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# FORMULATION AND EVALUATION OF GOAT FAT AND SHEA BUTTER BASED LIPOSPHERES OF BENZYL PENICILLIN

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## ABSTRACT

Lipospheres of benzyl penicillin were formulated using the conventional thin film hydration technique. Five different combinations of shea butter, surfactant (Span 80) and goat fat were the key variables employed in the formulations. The resultant lipospheres were evaluated with respect to surface morphology, particle size distribution, encapsulation efficiency, *in vitro* drug release, *in vivo* bioavailability and *in vitro* antimicrobial activity. Particle size was found to increase with increased drug loading, the average particle radius of the batches being 12.5 nm. The encapsulation efficiency was found to be high at all levels with encouraging values of 80.21 and 83.44 % for batch A and E respectively, with batch A containing shea butter and span 80 in the ratio of 1:1 and batch E containing shea butter, span 80 and goat fat in the ratio of 1:2:1. Batch A appear to exhibit sustained release *in vitro* with cumulative drug release being 60 % while batch E has a cumulative drug release of 98 % respectively. The presence of goat fat however seems to impact negatively on the *in vivo* stability of batch E liposphere. The test micro-organisms showed sensitivity to the two batches in marked contrast to the uncapsulated benzyl penicillin especially against the multiple-antibiotic resistant strains of *S. typhi, P. vulgaris* and *P. aereuginosa* used in the study.

**INTRODUCTION:** Numerous lipid based delivery systems such as liposomes, solid lipid nanoparticles, oily suspensions, submicron lipid emulsions, lipid implants, lipid microtubes and microcylinders, lipid microbubbles and lipid microspheres (lipospheres) have served as better alternatives to synthetic polymer carrier materials [1].

Lipospheres which represent novel drug delivery vehicles are water-insoluble lipid spheres forming a solid hydrophobic core, with a layer of phospholipids embedded on the surface of the core. Drugs or other biologically active agents may be contained in the hydrophobic core, adhered to the phospholipids, or a combination thereof.

Lipospheres have a number of advantages over some existing drug delivery systems for a number of reasons: possibility of controlled drug release, drug targeting, increased drug stability, high drug load, feasible incorporation of lipophilic and hydrophilic drugs, lack of bio-toxicity of the carrier, avoidance of organic solvents and no difficulties with large scale production and sterilization [2].

The goal of every drug delivery system is to deliver the precise amount of drug, at pre-programmed rate to the desired location in order to achieve tissue drug level necessary for treatment. The need for a better drug delivery system with a stable release profile, which could improve stability, is of importance. Lipospheres have therefore attracted increasing scientific and commercial attention during the last few years as possible means of achieving these goals [3] and as potential alternate colloidal drug delivery systems to liposomes. Lipospheres as carrier systems have been used to deliver anti-hypertensive enalapril maleate [1], anti-hyperglycemic glipizide [4], anti-bacteria ceftriaxone [5], anti-fungal miconazole [6], analgesic aceclofenac [7] and flurbiprofen [8].

The penicillins, a group of β-lactam antibiotics remain one of the safest group of drugs used till date. They are used in the treatment of bacterial infections caused by susceptible, usually Gram-positive, organisms. All penicillins possess the basic penam skeleton, which has the molecular formular R $–C_9HIN_20_4$, where R is a variable side chain. For benzyl penicillin, the R is a methyl group. Benzyl penicillin belongs to first generation penicillins. It is one of the most used antibiotics limited however because of its poor oral activity, short duration of action leading to high dosing frequency, hypersensitivity reactions and high level microbial resistance. Benzyl penicillin, commonly known as Penicillin G is typically formulated as powder for reconstitution before administration through parenteral route because it is unstable to gastric pH.

However, oral powders and tablet are formulated from a variant of the benzyl penicillin; phenoxymethyl penicillin otherwise known as Penicillin V. Like all Penicillins, benzyl penicillin is actively secreted, and about 80% of the penicillin dose is cleared within three to four hours of administration. Hence, the need to explore new drug delivery system, with the aim of improving its oral stability, reduce hypersensitivity reactions, improve stability, prolong therapeutic action and increase dosing convenience and patient compliance. In this research therefore, benzyl penicillin is formulated into different forms of liposphere. Various characteristics of the lipospheres were evaluated to identify the formulation(s) that has the most favourable biopharmaceutical and pharmaco-dynamic properties.

