IN VITRO AND IN VIVO CHARACTERISATION OF INDOMETHACIN-LOADED DIKA FAT BASED SOLID LIPID MICROPARTICLES


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ABSTRACT

Indomethacin–loaded solid lipid microparticles (SLMs) were formulated with 10 %w/w of lipid matrix consisting of Phospholipon® 90G a purified lecithin, and dika fat from Irvingia gabonensis, in the ratios of 1:1, 1:2 and 2:1 w/w respectively, 4 %w/v sorbitol and 2.5 %w/v of Tween 80 and enough distilled water to yield 100 %w/w. The SLMs were formulated by hot homogenization at 70 ± 1°C using Ultra turrax® homogenizer. The SLMs formulated were evaluated in terms of particle size and morphology, drug content and encapsulation efficiency. In vitro and in vivo studies were also evaluated. The result of the study indicated that indomethacin-loaded SLMs exhibited good release of drug in simulated intestinal fluid (SIF, pH 7.5). The particle size of the SLMs varied with lipid matrix ratio and amount of drug incorporated. The higher the ratio of dika fat, the faster the drug release from SLMs. The SLMs prepared had high encapsulation efficiency of up to 94 %. Pharmacodynamic studies showed that the indomethacin loaded SLMs had good anti-inflammatory properties, and also inhibited the ulcerogenicity of indomethacin. From the results, the performance of the lipid matrix ratios (Phospholipon® 90G: dika fat) could be ranked thus: 2:1 > 1:1 > 1:2.

Keywords: Dika fat, solid lipid microparticles, ulcerogenicity, anti-inflammation, NSAIDS.

INTRODUCTION

Numerous polymer-based particulate carriers have widely been studied as drug carriers in the field of drug delivery system 1–3. The use of synthetic polymer matrix materials often goes along with detrimental effects on incorporated drug during manufacturing of formulations or during the degradation of the polymers after application 4. The degradation of polymer might possibly cause systemic toxic effects through the impairment of reticulo endothelia system (RES) or after phagocytosis of particles by human macrophages and granulocytes 5. Therefore, alternative carrier substances have been investigated. Among them, lipidic materials have gained growing attention 5.

Dika fat is an edible vegetable fat derived from the kernel of Irvingia gabonensis Var excelcia 6. Dika fat has been evaluated as basis for drug delivery 6–9. The widening availability of lipic excipients with specific characteristics offer flexibility of application with respect to improving the bioavailability of poorly water soluble drugs and manipulating their release profile 10. Lipid based formulations have been shown to enhance the bioavailability of drugs administered orally 11–14. The proven safety (biocompatibility) of lipid based carriers makes them attractive candidates for the formulation of pharmaceuticals. Lipid formulations generally provide increased drug solubilization for water in-soluble drugs. Drug suspended in lipid matrix has been shown, in most cases to have a better absorption than conventional solid dosage forms. This could be due to the ease of wettting of hydrophobic drug particles in the presence of lipid matrix. The presence of surfactant in the formulation may further promote the wetting 15. Furthermore, lipids are non adhesive and therefore, do not adhere to intestinal walls unlike most polymers and so present a good matrix for the formulation of non-steroidal anti-inflammatory drugs (NSAIDS).

Solid lipid microparticles (SLMs) attract increasing attention as alternative delivery systems 16. SLMs combine the advantages of different traditional carriers; for example, they can be produced on a large industrial scale and allow control of drug release 17.

The mechanisms through which NSAIDs produce damage in the stomach can be subdivided into local (topical) actions and systemic actions 18. 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 18.

