

## Original Research Article

# *In vitro* and *In vivo* Characterisation of Piroxicam-Loaded Dika Wax Lipospheres

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## Abstract

**Purpose:** To formulate piroxicam-loaded lipospheres and evaluate their *in vitro* and *in vivo* properties.

**Method:** Piroxicam-loaded lipospheres were prepared by hot homogenization technique using dika wax and Phospholipon<sup>®</sup> 90G (1:1, 1:2 and 2:1) as the lipid matrix. Characterisation, based on particle size and morphology, pH, drug content and encapsulation efficiency, were carried out on the lipospheres. *In vitro* release was evaluated in simulated intestinal fluid (pH 7.5). Anti-inflammatory and ulcerogenic properties of the piroxicam-loaded lipospheres were studied using healthy, adult Wistar rats.

**Result:** Photomicrographs revealed spherical particles in the range of 1.66 – 3.56 µm. The results also indicated that lipospheres formulated with lipid matrix 1:1 and containing 0.25 % piroxicam had the highest encapsulation efficiency of 84 %. *In vitro* release data showed that lipospheres formulated with lipid matrix having higher concentration of dika wax exhibited the fastest drug release of drug with maximum release time between 60 - 70 min. The lipospheres exhibited good anti-inflammatory properties with 58.6 % oedema inhibition at 5 h. Piroxicam-loaded lipospheres had an ulcer index of zero while, the reference (plain piroxicam) had an ulcer index of 15.00 ± 1.23 ( $p < 0.05$ ).

**Conclusion:** Piroxicam lipospheres formulated with a mixture of dika wax and phospholipid exhibited good *in vitro* and *in vivo* properties.

**Keywords:** Dika wax, Lipospheres, Piroxicam, Phospholipid, Ulcerogenicity, Anti-inflammatory

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## INTRODUCTION

The rapid growth in the use of lipid-based drug delivery systems is primarily due to the diversity and versatility of pharmaceutical grade lipid excipients and their compatibility with liquid, semi-solid and solid drugs [1]. The increasing availability of lipidic excipients with specific characteristics offer flexibility of application with respect to improving the bioavailability of poorly water soluble drugs and manipulating their release profile [2].

Dika wax is an edible vegetable fat derived from the kernel of *Irvingia gabonensis* Var excelcia [3,4]. It is a completely biodegradable physiological lipid, and like other lipid excipients, it has a GRAS (generally regarded as safe) status. Therefore, the danger of use of synthetic polymer matrix forming materials which may be associated with detrimental effects on incorporated drug during manufacturing of formulations or during the erosion of the polymers after application is completely avoided [5].

Lipospheres are restricted to the stabilizing material of a phospholipid layer [6]. The combination of a solid inner core and a phospholipid exterior confers several advantages on the lipospheres compared with conventional microspheres, such as high dispersibility in an aqueous medium, and a release rate for the entrapped substance that is controlled by phospholipid coating and carrier [6,7].

Piroxicam is a non-steroidal anti-inflammatory drug (NSAID) with pronounced anti-inflammatory, analgesic and anti-pyretic effect. The study was aimed at evaluating piroxicam-loaded lipospheres formulated with dika wax and phospholipid (Phospholipon® 90G) both *in vitro* and *in vivo* in order to improve the oral bioavailability of piroxicam and reduce its ulcerative properties.

## EXPERIMENTAL

### Materials

The following materials were used as procured from their suppliers without further purification: piroxicam (Pfizer, Nigeria), n-hexane, ethyl acetate (Sigma-Aldrich, Germany), hydrochloric acid, sodium hydroxide, monobasic potassium phosphate and Tween 80 (Merck, Germany), purified lecithin (Phospholipon® 90G, Phospholipid GmbH, Köln, Germany), activated charcoal (Bio-Lab, London), and sorbitol (Across Organics, Germany). Dika fat was extracted from *Irvingia gabonensis* seed in our laboratory. All other reagents and solvents were of analytical grade.

