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Anti-inflammatory, antinociceptive and ulcerogenic properties of indomethacin tablets based on solidified reverse micellar solution (SRMS)

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The aim of the present study was to evaluate *in vivo* the anti-inflammatory, antinociceptive and ulcerogenic properties of indomethacin tablets based on solidified reverse micellar solution (SRMS). SRMS consisting of mixtures of phospholipid (Phospholipon® 90H) and triglyceride (Softisan® 154) were prepared in the ratios of 1:1, 2:1 and 1:2, respectively. SRMS based tablets containing 75 mg of indomethacin each were prepared using validated plastic mould. The physicochemical properties of the tablet formulations were studied using both official and unofficial tests. Anti-inflammatory, analgesic/antinociceptive and ulcerogenic properties of indomethacin tablets based on SRMS were studied. The results showed that the physicochemical properties of the tablet formulations were significantly affected by the composition/ratio of the lipid matrix used. The softening time in SIF ranged from 53.7 ± 0.5 to 102.6 ± 0.5 min. Results of analgesic/antinociceptive properties showed that indomethacin tablets formulated with the SRMS 1:1 had an increase in pain reaction time at 7 h significantly ($p < 0.05$) different from the results exhibited by tablets formulated with the lipid matrices, SRMS 1:2 and 2:1 and the reference, which showed a decrease in pain reaction time at 7 h. Indomethacin tablets based on SRMS had good anti-inflammatory properties and also inhibited the ulcerogenicity of indomethacin by 70 to 80%. Therefore, indomethacin tablets based on SRMS could be used for improved oral bioavailability of indomethacin and to enhance patient's compliance due to inhibition of gastric irritation effect of this drug.

Key words: Non steroidal anti-inflammatory drugs (NSAIDS), solidified reverse micellar solutions (SRMS), tablets, ulcerogenicity, antinociception, lipids.

INTRODUCTION

Efforts have recently been made to develop gastrointestinally safe non-steroidal anti-inflammatory drugs (NSAIDs) on the basis of reduced ability to interfere with the surface-active phospholipid layer in the gastrointestinal mucus. Lichtenberger et al. (1995) proposed that pre-associating nonsteroidal anti-inflammatory drugs (NSAIDs) with zwitterionic phospholipids prior to their administration should reduce the ability of the NSAIDs to associate with the phospholipids in the mucus gel, and should therefore reduce their ulcerogenicity. They

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demonstrated this to be the case by pre-associating aspirin and other NSAIDs with dipalmitoyl-phosphatidylcholine (DPPC) in evaluating their ulcerogenicity. The complexes produced significantly less damage in the gastrointestinal tract (GIT) than 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 (Wallace, 2000).

Lipid excipients are generally regarded as safe (GRAS) and have been proven to be non-toxic (Chime et al., 2012a). 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 dosage forms (Attama et al., 2009; Chime et al., 2012a). The widening availability of lipid excipients with specific characteristics offers flexibility of application with respect to improving the bioavailability of sparingly soluble drugs and manipulating their release profile (Attama and Nkemnele, 2005). Most lipids do not exert pharmacological effect and are relatively cheap and widely distributed in nature. For poorly water soluble drug molecules whose dissolution in water is likely the limiting step for overall oral absorption, the primary role of ingested lipids and their lipolytic products is to impact the drug dissolution step by forming with bile components different colloidal particles, which are able to maintain a larger quantity of hydrophobic drugs in solution via micellar solubilization (Porter et al., 2007). The primary mechanism of action which leads to improved bioavailability is usually avoidance or partial avoidance of slow dissolution process which limits the bioavailability of hydrophobic drugs from conventional solid dosage form (Pouton, 2000).

Solidified reverse micellar solution (SRMS) based carriers have been investigated and successfully employed to achieve controlled release of drugs (Umeyor et al., 2012; Schneeweis and Müller-Goymann, 2000; Friedrich and Müller-Goymann, 2003). SRMS consisting of phospholipid and solid lipid such as Softisan® 154, a completely hydrogenated palm oil transform into a lamellar mesophase after melting on contact with water. This transformation enables controlled release of solubilized drugs. SRMS also offer a high solubilization rate of different types of drugs (Friedrich and Müller-Goymann, 2003). SRMS carriers have recently been investigated as a sustained release matrix for both hydrophilic and hydrophobic NSAIDS (Chime et al., 2012b, c; Chime et al., 2013).

The objectives of the work were to formulate SRMS-based indomethacin in order to enhance the oral bioavailability of the drug and eliminate the severe gastric irritation often encountered with the use of indomethacin and to evaluate *in vivo*, the anti-inflammatory, anti-nociceptive and ulcerogenicity of the formulations.

