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# Improvement of Encapsulation Efficiency of Diclofenac Sodium in to Uncoated and Chitosan-Coated Liposomes

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**Semi synthetic phospholipid, dipalmitoylphosphatidylcholine, with or without cholesterol was used to study the encapsulation efficiency of diclofenac sodium into liposomes as well as to investigate its retention into liposomes. To improve encapsulation efficiency of diclofenac sodium into liposomes, natural phospholipids from *Triticum sp.* (wheat germ) and chitosan for coated liposomes were used. Diclofenac sodium was encapsulated into uncoated and coated liposomes using the thin film hydration method, with an efficiency of more than 90 %. Improvement in the encapsulation efficiency of diclofenac sodium into liposomes, was achieved by employing phosphatidylethanolamine, dicetyl phosphate and chitosan. The encapsulation efficiency reached a maximum when liposomes were prepared from *Triticum sp.* lipids and was 99 % compared to 59 % when dipalmitoylphosphatidylcholine was used. Results showed that the presence of cholesterol in the dipalmitoylphosphatidylcholine liposome bilayers produced a significant decrease in the encapsulation efficiency of diclofenac sodium. Chitosan-coated liposomes (dipalmitoylphosphatidylcholine, dipalmitoylphosphatidylcholine/cholesterol and *Triticum sp.* lipids) were prepared, and their encapsulation efficiency was studied. Encapsulation efficiency was affected by chitosan-coating, due to the presence of dicetyl phosphate rather than the presence of chitosan. The release of encapsulated diclofenac sodium from uncoated and coated liposomes in pH 7.4 normal saline at 37$^0$ was studied and DSC technique was employed to explain the results from drug release and the influence of cholesterol into uncoated liposome bilayers.**

Diclofenac sodium is a non-steroidal antiinflammatory, analgesic drug, which is widely used in the treatment of rheumatic disorders[1]. Exposure of the stomach to high levels of diclofenac sodium can cause gastric damage such as ulceration or bleeding[2]. To improve this disadvantage, sustained release or enteric-coated dosage forms have been developed employing microencapsulation technique involving the use of more than one polymer, which are oppositely charged[3,4]. The majority of liposome studies have involved the administration of liposomes intravenously, or intraperitoneally as drug carriers for the delivery of therapeutic or diagnostic agents to specific target tissues. However, there has been increasing interest in the potential use of liposomes for drug delivery by the oral route[5]. The main problems associated with orally-administration liposomes pertain to their relatively poor stabilities in the milieu of the GI tract and the low encapsulation efficiency of a high dosing orally administration drugs such as diclofenac sodium[6,7].

The aim of this work was to improve encapsulation efficiency of diclofenac sodium into liposomes by using liposomes with different lipid composition as well as to prepare chitosan coating liposomes, which was reported to enhance

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the accessibility and localization to the absorptive membrane via bioadhesion to improve the encapsulation efficiency of diclofenac sodium into liposomes and to improve liposomes stability into GI tract[8,9]. In this regard, the release of diclofenac sodium from uncoated and coated liposomes has been also determined. Differential scanning calorimetry technique has been employed to study the effect of cholesterol upon the lipid phase transition to explain the release of diclofenac sodium from uncoated liposomes.

## MATERIALS AND METHODS

Dipalmitoylphosphatidycholine (DPPC), dicetyl phosphate (DCP) and chitosan (CS), a deacetylated chitin (poly(N-deacetylglycosamine) were obtained from Sigma Chemical Co. St. Louis, USA. Cholesterol was obtained from Panreac Co. Barcelona, Spain. Diclofenac sodium was obtained from United Pharmaceutical Manufacturing Co. Amman, Jordan. Chloroform and methanol used were of spectroscopic grade. Silica gel H-60 was obtained from Merck KGaA, Darmstadt, Germany.

