Research Article

Chime S.A et al. /BioMedRx 2013,1(3), 227-234

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Diclofenac potassium–loaded dika fat solid lipid microparticles: *In vitro* and *in vivo* characterisation

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Received on:17-10-2012; Revised on: 19-11-2012; Accepted on:10-01-2013

ABSTRACT

The aim of the work was to formulate diclofenac potassium-loaded SLMs and evaluate the *in vitro* and *in vivo* properties of the solid lipid microparticles (SLMs). Diclofenac potassium-loaded SLMs were prepared by hot homogenization technique using admixtures of Phospholipon® 90G and dika fat (1:1, 1:2 and 2:1 %w/w) as the lipid matrix. Characterisation based on particle size, particle morphology, pH, drug content and encapsulation efficiency were carried out on the lipospheres. *In vitro* release was carried out in simulated intestinal fluid (SIF) without enzymes (pH 7.4) using the USP paddle method. Anti-inflammatory and ulcerogenic properties of diclofenac potassium-loaded SLMs were studied. From the results, the photomicrographs revealed spherical particles that ranged from 1.82 – 2.90 µm. SLMs formulated with lipid matrix 2:1 and containing 0.25 % diclofenac potassium had the highest encapsulation of 72 % and was significantly different from the other batches ($p < 0.05$). The *in vitro* release showed that SLMs having higher ratios of phospholipid (LM 2:1) i.e. batches C1 to C3 had more prolonged drug release rate with maximum drug release at 100 min, while SLMs formulated with lipid matrix having equal concentration of phospholipid and dika fat (A1 – A3) or having higher amount of dika fat (B1 – B3) exhibited faster release of drug with maximum drug release at 90 min. Diclofenac potassium SLMs prepared exhibited good anti-inflammatory properties and also inhibited the ulcerogenic potentials of diclofenac potassium. Therefore, diclofenac potassium-loaded SLMs exhibited good *in vitro* and *in vivo* properties.

Key words: Dika wax, solid lipid microparticles, diclofenac potassium, phospholipid, ulcerogenicity

INTRODUCTION

Recently, it has become more evident that the development of new drugs alone was not sufficient to ensure progress in drug therapy. Exciting experimental data obtained *in vitro* were very often followed by disappointing results *in vivo* because of the insufficient drug concentration due to poor absorption, rapid metabolism and elimination, poor water solubility and high fluctuation of plasma levels due to unpredictable bioavailability after oral administration. A promising strategy to overcome these problems involves the development of suitable drug carrier systems [1]. Solid lipid microparticles (SLM) are micro-scale drug carriers possessing matrix made from fatty acid, glyceride, fatty alcohol, and solid wax with high melting points [2]. SLM are complex multiphase systems and their properties are greatly influenced by their microstructures. For example, the solubility of a drug in an excipient affects the drug loading capacity and release performance [3]. There are several studies concerning the effects of production conditions, carrier materials and stabilizers on the morphological characterization, drug loading capacity, long-term stability as well as release performance of SLM [2].

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SLM combine many advantages of drug carrier systems. The amount of drug encapsulated can vary up to 80% for lipophilic compounds and they are well tolerated in living systems because they are made from physiological or physiologically related materials. The solid matrix protects loaded labile substance against degradation and they offer the possibility of controlled drug release and drug targeting. The suitability of lipid particles as a prolonged release formulation for lipophilic drugs has been demonstrated [4]. Compared to the polymer microparticles, SLM have the advantage of better biocompatibility which minimizes the hazards of acute and chronic toxicity. Besides, as SLM have solid cores, the mobility of incorporated drug and drug leakage from the carriers are reduced. Therefore, it is believed that SLM combine the advantages of many colloidal carriers and also overcome some of their disadvantages [2]. SLM can be produced on a large industrial scale and allow the control of drug release. SLM appear promising as drug carrier systems for topical applications. Occlusion properties as a result of film formation on the skin which can enhance the penetration of drugs through the stratum corneum have been reported for SLM [5]. SLM have been investigated as a taste–masking approach for a lipophilic weak base in suspension [6].

Dika fat is an edible vegetable fat derived from the kernel of *Irvingia gabonensis* Var excelcia [7]. Dika fat has been evaluated as basis for drug delivery [8-9]. Lipid based formulations have been shown to en-

hance the bioavailability drugs administered orally [10-13]. The widening availability of lipidic excipients with specific characteristics offer flexibility of application with respect to improving the bioavailability of sparingly soluble drugs and manipulating their release profile [14].