### Extraction and purification of dika wax from *Irvingia gabonensis*

*Irvingia gabonensis* seed were purchased from Nsukka market, Enugu State, Nigeria in the month of July, 2011. It was authenticated by Mr AO Ozioko, a consultant taxonomist with the International Center for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Nigeria, and a voucher specimen (no 151) was deposited in the herbarium of the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka.

Dried seeds of *Irvingia gabonensis* was milled using a hammer mill. The fat was extracted in a Soxhlet apparatus using n-hexane [8]. The solvent was allowed to evaporate at room temperature and boiled distilled water, which was about twice the volume of the latter, was poured into the molten fat in order to dissolve the hydrophilic gum in the fat. The dissolved gum

was removed with the aid of a separating funnel. Next, ethyl acetate was similarly used to remove the hydrophobic gum in 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 fat to 1 g column material. The fat was stored in a refrigerator until used [2].

### Preparation of lipid matrix

Mixtures (1:1, 1:2 and 2:1 w/w) of Phospholipon® 90G, a purified lecithin, and dika wax were melted in a beaker and stirred at a temperature of 70 °C using a magnetic stirrer, until a homogenous, transparent yellow liquid was obtained. The homogenous mixture was stirred at room temperature until congealed to ensure adequate mixing [9].

### Preparation of lipospheres

Piroxicam-loaded lipospheres were prepared using lipid matrix by hot homogenization technique using Ultra-Turrax® (T25 Basic Digital, Germany). Briefly, 5 g of the lipid matrix was melted at 70 °C in a crucible and the appropriate amount of piroxicam (0.25, 0.5 or 0.75 g) was incorporated into the lipid melt. Sorbitol (4 g) was dissolved in hot distilled water at the same temperature, mixed with Tween 80 (2.5 ml) and poured at 70 °C into the molten lipid phase under high shear homogenization of 5000 rpm for 5 min. An o/w emulsion was formed by phase inversion and allowed to cool at room temperature to generate the solid lipid microparticles (SLMs) [10]. The lipospheres (in emulsion form) were then stored in an amber coloured bottle until used.

### Determination of liposphere particle size and morphology

A small quantity of the lipospheres were placed on a microscope slide, the slide was covered with a cover slip and imaged under a Hund® binocular microscope (Weltzlar, Germany), fitted with a motic image analyzer (Moticam, China) at a magnification of x 400. Different portions of the SLMs from each batch were sampled and counted (n = 100) with the image analyzer, and the mean value computed.

### Determination of drug content

Beer's plot was obtained at the concentration range of 0.2 - 1.0 mg % for piroxicam in simulated intestinal fluid (SIF, pH 7.5). For each batch, 10 ml of SLMs was centrifuged (Chem. Lab. Instrument, UK) for 30 min at 1500 rpm.

The sediment was used in the analysis of drug content. A sample of 0.5 g of SLMs was triturated in a mortar and pestle with 10 ml of SIF (pH 7.5), transferred to a 100 ml volumetric flask and made up to volume with SIF. The solution was filtered through a filter paper (Whatman no. 1) and analyzed spectrophotometrically at a wavelength of 370 nm (Jenway 6305, UK). Drug concentrations were calculated with reference to the Beer's plot.

### Drug encapsulation efficiency

Encapsulation efficiency (EE) was calculated as in Eq 1.

$$EE (\%) = \frac{ADC}{TDC} \times 100 \dots\dots\dots (1)$$

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

### pH analysis

The pH of the SLMs were determined over a period of time (24 h, 1 week, 2 months and 3 months) using a pH meter (Suntex TS - 2, Taiwan).

### Evaluation of drug release studies

The USP paddle method was adopted in this study. The dissolution medium consisted of 900 ml of freshly prepared simulated intestinal fluid (SIF, pH 7.5) maintained at  $37 \pm 1$  °C. The polycarbonate dialysis membrane (MW 5000, Spectrum Labs, Brenda, Netherlands) selected was pretreated by soaking it in the dissolution medium for 24 h prior to use. A quantity of SLM equivalent to 0.020 g piroxicam was taken from each batch, 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 time intervals, 5 ml aliquots of the dissolution medium was withdrawn, appropriately diluted, and analyzed for drug content spectrophotometrically. Sink conditions were maintained by replenishing the medium with 5 ml of fresh dissolution fluid after each withdrawal. 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 piroxicam-loaded lipospheres was evaluated using the rat paw oedema test [11]. All animal experimental

