

## ***SOLID LIPID NANOPARTICLES FOR TOPICAL DELIVERY OF MELOXICAM: DEVELOPMENT AND IN VITRO CHARACTERIZATION***

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### **Abstract:**

Solid lipid nanoparticles (SLN) are colloidal carrier systems representing a promising approach as a drug delivery system for topical application. Therefore, the objective of this investigation was to develop Meloxicam Solid lipid nanoparticles (MLX SLNs) for topical delivery. The present study addresses the influence of different formulation compositions as lipid type and concentration in addition to surfactant concentration on the physicochemical properties and drug release profile of MLX SLNs. The nanoparticles were developed by modified high shear homogenization and ultrasonication technique using Geleol, Compritol 888 ATO or Precirol ATO 5 as solid core and poloxamer 188 as a surfactant. The results of the study revealed that MLX loaded SLNs showed extremely spherical shape having enriched core drug loading pattern with particle size (LD 90%) in the range of 325 to 1080 nm. A relatively high drug entrapment efficiency ranging from 61.94 to 85.33 % was obtained with zeta potential values lie between -17.6 to -38.6 mV indicating good stability. DSC examination revealed that MLX encapsulated in SLNs was in the amorphous state. According to the rheological study, all nanoparticulate systems exhibited non-Newtonian pseudoplastic flow with thixotropic behavior. In vitro release study showed a sustained release of MLX from the SLNs up to 48 h following Higuchi or zero order equations. Results of stability evaluation showed a long-term stability after storage at 4 °C for 12 months. In conclusion, SLNs with excellent physical stability, high entrapment efficiency and controlled drug release can be produced representing a promising carrier for topical delivery of meloxicam.

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**Key Words:** Meloxicam, Solid Lipid Nanoparticles, Topical delivery, DSC, in vitro release study

### **Introduction**

During the recent decades several studies have suggested that novel drug delivery systems based on lipid nanoparticles have the potential of increasing cutaneous drug delivery of both hydrophilic and lipophilic, compared to the other conventional vehicles (Mandawgade and Patravale, 2008; Liu et al., 2007a).

Solid lipid nanoparticles (SLNs) are colloidal carrier systems composed of a high melting point lipid/s as a solid core coated by surfactants (Mehnert and Mader, 2001; Wissing et al., 2004). Distinct advantages of solid lipid nanoparticles include negligible skin irritation, controlled release and protection of active substances (Jenning and Gohla, 2001; Mei et al. 2003; Jee et al., 2006; Muller et al., 2008). Because they are composed of physiologically tolerated, non-irritative and non-toxic lipids, SLNs seem to be well suited for use on inflamed and damaged skin (Muller et al., 2000). Moreover, the small size of the lipid particles ensures close contact to the stratum corneum increasing the amount of the drug penetrating into the mucosa or skin (Jenning et al., 2000a). These nanometer

sized particles with their solid lipid matrix may also allowing for sustained drug release (Cevc, 2004; Schafer-Korting et al., 2007).. After topical application, occlusive properties were also reported which decreases transepidermal water loss and favors the drug penetrating through the stratum corneum (Wissing and Muller, 2003; Muller et al., 2002; Wissing et al., 2001).

Meloxicam (MLX), a non-steroidal anti-inflammatory drug (NSAID), is a preferential inhibitor of cyclooxygenase-2 and has demonstrated potent analgesic and anti-inflammatory activity (Noble and Balfour, 1996). Considering the fact that most inflammatory diseases occur locally, topical application of MLX on the inflamed site can offer the advantage of delivering the drug directly producing its local effect. This occurs by avoiding gastric irritation, obtaining a substantial reduction of the systemic side effects, in addition to improvement of the patient compliance. In view of the characteristics of MLX including small oral dosage (7.5-15 mg/day), low molecular weight (354.1), lipid solubility and excellent tissue tolerability (Parfitt, 1999), it seems that there is a great need for investigating the MLX topical delivery system as an additional route for MLX administration. MLX performs very poorly in aqueous solubility and wettability, leading to difficulties in the design of pharmaceutical formulations. In order to overcome the formulation problems of MLX in addition to the barrier properties of the intact skin which limit the permeability of wide variety of pharmaceutical active agents, the development of a suitable vehicle system for optimum topical delivery of MLX is required.

Thus, the aim of the present study is to make the most benefits of solid lipid nanoparticles as drug delivery system for MLX through developing Meloxicam loaded Solid lipid nanoparticles (MLX SLNs) using high shear homogenization and ultrasonication method. Furthermore, the influence of some formulation variables on the characteristics of the MLX SLNs were also investigated.

## **Materials and methods**

### **Materials**

Meloxicam was supplied by Medical Union Pharmaceuticals, Abu-Sultan, Ismailia, Egypt. Geleol (glyceryl monostearate 40-55; 40-55 % monoglycerides - 30-45% diglyceride, m.p. 54.5-58.5 °C), Compritol 888 ATO (glyceryl behenate; 15-23 % monoglycerides - 40-60% diglyceride - 21-35% triglycerides, m.p. 69.0-74.0 °C) and Precirol ATO5 (glyceryl palmitostearate; 8-22 % monoglycerides - 40-60 % diglyceride - 25-35 % triglycerides m.p. 50-60 °C), kindly donated by Gattefossé, France. Poloxamer 188 (Pluronic<sup>®</sup> F68; a triblock copolymer of polyoxyethylene-polyoxypropylene), Methanol, Chromasolv<sup>®</sup> and Dialysis tubing cellulose membrane (molecular weight cut-off 12,000 g/mole) were purchased from Sigma Chemical Company, St. Louis, USA. All other chemicals and reagents used are of analytical grade.

### **Methods**

#### ***Preparation of solid lipid nanoparticles***

Solid lipid nanoparticles were prepared by a slight modification of the previously reported high shear homogenization and ultrasonication method (Mehnert and Mader, 2001; Venkateswarlu and Manjunath, 2004, 2005). Briefly, the lipid phase consisted of Geleol, Compritol or Precirol as solid lipid was melted 5 °C above the melting point of the lipid used and MLX (0.5% w/w) was dissolved therein to obtain a drug-lipid mixture. An aqueous phase consists of poloxamer 188 was heated up to the same temperature of the molten lipid phase. The hot lipid phase was poured onto the hot aqueous phase and homogenization was carried out at 25000 rpm for 5 minutes using Heidolph homogenizer (Heidolph Instruments, Germany). The resulted hot oil in water emulsion was sonicated for 30 minutes (Digital sonicator, MTI, USA). MLX loaded solid lipid nanoparticles were finally obtained by allowing hot nanoemulsion to cool to room temperature. Blank SLNs were prepared using the same procedure variables.

#### ***MLX entrapment efficiency***

The entrapment efficiency percent (E.E. %), which corresponds to the percentage of MLX encapsulated within the nanoparticles, was determined by measuring the concentration of free MLX in the dispersion medium. The untrapped MLX was determined by adding 500 µl of MLX loaded nanoparticles to 9.5 ml methanol and then this dispersion was centrifuged at 9000 rpm (Union 32R, Hanil Science Industrial, Korea) for 30 minutes. The supernatant was filtered through millipore membrane filter (0.2 µm) and analyzed for unencapsulated MLX at 360 nm by using validated UV-spectrophotometric method (Shimadzu, model 2401/PC, Japan) after suitable dilution.