### Lipid source and lipid isolation:

The total lipid fraction was isolated from *Triticium sp.* (wheat germ) which was commercially available as previously described by Maswadeh *et al.*[10]. In brief, the total lipid fraction was isolated from *Triticum sp.* using the Bligh and Dyer method, followed by quantitation and fractionation[11]. The lipid content of the extracted total lipids, was primary evaluated by thin layer chromatography TLC. TLC coupled with a flame ionization detector (FID, latroscan) was used to quantify the lipid classes[12]. The detected lipids were phospholipids, ceramides, free fatty acids and free sterols. The isolation of sterols and fatty acids was achieved after saponification of the total lipid fraction and extraction using hexane for the sterols and hexane after acidification of the remainder solution with 6N HCl. The lipid classes were isolated using vacuum liquid chromatography (VLC). The solvent mixtures for the isolation of the lipid classes was as follows: $CHCl_3$ 100% for neutral lipids, $CHCl_3$:MeOH 99:1 and 95:5 for the ceramides and MeOH 100 % for the phospholipids. The purified lipid classes were monitored by TLC, while commercially available lipids were used to identify lipid fractions. Further purification was made using preparative TLC and the solvents were $CHCl_3$:MeOH:$H_2O$ 90:10:1 and 65:25:4.

### Preparation of uncoated and chitosan-coated liposomes and diclofenac sodium encapsulation:

Different types of uncoated liposomes were prepared (*Triticum sp.* wheat germ lipids, DPPC, DPPC/cholesterol 90:10 and DPPC/cholesterol 70:30) using the thin film hydration method. In brief, the lipid film was prepared on the glass wall of a round bottom flask by dissolving the lipid mixture (10 mg phospholipid with or without cholesterol) with diclofenac sodium which was subsequently slowly evaporated in the rotatory evaporator. For chitosan-coated liposomes, the lipid film was prepared by dissolving the lipid mixture with diclofenac sodium and dicetyl phosphate (DCP) which was subsequently slowly evaporated in the rotary evaporator. Multilamellar vesicles ($MLV_s$) were prepared by adding 1 ml of 0.9 % NaCl. The sample was maintained at 30-40$^0$ for 10 min to allow the equilibrium of normal saline across the lipid bilayer[13].

Chitosan-coated liposomes were prepared by adding 1 ml of chitosan solution in 3 % acetate buffer (pH 4.4) followed by incubation at room temperature for about 1 h. The chitosan-coated liposomes were considered to be formed via ionic interaction between the positively charged chitosan and negatively charged DCP on the surface of the liposomes[14]. After cooling for 5 min, the vesicles (coated and uncoated liposomes) with encapsulated diclofenac sodium were separated from unentrapped diclofenac sodium by centrifugation for 10 min at 3500 rpm. After separation of liposomes, the normal saline was replaced with fresh normal saline and free diclofenac sodium was assayed by UV/Vis spectroscopy at 284 nm in ethanol[15].

### Drug release studies:

Release of diclofenac sodium from uncoated and coated liposomes in normal saline was assayed as follows: Liposomes containing diclofenac sodium were centrifuged for 10 min at 3500 rpm to remove free drug and replacing the normal saline with fresh normal saline, the sample was then placed in a plastic tube and incubated in water bath maintained at 37$^0$. Aliquots were removed at various times and the released diclofenac was separated from liposomes by centrifugation. Uncoated Liposomes were disrupted with ethanol and the entrapped diclofenac was assayed by UV/Vis spectroscopy at 284 nm[15,16].

### Differential scanning calorimetry (DSC):

Appropriate amounts of phospholipid with or without cholesterol at two concentrations (10 mol % and 30 mol %) were dissolved in spectroscopic-grade chloroform. The solvent was evaporated in a rotatory evaporator under vacuum (0.1 mm Hg) at a temperature above the transition temperature. For measurements this dry residue was dispersed in

appropriate amount of double distilled water by vortexing. Portions of the sample (5 mg) were sealed into stainless-steel capsules (Perkin Elmer). Thermograms were obtained on a Perkin Elmer DSC-7 calorimeter. Before scanning, the samples were held above their phase-transition temperature for 1-2 min to ensure equilibration. All samples were scanned at least twice until identical thermograms were obtained using a scanning rate of $2.5^0$ $min^{-1}$. The temperature scale of the calorimeter was calibrated using indium ($T_m$=156.6$^0$) as standard sample.

## RESULTS AND DISCUSSION

Photomicrographs of uncoated and coated liposomes were obtained by using optical microscopy as shown in fig. 1 and 2. In the second case, the particles were aggregated and appeared to be enclosed by a polymer shell. The vesicle size distribution for the liposomes was determined by laser particle sizer (Mastersizer). Size distribution histograms are shown in fig. 3 for liposomes prepared from *Triticum sp.* lipids, fig. 4 for DPPC/cholesterol (90:10) and fig. 5 for chitosan coated liposomes. The volume size distribution means of the three liposomal formulations were 7.03 μm (in the range 1-19 μm), 8.18 μm (in the range 1-19 μm) and 8.64 μm (in the range 1-22.5 μm), respectively. These differences between the three means were not significant, but variances were significant (Data were evaluated using a two-tailed unpaired t-test $P<0.05$).