protocols were carried out in accordance with the guidelines of the Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka and in compliance with the Federation of European Laboratory Animal Science Association and the European Community Council Directive of November, 1986 (86/609/EEC). The phlogistic agent employed in the study was fresh undiluted egg albumin. Adult Wistar rats of either sex (150 – 200 g) were divided into five groups. The animals were fasted and deprived of water for 12 h prior to the experiment. Lipospheres equivalent to piroxicam 10 mg/kg body weight was administered orally to the rats. The reference group received 10 mg/kg of pure sample of piroxicam, while the control group received normal saline 5 ml/kg. 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. The volume of distilled water displaced by treated right hind paw of the rats were measured using plethysmometer before egg albumin administration and at 30 min, 1, 2, 3, 4, 5 and 6 h after egg albumin injection. Inhibition of oedema was calculated using Eq 2.

$$\text{Inhibition (\%)} = \frac{V_o - V_t}{V_o} \times 100 \dots\dots\dots (2)$$

where  $V_t$  is the volume of oedema at any particular time and  $V_o$  is the volume of oedema in control rats at the same time [12,13].

### Ulcerogenicity of lipospheres

The ulcerogenicity of piroxicam-loaded lipospheres was determined using a method described by Chung-Chin *et al* [14]. The studies were carried out on healthy Wistar rats (180 – 220 g). The animals were divided into four groups ( $n = 5$ ). The control group received normal saline, the test group received SLMs equivalent to 10 mg/kg piroxicam (A1 and B1), while the reference group received 10 mg/kg pure sample of piroxicam orally. Prior to the test, the animals were fasted for 8 h but allowed free access to food and water, and sacrificed 8 h after administration of treatment. The gastric mucosa of each rat was examined under a microscope fitted with a 4 x binocular magnifier, and the number of lesions counted. The mean score of each treated group minus the mean score of the control group was taken as severity index of gastric damage.

### Statistical analysis

Statistical analysis of the data was carried out using SPSS, version 14.0 (SPSS Inc. Chicago, IL, USA). All values are expressed as mean  $\pm$  standard deviation (SD). Data were analysed by one-way ANOVA. Differences between means

were assessed by a two-tailed Student's t-test. Differences were considered statistically significant at  $p < 0.05$ .

## RESULTS

### Particle characteristics

The photomicrographs presented in Fig 1 shows that piroxicam-loaded lipospheres formulated were spherical in shape. Plain SLMs, i.e., without piroxicam, had particle sizes ranging from  $0.47 \pm 0.04$  to  $0.51 \pm 0.07 \mu\text{m}$  which were significantly ( $p < 0.05$ ) lower than those of SLMs containing piroxicam ( $1.66 \pm 3.18$  to  $3.56 \pm 2.95$ ), as presented in Table 1.

### pH of lipospheres

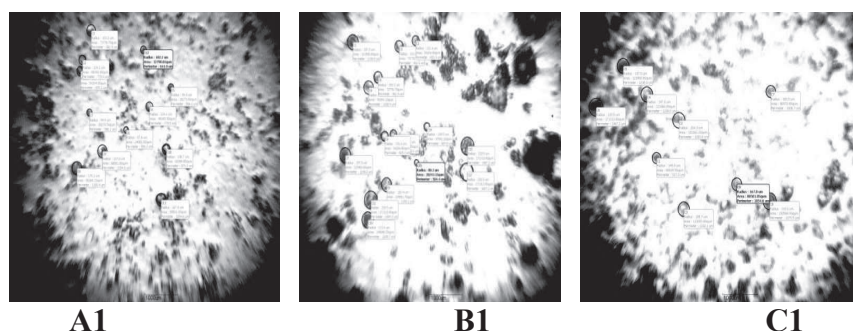
Table 1 shows pH values of the lipospheres over a period of 1 month. pH varied from between  $6.11 \pm 0.19$  and  $6.57 \pm 0.11$  within 24 h of preparation to between  $5.58 \pm 0.10$  and  $6.38 \pm 0.15$  over the next 1 month.