The E.E. % was calculated using the following equation (Hou et al., 2003; Souto et al., 2004):

$$\text{E.E. \%} = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100$$

Where “ $W_{\text{initial drug}}$ ” is the mass of initial drug used and the “ $W_{\text{free drug}}$ ” is the mass of free drug detected in the supernatant after centrifugation of the aqueous dispersion.

#### **Particle size analysis**

Particle size analysis of MLX loaded nanoparticles was performed by Laser diffraction particle size analyzer (LD, Master sizer X, Malvern Instruments, UK) at 25°C. The LD data obtained were evaluated using volume distribution as diameter values of 10%, 50%, 90% and Span values. The diameter values indicate the percentage of particles possessing a diameter equal to or lower than the given value. The Span value is a statistical parameter useful to evaluate the particle size distribution, the lower the Span the narrower is the particle size distribution. It is calculated applying the following equation (Teeranaachaideekul et al., 2007):

$$\text{Span} = \frac{\text{LD 90\%} - \text{LD 10\%}}{\text{LD 50\%}}$$

#### **Zeta potential ( $\zeta$ ) and pH measurement**

Zeta potential was measured in folded capillary cells using Laser Zetameter (Malvern Instruments, UK). Measurements were performed in distilled water adjusted with a solution of 0.1mM NaCl at 25 °C. The zeta potential values were calculated using the Smoluchowski equation.

The pH values of MLX lipid nanoparticles were measured at 25°C using digital pH meter (Jenway, UK).

#### **Transmission Electron Microscopy (TEM)**

The morphological examination MLX loaded SLNs was performed with TEM (model JEM-1230, Jeol, Tokyo, Japan). One drop of diluted sample was deposited on the surface of carbon coated copper grid and negatively stained with a drop of 2 % (w/w) aqueous solution of phosphotungstic acid for 30 s. Excess staining solution was wiped off by filter paper, leaving thin aqueous film on the surface. After being stained, samples were allowed to dry at room temperature for 10 minutes for investigation (Li et al., 2006).

#### **Differential scanning calorimetry (DSC) analysis**

DSC analysis was performed using Shimadzu Differential Scanning Calorimeter (DSC-50, Kyoto, Japan). About 10 mg sample was added in a 40  $\mu$ l aluminium pan which was sealed and heated in the range of 30-300°C at a heating rate of 10°C /min. An empty aluminium pan was used as reference standard. Analysis was carried out under nitrogen purge.

#### **Rheological study**

The rheological properties of the prepared lipid nanoparticles were measured using Brookfield’s viscometer (Brookfield LV-DV II+, USA). The sample (20 g) was placed in a beaker and allowed to equilibrate for 5 min. The measurements were carried at ambient temperature using the suitable spindle. The spindle speed rate was increased in ascending order from 1 to 100 rpm and then in descending order speed setting from 100 to 1 rpm with each kept constant for 10 seconds before a measurement was made.

#### **In vitro release study**

In vitro release of meloxicam was evaluated by the dialysis bag diffusion technique reported by Yang, et al. (Yang et al., 1999). The release studies of meloxicam from solid lipid nanoparticles were performed in phosphate buffer pH 5.5 and methanol (75: 25). The aqueous nanoparticulate dispersion equivalent to 2 mg of meloxicam was placed in a cellulose acetate dialysis bag and sealed at both ends. The dialysis bag was immersed in the receptor compartment containing 50 ml of dissolution medium, which was stirred in a water bath shaker at 100 rpm (Memmert GmbH, Germany) and maintained at  $32 \pm 2^\circ\text{C}$ . The receptor compartment was covered to prevent the evaporation of dissolution medium. A 2 ml sample of the receiver medium was withdrawn at predetermined time intervals (0.5, 1, 2, 3, 4, 5, 6, 8, 24 and 48 h) replaced by equivalent volume of fresh medium to maintain constant volume. The samples were analyzed for drug content spectrophotometrically at 360.5 nm. The data was analyzed using linear regression equations and the order of drug release from the different formulations was determined (zero order, first order or Higuchi diffusion model).

**Effect of storage on particle size**

MLX loaded SLN formulations were stored at 4°C for 12 months. Particle size was determined using Laser diffraction particle size analyzer (LD).

**Statistical analysis**

All experiments were repeated three times and data were expressed as the mean value  $\pm$  S.D. The statistical analysis of data was determined using one-way analysis of variance (ANOVA). Individual differences were evaluated using a nonparametric post hoc test. A difference of  $p < 0.05$  was considered to be statistically significant.

**Results and discussion****Preparation of Solid lipid nanoparticles**

SLNs have been prepared in various researches using different methods (Hu et al., 2002; Priano et al., 2007; Lva et al., 2009). In the present study, we had adopted an economical, simple and reproducible method for the preparation of SLN, i.e. homogenization followed by ultrasonication at above the melting point of the lipid (Fang et al., 2008; Chen et al., 2010). MLX loaded SLNs dispersions were composed of Geleol, Compritol 888 ATO or Precirol ATO 5 as core matrices used in different concentrations 5, 7.5 and 10 % (w/w). These lipid based carrier systems were stabilized by 0.5, 1, 2.5 and 5 % (w/w) Poloxamer 188. Meloxicam was incorporated at a constant concentration of 0.5% (w/w). The w/w percent composition of the investigated MLX SLNs is shown in table 1.

**Table I.** Composition of MLX SLNs (% w/w) of different lipids

Formula	Lipid		Surfactant conc. (Poloxamer 188) (%)
	Type	Conc. (%)	
SLN <sub>1</sub>	Geleol	5	0.5
SLN <sub>2</sub>			1
SLN <sub>3</sub>			2.5
SLN <sub>4</sub>			5
SLN <sub>5</sub>		7.5	0.5
SLN <sub>6</sub>		10	
SLN <sub>7</sub>	Compritol	5	0.5
SLN <sub>8</sub>			1
SLN <sub>9</sub>			2.5
SLN <sub>10</sub>			5
SLN <sub>11</sub>		7.5	0.5
SLN <sub>12</sub>		10	
SLN <sub>13</sub>	Precirol	5	0.5
SLN <sub>14</sub>			1
SLN <sub>15</sub>			2.5
SLN <sub>16</sub>			5
SLN <sub>17</sub>		7.5	0.5
SLN <sub>18</sub>		10	