MATERIALS AND METHODS

Chemicals

The following materials were used as procured from their suppliers without further purification: Indomethacin (Merck, Germany), Softisan® 154 (Schuppen, Condea Chemie GmbH, Germany), Phospholipon® 90H (Phospholipid GmbH, Köln, Germany), and distilled water (UNN Water Resources Management Lab. Ltd., UNN, Enugu State, Nigeria). Plastic mould used was constructed in the Faculty of Engineering, University of Nigeria, Nsukka. All other reagents and solvents were analytical grade and were used as supplied.

Preparation of solidified reverse micellar solutions (SRMS)

Mixtures of Phospholipon® 90H and Softisan® 154 (1:1, 1:2 and 2:1 w/w) were prepared by fusion. In each case the lipids were weighed, melted together and stirred at a temperature of 70°C using a magnetic stirrer, until a homogenous, transparent white melt was obtained. The homogenous mixture was stirred at room temperature until solidification (Attama et al., 2009; Chime et al., 2012).

Thermal analysis

Melting transitions and changes in heat capacity of Phospholipon® 90H, Softisan® 154, indomethacin, SRMS 1:1, 2:1 and 1:2, were determined using differential scanning calorimeter (Netzsch DSC 204 F1, Germany). About 10 mg of each sample was weighed into aluminum pan, hermetically sealed and the thermal behaviour determined in the range 20 to 500°C, at a heating rate of 10 K/min under a 20 ml/min nitrogen flux.

Validation of plastic mould

The plastic mould used for tablet production was validated by formulating bland or unloaded tablets using the lipid matrices. This test was performed in order to determine the amount of SRMS that would be used in the formulation of each tablet so as to ensure the reproducibility of the process and also to ensure the uniformity of weight and drug content of the tablets. A small amount of each of the lipid matrices, SRMS 1:1, 1:2 and 2:1 each was weighed out. This was melted at a temperature of 70°C and introduced into the wells of the plastic mould and allowed to solidify. The tablets were properly scraped and removed thereafter. The tablets were weighed and the average weight was recorded.

Preparation of indomethacin tablets based on SRMS

With reference to the average weight of the bland tablets prepared with 1:1, 1:2 and 2:1 w/w of the SRMS, the amount of indomethacin to be incorporated into each tablet was calculated. The composition of the tablets is shown in Table 1. Each of the SRMS was weighed out and placed in a crucible. This was melted at 70°C using a magnetic stirrer hot plate. The required amount of the active ingredient was weighed out and transferred quantitatively into the melted lipid matrix in the crucible with stirring until a homogenous mix was obtained (Umeyor et al., 2012). The homogenous mix was scooped into the wells of the mould with a clean stainless spoon. It was allowed to solidify, scraped and allowed to dry at room temperature. The tablets were pressed out of the plastic mould and

allowed to dry properly at room temperature.

Characterization of the tablets

Uniformity of weight

Twenty tablets were randomly selected from each batch. The tablets were weighed individually using an electronic balance (Ohaus Adventurer, China) and the individual weights recorded. The mean weight, standard deviation and percentage deviation were calculated.

Determination of surface morphology of the tablets

About 20 tablets were randomly selected and evaluated in terms of shape, colour and size. The shape and colour were determined visually by placing the tablets on a plain white sheath of paper; also the photographs of the tablets were taken. The size was determined by the use of venier caliper to determine the tablet thickness and diameter.

Softening/liquefaction time of the tablets

This test is important because the tablets will first of all soften or liquefy for a substantial amount of the drug to be released from the SRMS. The method described in the European Pharmacopoeia (Ph. Eur., 2005) was adopted. The test was carried out for each batch of the tablet using a beaker containing 250 ml of simulated intestinal fluid (SIF) (pH, 7.5) maintained at 37 ± 1°C. An inner compartment sealed at one end, containing a tablet, was tied with a thermo-resistant thread unto the clamp of a retort stand and immersed into the medium. The time taken for the tablets to soften, determined by an appreciable change in shape, was recorded.

Erosion time of tablets

A method described in the European Pharmacopoeia (Ph. Eur., 2005) was adopted. The test was carried out for each batch of the tablet using a beaker containing 500 ml of SIF (pH, 7.5) maintained at 37 ± 1°C. A thermometer was inserted into the medium to maintain the temperature. An inner compartment sealed at one end and containing 3 tablets from each batch was tied with a thermo-resistant thread unto a resort stand, and immersed into the medium. The medium was stirred at 100 rpm with a magnetic stirrer bar. The erosion time was taken as the time taken for the tablet to change in shape and erode appreciably. This test was repeated three times for each batch and the mean erosion time was determined. Erosion time is central to bioavailability, because the tablet should erode appreciably in order to enhance drug release and make the drug available for absorption.

