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Original Article

EFFECT OF LYOPHILIZATION ON THE PHYSICOCHEMICAL AND PHYSICOTECHNICAL PROPERTIES OF ASPIRIN-LOADED LIPOSPHERES

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ABSTRACT

Objectives: To formulate aspirin-loaded lipospheres and to study the effect of lyophilization on the physicochemical and physicotechnical properties.

Materials and methods: Lipospheres were formulated using lipid matrix (LM) made from goat fat (70 %) and Phospholipon® 90H (30 %) by hot homogenization. The formulations were lyophilized and analysed for particle size and morphology, percent drug content (PDC), and *in vitro* drug release. The micromeritic properties of the formulations were also studied.

Results and discussions: The unlyophilized lipospheres had particle size range of 35.9 ± 8.63 to 78.7 ± 3.30 µm, while the lyophilized formulations had particle size range of 16.6 ± 2.92 to 45.5 ± 2.72 µm. PDC of lipospheres ranged from 63.4 to 92 %. *In vitro* drug release showed about 92.4 and 91.3 % drug release at 5 h for A1 and B1lipospheres formulated with Poloxamer® 407 and Soluplus® respectively and containing 1 % of aspirin respectively, while 95 and 93 % was released at 8 h. The results of micromeritic studies showed that the lipospheres exhibited poor flowability.

Conclusion: Lyophilized aspirin-loaded lipospheres showed good properties and could be used orally twice daily.

Keywords: Aspirin, lipids, micromeritic, lyophilization, NSAIDs.

INTRODUCTION

Aspirin is a moisture sensitive drug hence is produced by dry granulation method which may be in form of direct compression or by slugging. It is used in treating pain, inflammation, and various heart diseases. Conventional aspirin tablets are reliably absorbed, therapeutically effective and relatively inexpensive. However, the usefulness may be limited by some problems such as achieving and maintaining therapeutic plasma drug concentrations and severe gastrointestinal tract side effects [1]. Because of these problems, alternative oral aspirin dosage forms, mostly in form of enteric coated aspirin that are intended to provide more stable plasma concentrations with less frequent oral administrations and reduced side effect has been formulated [1]. However, coating could subject aspirin tablets to both high temperatures and humidity [2]. These problems prompted the research into the field of application of lipospheres as a delivery system for aspirin. Researchers over time have discovered that lipospheres are good delivery system for both hydrophilic and lipophilic non steroidal anti-inflammatory drugs (NSAIDs) [3,4,5].

Lipospheres are restricted to the stabilizing material of a phospholipid layer [6], and have advantage over most particulate systems because the drug could be solubilized or dispersed in the lipid matrix carrier, high drug loading of up to 85 % could be obtained for both hydrophilic and lipophilic drugs, easy to manufacture and scale up, feasibility of controlled drug release and targeting, and high stability [6,7,8]. The solid inner core and a phospholipid exterior confers several advantages on the lipospheres compared with conventional microspheres, such as high dispersibility in an aqueous medium, and a release rate for the entrapped substance that is controlled by phospholipid coating and carrier [7,8]. Momoh et al.[4], Obitte et al.[5] and Chime et al.[3], independently proved that lipospheres can protect loaded labile drug from degradation and also found out that lipospheres inhibits gastric irritation caused by NSAIDs.

Lyophilization is a widely used process for water removal from sensitive samples. Cryoprotectants are often added to lyophilized formulations including lipospheres in order to preserve their shape and enhance stability. The addition of cryoprotectors is particularly important in order to decrease liposphere aggregation and to obtain a better redispersion of the dry product [9]. Typical cryoprotective agents are sorbitol, mannose, trehalose, glucose, and polyvinylpyrrolidone [9]. It can be considered as placeholders which prevent the contact between discrete lipid particles. Some cryoprotectants act by interacting with the polar head group of the surfactant and serve as a kind of 'pseudo hydration shell' [10,11]. Freezing of lipospheres may however, lead to stability problems because of the freezing-out effect, which results in changes in the osmolarity and the pH. The second transformation, resolubilization, involves, at least in its initial stages, situations that favor particle aggregation (low water and high particle content and high osmotic pressure). The protective effect of the surfactant can also be compromised by lyophilization [12]. It has been found that, to prevent an increase in particle size, the lipid content of the liposphere dispersion should not exceed 5%. Direct contact of lipid particles have been found to decreased in diluted samples. Furthermore, diluted liposphere dispersions will also have higher sublimation velocities and a higher specific surface area [9,13].