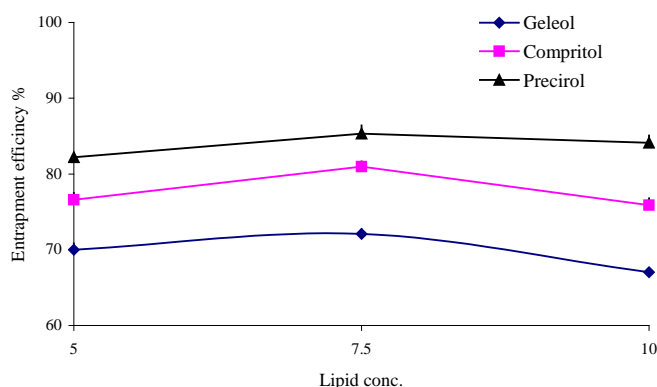
**MLX entrapment efficiency**

The entrapment efficiencies of all SLN formulations are shown in table 2. The data clearly shows that all formulations possessed high entrapment efficiency (E.E. %) ranged from  $61.94 \pm 1.31$  to  $85.33 \pm 1.07$  %. The results might be related to the structure of the lipid which had a great influence on the capacity for drug incorporation. Lipids which form highly crystalline particles with a perfect lattice (e.g. monoacid triglycerides) lead to drug expulsion (Westesen et al., 1997). More complex lipids as Geleol, Compritol 888 ATO and Precirol ATO 5; being mixtures of mono-, di- and triglycerides form less perfect crystals with many imperfections offering space to accommodate the drugs (Muller et al., 2000). The data clearly shows that Geleol SLNs exhibited the lowest entrapment of meloxicam compared to Compritol and Precirol SLNs (Figs. 1 and 2). This can be attributed to the difference in composition and chain length of the three lipids used. The higher drug E.E % noticed with Compritol and Precirol was attributed to the high hydrophobicity due to the long chain fatty acids attached to the triglycerides resulting in increased accommodation of lipophilic drugs (Jenning and Ghola, 2000).

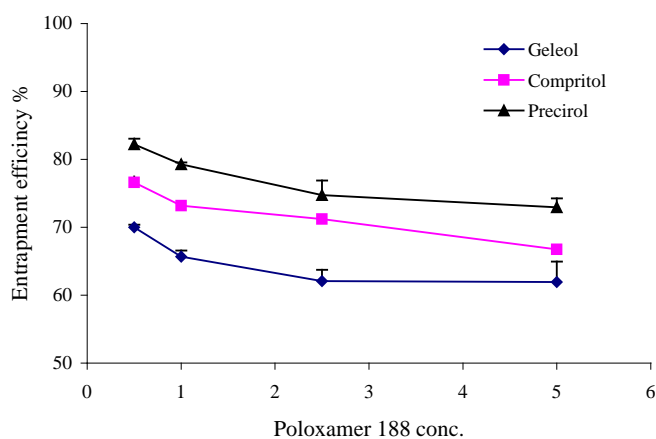
As observed in Fig. 1, at a constant amount of Poloxamer 188 (0.5 % w/w) increasing the lipid concentration from 5 to 7.5 % (w/w) resulted in a consequent increase in E.E. % ( $p < 0.05$ ), while decreased upon further lipid increase to 10 % (w/w). This is in accordance with the study done by Abdelbary and Fahmy on diazepam-loaded SLN, in which they found that increasing the Compritol concentration to 10% (w/w) consequently resulted in a decrease in the amount of diazepam entrapped. A possible explanation is that during the crystallization of the lipid phase a partial expulsion of the drug on the particle surface may occur. Furthermore, the higher viscosity at the interface produced by higher lipid concentration may cause a decrease in diffusion and hence fewer lipid molecules will be carried into the aqueous phase. Therefore, the formation and stabilization of lipid aggregates at these higher concentrations are reduced (Abdelbary and Fahmy, 2009).

**Table II.** Physicochemical characterization of the MLX loaded SLNs

Formula	LD 10	LD 50	LD 90	Span	E.E %	Z potential ( $\zeta$ ) (mV)	pH
SLN <sub>1</sub>	235 ± 53.03	350 ± 56.57	525 ± 35.36	0.83	70.00 ± 0.82	-19.0	5.50 ± 0.68
SLN <sub>2</sub>	260 ± 28.28	350 ± 14.14	420 ± 14.14	0.46	65.67 ± 0.26	-20.3	6.03 ± 0.14
SLN <sub>3</sub>	210 ± 30.90	270 ± 42.43	325 ± 7.07	0.43	62.07 ± 2.17	-23.7	6.39 ± 0.23
SLN <sub>4</sub>	150 ± 14.14	230 ± 28.28	335 ± 7.07	0.80	61.94 ± 1.31	-24.7	5.60 ± 0.02
SLN <sub>5</sub>	235 ± 77.78	405 ± 53.03	755 ± 21.21	1.28	72.08 ± 0.60	-27.2	5.88 ± 0.02
SLN <sub>6</sub>	185 ± 7.07	385 ± 35.36	850 ± 28.28	1.73	67.03 ± 0.30	-38.2	5.40 ± 0.02
SLN <sub>7</sub>	195 ± 7.07	395 ± 21.21	680 ± 14.14	1.23	76.60 ± 0.88	-18.7	5.37 ± 0.17
SLN <sub>8</sub>	205 ± 3.54	405 ± 7.07	585 ± 7.07	0.94	73.18 ± 0.24	-17.6	5.37 ± 0.02
SLN <sub>9</sub>	215 ± 25.80	355 ± 77.78	475 ± 21.21	0.73	71.22 ± 0.56	-22.9	6.10 ± 0.05
SLN <sub>10</sub>	235 ± 20.21	300 ± 70.71	415 ± 7.07	0.60	66.74 ± 0.16	-22.9	5.92 ± 0.04
SLN <sub>11</sub>	185 ± 7.07	395 ± 21.21	1025 ± 21.21	2.13	80.96 ± 0.68	-22.8	6.33 ± 0.06
SLN <sub>12</sub>	375 ± 75.77	505 ± 70.50	1080 ± 14.14	1.40	75.88 ± 0.91	-37.8	6.21 ± 0.09
SLN <sub>13</sub>	190 ± 14.14	380 ± 56.57	675 ± 35.36	1.28	82.22 ± 0.39	-20.5	6.05 ± 0.23
SLN <sub>14</sub>	185 ± 7.07	355 ± 7.07	565 ± 7.07	1.07	79.29 ± 0.90	-23.3	6.07 ± 0.02
SLN <sub>15</sub>	155 ± 3.54	270 ± 14.14	440 ± 14.14	1.06	74.73 ± 1.68	-23.1	5.82 ± 0.29
SLN <sub>16</sub>	145 ± 7.07	220 ± 14.14	325 ± 21.21	0.82	72.94 ± 2.99	-28.9	5.69 ± 0.15
SLN <sub>17</sub>	185 ± 21.21	335 ± 7.07	1005 ± 35.36	2.45	85.33 ± 1.07	-32.1	5.77 ± 0.08
SLN <sub>18</sub>	385 ± 50.91	515 ± 33.35	1000 ± 28.28	1.19	84.12 ± 0.94	-38.6	5.37 ± 0.06