## MATERIALS AND METHODS:

**Extraction of Shea Butter:** The yellow shea butter sourced from Nsukka market in Enugu State, South-East of Nigeria, was extracted using n-hexane. Exactly 10 kg of shea butter was extracted using 1.5 L of n-hexane and the solution was left for 24 h. The supernatant was decanted from the mixture and allowed to evaporate until the yellow semi-solid emerged.

**Preparation of Phosphate Buffer Solution:** A 3.63 g quantity of potassium hydrogen phosphate ($K_2HPO_4$) and 5.68 g of disodium phosphate ($Na_2PO_4$) were dissolved in 100 ml of distilled water. The resultant solution was then made up to 1000 ml as stipulated in the British Pharmacopoeia [9]. The solution was adjusted to PH 7.4 using sodium hydroxide.

**Formulation of Benzylpencillin Lipospheres:** Different batches of benzyl penicillin lipospheres as shown in **table 1** were prepared. The corresponding ingredients are weighed into a beaker containing 10 ml of chloroform. The resultant solution was stirred vigorously using a vortex mixer for 3 minutes, and concentrated to a lipid film using hot plate at low heat. The pure benzyl penicillin powder for each batch was weighed and dissolved in 10 ml of the phosphate buffer. This was then added to the thin film formed previously from the lipid(s) and the surfactant. The mixture was then agitated for 3 h on a magnetic stirrer. The resultant liposphere were centrifuged at 3000 rpm for 30 minutes to allow for separation.

**TABLE 1: FORMULA FOR DIFFERENT BATCHES OF THE LIPOSPHERES**

| Lipospheres batch | Ingredients (mg) | | | |
|---|---|---|---|---|
| | Shea butter | Span 80 | Goat fat | Drug |
| A | 50 | 50 | -- | 100 |
| B | 100 | 50 | -- | 100 |
| C | 50 | 100 | -- | 100 |
| D | 50 | 50 | -- | 200 |
| E | 25 | 50 | 25 | 100 |
| F | 50 | 50 | 25 | 100 |

**Benzyl Penicillin Lipospheres Morphology and Particle Size Analysis:** The morphology and particle sizes of the lipospherical formulations were obtained using a Maddox photo micrograph (Maddox- 207R 11 India)**.** Few drops of the formulations are placed on the

sample slide and the slide mounted on the Maddox photo micrographic instrument. The readings were obtained automatically from the photomicrograph.

**Entrapment Efficiency of Lipospheres:** The resulting supernatant of each batch obtained after centrifugation was analyzed spectrophotometrically at 209 nm for its free drug content. The absorbencies obtained were converted to concentrations using a calibration curve prepared for the purpose. These concentrations were used to calculate the respective entrapment efficiencies.

***In vitro* Drug Release Studies of Benzyl Penicillin Lipospheres:** The release study was carried out over a period of 3 h. One ml volume of the sediment of the liposphere of each batch formulated was introduced into 20 ml of phosphate buffer using a 1 ml pipette. A 0.5 ml of the suspension was withdrawn at stipulated time intervals and replaced with an equivalent quantity of the buffer. The withdrawn portions were then analyzed spectrophotometrically at 209 nm and corresponding concentration obtained at the stipulated time intervals using the calibration curve.

***In vivo* Bioavailability Study:** A total of 20 albinos Wister rats weighing 200-220g (4 rats per treatment including the control) were used for this study. The bioavailability study was carried out over a period of 24 h. A 0.1 ml of each batch and the control were administered orally using an intubation set constructed from a syringe and infusion set tube. 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 for usage of animals in experiments. The blood samples were then left for 30 min at room temperature to allow for separation.

The sera were then diluted and assayed spectrophotometrically at 320 nm to obtain the serum blood concentration for the rats in each batch. The results obtained were further analyzed and pharmacokinetic parameters obtained using the Winnolin Pharmacokinetic program, version 5 (Pharsight corporation, Mountain View California).

