Application of *Cyperus esculentus* oil in the development of sustained release diclofenac sodium-loaded nanostructured lipid carrier

Salome A. Chime[1]*, Calister E. Ugwu[1], Emmanuel U. Ejiofor[1] and Godswill C. Onunkwo[1]

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

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*Corresponding author: Salome.chime@unn.edu.ng

Abstract

The aim of the work was to develop sustained release diclofenac sodium nanostructured lipid carrier (NLC) using tigernut oil (TNO), solid lipids and polyethylene glycol 4000 (PEG 4000) and to evaluate the properties of the formulations. Structured lipids containing tiger nut oil, Softisan®154 and Phospholipon® 90H at varying ratios were prepared by fusion and used as the lipid carrier in formulating the NLC. PEGylated lipid carriers were also employed and the physicochemical properties of these formulations were studied using standard methods including particle size and polydispersity index, encapsulation efficiency, loading capacity and drug release. The results revealed some monodispersed nano-sized formulations that were stable over time. Particle size ranged from 75.22 ± 22.72 nm to 78.11 ± 32.73 nm. High encapsulation efficiency of about 92 % was obtained confirming the suitability of the TNO based carrier. *In vitro* drug release in simulated intestinal fluid (pH 7.2) revealed that PEGylated diclofenac sodium-loaded NLC exhibited significantly higher sustained release properties than the non-PEGylated formulations ($p < 0.05$). The results of the drug release kinetics models revealed that the NLC followed a mixed order release kinetic. Hence, the findings in this work showed that TNO could be used as a lipid carrier matrix in combination with other solid lipids for the development of sustained release diclofenac sodium-loaded nanostructured lipid carrier.

Keywords: *Cyperus esculentus*, diclofenac sodium, nanoparticles, PEGylation, Tiger nut oil.

Introduction

Cyperus esculentus L. var. sativus (Igbo name- aki Hausa, Yoruba name- imumu) commonly called tiger nut, chufa or earth almond is rhizome and a perennial herb (De Vries, 1991). These nutshave been used as culinary and medicinal purposes, especially by the ancient Egyptians (Negbi, 1992; Eze *et al.*, 2014). Specifically, the use of tigernut by Egyptians dated back to the 5th millennium BC, however, these tubers are now cultivated in many countries including Nigeria, Spain, America, China etc. Tiger nuts can be ground for beverages, roasted and majorly eaten raw by most people (Tortajada., 2010).

Tiger nut tubers contain about 15.9 to 41.2 % oil (Lasekan and Abdulkarim, 2012; Yeboah et al., 2012); the yield of oil is affected by origin of the tubers, age of the tissue or genetic history (Eteshola and Oraedu, 1996; Eze et al, 2014). This oil is obtained majorly by soxhlet extraction using

n- hexane (Yeboah et al., 2012), however, there are other extraction methods outside the scope of this work. TNO is comparable to olive oil in terms of the fatty acid composition (Cos¸kuner *et al*., 2002; Arafat, et al., 2009; Sánchez-Zapata *et al*., 2012;), with oleic acid as the most component fatty acid (Eze *et al*., 2014). Since tiger nut is grown in Nigeria, provide local names for easier identification

The use of oleic acid containing lipids from plant origin cannot be over emphasized as they are beneficial for the overall well-being of the heart especially in geriatrics (WHO, 2003). Recent research revealed that dietary unsaturated fatty acids play major roles in preventing the development of most terminal diseases including diabetes, hypertension and cancer (Lunn and Theobald, 2006). This is possible due to their content of essential fatty acids and vitamins and phenolic compounds. TNO is rich in Vitamin E (tocopherols) which is responsible for their antioxidant properties, hence is very good excipient for the formulation of pharmaceuticals (Ali Rehab et al., 2012). TNO also contains about 5.4 % phospholipids including choline, serine glycerophospholipid, ethanolamine and inositol (Kim *et al*., 2007; Oderinde and Tairu, 1992). TNO also contains 3.0 % unsaponifiable matter consisting of hydrocarbons, sterols, waxes, higher alcoholic esters and triterpene alcohols (Oderinde and Tairu, 1992; Eze *et al.*, 2014).

Our focus in this paper is therefore, to formulate sustained release diclofenac sodium for enhanced oral delivery in the treatment of various degrees of pain and inflammation using tigernut oil structured lipid carrier. Formulating NSAIDs using suitable zwitterionic phospholipids reduces their ability to associate with the mucosal phospholipids thereby reducing gastrointestinal irritation (Lichtenberger *et al*., 1995; Wallace, 2000). Lipid based formulations have proven gastroproctective potentials against non-steroidal anti-inflammatory drugs (NSAIDS) induced gastric ulcers (Obitte *et al*., 2012; Obitte *et al*., 2013; Momoh *et al*., 2013; Chime *et al*., 2020). Hence, developing diclofenac sodium-entrapped nanostructured lipid carrier (NLC) would impart gastroprotection as well as sustained release to this NSAID. In addition, NLC, a second generation of lipid nanocarriers were chosen due to their advantages such as cost effectiveness, nontoxicity over the other types of lipid carriers such as solid lipid nanocarriers, liposomes and enhanced drug loading. TNO were chosen for this formulation in order to incorporate its potentials and rich micronutrients into these formulations. To the best of our knowledge, this is the first report that shows the excipient potentials of tiger nut oil in the development of NLC. The NLC developed were evaluated for their physicochemical properties such as particle size, zeta potential, encapsulation, loading capacity, which are the indicators of formulation stability and performance.

Materials and methods

Chemicals

Diclofenac sodium, Tween® 80, sorbitol, polyethylene glycol 4000 (Merck, Darmstadt, Germany), Softisan® 154 (Cremer Oleo GmbH, Germany), sorbitol (Wharfedale Laboratories, England), Phospholipon® 90H (Phospholipid GmbH, Köln, Germany), activated charcoal (Bio–Lab. (UK) Limited, London), sodium hydroxide, monobasic potassium phosphate, n-hexane (BDH, Poole, England). Tiger nut

oil 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 Tiger nut oil from Cyperus esculentus tubers

Dried *Cyperus esculentus* tubers was purchased from Nsukka market, Enugu Nigeria and authenticated by Mr. A.O. Ozioko, a consultant taxonomist at the International Center for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Nigeria. The tubers were milled in an equipment of the hammer mill type and TNO was extracted in a Soxhlet using n–hexane. The n-hexane was allowed to completely evaporate at room temperature. The extracted oil was further purified by passing it through a column of activated charcoal and bentonite (2:1) at a ratio of 10 g of oil and 1g of the column material. The oil was stored in an air tight glass container (Onyishi *et al*., 2015).