The mechanisms through which NSAIDs produce damage in the stomach can be subdivided into local (topical) actions and systemic actions [15]. The topical actions of NSAIDs on the gastric epithelium may involve several mechanisms. Some NSAIDs, particularly those of acidic nature, can directly kill epithelial cells. Various mechanisms have been proposed for this cytotoxic action, including the induction of osmotic lysis subsequent to trapping of charged NSAIDs with the epithelial cells, and death of the epithelial cell subsequent to uncoupling of oxidative phosphorylation. NSAIDs can also reduce mucus and bicarbonate secretion, thereby decreasing the effectiveness of the juxtamucosal pH gradient in protecting the epithelium. NSAIDs can also disrupt the layer of surface-active phospholipids on the mucosal surface, independent of effects on prostaglandin synthesis. Such an action would render the mucosa less able to resist damage induced by luminal acid [15].

It has been shown that the gastric mucosal barrier prevents self digestion by pepsin and acid secretion by several components. These are mucin, bicarbonate secretion, mucosal blood flow and the cytoprotectivity of gastric mucosa [16]. It is generally accepted that back diffusion of hydrogen ions is prevented by these components. Phospholipid content of gastric mucosa has been proposed as another component of the barrier [16]. Gastric phospholipids forming a hydrophobic mucosal surface between luminal secretions and the epithelium may repel H^+ and keep the surface of the epithelium dry by preventing the contact of luminal acid. Indeed the repelling of H^+ from the gastric epithelium is an important hypothesis to explain the protective mechanism of the mucosal phospholipid component of the gastric barrier [16].

Attempts have been made to produce NSAIDs with reduced topical irritant effects. These include formulations in slow-release or enteric coated tablets, as well as the preparation of the drug as a pro-drug that requires hepatic metabolism in order to be active [15]. Efforts have recently been made to develop gastrointestinally safe NSAIDs on the basis of a reduced ability to interfere with the surface-active phospholipid layer in the gastrointestinal mucus. Lichtenberger *et al* [17] proposed that pre-associating NSAIDs with zwitterionic phospholipids prior to their administration should reduce the ability of the NSAIDs to associate the phospholipids in the mucus gel, and should therefore reduce their ulcerogenicity. They demonstrated this to be the case by pre-associating aspirin and other NSAIDs with dipalmitoylphosphatidylcholine (DPPC) and demonstrating that the complex produced significantly less damage in the gastrointestinal tract than did the parent drug. Importantly, the pre-association of aspirin with DPPC did not interfere with the effectiveness of the aspirin to reduce fever or inflammation [15].

Diclofenac potassium is a non-steroidal anti-inflammatory drug (NSAID) with prominent anti-inflammatory, analgesic and antipyretic properties, but just like other NSAIDS, it causes severe gastric ulceration. Commercial formulations of diclofenac potassium have not been able to protect the gastric mucosa from erosion caused from diclofenac potassium. This study aimed at evaluating the *in vitro* and *in vivo* properties of diclofenac potassium-loaded SLMs formulated with dika fat and phospholipid (Phospholipon® 90G). The effect of the formulation on the gastrointestinal tract was studied in order to determine their gastro-protective potentials.

MATERIALS AND METHODS

The following materials were used as procured from their local suppliers without further purification: n–hexane, ethylacetate (Sigma–Aldrich, Germany), hydrochloric acid, sodium hydroxide, monobasic potassium phosphate, Tween 80 (Merck, Germany), diclofenac potassium (Healthy Life Pharma, India), Phospholipon® 90G (Phospholipid GmbH, Köln, Germany), activated Charcoal (Bio–Lab. (UK) Limited, London). Sorbitol (Wharfedale Laboratories, England), distilled water (Lion Water, Nsukka, Nigeria). Dika fat was obtained from a batch processed in our laboratory. All other reagents and solvents were analytical grade and were used as supplied.

Extraction and purification of dika fat from *Irvingia gabonensis*

Dika fat was extracted by soxhlet extraction from *Irvingia gabonensis* [18]. *Irvingia gabonensis* was milled in an equipment of the hammer mill type; the dika fat was extracted in a soxhlet using n–hexane. The n-hexane was allowed to completely evaporate at room temperature. Boiled distilled water which was twice the volume of the fat was poured into the molten fat in order to dissolve the hydrophilic gum contained in the fat. The hydrophilic gum was removed using a separating funnel. Ethyl acetate was equally poured into the molten fat in order to remove the hydrophobic gum from the fat. The extracted fat was further purified by passing it through a column of activated charcoal and bentonite (2:1) at 100 °C at a ratio of 10 g of fat and 1g of the column material. The fat was stored in a refrigerator until used [14].

