

# Development, Physicochemical and *In-Vitro* Evaluation of Dexamethasone-Containing Liposomes

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## ABSTRACT

The purpose of this research was to develop dexamethasone-containing liposomes (DCL) based on different combination of cholesterol and soy L- $\alpha$ -lecithin by lipid film hydration method. Although many studies are available on DCL, none of them provides sufficiently convincing technologies for manufacturing DCL with all the standardized process parameters such as amount of drug loading, drug-release, liposome size, etc. Therefore, more research is required in the field. Different process parameters such as drug-excipient interaction (by FTIR study), surface morphology by scanning electron microscope (SEM), particle size analysis and *in-vitro* drug release study were done. The average particle size of the liposomes was 1  $\mu$ m. It was found that drug loading of the formulations were between 1.39% w/w and 1.49% w/w. *In vitro* drug release study shows that between 87% and 96% release of drug obtained from the different experimental liposomes in 500 min. Drug release was found to follow Korsmeyer kinetics.

**Keywords:** cholesterol, FTIR-spectroscopy, release kinetics, soy L- $\alpha$ -lecithin, sustained release

## INTRODUCTION

With the advancement in drug delivery technology, new drugs and novel delivery systems are constantly in pursuit. Interest in liposomes has focused on devising ways to improve delivery methods, thereby reducing toxicity and consequently improving the therapeutic index (Bangham *et al.* 1965). Liposomes can be used as delivery and targeting agents for the administration of drugs at lower doses with reduced toxicity (Kohno *et al.* 1998; Huwyler *et al.* 2008). Liposomes are the unilamellar or multilamellar spherical structures consisting of lipid bilayers arranged in a concentric fashion enclosing an equal number of aqueous compartments (Xiang *et al.* 2006). Liposomes are able to encapsulate both hydrophilic and lipophilic molecules (Dodov *et al.* 2004). Lipophilic dexamethasone is a glucocorticoid that is used clinically as an anti-inflammatory and immunosuppressive agent (Tsotas *et al.* 2007). Glucocorticoids are inhibitory to cellular inflammation processes as well as smooth muscle cell proliferation and collagen formation, but the great number of side effects such as: hypertension, hydro-electrolytic disorders, hyperglycemia, peptic ulcers and glucosuria restricts the use of dexamethasone in prolonged therapy. In the last years much interest has been focused on liposomes, as drug delivery systems, due to their possibilities of increasing drug efficacy, reducing toxicity and controlling drug release. However, few data are available about the entrapment of dexamethasone in liposomes (Beck *et al.* 2003). Depending on cholesterol/phospholipids molar ratio DCL have been studied widely. Further, none of the available studies related to manufacturing and standardization of process parameters is sufficient enough to bring them in a large-scale industrial production. Thus more studies in this area are required. Treatment of arthritis or osteoarthritis has significantly improved in recent years, but therapies are still symptomatic (Lee *et al.* 2008). Current treatment mainly involves analgesics or anti-inflammatory drugs (both non-steroidal and steroidal agents), administered either orally or intra-articularly (i.a.). Thus, corticosteroid-containing drug delivery systems are of major interest in the intra-articular administration of corticosteroids, ensuring a long and con-

trolled release of the active substance in the region of interest (Butoescu *et al.* 2009). The formulations were prepared here taking cholesterol and phospholipid at different ratios to develop the most suitable formulation out of the prepared ones, which would deliver dexamethasone in a controlled manner over a prolonged period of time. To investigate the effect of different process parameters on the liposomes, physicochemical evaluation such as FTIR study, surface morphology by scanning electron microscope (SEM), particle analysis study and *in-vitro* drug release from the formulations were conducted.

## MATERIALS

Dexamethasone was obtained as a gift sample from Bio-ethicals pharma Ltd. (Mumbai, India). Soya L- $\alpha$ -lecithin from HiMedia Laboratories Pvt. Ltd. (Mumbai, India) and cholesterol from Merck (Mumbai, India), butylated hydroxy anisole (BHA) from Qualigens Fine Chemicals (Mumbai, India) and chloroform (Merck) were purchased. All other chemicals used were of analytical grade.