**Fig.1.** Effect of lipid concentration and type on the E.E% of MLX SLNs



**Fig.2.** Effect of surfactant concentration on the E.E% of MLX SLNs

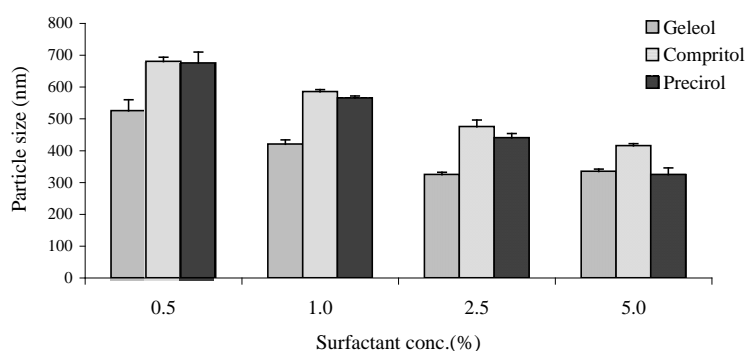
It was also evident that increasing the amount of surfactant from 0.5, 1, 2.5 to 5 % (w/w) at a constant amount of lipid (5 % w/w), resulted in a gradual significant decrease in the E.E. % of the produced SLNs ( $p < 0.05$ ) (Fig. 2). This observed decrease in E.E. % could be explained by partition phenomenon. High surfactant level in the external phase might increase the partition of drug from internal to external phase of the medium. This increased partition is due to the increased solubilization of the drug in the external aqueous phase so more drug can disperse and dissolve in it (Rahman et al., 2010). However, in case of Geleol SLNs no further decrease in E.E. % was observed upon increasing the poloxamer 188 concentration above 2.5% (w/w) ( $p > 0.05$ ) suggesting that an optimum concentration of surfactant was reached sufficient to cover the surface of nanoparticles effectively.

#### **Particle size analysis**

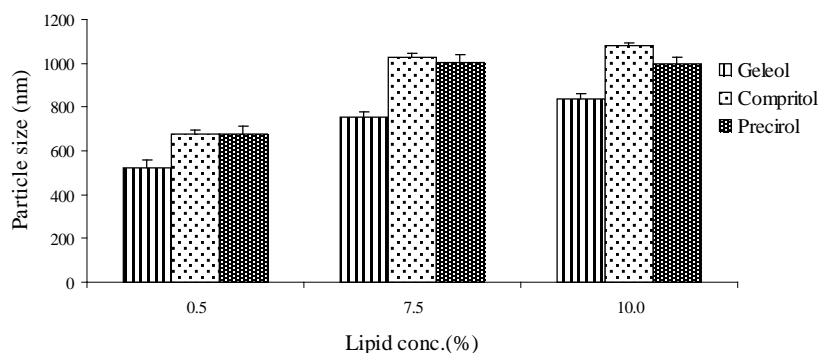
Table 2 depicts LD data of resulting MLX SLNs where LD 90% was used for comparing the influence of different parameters on the size of the nanoparticles. The LD 90% of all formulations ranged from  $325 \pm 7.07$  to  $1080 \pm 14.14$  nm with low Span values indicating narrow particle size distribution. The results clearly showed that there was a gradual decrease in particle size with an increase in surfactant concentration from 0.5, 1, 2.5 to 5 % (w/w) ( $p < 0.5$ ) (Fig. 3). This decrease in size at high surfactant concentrations might be due to effective reduction in interfacial tension between the aqueous and lipid phases leading to the formation of emulsion droplets of smaller size

(Liu et al., 2007b). Higher surfactant concentrations effectively stabilized the particles by forming a steric barrier on the particle surface and thereby protect smaller particles and prevent their coalescence into bigger ones (Rahman et al., 2010).

As revealed in Figs. 3 and 4, Compritol 888 ATO showed the largest particle sizes followed by Precirol ATO 5 then Geleol SLNs. A possible explanation for the differences in sizes may be due to differences in chain lengths and viscosities of lipids used (Ahlin et al., 1998). Compritol 888 ATO (m.p. 69.0-74.0 °C) is a solid lipid based on glycerol esters of behenic acid (C22), where the main fatty acid is behenic acid > 85% but other fatty acids (C16-C20) are also present. Precirol ATO 5 (m.p. 50.0-60.0 °C) and Geleol (m.p. 54.5-58.4 °C) are composed mainly of palmitic (C16) and stearic acid (C18) > 90 %. High melt temperature resulting in higher viscosity plus the long hydrocarbon chain length of Compritol 888 ATO might result in larger particle size compared to Precirol ATO 5 and Geleol SLNs.



**Fig.3.** Effect of surfactant concentration on particle size measured by LD 90 % of different SLNs



**Fig.4.** Effect of lipid concentration and type on particle size measured by LD 90 % of MLX SLNs

Lipid concentration seems to be one of the most important factors influencing the particle size. According to Fig. 4, increasing the lipid content from 5, 7.5 to 10 % (w/w) resulted in a subsequent increase in particle size. It was noticeable that SLN11, SLN12, SLN17 and SLN18 showed larger particle size exceeding the nanometer range. This increase in particle size may partially be related to the viscosity of the samples. The use of a low viscous lipid phase improves size reduction and enhances stability in SLN production (Manjunath et al., 2005). At higher lipid contents, the efficiency of homogenization decreases due to a higher viscosity of the sample, resulting in larger particles. Also, a high particle concentration at high lipid contents increases the probability of particle contact and subsequent aggregation (Freitas and Muller, 1998).

#### **Zeta potential analysis ( $\xi$ ) and pH measurements**

Table 2 shows the measured zeta potential ( $\xi$ ) values of MLX SLNs. As depicted from the table, all formulations were negatively charged, the zeta potential varied from -17.6 to -38.6 mV



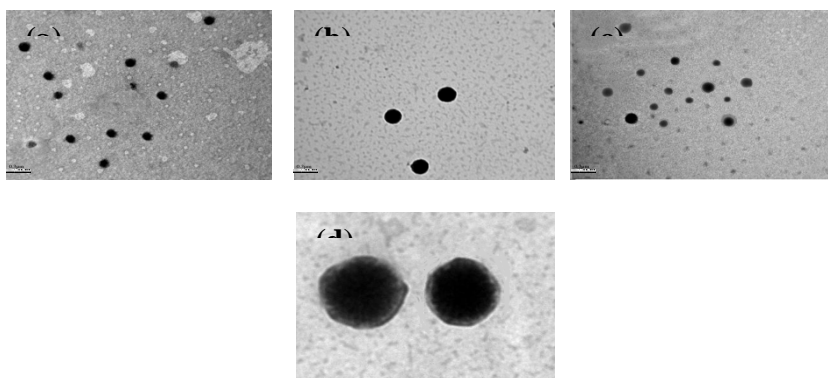
indicating a relatively good stability and dispersion quality. It was noticeable that as the amount of surfactant increased in the formulation, the zeta potential became more negative. A similar finding was previously reported upon increasing Tween 80 concentration from 0.5 to 1 % which was attributed to the formation of denser surfactant film (Estella-Hermoso de Mendoza et al., 2008). Poloxamer 188 being non-ionic surfactant succeeded in the production of relatively stable dispersions. Although non-ionic surfactant could not ionize into charging group like ionic ones, but still demonstrated its zeta potential. The reason might be due to molecular polarization and the adsorption of emulsifier molecule on the charge in water, it was absorbed to the emulsifier layer of particle/water interface and electric double layer similar to ionic was formed. Liu et al. reported that poloxamer 188 was found to be one of the most effective non-ionic surfactants to avoid the formation of aggregates (Liu et al., 1996). Poloxamer 188 can provide additional steric stabilization of particles; so we can expect combined electrostatic and steric stabilization of SLN formulations (Schwarz and Mehnert, 1999; Lim and Kim, 2002).