Preparation of lipid matrix

Mixtures of (1:1, 1:2 and 2:1 w/w) Phospholipon® 90G, a purified lecithin and dika fat were melted and stirred at a temperature of 70 °C using a magnetic stirrer, until a homogenous, transparent yellow melt was obtained. The homogenous mixture was stirred at room temperature for solidification [19-20].

Preparation of SLMs

Appropriate quantities of lipid matrix, Tween 80, Sorbitol, diclofenac potassium and distilled water as presented in Table 1, were used for the formulation. Diclofenac potassium-loaded SLMs were prepared using 1:1, 1:2 and 2:1 (w/w) of the lipid matrix by hot homogenization techniques using Ultra-Turrax® (T25 Basic Digital, Germany). In each case 5 g of the lipid matrix was melted at 70 °C in a crucible and an appropriate amount of drug was incorporated into the lipidic melt. Sorbitol was dissolved in hot distilled water at the same temperature together with Tween 80. The aqueous phase at 70 °C was poured into the lipidic melt under high shear homogenization at 5000 rpm for 5 min. An o/w emulsion was finally formed by phase inversion [21].

EVALUATION OF THE SLMS

Determination of particle size and morphology of SLMs

Small amount of SLMs were placed on a microscope slide, the slide was covered with a cover slip and imaged under a Hund® binocular microscope (Weltzlar, Germany), attached with a motic image analyzer (Moticam, China) at a magnification of x 100. Different particles of the SLMs from each batch were counted (n=100), and the mean value taken.

Drug Content of SLMs

Beer's plot was obtained at the concentration range of 0.2 - 1.0 mg % for diclofenac potassium in simulated intestinal fluid (SIF, pH 7.4). Each batch of the SLMs was centrifuged at 5000 rpm for 10 min; the sediment was used in the analysis of drug content. A 0.5 g of the SLMs (each containing 0 %, 0.25 %, 0.5 % and 0.75 % of diclofenac potassium) was triturated using mortar and pestle with 10 ml of SIF and the solution was placed in a 100 ml volumetric flask. The flask was made up to volume and the solution filtered through a filter paper (Whatman No.1) and analyzed spectrophotometrically at predetermined wavelength of 303 nm (Jenway 6305, Borloworld Scientific Ltd.). This was repeated five times for all the batches. The drug concentrations were calculated with reference to Beer's plot.

Table 1: Quantities of material used for SLMs formulation

Batch	LM ratio	Tween 80 (ml)	LM (%)	Sorbitol (%)	Diclofenac potassium (%)	Distilled water q.s (% w/w)
A1	1:1	2.5	5.0	4	0.25	100
A2	1:1	2.5	5.0	4	0.50	100
A3	1:1	2.5	5.0	4	0.75	100
B1	1:2	2.5	5.0	4	0.25	100
B2	1:2	2.5	5.0	4	0.50	100
B3	1:2	2.5	5.0	4	0.75	100
C1	2:1	2.5	5.0	4	0.25	100
C2	2:1	2.5	5.0	4	0.50	100
C3	2:1	2.5	5.0	4	0.75	100
A0	1:1	2.5	5.0	4	0	100
B0	1:2	2.5	5.0	4	0	100
C0	2:1	2.5	5.0	4	0	100

LM: lipid matrix, A0 – A3: contain LM 1:1, B0- B3: contain LM 1:2, C0 – C3: contain LM 2:1 and A0, B0 and C0: contain no drug.

Drug encapsulation efficiency

The quantities of the drug theoretically contained in the SLMs were compared with the quantity actually gotten from the drug content studies. This was calculated using the equation below:

$$\text{Encapsulation efficiency (EE \%)} = \frac{ADC}{TDC} \times 100 \qquad (1)$$

where ADC is the actual drug content and TDC is the theoretical drug content.

pH analysis

The pH of the SLMs were determined in time dependent manner (24 h, 1 week, 2 months and 3 months) using pH meter (Suntex TS – 2, Taiwan).

