AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Piroxicam Solid Lipid Microparticles: *In vitro* and *in vivo* Evaluation

NC Obitte[1*], SA Chime[1], DC Ibe[2], OR Nweke[1], TC Ugwudah[1].

1.Department of Pharmaceutical Technology and Industrial Pharmacy, University of Nigeria, Nsukka 410001, Nigeria

2. Department of Public Health, Federal University of Technology, Owerri, Nigeria.

ABSTRACT

The aim of this work was to investigate the potential of solid lipid microparticles (SLM) to offer gastroprotection and improve piroxicam's anti-inflammatory property. The effects of NaCl and cabosil® on the properties of the SLM were also evaluated. The SLM were prepared by the hot homogenization technique using Ultra turrax (T25 Basic digital). Phospholipon® 90G and *Capra hircus* fat constituted the lipid matrix. The particle size, drug content, encapsulation efficiency, *in vitro* drug release, anti-inflammatory and ulcerogenic studies were carried out on the formulations. Results showed that carbosil® significantly ($p<0.05$) contributed to increase in particle size of the SLM. Drug-load caused significant ($p<0.05$) decrease in EE % whereas significant ($p<0.05$) increase in EE % was associated with carbosil®. Significant ($p<0.05$) reduction in drug release rate was occasioned by carbosil®. SLM batches did not show any presence of ulcer lesions and indicated satisfactory antiinflammatory property of piroxicam. In conclusion piroxicam SLM promoted gastroprotection and satisfactory antiinflammatiory activity of piroxicam.

Keywords: Solid lipid microparticles; Piroxicam; Ulcerogenicity; Anti-inflammation.

*Corresponding Author Email: obittenick@yahoo.com

Received 19 March 2013, Accepted 8 April 2013

Please cite this article in press as: Obitte NC. *et al.*, Piroxicam Solid Lipid Microparticles: *In vitro* and *in vivo* Evaluation. American Journal of PharmTech Research 2013.

INTRODUCTION

Some known approaches to improve the aqueous solubility of some poorly soluble drugs include liquisolid compacts, solid dispersions, micronization and salt formation. Amongst these, solid dispersion has been deemed a robust approach in the improvement of the solubility of piroxicam[1] and indomethacin[2]. However, lipid carriers may be advantageous in improving the solubility, dissolution and pharmacodynamic properties of these drugs [3, 4]. Solid lipid microparticles (SLM) are lipid-based micro-scale drug carriers possessing matrix made from fatty acid, glyceride, fatty alcohol, and solid wax with high melting points [5]. It is believed that SLM combine the advantages of many colloidal carriers while overcoming some of their disadvantages [5]. Besides being well tolerated in living systems the encapsulation efficiency can be as high as 80% for lipophilic compounds. The solid matrix protects loaded labile substances against degradation and they offer the possibility of targeting or controlled drug release [6]. The solid core structure averts or mitigates the mobility of incorporated drug and drug leakage from the carriers.

Gastro-duodenal ulceration and bleeding are the major limitations to the use of non-steroidal anti-inflammatory drugs (NSAIDs). They can cause damage to the gastro-duodenal mucosa via several mechanisms, including the topical irritant effect of these drugs on the epithelium, impairment of the barrier properties of the mucosa, suppression of gastric prostaglandin synthesis, reduction of gastric mucosal blood flow and interference with the repair of superficial injury [7]. Piroxicam (P, 4-hydroxy-2-methyl-N-(2-piridyl) 2H-1, 2-benzothiazine-3-carboxamide-1, 1-dioxide) is a potent non-steroidal anti-inflammatory and analgesic drug which is insoluble in water [1, 8] and has gastrointestinal adverse effects. After oral ingestion more than 2 h are required to attain maximum concentration, which implies slow absorption rate [9]. Piroxicam enteric-coated pellets using nonpareil seeds by powder layering technique have been prepared to minimize gastrointestinal adverse effects [10]. On the other hand attempts to resolve the poor water solubility have been documented, including microparticles, solid dispersion and microemulsion [11, 12]. All these techniques as good as they are may not compare with SLM vis-a-viz the functionality of phospholipids used in the latter. Since phospholipids uniquely possess gastroprotective property our present investigation was therefore aimed at evaluating the potential of SLM to offer gastroprotection to piroxicam while improving anti-inflammatory characteristic.