***In vitro* Antimicrobial Study:** The agar-cup diffusion technique as described earlier [10] was used for this study. With the aid of sterile wire loop, two loopfuls of the broth culture (0.5 McFarland standards) of the test organisms were introduced into a sterile Petri-dish. Sterile molten nutrient agar at 40$^{o}$C was added. It was then gently rocked 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. The plates were marked into segments and labelled at the back with permanent marker. Thereafter, 0.1 ml of the two fold serial dilutions of each batch were added into the holes according to the labels and allowed to stand for 15-20 minutes for proper diffusion. The plates were incubated at 37$^{o}$C for 24 h. The inhibition zone diameters (IZD) were determined in millimetre (mm).

**RESULTS AND DISCUSSIONS:** The photomicrograph (**Figure 1**) revealed that the lipospheres were spherical with some extensions on the surface. The extensions could be as a result of drug crystals on the surface of the lipospheres. This observation had been noted with lipospheres in separate studies [11, 12]. On the average, the photograph revealed that the batches exhibited a narrow size distribution, with average particles radius of 12.50 nm. The extent of loading appears to affect the amount of the drugs. This is in line with an earlier finding that particle size decreased with decrease loading [13].

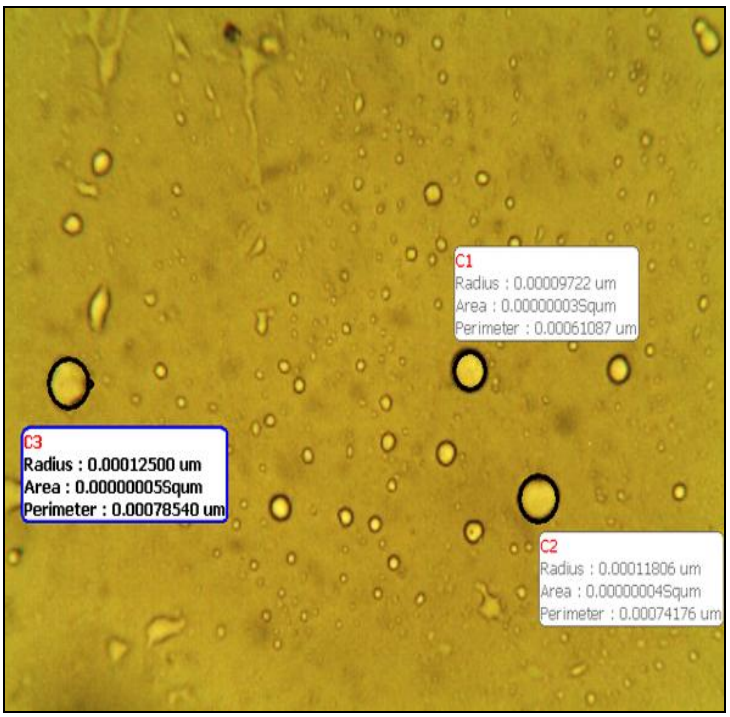


**FIGURE 1: PHOTOMICROGRAPH OF LIPOSPHERE OF ONE OF THE BATCHES (FORMULATION E) CONTAINING BENZYLPENICILLIN 50%, SHEA BUTTER 12.5%, GOAT FAT 12.5% AND SPAN 80 25%**

The entrapment efficiencies of the lipospherical formulations of benzyl penicillin are shown in **figure 2**. It was observed that using a constant drug concentration of 100 mg and a lipid: surfactant ratio of 1:1, yields of high entrapment efficiency were obtained (80.21% and 83.44%). Increasing lipid concentration did not improve entrapment efficiency but rather reduced entrapment (65%). Increasing the lipid concentration may have reduced the solubility of the hydrophilic drug molecules by reducing the partitioning of the drug in the lipid phase, hence reducing entrapment efficiency.

