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PIROXICAM-LOADED P90GYLATED TALLOW FAT-BASED SOLID LIPID MICROPARTICLES: CHARACTERIZATION AND *IN VIVO* EVALUATION

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Abstract
The purpose of this study was to develop solid lipid microparticles (SLMs) loaded with piroxicam, intended to be orally administered, able to improve the solubility of the drug yet devoid of adverse effects of piroxicam especially upon chronic use. Piroxicam-loaded P90Gylated tallow fat SLMs were prepared by the hot homogenization method. Here we report SLMs prepared from a combination of P90G and homolipid from *Bos indicus* (tallow fat) and evaluated for intestinal delivery of piroxicam. The investigated properties of the SLMs included particle size and morphology, thermal properties, drug loading efficiency, storage stability studies and injectability. The result dose-dependently showed high encapsulation efficiency up to 57 % and 92.49 % protection (inhibition) against electrical heat-induced pain (antinociception) where as 96.33 % inhibition of pedal oedema (anti-inflammatory activity) was recorded in the animal models.

Keywords: Piroxicam ; P90Gylated solid lipid microparticles ; tallow fat, anti-inflammatory activity.

INTRODUCTION

Piroxicam, a non-steroidal anti-inflammatory drug (NSAID), is used in the treatment of dysmenorrhea, various acute and chronic musculoskeletal disorders like rheumatoid arthritis, osteoarthritis etc., and also as a potent analgesic (Andersson, 1999) However, the use of piroxicam has been associated with a number of gastrointestinal disorders (Schiantarelli and Cadel, 1981) Enhanced bioavailability in a targeted delivery system based on improvement of solubility is an alternative form, but requires a formulation which ensures total solubilisation of piroxicam in the host material. Solid lipid microparticle is such a system that can enhance the performance of piroxicam *in vivo* in a is released *in vivo* and will therefore cause less adverse effects normally associated with the drug in conventional dosage forms. Several researchers have successfully delivered piroxicam via alternative forms like organogel (Agrawal *et al.*, 2004), buccal gel (Attia *et al.*, 2007), mucoadhesive system (Cilurzo *et al.*, 2005), microspheres based drug delivery (Raman *et al.*, 2005; Berkland *et al.*, 2004; Georgeta *et al.*, 2004), iontophoresis (Curdy *et al.*, 2001), cyclodextrin based enhancement (Curdy *et al.*, 2004) and gel based formulation (Shin *et al.*, 2000; Santoyo *et al.*, 1995).

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Lipid-based drug delivery systems have recently received increasing attention for a number of reasons (Gao *et al*., 2004; Jaspart *et al.*, 2007; Stuchlík and Zak, 2001). A number of biodegradable and biocompatible excipients have been employed in SLMs production (Trotta *et al.*, 2005; Jaspart *et al*., 2005). Natural fats have been shown to possess better biocompatibility and lower *in vivo* toxicity than semi-synthetic lipids (Kim *et al*., 2005). A homolipid from *Capra hircus* (goat fat) has been employed in numerous drug delivery systems due to its good crystal properties (Attama *et al.*, 2003; Attama *et al*., 2007). The homolipid from *Bos indicus* (tallow fat) has been shown to possess similar fatty acid composition (C16:0, C18:0 and C18:1) with goat fat although the location of these fatty acids in their triglycerides differ (Attama and Müller-Goymann, 2006). Phospholipon® 90G used in this study contains linoleic, oleic, stearic and palmitic acids, which are fatty acids of different chain lengths and degrees of saturation (Stuchlík and Žák, 2001). The interaction of these fatty acids with the diverse fatty acids present in tallow fat may result in a partly amorphous system with numerous spaces to accommodate the drug (Kameda, 2004). Highly ordered crystalline lipid matrices tend to expel any incorporated drug within its domain upon crystallization of the previously molten matrices (Radtke, 2005). Surface modification of the particles by physical structuring has been shown to produce highly disordered matrices with many imperfections in the crystal lattices offering numerous spaces for drug localization (Sastry, 2000; Attama *et al.*, 2006). The phospholipids bilayer structure formed around the lipid core may increase the drug loading capacity, as biologically important molecules can be anchored on the colloidal particles especially when generation of the microparticles is carried out in an aqueous medium (Sastry, 2000). We report here the surface functionalization of SLMs with P90G and their use to deliver piroxicam in fresh egg albumin-induced acute inflammation of rat hind paw. The modified SLMs are found to be biocompatible and exhibit *in vivo* anti-inflammatory efficiencies that are comparable to a commercially available agent Feldene but surpassed it in terms of the prolonged duration of release of the entrapped drug.

MATERIALS AND METHODS

Materials

Phospholipon 90G® (P90G) (Phospholipid GmbH Köln, Germany), is a purified, deoiled and granulated soy lecithin with phosphatidylcholine content of at least 90 %. Piroxicam was a kind gift from Juhel Pharmaceutical Nigeria Limited. Sorbic acid, sorbitol (BDH, England), and polysorbate 80 (Tween 80®, Uniqema, Belgium) were used as procured from their manufacturers without further purification. Tallow fat was from a batch prepared from our Pharmaceutics Laboratory of the University of Nigeria (Nsukka, Nigeria) Distilled water (Lion water, Nigeria) was used for SLM preparation.

Preparation of the P90Gylated tallow fat matrices

The optimized lipid matrix (Nnamani, 2010a; Nnamani, 2010b) corresponded to 5 % w/v in a 4:1 ratio combination of tallow fat and P90G and was prepared by fusion. The lipids were carefully weighed with an electronic balance (Mettler H8, Switzerland), melted together at 60 ºC on a thermo-regulated water bath shaker (Heto, Denmark) and stirred until solidification.

Characterization of lipid matrices

The bulk material of tallow fat was subjected to DCS to determine the melting transitions and changes in heat capacity using a calorimeter (DSC) (NETZSCH DSC 204 F1, Germany). Approximately, samples of 3 – 5 mg of the lipid matrix was weighed (Mettler M3 Microbalance) into an aluminium pan, hermetically sealed and the thermal behaviour determined in the range of 35-190 ºC under a 20 ml/min nitrogen flux at a heating rate of 10 ºC/min. The thermal property of the P90Gylated tallow fat matrix was also determined. All determinations were base-line corrected.