Diclofenac sodium was encapsulated into different types of liposomes as shown in Table 1. The encapsulation efficiency of diclofenac sodium into uncoated liposomes composed of DPPC, DPPC/cholesterol (90:10) and DPPC/cholesterol (70:30) was 59 %, 48 % and 40 %, respectively. The encapsulation efficiency gradually increased with decreasing cholesterol concentration, this may be attributed to antagonism of cholesterol and diclofenac sodium to the same position into bilayer.

Table 1 shows that the encapsulation efficiency of diclofenac sodium into liposomes was highly depends on lipid composition and was greater for liposomes prepared from *Triticum sp.* lipids (PC/PE/L-PC/cholesterol)[10], DPPC/cholesterol/DCP, DPPC/cholesterol/DCP/chitosan. More specifically, diclofenac sodium was encapsulated into liposomes prepared from *Triticum sp.* lipids, with high encapsulation efficiency >99 % due to its content of phosphatidylethanolamine (PE). The presence of PE increased the encapsulation efficiency into liposomes due to the ionic interaction between the positively charged PE and negatively charged diclofenac on the surface of the liposomes.

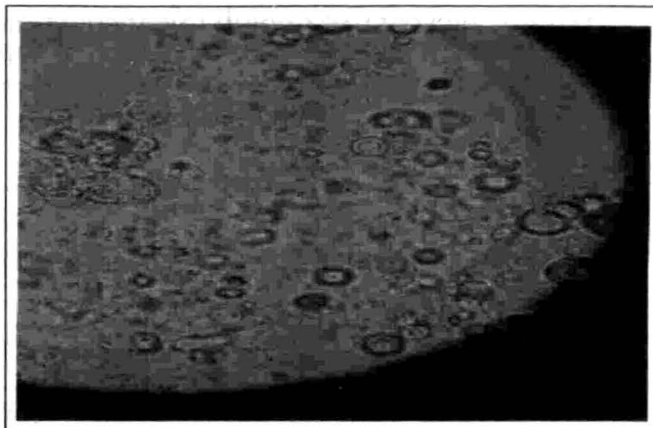

**Fig. 1: Photomicrographs from optical microscope showing the microstructure of uncoated liposomes.**

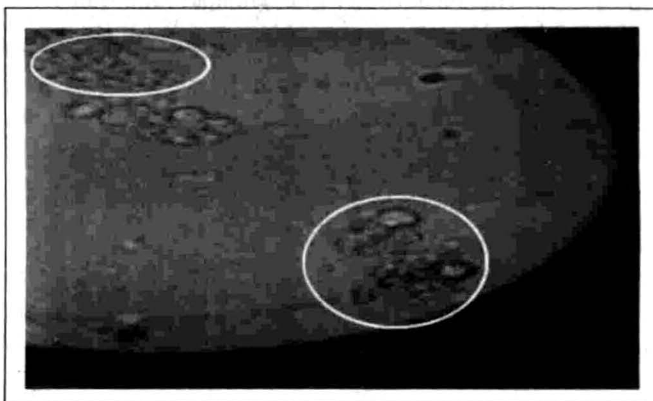

**Fig. 2: Photomicrographs from optical microscope showing the microstructure of chitosan-coated liposomes.**

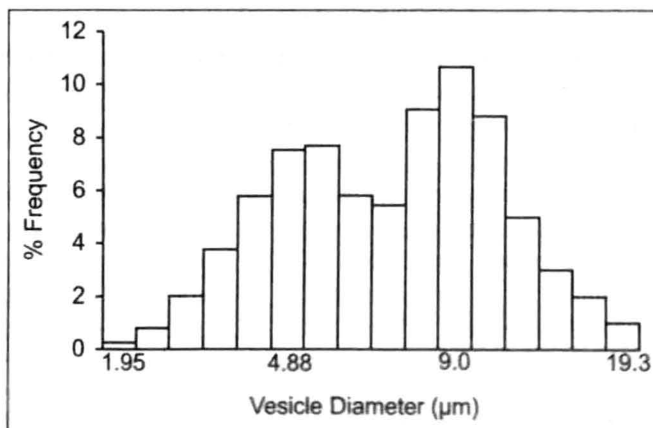


**Fig. 3: Size distribution histogram of uncoated liposomes prepared from *Triticum sp.* lipids.**

**Size distribution of uncoated liposomes prepared from *Triticum sp.* lipids were determined by Mastersizer (laser partical sizer).**