### Encapsulation efficiency (EE)

As indicated in Table 1, EE of the lipospheres ranged from 24 % for batch C3 to 84 % for batch A1. Piroxicam-loaded lipospheres containing 0.25 % of piroxicam had the highest encapsulation efficiency of 76 % for batch B1 lipospheres and 84 % for batch A1 lipospheres.

### Drug release from piroxicam-loaded lipospheres

The drug release profile presented in Fig. 2 show that all the piroxicam-loaded lipospheres exhibited good drug release. The lipospheres formulated with higher content of phospholipid (2:1) in the lipid matrix, i.e., batches C1 to C3 greater prolonged drug release behaviour with maximum drug release at 120 min. However, piroxicam-loaded lipospheres formulated with LM containing phospholipid: dika wax in 1:1 showed maximum drug release ranging from 70 min for batches A1 and A3 to 80 min for batch A2.



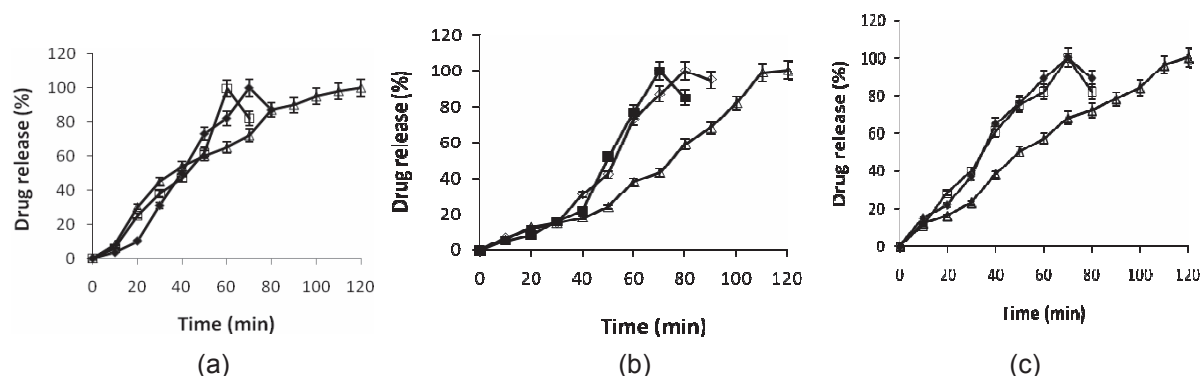
**Fig. 1:** Photomicrographs of representative samples of piroxicam-loaded lipospheres formulated with 1:1, 1:2 and 2:1 %w/w (A1, B1 and C1) of lipid matrix (Phospholipid: dika wax) and containing 0.25 % piroxicam respectively, magnification x 400.