Solid lipid microparticle preparation

Solid lipid microparticles were formulated with hot homogenization technique (Jaspert *et al.,* 2007). To the lipid phase heated at 60 ºC, the aqueous phase containing 1.4 g of Tween 80, 4 g of sorbitol and 0.1 g of sorbic acid heated at the same temperature was added. The mixture was stirred with a high speed mixer (Silverson Adelphi Manufac. Co., England) at 6,200 rpm for 5 min. The emulsions were collected in pre-warmed containers and allowed to cool to room temperature.

By adding piroxicam (increasing concentrations of 250, 500, 750 and 1000 mg %) to the lipidic phase and melting together and following the above described procedure, piroxicam-loaded solid lipid microparticles (SLM-0 as blank without drug and SLM 1-4 as drug-loaded) were obtained.

Evaluation of SLMs

Differential Scanning Calorimetry (DSC) of drug and drug-loaded SLMs

The thermal properties of pure piroxicam were evaluated by DSC at different scan ranges of 35 and 350 ºC under a 20 ml/min nitrogen flux. The thermal behaviours of the piroxicam-containing SLM formulations were also investigated as well as the zero-drug containing SLMs.

Morphology and Particle size analysis

Particle size analysis was carried out on the SLM after production using a digital light microscope (Leica Diestar, Germany) and images captured with Moticam 1000 after preparation. The morphology and sizes of the particles were also noted. The SLMs were also subjected to time-resolved particle size analyses for 12 months at 6 months interval to check the effect of storage on the particle size.

Drug encapsulation efficiency

Approximately 6 ml of the piroxicam-loaded SLMs was added into a microconcentrator (5, 000 MWCO Vivascience, Germany). This was centrifuged (TDL-4 B. Bran Scientific and Instru. Co., England) at 3,000 rpm for 120 min. The drug concentration of the aqueous continuous phase was indirectly determined by UV/Vis Spectrophotometer (Unico 2102, England) at 332 nm. The amount of drug encapsulated in the microparticles was calculated with reference to a standard Beer's plot to obtain the % encapsulation efficiency (EE) using the formula below:

$$EE\ (\%) = \frac{\textit{Real drug loading}}{\textit{Theoretical drug loading}} \times 100 \quad \ldots (1)$$

Preparation of experimental rats

Clinically normal male Sprague-Dawley albino rats weighing 230 ± 10 g and normal male albino Wistar mice weighing 20-25 g were used for the experiment. The animals were kept and maintained under laboratory conditions of temperature, humidity and light; and allowed free access to food (standard pellet diet) and water *ad libitum*. All the animals

were fasted for 16 h, but still allowed free access to water, before commencement of the experiments. The mice were used for the antinociceptive evaluation of the piroxicam-loaded SLMs while the rats were used for the anti-inflammatory investigation of the SLMs.

Evaluation of antinociceptive activity

The hot - plate (thermal) test method was used in this study. This method was modified from those described elsewhere (Lanhers *et al.*, 1992; Williamson *et al.*, 1996). Briefly, a 600 ml glass beaker was placed on a hot-plate (Heidolph® MR 2002) with adjustable temperature. The temperature of the hot-plate was then regulated to 45 ± 1 ºC. Each mouse was placed in the glass beaker (on the hot-plate) in order to obtain the animal's response to electrical heat-induced nociceptive pain stimulus (licking of the forepaw and eventually jumping out of the glass beaker). Jumping out of the beaker was taken as an indicator of the animal's response to heat-induced nociceptive pain stimulus. The time taken for each mouse to jump out of the beaker (i.e. reaction time) was noted and recorded in seconds. Each mouse served as its own control. Thus, before treatment, its reaction time was determined thrice at 1 h intervals. The mean of these determinations constituted the 'initial reaction time' that is reaction time before treatment of the mouse. The mean reaction time for all the mice were pooled to get the final, 'control' mean reaction time (T_b).

Each of the test mice was thereafter treated with either orally administered distilled water, loaded-SLMs (SLM 1-4) or unloaded (SLM-0) with piroxicam, commercial sample (Feldene®) and pure piroxicam solution (i.p). Twenty minutes after i.p. treatment with piroxicam, and oral treatment with SLM formulations, commercial sample and distilled water, the reaction time was again evaluated. This value was pooled for the mice used in each treatment group, and the final 'test' mean reaction time value (T_a) for each treatment group was calculated. This final 'test' mean reaction time value represented 'after treatment reaction time' (T_a) for each group of treated mice. This 'test' mean reaction time value (T_a) was subsequently used to determine percentage thermal pain stimulus relief or protection, by applying the formula:

$$(\%)\ Protection = \frac{test\ mean - control\ mean}{Control} \quad \ldots\ldots (2)$$

$$= \frac{T_a - T_b}{T_b} \times 100 \quad \ldots\ldots (3)$$

Groups of eight mice each were used. The piroxicam-loaded SLMs 1-4 were tested at doses of 2.5, 5.0, 7.5, and 10 mg/kg p.o. respectively while the blank formulation (SLM-0) was given at 3 ml/kg p.o. The commercial sample (S) was used at a dose of 10 mg/kg p.o; the pure piroxicam powder (DW-P) was used at a dose of 10 mg/ml i.p. only while distilled water (DW) was given at 3 ml/kg p.o. only.

Evaluation of anti-inflammatory property of the SLM

The rats were divided into eight groups (DW, DW-P, S, and SLM 0-4) of five rats per group. The SLM-0 rat group was used as the untreated control. The rats in this group received only the blank SLM (without piroxicam). Each of the DW rat group received distilled water (3 ml/kg p.o.) only while the rats in the S-group received 10 mg/kg of Feldene®. Each test rat in the groups marked SLM 1-4 received graded doses of piroxicam-loaded SLMs in the range of 2.5, 5.0, 7.5, and 10 mg/kg respectively. The group marked DW-P received pure piroxicam powder in distilled water (10 mg/kg i.p.).