Considering the effect of lipid type and concentration on the zeta potential ( $\xi$ ) of the produced SLN formulations, the results showed no direct relationship between the type of lipid used and the measured  $\xi$  values. On the contrary, as the lipid concentration increased the zeta potential was found to be more negative. Rahman et al. reported the same observation when studying the effect of increasing Compritol amount in final formulation (Rahman et al., 2010).

The pH of different MLX SLN formulations was found to be within acceptable limits for topical application ranging from  $5.37 \pm 0.02$  to  $6.39 \pm 0.23$  (table 2).

#### **Transmission Electron Microscopy (TEM)**

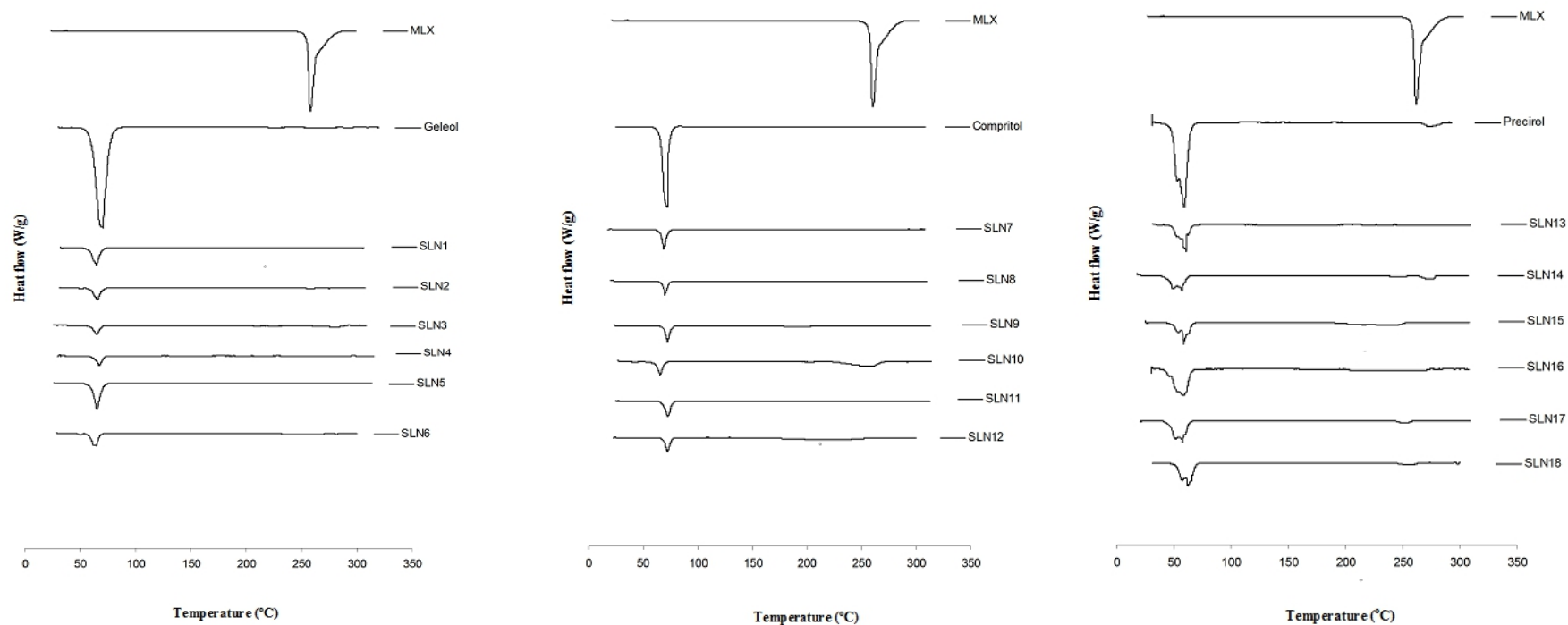
TEM was conducted to investigate the morphology of MLX loaded SLNs. It was evident from TEM images that nanoparticles were almost spherical with smooth morphology, appeared as black dots, well dispersed and separated on the surface (Fig. 5). This description agrees with a previous observation that the use of chemically heterogeneous lipids in combination with heterogeneous surfactants favors the formation of ideally spherical lipid nanoparticles (Mehnert and Mader, 2001). Fig. 5d illustrates the presence of a very thin layer surrounding the particles which postulate a drug-enriched core model. This model can be achieved if during the lipid solidification process; the drug precipitates first, which results in a drug-enriched core covered with a lipid shell which has a lower drug concentration. This drug distribution within the nanoparticles will have its impact on in vitro drug release profile discussed later on.



**Fig.5.** Transmission electron micrographs of MLX loaded SLN dispersions (a) SLN4 (b) SLN10 (c) SLN16 and (d) SLN representing the core and shell theory

***Differential scanning calorimetry (DSC) analysis***

DSC is a tool to investigate the melting and recrystallization behavior of crystalline material like SLNs (Liu et al., 2005). Fig. 6 shows the DSC thermograms of pure MLX, bulk lipids (Geleol, Compritol 888 ATO and Precirol ATO 5) and MLX loaded SLNs. Pure MLX showed a sharp endothermic peak at 259.54 °C corresponding to its melting point, indicating its characteristic crystalline nature. Bulk Geleol showed distinctive melting peak at 66.01 °C, while a sharp peak at 74.22 °C was observed for Compritol 888 ATO. The bulk Precirol ATO 5 exhibits a sharp endothermic event, ascribing to the melting, around 63.35 °C, with a small but well defined shoulder at 57.37 °C which might be due to the melting of  $\alpha$  polymorphic form (Araujo et al., 2010). These sharp melting endothermic peaks of bulk lipids indicate that the starting materials were crystalline. As observed in Fig. 6, the thermograms of all investigated SLN systems did not show the melting peak of MLX around 259.54 °C indicating the conversion of crystalline MLX to the amorphous form which could be attributed to complete dissolution of the drug in the molten lipid matrix. The melting points of Geleol, Compritol 888 ATO and Precirol ATO 5 in SLN form were depressed showing slight shift to lower temperature side when compared to the corresponding bulk lipids. This melting point depression could be due to the small particle size (nanometer range), the high specific surface area, and the presence of surfactant - in other words, the depression can be attributed to the Kelvin effect (Jenning et al., 2000b). Kelvin realized that small, isolated particles would melt at a temperature lower than the melting temperature of bulk materials. In the same way, the melting enthalpy values of different lipids in SLN formulations showed drastic depression compared to their bulk lipids. These lower melting enthalpy values should suggest less ordered lattice arrangement of the lipid within nanoparticles compared to the bulk materials (Hou et al., 2003). For the less-ordered crystal or amorphous state, the melting of the substance requires less energy than the perfect crystalline substance, which needs to overcome lattice force. Lipid nanoparticles seem to loose part of their crystalline state transforming from a mixture of  $\beta'$  and  $\beta$  polymorphs to the most stable  $\beta$  polymorph, permitting MLX to fit in the molecular gaps. Therefore this decrease in the melting point and enthalpy values is associated with numerous lattice defects and the formation of amorphous regions in which the drug is located