Preparation of lipid matrix

Lipid matrix carrier was prepared by fusion using optimized ratios of the individual lipids comprising TNO (30 %): S154 (20 %): P90H (50 %) as earlier described (Umeyor *et al.*, 2012).

Preparation of NLC

Drug loaded and bland NLC were prepared by hot homogenization at 70 ^{o}C using a high speed homogenizer (Ultra TurraxT25 Basic Digital, Germany) and a digital magnetic stirrer hot plate (IKA® RCT Basic, Germany). About 8 g of the lipid matrix was used after optimization, melted in a 250 ml beaker, and diclofenac sodium (2 %) was dispersed in it. Water containing the surfactant, Tween® 80 (1.5 %) and preservative, Sorbic acid (0.1 %) at the same temperature with the molten lipid was transferred to the lipid dispersion and homogenized at 18 000 rpm for 30 min (A1-A3). Also, polyethylene glycol (PEG 4000, 1 %, 3 % and 5 %) was incorporated into the lipid matrix and used to formulate other batches (B1-B3) using same procedure, the NLC emulsions formed were stored in containers at room temperature.

Mean particle size and zeta potential

Photon correlation spectroscopy using a Zeta-sizer Nano (Malvern Instruments, Worcestershire, UK) at 25°C (REF23) was employed for the analysis of the mean particle size and polydispersity index (PI). The particle size and PI values were obtained at an angle of 90 degrees with respect to the incident beam in 10 mm disposable polystyrene cells. The zeta potential was measured in disposable plain folded capillary zeta cells by determining the electrophoretic mobility on the particle surface using the same instrument (Zhang *et al.*, 2014)).

Encapsulation Efficiency

Beer's plots of what of diclofenac sodium was obtained over a concentration range of 0.001 to 0.01 mg/ml in distilled water at 278 nm. The drug content was determined in the NLC emulsion. In each case, a 5 ml quantity of NLC from each of the batches was centrifuged at 1,252 × g for 30 min (Chem. Lab. Instrument, UK) using a microconcentrator (Vivaspin 6, Vivascience Honover, Germany). The supernatant was diluted with water (Whatman No. 1) and analyzed in a spectrophotometer (UNICO 2102 PC UV/Vis Spectrophotometer, USA) at a wavelength of 278 nm.

$$\text{Encapsulation efficiency (EE \%)} = \frac{\text{Actual drug content}}{\text{Theoritical drug content}} \times 100 \quad (1)$$

Drug loading capacity (LC)

LC expresses the ratio between the entrapped active pharmaceutical ingredient (API) and the total weight of the lipids and was determined using the relationship (Gugu *et al*., 2015):

$$LC = \frac{Wa - Ws}{Wa - Ws + Wl} \; x \; 100 \quad (2)$$

Where Wl is the weight of lipid in the formulation, Wa is the weight of diclofenac sodium added to the formulation and Ws is the actual amount of diclofenac sodium entrapped in the SLN.

pH study

The pH of the NLC emulsions was determined in time -dependent manner (24 hours, 1 week, and 1 month) using pH meter (Suntex TS-2, Taiwan). No description and no reference

***In vitro* drug release analysis**

The USP XXII rotating paddle apparatus (Erweka, Germany) was employed for this release study. The dissolution medium consisted of 500 ml of freshly prepared simulated intestinal fluid (SIF pH 7.2) maintained at 37 ± 1 ^{o}C. The polycarbonate dialysis membrane selected as release barrier was pretreated by soaking in the dissolution medium for 24 h prior to use (MWCO 6000-8000, Spectrum Labs, Brenda, The Netherlands). A 10 ml quantity of the NLC was placed in the 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-portion of the dissolution medium was withdrawn, appropriately diluted, and analyzed for the content of the drug in a spectrophotometer (UNICO 2102 PC UV/Vis Spectrophotometer, USA) at a predetermined wavelength of 278 nm. Sink condition was maintained by replacing the withdrawn medium with an equal volume of the fresh medium. The amount of drug released at each time interval (0.25 h to 6 h) was determined with reference to Beer's Plot.

Evaluation of drug release kinetics and mechanisms

The drug release data were analyzed using different drug release kinetics models. including zero-order kinetics which describes the systems where the drug release rate is independent of its concentration (Eq. 3). The first order Equation (Eq. 4) which describes the release from systems where release rate is concentration -dependent. Higuchi model (1961, 1963), described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion (Eq. 6). Ritger-Peppas (1987) derived a simple relationship which described drug release from both swellable and non-swellable systems. To find out the mechanism of drug release, the first 60 % drug release data were fitted in Ritger–Peppas model.

$$Q = k_0 t \quad (3)$$

$$\log Q_0 - \log Q_t = k_1 t / 2.303 \quad (4)$$

$$Q = K_2 t^{1/2} \quad (5)$$

$$M_t/M_\infty = K_3 t^n \quad (6)$$

Where, Q is the amount of drug released or dissolved at time t, Q_0 is the initial concentration of drug, k_0, k_1, k_2 and k_3 are zero-order, first-order, Higuchi and Ritger-Peppas kinetic constant respectively. M_t/M_∞ is fraction of drug released at time *t*, *n* is diffusion exponent and is indicative of the mechanism of transport of drug through the matrix (Kalam *et al*., 2007). The following plots were made: cumulative drug release versus time (zero-order), log cumulative of % drug remaining vs. time (first order kinetic model), cumulative % drug release vs. square root of time (Higuchi model) and the integral form of Higuchi, log cumulative % drug release vs. log time and log fraction of drug release versus log time (Kalam *et al*., 2007).