Release studies of SLMs

The USP paddle method was adopted in this study. The dissolution medium consisted of 900 ml of freshly prepared medium (SIF, pH 7.4) maintained at 37 ± 1 °C The polycarbonate dialysis membrane MWCO 5000 (Spectrum labs, Brenda, Netherlands) selected was pretreated by soaking in the dissolution medium for 24 h prior to use. A quantity of SLM equivalent to 0.025 g diclofenac potassium was weighed from each batch and placed in a polycarbonate dialysis membrane containing 2 ml of the dissolution medium, securely tied with a thermo–resistant thread and placed in the appropriate chamber of the release apparatus. The paddle was rotated at 100 rpm, and at predetermined timed intervals, 5 ml portions of the dissolution medium was withdrawn, appropriately diluted, and analyzed for drug content in a spectrophotometer. The volume of the dissolution medium was kept constant by replacing it with 5 ml of fresh medium after each withdrawal to maintain sink condition. The amount of drug released at each time interval was determined with reference to Beer's plot.

Anti-inflammatory studies

The anti–inflammatory activity of the SLMs formulated was carried out using the rat paw oedema test [22]. All animal experimental protocols were carried out in accordance with guidelines of the Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. The philogistic agent employed in the study was fresh undiluted egg albumin [23]. Adult Wistar rats of either sex (150 – 200 g) were divided into five experimental groups of five rats per group. The animals were fasted and deprived of water for 12 h before the experiment. The deprivation of water was to ensure uniform hydration and to minimize variability in oedematous response [24]. The diclofenac potassium–loaded SLMs equivalent to 5 mg/kg body weight was administered orally to the rats. The reference group received 5 mg/kg of pure sample of diclofenac potassium, while the control group received normal saline. Thirty minutes post treatment; oedema was induced by injection of 0.1 ml fresh undiluted egg–albumin into the sub plantar region of the right hind paw of the rats [25]. The volumes of distilled water displaced by treated right hind paw of the rats were measured using plethysmometer before and at 30 min, 1, 2, 3, 4, 5 and 6 h after egg albumin injection.

The percent inhibition of oedema was calculated using the relationship [26],

$$\% \text{ inhibition of oedema} = \frac{Vo - Vt}{Vo} \times 100 \qquad (2)$$

where, Vt is the volume of oedema at corresponding time and Vo is the volume of oedema in control rats at the same time [23-27].

Ulcerogenicity of SLMS

The ulcerogenicity of SLMs formulated was determined using a method described by Chung-Chin *et al* [28]. The studies were carried

out on healthy Wistar rats (150 – 210 g). The animals were divided into five experimental groups of five animals per group. The control group received normal saline while the reference group received 5 mg/kg pure sample of diclofenac potassium orally. The animals were fasted for 8 hours prior to a single dose of either the control or the test compounds, given free access to food and water, and sacrificed 8 hours later. The gastric mucosae of the rats were examined under a microscope using a 4 x binocular magnifier. The lesions were counted. The mean score of each treated group minus the mean score of the control group was considered as severity index of gastric damage.

Statistical analysis

Statistical analysis was done using SPSS version 14.0 (SPSS Inc. Chicago, IL.USA). All values are expressed as mean ± SD. Data were analysed by one-way ANOVA. Differences between means were assessed by a two-tailed student's T-test. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Particle size analysis and morphology

Figure 1 shows the photomicrographs of the representative batches of diclofenac potassium-loaded SLMs; A1 was formulated with LM 1:1, B1 contain LM 2:1, while C1 was formulated with LM 2:1 (phospholipid:dika fat). A1, B1 and C1 contain 0.25 % diclofenac potassium. From the photomicrogaphs, it could be seen that the SLMs were spherical in shape. Particle size may be a function of either one or more of the following: formulation excipients, degree of homogenisation, homogenisation pressure, rate of particle size growth, crystalline habit of the particle etc. [19]. From the results of particle size presented in Table 2, it was seen that the particle size of diclofenac potassium SLMs increased with increase in encapsulation efficiency, except for batches B1 – B3 containing higher amount of dika fat, whose particle size did not increase with increase in encapsulation efficiency. However, batches B1 – B3 formulated with LM 1:2 (phospholipid: dika fat) generally showed higher particle size values than other formulations. This may be due to increase in entrapement of diclofenac potassium in the hydrophobic fat core of the system when the ratio of dika fat was increased. The particle size however, varied significantly ($p < 0.05$) within the sub-batches and across the batches. The amount of drug loaded into each formulation affected the size of the SLMs, optimum particle size and higher encapsulation efficiency was obtained in SLMs batches containing 0.25 % diclofenac potassium. Increasing the ratio of drug loaded above 0.25 % caused a decrease in both the particle size and encapsulation efficiency as shown in Table 2. This may be due to saturation of the lipid matrix at increased drug loading.