Rat hind paw oedema was used as a model of acute inflammation. The rat hind paw oedema was induced by intra-plantar injection of fresh egg albumin (0.5 ml/kg), as a cheap phlogistic agent (Ekpendu *et al.*, 1996; Muko and Ohiri, 2000). Acute inflammation of the hind paw was induced in each of the rats by injecting 0.5 ml/kg of fresh egg albumin into the subplantar surface of the right hind paw. Pedal inflammation (oedema) was evident within 5-8 min following fresh egg albumin injection. Two different test methods were used to assess the degree of inflammation and protection.

Linear diameter measurement

Here, the linear diameter of the injected paw was measured for 3 h at 30 min intervals after the administration of phlogistic agent. Increases in the linear diameter of the right hind paws were taken as an indicator of paw oedema. Oedema was assessed in terms of the difference in the 'zero time' (C_o) linear diameter at time t, (C_t – that is 30, 60, 90, 120, 150, and 180 min) following fresh egg albumin administration. The increase in the right hind paw diameters induced by injections of fresh egg albumin were compared with those of the contra-lateral, non – injected left hind paw diameters (Oriowo, 1982).

Graded doses of piroxicam-loaded SLMs (SLM 1-4) were separately administered to each of the rats in the test groups, 20 min before inducing inflammation with the fresh egg albumin. Rats in the reference comparative 'test' group marked DW-P received piroxicam (10 mg/kg i.p.) in distilled water; while the rat groups marked SLM-0 received blank (untreated) SLM; the rats in the group marked S received 10 mg/kg p.o. Feldene® and DW-rats received distilled water (3 ml/kg p.o.) only. Percentage inflammation (oedema) were calculated from the formula

$$\% \ oedema = \frac{C_o}{C_t} X\ 100 \quad \ldots (4)$$

while percentage inhibition of the oedema was calculated from the formula:

$$\% \ inhibition = \frac{\frac{C_o}{C_t} X\ 100}{C_o} \quad \ldots\ldots (5)$$

where C_o is the average inflammation (hind paw oedema) of the control SLM-0 (blank SLM) at any given time, and C_t is the average inflammation of the control DW (distilled water) minus [SLM 1-4 (piroxicam-loaded SLMs) or S (Feldene®) or DW-P (pure piroxicam powder treated rats)] at the same time.

Volume displacement method

Here, the volume of water displaced from 7.4 ml measuring cylinder was measured immediately before the administration of the phlogistic agent and at 30 min for 3 h thereafter. For routine drug targeting, the increase in volume of water displaced 3 h after administration of the egg albumin was adopted as the parameter for measuring inflammation.

Thus inflammation was assessed as the difference between zero time volume displacement and 3 h after egg albumin administration. Exactly, 1 h prior to the administration of the egg albumin, the SLMs 1-4 rat groups received 2.5, 5.0, 7.5, and 10.0 mg/kg p.o. respectively. The control groups marked SLM-0 and DW respectively received blank SLMs and distilled water 3 ml/kg p.o. The DW-P group received pure piroxicam powder (10 mg/kg i.p.) in distilled water while the S group received Feldene® 10 mg/kg p.o.

Percentage inflammation was calculated for each dose using the formula

$$\% \ Inflammation = \frac{Av.\ Inflammation\ at\ t}{Av.\ Inflammation\ of\ control} \times 100 \quad \ldots\ldots\ldots (6)$$

The initial volume of paw displacement was measured as V_i. At subsequent 30 min intervals, the paw displacements were measured as V_f. The percentage oedema variation and inhibition were also calculated.

Storage stability studies of the SLMs

The physical stability of the SLMs was evaluated for 12 months under different temperature conditions. Exact volumes of each SLMs was put in closed tubes and stored at 25 ^oC and 4-6 ^oC out from direct light. Aliquot samples were withdrawn every 6 months to determine particle size and morphology as described above.

Determination of injectability

Injectability, defined as the smallest needle gauge that a microparticulate sample can pass through, was determined according to the method of Nnamani, (2010). This was carried out by pushing 4 ml of sample from a 5-ml plastic disposable syringe through hypodermic needles ranging from 18 to 27 within 20 sec. The formulation was first tested using the smallest needle (27 G). If the entire content of the sample passed through a 27 G needle, its injectability was recorded as 27, otherwise the study was repeated using 25 G needle, followed by the next smaller gauge needle.

Statistical analysis

All experiments were performed in replicates for validity of statistical analysis. Results were expressed as mean ± S.D. ANOVA and student's t-test was performed on the data sets generated using SPSS. Differences were considered significant for p-values < 0.05– 0.001.

RESULTS AND DISCUSSION

Characterization of lipid matrices

Tallow fat showed a pretransition below the final stable melting temperature which took place with maximum peak at 54.5 ^oC with an enthalpy of -5.067 mW/mg according to our earlier report (Nnamani *et al.*, 2010a). The lower melting peak would belong to unstable modification, while the higher peak belongs to stable modification. The P90Gylated tallow fat matrix showed a peak melting temperature at 52.2 ^oC with an enthalpy of -5.501 mW/mg, as reported previously (Nnamani *et al.*, 2010a). P90Gylation of bulk crystalline matrices generally produce matrices with lower melting endotherms as well as enthalpies (Attama and Müller-Goymann, 2006). This is because it has recently been shown that P90G is a good surface modifier for solid lipid particles (Schubert and Müller-Goymann, 2005; Schubert *et al.*, 2005) with resultant improvement in targeting and pharmacokinetics (Huang *et al.*, 1992; Heiati, 1998). The phospholipids bilayer structure formed around the lipid core may increase the drug loading capacity (Attama *et al.*, 2006).

Morphology and Particle size analysis

Particle size of the SLMs determined after 1 week of preparation as presented in Table 1 shows very small particles of 10.2±0.4, 22.95±0.8, 50.50.5±0.9, 90.5±1.2 and 106.8±3.7 µm for SLM-0, SLM-1, SLM-2, SLM-3 and SLM-4, respectively corresponding to different piroxicam concentrations of 0.0, 0.25, 0.5, 0.75, and 1.0 % w/w. It simply shows that the size of the microparticles increased with increase in drug concentration,

which agrees with an earlier report (Sanna *et al.*, 2004).