MATERIALS AND METHOD

The following materials were used as procured from their local suppliers without further purification: hydrochloric acid, sodium hydroxide, potassium dihydrogen phosphate, Tween 80,

Sorbitol (Merck, Germany), piroxicam (Pfizer, Nigeria), Phospholipon® 90G (Phospholipid GmbH, Köln, Germany), activated Charcoal (Bio–Lab. (UK) limited, London), Distilled water (Lion water, Nsukka, Nigeria). Goat fat was obtained from a batch processed in our laboratory. All other reagents and solvents were of analytical grade and used as such.

Extraction and purification of *Capra hircus* fat

Capra hircus fat was extracted from the adipose tissue by wet rendering [13]. The adipose tissue was grated and subjected to moist heat by boiling with about half its weight of water in water bath for 45 min. The molten fat was separated from the aqueous phase after filtering with a muslin cloth. Further purification was effected by heating a 2% w/w suspension of a 1:9 ratio blend of activated charcoal and bentonite in the lipid at 80-90^0 C for 1h. Subsequently vacuum-filtration was carried out to recover the purified fat [14].

Preparation of the lipid matrix (LIM)

A 30:70 ratio blend of Phospholipon® 90G (a purified soy lecithin) and *Capra hircus* fat were melted and stirred at 70^0 C using a magnetic stirrer, until a homogenous melt was obtained. The resultant homogenous mixture was continuously stirred until it solidified [15].

Preparation of SLM

Piroxicam SLMs were prepared by hot emulsion-homogenization technique using an Ultra-turrax homogenizer (T25 Basic digital, Ika/Staufen, Germany). Cabosil® was dispersed in the LIM of some batches as presented in Table 1) prior to melting at 70^0 C; this was followed by the addition of piroxicam. On the other hand sorbitol, Tween 80, with or without NaCl was dissolved in a hot (70^0 C) aqueous phase. Subsequently the hot aqueous phase was introduced into the lipid phase and immediately homogenized (Ultra turrax, T25 Basic digital, Ika/Staufen, Germany) at 5000 rpm for 10 min [16].

Particle size determination

A small dispersion of the SLM was placed on a microscope slide and covered with a cover slip. This was imaged under a Hund® binocular microscope (Weltzlar, Germany) attached with a motic image analyzer (Multicam, China) at x 40 magnification.

Encapsulation efficiency

The SLM was centrifuged at 3000 rpm for 30 min and the supernatant decanted. A 0.5 g quantity of each batch of the SLMs was triturated with 50 ml of phosphate buffer and later transferred to a 100 ml volumetric flask. The flask was made up to volume with the medium, shaken, filtered (Whatman No. 1) and content of piroxicam determined spectrophotometrically (Model SP6 - 450

UV/Vis Pye Unicam) at a predetermined wavelength of 252 nm. This was repeated in triplicates for all the batches. From the drug content values the encapsulation efficiency was calculated thus:

Table 1: Quantities of materials for producing the various SLM batches

Batch	LM (g)	Piroxicam (g)	Tween 80 (ml)	Sorbitol (g)	Thiomersal (ml)	Sodium chloride (g)	Cabosil (g)	Water q.s
S1	5.0	0.5	2.5	4.0	0.0025	-	-	100
S2	5.0	1.0	2.5	4.0	0.0025	-	-	100
S3	5.0	1.5	2.5	4.0	0.0025	-	-	100
S4	5.0	2.0	2.5	4.0	0.0025	-	-	100
S5	5.0	0.5	2.5	4.0	0.0025	0.9	-	100
S6	5.0	1.0	2.5	4.0	0.0025	1.2	-	100
S7	5.0	1.5	2.5	4.0	0.0025	1.5	-	100
S8	5.0	2.0	2.5	4.0	0.0025	2.0	-	100
S9	5.0	0.5	2.5	4.0	0.0025	-	0.1	100
S10	5.0	1.0	2.5	4.0	0.0025	-	0.2	100
S11	5.0	1.5	2.5	4.0	0.0025	-	0.5	100
S12	5.0	2.0	2.5	4.0	0.0025	-	1.0	100
S13	5.0	0.5	2.5	4.0	0.0025	0.9	0.1	100
S14	5.0	1.0	2.5	4.0	0.0025	1.2	0.2	100
S15	5.0	1.5	2.5	4.0	0.0025	1.5	0.5	100
S16	5.0	2.0	2.5	4.0	0.0025	2.0	1.0	100
S17	5.0	-	2.5	4.0	0.0025	-	0.1	100
S18	5.0	-	2.5	4.0	0.0025	0.9	-	100