NSAIDs induce injury/bleeding via three key pathways: inhibition of cyclooxygenase (COX)-1 activity, inhibition of COX-2 activity, and direct cytotoxic effects on the epithelium. Effects produced via only one of these
pathways (e.g., selective inhibition of COX-1 or of COX-2) are unlikely to produce significant damage. NSAIDs can also diminish the ability of epithelial growth factors (EGF) to promote epithelial repair. Thus inhibition of epithelial proliferation has been observed when the cells are exposed to NSAIDs, and this appears to involve a reduction of EGF binding to its receptor and inhibition of EGF signaling pathways. The most important of the systemic effects of NSAIDs, in terms of inducing gastric ulceration, is their ability to suppress prostaglandin synthesis. The gastric mucosa is normally protected by a range of mechanisms. Indomethacin and other non-selective NSAIDs disrupt the mucosal barrier, reduce bicarbonate production, directly damage the gastric mucosal epithelium and reduce the production of prostaglandins, thus impairing the repair processes. Once the initial defense mechanisms have been breached, gastric acid and pepsin augment the mucosal injury.

The study was carried out to evaluate the in vitro properties of indomethacin-loaded SLMs formulated with dika fat and lecithin; and also evaluate the anti-inflammatory and ulcerogenic properties of indomethacin-loaded SLMs formulated.

**MATERIALS AND METHODS**

The following materials were used as procured from their suppliers without further purification: n-hexane, ethylacetate (Sigma–Aldrich, Germany), hydrochloric acid, sodium hydroxide, monobasic potassium phosphate, Tween 80, indomethacin (Merck, Germany), Phospholipon 90G (Phospholipid GmbH, Köln, Germany), activated Charcoal (Bio–Lab. UK). 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***

*Irvingia gabonensis* were purchased from Nsukka market, Enugu State, Nigeria in the month of July, 2011. The seed material was authenticated by Mr. A.O. Ozioko, a consultant taxonomist with the International Center for Ethnomedicine and Drug Development (InterCEDD) Nsukka and the voucher specimen was deposited in the herbarium of the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka.

Dika fat was extracted by soxhlet extraction, *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 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.

**Preparation of lipid matrix**

Mixtures of Phospholipon 90G and dika fat (1:1, 1:2 and 2:1 w/w) 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 until solidification.

**Preparation of SLMs**

Appropriate quantities of lipid matrix, Tween 80, sorbitol, drug and distilled water as presented in Table 1, were used for the formulation. Indomethacin-loaded SLMs were prepared using the 1:1, 1:2 and 2:1 (w/w) of the lipid matrix by hot homogenization techniques using Ultra-turrax T25 Basic, Digital. In each case, 5 g of the lipid matrix was melted at 70°C in a crucible and an appropriate amount of indomethacin 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 with ultra-turrax at 5000 rpm for 5 min. An o/w emulsion was finally formed by phase inversion and was allowed to cool at room temperature to generate the SLMs.

| Table 1: Quantities of material used for SLMs formulation |
|----------------|----------------|----------------|----------------|----------------|----------------|
| Batch | Lipid matrix ratio | Tween 80 (ml) | Lipid matrix (g) | Sorbitol (g) | Indomethacin (g) | 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 |

A₁, A₂, C₁ contain LM 1:1, B₁, B₂, B₃ contain LM 1:2, C₂, C₃ contain LM 2:1 and A₀, B₀ and C₀ contain no drug.
Evaluation of the SLMs

Determination of particle size and morphology of SLMs

SLMs (2 drops) 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 400. 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 to 1.0 mg % for indomethacin in SIF. 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 (containing 0 %, 0.25 %, 0.5 % and 0.75 % of indomethacin) was triturated using mortar and pestle with 10 ml of SIF (pH 7.5) and the solution placed in a 100 ml volumetric flask. The flask was made up to volume; the solution was filtered through a filter paper (Whatman No.1) and analyzed spectrophotometrically at predetermined wavelength of 298 nm (Jenway 6305, Borloworld scientific Ltd. UK). This was repeated five times for all the batches. The drug concentrations were calculated with reference to Beer’s plot.

Drug encapsulation efficiency

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

\[
\text{Encapsulation efficiency (EE)} = \frac{\text{ADC}}{\text{TDC}} \times 100
\]

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 a 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 7.5) 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 indomethacin 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 chamber of the release apparatus. The paddle was rotated at 100 rpm, and at predetermined timed intervals, 5 ml portions of the dissolution medium were withdrawn, appropriately diluted, and analysed for drug content in a spectrophotometer (Jenway 6305, Borloworld scientific Ltd. UK). 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 test. This test was repeated three times for all the batches.