## METHODS

Liposomes were prepared by lipid film hydration method (Mukherjee *et al.* 2007). Briefly, the weighed amounts of dexamethasone, soya L- $\alpha$ -lecithin and cholesterol were taken as per their experimental combinations (Table 1) containing butylated hydroxyl anisole (BHA) (2% w/w of lipid) in 250 ml round bottom flask and were dissolved in chloroform. The mixture was placed in a rotary vacuum evaporator with an aspirator A 3S (Tokyo Rikakikai Co. Ltd., Tokyo, Japan). The rotary vacuum evaporator was fitted

**Table 1** Lipid compositions of the various formulations and % loading.

Formulations	Lipid compositions of the formulations (molar ratio)	% Loading
F1	SPC:CH = 100:5	1.47 $\pm$ 0.034
F2	SPC:CH = 100:11.2	1.41 $\pm$ 0.052
F3	SPC:CH = 100:14	1.39 $\pm$ 0.045
F4	SPC:CH = 100:20	1.49 $\pm$ 0.039

with a cold water circulating bath (Spac-N Service, Kolkata, India) and was rotated at 150 rpm. The temperature of the water bath was maintained at 32°C to evaporate the solvent. After the initial evaporation, the flask was kept in a vacuum desiccator overnight for complete removal of residual chloroform. The film was hydrated in a rotary vacuum evaporator maintained at 60°C and rotated at 100 rpm until the lipid film was dispersed in the aqueous phase. The sizes of the vesicles were reduced by a bath-sonicator (Instrumentation India, Kolkata, India) at 60°C for 1 h. After sonication, the preparations were kept at room temperature for 1h for vesicle formation and then they were kept at 4°C in an inert atmosphere for 24 h. The preparation was then centrifuged at 5000 rpm at 4°C for 5 min. The supernatant containing the vesicles in each case was taken for further studies as a suspended formulation. The formulations were lyophilized using a lyophilizer (Instrumentation India Ltd, Kolkata, India). Primary drying was conducted at -35°C for 8 h under vacuum after the standard prefreezing.

## Characterization of dexamethasone liposomes

### 1. Fourier transforms infra-red spectroscopy (FTIR) study

The pure drug dexamethasone, mixture of dexamethasone with cholesterol and soya L- $\alpha$ -lecithin and a mixture of cholesterol and soya L- $\alpha$ -lecithin were mixed separately with IR grade KBr in the ratio 1: 100 and corresponding pellets were prepared by applying 5.5 metric tons of pressure in a hydraulic press. The pellets were scanned in an inert atmosphere over a wave number range of 4000 to 400  $\text{cm}^{-1}$  in Magna IR 750 Series II (Nicolet, USA) FTIR spectroscope (Ghosh *et al.* 2009).

### 2. Scanning electron microscopic study

Surface morphology was determined by scanning electron microscopy (JEOL, JSM5200, and Tokyo, Japan). The samples were spreaded on metal stubs and gold coating was done by using ion-sputtering device. The gold coated samples were vacuum dried and then examined (De *et al.* 2009).

### 3. Liposome size distribution study

Liposome size distribution was performed by the instrument Zetasizer nano ZS with DTS software (Malvern Instrument Limited, UK). NIBS<sup>®</sup> (non-invasive backscatter optics) technology was used for measurement of vesicles. The formulations were taken in lyophilized form in microcentrifuge tubes, suspended in phosphate buffer, pH 7.4 and introduced in the instrument to read the results (Mukherjee *et al.* 2008).

### 4. Drug loading study

The liposome vesicles were lysed with chloroform and the free drug was partitioned into PBS buffer, pH 7.4. The absorbance of the buffer was noted using the UV/VIS spectrophotometer (Beckman, USA) at 240 nm against blank (PBS buffer). The same procedure was performed using the formulations without drug as a control. The loading was calculated using the following formula (Mukherjee *et al.* 2007)

$$\% \text{ Loading} = \frac{\text{Amount of the drug in liposomes}}{\text{Amount of liposomes}} \times 100$$