A – D: various formulations of piroxicam-loaded SLM; LM: lipid matrix; S17: Placebo containing carbosil® without sodium chloride; S18: Placebo containing NaCl without carbosil®

$$\text{Encapsulation efficiency} = \frac{\text{Amount of drug encapsulated}}{\text{Amount of drug incorporated}} \text{ x } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Release studies

The release of piroxicam from the SLM batches was studied using the USP paddle method. The dissolution medium consisted of 900 ml of freshly prepared phosphate buffer (pH 7.4) maintained at 37 ± 0.5^0 C. A polycarbonate dialysis membrane was pretreated by soaking in the medium for 24 h prior to use. Amount of SLM equivalent to 20 mg of piroxicam was placed in the membrane containing 2 ml of the dissolution medium, securely tied with a thermo-resistant thread and then placed in the dissolution apparatus. The paddle was rotated at 50 rpm. A 5 ml quantity of the dissolution medium was sampled at intervals, diluted appropriately and absorbance determined spectrophotometrically as already described. The volume of the dissolution medium was refreshed with 5 ml of fresh medium.

Anti-inflammatory studies

Egg-albumin-induced-rat-paw-oedema method was adopted in this study. All experimental protocols were approved by the animal ethics committee of the faculty of pharmaceutical sciences, university of Nigeria, Nsukka. Acute inflammation was measured in terms of change in volume of the rat hind paw induced by sub planter injection of egg-albumin [17, 18]. Wistar rats (120 – 185 g) of both sexes were divided into 4 experimental groups of 5 per group. The rats were fasted for 6 h with no access to water during experiment. The deprivation of water was to ensure uniform hydration and to minimize variability in oedematous response [19]. Dose of SLM equivalent to 1 mg/kg of piroxicam was administered orally to the rats. The control groups received normal saline while the reference group received pure sample of piroxicam 10 mg/kg. Thirty minutes post-treatment, oedema was induced with 0.1 ml fresh undiluted egg-albumin injection into the sub planter region of the right hind paw of the rats. The volume of distilled water displaced by the treated paw was measured with plethysmometer before and at 1, 2, 3, 4, and 5 h post induction of oedema. The anti-inflammatory activity was calculated at each time as percent inhibition of oedema using the equation:

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

where, Vt is the volume of oedema at corresponding time and Vo is the volume of oedema in control rats at the same time [20, 21, 22].

Ulcerogenic studies

The method described by some workers [22] was used in this study. All experimental protocols were approved by the animal ethics committee of the faculty of pharmaceutical sciences, university of Nigeria, Nsukka. Healthy Wistar rats (110 – 150 g) of five rats per group were fasted for 12 h. SLM equivalent to 10 mg/kg of piroxicam was administered orally to the rats. The control group received normal saline, while the reference group received piroxicam pure sample (10 mg/kg). Five hours post treatment, the animals where sacrificed by ether anesthesia, the gastric mucosa was removed, cut along the lesser curvature and opened up to expose the mucosal surface [22]. The mucosa was washed with normal saline and observed with magnifying glass (x 10). The number of ulcers were counted and the ulcer index calculated using the method described by Main and Whittle [23].

Statistics

All statistical analysis was carried out using (Graph pad instat, demo version). Mean values and standard deviation were tested for their level of significance at ($p<0.05$).