The need to study the physicochemical and physicotechnical formulation aspect of lyophilized aspirin-loaded lipospheres cannot be over emphasized. Lipid based drug delivery system enhances the absorption of drugs and can be used for controlled drug release. Therefore, there is need to tailor the liposphere formulation to the final dosage form, i.e. the form in which the formulation would be presented to the patient. Lyophilized lipospheres formulations may be presented as dried powders for reconstitution, they may beencapsulated or tabletted as the case may be. Hence, there is need to study the effect of lyophilization on some physicochemical properties of aspirin-loaded lipospheres. Micromeritic studies are also vital as differences in particle flow are detrimental to encapsulated powder, tabletting and powder dosage forms generally. The aims of the present study are to investigate the physicochemical properties of aspirin-loaded lipospheres and also to evaluate the flow behaviour of the formulations for possible presentation as encapsulated formulations and tablets.

MATERIALS AND METHODS

Materials

Phospholipon® 90H (Phospholipid GmbH, Köln, Germany), sorbic Acid (Sigma® Chemical company, USA), sorbitol (Qualikems Laboratory reagent, India), Poloxamer® 407 (Synochem City,

Germany), Soluplus®, sodium hydroxide, monobasic sodium phosphate (Merck, Darmstadt, Germany), goat fat (Quarter market, Awka, Nigeria), activated charcoal (Bio–Lab. UK), aspirin (Evans pharmaceutical Ltd., England), and distilled Water (Lion water, Nsukka, Nigeria). All the materials were used just as supplied by the local distributors.

Extraction of goat fat

A method described by Attama and Nkemnele [14] was adopted. The fat was extracted by grating the adipose tissue prior to boiling with half its weight of water on a water bath for 45 min. Molten fat was separated from the aqueous phase using a muslin cloth. Further purification was carried out by heating a 2% w/w suspension of a 1:9 ratio blend of activated charcoal and bentonite in the lipid at 80^{o}C for 1 h. Thereafter, the suspension was vacuum-filtered using a Buchner funnel [15].

Preparation of lipid matrix (LM)

The lipid matrix was prepared by fusion using Phospholipon® 90H (30 g) and purified goat fat (70 g). Also according to the method described by Attama and Nkemnele and Attama et al., [14].

Formulation of the lipospheres

The aspirin-loaded lipospheres were prepared using the hot homogenization technique [16]. In each case, 5 g of the lipid matrix was melted at 80 ^{o}C on a water bath and an appropriate amount of aspirin (1, 3, 5 and 0%) was incorporated into the lipidic melt. About 4 % of sorbitol, 4 % of Poloxamer 407 (for batches A1, A2, A3 and A4) or 2 % of Soluplus (for batches B1, B2, B3 and B4) as the case may be were dissolved in hot distilled water at the same temperature with the lipidic melt. The hot aqueous phase was transferred into the moulten lipid and immediately homogenized with Ultra-Turrax (T25 Basic, Digital, Ika Staufen, Germany) at 7200 rpm for 10 min. An oil in water emulsion was formed by phase inversion [17].

Lyophilization

A 50 ml quantity of the lipospheres was lyophilized using a freeze-dryer (Amsco/Finn-Aqua Lyovac GT3, Germany), the sample formulation was added to 250 ml quick fitted conical flask and attached to the vacuum pressure pump of the machine. The set up was allowed until the total removal of aqueous phase.

Particle size and morphology determination

The particle sizes and morphology of both the lyophilized and unlyophilized lipospheres were determined by computerized image analysis of at least 100 microparticles using a binocular microscope (Lieca, Germany) attached with a Motic image analyzer (Moticam, China), at a magnification of x 400.