Table 2*:* Properties of diclofenac potassium-loaded lipospheres

Batch	LM	pH			Particle size (μm ± SD)[a]	ADC(% ± SD)[b]	TD(%)	EE (%)
		24 h	1 week	1 month				
A0	1:1	6.34 ± 0.21	6.10 ± 0.27	5.98 ± 0.13	0.47 ± 0.04	0.00	0.00	-
A1	1:1	7.55 ± 0.20	7.00 ± 0.09	7.03 ± 0.11	2.75 ± 1.22	0.17 ± 0.12	0.25	66.0
A2	1:1	7.95 ± 0.13	7.45 ± 0.21	7.00 ± 0.10	1.90 ± 2.11	0.31 ± 0.24	0.50	62.0
A3	1:1	8.21 ± 0.27	8.00 ± 0.17	7.91 ± 0.15	1.82 ± 2.00	0.37 ± 0.179	0.75	49.0
B0	1:2	6.11 ± 0.19	6.07 ± 0.11	6.00 ± 0.25	0.50 ± 0.03	0.00	0.00	-
B1	1:2	7.63 ± 0.07	7.21 ± 0.31	8.21 ± 0.12	2.27 ± 1.14	0.13 ± 0. 23	0.25	52.0
B2	1:2	8.15 ± 0.13	7.98 ± 0.23	8.30 ± 0.10	2.56 ± 1.27	0.29 ± 0.17	0.50	58.0
B3	1:2	8.31 ± 0.19	8.00 ± 0.24	7.53 ± 0.15	2.32 ± 2.10	0.32 ± 0.23	0.75	43.0
C0	2:1	6.12 ± 0.17	6.08 ± 0.20	5.97 ± 0.17	0.51 ± 0.07	0.00	0.00	-
C1	2:1	7.64 ± 0.09	7.51 ± 0.24	6.53 ± 0.29	2.90 ± 2.10	0.18 ± 0.17	0.25	72.0
C2	2:1	8.01 ± 0.12	7.90 ± 0.11	7.54 ± 0.19	1.89 ± 2.23	0.32 ± 0.13	0.50	64.0
C3	2:1	8.14 ± 0.14	8.25 ± 0.17	7.22 ± 0.23	2.15 ± 1.27	0.39 ± 0.27	0.75	52.0

[a]n = 100, SD = Standard deviation, [b]n = 5, A0 _ A3: contain LM 1:1, B0- B3: contain LM 1:2, C0 _ C3: contain LM 2:1 and A0, B0 and C0: contain no drug; ADC: actual drug content; TDC: theoretical drug content.

pH analysis of SLMs

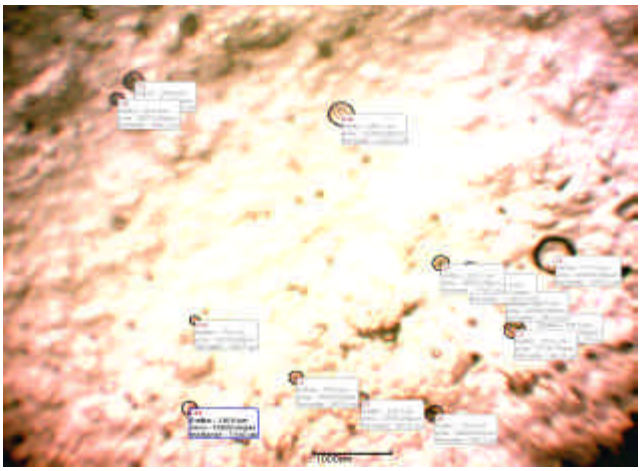
Table 2 shows the pH values of all the batches of diclofenac potassium-loaded and unloaded SLMs formulated. pH change could be a function of degradation of the API or excipients. A prior stable API may be affected by degradation of excipients with storage through generation of unfavorable pH (increase or decrease) or reactive species for the API [19]. Table 2 showed that there was a slight decrease in pH from 24 h to 1 month. For batch A1 containing 0.25 % diclofenac potassium, the pH showed slight change from between 7.55 ± 0.20 at 24 h to between 7.03 ± 0.11 at 1 month of preparation. The pH change in the diclofenac potassium-loaded SLMs was not due to degradation of the drug since there was also a fall in pH of the unloaded SLMs. Degradation of the free fatty acids may be implicated in the fall of pH [19].