TABLE 1: DICLOFENAC SODIUM ENCAPSULATION EFFICIENCY INTO UNCOATED AND CHITOSAN COATED LIPOSOMES

| Lipid composition | % Encapsulation efficiency | |
|---|---|---|
| | Uncoated liposomes | Coated liposomes |
| DPPC | 59 | 93 |
| DPPC/cholesterol (90:10)* | 48 | 91 |
| DPPC/DCP 1.5 mg/Cholesterol (90:10)* | 82 | - |
| DPPC/DCP 0.5 mg/Cholesterol (90:10)* | 82 | - |
| DPPC/cholesterol (70:30)* | 40 | 90 |
| *Triticum sp.* lipids/cholesterol (50:50)** | >99 | >99 |

*molar concentration, ** % w/w. Comparison of diclofenac sodium encapsulation efficiency into uncoated and coated liposomes. The initial Drug / Phospholipid ratio was 1/10. In all cases, the relative S.D. was less than 2 %.

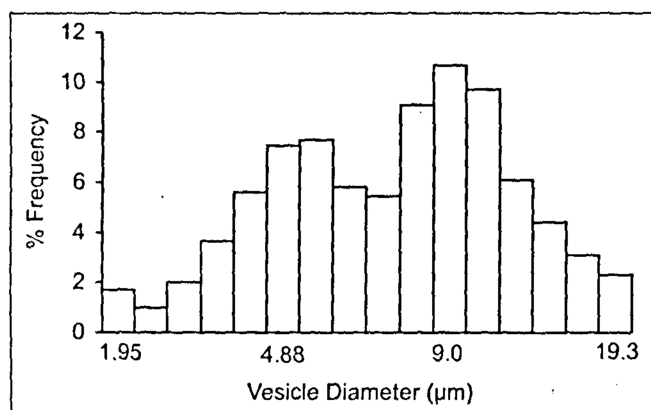


**Fig. 4: Size distribution histogram of uncoated liposomes prepared from DPPC/cholesterol (90:10).**

**Size distribution of uncoated liposomes prepared from DPPC/cholesterol (90:10) were determined by Mastersizer (laser partical sizer).**

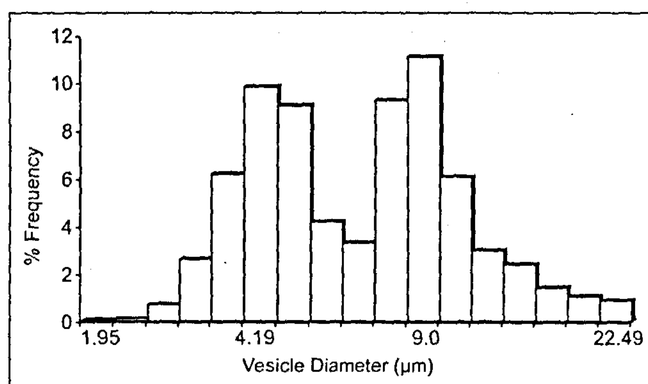


**Fig. 5: Size distribution histogram of chitosan coated liposomes prepared from DPPC/cholesterol/DCP/chitosan.**

**Size distribution of chitosan-coated liposomes prepared from DPPC/cholesterol/DCP/chitosan were determined by Mastersizer (laser partical sizer).**

To prepare coated liposomes, dicetyl phosphate DCP and chitosan were used. The encapsulation efficiency of chitosan-coated liposomes was highly increased for all liposome formulation due to the presence of DCP and chitosan. As shown in Table 1, the encapsulation efficiency was improved to 82 %, by adding 0.5 mg or 1.5 mg DCP, due to the ionic interaction between the positively charged DCP and negatively charged diclofenac on the surface of the liposomes, independent on the DCP concentration used. By adding chitosan to preformed liposomes DPPC/cholesterol/DCP, only 9-10 % improvement of encapsulation efficiency was achieved due to the presence of chitosan. The ability of uncoated liposomes to retain diclofenac sodium appears to be related to time and strongly dependent on vesicle composition. Fig. 6 shows that 64 %, 69 %, 18 % and 62 % diclofenac remained encapsulated into DPPC, DPPC/cholesterol (70:30), DPPC/cholesterol (90:10) and *Triticum sp.* lipid vesicles within 2 h, while at 24 h only 20 %, 49 %, 6 % and 40 % were still encapsulated.