Percentage drug content (PDC)

Beer-Lambert's plot was obtained for aspirin in water in concentration range of 0.1 – 0.8 mg% at a predetermined wavelength of 300 nm. The drug content was determined using the lyophilized formulations. A 5 00 mg quantity of lyophilized liposphere from each of the batches was extracted with distilled water, filtered (Whatman no 1) and analyzed in spectrophotometer (UNICO 2102 PC UV/Vis Spectrophotometer, USA). The actual amount of aspirin in the formulations was determined using the formula:

$$\text{PDC} = \frac{\text{Amount of drug in the lyophilized formulations}}{\text{Actual amount of drug incorporated in the formulations}} \times 100 \quad (1)$$

Loading capacity (LC)

LC expresses the ratio between the entrapped active pharmaceutical ingredient (API) and the total weight of the lipids. LC was determined using the relation [17]:

$$\text{LC} = \frac{\text{Amount of API encapsulated}}{\text{Weight of lipid}} \times 100 \quad (2)$$

***In vitro* release studies**

The USP paddle method was adopted in this study. The dissolution medium consisted of 900 ml of freshly prepared simulated intestinal fluid (SIF, pH 7.2) maintained at 37 + 1^{o}C. The polycarbonate dialysis membrane (MWCO 6000–8000, Spectrum Labs, Breda, Netherlands) selected was pre-treated by soaking in the dissolution medium for 24 h prior to use. A 500 mg quantity of lyophilized lipospheres was weighed from each batch and placed in a polycarbonate dialysis membrane containing 2 ml of the dissolution medium, securely tied with a thermo-resistant thread and placed in the appropriate chamber of the release apparatus. The paddle was rotated at 100 rpm. About 5 ml was withdrawn from the dissolution medium at 0.5, 1.0, 1.5, 2.0, 3, 5 and 8 h, filtered with a non adsorbent filter paper (Whatman no. 1) and analyzed using a spectrophotometer (UNICO 2102 PC UV/Vis Spectrophotometer, USA) at 300 nm. An equal volume of the withdrawn sample was replaced with a fresh medium to maintain sink condition in each case. The amount of drug released at each time interval was determined with reference to the standard Beer's plot for each drug.

***In vitro* release kinetics**

In vitro release kinetics and mechanism of drug release was analysed using the first order, Higuchi square root equation and Ritger-Peppas empirical model as shown in Equations 3-5.

$$Q = 100(1 - e^{-k_1 t}), \quad (3)$$

$$Q = K_2(t)^{1/2}, \quad (4)$$

$$M_t/M_\infty = K_3 t^n \quad (5)$$

where Q is the release percentage at time, t and K_1, K_2 and K_3 are the rate constants of first-order, Higuchi and Ritger-Peppas models, respectively. M_t/M_∞ is fraction of drug released at time t, n is diffusion exponent and is indicator of the mechanism of transport of drug through the polymer, k is kinetic constant (having units of t^{-n}) incorporating structural and geometric characteristics of the delivery system [18,19,20].

Micromeritic evaluation

A 5 g quantity of each batch of lyophilized lipospheres was placed in a 25 ml measuring cylinder. The volume occupied by the sample was noted and the bulk density was calculated using the formula [21,22]:

$$\text{Bulk density } (\ell_B) = \frac{\text{Mass of powder (M)}}{\text{Bulk volume of powder } (V_B)} \quad (6)$$

The cylinder was tapped on a wooden platform by until there a consolidated volume was achieved and the tapped density was calculated using the formula:

$$\text{Tapped density } (\ell_T) = \frac{\text{Mass of powder (M)}}{\text{Tapped volume of powder } (V_T)} \quad (7)$$

Carr's compressibility indices (%) of the granules were obtained using the formula: Carr's index (%) = $\frac{\ell_T - \ell_B}{\ell_T} \times 100$ (8)

While Hausner's ratio was obtained thus:

$$\text{Hausner's ratio} = \frac{\ell_T}{\ell_B} \quad (9)$$

Data and statistical analysis

Data were analyzed using SPSS Version 16.0 (SPSS Inc. Chicago, IL.USA). All values were expressed as mean ± SD. Data were analysed by one-way ANOVA. Statistical significance was set at $P < 0.05$.