Statistical analysis

Statistical analysis was performed using SPSS version 16.0 (SPSS Inc. Chicago, IL.USA). Data were analyzed by one-way ANOVA. Differences between means were assessed using student's t-test.

Results

Particle size and Polydispersity index

The results of the particle size of the optimized NLC shown in Table 1 showed that Z-average of about 72 nm was recorded for non-PEGylated NLC (Batches A1, A2 and A3). However, for the PEGylated NLC (Batches B1, B2 and B3) particle size ranged from 75.22 ± 22.72 nm to 78.11 ± 32.73 nm. The results also revealed that the particles were monodispersed with PI values of about 0.2 to 0.28.

Encapsulation efficiency and Loading capacity

Table 2 shows the results of EE of diclofenac sodium-loaded NLC. PEGylated diclofenac sodium-loaded NLC had EE range of 91-92 % while, non-PEGylated NLC had EE range of 84 -91 %. However, formulations containing 2 % diclofenac sodium generally had higher EE values of about 92 %. Loading capacity of the lipid matrix ranged from 87 to 93 mg API/100mg lipid, which confirmed their suitability for encapsulating diclofenac sodium.

Table 1: Properties of diclofenac sodium-loaded NLC

Batch	TDC (%)	ADC (%)	EE (%)	LC(mg API/100mglipid)	Z-average (nm ± SD)	PDI
A1	1.0	0.89	89.4 ± 0.18	87.0	-	-
A2	1.5	1.27	84.2 ± 0.19	90.5	-	-
A3	2.0	1.82	91.1 ± 0.29	93.18	72.31 ± 24.43	0.252 ± 4.09
B1	2.0	1.83	91.3 ± 0.15	93.2	75.22 ± 22.72	0.281 ± 3.27
B2	2.0	1.85	92.4 ± 0.21	93.3	77.39 ± 17.28	0.213 ± 2.19
B3	2.0	1.84	91.8 ± 0.29	93.2	78.11 ± 32.73	0.232 ± 3.234

Batches A1-A3, contain 1, 1.5 and 2 % DS; while Batches B1-B3 contain 2 % DS and 1, 3 and 5 % of PEG 4000 respectively. TDC: Theoretical drug content, ADC: Actual drug content; EE: Encapsulation efficiency; LC: Loading

capacity; API: Active Pharmaceutical ingredient; PDI: Polydispersity index; DS: Diclofenac sodium; SD: Standard deviation

pH study

The results of the pH of diclofenac-loaded and bland NLC (Batch A4) are shown in Fig. 1 and revealed that the formulations had neutral pH. Bland NLC had pH of 7.4 on day 1 and 6.9 at 1 month (Batch A4), while the Batch A3 containing 2% of diclofenac sodium and non-PEGylated had Ph of 6.5 and 6.3 on day 1 and I month respectively. Also the PEGylated NLC (Batches B1 to B3) 6.5 at day 1 and 6.3 at 1 month for Batch B1 prepared with 1% of PEG 4000. Hence, PEGylation had so effect on the Ph as shown in Fig. 1

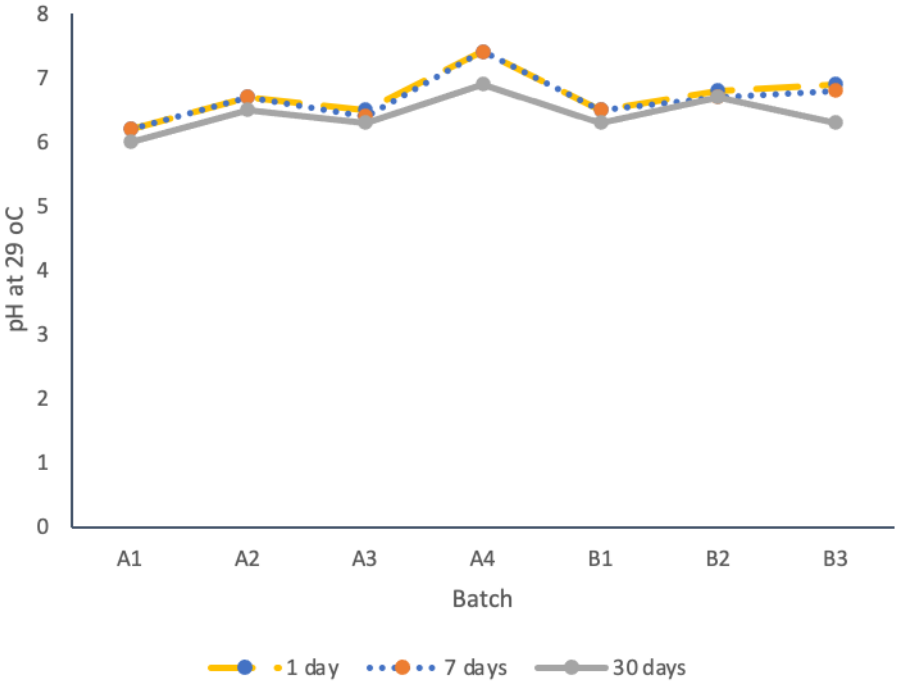

Fig. 1: The pH stability of diclofenac sodium NLC. Batches A1-A3, contain 1, 1.5 and 2 % DS; A4 is placebo non-PEGylated NLC, while Batches B1-B3 contain 2 % DS and 1, 3 and 5 % of PEG 4000 respectively; DS: Diclofenac sodium.