RESULTS AND DISCUSSION

Particle size determination

Figures 1-5 show the photomicrographs and Figure 6 the particle sizes of the various SLM batches. The photomicrographs reveal spherical particles ranging between 37-100 µm. In the absence of carbosil® NaCl did not have impact on particle size variation. However, in the presence of carbosil® significant ($p<0.05$) increase in particle size was observed. Drug-load had a significant ($p<0.05$) reduction effect on particle size in the absence of carbosil® and NaCl (batches S1-4). However when carbosil® was introduced the variation followed no regular trend. Contrary to this observation some workers [24] had reported that increase in drug-load caused a corresponding increase in particle size. However, the particle sizes of the placebo batches (without drug-load) were significantly ($p<0.05$) higher than most of the drug-loaded batches.

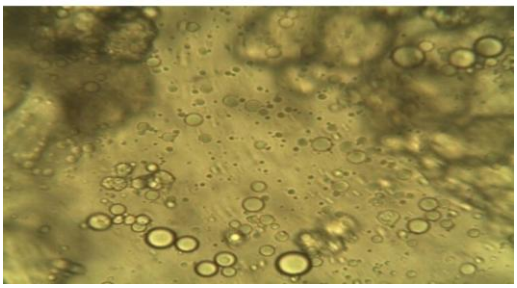

Figure 1: Photomicrograph of batch S1

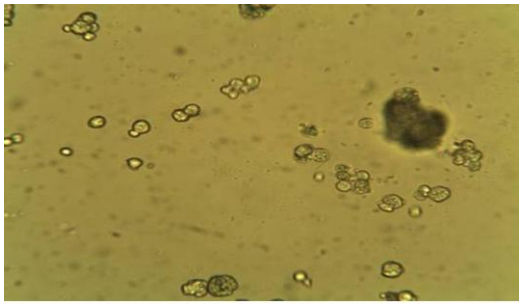

Figure 2: Photomicrograph of batch S7

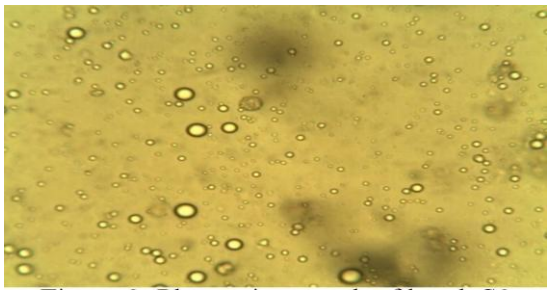

Figure 3: Photomicrograph of batch S9

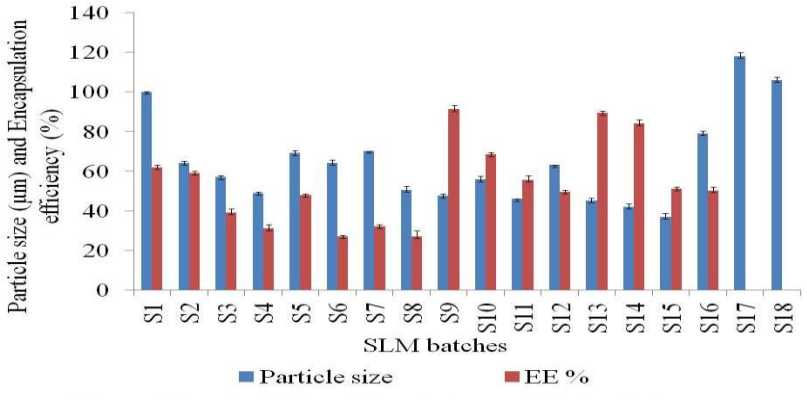

Figure 6:Particle size and encapsulation efficiency(%) values of SLM batches

Sphericity of particles may be attributed to high shear force of the homogenizer. Typically, an important functional role of carbosil® may have impelled the observed particle size variation in some of the batches. Owing to its lipophilicity, carbosil® dispersion in the lipid matrix may have enhanced particle adhesion and size increase during the homogenization phase. The placebo batches recorded high particle sizes because of drug absence. Certainly the presence of piroxicam, a hydrophobic drug was expected to be entrapped within the hydrophobic hydrocarbon tail of the phospholipid amphiphile. Drug entrapment may have actuated bending and tilting of the hydrocarbon chain with consequent size reduction [24, 25]. Therefore without drug entrapment, little or no stress impinged on the amphiphilic particle. Hence the observed larger particle sizes.