RESULTS

Effect of lyophilization on the particle size of lipospheres

The results of particle size of lyophilized and unlyophilized lipospheres are shown in Fig. 1 and show that the unlyophilized lipospheres had particle size range of 35.9 ± 8.63 to 78.7 ± 3.30 µm, while the lyophilized formulations had particle size range of 16.6 ± 2.92 to 45.5 ± 2.72 µm. The results therefore, revealed that the unlyophilized formulations exhibited significantly higher particle

size than the lyophilized lipospheres ($p < 0.05$). The results of the particle morphology of the unlyophilized lipospheres (Fig. 2) and the lyophilized lipospheres (Fig. 3) showed that the formulations were spherical and smooth.

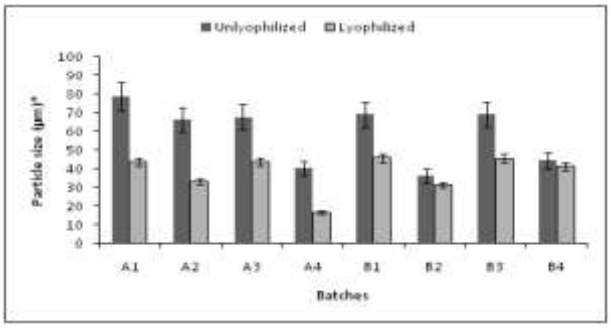

Fig. 1: Particle size of lyophilized and unlyophilized lipospheres; A1, A2, A3 and A4 were formulated with Poloxamer 407 and contain 1, 3, 5 and 0 % aspirin respectively, while, B1, B2, B3 and B4 were formulated with Soluplus and contain 1, 3, 5 and 0 % aspirin respectively.

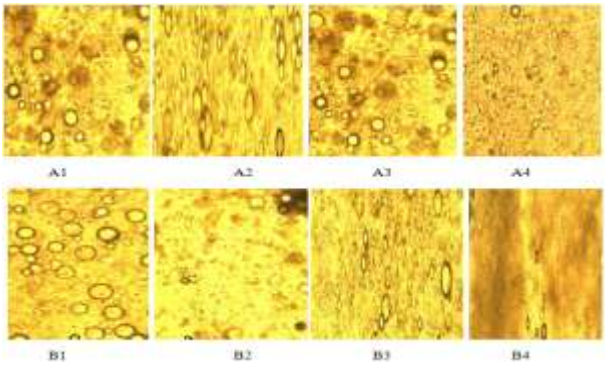

Fig. 2: Photomicrographs of unlyophilized aspirin-loaded and bland lipospheres; A1, A2, A3 and A4 were formulated with Poloxamer 407 and contain 1, 3, 5 and 0 % aspirin respectively, while, B1, B2, B3 and B4 were formulated with Soluplus and contain 1, 3, 5 and 0 % aspirin respectively.

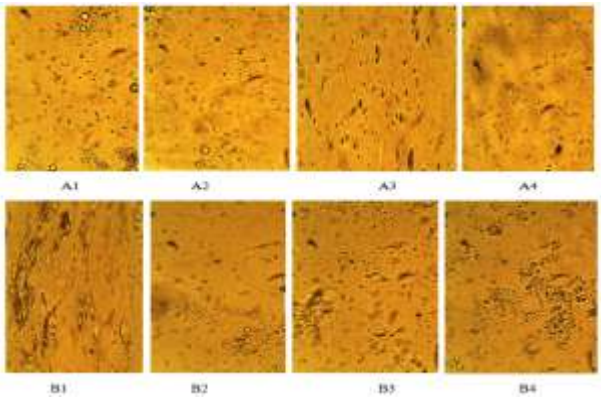

Fig. 3: Photomicrograph of lyophilized aspirin-loaded and bland lipospheres; A1, A2, A3 and A4 were formulated with Poloxamer 407 and contain 1, 3, 5 and 0 % aspirin respectively, while, B1, B2, B3 and B4 were formulated with Soluplus and contain 1, 3, 5 and 0 % aspirin respectively.