The photomicrographs of SLMs as shown in Fig. 1 reveal smooth and spherical particles with a thick surfactant ring shielding the inner lipid core.

Table 1: Properties of the formulated SLMs

Formu-lations	Drug composition (g w/w)	Average particle size (µm)		Drug encapsulation efficiency (%)	Injectability (Guage)		
		After preparation	After 6 months storage		1 week of preparation	After 6 months	After 12 months
SLM-0	0.00	10.2±0.4	7.4±2.3	-	27	25	25
SLM-1	0.25	22.95±0.8	25.70±5.2	28.57 ± 10.30	27	25	25
SLM-2	0.50	50.50±0.9	153.90±28.3	50.00 ± 20.30	27	18	18
SLM 3	0.75	90.5±1.2	273.3±10.1	53.30 ± 23.20	27	23	23
SLM-4	1.0	106.5±3.7	378.70±25.7	57.14 ± 20.50	27	23	23

The chalky-appearances however depict some degree of lipid crystallization from the previously molten matrices. Yet the core of the microparticles maintained the pale-yellow colour of piroxicam as is evidently seen in SLMs stored at 4 – 6 ^{o}C (Fig. 2). The SLMs increased in size within the first 6 months of storage after which they maintained a steady particle size (Fig.1). Upon 1 week of preparation, all the SLMs had a syringeability of 27 G but varied upon storage. The SLMs were best stored at 4-6 ^{o}C.

Drug encapsulation efficiency

Drug encapsulation efficiency of 57.14 % was recorded for the highest piroxicam loading into SLM-4 (Table 1) but the SLMs prepared without P90G had insignificant loading efficiency ($p<0.05$, data not shown). This shows that the P90G-containing matrices possessed spaces which housed the drug (evident in Fig. 2) and the drug loading did not affect the morphology of the SLMs. This could further be explained on the basis that the highly lipophilic portion of the drug was completely housed within the lipid bilayer of the lipids.

DSC properties of piroxicam and SLM formulations

DSC is a highly useful means of detecting drug-excipient incompatibility in a formulation. It gives insight into the capacity of the SLMs to entrap high amounts of the drug. Piroxicam had a sharp endothermic peak at 203.1 ^{o}C with an enthalpy of -6.354 mW/mg (Fig. 3). DSC thermogram of SLM dispersions without drug (Fig. 4) showed two endothermic peak transitions with peak minima at 104.8 ^{o}C (-13.31 mW/mg) and 108.8 ^{o}C (-14.67 mW/mg). The lower melting peak would belong to unstable modification while the higher peak belongs to stable modification (Attama and Müller-Goymann, 2006).

