

Formulation and Evaluation of Niosomes of Benzyl Penicillin

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ABSTRACT

Benzylpenicillin niosomes were formulated using thin film hydration technique. The resultant niosomes were evaluated using surface morphology, particle size and its distribution, encapsulation efficiency, *In vitro* drug release, in vivo bioavailability and *In vitro* antimicrobial activity parameters. Both short (3 h) and long term (24 h) stability studies were carried out on the formulations. The lipid-surfactant ratio and the presence of co-surfactant were identified as the key variables that affect performance of the formulations. The niosomes particle sizes were between 1.67µm to 2.22 µm. The encapsulation efficiency was found to be highest in batch A with value of 82.42 %. Batches B and C exhibited slow release, oral stability and good bioavailability in vivo. For *In vitro* and in vivo studies, batch B containing span 80, Tween 65 and cholesterol was particularly stable and released its drug content in a controlled manner. The C_{max} for the batches were higher than that of pure drug which has value of 55.04 mg/ml in vivo. The IZD of the batches were high against the test micro organisms and all the batches exhibited antimicrobial activities greater than the unformulated drug against *S. typhi*, *P. vulgaris* and *Ps. Aereuginosa*.

INTRODUCTION

Niosomes are microscopic lamellar structures formed from non-ionic surfactant of dialkyl polyglycerol ether class and cholesterol and subsequently hydrated in aqueous media (Rajera *et al*, 2011). Niosomes are similar to liposomes structurally because they are made up of bilayers. Niosomes are made up of non-ionic surface active agents rather than phospholipids as seen in liposomes. Some surfactants will yield bilayer vesicles commonly known as niosomes, while other surface active agents will yield micellar structures on immersion in aqueous environment (Reddy *et al*, 2012; Srinivas *et al*, 2010). As drug delivery systems, niosomes have advantages which include the possibility of controlled drug release, drug targeting, increased drug stability, high drug loading, feasible incorporation of lipophilic and hydrophilic drugs, lack of biotoxicity and ease of large scale production with attendant low cost of production and sterilization (Pratap *et al*, 2009). Most often, the goal of drug delivery systems is to deliver the precise amount of drug to achieve the drug level necessary for treatment.

Therefore, the need for an optimal drug delivery system with a stable release profile and improved stability is of importance. The new drug carriers like niosomes have therefore attracted increasing scientific and commercial attention in recent time as possible means of achieving these goals (Gupta and Kompella, 2006; Shatalebi *et al*, 2010; El-Ridy *et al*, 2012; Masotti, 2013). Benzyl penicillin which belongs to the group of β-lactam antibiotics remains one of the safest groups of drugs used till date. However, its clinical applicability is significantly limited by its poor oral activity, short duration of action leading to high dosing frequency and hypersensitivity and reduced activity because of increased bacterial resistance (Sanjib, 2010). The objective of this study therefore is to formulate niosomes of benzylpenicillin with potentials for high oral activity, long duration of action and enhanced antimicrobial activity.

MATERIALS AND METHODS

Formulation of benzylpenicillin niosomes

Benzyl penicillin niosomes were prepared by employing film hydration method. Span 80 and cholesterol or shea butter are weighed (see Table 1) and dissolved in 10 ml of chloroform for each batch. The resultant solution was stirred vigorously using a

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vortex mixer for 3 min and concentrated to a lipid film using hot plate at low heat. Pure benzyl penicillin powder for each batch was weighed and dissolved in 10 ml of phosphate buffer. This was then added to the thin film formed previously from the lipid and the surfactant(s) as the case may be. The mixture was then agitated for 3 h on a magnetic stirrer and the resultant niosomes centrifuged at 3000 rpm for 30 min to allow for separation of supernatant and sediment, the sediment being the niosomes (Chawda *et al.*, 2011).

Morphology and particle size analysis

The morphology and particle sizes of the niosomal formulations were obtained using a Maddox photomicrograph. Aliquots of various formulations prepared were mounted on sample slide of the Maddox photomicrograph and the readings obtained automatically from the photomicrograph.

Determination of entrapment efficiency of niosomes

The resultant supernatant of each preparation were obtained and analyzed spectrophotometrically at 209 nm for its free drug content. The absorbencies obtained were converted to concentrations and were used to calculate their respective entrapment efficiencies.