Percent drug content (PDC) and LC

The results of the PDC are shown in Fig. 4 and show that the lipospheres had overall PDC range of 63.4 to 92 %. Hence the aspirin was neither denatured by the carrier matrix nor the formulation technique adopted. The results of the LC of the formulations also shown in Fig. 4 showed that LC increased with drug content.

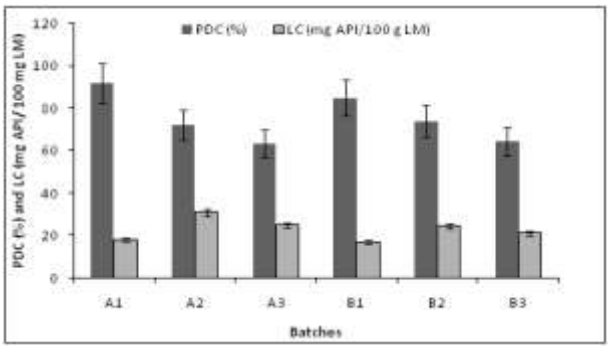

Fig. 4: The results of percent drug content (PDC) and loading capacity (LC); A1, A2 and A3 were formulated with Poloxamer 407 and contain 1, 3 and 5 % aspirin respectively, while, B1, B2 and B3 were formulated with Soluplus and contain 1, 3 and 5 % aspirin respectively.

***In vitro* drug release**

The results of the *in vitro* drug release of aspirin from lipospheres are shown in Fig. 5 and show that about 72.3 and 60.2 % of aspirin were released from batches A1 formulated with Poloxamer 407 and B1 formulated with Soluplus respectively at 1 h. At 5 h, about 92.4 and 91.3 were released from A1 and B1 respectively, while 95 and 93 % was released at 8 h.

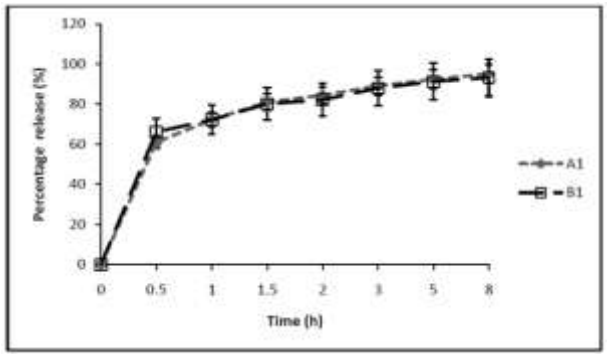

Fig. 5: *In vitro* release of aspirin from lipospheres in simulated intestinal fluid (pH, 7.2); A1 was formulated with Poloxamer 407 and contains 1% aspirin, while, B1 was formulated with Soluplus and contains 1aspirin.

Drug release kinetics

The results of the drug release kinetics and mechanisms of release are shown in Table 1 and show that the first order plots were linear ($r^2 = 0.9$). The Higuchi and Ritger-Peppas models were also linear as shown in Table 1. The results therefore showed that the lipospheres followed first order release kinetics.

Table 1: Drug release kinetics

Batch	First order	Higuchi	Ritger-Peppas	
	r^2	r^2	n	r^2
A1	0.911	0.944	0.183	0.951
B1	0.984	0.924	0.171	0.982

A1 was formulated with Poloxamer 407 and contains 1% aspirin, while, B1 was formulated with Soluplus and contains 1aspirin.

Micromeritic properties

The results of the bulk and tapped densities of aspirin-loaded and bland lipospheres are shown in Fig. 6 and show that the formulations showed bulk density range of 13 to 18 mg/ml and tapped density range of 9.3 to 13 mg/ml.