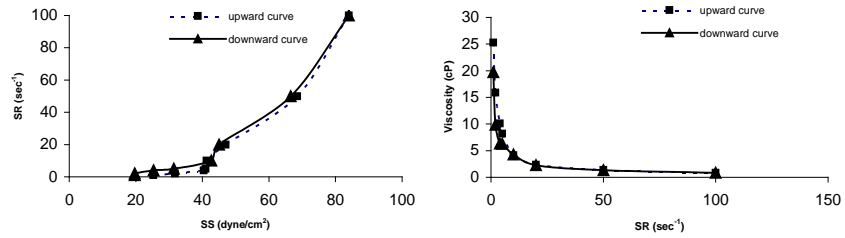
**Fig.6.** DSC curves of pure drug (MLX), bulk lipids (Geleol, Compritol and Precirol) and MLX loaded SLNs

***Rheological Study***

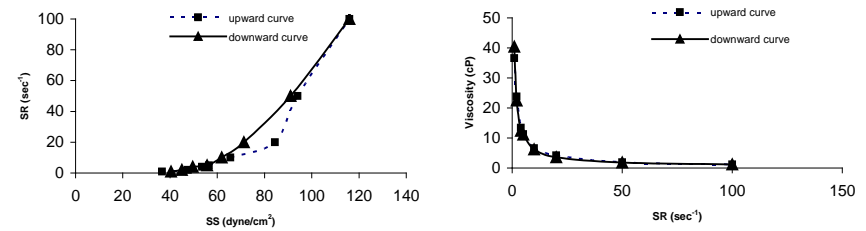
As for other disperse liquid and semisolid systems, the rheological properties of lipid nanoparticles influence their potential for dermal application in a fundamental way (Muller et al., 2002; Lippacher et al., 2001, 2002). Therefore, rheological behavior of different lipid nanoparticles formulations was studied and presented by plotting the shear stress versus shear rate (flow curves) and the viscosity versus the shear rate (viscosity curves) (Illinga and Unruh, 2004; Liu et al., 2008). The rheograms of different SLN formulations were shown in Fig. 7.

All SLN dispersions revealed non-Newtonian flow where the viscosity of non-Newtonian fluids changes according to the shear rate i.e. has no constant viscosity (49-Barnes, 1997). This flow was characterized by shear-thinning behavior in which the viscosity of the SLN dispersions decreased with the increase of shear rate. This dependent change in viscosity is a desired property in the pharmaceutical formulations due to their requirement of flexibility in topical drug delivery (50-Sheth, 2007). When the preparation is subjected to a shear force, its network structure breaks down leading to a gradual decrease in viscosity in order to spread on the skin. When the shear force is removed, the viscosity recovers slowly and the increased viscosity keeps the preparation on the skin.

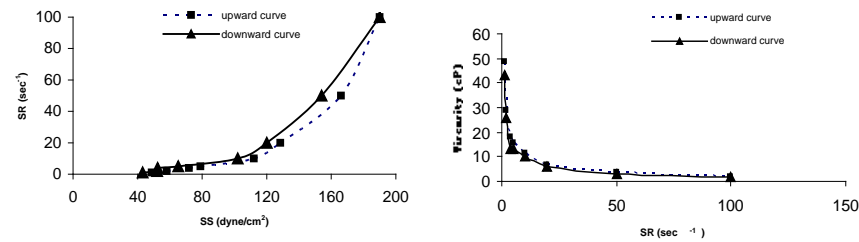
Comparing the viscosity curves of various SLN formulations, it was observed that, for each type of lipid, as the concentration of lipid increased the viscosity increased (Fig. 7). This may be related to the density of network structure, meaning that the network structure of 10 % (w/w) lipid was more viscous than that of 7.5 and 5 % (w/w) lipid, respectively, which was due to the higher amount of lipid incorporated into the system inducing an increase in the interaction between the lipid particles (Seetapan et al., 2010). In addition, the type of lipid affected the viscosity of the final product; Geleol SLNs showed lower viscosities compared to Precirol ATO 5 and Compritol 888 ATO SLNs. However, increasing the surfactant concentration did not result in considerable change in viscosity (data not represented).



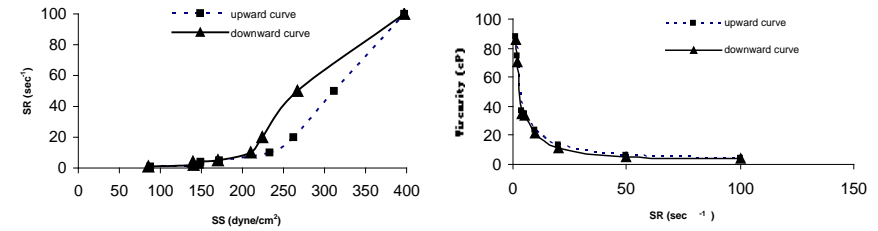
(SLN1)



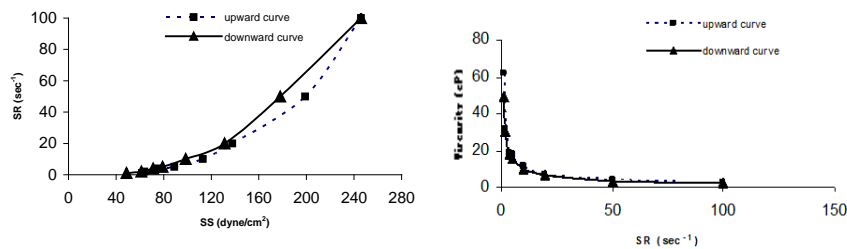
(SLN7)



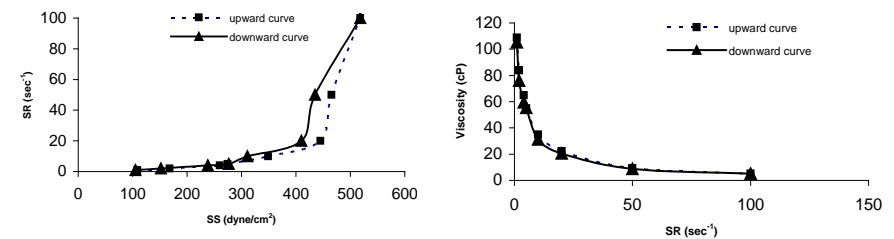
(SLN5)



(SLN11)