The release of diclofenac sodium from coated liposomes in 0.9 NaCl at $37^0$ is described in fig. 7. Diclofenac sodium was rapidly released from uncoated liposomes DPPC/cholesterol (90:10), about 70 % being released within 30 min, and the remaining being gradually released over a period of 6 h. In contrast, chitosan-coated liposomes yielded de-

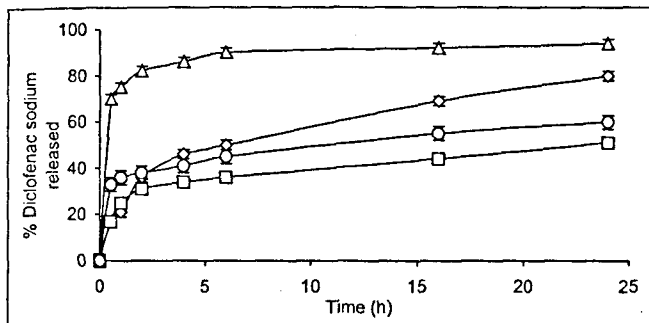


**Fig. 6: Release of diclofenac sodium from uncoated liposomes.**

**Release of diclofenac sodium from uncoated DPPC (◊), DPPC/cholesterol 90:10 (Δ), DPPC/cholesterol 70:30 (□) and *Triticum sp.* lipids (o) liposomes at 37° in 0.9% NaCl. Incubation condition and determination of free and liposome-associated diclofenac sodium were determined as described in Materials and Methods. Each point represents the mean of three independent experiments (bars represent s.d.).**

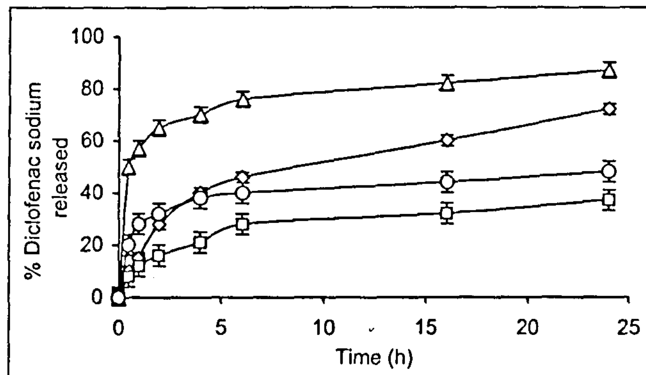


**Fig. 7: Release of diclofenac sodium from chitosan coated liposomes.**

**Release of diclofenac sodium from coated DPPC (◊), DPPC/cholesterol 90:10 (Δ), DPPC/cholesterol 70:30 (□) and *Triticum sp.* lipids (o) liposomes at 37° in 0.9% NaCl. Incubation condition and determination of free and liposome-associated diclofenac sodium were determined as described in Materials and Methods. Each point represents the mean of three independent experiments (bars represent s.d.).**

creased release of diclofenac sodium to the extent of about 50 % within 30 min, followed by continuous, slow release as shown in fig. 7. For coated liposomes composed from *Triticium sp.* lipids, about 20 % being released within 30 min, and 28 % of the remaining diclofenac sodium being gradually released over a period of 24 h. In contrast, uncoated *Triticum sp.* lipids liposomes yielded increased release of diclofenac sodium to the extent of about 33 % within 30 min and 60 % within 24 h. These different results are interpreted to mean that the addition of chitosan to preformed liposomes coated the outer bilayer and plays an important role in the polymer film acting as barrier to diclofenac sodium release.

The results from DSC fig. 8 and Table 2 can be used to explain the release of diclofenac from uncoated liposomes DPPC, DPPC/cholesterol (90:10) and DPPC/cholesterol (70:30). The bilayers of liposomes composed from DPPC existed in the gel phase at temperature lower than 35° and in the liquid crystalline phase at temperature higher than 42°. The addition of 10 % cholesterol in DPPC phospholipids bilayers results in abolishment of the pre-transition, lowering of ΔH and marginal decrease in $T_m$, with increasing the bilayer fluidity and increasing drug permeability. The presence of 30 % cholesterol results in abolishment of both the pre-transition and the mean transition, with decreasing the bilayer fluidity and decreasing in drug permeability. These results are in good agreement with the results of previous studies[14].