### 5. Release study

*In-vitro* release of dexamethasone from liposomes was conducted by dialysis in a dialysis sac (Sigma, 12000 MW cut off) with 100 ml of phosphate buffer saline (PBS) pH 7.4 at 37°C following the method published elsewhere (Zhang *et al.* 2005). In a 250-ml conical flask, 180 ml of PBS was taken. 1 ml (250 mg freeze-dried formulation/ml) of a formulation was taken into a dialysis bag. Two ends of the dialysis sac were tightly bound with threads. The sac was hanged inside a conical flask with the help of a glass rod so that the portion of the dialysis sac with the formulation dipped into the buffer. The flask was kept on a magnetic stirrer. Stirring was maintained at 300 rpm with the help of a magnetic bead and the

temperature of the water was maintained at 37°C with a thermostatic control. Sampling was done by withdrawing 1ml from the released medium with the help of micropipette and 1 ml of fresh buffer was added in each case. Samples were analyzed using a spectrophotometer at a wave length of 240 nm. With the help of the standard curve prepared earlier, drug concentration was measured. In case of the freeze-dried formulations, reconstitution was done with PBS buffer just before putting into a dialysis bag for release study.

## RESULTS AND DISCUSSION

Evaluation of drug-excipient interaction is an important study, which depicts much information including the stability of formulations, drug release from them and drug availability pattern (Lia *et al.* 2008). The interaction between drug and excipients can be determined by various methods such as IR and FTIR spectroscopy, differential scanning calorimetry etc. (Ghosh *et al.* 2009; Roy *et al.* 2009). Here the study was conducted using FTIR spectroscopy. The spectral analysis (Figs. 1-3) show that there were interaction in the wave number range between 3300 and 3450  $\text{cm}^{-1}$  which is a stretching zone of hydrogen-bonded alcohols, phenols and carboxylic acid (Gustafsson 1999; Williams 2002). This suggests that there were the formations of weak bond such as hydrogen bond or bond due to Van der Waals force or due to dipole-dipole interactions between -OH group of cholesterol and methyl or ketonic group of the drug. There might be a formation of similar bonds between the -NH<sub>2</sub> group of soya L- $\alpha$ -lecithin (stretching region of -NH<sub>2</sub> 3300-3450  $\text{cm}^{-1}$ ) and -OH group or -CH<sub>3</sub> group or C=O group present in the drug (Gustafsson 1999; Williams 2002). There were also some interactions between 400  $\text{cm}^{-1}$  and 1000  $\text{cm}^{-1}$  which is stretching region of alkanes and aromatic rings (Mukherjee *et al.* 2005). So there may be possibly physical bond formation between the -C- or -H of the aromatic ring present in drug and alkyl group of soya lecithin. No shifting of characteristic peaks of the compounds in spectral analysis suggests that there was no chemical interaction between the drug and excipients used. Various physical interactions have been reported to produce stable liposomes (Gokhale *et al.* 1996; Cabens *et al.* 1998) and are responsible factor of drug release as well as shape and size of liposomes. SEM photograph (Fig. 4) shows that the prepared liposomes had smooth surface and were spherical in shape and maximum vesicle distribution was in nano range size as compared to the larger vesicles which were also approximately 1  $\mu\text{m}$  in size. There was a clear distribution of both the small and large vesicles and they were not conglomerated. The results of liposomal vesicles size analysis by laser diffraction showed that vesicle size varied from 850 nm to 1  $\mu\text{m}$  (Fig. 5). Percentage of loading (w/w) of dexamethasone was between 1.39 and 1.49%. Loading of variation of dexamethasone was very little due to the variable amounts of cholesterol in the formulation. When the amount of cholesterol was nearly double, triple or enhanced by four times, dexamethasone loading was initially dropped to 4.08 and 5.44%, respectively in case of the double and triple amount of cholesterol in the formulation as compared to the formulation F1 (Table 1). Incorporation of cholesterol at low concentration into the lipid bilayers of liposomes leads to an increase in *trans*-membrane permeability, whereas incorporation of higher amount of cholesterol (> 30%) eliminates phase-transition and decreases the membrane permeability at a temperature greater than phase transition temperature (Corvera *et al.* 1992). That is why, when the amount of cholesterol was enhanced, dexamethasone loading was dropped. However the amount of loading was enhanced by 1.36% in the case of the formulation having four times of the amount of cholesterol as compared to formulation F1. The reason is not clear. However, the difference of loading was not statistically significant. Therefore data suggest that due to the variation of cholesterol dexamethasone loading was not improved significantly ( $P < 0.05$ ). *In-vitro* drug release study shows that