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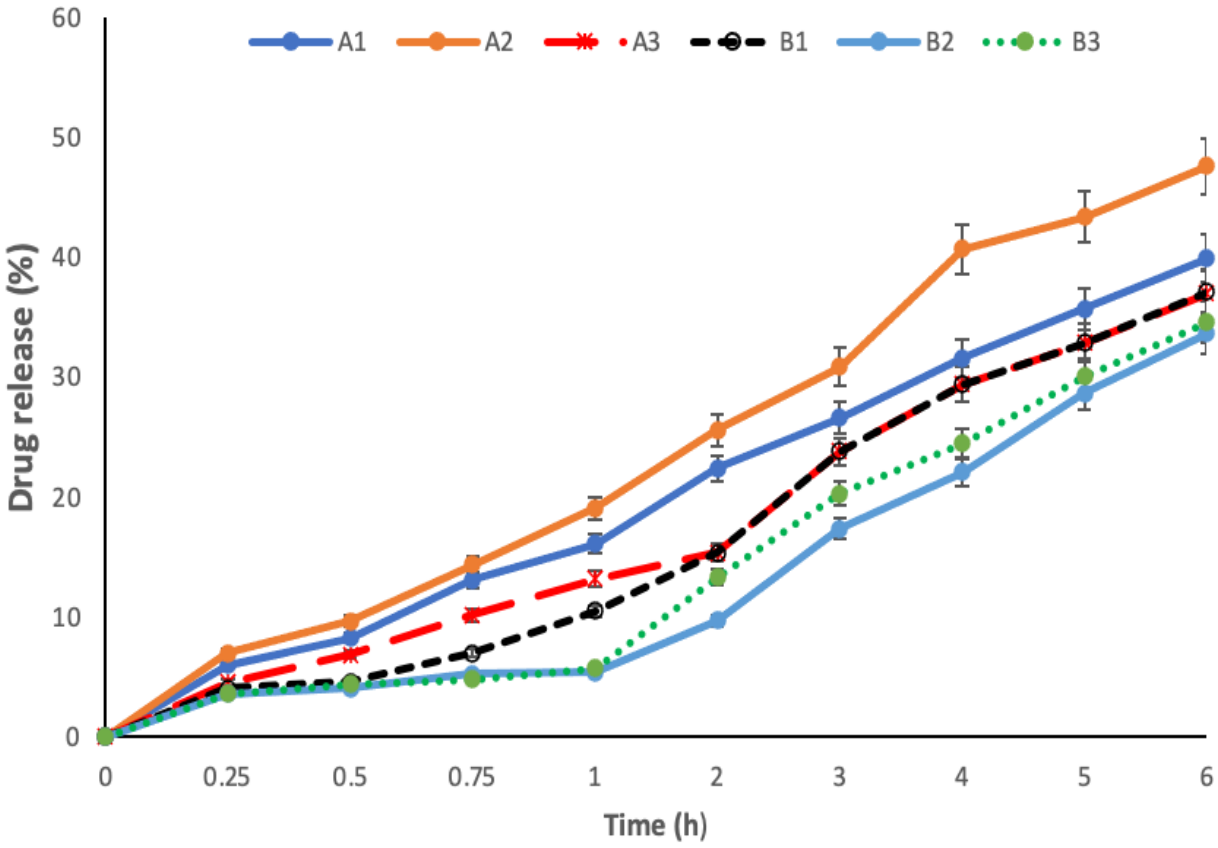

Fig. 2: *In vitro* drug release kinetics of diclofenac sodium-loaded NLC. Batches A1-A3, contain 1, 1.5 and 2 % DS; while Batches B1-B3 contain 2 % DS and 1, 3 and 5 % of PEG 4000 respectively; DS: Diclofenac sodium.

In vitro drug release

The results of the *in vitro* drug release of diclofenac sodium from the NLC are shown in Fig. 2. At 0.25 h, about 5-6 % of diclofenac sodium were released from the non-PEGylated NLC (Batches A1 to A3), while the PEGylated DS-loaded NLC (Batches B1 to B3) had 4 % release. PEGylated DS-loaded NLC had significantly lower initial drug release between 0.25 h to 3 h. However, drug release became similar to non-PEGylated NLC after 5 h, which showed that PEGylated lipid matrix presented a more stable carrier that prevented initial drug release, hence, dose dumping was eliminated as well as early release of drug which when given orally will be able inhibit drug release in the stomach thereby preventing gastric ulceration.

Drug release kinetics and mechanisms

The results of the drug release kinetics are shown in Figs. 3-6. The results of the zero order release kinetics of the DS-loaded NLC are shown in Fig. 3) and revealed that drug

release followed zero order release kinetic model characteristic for sustained release dosage forms (r^2 = 0.9931). Also the First order release kinetics models (Fig. 4) also showed that the drug release also followed first order release kinetics models (r^2 = 0.9959). Higuchi models (Fig. 5 and 6) showed that drug release followed diffusion controlled process ($n \geq 0.5$) (Higuchi, 1963). The Ritger-Peppas plots were also linear and used to analyze the release processes (Fig. 7).