Content of active ingredient

Beer's calibration curve was obtained at a concentration range of 0.1 to 1.0 mg% for indomethacin in SIF (pH 7.5) at a predetermined wavelength of 298 nm. Twenty tablets were randomly selected from each batch of the tablets. The tablets were weighed together and crushed in a mortar with a pestle. An amount equivalent to the average weight of the crushed tablet was weighed out in an analytical balance and dispersed in distilled water. The dispersion was heated for 30 min at 70°C using a magnetic stirrer hot plate, to enhance dispersion. This dispersion was allowed to cool, filtered and an aliquot of the filtrate was assayed using spectrophotometer (Jenway 6305 spectrophotometer, Barloworld Scientific Ltd., Essex CMB 31BWL, UK). The absorbance was recorded and the concentration of indomethacin in each tablet was calculated with reference to Beer's plot.

Hardness/crushing strength test

This test was carried out using Monsanto-Stokes hardness tester (Manesty, England). Ten tablets from each batch were randomly selected. Each tablet was placed between the jaws of the hardness tester and force was applied by adjusting the knob of tester until the tablet integrity failed. The results were recorded in kgf.

Tablet friability test

Twenty tablets were randomly selected from each batch of the tablet. The tablets were dedusted and weighed. The tablets were placed into the drum of the friabilator (Erweka GmbH, Germany) and rotated 100 times at 25 rpm for 4 min. The tablets were removed from the friabilator, dedusted again and reweighed. The friability result was expressed as loss of mass expressed as a percentage of the initial mass (BP, 2009). The percentage friability was calculated from the equation 1:

$$\text{Friability (\%)} = \frac{W_i - W_f}{W_i} \times 100 \quad (1)$$

Where, W_i and W_f are the initial weight and final weight of the tablets, respectively.

Anti-inflammatory properties

The anti-inflammatory activity of the indomethacin tablets based on SRMS was carried out using the rat paw oedema test (Winter et al., 1962). 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 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 (Anosike et al., 2009). Adult Wistar rats of either sex (150 to 200 g) were divided into six experimental groups of five rats per group for each drug, respectively. The rats 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 (Winter et al., 1963). The tablet was crushed using mortar and pestle. The dose of indomethacin tablet formulation based on SRMS equivalent to 10 mg/kg of indomethacin was weighed out and dispersed in 0.5 ml of water. This was administered orally to the rats using a 1 ml syringe. The negative control group received normal saline while the positive control group received 10 mg/kg of indomethacin pure sample. 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 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, 6, 7 and 8 h after injection of egg albumin. Average oedema at every interval was assessed in terms of difference in volume displacement of injected paw (V_t - V_o) (Ajali and Okoye, 2009). The percent inhibition of oedema was calculated using the relationship

(Parez, 1996):

$$\% \text{ Inhibition of oedema} = 1 - \left(\frac{a - x}{b - y} \right) 100 \quad (2)$$

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.

Analgesic properties

Analgesic activity was tested in rats using the hot plate method described by Nkomo et al. (2010). Adult Wistar rats of either sex (120 to 205 g) were divided into six experimental groups of five rats per group. The tablet was crushed using mortar and pestle. Each indomethacin tablet formulation equivalent to 10 mg/kg of indomethacin was weighed out, dispersed in 0.5 ml of water and administered orally to the rats using a 1 ml syringe. The control groups received normal saline 5 ml/kg while the reference group received 10 mg/kg of indomethacin pure sample. Rats were placed on hot plate maintained at 55 ± 1°C and the reaction latency in seconds for licking of hind paw or jumping was recorded. Recordings were taken before treatment with the different drugs and 30, 45 min and at 1, 2, 3, 4, 5, 6 and 7 h post treatment. Results were expressed as difference between the baseline reaction latency and the reaction latency at the different time intervals (Nkomo et al., 2010).

Ulcerogenicity of the tablet formulations

The ulcerogenic potentials of the indomethacin tablet formulations based on SRMS were determined using a method described by Chung-Chin et al. (2009). The studies were carried out on healthy Wistar rats (150 to 200 g). The animals were divided into six groups of five animals each. The tablet was crushed using mortar and pestle, the required dose was weighed out and dispersed in 0.5 ml of water. The control group received normal saline, the test group received indomethacin tablets based on SRMS equivalent to 10 mg/kg of indomethacin, while the reference group received pure sample of indomethacin 10 mg/kg orally. The animals were fasted 8 h prior to a single dose of either the control or test compounds, given free access to food and water and sacrificed 17 h later. The gastric mucosa of the rats was examined under a microscope using a 4× binocular magnifier. The lesions were counted and divided into large (greater than 2 mm in diameter), small (1 to 2 mm) and punctiform (less than 1 mm). For each stomach, the severity of mucosal damage was assessed according to the following scoring system: 0 – no lesions or one punctiform lesions; 1 – two to five punctiform lesions; 2 – one to five small ulcers; 3 – more than five small ulcers or one large ulcer; 4 – more than one large ulcers.