In vitro drug release studies of benzyl penicillin niosomes

The release study was carried out over a period of 3 h. A 1.0 ml of the niosome of different batches of the drug formulated was introduced differently into 20 ml of phosphate buffer using a 1ml pipette. A 0.5 ml of the suspension was withdrawn at stipulated time intervals and replaced with an equivalent quantity of the buffer solution. The withdrawn portions were then analyzed spectrophotometrically at 209 nm and corresponding concentration obtained at the stipulated time intervals using a Beer's plot constructed with the pure drug sample.

In vivo bioavailability study

A total of 25 albino rats (males and non pregnant females) weighing between 200-220 g were used for this study. Five rats per formulation/treatment group and control were employed for the study. The bioavailability study was carried out over a period of 24 h. A 0.1 ml quantity of each formulated batch was administered orally using an oral intubation tube constructed from syringe and drip set. Blood samples were withdrawn at predetermined time intervals of 0, 0.5,1,2,3,5,8,18 and 24 h by retro orbital puncture while observing all standards pertaining to use of animal in experiments. The blood samples were then left for 30 min at room temperature to allow for separation. The supernatants were then diluted and assayed spectrophotometrically at 320 nm to obtain the serum blood concentration for each of the rats in a batch. The result obtained was further analyzed graphically and certain pharmacokinetic parameters obtained using the Winnolin Pharmacokinetic program, version 5 (Pharsight corporation, Mountain View California).

Antibacterial evaluation of the formulations

The agar-cup diffusion technique as described by Ofokansi and Esimone (2005) was used for this study. Two loopfuls of the standardized broth culture (0.5 McFarland standards) of the test organisms were introduced into a sterile petri dish and sterile molten nutrient agar at 40 °C was added. The mixture was then shaken to ensure even distribution of the organisms over the media. The seeded agar plates were allowed to solidify. A sterile cork borer of 8 mm diameter was used to bore holes in the solidified agar plate and 0.1 ml of the two fold serial dilutions of each formulated batch were added into the holes and allowed to stand for 20 min to allow for diffusion. The plates were incubated at 37 °C for 24 h and the inhibition zone diameters (IZD) were determined in millimeter (mm).

RESULTS AND DISCUSSIONS

Morphology and particle size analysis

The photomicrographs (Figures 1a-c) revealed that the niosomes were spherical with some extensions on the surface. Such extensions have been observed in lipospheres of bupivacaine formulations and have been attributed to drug crystals on the surface of the niosomes (Toongsuwan *et al.*, 2004). On the average, the niosome batches exhibited a narrow size distribution range, with particles diameter ranging from 1.67µm to 2.22µm. The extent of loading appears to affect the size of the niosomes. This had earlier been observed by Kilicarslan and Baykara (2003) who found that particle size decreased with decreased loading.

Entrapment efficiencies of the niosomes of benzyl penicillin

The entrapment efficiencies of the niosomal formulations of benzyl penicillin are shown in Table 1. It is apparent from the table that cholesterol: surfactant ratio of 1:1 gave the highest entrapment efficiency (82.42%). This is followed by similar ratio of shea butter to surfactant with entrapment efficiency of 64.97 %. Combining two surfactants (Span 80 and Tween 65) appear to reduce the entrapment efficiency since batch B with such combination of surfactants had a reduced entrapment efficiency of 60.51 %. High entrapment efficiencies have consistently been observed in lipospheres formulations involving equal ratios of lipids and surfactants (Hagalavadi *et al.*, 2007; Esimone *et al.*, 2012).

In vitro drug release

The *In vitro* drug release data are presented in figure 2. The figure indicates that formulation A released none of its drug content within the first 10 min; the percentage drug released however increased with time. Formulation B released a consistently low percentage of its drug content throughout the 3 h of study. The slow release pattern of the entrapped drug may indicate high stability of the niosomal formulation. The high stability of formulation B may possibly be due to the presence of the co-surfactant, Tween 65 and its particle size. The presence of Tween 65 may have resulted in high partitioning between the lipid phase and the aqueous phase resulting to the formation of a stable

lipid based system. Halagalavadi *et al* (2007) reported that stearic acid when used as wax modifier, rendered the formulation microporous. Attia *et al* (2007) inferred that the presence of high levels of cholesterol and surfactant explains this high stability. Formulation B was found to contain the lipid and surfactants in the ratio 1:1 which is in agreement with the findings of Santucci *et al* (1996). They identified the ideal lipid:surfactant ratio as 1:1 for optimal niosomal formulation. Formulations A and C also gave up approximately 30% of their drug content, as clearly depicted in figure 2. Analysis of the release data showed that formulations A, B and C yield less than 40 % of their drug contents *In vitro* in 3h. The formulations obeyed Higuchi's principle (plot not shown), so their release patterns *in vivo* are diffusion dependent. The niosomes formulations exhibited slower release indicating stability which is likely conferred by the cholesterol.