Encapsulation efficiency (EE%)

Drug-load was basically responsible for significant ($p<0.05$) decrease in EE % in some of the formulations. This may be attributed to saturation effect. The hydrophobic tail of the amphiphile may have a limit to its drug entrapment capacity; once it was overshot the remaining drug probably escaped into the aqueous phase. Obviously from our results increase in drug load was unnecessary and potentially uneconomical. However the presence of carbosil® significantly ($p<0.05$) increased the EE%. Particle adhesion and impedance of drug migration into the aqueous phase may explain this behavior.

Release studies

Figures 7 and 8 represent the release profiles of piroxicam from the SLM batches. Figure 7 constitutes batches (S1-S8) with or without NaCl while Figure 8 consists of batches (S9-S16) with carbosil® or carbosil® and NaCl. Carbosil-containing batches remarkably recorded higher T50 and T85 than batches without it. Whereas T50/T85 was within 3-10/11-39 min for S1-S8 batches, S9-S12 batches recorded T50/T85 values that were within 42-56/50-92 min. S1-8

batches witnessed burst release (40-60%) within 5 min, with the rest of drug release taking place within 15-50 min. On the other hand S9-13 batches had a biphasic release, with the first phase involving 25-50 % drug release within 40-60 min and the second phase 25-100 % drug release within 40-110 min.

S1-S8 batches are likely to have quicker *in vivo* onset of action than the rest batches based on the T50 values. Apparently sodium chloride and drug-load did not minimize drug release rate as carbosil® did. Carbosil® disperses in oils and lipids to form gels. This gel network may have restricted permeation of aqueous medium during dissolution studies. Hence the observed reduction in drug release rate in carbosil-containing batches.

Figures 7 and 8 represent the release profiles of piroxicam from the SLM batches. Figure 7 constitutes batches (S1-S8) with or without NaCl while Figure 8 consists of batches (S9-S16) with carbosil® or carbosil® and NaCl. Carbosil-containing batches remarkably recorded higher T50 and T85 than batches without it. Whereas T50/T85 was within 3-10/11-39 min for S1-S8 batches, S9-S12 batches recorded T50/T85 values that were within 42-56/50-92 min. S1-8 batches witnessed burst release (40-60%) within 5 min, with the rest of drug release taking place within 15-50 min. On the other hand S9-13 batches had a biphasic release, with the first phase involving 25-50 % drug release within 40-60 min and the second phase 25-100