The drug-loaded SLMs (Fig. 5) showed low crystalline

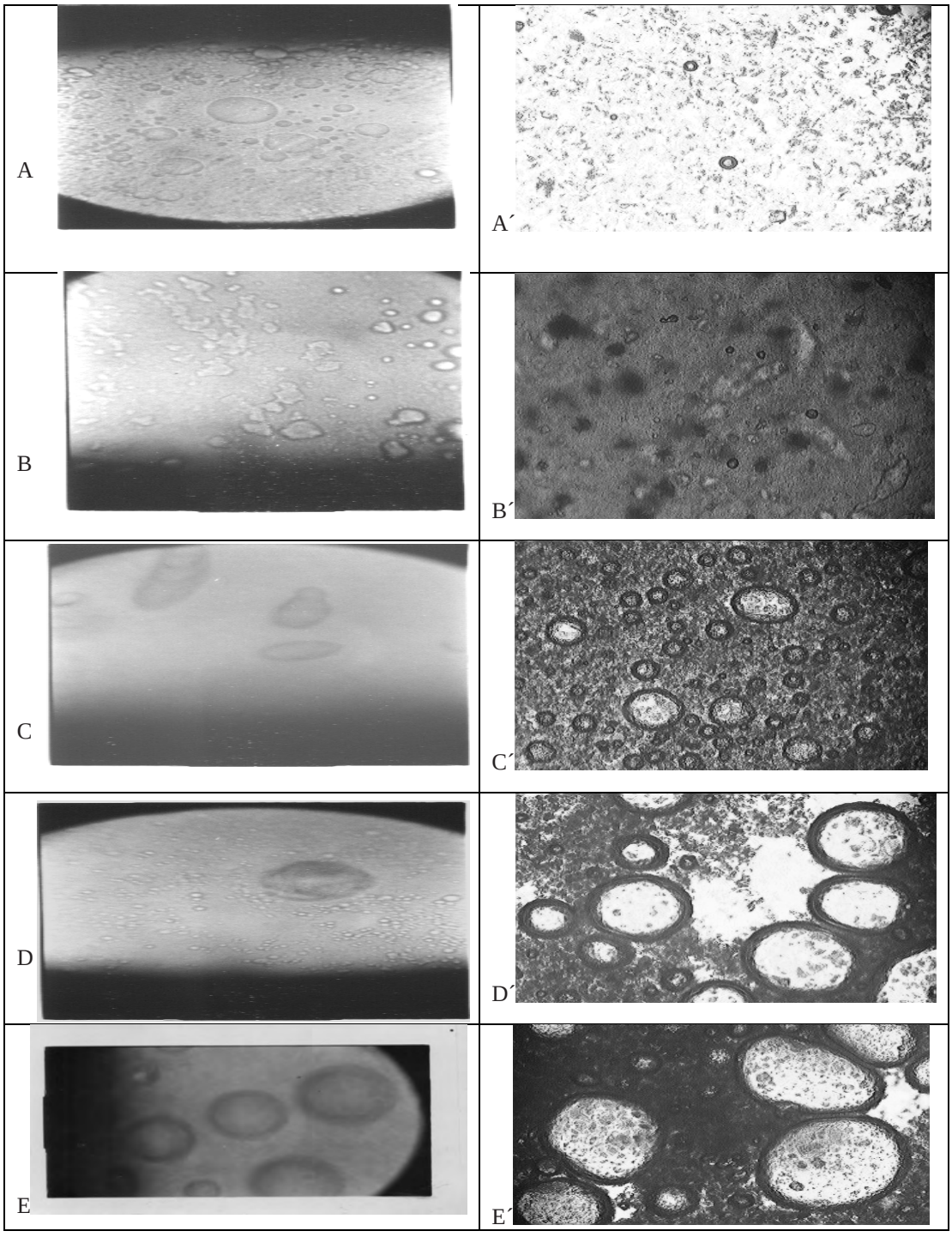

Fig. 1: Photomicrograph of the SLM dispersions: (A) Blank SLM; (B) 0.25 % w/w, (C) 0.5 % w/w, (D) 0.75 %w/w, and (E) 1.0 % w/w piroxicam-loaded SLM after one week of preparation and their corresponding photomicrographs after storage for six months at 25 ^{o}C denotated as A´, B´, C´, D´, and E´.

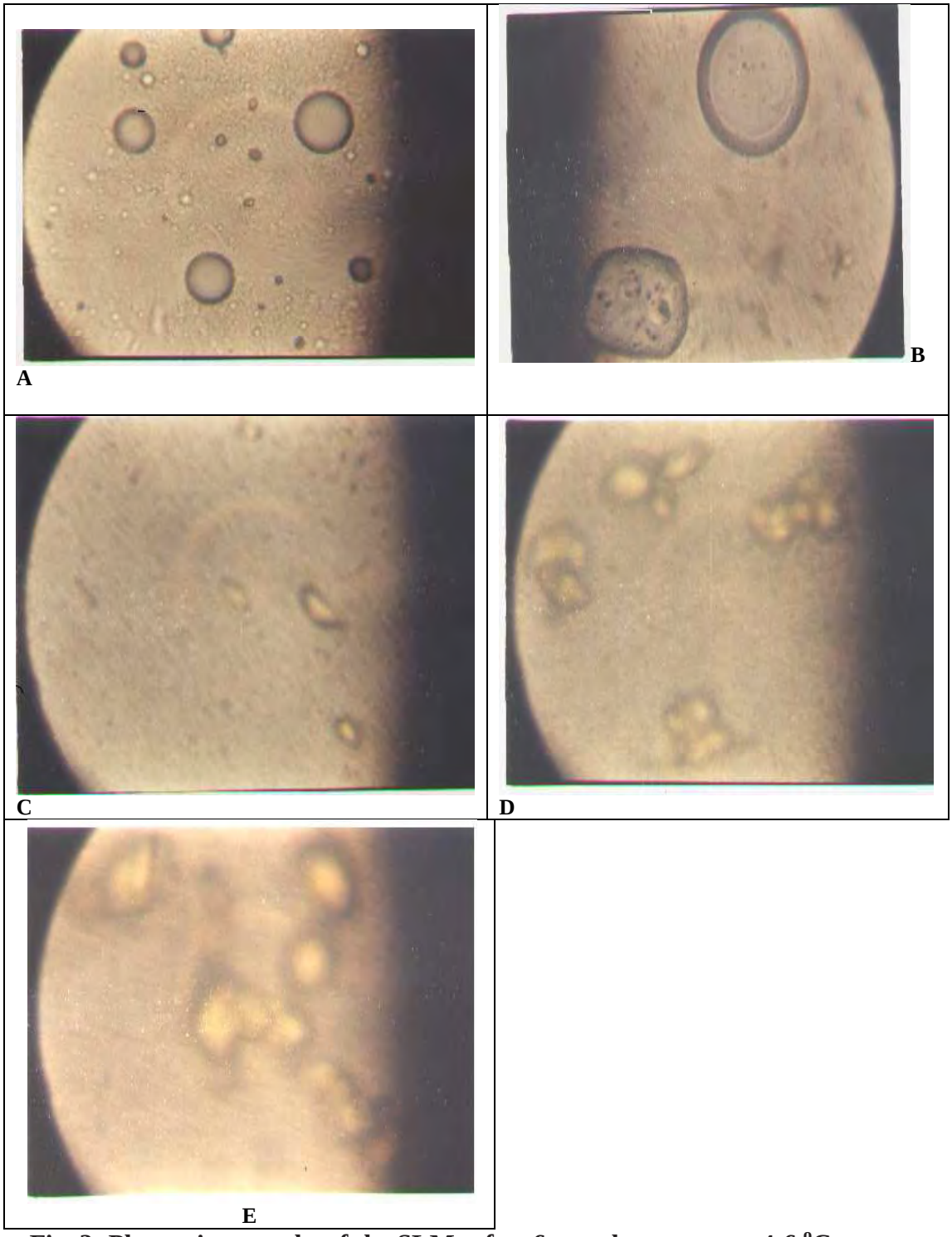

Fig. 2: Photomicrographs of the SLMs after 6 months storage at 4-6 ^{o}C
[A. blank; B. 0.25 g; C. 0.5 g; D.0.75 g and E. 1.0 g w/w of piroxicam]

state except for SLM-1. However, the SLM-1, SLM-2, SLM-3 and SLM-4 respectively containing 0.25 g; 0.5 g; 0.75 g and 1.0 g of the drug showed endothermic peaks (with enthalpies in parenthesis) at 109.8 ºC (-16.12 mW/mg); 95.0 ºC (-9.782 mW/mg); 114.8 ºC (-12.17 mW/mg) and two endothermic transitions for the 1.0 g drug-loading (SLM-4), which occurred at 78.8 ºC (-9.155 mW/mg) and 106.9 ºC (-6.717 mW/mg) respectively.