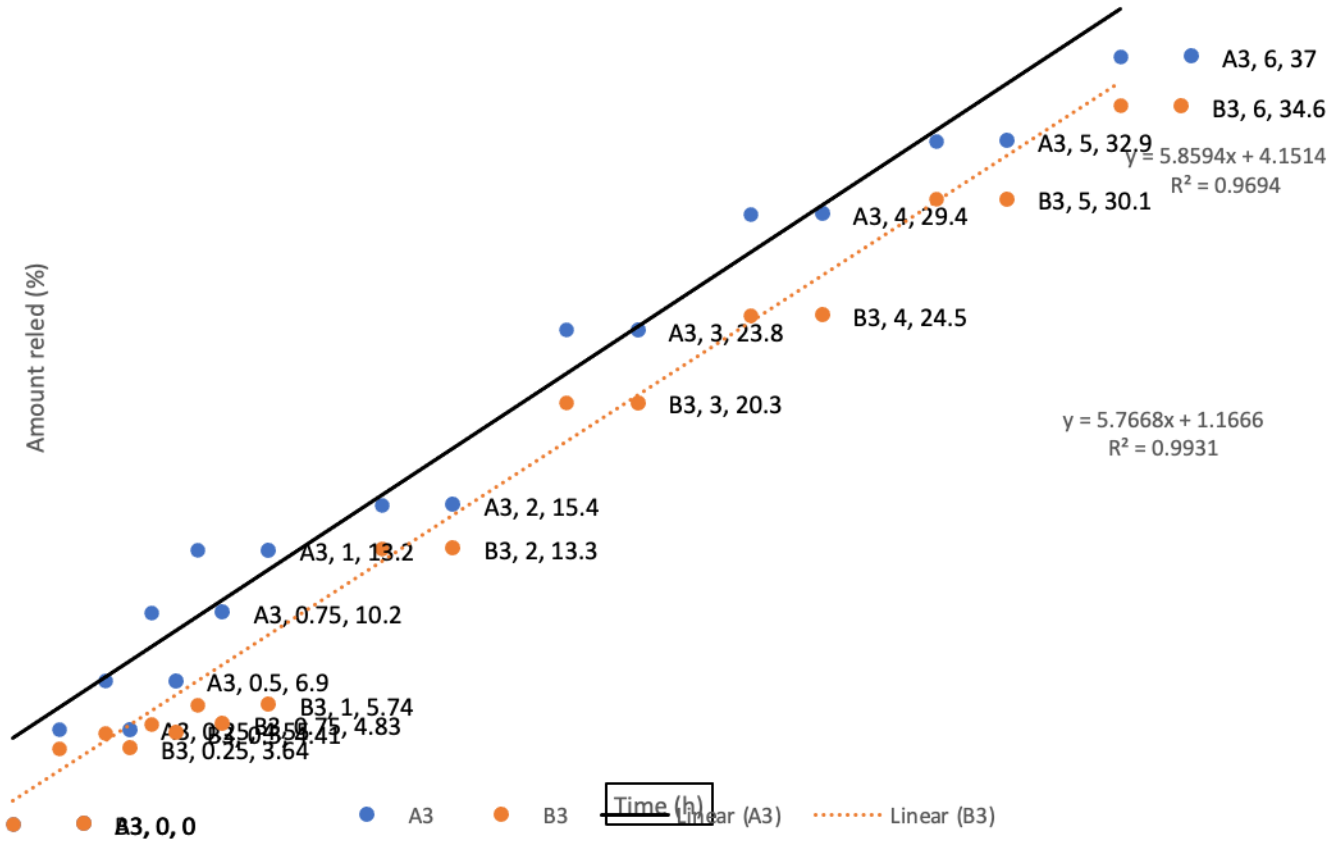

Fig. 3: Zero-order plots of diclofenac sodium-loaded NLC

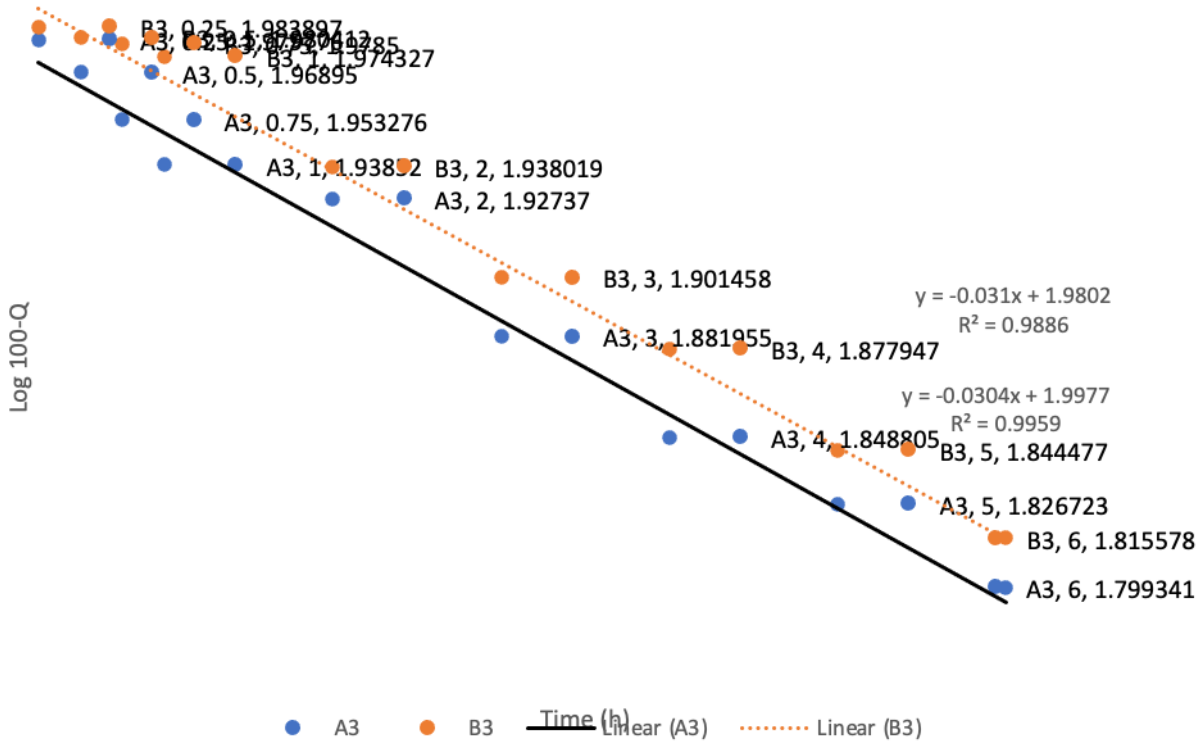

Fig. 4: First order plots of diclofenac sodium-loaded NLC

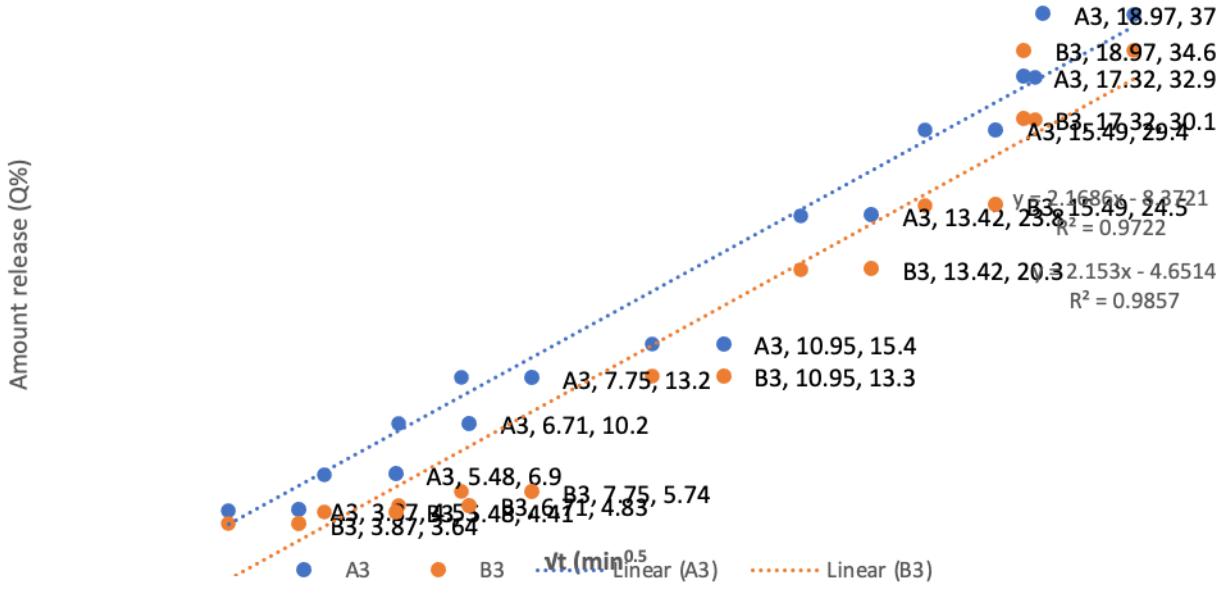

Fig. 5: Higuchi plots of diclofenac sodium-loaded NLC

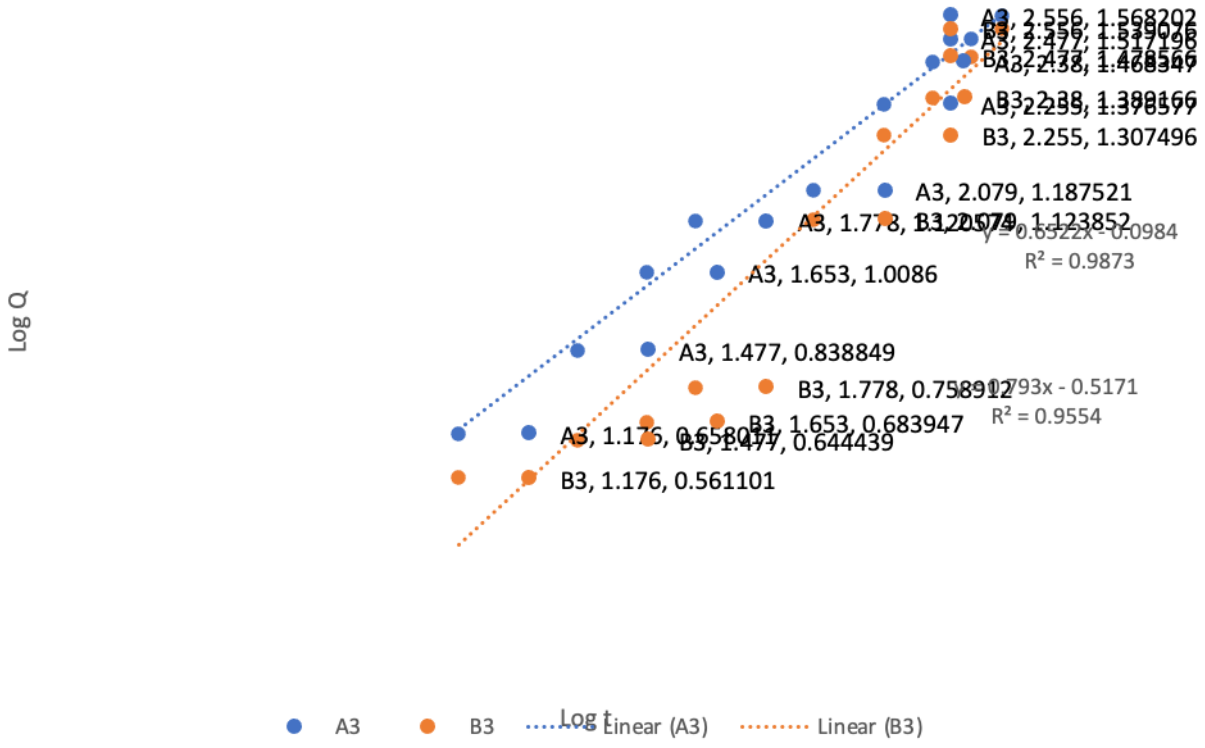

Fig 6: Higuchi integral plots for diclofenac sodium-loaded NLC

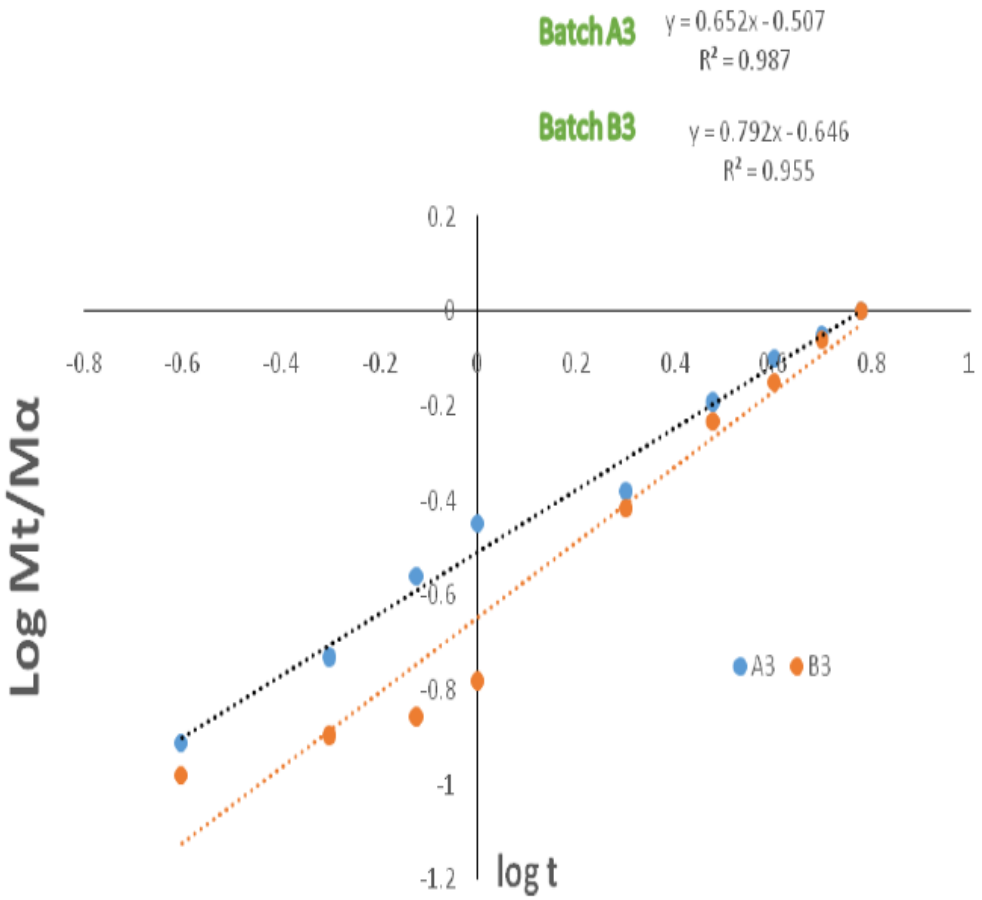

Fig. 7: Ritger-Peppas plot of diclofenac sodium-loaded NLC

Discussion

The results of the particle size of the optimized NLC shown in Table 1 showed that particle size varied directly with the amount of PEG 4000 for Batches B1, B2 and B3 respectively containing 1, 3 and 5 % of PEG 4000 however, results revealed that PEGylation had an insignificant increase in the particle size of NLC as shown in Table 1 ($p < 0.05$). The results also revealed that the particles were monodispersed; PI values < 0.05 to 0.7 are more common to monodisperse samples, while values > 0.7 are common to a broad size (polydispersed) distribution of particles (Thilak *et al*., 2019). Hence, PEGylated TNO-based diclofenac sodium NLC exhibited nanometer sized monodispersed nanoparticles.

The results of EE of diclofenac sodium-loaded NLC showed that generally, the imperfect lipid matrices generated by structuring three different lipids viz TNO, P90H and S154 created real spaces for drug encapsulation which resulted to high entrapment efficiency obtained in all the formulations. PEGylated diclofenac sodium-loaded NLC showed that PEGylation had an

insignificant effect on EE. Highest EE was obtained in formulations containing 2 % diclofenac sodium, hence this batch was taken as the optimized batch for further studies. Batches B1-B3 prepared with PEGylated lipid matrices (1, 3 and 5 % PEG 4000) all had insignificantly higher encapsulation efficiencies than the non-PEGylated Batches ($p < 0.05$) (A1-A3). The results of the LC of the lipid matrix showed that the lipid exhibited generally high LC. The results also showed that LC increased with increasing drug loading in agreement with previous research (Kenechukwu *et al*., 2014; Gugu *et al.,* 2015).