Statistical analysis

Statistical analysis was done using statistical package for social sciences (SPSS) version 14.0 (SPSS Inc. Chicago, IL.USA). All values are expressed as mean ± standard deviation (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

Thermal analysis

The thermograms of the materials are shown in Figure 1 and from the results, the differential scanning calorimetry (DSC) curve of Softisan® 154 showed a narrow endothermic peak, with melting peak at temperature of 61.4°C. Phospholipon® 90H showed a curve with the melting peak at temperature of 124°C. Also, indomethacin DSC curves showed a melting peak at 162.2°C. The sharp peak showed the presence of pure crystalline indomethacin. This value is comparable to the melting temperature recorded for indomethacin in BP (2009). The DSC results of the lipid matrices showed that the structuring of Softisan® 154 with P90H generally produced matrices with low enthalpies.

Surface morphology

The results of dimensional properties of the tablets are shown in Table 2. From the results indomethacin tablets had stable dimensional properties. The tablets had uniform diameter of 1.20 cm while the thickness of the tablets ranged from 0.33 ± 0.01 for N_2 tablets to 0.35 ± 0.01 for N_3 tablets.

Softening/liquefaction time

For a substantial amount of the drug to be released from the lipid matrix, the tablets will first of all soften or liquefy. Generally from the result of softening time presented in Table 2, the indomethacin tablets prepared with the lipid matrix, SRMS 2:1 exhibited the highest softening time of 102.6 min, significantly different form tablets formulated with the lipid matrix, SRMS 1:1 and 1:2 ($p < 0.05$), respectively. This may be due to structural enhancement at 2:1 ratio which generated more compact matrix. The lipid matrix, SRMS 2:1 (Phospholipon® 90H:Softisan®154) is particularly good for sustained release oral and parenteral preparations. This is because of absolute elimination of dose dumping often encountered with sustained release dosage formulations.

Weight uniformity

The result of tablet weight uniformity test presented in Table 2 showed that indomethacin tablets formulated complied with BP specifications and their percentage deviations were significantly lower than 5% ($p < 0.05$).