The results of the Carr's compressibility indices of aspirin-loaded and bland lipospheres are shown in Fig. 7 and show that ranges of 31.5 to 59.3 % were recorded for the lipospheres formulations. The

results of Hausner's quotient of the aspirin-loaded formulations are shown in Fig. 8 and show that the formulations exhibited a range of 0.63 to 0.72.

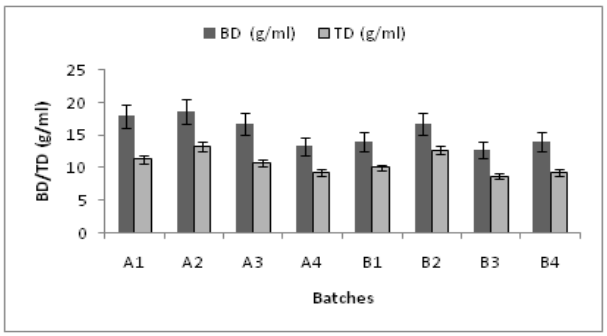

Fig. 6: Bulk and tapped densities of aspirin-loaded lipospheres; A1, A2, A3 and A4 were formulated with Poloxamer 407 and contain 1, 3, 5 and 0 % aspirin respectively, while, B1, B2, B3 and B4 were formulated with Soluplus and contain 1, 3, 5 and 0 % aspirin respectively.

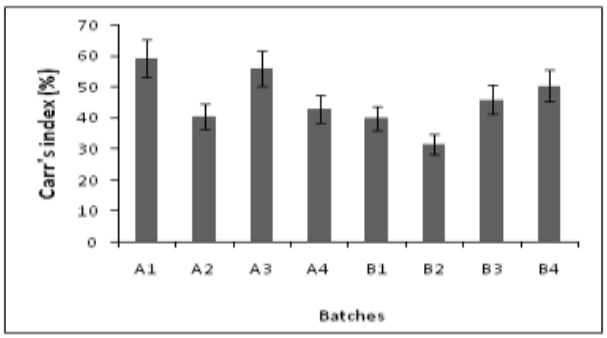

Fig. 7: The results of Carr's compressibility indices of aspirin-loaded lipospheres; A1, A2, A3 and A4 were formulated with Poloxamer 407 and contain 1, 3, 5 and 0 % aspirin respectively, while, B1, B2, B3 and B4 were formulated with Soluplus and contain 1, 3, 5 and 0 % aspirin respectively.

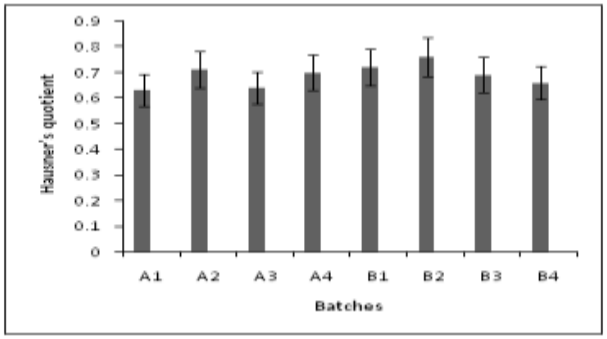

Fig. 8: The results of Hausner's quotient of aspirin-loaded lipospheres; A1, A2, A3 and A4 were formulated with Poloxamer 407 and contain 1, 3, 5 and 0 % aspirin respectively, while, B1, B2, B3 and B4 were formulated with Soluplus and contain 1, 3, 5 and 0 % aspirin respectively.