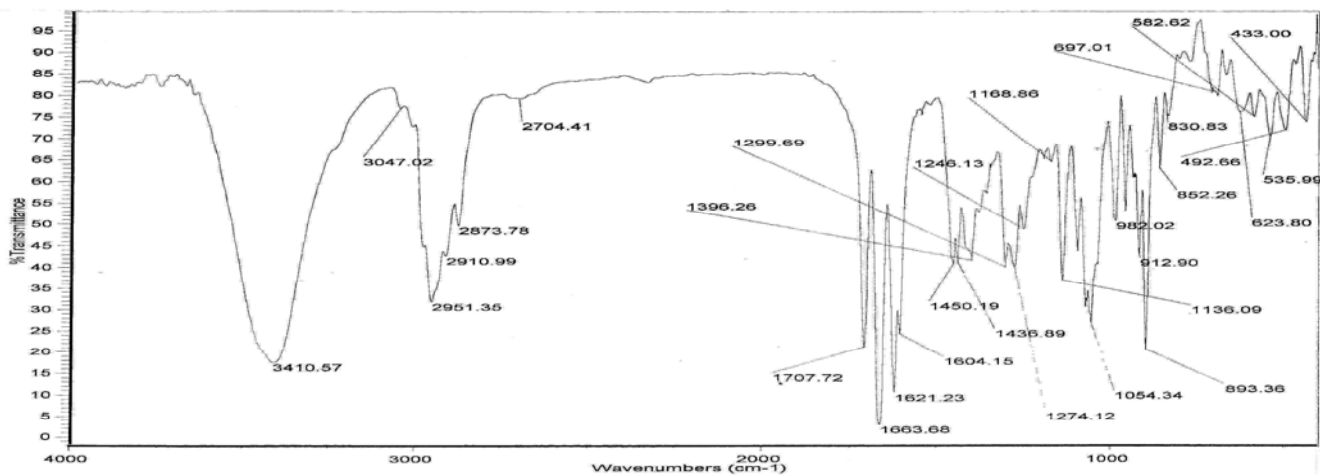


Fig. 1 IR spectrum of dexamethasone.

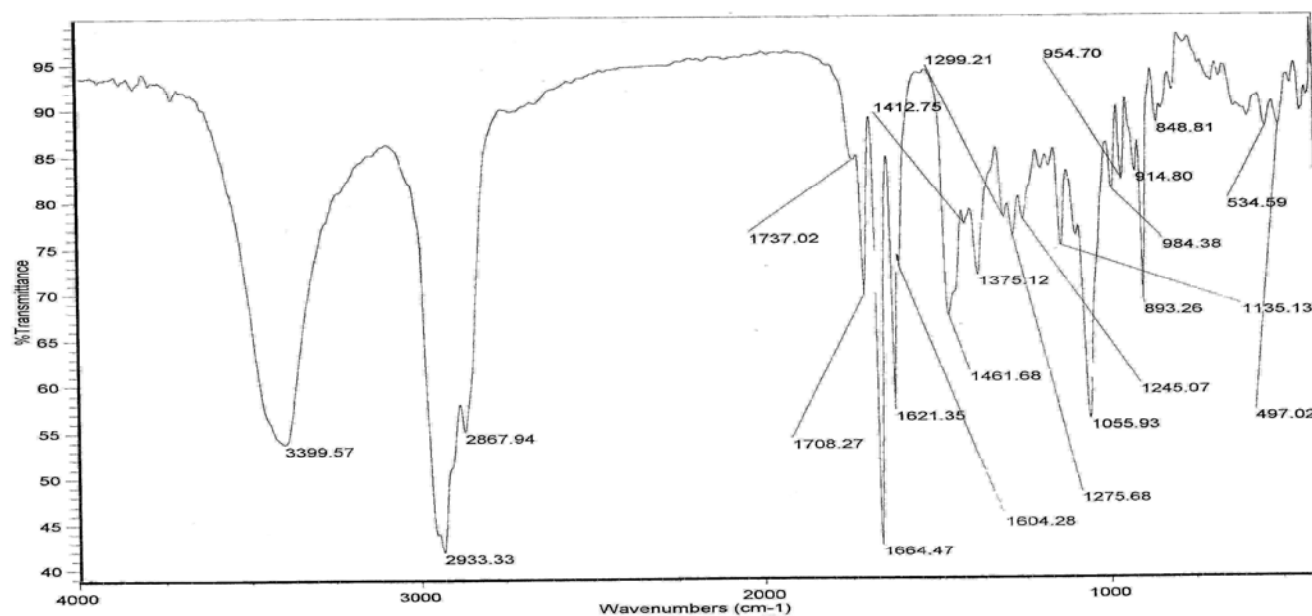


Fig. 2 IR spectrum of dexamethasone and excipients.