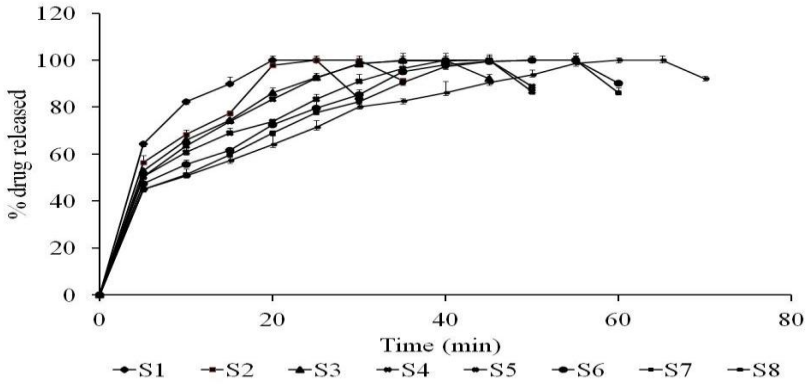

Figure 7:Release profile of piroxocam from SLM prepared with or without NaCl

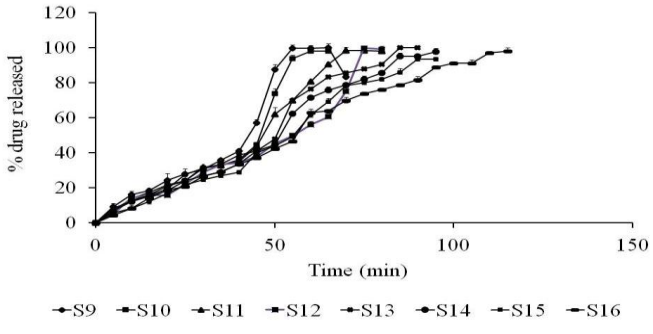

Figure 8:Release profile of piroxocam from SLM prepared with carbosil and NaCl

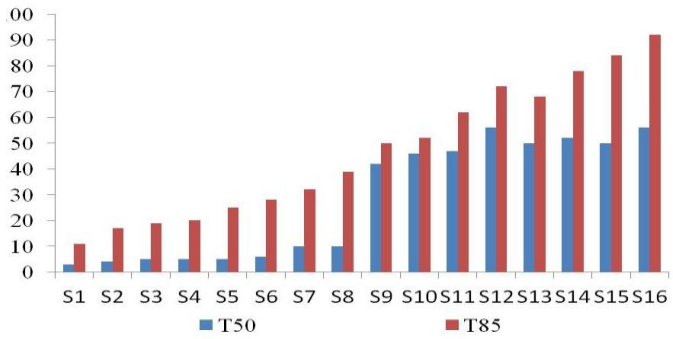

Figure 9: T50 and T85 values of S1-S16

Response:S1-S8 batches are likely to have quicker *in vivo* onset of action than the rest batches based on the observed T50 values. The T85 values additionally portray their immediate release characteristics. For immediate release formulations about 85% of drug should be released within 30 min. In contrast S1-S9 batches had values that fell outside this bench mark. Apparently sodium chloride and drug-load did not minimize drug release rate as carbosil® did. Sodium chloride did not exert any effect on drug release probably because it was only a constituent of the aqueous phase. Carbosil® on the other hand disperses in oils and lipids to form gels. During preparation of the SLMs while sodium chloride was dissolved in water carbosil® was dispersed in the lipid matrix. This gel network created by carbosil® may have restricted permeation of aqueous medium during dissolution studies. Hence the observed reduction in drug release rate in carbosil-containing batches.

Cyclosporin A incorporated in a lecithin formulation had improved bioavailability that was bioequivalent to Sandimmun Neoral® (a microemulsion formulation of it) [25, 26]. Response: The above mentioned works were not done by us. They were done by workers in References 25 and 26.

Anti-inflammatory studies

Table 2 shows the anti-inflammatory studies results. Compared to the control S9 caused significant ($p<0.05$) reduction in edema from the 2nd h while S1 and Reference did so from the 3rd h. S9 largely performed best, in view of highest % edema inhibition from the 2nd h. The phospholipid component of the SLM was responsible for improved anti-inflammatory activity. Cyclosporin A incorporated in a lecithin formulation had improved bioavailability that was bioequivalent to Sandimmun Neoral® (a microemulsion formulation of it) [25, 26]. The phospholipid constituent (lecithin) was believed to facilitate drug absorption via lymph circulation. Lipids from lymph lipid precursor pool (LLPP) are known to assemble into lipoproteins which are transported from the enterocyte via the lymphatic system into the systemic circulation.

Phospholipids increase the mass of LLPP and significantly increase the chances of co-administered lipophilic drug to be transported through the lymphatic system [27]. This may explain the superior anti-inflammatory activity of S9. In addition the nanoparticulate size of carbosil® may have enhanced rapid enterocyte absorption and subsequent lymphatic transport.

Table 2. Anti-inflammatory results

Paw volume oedema (ml ± SD)[a] and percentage inhibition of oedema (%)						
Batch	**0.5 h**	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
S1	1.30 ± 0.12 (0)	1.30 ± 0.07 (0)	1.20 ± 0.05 (7.6)	1.50 ± 0.09* (37)	1.03 ± 0.24* (38)	0.93 ± 0.07* (44)
S9	1.28 ± 0.06 (1.5)	1.25 ± 0.09 (3.8)	1.03 ± 0.07* (20)	1.0 ± 0.05* (40)	0.93 ± 0.11* (44)	0.93 ± 0.12* (44)
P (Reference)	1.30 ± 0.17 (0)	1.30 ± 0.12 (0)	1.20 ± 0.21 (7.6)	1.05 ± 0.03* (37)	1.04 ± 0.07* (38)	0.98 ± 0.05* (41)
Control	1.30 ± 0.06	1.30 ± 0.21	1.30 ± 0.07	1.67 ± 0.12	1.66 ± 0.03	1.66 ± 0.05

*****Reduction in oedema 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 calculated relative to control. S1 and S9: piroxicam-loaded SLMs.