The results of the pH of diclofenac-loaded and bland NLC (Batch A4) shown in Fig. 1 revealed that the formulations had neutral pH. The NLC exhibited an insignificant pH reduction over the study period of 1 day to 1 month ($p< 0.05$) for both bland or placebo formulation (Batch A4) and formulations loaded with diclofenac sodium. The slight reduction is always attributable to release of fatty acids from the lipids and is in agreement with previous research (Umeyor *et al.,* 2012). Hence, stable NLC were developed from the TNO - loaded with diclofenac sodium**.**

The results of the *in vitro* drug release of diclofenac sodium from the NLC shown in Fig. 2 showed that the NLC had no burst release. However, the PEGylated DS-loaded NLC had significantly higher sustained drug release properties than the non-PEGylated NLC. PEGylated NLC also circumvented dose dumping which is always a problem encountered in some sustained release preparation. Hence, PEGylation may be another alternative to enteric coating of diclofenac sodium in order to protect patients from gastric ulceration.

The results of the drug release kinetics revealed that drug release followed both zero order release kinetic model characteristic for sustained release dosage forms and First order release kinetics models which confirming that dissolution was also implicated as one of the mechanisms of drug release. Hence drug release was of mixed order release kinetics. The mechanisms of drug release studied using Higuchi models (Fig. 5 and 6) showed that drug release followed diffusion controlled process ($n \geq 0.5$) (Higuchi, 1963). The Ritger-Peppas plots were also linear and used to analyze the release processes (Fig. 7). This model also confirmed the results obtained in the integral form of Higuchi confirming that diffusion was the dominant mechanism of drug release. Drug release strictly followed the diffusion and erosion processes or non-Fickian diffusional release process ($0.43 < n < 1.00$) (anomalous) (non-swellable spherical matrix) (Ritger and Peppas, 1987).

Conclusion

Diclofenac sodium-loaded NLC prepared with tiger nut oil showed zero-order controlled release process and could be a better alternative to coated formulations. Additionally, PEGylation conferred enhanced physical stability on the NLC, which resulted to significantly higher controlled drug release over time compared to non-PEGylated NLC. Formulations also presented potentials of circumventing the gastric ulceration often encountered with the use of diclofenac sodium. Furthermore, the presence of tiger nut oil in the formulation would generate additional micronutrients and fatty acids required for good functioning of the body especially in geriatrics. The formulation processes and materials involved in formulating NLC are also relatively cheap and environmentally

friendly. Lipids from renewable sources are readily available and could be a cheaper and safer alternative to coated tablets of diclofenac sodium.

Conflict of interest

The authors state no conflict of interest.

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References

Ali Rehab FM, El Anany AM (2012). Physicochemical studies on sunflower oil blended with cold pressed tiger nut oil during deep frying process. *Gra Aceit,* 63, 455–465.

Arafat SM, Gaafar AM, Basuny AM, Nassef SL (2009). Chufa tubers (*Cyperus esculentus* L.): As a new source of food. *Wd Appl Sci J,* 7: 151–156.

Chime SA, Akpa PA, Ugwuanti C, Attama AA (2020) Anti- inflammatory and gastroprotective properties of aspirin – entrapped solid lipid microparticles. *Rec Pat Inflam Aller Drug Dis*, 14 (1): 78–88.