Drug content

The active ingredient contents varied from that loaded into the SLMs. This may be due to the solubility of the drug in the lipid**.** The drug content was significantly ($p < 0.05$) affected by the amount of drug loaded into the SLMs and the ratio of two lipids used in formulating the lipid matrix. Increasing the amount of diclofenac potassium loaded into the SLMs decreased the actual drug content of the SLMs, this may be due saturation of the LM when the amount of drug was increased. SLMs formulated with lipid matrix having higher ratio of phospholipid (batches C1 – C3) had higher drug content than other batches. This may be due to the increase in the stability of the SLMs with increased phospholipid concentration leading to higher degree of structural modification at the surface of the microparticle. The low standard deviation of the drug content attests to the reproducibility and reliability of the formulation.

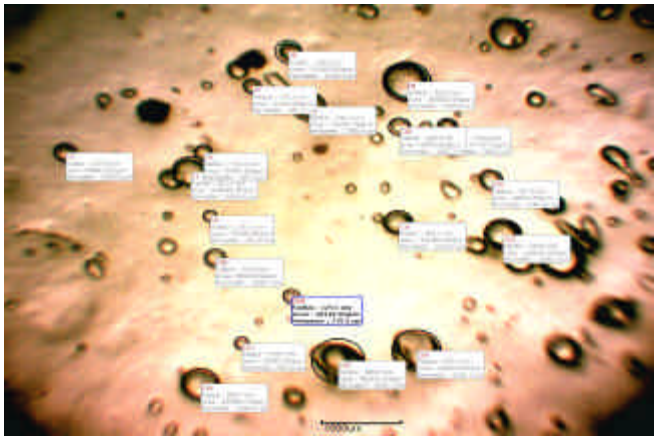
Encapsulation efficiency (EE%)

Table 2 shows the EE% of the various formulations of diclofenac potassium-loaded SLMs. The varied EE% may be as a result of API and matrix physicochemical and material related factors [19]. EE% ranged from 43 % for batch B3 SLMs to 72 % for batch C1 SLMs. Generally, the EE% was affected by the lipid composition/ratio used in formulating the SLMs. Diclofenac potassium-loaded SLMs formulated with Lipid matrix (2:1) i.e. batches C1 – C3 with lower ratio of dika fat showed higher EE% values significantly different ($p < 0.05$) from other batches with higher ratios of dika fat as shown in Table 2. The reason may be due to higher degrees of structural modifications and changes in microstructure of the SLMs. EE% of diclofenac potassium-loaded SLMs was also affected by the total amount of API in the dispersion. Diclofenac potassium-loaded SLMs containing 0.25 % of drug had the highest encapsulation efficiency of 66 % to 72 % for batches A1and C1 SLMs respectively. However, batch B1 had low EE% of 52 %. EE% depends on several parameters, such as the lipophilic properties of the API, screening of the most appropriate lipid composition/ratio and surfactant combination, as well as the production procedure used [19].