Tablet friability

From the results of tablet friability test presented in Table

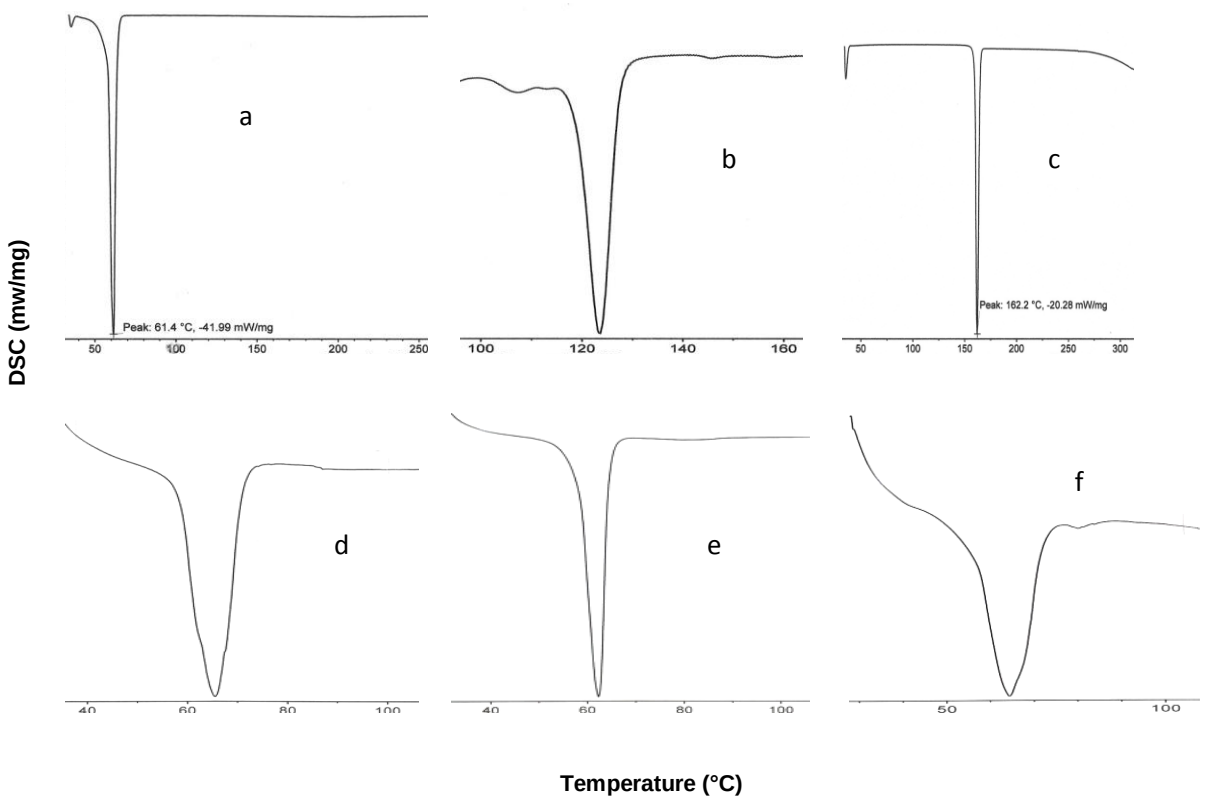

Figure 1. DSC thermograms of: (a) Softisan® 154, (b): Phospholipon® 90H, (c): Indomethacin, (d): SRMS 1:1, (e): SRMS 2:1, (f): SRMS 1:2.

Table 1. Composition of indomethacin tablets.

Batch	LM (Phospholipon 90H:Softisan® 154)	Indomethacin (mg)	Ratio of drug to lipid matrix (Drug: LM)
N_1	1:1	75.0	1:3.9
N_2	2:1	75.0	1:4.0
N_3	1:2	75.0	1:4.1

LM = lipid matrix, N_1 - N_3: Indomethacin tablets.

2, all the batches of indomethacin tablets formulated complied with BP (2009) standards for tablet friability test with friability results significantly lower than 1% ($p < 0.05$).

Crushing strength of tablets

The crushing strength test was performed on the tablets to determine the hardness profile of the tablets. From the crushing strength results presented in Table 2, all the batches of indomethacin tablets complied with the BP (2009) specification for crushing strength test of approximately 5 kgf.

Erosion time of tablets

Erosion time is important because the tablet should erode appreciably in order to enhance drug release and make the drug available for absorption. From the results of erosion time test presented in Table 2, all the tablet batches prepared with different ratios of lipid matrix passed the erosion time test. However, indomethacin tablets formulated with lipid matrix, SRMS 2:1 had the highest erosion time of 180.40 ± 1.06 min for N_2 tablets.

Content of active ingredient

The drug content of the formulated tablets were studied

Table 2. Properties of indomethacin tablets.

Batch	Diameter (cm)*	Thickness (cm)*	Weight (mg±CV)*	Hardness (kgf)[a]	Friability (%)*	Softening time (min)[a]	Erosion time (min)[a]	Drug content (mg±CV)*
N_1	1.20±0.05	0.34±0.01	369.00±0.18	5.10±0.24	0.07	64.8±0.4	134.0±2.8	75.27±0.39
N_2	1.20±0.01	0.34±0.01	369.00±0.37	5.60±0.36	0.06	102.6±0.5	180.0±1.1	75.12±1.10
N_3	1.20±0.01	0.35±0.01	379.00±0.29	5.00±0.39	0.09	53.7±0.5	124.0±0.5	75.20±0.75

*Mean for 20 tablets ± SD, [a]Mean for 10 tablets ± SD, CV: Coefficient of variation SD: Standard deviation, LM = lipid matrix, N_1 - N_3: Indomethacin tablets prepared with LM 1:1, 2:1 and 1:2, respectively.

Table 3. Result of anti–inflammatory properties of indomethacin tablets.

Batch	Paw volume (oedema) (ml) and percentage inhibition of oedema (%)								
	30 min	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h
N_1	0.83±0.03(24.5)	0.8±0.04 (27.3)	0.62±0.03 (27.9)	0.56±0.00 (30.0)	0.42±0.03* (44.0)	0.27±0.03* (61.4)	0.15±0.01 (78.6)	0.12±0.03 (82.6)	0.12±0.00 (82.6)
N_2	0.87±0.03 (13.0)	0.78±0.04 (29.0)	0.61±0.04 (29.0)	0.56±0.03 (30.0)	0.44±0.05* (41.3)	0.39±0.02* (44.3)	0.29±0.02 (58.6)	0.17±0.03 (75.4)	0.12±0.00 (80.0)
N_3	0.90±0.01 (10.0)	0.90±0.04 (10.0)	0.74±0.02 (14.0)	0.63±0.01 (21.3)	0.52±0.03* (30.7)	0.41±0.02* (41.4)	0.28±0.03 (60.0)	0.64±0.02 (76.8)	0.11±0.00 (82.3)
R_2	0.91±0.02 (9.0)	0.93±0.03 (15.5)	0.70±0.01 (17.6)	0.60±0.04 (25.0)	0.50±0.04* (33.3)	0.36±0.05* (48.6)	0.19±0.02 (73.4)	0.12±0.03 (82.6)	0.10±0.00 (83.2)
O	1.0 ±0.02	1.10±0.01	0.86±0.05	0.80±0.07	0.75±0.01	0.70±0.01	0.70±0.05	0.69±0.03	0.62 ±0.04

*Reduction in oedema 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. N_1 to N_3: indomethacin tablets, R_2: pure indomethacin, O: control.

in order to determine whether they complied with BP (2009) standards, and to determine if the drug was lost either by physical or chemical treatments. From the results of drug content presented in Table 2, all the tablet batches complied with the BP (2009) standard for assay of active ingredient. All the tablets were within the range of 90 to 110% of the average value.