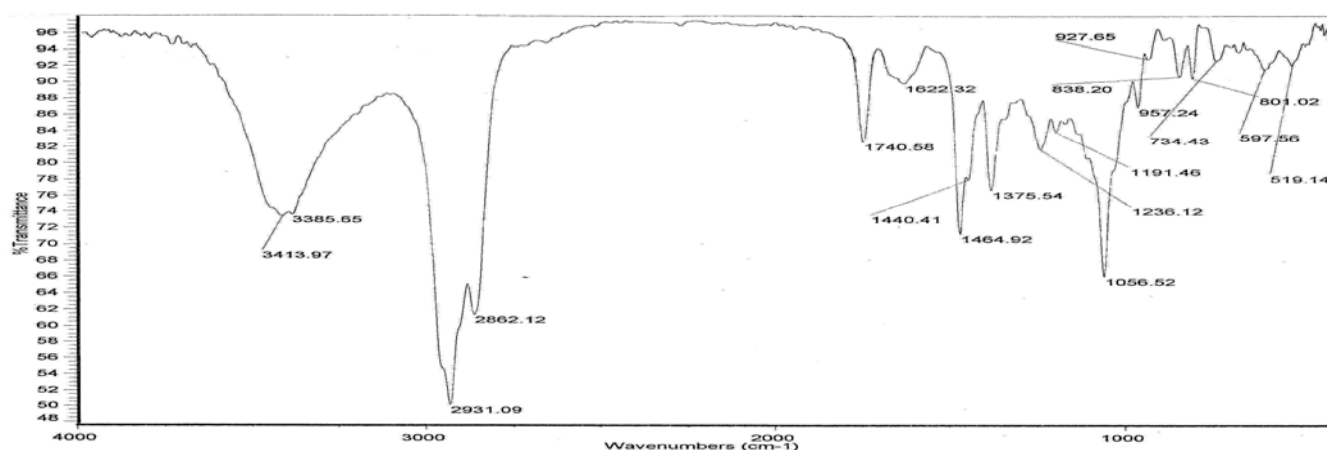


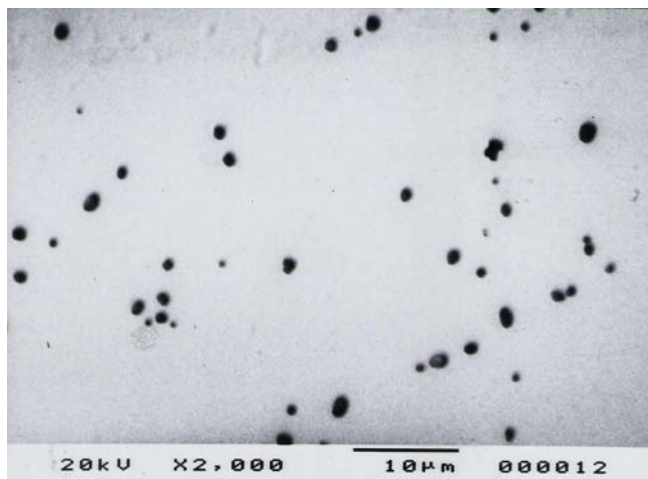
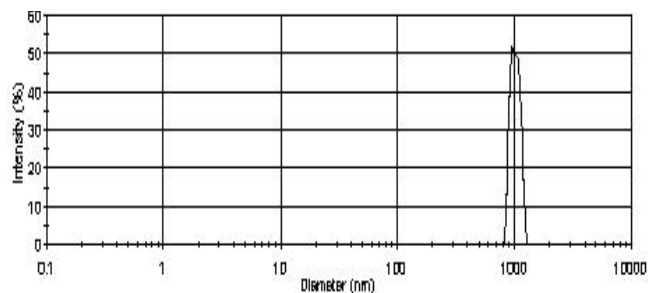
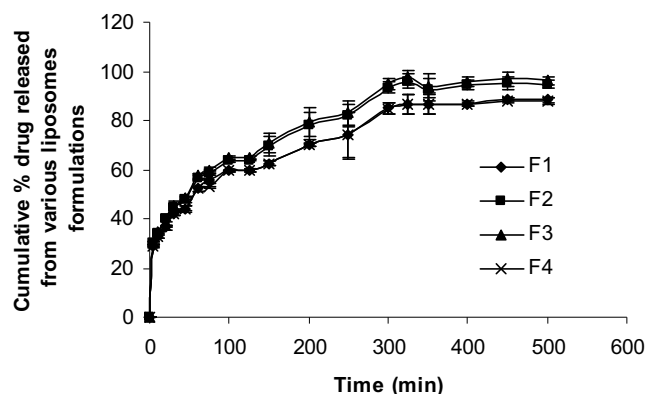
Fig. 3 IR spectrum of excipients.