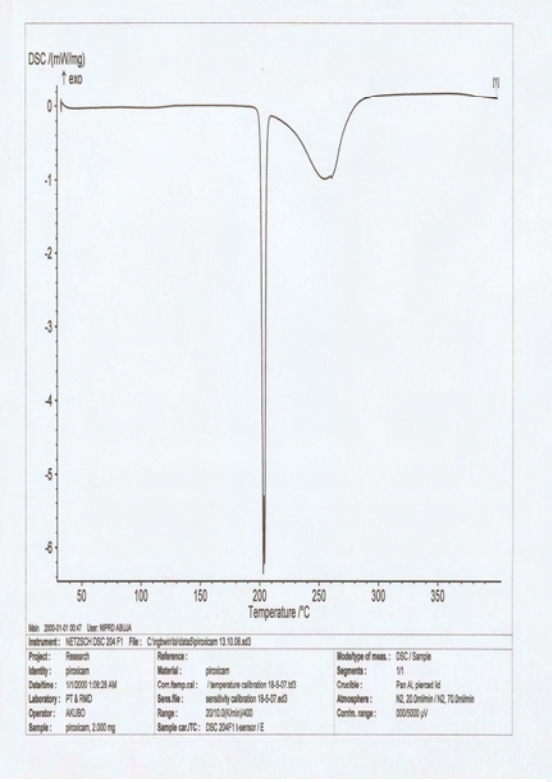

Fig. 3: DSC thermogram of piroxicam

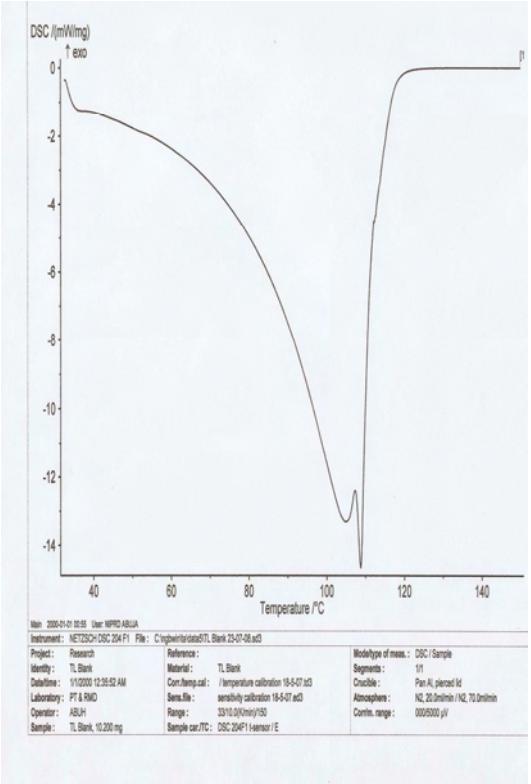

Fig. 4: DSC thermogram of SLM dispersion without drug

This confirms the high encapsulation efficiency recorded for SLM-4 since it possessed the

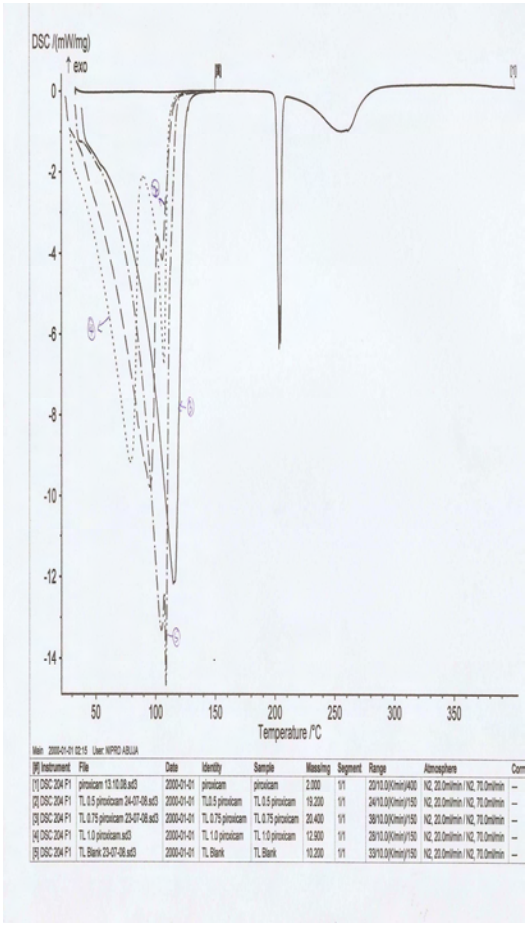

Fig. 5: DSC thermograms of drug-loaded SLMs

least enthalpy indicating that the lattice arrangement was highly disordered (imperfect) with numerous spaces which accommodated the drug. The increase in enthalpy as seen in SLM-1 confirms higher amounts of crystals upon storage (as is evident in Fig. 1) due to delayed crystallization from fractions of a cooled amorphous melt.

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This agrees with the observation of Kameda (2004).

administration. The piroxicam-loaded SLM (SLM 1-4) produced significant reductions ($p<0.05 – 0.001$) in the fresh egg albumin-induced acute

Table 2: Effect of piroxicam-loaded SLMs on electrical heat-induced pain.

Formulations	Dose	Mean reaction time (s)	% protection
SLM-0	2 ml/kg	10.57 ± 1.32	0.75 NS
DW	3.0 ml/kg	10.65 ± 1.40	0.00
SLM-1	2.5 mg/kg	15.2 ± 1.42	42.72
SLM-2	5.0 mg/kg	16.82 ± 1.65[b]	57.93[b]
SLM-3	7.5 mg/kg	18.64 ±1.70[b]	75.02[b]
SLM-4	10 mg/kg	20.50 ± 2.30[a]	92.49[a]
DW-P	10 mg/kg	21.25 ± 2.0[a]	99.53[a]
Feldene®	10 mg/kg	21.23 ± 2.0[a]	99.34[a]

Each value represents the mean (± SEM) of five observations.
NS= $p<0.005$; [a]$p<0.001$ Vs control; [b]$p<0.01$

In vivo release studies of piroxicam-loaded SLMs

Antinociceptive property

The piroxicam-loaded SLMs produced a dose-related significant ($p<0.05 – 0.001$) nociception in mice (Table 2). The piroxicam-loaded SLM dose-dependently delayed the reaction times of the mice to electrical heat-induced pain.