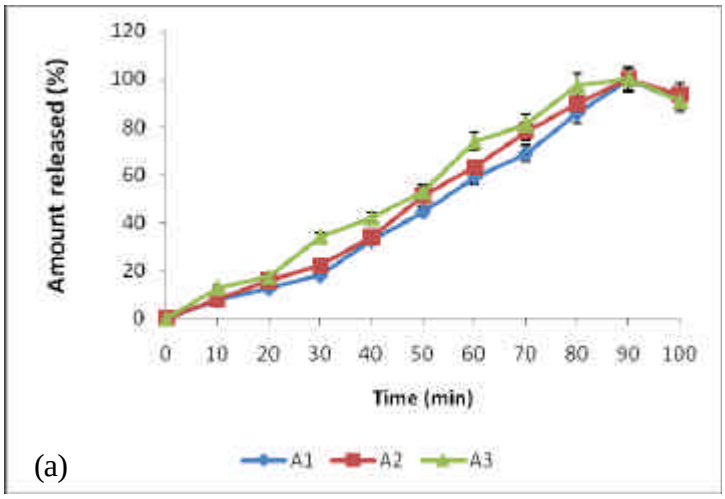
A1

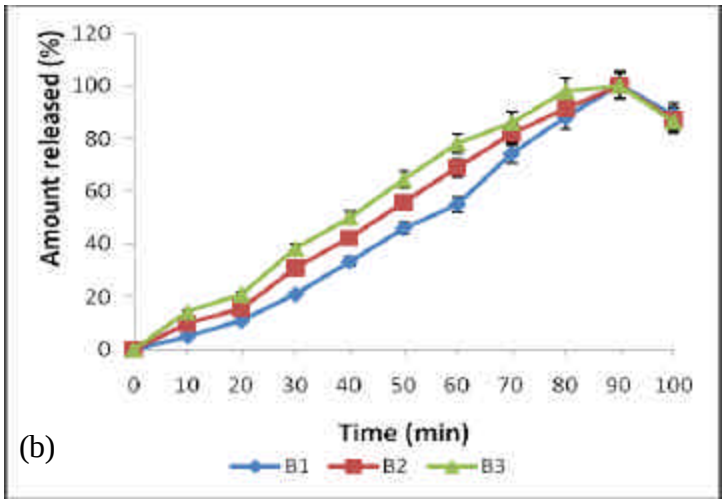
B1



C1

Fig. 1: Photomicrographs of diclofenac potassium-loaded lipospheres formulated with 1:1, 1:2 and 2:1 %w/w of lipid matrix (Phospholipid: dika wax) and containing 0.25 % diclofenac potassium respectively.





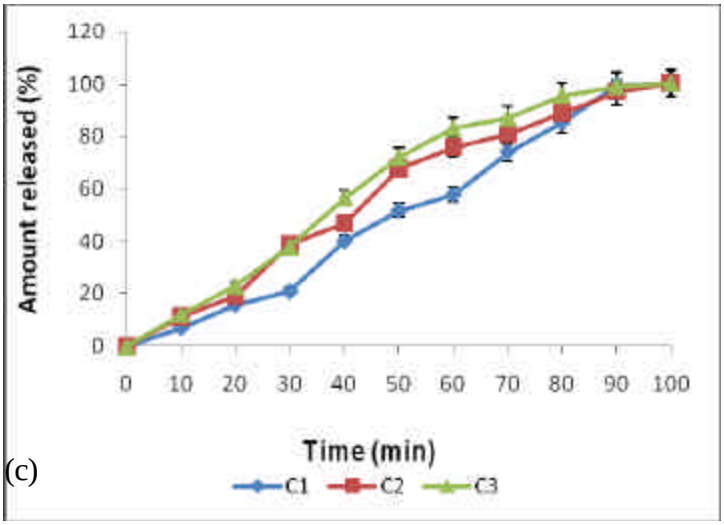


Fig. 2: Release profile of diclofenac potassium-loaded SLMs (a): formulated with LM 1:1 and containing 0.25 %, 0.5 % and 0.75 % diclofenac potassium respectively (A1, A2, A3); (b): formulated with LM 1:2 and containing 0.25 %, 0.5 % and 0.75 % diclofenac potassium respectively (B1, B2, B3); (c): formulated with LM 2:1 and containing 0.25 %, 0.5 % and 0.75 % diclofenac potassium respectively (C1, C2 and C3).

Drug release from diclofenac potassium-loaded lipospheres

The drug release profile presented in Fig. 2 showed that diclofenac potassium-loaded SLMs exhibited good release of drug from SLMs. However, the rate of drug release was affected by the ratio/combination of the lipids used in preparation of the lipid matrix. Diclofenac potassium-loaded SLMs formulated with higher ratios of phospholipid (LM 2:1) i.e. batches C1 to C3 showed maximum drug release (T100) at 100 min. This may be due to enhanced physical stability caused by an increase in outer phospholipid coating of the SLMs. Diclofenac potassium-loaded SLMs formulated with lipid matrix having equal concentration of phospholipid and dika fat (A1 – A3) or having higher ratio of dika fat (B1 – B3) exhibited the faster release of drug with maximum drug release at 90 min. Therefore, SLMs containing diclofenac potassium exhibited reproducible drug release in all the batches, without any form of burst effect.