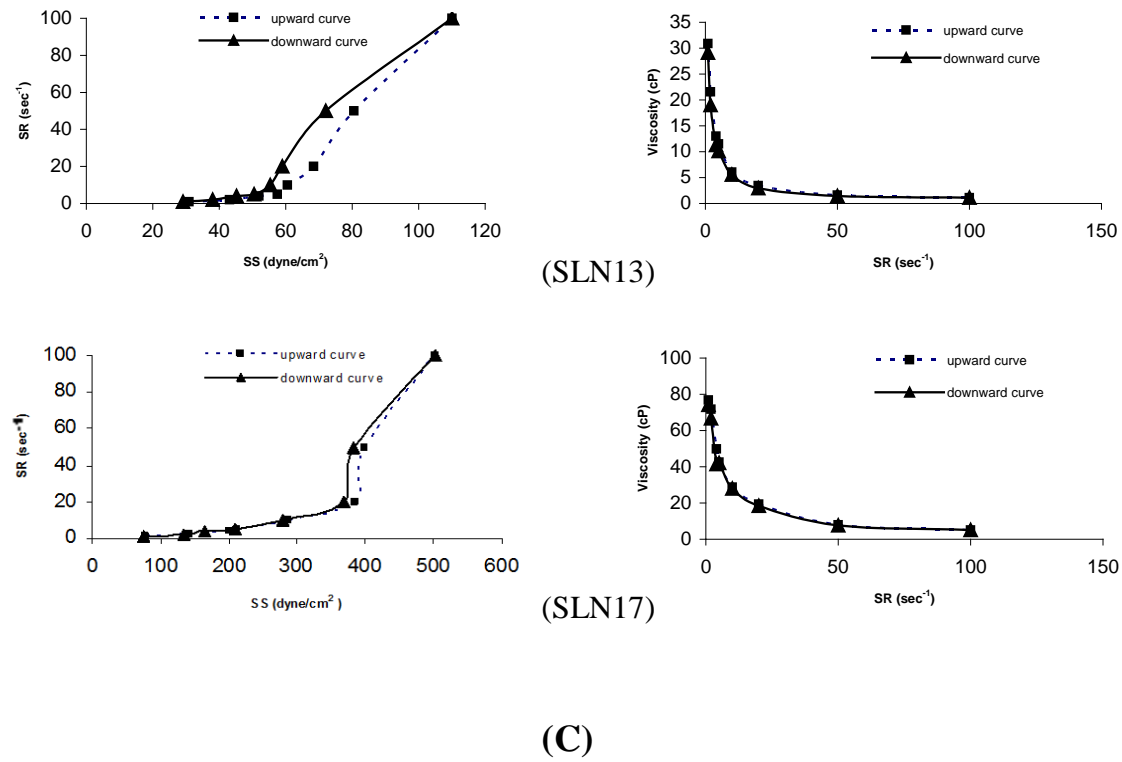
(SLN6)



(SLN12)

(A)

(B)



**Fig.7.** Rheograms of (A) Geleol SLNs (B) Compritol SLNs (C) Precirol SLNs

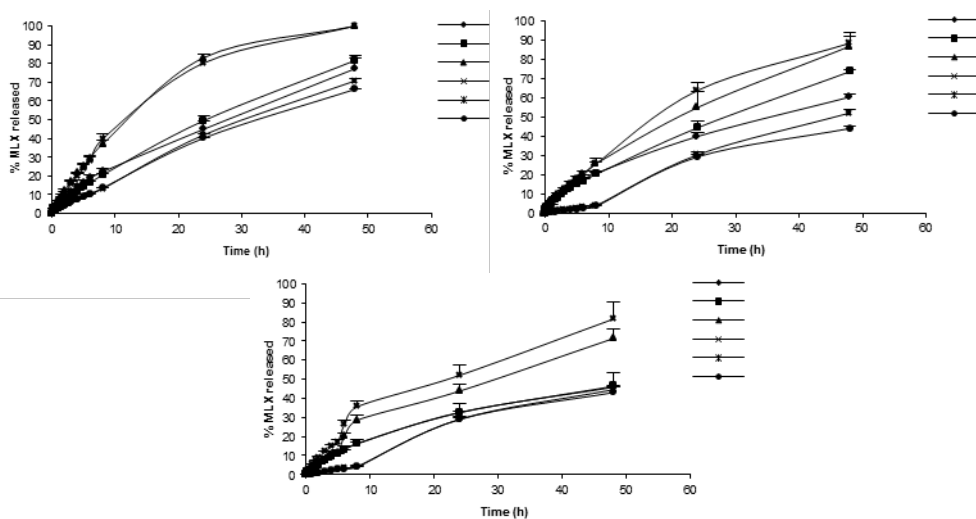
### ***In vitro Release Study***

To elucidate the mechanism of MLX release from SLNs for topical administration, *in vitro* release study using dialysis bag diffusion technique has been performed over 48 hrs, each sample was analyzed in triplicate and the release curves are shown in Fig. 8. The release data was fitted into zero order, first order and Higuchi equations which are widely used in determining the release kinetics of lipid nanoparticles. The release pattern of the drug from almost all SLN formulations followed Higuchi equation with some fitted to zero order equation. The previous result is in agreement with many studies which reported that drug loaded SLN provide a controlled release pattern following Higuchi's square root model (Tiyaboonchai et al., 2007; Vivek et al., 2007).

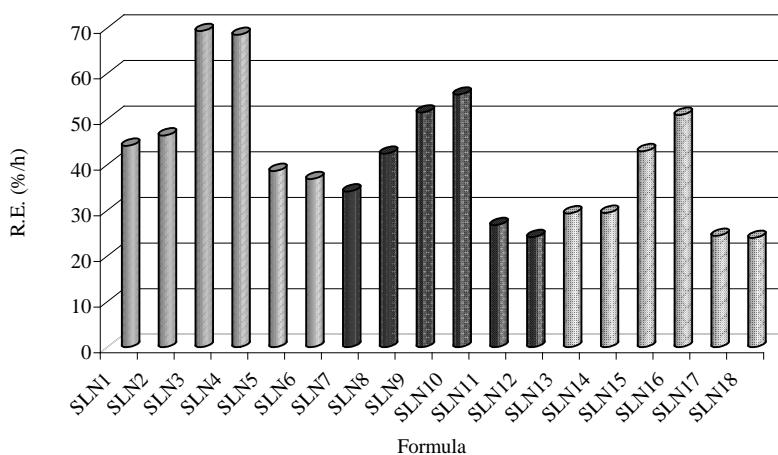
As shown in Fig. 8, MLX SLN formulations were able to release MLX in controlled manner and the percentage of MLX released up to 48 hours ranged from 43.11% to 100%. Interestingly, the amount of poloxamer 188 used had a great influence on the release pattern of SLNs. Increasing the poloxamer 188 concentration from 0.5, 1, 2.5 to 5% (w/w) led to corresponding increase in the percentage of MLX released as well as the release efficiency % (R.E. %) after 48 h, which was noticeable for all three lipids used (Figs. 8 and 9). However, in case of Geleol (SLN1& SLN2) and Precirol ATO 5 (SLN13 & SLN14) no significant increase in R.E. % was found upon increasing poloxamer 188 concentration from 0.5 to 1 % ( $p > 0.5\%$ ). The fast or rapid release and higher release efficiency noticed at higher surfactant concentration could be explained by the partitioning effects of the drug between the melted lipid phase and the aqueous surfactant phase during particle production. During particle production by the hot homogenization technique, drug partitions from the liquid oil phase to the aqueous water phase. The amount of drug partitioning to the water phase will increase with the increase of the drug solubility in the water phase, which means with increasing temperature of the aqueous phase and increasing surfactant concentration. The higher the temperature and surfactant concentration, the greater is the solubility of the drug in the water phase so the amount of drug in the outer shell increased and released in a relatively rapid way (Zur Muhlen and Mehnert, 1998).