Ulcerogenic studies

The ulcerogenic results presented in Table 3 shows that the piroxicam SLM completely inhibited the ulcerogenic potential of the NSAID, evidenced by total absence of lesions on the gastric mucosa of the animals. In contrast several lesions were observed on the animals that received the reference drug (piroxicam pure sample). Non-selective NSAIDS, including piroxicam inhibit COX-2 to exert anti-inflammatory effect and inhibit COX-1 responsible for prostaglandin synthesis [28]. Typically, phospholipid produced by gastric mucosa stimulates prostaglandin release which imparts hydrophobic characteristic to mucosal surface [25]. It is important to note that both endogenous and exogenous phospholipids are capable of stimulating prostaglandin release. Therefore the simultaneous counter process of inhibition of prostaglandin by COX-1and its synthesis by phosphatidylcholine seemed to have favored gastroprotection in our present investigation. Hence the absence of lesions in the SLM formulations evaluated.

Table 3. Ulcerogenic results

Batch	**Ulcer index (Mean ± SD)a**
S1	0.00 ± 0.00*
S9	0.00 ± 0.00*
P (Reference)	15.00 ± 1.17*
Control	0.00 ± 0.00

*** Significant at $p < 0.05$ compared to control. [a]n = 5; B_1: piroxicam-loaded SLMs; P: pure piroxicam sample.**

CONCLUSION

The anti-inflammatory enhancement and gastroprotective potential of piroxicam SLM were evaluated in this study. *In vitro* findings showed that drug load impinged on some of the properties of the SLMs. Cabosil® imparted higher encapsulation efficiency and delayed drug release. Evident gastroprotection and improved anti-inflammatory effect were observed in the SLM formulations. Therefore SLM is an ideal gastroprotective formulation for the delivery of piroxicam considering the drug's gastric irritation potential.

REFERENCES

1. Sharma DK, Gupta VB, Purohit S.. Industry feasible method to improve solubility of Piroxicam with Crospovidone: reparation, characterization and tableting consideration. Der Pharmacia Lettre 2010; 2(3):123-135.
2. Mesnukul A, Yodkhum K, Phaechamud T. Solid dispersion matrix tablet comprising indomethacin-PEG-HPMC fabricated with fusion and mold technique. Indian J Pharm Sci 2009; 71(4): 413–420.
3. Nanjwade BK, Patel DJ, Udhani RA, Manvi FV. Functions of lipids for enhancement of oral bioavailability of poorly water-soluble drugs. Sci Pharm 2011; 79: 705–727.
4. Obitte NC, Chime SA, Magaret AA, Attama AA, Onyishi IV, Brown SA. Some *in vitro* and pharmacodynamic evaluation of indomethacin solid lipid microparticles. Afr J Pharm Pharmacol 2012; 6 (30): 2309-2317.
5. Long C, Zhang L, Qian Yu. Preparation and Crystal modification of Ibuprofen loaded solid lipid microparticles. Chin J Chem Eng 2006; 14 (4): 518 – 525.
6. Eradel MS, Gungor S, Ozsoy Y, Araman A. Preparation. And in vitro evaluation of indomethacin loaded solid lipid microparticles. Acta Pharmaceiutical Sciencia 2009; 51: 203 – 210.
7. Wallace J. How do NSAIDs cause ulcer disease? Bailliere's Clinical Gastroenterology 2008; 14 (1): 147 – 159.
8. Dixit M, Kini AG, Kulkarni PK. Preparation and characterization of microparticles of piroxicam by spray drying and spray chilling methods. Res Pharm Sci 2010; 5(2): 89–97.
9. Gwak HS, Choi JS, Choi HK. Enhanced bioavailability of piroxicam via salt formation with ethanolamines. Int J Pharm 2005; 297: 156-161.
10. Varshosaz J, Tavakoli N, Serri A. Preparation and *in vitro* characterization of piroxicam enteric coated pellets using powder layering technique. Pharm Dev Tech 2009; 14(3):

305-311.