Anti-inflammatory studies

The anti-inflammatory activity of the indomethacin–loaded SLMs was carried out using the rat paw oedema test. 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 phlogistic agent employed in the study was fresh undiluted egg albumin. Adult Wistar rats of either sex (120 – 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. The indomethacin–loaded SLMs equivalent to 10 mg/kg body weight was administered orally to the rats. The reference group received 10 mg/kg of pure sample of indomethacin, while the control group received normal saline. Thirty minutes post treatment; oedema was induced by injection of 0.1ml fresh undiluted egg–albumin into the sub plantar region of the right hind paw of the rats. 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.

Average oedema at every interval was assessed in terms of difference in volume displacement (V₀ – Vₓ) 22. The percent inhibition of oedema was calculated using the relation:

\[
\% \text{Inhibition of oedema} = 1 - \left( \frac{a - x}{b - y} \right) \times 100
\]

where, a is the mean paw volume of treated rats after egg albumin injection, x is the mean paw volume of treated rats before egg albumin injection, b is the mean paw volume of control rats after egg albumin injection and y is the mean paw volume of control rats before egg albumin injection.

Ulcerogenicity of SLMs

The ulcerogenicity of SLMs formulated was determined using a method described by Chung-Chin et al. The studies were carried out on healthy Wistar rats (180 – 200 g). The animals were divided into five experimental groups of five animals per group. The control group received normal saline, the test group received indomethacin–loaded SLMs equivalent to 10 mg/kg pure sample of indomethacin, while the reference group received 10 mg/kg pure sample of indomethacin 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 objective 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 analysis of variance (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 and morphology**

From the photomicrographs presented in Fig 1, 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. 

Table 2 shows the particle sizes of all the batches of indomethacin SLMs formulated. The ratio of drug to lipid affected the size of the SLMs produced. Increase in the concentration of the loaded drug increased the particle size of the SLMs in all the batches. This result was in agreement with the work done by Barakat et al., who prepared carbamazepine–loaded precific lipospheres and found out that the particle size of lipospheres increased with increase in drug loading, and Joseph et al., who prepared piroxicam loaded polycarbonate microspheres.

Also the ratio of the two lipids used affected the size of the SLMs. From the result in Table 2, lipid matrix 1:2 had the largest particle size of 0.56 - 2.50 µm depending also on the amount of drug loaded. Generally, lipid matrix 1:1 and 2:1 had the lowest particle size respectively. Therefore, increasing the ratio of dika fat increased the particle size of the SLMs produced. This suggested that the encapsulated drug is located in the fat core of the lipospheres.

**pH analysis of SLMs**

The pH of different batches of indomethacin-loaded SLMs were measured in time dependent manner, 24 h, 1 week and 1 month after preparation to determine the change of pH with time. Table 2 shows the pH values of all the batches of indomethacin-loaded and unloaded SLMs formulated. The pH varied from between 4.03 ± 0.09 and 4.77 ± 0.12 within 24 h of preparation to between 2.67 ± 0.07 and 3.87 ± 0.05 within 1 month of preparation. 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. The 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.

The pH change in the indomethacin-loaded SLMs was not due to degradation of the drug since there was also a fall in pH from 24 h to 1 month. The pH change in the indomethacin-loaded SLMs was not due to degradation of the drug since there was also a fall in pH from 24 h to 1 month.