Concerning the type of lipid matrix, the results clearly show that among the glycerides used, the highest release was achieved with Geleol compared to Compritol 888 ATO and Precirol ATO 5. Being the lipid of highest monoglyceride content; Geleol had shown the highest release efficiency and consequently lower  $t_{50\%}$ , while in case of Compritol 888 ATO and Precirol ATO 5 the relatively slow release and higher  $t_{50\%}$  can be attributed to the hydrophobic long chain fatty acids of the triglycerides that retain the lipophilic drug resulting in more sustained release effect (Reddy and Murthy, 2005; Kumara et al., 2007) (Figs. 8-10). Furthermore, the lower melting point of Geleol (54.5-58.5°C) may result in a higher mobility at the temperature used in the release experiment. It is well known that the melting point of colloidal structures may be lower than that of the bulk due to the influence of surface energy (Mader, 2006). A difference in release profiles caused by a difference in lipid melting points was also suggested by Paolicelli et al. in a study with ibuprofen and acylglycerols differing in melting points (Paolicelli et al., 2009). The higher amount released from Geleol particles may also reflect the smaller size of these particles as the mean diameter of Geleol nanoparticles represent the smallest of the SLN tested.

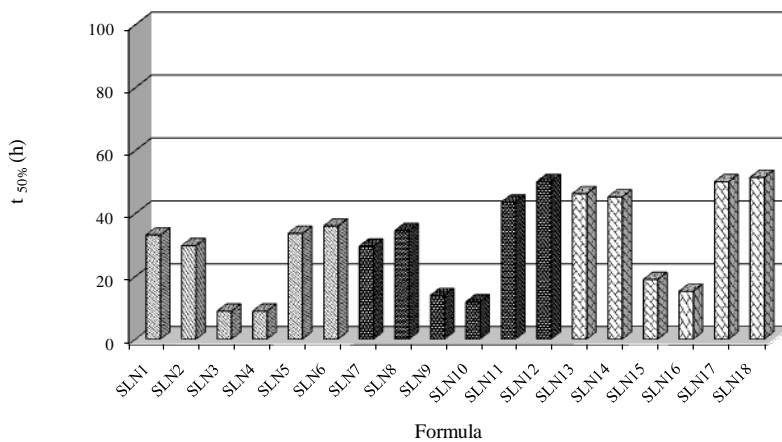
The results also pointed to the effect of lipid concentration on SLNs release profile. Increasing the lipid concentration from 5, 7.5 to 10 % (w/w) resulted in a corresponding decrease in percentage of MLX released, R.E. % after 48 h and a consequent increase in  $t_{50\%}$  (Figs. 8-10). However, for Geleol and Precirol SLNs further increase of the lipid above 7.5% didn't result in a significant decrease in R.E. % ( $p > 0.5\%$ ). This decrease in release profile observed can be attributed to the higher lipid content encapsulating the drug thus reducing drug partition in the outer phase and consequently its release in the receiver media. The release profiles of these SLNs resemble the drug enriched core model (Wissing et al., 2004). In such a model, the drug enriched core is surrounded by a practically drug-free lipid shell. Due to the increased diffusional distance and hindering effects by the surrounding solid lipid shell, the drug has a sustained release profile.



**Fig.8.** In vitro release percentage (%) - time profiles of MLX from SLNs. Data are expressed as the mean  $\pm$  S.D. (n = 3)



**Fig.9.** Release efficiency (R.E. %) of different MLX SLNs formulations



**Fig.10.**  $t_{50\%}$  of different MLX SLNs formulations



### Effect of storage on particle size

In order to evaluate the stability of lipid nanoparticles, the study of size was generally used as a characterization tool. The stability of the MLX loaded SLNs was studied for particle size after 12 months of storage at 4 °C. As previously mentioned, LD 90% was used for comparing the change in particle size of different SLNs.

As illustrated in Fig. 11, SLNs prepared from different lipids showed non significant change in particle size indicating good stability during the period of study. No obvious change of clarity or degradation was observed. All samples were in the nanometer range except for SLN11, SLN12, SLN17 & SLN18 which was probably due to higher amounts of lipid added to these formulations leading to increment in viscosity and subsequent aggregation of the nanoparticles.

The increase in particle size was observed to be more pronounced at low surfactant concentration( 0.5-2.5% ) for the three surfactants used , while 5% SLN showed the highest stability and particle size was almost unchanged allover the course of this investigation. This good stability might be attributed to the strong repulsion by sterical stabilization of the surfactants used at higher concentration (Freitas and Muller, 1999), emphasizing the importance of surfactant concentration for SLN stabilization.

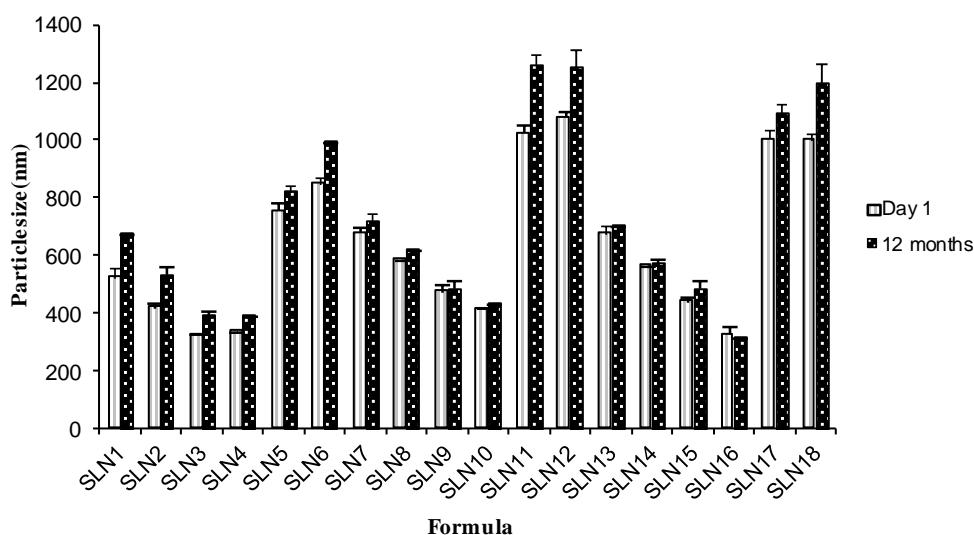


Fig.11. Particle size measured by LD 90 % of MLX SLNs after 1 day and 12 months of storage at 4 °C

It was noticeable that the role of surfactant in SLN systems varies depending on the lipid matrix used. SLN prepared with Geleol showed lower physical stability represented in higher percentage increase in particle size compared to Compritol 888 ATO and Precirol ATO 5. This could be explained by increased amount of partial glycerides like monoglycerides (40–55 % for Geleol, 15-23 % for Compritol 888 ATO and 8-22 % for Precirol ATO 5) which might be responsible for this physical destabilization. These results are in agreement with those reported by Jenning and Gohla (Jenning and Gohla, 2000).

### Conclusion

In the present work, MLX-loaded SLNs were successfully prepared by high shear homogenization and ultrasonication technique.. The various physicochemical properties, rheological properties and the in vitro release behavior, were greatly affected and can be controlled by optimizing the compositional variables represented in the concentration of surfactant and lipid as well as the type of lipid used. The sustained release behaviour of MLX loaded SLNs with favourable physicochemical characteristics can form a foundation for further clinical studies using these nanoparticles for the topical delivery of meloxicam.

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