Anti–inflammatory properties

The results of anti-inflammatory properties of indomethacin tablets formulations presented in Table 3 and Figure 3 show that indomethacin tablets prepared with varying ratios of lipid matrix had significant anti-inflammatory properties comparable to the reference drug and varied significantly from the control from 1 to 8 h ($p < 0.05$). At 30 min, indomethacin tablets formulated with lipid matrices, SRMS 1:1, 2:1 and 1:2 (N_1, N_2 and N_3) inhibited the oedema by 24.5, 10 and 13%, respectively. At 8 h, the indomethacin tablets prepared with the lipid matrices, SRMS 1:1, 1:2 and 2:1 showed percentage oedema inhibition of 82.6, 82.3 and 80%, respectively as shown in Figure 3. The results are comparable to the effect of the reference drug, indomethacin pure sample which exhibited 83.2% oedema inhibition at 8 h.

Results of analgesic/antinociceptive properties

The results of analgesic properties presented in Table 4 showed that indomethacin tablets formulated with the lipid matrix, SRMS 1:1 had an increase in pain reaction time at 7 h significantly ($p < 0.05$) different from the results exhibited by tablets formulated with the lipid matrices, SRMS 1:2 and 2:1, which showed a decrease in pain reaction time at 7 h as shown in Figure 4. The tablet formulations varied significantly ($p < 0.05$) from the control (normal saline). However, the tablets formulated with the lipid matrices, SRMS 2:1 and 1:2 (N_2 and N_3) showed maximum analgesic effect

Table 4. Analgesic/antinociceptive properties of indomethacin tablets.

Group	Pain reaction time (s)								
	30 min	45 min	1 h	2 h	3 h	4 h	5 h	6 h	7 h
1:1 (N_1)	3.83±1.16	5.04±0.51	6.41±0.70	7.62±0.56*	9.84±1.70*	12.01±1.05*	14.46±1.41*	16.02±1.22*	16.46±0.72*
2:1 (N_2)	3.26±1.36	4.50±1.66	5.86±1.69	8.05±1.88*	11.75±2.50*	13.84±2.16*	17.35±1.48*	19.21±1.10*	14.41±1.12*
1:2 (N_3)	2.84±1.21	5.37±0.67	6.46±1.01	8.03±1.77*	9.76±1.75*	11.69±1.59*	13.77±1.25*	16.34±1.15*	15.44±0.56
(R_2) Ref.	3.82±1.12	4.86±0.67	6.40±1.52	9.10±1.14*	11.09±1.16*	13.49±1.08*	16.01±1.22*	18.83±1.40*	14.92±2.44
(O) Cont.	2.71±0.66	2.77±0.89	2.76±0.90	3.55±0.41	3.07±0.50	3.33±0.49	4.01±0.93	3.70±1.10	3.74±0.91

*Significant at $p < 0.05$ compared to control. Values shown are mean ± SD (n = 5), N_1 to N_3: indomethacin tablets, R_2: pure indomethacin, O: normal saline.

at 6 h.

Ulcerogenicity studies

Ulcerogenicity of indomethacin tablet formulations was studied in order to determine the effect of these formulations on the GIT. Indomethacin tablet formulations based on SRMS reduced the ulcerogenicity of the indomethacin by 70 to 80% as shown in Table 5.

DISCUSSION

The DSC measurements were carried out in order to determine the melting points of indomethacin, Softisan®, Phospholipon® 90H, SRMS 1:1, 2:1 and 1:2. The narrow melting peak of Softisan®154 indicated that it is a high purity lipid. The DSC thermogram of Phospholipon® 90H revealed that it consists entirely of stable form because of the sharp melting peak seen. Thermograms of the lipid matrices showed that (Figure 2d, e and l) SRMS 1:1, 2:1 and 1:2 generated imperfect matrices (due to distortion of crystal arrangement of individual lipids after melting and solidification), which may have created numerous spaces for drug localization (Umeyor et al., 2012; Chime et al., 2012). That was the reason for low enthalpies they exhibited. The varied fatty acid contents of these lipids may have interacted in such a manner as to partly disorder the crystal arrangement of the individual lipids (Sanna et al., 2004; Jaspart et al., 2005; Attama and Muller-Goymann, 2007; El-Kamel et al., 2007; Umeyor et al., 2012). Reduction in enthalpy generally suggests less crystallinity of lipid matrices (Umeyor et al., 2012; Attama et al., 2006).