Table 3: Anti-inflammatory properties of diclofenac potassium-loaded SLMs

	Paw volume oedema (ml ± SD)[a] and percentage inhibition of oedema (%)					
Groups	**0.5 h**	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
A1	1.10 ± 0.12* (15.4)	1.15 ± 0.17* (25.2)	1.00 ± 0.11* (30.6)	0.90 ± 0.23* (37.9)	0.80 ± 0.17* (44.4)	0.75 ± 0.13* (47.2)
B1	1.23 ± 0.24 (5.7)	1.30 ± 0.32* (9.7)	1.10 ± 0.41* (23.6)	1.00 ± 0.37* (31.0)	0.90 ± 0.21* (37.5)	0.29 ± 0.21* (43.7)
C1	1.25 ± 0.11 (3.85)	1.30 ± 0.27* (9.7)	1.15 ± 0.26* (20.1)	1.10 ± 0.23* (24.1)	1.00 ± 0.11* (30.6)	0.80 ± 1.31* (43.7)
D (ref.)	1.00 ± 0.21* (23.0)	1.00 ± 0.13* (30.6)	0.90 ± 0.11* (37.5)	0.85 ± 0.23* (41.4)	0.81 ± 0.51* (43.8)	0.70 ± 0.39* (50.7)
E(Cont.)	1.30 ± 0.17	1.44 ± 0.19	1.44 ± 0.23	1.45 ± 0.34	1.44 ± 0.32	1.42 ± 0.14

***Significant at p < 0.05 compared to control. Values of oedema shown are mean ± SD ([a]n = 5). Values in parenthesis are percent inhibition of oedema; A1, B1 and C1: diclofenac potassium SLMs; D: pure sample of diclofenac potassium; E: normal saline.**

Anti-inflammatory properties

Table 3 shows the result of anti-inflammatory properties of diclofenac potassium-loaded SLMs formulated with varying ratios of lipid matrix and containing 0.25 % of diclofenac potassium. The SLMs containing diclofenac potassium exhibited good anti-inflammatory properties significantly different from the control ($p < 0.05$). Diclofenac potassium-loaded SLMs had 24.1 %, 31.0 % and 37.9 % oedema inhibition at 3 h, while the reference drug had 41.4 % oedema inhibition at 3 h. Also, diclofenac potassium-loaded SLMs had 43.7 % and 47.2 % oedema inhibition at 5 h, while the reference drug showed 50.7 % oedema inhibition at 5 h. Generally, diclofenac potassium SLMs prepared exhibited good anti-inflammatory properties significantly different ($p < 0.05$) from the reference drug-diclofenac potassium pure sample (5 mg/kg).

Ulcerogenic studies

The ulcerogenic results presented in Table 4 showed that diclofenac potassium-loaded SLMs inhibited the ulcerogenic potentials of the NSAID. There was absence of lesions on the gastric mucosa of the animals that received the SLMs containing diclofenac potassium, unlike the animals that received the reference drug-diclofenac potassium pure sample, which had many lesions on the gastric mucosa of the animals. Therefore, the SLM inhibited the ulcerogenic potentials of the highly ulcerogenic diclofenac potassium. This may be due to the presence of phospholipid in the formulations which may provide a gastro protective effect on the gastrointestinal tract.

Table 4: Ulcerogenic properties

Batch	Ulcer index (Mean ± SD)[a]
C1	0.00 ± 0.00
M (Reference)	12.00 ± 2.23*
N (Control)	0.00 ± 0.00

***Significant at $p < 0.05$ compared to control. [a]n = 5; C1: diclofenac potassium-loaded SLMs; M: indomethacin pure sample;N: normal saline.**

CONCLUSIONS

Results of this study indicated that diclofenac potassium-loaded SLMs formulated with higher ratios of phospholipid (LM 2:1) i.e. batches C1 to C3 showed maximum drug release (T100) at 100 min, while SLMs formulated with lipid matrix having equal concentration of phospholipid and dika fat (A1 – A3) or having higher ratio of dika fat (B1 – B3) exhibited faster release of drug with maximum drug release at 90 min. EE% ranged from 43 % for batch B3 SLMs to 72 % for batch C1 SLMs. Generally, the EE% was affected by the lipid composition/ratio used in formulating the SLMs. Diclofenac potassium-loaded SLMs formulated with Lipid matrix (2:1) i.e. batches C1 – C3 with lower ratio of dika fat showed higher EE% values significantly different ($p < 0.05$) from other batches with higher ratios of dika fat. Generally, diclofenac potassium SLMs exhibited good anti-inflammatory properties significantly different ($p < 0.05$) from the reference drug-diclofenac potassium pure sample. Also, the SLM containing diclofenac potassium inhibited the ulcerogenic potentials of the highly ulcerogenic NSAID.

ACKNOWLEDGEMENTS

We thank Phospholipid GmbH, Köln, Germany for providing samples of Phospholipon 90G.

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Source of support: Nil, Conflict of interest: None Declared