.4±1.7
PC:CHOL:SA (16:8:8µ moles)	60.27±1.22	53.1±2.4
PC:CHOL:DCP (16:8:4µ moles)	-61.67±4.76	-57.2±1.8
PC:CHOL:DCP (16:8:8µ moles)	-58.50±2.59	-52.6±1.3

Encapsulation efficiency

Encapsulation efficiency depends on the concentration ratio of the lipids. Percentage of drug encapsulated of PC (16µ moles) is higher than PC (8µ moles), which may be due to increased concentration.

Table 4: Encapsulation efficiency (%) of drug loaded liposomes

Composition-Drug loaded	Encapsulation efficiency
PC (8µ moles)	69.81±1.43
PC (16µ moles)	83.5±1.68
PC:CHOL (16:8µ moles)	44.37±3.31
PC:CHOL (16:16µ moles)	77.7±2.38
PC:CHOL:SA (16:8:4µ moles)	67.93±3.09
PC:CHOL:SA (16:8:8µ moles)	63.93±1.46
PC:CHOL:DCP (16:8:4µ moles)	32.34±0.87
PC:CHOL:DCP (16:8:8µ moles)	46.4±6.24

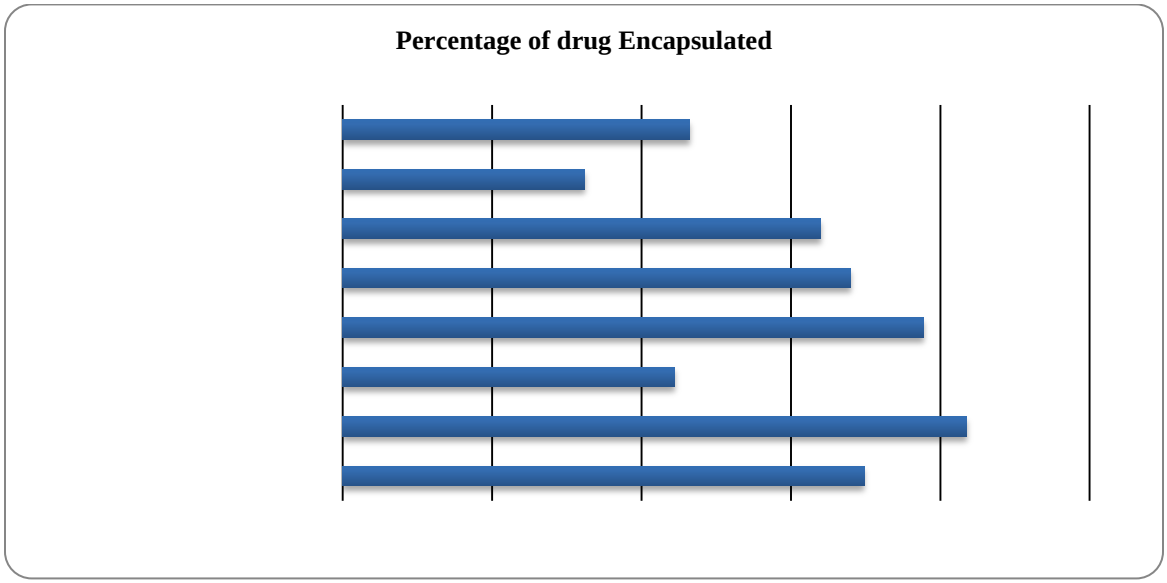

Figure 2: Percentage of drug (Diclofenac sodium) encapsulated of drug loaded liposomes containing phosphatidyl choline (PC), cholesterol (CHOL), stearylamine (SA), and dicetylphosphate (DCP) was drawn against compositions.

In-vitro Drug release study

Table 5: in-vitro drug release of drug loaded liposomes

Sample time	*PC:CHOL:DS* % Drug release	*PC:CHOL:SA:DS* % Drug release	*PC:CHOL:DCP:DS* % Drug release
0.5	32.46±7.2	3.46±0.8	0.62±1.3
1	40.86±2.9	8.96±1	4.1±1.6
2	53.1±3.5	14.7±1.2	7.96±1.9
4	70.1±5.2	22.3±0.9	14.13±2.1
6	77.3±7.5	23.9±1.4	15.25±2.3
8	78.2±8.1	26.2±1.6	17.9±2.4
24	76.86±9.75	36.7±4.2	35.5±4.8