The dimensional properties of indomethacin tablets based on SRMS showed that the tablets were smooth and spherical with no form of depressions or cracks seen in any of the batches as shown in Figure 2. The nature of drug contributed to the smooth and uniform surface seen in the tablets. Indomethacin solubilized in the lipid matrix and gave light yellow tablets. The low standard deviation of the dimensional properties confirmed the reproducibility of the method of production and reliability of this formulation. Weight uniformity test was performed on the tablets so as to determine its compliance with BP specifications (2009). Variation in weight of tablets causes variation in drug content which will also affect the bioavailability of the drug. The low coefficient of variation of tablet weight uniformity confirms the reproducibility of the formulation. The results showed that batch N_3 formulated with lipid matrix 1:2 (Phospholipon® 90H: Softisan® 154) exhibited higher mean weight than other batches of the tablets. This may be due to increase in density of the lipid matrix with increased Softisan® 154 ratio. The results of tablets friability showed that the tablets can withstand handling, packaging and transportation without affecting the integrity of the products. The results of crushing strength test also showed that the mechanical properties of the tablet will not be compromised during long term storage. The erosion time of the tablets studied in SIF (pH 7.5) showed that the formulations showed properties as sustained release tablets. Sustained release preparation and enteric coated tablets are expected to disintegrate or erode appreciably in SIF within 2 h (Ofoefule, 2002). However, batch N_2 had erosion time of up to 180 min; this may be due to the lipophilicity of the drug in the lipid matrix and formation of more compact matrix seen in lipid matrix, SRMS 2:1. Therefore, these results showed that indomethacin tablets based on SRMS could have good sustained release contents of the tablets studied showed that the drug was

Table 5. Result of ulcerogenicity of indomethacin tablets.

Group	Ulcer score	Percentage ulcer inhibition (%)	Ulcer diameter (mm)
1:1 (N_1)	0.80±0.90*	80.00	Lesion<1
2:1 (N_2)	1.20±0.58*	70.00	Lesion<1
1:2 (N_3)	1.00±0.71*	75.00	Lesion<1
Reference (R_1)	4.00±0.52*	0.00	Lesion>2
Control (O)	0.00±0.00	100.00	No lesion

Values shown are mean ± SD. (n = 5). *Significantly different from control at $p < 0.05$, N_1 to N_3: indomethacin tablets, R_1: pure indomethacin, O: normal saline.

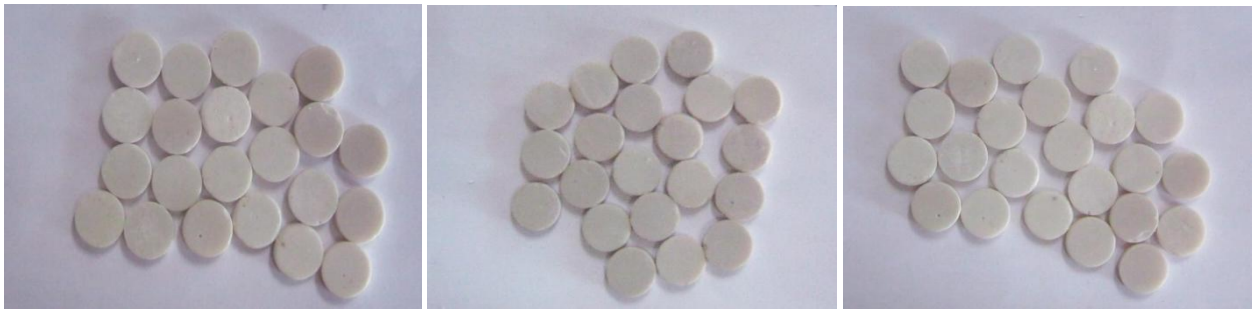

Figure 2. Indomethacin tablets formulated with lipid matrix ratio (SRMS) 1:1, 2:1 and 1:2 (N_1, N_2, and N_3), respectively.

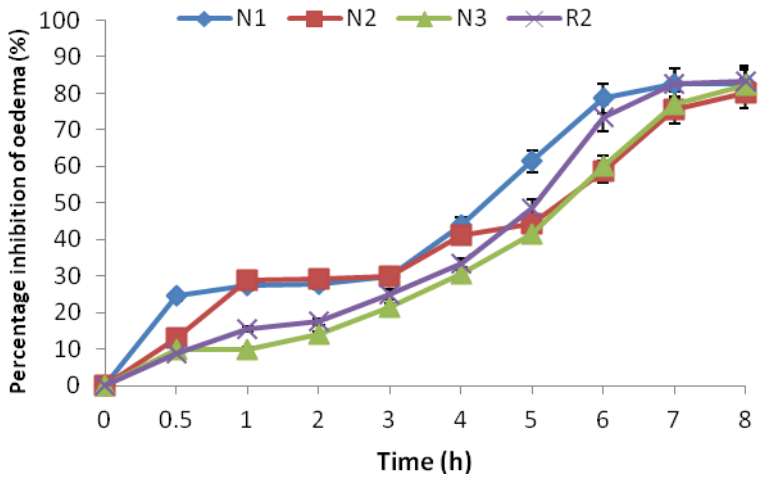

Figure 3. Plot of percentage oedema inhibition against time for indomethacin tablets formulated with SRMS 1:1, 2:1 and 1:2, respectively (N_1, N_2 and N_3) and reference drug (R_2- indomethacin pure sample, 10 mg/kg).