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![Figure 1: Photomicrographs of indomethacin-loaded SLMs formulated with LM 1:1 (phospholipid:dika wax) and containing 0.25 %, 0.5 %, and 0.75% (A, A2, A3). MAGNIFICATION X 400](image-url)

**Table 2:** Physicochemical properties of SLMs

<table>
<thead>
<tr>
<th>Batch</th>
<th>LM</th>
<th>pH 24 h</th>
<th>pH 1 week</th>
<th>pH 1 month</th>
<th>Particle size (µm ± SD)</th>
<th>ADC (%)</th>
<th>TDC (%)</th>
<th>EE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>1:1</td>
<td>4.51 ± 0.12</td>
<td>4.54 ± 0.09</td>
<td>3.47 ± 0.05</td>
<td>0.49 ± 0.05</td>
<td>0.17 ± 0.23</td>
<td>0.25</td>
<td>68.0</td>
</tr>
<tr>
<td>A2</td>
<td>1:1</td>
<td>4.36 ± 0.07</td>
<td>4.34 ± 0.02</td>
<td>2.78 ± 0.07</td>
<td>0.53 ± 0.15</td>
<td>0.43 ± 0.29</td>
<td>0.50</td>
<td>86.0</td>
</tr>
<tr>
<td>A3</td>
<td>1:1</td>
<td>4.63 ± 0.11</td>
<td>4.63 ± 0.08</td>
<td>3.79 ± 0.02</td>
<td>0.55 ± 0.10</td>
<td>0.61 ± 0.55</td>
<td>0.75</td>
<td>81.3</td>
</tr>
<tr>
<td>B1</td>
<td>2:1</td>
<td>4.03 ± 0.09</td>
<td>4.05 ± 0.10</td>
<td>3.87 ± 0.05</td>
<td>0.56 ± 0.08</td>
<td>0.15 ± 0.37</td>
<td>0.25</td>
<td>60.0</td>
</tr>
<tr>
<td>B2</td>
<td>2:1</td>
<td>4.31 ± 0.12</td>
<td>4.35 ± 0.14</td>
<td>2.67 ± 0.07</td>
<td>1.70 ± 0.05</td>
<td>0.39 ± 0.42</td>
<td>0.50</td>
<td>78.0</td>
</tr>
<tr>
<td>B3</td>
<td>2:1</td>
<td>4.75 ± 0.01</td>
<td>4.77 ± 0.17</td>
<td>2.97 ± 0.07</td>
<td>2.50 ± 0.14</td>
<td>0.54 ± 0.20</td>
<td>0.75</td>
<td>72.0</td>
</tr>
<tr>
<td>C1</td>
<td>2:1</td>
<td>4.13 ± 0.17</td>
<td>4.11 ± 0.20</td>
<td>3.79 ± 0.11</td>
<td>0.50 ± 0.07</td>
<td>0.21 ± 0.25</td>
<td>0.25</td>
<td>84.0</td>
</tr>
<tr>
<td>C2</td>
<td>2:1</td>
<td>4.47 ± 0.05</td>
<td>4.42 ± 0.13</td>
<td>3.86 ± 0.06</td>
<td>0.54 ± 0.12</td>
<td>0.47 ± 0.17</td>
<td>0.50</td>
<td>94.0</td>
</tr>
<tr>
<td>C3</td>
<td>2:1</td>
<td>4.77 ± 0.12</td>
<td>4.57 ± 0.15</td>
<td>3.24 ± 0.02</td>
<td>0.55 ± 0.011</td>
<td>0.69 ± 0.73</td>
<td>0.75</td>
<td>92.0</td>
</tr>
<tr>
<td>A0</td>
<td>1:1</td>
<td>6.34 ± 0.05</td>
<td>6.10 ± 0.07</td>
<td>5.98 ± 0.03</td>
<td>0.47 ± 0.04</td>
<td>0.00</td>
<td>0.00</td>
<td>-</td>
</tr>
<tr>
<td>B0</td>
<td>1:2</td>
<td>6.11 ± 0.09</td>
<td>6.07 ± 0.11</td>
<td>6.00 ± 0.05</td>
<td>0.50 ± 0.03</td>
<td>0.00</td>
<td>0.00</td>
<td>-</td>
</tr>
<tr>
<td>C0</td>
<td>2:1</td>
<td>6.12 ± 0.17</td>
<td>6.08 ± 0.20</td>
<td>5.97 ± 0.07</td>
<td>0.51 ± 0.07</td>
<td>0.00</td>
<td>0.00</td>
<td>-</td>
</tr>
</tbody>
</table>