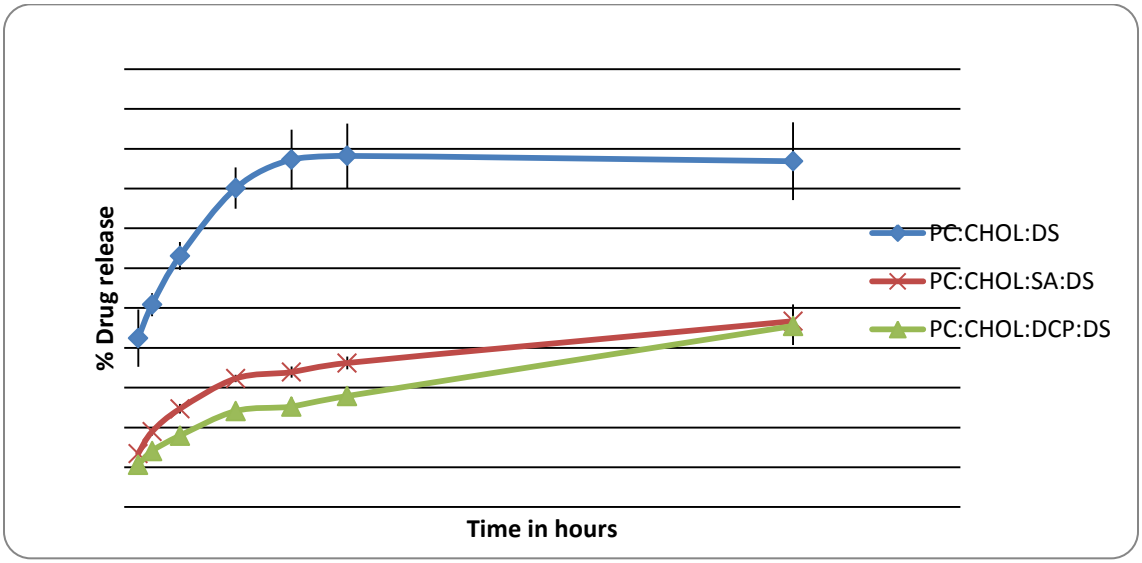

Figure 3: The drug loaded liposomes of the compositions PC:CHOL:DS, PC:CHOL:SA:DS, PC:CHOL:DCP:DS were being studied for the amount of drug released, and the graph was plotted by taking time in hours on X-axis and % drug released on Y-axis.

Stability studies

It can be observed from that the size of the vesicles was not much varied upon storing them for a period of 21 days at three different temperatures.

Table 6: Standard zetapotential values and their related stability behaviour

Zeta potential (mV)	Stability behaviour of the colloid
From 0 to ±5	Rapid flocculation
From ±10 to ±30	Incipient instability
From ±30 to ±40	Moderate stability
From ±40 to ±60	Good stability
More than ±61	Excellent stability

Table 7: data of the particle size and zeta potential during stability

Particle size													
	0^0C temperature				**25^0C temperature**				**40^0C temperature**				
Composition	0^{th} day	7^{th} day	14^{th} day	21^{st} day	0^{th} day	7^{th} day	14^{th} day	21^{st} day	0^{th} Day	7^{th} day	14^{th} day	21^{st} day	
PC:CHOL:DS	6.4±0.3	6.4±0.4	6.8±0.5	8.8±1.2	6.4±0.3	7.0±0.8	13.2±2	30.9±7	6.4±0.3	8.6±5.6	10.6±6	8.9±1.5	
PC:CHOL:SA:DS	5.8±0.1	5.7±0.3	5.5±0.1	5.6±0.3	5.8±0.1	5.8±0.4	5.6±0.2	6.3±1.1	5.8±0.1	5.5±0.1	5.83±0.5	6.2±0.8	
PC:CHOL:DCP:DS	5.7±0.6	5.6±0.4	5.8±0.1	5.7±0.5	5.6±0.6	5.7±0.6	6.7±1.7	10.2±4	5.7±0.6	6.5±3.2	7.6±2	6.6±0.2	

Zeta potential

Composition	0^0C temperature				25^0C temperature				40^0C temperature			
	0^{th} day	7^{th} day	14^{th} day	21^{st} day	0^{th} day	7^{th} day	14^{th} day	21^{st} day	0^{th} day	7^{th} day	14^{th} day	21^{st} day
PC:CHOL:DS	-1.5±2.2	-2.83±0.4	-15.7±4.2	-24.2±1.0	-1.5±0.2	-18.8±3.3	-33.5±0.8	-34.5±3.3	-1.5±0.2	-29.3±4.3	-38.2±1.8	-24.6±7.0
PC:CHOL:SA:DS	59.3±1.8	52.2±2.1	51.4±2.6	51.3±3.5	59.3±1.8	48.1±5.9	49.6±4.0	47.2±4.5	59.3±1.8	44.4±1.4	39.7±5.6	42.8±1.8
PC:CHOL:DCP:DS	-57.2±1.8	-59.7±2.9	-63.5±4.0	-61.6±2.3	-57.2±0.8	-57.2±4.2	-60.8±3.0	-66.7±4.9	-57.2±1.8	-61.5±5.8	-68.5±2.5	-67.8±2.5

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