In vivo bioavailability

Table 2 shows the pharmacokinetics of the formulations. Formulation A containing lipid: surfactant in the ratio of 1:1, showed a high degree of instability *in vivo*, releasing quite a larger proportion of its drug content within the first three hours of the 24 h study. In spite of its high stability *In vitro*, it undergoes fast degradation in the gastric environment. Formulations B and C exhibited retarded release which indicates high stability *in vivo* in the 24 h of study.

The high Cmax and long half life of formulation B depicts its high stability and long time availability *in vivo*. For formulations A, B and C, Tmax were rather long; and appears in the following descending order: A>B>C. All test formulations showed higher concentrations (Cmax) than the pure drug sample *in vivo*, showing improved availability *in vivo*. This could be as a result of improved absorption. All the formulations showed improvement over the pure drug sample in terms of oral bioavailability as depicted by their higher AuC. The increase in bioavailability could be as a result of protection of the drug offered by the niosomes enabling greater quantities of the drug to be available in systemic circulation. More so, the increase in mean residence time (MRT) of formulations B and C over the free drug reflects sustained release.

Antibacterial activity of the formulations

The inhibition zone diameters (IZD) of the formulations against some penicillin-susceptible and penicillin-resistant bacteria are shown in table 3. The results showed that all the batches exhibited inhibitory actions against all the test organisms showing retention of antimicrobial activity of benzyl penicillin in niosomal formulations. A noticeable improvement was also observed as two of the test organisms resistant to the pure drug sample (*Ps. Aeruginosa* and *Sal. Typhi*) were found to be sensitive to all the formulations.

Table. 1: Formula for different batches of the niosomes and their entrapment efficiencies

Niosomes batch	Ingredients (mg)						E.E(%)
	Cholesterol	Shea butter	Span 80	Tween 65	Drug		
A	50	---	50	---	100	82.42	
B	50	---	25	25	100	60.51	
C	---	50	50	---	100	64.49	

E.E = Entrapment Efficiency

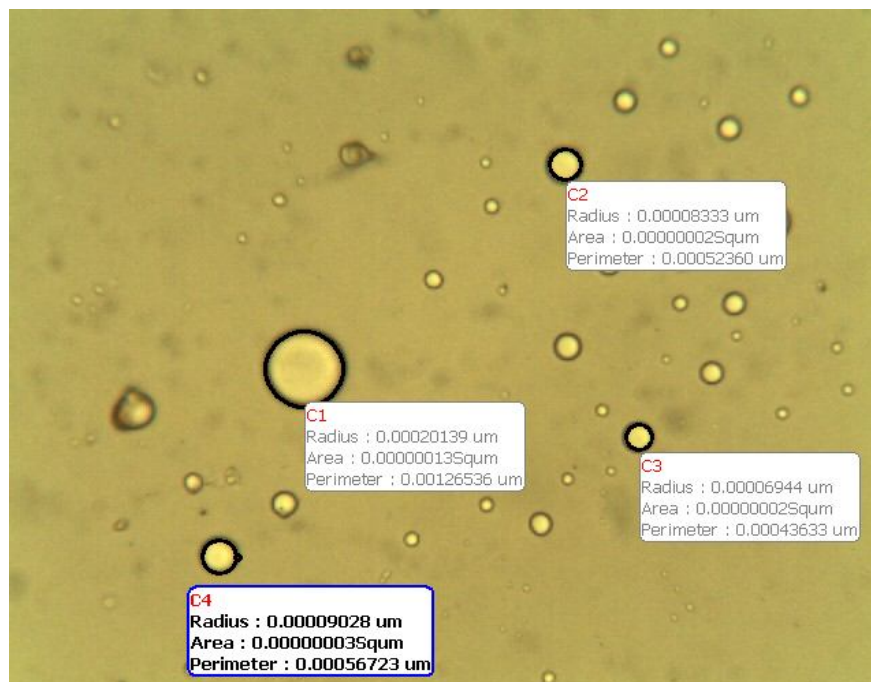


Fig. 1a: Photomicrograph of Niosome formulation A containing Benzyl penicillin; 50%, Span 80; 25 % and Cholesterol; 25 %.