**Table 1:** Some physicochemical properties of piroxicam-loaded lipospheres

Batch	LM	pH			Particle size ( $\mu\text{m} \pm \text{SD}$ ) <sup>a</sup>	ADC (% $\pm$ SD) <sup>b</sup>	TDC (%)	EE (%)
		24 h	1 week	1 month				
A0	1:1	$6.34 \pm 0.21$	$6.10 \pm 0.27$	$6.00 \pm 0.13$	$0.47 \pm 0.04$	0.00	0.00	-
A1	1:1	$6.37 \pm 0.17$	$6.00 \pm 0.12$	$5.58 \pm 0.10$	$3.13 \pm 3.00$	$0.21 \pm 0.27$	0.25	84.0
A2	1:1	$6.46 \pm 0.14$	$6.32 \pm 0.17$	$6.15 \pm 0.11$	$2.09 \pm 3.13$	$0.12 \pm 0.32$	0.50	24.0
A3	1:1	$6.48 \pm 0.27$	$6.42 \pm 0.15$	$6.38 \pm 0.15$	$3.08 \pm 2.88$	$0.13 \pm 0.47$	0.75	17.3
B0	1:2	$6.11 \pm 0.19$	$6.07 \pm 0.11$	$6.00 \pm 0.25$	$0.50 \pm 0.03$	0.00	0.00	-
B1	1:2	$6.37 \pm 0.11$	$6.15 \pm 0.20$	$6.04 \pm 0.23$	$2.89 \pm 3.03$	$0.19 \pm 0.21$	0.25	76.0
B2	1:2	$6.43 \pm 0.10$	$6.38 \pm 0.17$	$6.09 \pm 0.15$	$2.89 \pm 3.03$	$0.15 \pm 0.39$	0.50	30.0
B3	1:2	$6.57 \pm 0.11$	$6.24 \pm 0.21$	$6.00 \pm 0.11$	$3.56 \pm 2.95$	$0.24 \pm 0.27$	0.75	31.0
C0	2:1	$6.12 \pm 0.17$	$6.08 \pm 0.20$	$6.00 \pm 0.17$	$0.51 \pm 0.07$	0.00	0.00	-
C1	2:1	$6.38 \pm 0.12$	$6.20 \pm 0.16$	$6.10 \pm 0.12$	$3.10 \pm 1.02$	$0.08 \pm 0.23$	0.25	31.5
C2	2:1	$6.41 \pm 0.07$	$6.37 \pm 0.17$	$6.10 \pm 0.13$	$3.24 \pm 2.91$	$0.14 \pm 0.19$	0.50	27.0
C3	2:1	$6.51 \pm 0.09$	$6.18 \pm 0.12$	$5.89 \pm 0.27$	$1.66 \pm 3.18$	$0.18 \pm 0.24$	0.75	24.0

<sup>a</sup> $n = 100$ , SD = Standard deviation; <sup>b</sup> $n = 5$ ; A0–A3 contain LM 1:1; B0–B3 contain LM 1:2; C0–C3 contain LM 2:1; A0, B0 and C0: contain no drug; LM = lipid matrix





**Fig. 2:** Release profile of piroxicam loaded lipospheres: (a)  $\blacklozenge$  A1,  $\square$  B1 and  $\triangle$  C1 contain LM 1:1, 1:2 and 2:1, respectively, as well as 0.25 % piroxicam; (b)  $\diamond$  A2,  $\blacksquare$  B2,  $\triangle$  C2 contain 0.5 % piroxicam; (c)  $\blacklozenge$  A3,  $\square$  B3 and  $\triangle$  C3 contain 0.75 % piroxicam.

**Table 2:** Anti-inflammatory properties of piroxicam-loaded lipospheres

Group	Paw oedema volume (ml $\pm$ SD) <sup>a</sup>					
	0.5 h	1 h	2 h	3 h	4 h	5 h
A1	0.58 $\pm$ 0.23* (27.5)	0.55 $\pm$ 0.27* (35.3)	0.48 $\pm$ 0.19* (40.7)	0.42 $\pm$ 0.29* (46.8)	0.35 $\pm$ 0.12* (53.3)	0.30 $\pm$ 0.17* (57.1)
B1	0.56 $\pm$ 0.37* (30.0)	0.58 $\pm$ 0.45* (31.8)	0.50 $\pm$ 0.13* (38.3)	0.40 $\pm$ 0.32* (49.4)	0.35 $\pm$ 0.24* (53.3)	0.29 $\pm$ 0.21* (58.6)
C1	0.60 $\pm$ 0.52* (25.0)	0.55 $\pm$ 0.47* (35.3)	0.51 $\pm$ 0.54* (37.0)	0.43 $\pm$ 0.39* (43.0)	0.37 $\pm$ 0.45* (50.7)	0.30 $\pm$ 1.52* (57.1)
D (ref.)	0.60 $\pm$ 0.24* (25.0)	0.57 $\pm$ 0.43* (33.0)	0.50 $\pm$ 0.57* (38.3)	0.45 $\pm$ 0.51* (43.0)	0.40 $\pm$ 0.23* (46.7)	0.31 $\pm$ 0.27* (55.7)
E(Cont.)	0.80 $\pm$ 0.35	0.85 $\pm$ 0.17	0.81 $\pm$ 0.27	0.79 $\pm$ 0.13	0.75 $\pm$ 0.21	0.70 $\pm$ 0.12

\* significant difference at  $p < 0.05$  compared to control. values are mean  $\pm$  SD ( $n = 5$ ); <sup>a</sup> values in parenthesis are % oedema inhibition; A1 – C2 = piroxicam-loaded lipospheres, D = pure piroxicam (reference); E = negative control (normal saline).