DISCUSSIONS

The results of the particle size of the aspirin-loaded and the bland lipospheres are showed that the particle size of both the unlyophilized and the lyophilized formulations were within the micrometer limit for lipospheres. The two surfactant used did not show significant effect on the particle size of the lipospheres ($p < 0.05$). However, the lyophilized formulations showed significantly lower particle sizes both in the bland and aspirin-loaded formulations. This may be due to formation of a protective capping layer around the lipospheres by the cryoprotectant, sorbitol (4 %) used in the study [23]. It can be considered as placeholders which prevent the contact between discrete lipid particles. Some cryoprotectants act by interacting with the polar head group of the surfactant and serves as a kind of 'pseudo hydration shell' [10]. The lower particle size exhibited by the lipospheres is more desirable than particle aggregation often encountered in liposphere formulations [11]. Freezing of lipospheres may cause stability problems because of the freezing-out effect, which results in changes of the osmolarity and the pH. The second transformation, resolubilization, involves, at least in its initial stages, situations that favor particle aggregation (low water and high particle content and high osmotic pressure). The protective effect of the surfactant can be compromised by lyophilization [12]. It has been found that, to prevent an increase in particle size, the lipid content of the liposphere dispersion should not exceed 5%. Direct contact of lipid particles is decreased in diluted samples. Furthermore, diluted liposphere dispersions will also have higher sublimation velocities and a higher specific surface area [9,13]. The addition of cryoprotectors is particularly important in order to decrease liposphere aggregation and to obtain a better redispersion of the dry product. Typical cryoprotective agents are sorbitol, mannose, trehalose, glucose, and polyvinylpyrrolidone [9].

The results of the percentage drug content of the formulations showed that the lipospheres protected aspirin from hydrolysis after lyophilization. The lipospheres generally exhibited high percent drug content. However, PDC was significantly affected by the amount of drug incorporated. Lipospheres having 1 % of aspirin exhibited the highest PDC than other formulations containing 3 and 5 % of aspirin. The possible reason may be due to saturation of the lipid matrices with increased drug incorporation leading to lower amount of encapsulated drug in the inner lipid core of the liposphere. The unencapsulated drugs were probably degraded by hydrolysis leading to lower PDC in those batches. Loading capacity increased with increase in drug content in agreement with previous research [14,17].

The results of the *in vitro* release of aspirin from lipospheres showed that the formulations exhibited a higher initial release an effect like burst release, however, this was caused by drugs in the periphery of the lipospheres. This effect was desirable in order to provide a minimum effective dose needed in the treatment of patients. The release was now sustained over time. Therefore, this formulation could be used orally twice daily.

The results of the *in vitro* release kinetics of the lipospheres showed that the formulations followed first order release kinetics. However, the results of the Higuchi plots revealed that the release mechanism involved diffusion controlled process. The Ritger-Peppas models showed that the formulations followed Fickian diffusion release mechanism ($n \leq 0.43$, non swellable spheres) [18-20].

The results of the micromeritic properties of the lyophilized aspirin-loaded lipospheres showed that the formulations generally exhibited poor flowability. Hence filling into capsule will be difficult and flow from hopper to die cavity during tabletting may be erratic without addition of some flow aids. Powder fluidity is important in capsule filling because variability in flow rate will automatically cause variability in capsule filled weight and active ingredient variation in both tablets and capsules. An increase in bulk density causes an increase in interparticulate contact leading to poor flow. The results of bulk and tapped densities were further applied to other flow indices that helped to analyze their flow behaviours. Hausner's ratio ≤ 1.25 indicates good flow, while values > 1.25 indicates poor flow [21]. Therefore all the liposphere formulations exhibited extremely poor flow. Carr's index in the range of 5 – 16 indicates good flow, 18 – 21 shows fair flow, while values above 38 shows very poor flow [21]. Therefore, all the batches of the aspirin-loaded and the bland lipospheres exhibited good flowability. The possible reason may be due to the cryoprotectant used. The use of sucrose as cryoprotectant may result in the formation of sticky glassy dried mass that is very difficult to handle after lyophilization [10]. Therefore, the problem of poor flowability encountered may be solved by using trehalose as cryoprotectant, this is because trehalose have been found to give dried free flowing lipospheres

[9,10]. Also, some flow aids like talc and carbosil may be added in order to enhance the flow.

CONCLUSION

Liposphere is a promising delivery system for aspirin, a moisture sensitive drug. The results revealed good physicochemical properties, however, the micromeritic properties of the formulations needs to be improved by better selection of excipients. The field therefore, requires further research in order to make liposphere formulations of aspirin available in the market.

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COMPETING INTERESTS

The authors state no conflicts of interest.

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