87-96% release of drug (Fig. 6) was obtained in four liposome formulations in 500 min. Presence of rigid cholesterol nucleus along with the acyl chain of phospholipids is known to reduce the freedom of motion of acyl chain which ultimately caused the membrane to condense, decrease its fluidity, act as barrier to the entrapped drug and finally might retard the drug release (Damel *et al.* 1976) for such a prolonged period.

To evaluate the drug-release kinetic patterns, drug-release data were assessed using zero order, first order, Higuchi, Korsmeyer and Hixon-Crowell kinetic models (Mukherjee *et al.* 2009). Calculated  $R^2$  values for the studied kinetics were tabulated (Table 2). The corresponding plot (log cumulative percent drug release vs time) for the Korsmeyer equation indicated a good linearity ( $r^2 = 0.98$ ). The evaluation suggests that the drug release from all the lipo-

**Table 2** Release kinetics of the various liposome formulations

Formulations	Zero order kinetics	First order kinetics	Higuchi kinetics	Korsmeyer <i>et al.</i> kinetics	Hixon Crowel kinetics
F <sub>1</sub>	y=0.1352x+35.91 R <sup>2</sup> = 0.7984	y= -0.0018x+1.8325 R <sup>2</sup> = 0.945	y=3.4915x+19.735 R <sup>2</sup> = 0.9397	y=0.2649x+1.2434 R <sup>2</sup> = 0.9842	y=-0.0043x+4.0367 R <sup>2</sup> = 0.9149
F <sub>2</sub>	y=0.1482x+38.472 R <sup>2</sup> = 0.7945	y= -0.0027x+1.8453 R <sup>2</sup> = 0.9312	y=3.8382x+20.622 R <sup>2</sup> = 0.94	y=0.2748x+1.2558 R <sup>2</sup> = 0.9864	y=-0.0057x+4.0158 R <sup>2</sup> = 0.9193
F <sub>3</sub>	y=0.1515x+38.88 R <sup>2</sup> = 0.7952	y= -0.0031x+1.8602 R <sup>2</sup> = 0.9249	y=3.9217x+20.641 R <sup>2</sup> = 0.9408	y=0.2781x+1.2552 R <sup>2</sup> = 0.985	y=-0.0061x+4.0245 R <sup>2</sup> = 0.9228
F <sub>4</sub>	y=0.1351x+35.652 R <sup>2</sup> = 0.8013	y= -0.0018x+1.8337 R <sup>2</sup> = 0.9441	y=3.4868x+19.523 R <sup>2</sup> = 0.9412	y=0.265x+1.2412 R <sup>2</sup> = 0.9842	y=-0.0043x+4.0416 R <sup>2</sup> = 0.9149

**Fig. 4** Scanning electron microscopy of liposome formulation (sample F1).**Fig. 5** Size distribution pattern of liposome formulation (F1).**Fig. 6** *In vitro* release of dexamethasone from various liposome formulations. Data show mean  $\pm$  SD (n=3).

some formulations followed Korsmeyer kinetics. The release exponent “n” was about 0.26 to 0.27, which appears to indicate a coupling of the diffusion and erosion mechanism—so-called anomalous diffusion—and may indicate that drug release is controlled by more than one process (Korsmeyer *et al.* 1983; Hixson *et al.* 1931).

## CONCLUDING REMARKS

Lipophilic dexamethasone has been successfully formulated in liposomes (average size around one micron) based on cholesterol and soya L- $\alpha$ -lecithin with a drug loading of around 1.5%. Dexamethasone release study suggested that the drug released from the formulations in a sustained manner for about 8 h and drug release profile followed Korsmeyer kinetics, indicating involvement of effusion and erosion mechanism.

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