Scale: 100,000

Magnification: 10

Particle size: 2.22 μ m Particle size: 2.22 μ m

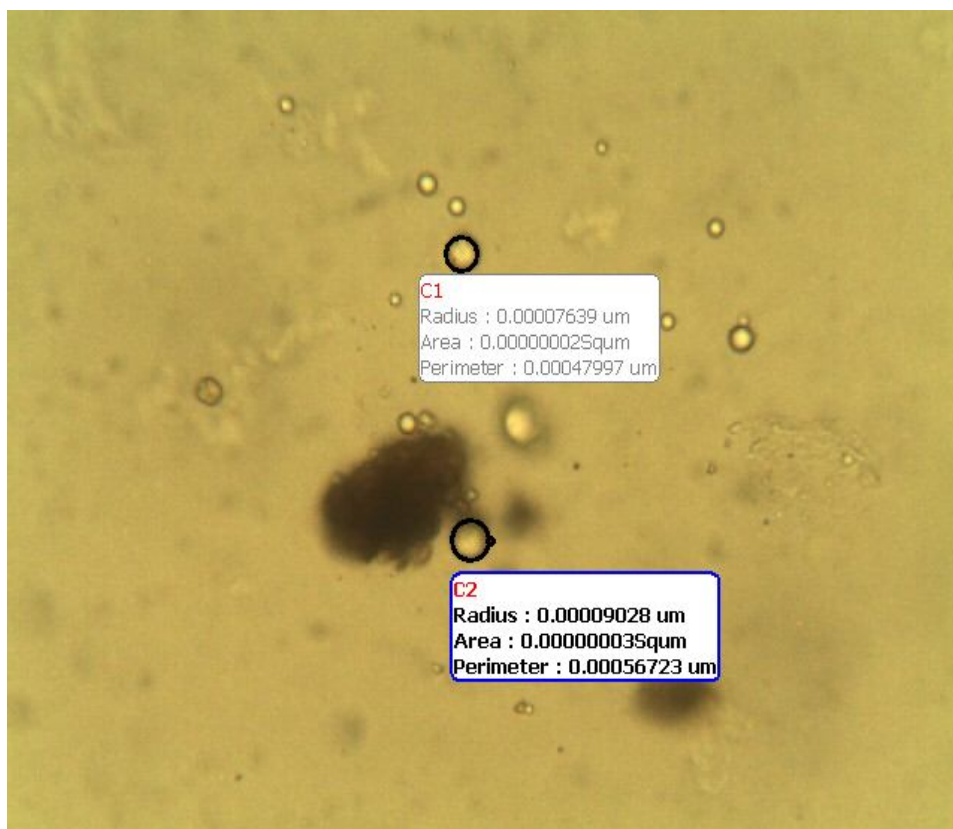


Fig. 1b: Photomicrograph of Niosome formulation B containing Benzyl penicillin; 50 %, Cholesterol; 25 %, Span 80; 12.5 % and Tween 65; 12.5%.
 Scale: 100,000
 Magnification: 10
 Particle size: 1.67µm