Cos¸kuner Y, Ercan R, Karababa E, Nazlıcan AN (2002). Physical and chemical properties of chufa (*Cyperus esculentus* L) tubers grown in the Çukurova region of Turkey. *J Sci Food Agric*, 82: 625–631.

De Vries FT (1991). Chufa (*Cyperus esculentus*, Cyperaceae): A weedy cultivar or a cultivated weed? *Econ Bot* 45: 27–37.

Eteshola E, Oraedu ACI (1996). Fatty acid compositions of tigernut tubers (*Cyperus esculentus* L.), baobab seeds *(Adansonia digitata* L.), and their mixture. *J Am Oil Chem Soc* 73: 255–257.

Ezeh O, Gordon MH and Niranjan K (2014). Tiger nut oil (Cyperus esculentus L.): A review of its composition and physico-chemical properties. Eur. J. Lipid Sci. Technol. 2014, 116, 783–794.

Gugu T.H., Chime S.A., Attama A.A. (2015). Solid lipid microparticles: An approach for improving oral bioavailability of aspirin. *As J Pharm Sci,* 10(5): 425-432.

Higuchi T (1963). Mechanism of sustained-action medication: Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *J Pharm Sci*, 52: 1145-9.

Higuchi T (1961). Rate of release of medicaments from ointment bases containing drugs in suspension. *J Pharm Sci*, 50: 874-5.

Kalam M.A., Humayun M, Parvez N, Yadav S, Garg A, Amin S, *et al.* (2007). Release kinetics of modified pharmaceutical dosage forms: A review. Continental J. Pharm. Sci., 1: 30-5.

Kenechukwu FC, Umeyor CE, Momoh MA, Ogbonna JDN, Chime SA, Nnamani PO, *et al.* (2014). Evaluation of gentamicin-entrapped solid lipid microparticles

formulated with a biodegradable homolipid from *Caprahircus. Trop J Pharm Res,* 13(8): 1999-205.

Kim M, No S, Yoon S (2007). Stereospecific analysis of fatty acid composition of chufa (*Cyperus esculentus* L.) tuber oil. *J Am Oil Chem Soc,* 84, 1079–1080.

Lasekan, O, Abdulkarim, SM (2012). Extraction of oil from tiger nut (*Cyperus esculentus* L.) with supercritical carbon dioxide (SC-CO2). *LWT– Fd Sci.Technol,* 47: 287–292.

Lichtenberger LM (1995). The hydrophobic barrier properties of gastrointestinal mucus. *Ann Rev Physio,* 57:565-583.

Lunn J, Theobald H (2006). The health effects of dietary unsaturated fatty acids. *Nutr Bull,* 31: 178–224.

Momoh MA, Akpa PA, Attama AA (2013). Phospholipon 90G based SLMs loaded with ibuprofen: an oral anti-inflammatory and gastrointestinal sparing evaluation in rats. *Pak J Zool,* 44(6): 1657-64.

Negbi MA (1992). Sweetmeat plant, a perfume plant and their weedy relatives: A chapter in the history of *Cyperus esculentus* L. and C. *rotundus* L. *Econ Bot* 46: 64–71.

Obitte NC, Chime SA, Magaret AA, Attama AA, Onyishi IV, Brown SA (2012). Some *in vitro* and pharmacodynamic evaluation of indomethacin solid lipid microparticles. *Afr J Pharm Pharmacol,* 6(30): 2309-17.

Obitte NC, Ofokansi KC, Chime SA, Odimegwu DC, Ezema AO, Odoh UE (2013). A preliminary attempt to address indomethacin's poor water solubility using solid self-emulsifying drug delivery system as a carrier. *Afr J Pharm Pharmacol,* 7(46): 2918-27.

Oderinde, R, Tairu A (1992). Determination of the triglyceride, phospholipid and unsaponifiable fractions of yellow nutsedge tuber oil. *Food Chem,* 45, 279–282.

Onyishi VI, Chime SA, Ogudiegwu EO (2015). Formulation of novel sustained release rifampicin-loaded solid lipid microparticles based on structured lipid matrices from *Moringa oleifera*. *Pharm Dev Tech,* 20(5): 546-554.

Ritger PL, Peppas NA (1987). A simple equation for description of solute release 1. Fickian and non- Fickian release from non swellable device in the form of slabs, spheres, cylinders and discs. *J Con Rel,* 5: 23-36.

Sánchez-Zapata E, Fernández-López J, Angel P, Alvarez J (2012). Tiger nut (*Cyperus esculentus*) commercialization: Health aspects, composition, properties, and food applications. *Comp Rev Fd Sci Fd Saf,* 11: 366–377.

Forner TD, Conde JAN (2010). Tiger nut milk base concentrate and method for obtaining a cosmetic product from said base concentrate. *U.S. Patent 20100183526A1*.

Thilak M, Haiou Q, Desiree V, Siyam MA, Angel P (2019). Characterization of Nanomaterials. *Taylor Ingle,* 313-353.

Umeyor EC, Kenechukwu FC, Ogbonna JD, Chime SA and Attama AA (2012). Preparation of novel solid lipid microparticles loaded with gentamicin and its evaluation *in vitro* and *in vivo*. *J Microencapsul,* 29(3): 296-307.

Wallace JL (2000). Pathogenesis of NSAID ulceration. *Bail Clin Gastro*, 14(1): 147 – 159.

World Health Organisation, Geneva (2003). Diet, nutrition and the prevention of chronic diseases, report of the jointWHO/FAO expert consultation, *in: World Health Organ Tech Rep Ser, (WHO)*, p. 149.

Yeboah, SO, Mitei YC, Ngila, JC, Wessjohann, L, Schmidt J (2012). Compositional and structural studies of the oils from two edible seeds: Tiger nut, *Cyperus esculentus*, and *Asiato, Pachira insignis*, from Ghana. *Fd Res Int*, 47: 259–266.

Zhang W, Li X, Ye T, Yu S, Chen J, Xinggang Y, Na Y, Jinsong Z, Jinlu L , Weisan P, Jun K, (2014). Nanostructured lipid carrier surface modified with Eudragit RS100 and its potential ophthalmic functions. *Int J Nanomed*, 9: 4305–4315.