(\(n = 100\). SD = Standard deviation, \(n = 5\)). A, A2, A3 contain LM 1:1, B, B2 contain LM 1:2, C, C2 contain LM 2:1 and A0, B0 and C0 contain no drug. TDC – Theoretical drug concentration, ADC – Actual drug concentration.
Drug content

The drug content varied significantly with the particle size of the SLMs and the ratio of the lipid matrix used in the formulation respectively (p < 0.05). From the values in Table 2, indomethacin SLMs prepared with lipid matrix 2:1 showed no significant variation from the TDC. Also amount of indomethacin loaded affected the ADC; lipid matrix 2:1 showed good release of drug with maximum release at 150 min. The inclusion of Tween 80 in the formulation may improve the in vivo bioavailability of this drug by enhancing the rate and/or extent of drug solubilization in the aqueous intestinal fluids.

Drug encapsulation efficiency (EE %)

From the values in Table 2, it can be seen that encapsulation efficiency of the SLMs loaded with 0.5% of indomethacin were higher than the lipid matrix loaded with 0.25 % and 0.75 % of drug. Also lipid matrix 2:1 having higher ratio of phospholipid to dika fat had the highest encapsulation efficiency for all concentrations of the drug. Therefore, the amount of drug loaded and the ratio of LM affected the encapsulation efficiency. However, all the batches showed high encapsulation efficiency which may be due to the lipophilicity of indomethacin.

Release studies of SLMs

The result of the release studies presented in Figs. 2 – 4 showed that SLMs prepared with lipid matrix 1:2 showed the fastest release with T50 of 60 min for B3 and B2 containing 0.25 % and 0.5 % indomethacin respectively, while B2 containing 0.75 % of indomethacin gave T50 of 90 min. This was followed by SLMs prepared with lipid matrix 1:1 which had maximum release at 90 min for batches A1 and A2 loaded with 0.25 % and 0.5 % of indomethacin respectively and 120 min for batch A3 containing 0.75 % of indomethacin. Indomethacin-loaded SLMs prepared with 2:1 ratio of the lipid matrix had a gradual but, steady release of the incorporated drug with maximum release at 150 min for batches C1 and C2 loaded with 0.25 % and 0.75 % of indomethacin respectively and 120 min for batch C3 containing 0.5 % of indomethacin. However the high amount of drug released seen in SLM loaded with 0.75 % indomethacin could be due to peripheral encapsulation of indomethacin by the lipospheres resulting from high drug concentration. Considering the fact that indomethacin is a non-steroidal anti-inflammatory drug with high gastric irritation tendency, the indomethacin-loaded SLMs formulated with lipid matrix ratio 2:1 showed good release of drug with maximum release at 150 min. The inclusion of Tween 80 in the formulation may improve the in vivo bioavailability of this drug by enhancing the rate and/or extent of drug solubilization in the aqueous intestinal fluids.

Table 3: Anti-inflammatory properties of indomethacin-loaded SLMs

<table>
<thead>
<tr>
<th>Groups</th>
<th>0.5 h</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>5 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2</td>
<td>1.71 ± 0.17* (22.7)</td>
<td>1.74 ± 0.50* (27.1)</td>
<td>1.76 ± 0.12* (30.7)</td>
<td>1.38 ± 0.55* (55.7)</td>
<td>1.27 ± 0.71* (60.0)</td>
<td>1.04 ± 0.14* (72.0)</td>
<td>1.00 ± 0.62* (77.0)</td>
</tr>
<tr>
<td>B2</td>
<td>1.70 ± 0.51 (25.8)</td>
<td>1.75 ± 0.80* (28.6)</td>
<td>1.72 ± 0.31* (35.3)</td>
<td>1.39 ± 0.51* (57.0)</td>
<td>1.18 ± 0.42* (66.0)</td>
<td>1.07 ± 0.90* (75.0)</td>
<td>1.02 ± 0.75* (78.0)</td>
</tr>
<tr>
<td>C2</td>
<td>1.20 ± 0.92* (37.5)</td>
<td>1.23 ± 0.71* (40.7)</td>
<td>1.26 ± 0.53* (42.7)</td>
<td>0.98 ± 0.85* (61.3)</td>
<td>0.92 ± 0.45* (62.3)</td>
<td>0.78 ± 1.10* (70.3)</td>
<td>0.72 ± 0.95* (74.6)</td>
</tr>
<tr>
<td>D1 (ref.)</td>
<td>1.60 ± 0.83 (29.7)</td>
<td>1.65 ± 0.72* (32.1)</td>
<td>1.60 ± 0.90* (40.0)</td>
<td>1.50 ± 0.55* (46.3)</td>
<td>1.20 ± 0.72* (63.8)</td>
<td>1.15 ± 0.80* (64.8)</td>
<td>1.00 ± 0.52* (77.0)</td>
</tr>
<tr>
<td>E1(Cont.)</td>
<td>2.0 ± 0.51</td>
<td>2.12 ± 0.50</td>
<td>2.22 ± 0.71</td>
<td>2.21 ± 0.82</td>
<td>2.10 ± 0.70</td>
<td>2.00 ± 0.92</td>
<td>1.98 ± 0.27</td>
</tr>
</tbody>
</table>