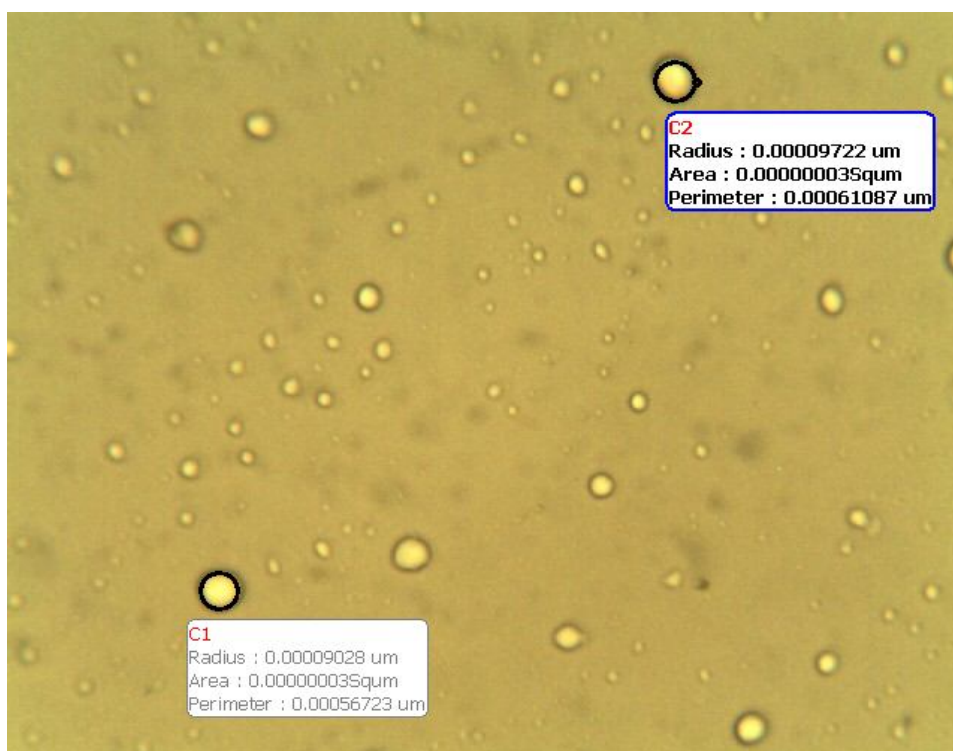


Fig. 1c: Photomicrograph of Niosome formulation C containing Benzyl penicillin; 50 %, Shea butter; 25 % and span 80; 25 %
 Scale: 100,000
 Magnification: 10
 Particle size: 1.88 µm

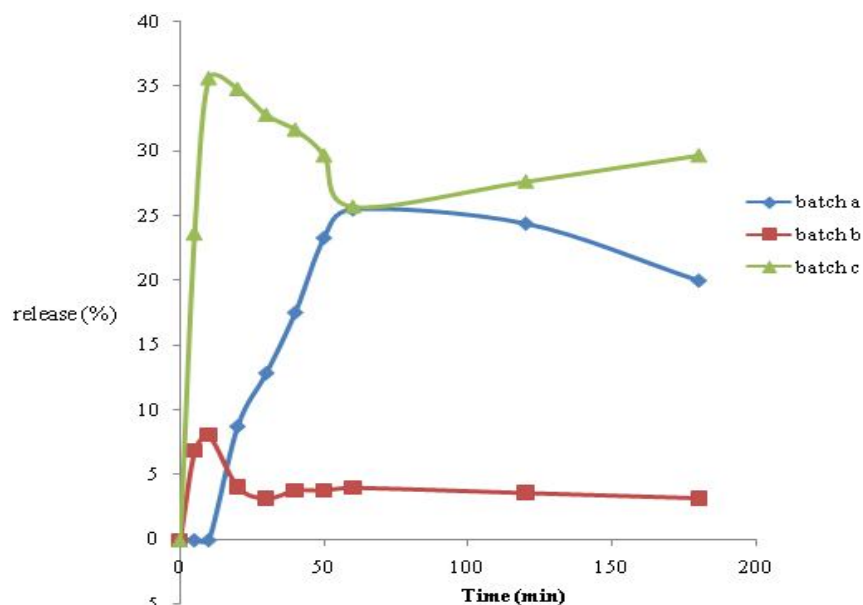


Fig. 2: *In vitro* drug release.

Table. 2: Pharmacokinetic data of *in vivo* drug release.

Pharmacokinetics	Batch			
	A	B	C	Control (Pure drug)
T _{max} (h)	10.40±4.60	7.90±4.20	5.50±3.50	4.00±3.50
C _{max} (mg/ml)	119.66±22.54	75.24±14.17	57.55±4.03	55.04±12.05
T _{1/2} (h)	15.97±6.86	46.24±23.47	19.73±11.03	36.41±16.58
AUC (mgh/ml)	1045.75±175.17	980.98±154.52	807.09±41.71	674.88±93.55
MRT (h)	11.28±1.142	12.90±1.143	12.18±0.861	11.99±0.748

Table. 3: Inhibition zone diameters (mm) of the niosomes.

Organism	Batch A	Batch B	Batch C	Control (Pure drug)
<i>S. aureus</i>	15	14	16	17
<i>B. subtilis</i>	7	7	7	15
<i>Ps. aeruginosa</i>	12	11	13	0
<i>Sal. typhi</i>	27	21	25	0
<i>K. pneumonia</i>	8	8	10	18

CONCLUSION

The niosomes sizes ranges from 1.67 μ m to 2.22 μ m. Niosomes with cholesterol to surfactant ratio of 1:1 gave the highest entrapment efficiency of 82.42 %. Similar ratio of shea butter to surfactant (span 80) gave entrapment efficiency of 64.97 %. However, combining two surfactants (Span 80 and Tween 65) reduces the entrapment efficiency to 60.51 %. The niosomes formulations exhibited *In vitro* slow release indicating stability while only batches B and C show stability *in vivo*. All the three batches showed improved availability *in vivo* with Batches B and C depicting sustained release. The formulated niosomes retained the benzylpenicillin antibacterial activity against the test organisms and specifically to the bacteria that are resistant to the pure drug.

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