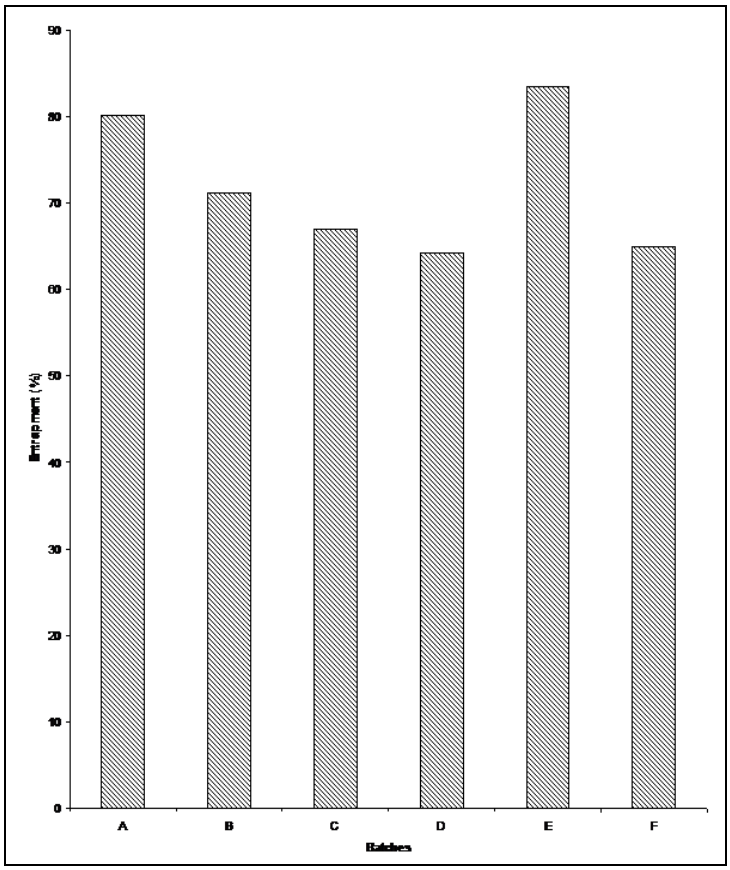


**FIG. 2: ENTRAPMENT EFFICENCY OF BENZYL PENICILLIN LIPOSPHERES**

Also, increasing the surfactant concentration yielded lower entrapment efficiency (67.03%). This could be as a result of increased hydrocarbon head of the surfactant at high concentration, leading to reduced solubility of the drug. This caused inadequate dispersion of the drug in the lipid, resulting in lower drug encapsulation at higher span 80 levels. Shivakumar *et al.,* [12] reported a lower drug encapsulation at higher stearic acid levels.

Furthermore, doubling the drug concentration yielded less entrapment efficiency than the previous two variations (64.31%). This may be as a result of saturation of the surfactant moieties leading to reduced solubility of the drug in the lipid. Toongsuwan *et al.,* [11] reported that higher drug loading led to the insufficient coating of the drug particle by the lipophilic material. However, the addition of goat fat while maintaining lipid: surfactant ratio at 1:1 improved entrapment, increasing it to 83.44% which was the highest value obtained. This is in line with an earlier observation. Attama *et al.,* [14] reported that mixtures of beeswax and goat fat produced matrices composed of mixtures of crystals alongside mixed crystals with imperfections necessary for increased drug loading and retention capacity.

It was also observed however, that still in the presence of goat fat, an increase in lipid concentration to obtain a lipid: surfactant ratio of 2:3, reduced entrapment efficiency greatly (65%), second only to that obtained from doubling drug concentration. This is likely due to reduction in the wetting of the hydrophilic drug and a resultant reduction in solubility. Hence, maintaining a lipid: surfactant ratio of 1:1 was found to be best for high entrapment in lipospheres.

From these preparations, two formulations (batch A and E) were chosen for further analysis based on the batch with best entrapment efficiency.

Batch A releases approximately 29 % of its drug content *in vitro* while batch E released 33 % of its drug content within the first five minutes (**Figure 3**). The release data also showed that batch A released close to 60 % of its drug within three hours. Batch E on the other hand, released 98 % of its drug contents within three hours. Invariably, the introduction of goat fat in a ratio of 1:1 with the shea butter in batch E, compared to its absence in batch A, compromised the texture of the shea butter allowing more drugs molecules easy access through the matrix.

The two batches showed an initial burst immediately after oral administration, thereby releasing the highest percentage of drug content within 1 h (**Figure 4**). This initial burst may provide an advantage since this may serve to initiate dosing followed by a sustained release. However, batch A seems to be more stable *in vivo*

since it releases and sustains higher concentration of the drug (Figure 4) than batch E, which appears to be more stabilized *in vitro* as depicted by higher concentration of drug released and sustained (Figure 3). On the other hand, Batch E *in vivo* release performance seems to fall short of the control.