Evaluation of anti-inflammatory properties

Subplantar injection of fresh egg albumin (0.5 ml/kg) provoked marked time-related increases in the hind paw diameters of the rat control group that received blank SLM (SLM-0). Although pedal inflammation (oedema) was evident within 5-8 min following fresh egg albumin injection, maximal swelling and/or oedema occurred approximately 90 min following fresh egg albumin

inflammation of the rat hind paws (Table 3 and 4). The blank SLM-0 (2 ml/kg p.o.) neither modified responses to nociceptive stimuli in mice, nor do the rat hind paw oedema induced by fresh egg albumin administration. The reduction of the inflammatory process obtained within the pedal surface is displayed in Table 3 (linear diameter measurement) and Table 4 (the volume displacement method).

It is therefore evident that the drug release from the phospholipids-containing SLMs maintained a dose-dependent steady release of piroxicam ($p<0.05$ - 0.001) in both nociceptive and inflammatory conditions in the mammalian laboratory animal models. However, the possible mechanism of action of these systems aside from the drugs' intrinsic mechanism of action is based on their small particle sizes because such small sized particles are easily recognized by the immune

system as danger signals from where they generally get internalized by antigen-sampling membranous (M) cells in intestinal Peyer's patches (Fig. 6).

These M cells (specialized epithelial cells) have a thinner glycocalyx and less organized microvilli than

Table 3: Linear diameter measurement from SLM-treated oedematous rats

Formu-lations	Dose	Time (min) and paw diameter (mm)						% inhibition
		30	60	90	120	150	180	
SLM-0	2 ml/kg	10.36±0.2	12.50±0.4	15.27±0.5	13.50±0.4	12.40±0.4	11.45±0.4	0.25
DW	3 ml/kg	10.36±0.4	12.40±0.3	15.42±0.5	13.6±0.40	12.35±0.4	11.42±0.4	-
SLM-1	2.5mg/kg	10.0±0.39	11.0±0.35	12.67±0.5	10.76±0.3	9.3±0.30	8.24±0.37	28.25[b]
SLM-2	5.0 mg/kg	9.3±0.33	9.58±0.4	11.35±0.3	9.47±0.26	8.30±0.28	7.1±0.25	38.03[b]
SLM-3	7.5 mg/kg	8.15±0.25	6.8±0.25	5.2±0.34	3.39±0.31	2.30±0.21	1.85±0.23	84.30[a]
SLM-4	10 mg/kg	7.3±0.35	4.10±0.30	3.53±0.35	2.10±0.30	0.9±0.06	0.4±0.04	96.33[a]
DW-P	10 mg/kg	5.14±0.28	2.5±0.51	0.42±0.01	-	-	-	100.00[a]
Sample	10 mg/kg	5.10±2.0	3.0±0.45	0.35±0.25	0.35±0.25	-	-	96.84[a]

Each value represents the mean (±SEM). [b]$p<0.05$; [a]$p<0.01$ Vs control

Table 4: Volume displacement measurements from SLM-treated oedematous rats

Formu-lations	Dose	Time (min) and volume displacement (ml)						% inhibition
		30	60	90	120	150	180	
SLM-0	2 ml/kg	2.0 ± 0.5	2.3 ±0.6	2.2 ± 0.5	2.2 ± 0.4	2.1 ± 0.7	2.1 ± 0.7	-5
DW	3 ml/kg	2.0 ± 0.4	2.2 ± 0.3	2.2 ± 0.4	2.1 ± 0.3	2.2 ± 0.4	2.0 ± 0.4	-
SLM-1	2.5mg/kg	1.90 ± 0.5	1.9 ± 0.3	1.7 ± 0.6	1.6 ± 0.5	1.5 ± 0.5	1.45 ±0.6	27.5 ± 0.31
SLM-2	5.0 mg/kg	1.9 ± 0.7	1.9 ± 0.6	1.6 ± 0.4	1.5 ± 0.5	1.4 ± 0.3	1.25 ± 0.2	37.5 ± 0.26
SLM-3	7.5 mg/kg	2.0 ± 0.6	2.3 ± 0.5	1.7 ± 0.5	1.5 ± 0.4	1.0 ± 0.5	0.3 ± 0.2	85.0 ± 0.23
SLM-4	10 mg/kg	2.2 ± 0.5	2.0 ± 0.4	1.9 ± 0.5	1.0 ±0.6	0.1 ± 0.5	0.08 ± 0.2	96. 0± 0.03
DW-P	10 mg/kg	1.60 ± 0.6	1.0 ± 0.5	0.01 ± 0.4	0.01 ± 0.2	-	-	99.5 ± 0.01
Feldene®	10 mg/kg	2.1 ± 0.8	1.0 ± 0.3	0.08 ± 0.5	0.08 ± 0.5	-	-	96.0 ± 0.02

Each value represents the mean (±SEM).

enterocytes and are known to internalize and transcytose particles

to underlying lymphocytes and antigen-presenting cells (Clark *et al.*, 2001; Eldridge *et al.*, 1990; Andrianov and Payne, 1998; Swartz, 2001; Kreuter, 1991; Florence *et al.*,

lymph nodes, spleen, and lymphoid tissues associated with the mucosa, like the gut-associated lymphoid tissues such as tonsils, Peyer's patches, and appendix, which are sites of lymphocyte activation by antigens. Particles up to 10 µm in diameter can be internalized into Peyer's patches and particles less than 5 µm can be transported to draining lymph nodes