not lost either by physical or chemical means. The low coefficient of variation obtained in the study attests to the reproducibility and reliability of the formulation process.

The anti-inflammatory properties of the indomethacin tablets based on SRMS showed that the formulations exhibited good anti-inflammatory properties. However, the ratio of phospholipids affected the anti-inflammatory properties. Indomethacin tablets formulated with lipid matrix, SRMS 2:1 (N_2), containing higher amount of phospholipid showed lower anti-inflammatory properties

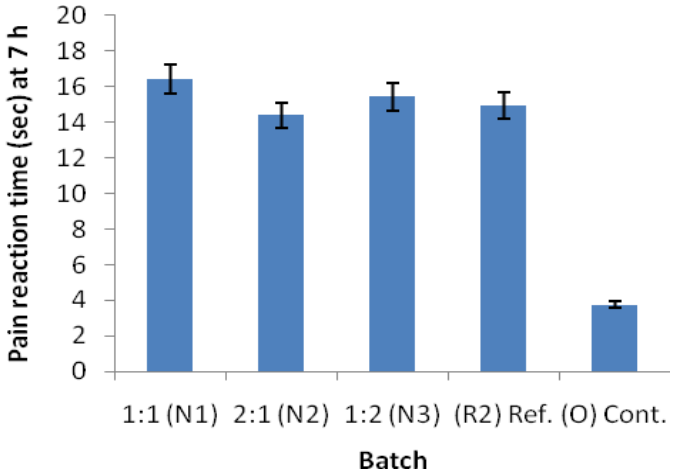

Figure 4. Antinociceptive properties of indomethacin tablet formulations at 7 h. N_1 to N_3: indomethacin tablets, R_2: pure indomethacin, O: normal saline.

from 6 to 8 h significantly different from other batches ($p < 0.05$). This may be due to the hardness, high softening and erosion time exhibited by these batches.

The antinociceptive/analgesic properties of indomethacin tablets showed that the formulations exhibited higher analgesic properties than the reference drug, due to enhanced oral absorption of indomethacin in the presence of lipids. The tablets formulated with the lipid matrices, SRMS 2:1 and 1:2 (N_2 and N_3) showed faster maximum analgesic effect than other formulations. This may be due to the form in which the drug was administered; the tablet was crushed before administration. This may have affected some of the mechanical properties of the tablets for example, the hardness, softening and erosion time of tablets. Therefore, indomethacin tablets prepared with varying ratios of lipid matrix showed good analgesic/anti-nociceptive properties. The results also revealed that the indomethacin tablets based on SRMS may have sustained release properties in addition to other properties.

The ulcer inhibition properties of the indomethacin tablets based on SRMS showed that the SRMS inhibited the ulcerogenic potentials of the highly ulcerogenic indomethacin. Also, the ratios of phospholipid used in the formulations had significant effect on the result as shown in Table 5. The N_1 tablets, formulated with the lipid matrix, SRMS 1:1 exhibited up to 80% ulcer inhibition. The formulations therefore, showed good gastro-protective potentials. The result is in agreement with the work done by Lichtenberger et al. (1995), who proposed that pre-associating NSAIDs with zwitterionic phospholipids prior to their administration should reduce the ability of the NSAIDs to associate with the phospholipids in the mucus gel, and should therefore reduce their ulcerogenicity.

Conclusion

Solidified reverse micellar solutions consisting of phospholipid and triglyceride presented good matrices for the delivery of indomethacin. The results of the *in vitro* properties of the tablets showed that the tablets had good physicochemical properties that complied with specifications. The results of the *in vivo* studies showed that SRMS inhibited the ulcerogenicity of the highly ulcerogenic indomethacin. The formulations also exhibited good anti-inflammatory and antinociceptive/ analgesic effect. Therefore, incorporating indomethacin into the SRMS enhanced the *in vivo* properties of indomethacin due to enhance absorption caused by the presence of the lipids. Indomethacin tablets based on SRMS have advantages over the commercial indomethacin which include: low cost of ingredients, low cost of technologies, little or no ulcerogenicity and better control of pain and inflammation. Further research into this field of study is highly encouraged in order to effectively scale up all its aspects and finally make this product available in the market.

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