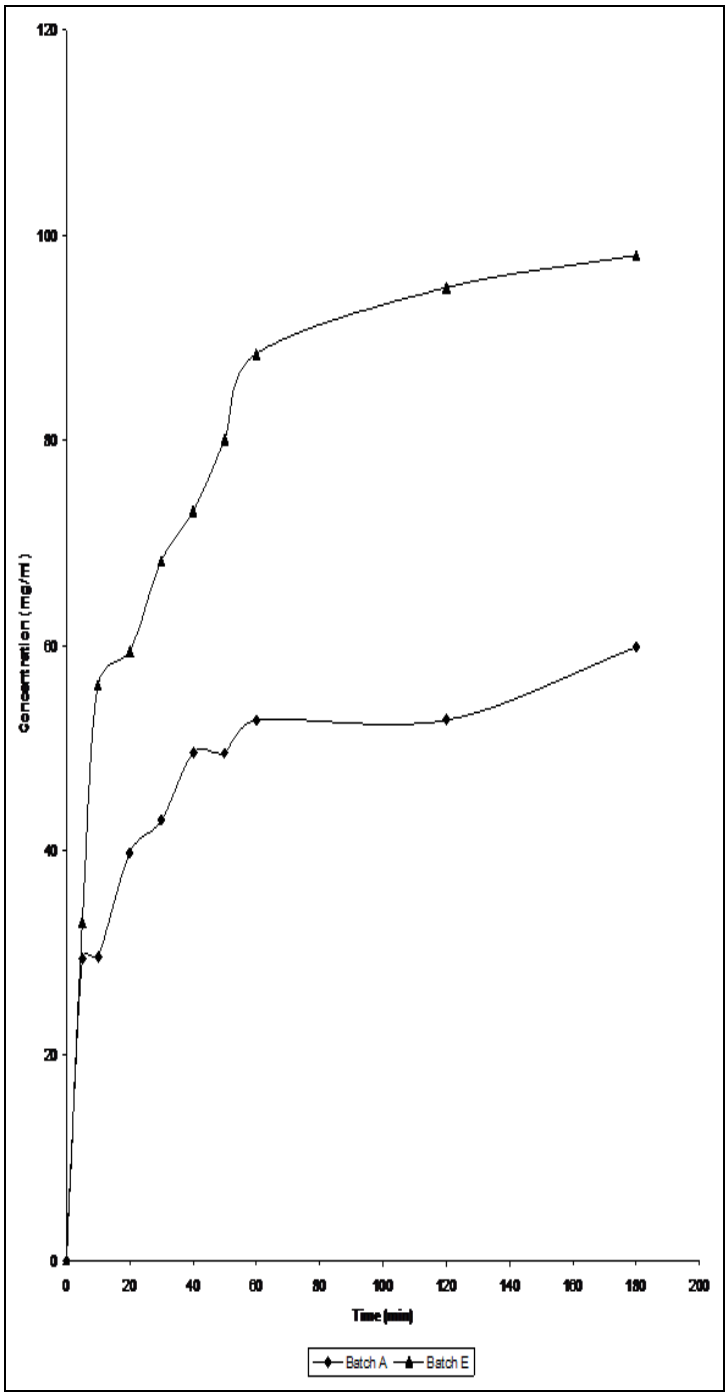


**FIG. 3: *IN-VITRO* RELEASE OF BENZYL PENICILLIN LIPOSPHERES**

Further analyses showed that *in vitro* release of batch A is highly correlated to the *in vivo* release with a coefficient of correlation (r) of 0.92, while that of batch of E has a value of 0.94.