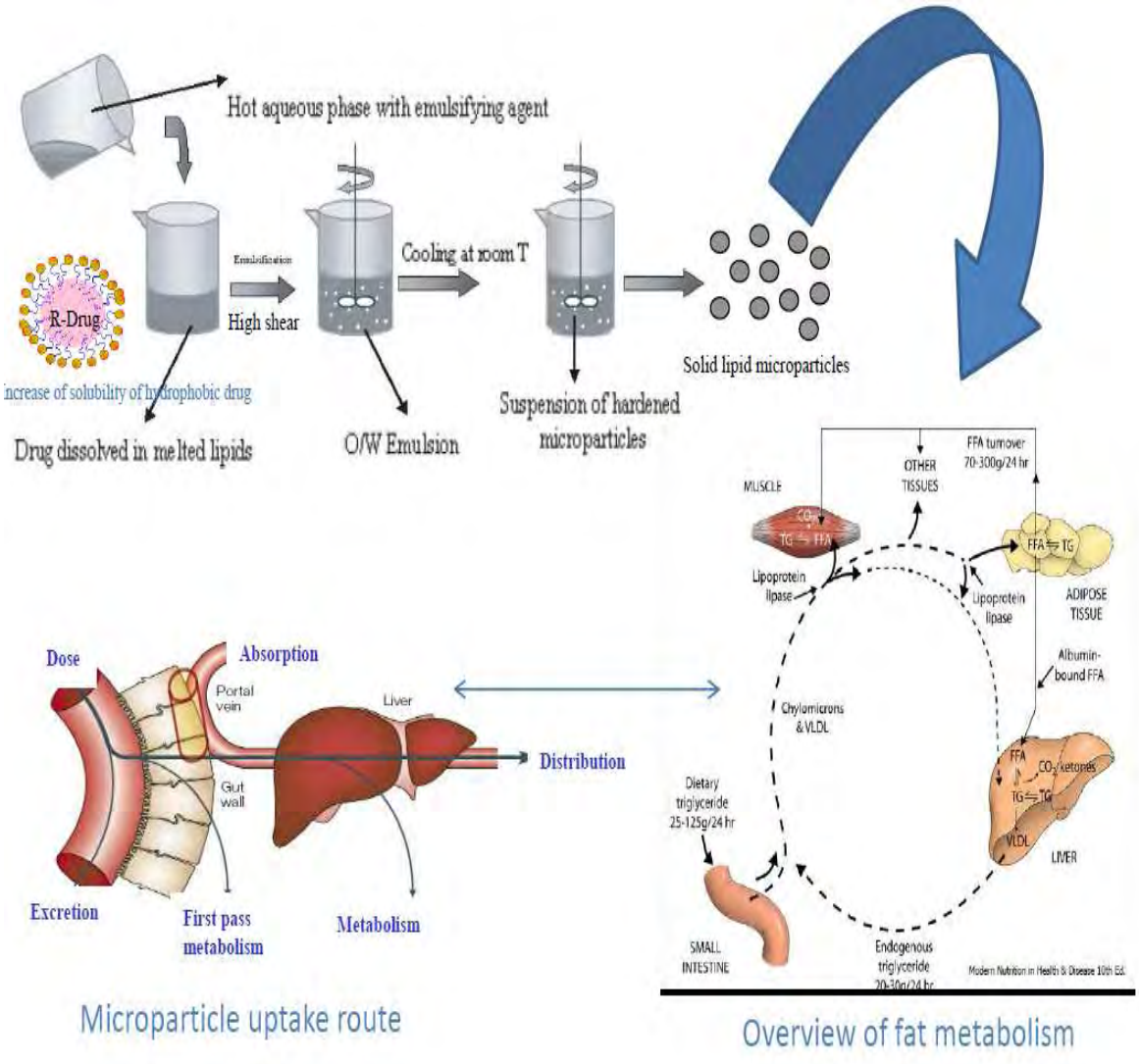

Fig. 6: General mechanism of *in vivo* SLM uptake

1995; Cavalli *et al.*, 2003). It is noteworthy to recall that lymphocytes arise from stem cells in bone marrow and differentiate centrally into B-cells and T-cells (thymus) from where they move through the bloodstream to the peripheral lymphoid tissues – the

and the spleen (Clark *et al.*, 2001; Eldridge *et al.*, 1990). Draining lymph carries these particles from the tissues (extracellular fluid as lymph) via the afferent lymphatics vessels into the thoracic duct, which returns the lymph

to the bloodstream by emptying into the left subclavian vein. Although the organization of the spleen is similar to that of a lymph node (like Peyer's patches), antigen enters the spleen from the blood (via trabecular artery into the central arteriole from where they enter the marginal sinus and drain into a trabecular vein) rather than from the lymph.

Another side to this analogy is that exogenously administered triglycerides are digested by the action of pancreatic lipase/colipase digestive enzymes in the small intestine and absorbed into enterocytes. After absorption, long-chain fatty acids or lipids are biosynthesized into triglyceride-rich lipoprotein particles (chylomicrons), which are secreted into intestinal lymph (Fig. 6). The size of intestinal lipoproteins precludes their absorption into the blood capillaries, and therefore they are secreted into the lymph. Secondly, the cellular lining of the gastrointestinal tract is composed of absorptive enterocytes interspersed with membranous epithelial (M) cells. M cells that cover lymphoid aggregates, known as Peyer's patches, take up microparticles by a combination of endocytosis or transcytosis (Eldridge *et al.*, 1990). The important characteristics of nanoparticles for their uptake are optimum size (10-100 nm), hydrophobicity, and surface charge (Andrianov and Payne, 1998). The uptake of fluorescent polystyrene microparticles of size ranging from 0.1 to 3.0 μm into Peyer's patches of rats was dependent on both the size and the nonionic nature of the particles. Uptake of many colloidal polymeric carriers across the intestinal mucosa (Swartz, 2001) has been shown to occur via Peyer's patches or isolated lymphoid follicles after oral administration (Kreuter, 1991). In addition to the size of these SLMs within one week of preparation, their hydrophobic surface, imparted by phosphatidylcholine, might have influenced the SLM uptake by Peyer's patches (Cavalli *et al.*, 2003).

CONCLUSION

Here we conclude that it is possible to formulate piroxicam in solid lipid microparticles with good properties using surface-modified tallow fat prepared by hot homogenization technique. Piroxicam-loaded SLMs displayed significant anti-inflammation against acute egg albumin-induced pedal inflammation in rats quite comparable to Feldene® with a sustained *in vivo* release and anti-inflammatory activity and may be considered as an alternative dosage form for this drug to overcome its bioavailability problems.

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