### Anti-inflammatory properties

The anti-inflammatory results, presented in Table 2, show that piroxicam-loaded lipospheres exhibited good anti-inflammatory activity that was significantly higher than that of the control group ( $p < 0.05$ ). The oedema inhibition ranged from 35.3 to 53.3 % for piroxicam-loaded lipospheres (A1) while the reference drug showed inhibition of 25 to 47 %.

### Ulcerogenic properties

The results of ulcerogenic studies are presented in Table 3. Piroxicam lipospheres did not show ulcer-inducing potentials. However, the reference drug (plain piroxicam) showed a high ulcer index of  $15.00 \pm 1.23$  which was significantly higher than that of the piroxicam-loaded lipospheres and negative control ( $p < 0.05$ ).

## DISCUSSION

Increase in the amount of piroxicam encapsulated increased also the particle size of the lipospheres. This is in agreement with the findings of Barakat *et al* [15] who evaluated carbamazepine-loaded precifac lipospheres.

Joseph *et al* [16], who studied piroxicam-loaded polycarbonate microspheres made a similar observation. The formulations also exhibited stable pH from 24 h to 1 month. Therefore, there was no degradation of the API and excipients.

**Table 3:** Ulcerogenic properties of piroxicam lipospheres

Batch	Ulcer index (mean $\pm$ SD) <sup>a</sup>
A1	0.00 $\pm$ 0.00
B1	0.00 $\pm$ 0.00
D (reference)	15.00 $\pm$ 1.23*
E (control)	0.00 $\pm$ 0.00

\*Significant at  $p < 0.05$  compared to control. <sup>a</sup> $n = 5$ ; A1 and B1: piroxicam-loaded SLMs; D: piroxicam pure sample; E: normal saline.

Generally, encapsulation efficiency (EE) was affected by the lipid composition/ratio used in formulating the lipospheres. The reason may be the presence of small amounts of fat in the inner core of the lipospheres which can lead to saturation of the fat core of the lipospheres by the drug incorporated in the dispersion. EE of piroxicam-loaded lipospheres was also inversely

affected by the total amount of drug in the dispersion.

The SLMs had reproducible drug release in all the batches, without any evidence of 'burst effect'. However, the rate of drug release was affected by the ratio/combination of the lipid used in preparation of the lipid matrix. Lipid matrices having higher amount of dika wax showed faster release of drug than lipospheres with higher phospholipid concentration. This may be due to diffusion of the drug from the inner core of the lipospheres through the phospholipid coating to the surface of the lipospheres.

The piroxicam-loaded lipospheres showed higher inhibition of oedema than the reference (plain piroxicam). This may be due to increased absorption of drug *in vivo* in the presence of the lipid matrix carrier. Drugs suspended in lipid matrix have been shown, in most cases, to be better absorbed than those incorporated in conventional solid dosage forms [1]. This could be due to the ease of wetting of hydrophobic drug particles in the presence of lipid matrix [2]. The presence of surfactant in the formulation may further promote drug release [2].

Piroxicam-loaded lipospheres drastically reduced the ulcerogenic potentials of piroxicam. This may be due to the presence of the phospholipid in the formulations which might have helped to shield or protect the gastric mucosa from irritation by the drug which is a non-steroidal anti-inflammatory drug (NSAID). Our finding is in agreement with that of Lichtenberger *et al* [17], who 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 thus reduce their ulcerogenicity.

## CONCLUSION

Dika wax and Phospholipon® 90G presented good matrix for the formulation of piroxicam-loaded lipospheres. *In vitro* and *in vivo* studies undertaken with the lipospheres provided positive results, thus indicating the need for further development and optimization of the formulation. The lipospheres exhibited good anti-inflammatory properties as well as minimal ulcerogenic potentials. Piroxicam-loaded lipospheres based on dika wax matrices have advantages over the commercial formulations of piroxicam, include low excipient and processing costs.

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