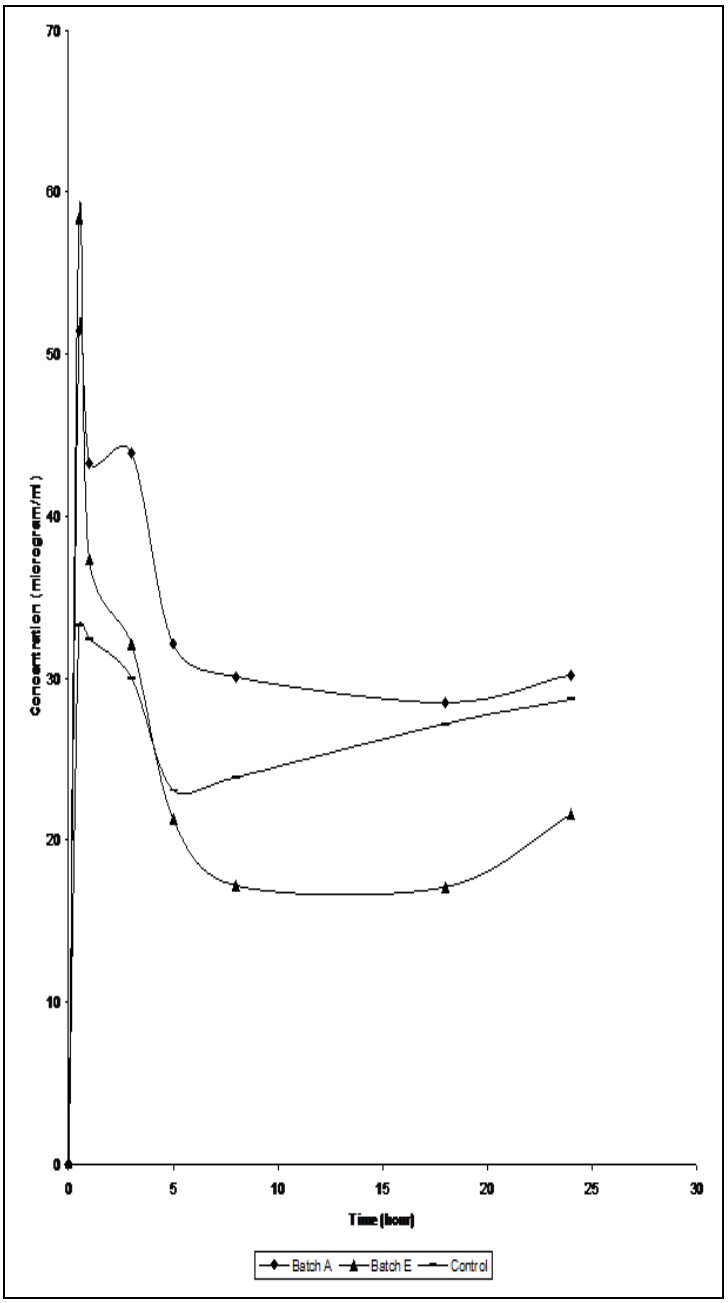


**FIG. 4: *IN-VIVO* BIOAVAILABILITY OF BENZYL PENICILLIN LIPOSPHERES**

The inhibition zone diameters (IZD) of the batches against some susceptible organisms are shown in **table 2**. The results showed that the two batches exhibited inhibitory actions against all the test organisms used. A noticeable improvement was also observed as three of the test organisms resistant to the pure drug sample were found to be sensitive to the test formulations. This could be as a result of the synergistic action between the penicillin and surfactants. Surfactants have been shown to possess anti bacterial activities [15] with the molecules of the surfactants dislodging the cell membranes thereby ensuring higher influx of the drug.

**TABLE 2: INHIBITION ZONE DIAMETERS (mm) OF LIPOSPHERES**

| Bacteria Species | Inhibition Zone Diameters (mm) | | |
|---|---|---|---|
| | Batch A | Batch E | Control |
| *S. aureus* | 16 | 16 | 17 |
| *B. subtilis* | 7 | 7 | 15 |
| *P. Aeroginosa* | 13 | 9 | 0 |
| *P. vulgaris* | 11 | 12 | 0 |
| *S. Typhi* | 25 | 24 | 0 |
| *K. pheumonia* | 10 | 9 | 18 |

**CONCLUSION:** The study has shown that benzyl penicillin is successfully encapsulated in shea butter based lipospheres. The lipospheres particle sizes fall within the nanometer range. It was observed that the best lipid: surfactant ratio for good *in vitro* stability is 1:1. The presence of goat fat in the lipospheres increased entrapment efficiency and improves *in vitro* and but reduced *in vivo* stability. The resultant lipospheres improved the antimicrobial activity of benzyl penicillin against some microorganisms. The pure drug resistant microbe species all showed sensitivity to the two best formulations.

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