The results of this study demonstrate a clear effect of the presence of phosphatidylethanolamine (PE), dicetyl phosphate (DCP) and chitosan in improving the encapsulation efficiency of diclofenac sodium into coated and uncoated liposomes. In all cases a major improvement in the encap-

TABLE 2: QUANTITATIVE THERMAL DATA FOR DPPC BILAYERS WITH OR WITHOUT CHOLESTEROL

| Sample | $T_{pretrans}^{0}$ | $T_m^{0}$ | $T_m^{½ 0}$ | ΔH (cal g⁻¹) |
|---|---|---|---|---|
| DPPC | 34.8 | 41.2 | 2.8 | 1.11 ± 0.04 |
| DPPC/cholesterol (90:10)* | | 39.5 | 1.1 | 6.65 ± 0.44 |
| DPPC/cholesterol (70:30)* | | 41.7 | 16 | 3.02 ± 0.15 |

*molar concentration. Values of pretransition temperature ($T_{pretrans}$), half-width temperature ($T_m^{½}$), peak temperature ($T_m$) and enthalpy change (ΔH) of phospholipids bilayers without and with cholesterol.

sulation efficiency was due to the ionic interaction between the positively charged diclofenac and negatively charged DCP or PE on the surface of the liposomes. Adding chitosan to preformed liposomes only coated the outer bilayers and had a small influence in the encapsulation efficiency of diclofenac sodium. From the other hand chitosan coated liposomes released entrapped drug, such as diclofenac sodium, at a much slower rate than uncoated liposomes.

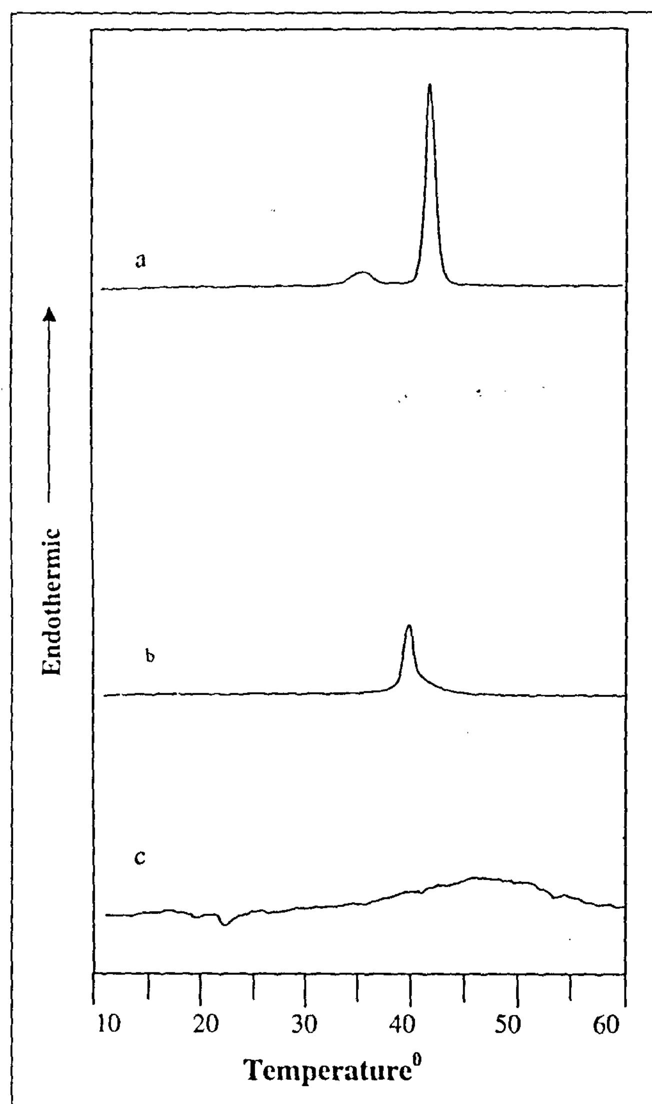


**Fig. 8: A DSC calorimetry scan.**

**A DSC calorimetry scan of: DPPC (a); DPPC/cholesterol (90:10) (b); and DPPC/cholesterol (70:30).**

The main significance of these studies is the possibility of developing a liposome system which possesses considerably greater encapsulation efficiency of diclofenac sodium with great stability in sodium cholate due to the presence of chitosan. This could have an important implication in the design of a liposome drug delivery system for oral administration.

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