*Significant at p < 0.05 compared to control. Values of oedema shown are mean ± SD (n = 5). Values in parenthesis are percent inhibition of oedema, A2 – C2: indomethacin-loaded SLMs, D1: pure indomethacin, E1: normal saline.

Figure 2: Release profile of indomethacin from SLMs formulated with lipid matrix 1:1, loaded with 0.25, 0.5 and 0.75% drug (A1, A2 and A3) respectively and reference, pure sample of indomethacin (D).

Figure 3: Release profile of indomethacin from SLMs formulated with lipid matrix 1:2, loaded with 0.25, 0.5 and 0.75% drug (B1, B2 and B3) respectively and reference, pure sample of indomethacin (D).
properties with up to triazone releasing from –
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Figure 4: Release profile of indomethacin from SLMs formulated with lipid matrix 2:1, loaded with 0.25, 0.5 and 0.75% drug (C1, C2 and C3) respectively and reference, pure sample of indomethacin (D).

Anti-inflammatory properties

From Table 3, the SLMs exhibited good anti-inflammatory properties comparable to the reference drug (indomethacin). The formulations exhibited oedema inhibition values significantly different from control (p < 0.05). At 0.5 h, the SLMs inhibited the size of the oedema by 23-37% comparable to the reference drug which had 29.7% oedema inhibition at 0.5 h. The SLMs formulated showed more than 55% inhibition of oedema at 3 h. Generally, all the batches of indomethacin SLMs prepared exhibited good anti-inflammatory properties with up to 78% oedema inhibition at 6 h, comparable to the reference drug which had 77% oedema inhibition at 6 h which were less than 1mm in diameter, and inhibited ulcer by 75%. Therefore, the SLMs inhibited the ulcerogenic potentials of indomethacin, a highly ulcerogenic drug to zero i.e. no lesions. The ulcer inhibition potentials of the LM could be ranked thus: 1:2 < 2:1=1:1. This may be due to protective actions of the phospholipid on the intestinal mucosa of the animals.

Therefore, the result of the study showed that indomethacin-loaded SLMs inhibited the ulcerogenic potentials of indomethacin. This formulation method could be very useful in the formulation of most NSAIDS in order to reduce the gastric irritation potentials of this class of drug.

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CONCLUSION

The result of the study showed that indomethacin-loaded SLMs formulated with dika fat had high drug encapsulation efficiency. The formulations exhibited good release of drug in SIF. However, the result of this study indicated that in vitro drug release varied with the ratio of LM contained in the SLMs and the particle size of SLMs; the higher the ratio of dika fat, the larger the particle size of SLMs and the faster the drug release. Indomethacin SLMs exhibited good anti-inflammatory properties. The formulation inhibited the ulcerogenic potentials of the non steroidal anti-inflammatory drug-indomethacin. This formulation method should be very useful in the formulation most NSAIDS in order to reduce the gastric irritation potentials of this drug.

REFERENCES