11. Abd-Allah FI, Dawaba HM, Ahmed AMS. Development of a microemulsion-based formulation to improve the availability of poorly water-soluble drug. Drug Discov Ther 2010; 4(4): 257-266.
12. Dixit M, Kulkarni PK, Selvam RP, Kini AG, Shivakumar HG. Preparation and characterization of spherical agglomerates of piroxicam by neutralization method. Amer J Drug Disc Dev 2011; 1(3): 188-199.
13. Attama, AA, Nzekwe IT, Nnamani PO, Adikwu MU, Onugu CO. The use of self-emulsifying systems in the delivery of diclofenac. Int J Pharm 2003; 262: 23 – 28.
14. Obitte NC, Chukwu A. The excipient usefulness of carbosil® and *Landolphia owariensis* in two oil-based selfemulsifying formulations. As J Pharm 2010; 4(4): 213-219.
15. Friedrich I., Muller Goymann CC. Characterization of solidified reverse micellar solutions (SRMS) and production development of SRMS-based nanosuspension. Eur J Pharm Biopharm 2003; 56:111-119.
16. Jaspert S, Bertholet P, Piel G, Dogne JM, Delattre L, Evrard B. Solid lipid microparticles as a sustained release system for pulmonary drug delivery. Eur J Pharm Biopharm 2007; 65: 47 – 56.
17. Ekpendu JO, Akah PA, Adesomoju AA, Okogun JI. Anti-Inflammatory and Anti-microbial activities of *Mitracarpus scaber* extracts. Int J Pharmacognosy 1994; 32: 991-2196.
18. Osadebe PO, Okoye FBC. Anti-Inflammatory Effects of Crude Methanolic Extract and Fractions of *Alchornea cordifolia* leaves. J Ethnopharmacology 2003; 89: 19-24.
19. Winter EA, Risley EA, Nuss GU. Anti-inflammatory and antipyretic activities of indomethacin. J Pharm Exp Ther 1963; 141: 367-376.
20. Parez GRM. Anti-Inflammatory activity of *Ambrosia artemisiaefolia* and *Rheo spathaceae*. Phytomedicine 1996; 3: 163-164.
21. Ahmed MM, Qureshi S, Al-bekairi AM, Shah AH, Rao RM. Anti- inflammation activity of *Caralluma tuberculata* alcoholic extract. Fitoterapia 1993; 64: 359-362.
22. Ajali U, Okoye FBC.). Antimicrobial and anti-inflammatory activities of Olax viridis root bark extracts and fractions. Int J Applied Res Nat Prod 2009; 2(1): 27-32.
23. Main IHM, Whittle NB (Jnr).. Investigation of vasodilator and antisecretory role of prostagladin in the rat mucosa by use of NSAIDs. British J Pharmacol 1975; 53: 217 – 224.

24. Barakat SN, Yassin EB. *In vitro* characterization of carbamazepine-loaded precifac lipospheres. Drug Deliv 2006; 13: 95 – 104.
25. Fricker G, Kromp T, Wendel A, Blume A, Zirkel J, Rebmann H, Setzer C, Quinkert R, Martin F, Müller-Goymann C. Phospholipids and lipid-based formulations in oral drug delivery. Pharm Res 2010; 27:1469-1486.
26. Gershanik T, Benzeno S, Benita S. Interaction of a self emulsifying lipid drug delivery system with the everted rat intestinal mucosa as a function of droplet size and surface charge. Pharm Res. 1998; 15: 863–869.
27. Trevaskis NL, Porter CJH, Charman WN. An examination of the interplay between enterocyte-based metabolism and lymphatic drug transport in the rat. Drug Met Dep 2006; 5 (34):729-733.
28. Bhupinderjit SA, Jim JR, Sudershan KS, Lenard ML Phospholipid association reduces the gastric mucosal toxicity of aspirin in human subjects. The Amer J Gastroent 1999; 94: 1818-1822.