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***Journal of Scientific and Engineering Research*, 2023, 10(1):11-18**

**Research Article**

**ISSN: 2394-2630**
**CODEN(USA): JSERBR**

# Formulation and Characterization of Rice Bran Oil Loaded Albumin Nanopaticles

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**Abstract** Niosomes (the nonionic surfactant vesicles), considered as novel drug delivery systems, can improve the solubility and stability of natural pharmaceutical molecules. They are established to provide targeting and controlled release of natural pharmaceutical compounds. Many factors can influence on niosome construction such as the preparation method, type and amount of surfactant, drug entrapment, temperature of lipids hydration, and the packing factor. The present review discusses about the most important features of niosomes such as their diverse structures, the different preparation approaches, characterization techniques, factors that affect their stability, their use by various routes of administration, their therapeutic applications in comparison with natural drugs, and specially the brain targeting with niosomes-ligand conjugation. It also provides recent data about the various types of ligand agents which make available active targeting drug delivery to the central neuron system. This system has an optimistic upcoming in pharmaceutical uses, mostly with the improving availability of innovative schemes to overcome blood-brain barrier and targeting the niosomes to the brain.

**Keywords** Formulation, Characterization, Rice Bran Oil, Albumin, Nanopaticles

## Introduction

The last few years have witnessed the discovery and development of a wide spectrum of large scale manufacturing and production of novel materials that lie with in the nanometer scale. Nanoscience is one of the most important research and development frontiers in modern science, the word ‘nano’ means one billionth, and nanometer, defines the length scale that is used to measure system being studied in Nanoscience. The aim of Controlled drug delivery system is releasing the correct dose of a therapeutic directly in the desired zone at required period of time. It allows improving the efficacy of the therapeutic, patient compliance and reducing the possible side effects, frequency of drug administration and fluctuation of drug level in blood [1]. Controlled drug delivery system has many advantages over the conventional drug delivery system not only in clinical purpose but it has Commercial / Industrial Advantages like Illustration of innovative/technological leadership, life-cycle extension of product, Product differentiation and Patent extension [2,3]. Niosomes are of nonionic multilameller vesicular structure of surfactants, which similar to liposomes. Niosomes are composed of non-ionic surface active agents instead of phospholipids whichare the components of liposomes. There are various types of surface active agents or surfactants have been reported to form vesicles, and have the capacity to entrap and retain the hydrophilicandlipophilic substance. Now a day’s niosomes have been comprehensively studied for their probable to serve as a carrier for the delivery of drugs, antigens and hormones. Besides this, niosome has been used to solve the problem of rapid degradation of drugs, insolubility and in stability of active pharmaceutical ingredients [4].

The nature of niosomes are ampiphillic in which the core cavity entrapped the hydrophilic drug and -polar region present within the bilayer entrapped hydrophobic drugs (Figure 1) hence niosone incorporated both hydrophilic and hydrophobic drugs [5-10]. In 1975 first niosome formulations were developed and patented by L'Oreal and an alternative to liposomes because they alleviate the disadvantages associated with liposomes such as chemical instability. The main purpose of development of niosomal system is biodegradability, biocompatibility, chemical stability, low production cost, easy storage, chemical stability, handling and low toxicity. Niosomes can provide admistration of drugs through various routes such as oral, parenteral, topical and used as a carrier to deliver different types of drugs such as synthetic and herbal, antigens, hormones and other bioactive compounds [10-15].

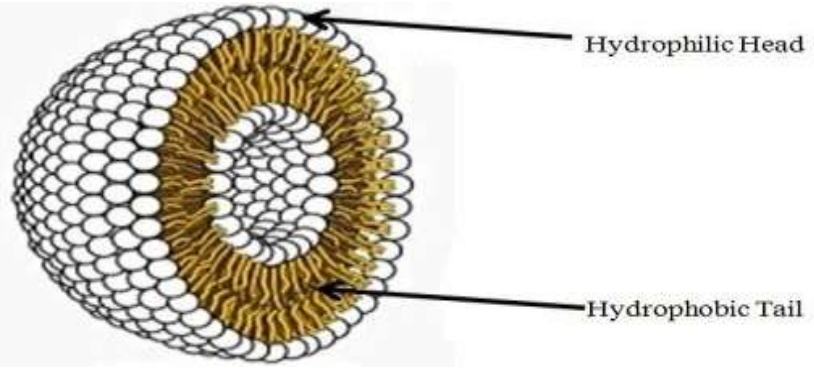


*Figure 1: Structure of Niosone*

Salient features of niosomes:-

1. Niosomes are osmotically active and stable
2. Niosomes composed by amphiphilic molecules i.e. hydrophobic as well as hydrophilic and together thus likewise necessitate the medication atoms with an wide-ranging variety of dissolvability.
3. Niosome release the drug in a controlled way due to its bilayer which give supported arrival of the enclosed medication and act as medication warehouse in the body.
4. Niosome provide targeted drug delivery or called smart drug delivery where medication to a patient in a manner that increases the concentration of the medication in some parts of the body relative to others.
5. Niosome enhances the solubility as well as oral bioavailability of poorly soluble drugs and when applied topically also improve the skin permeability of drugs.
6. According to the desired situation niosomes provide flexibility in their structural characteristics like composition, fluidity and size.
7. Niosome protecting the drug molecules from biological environment improve availability of drug to the particular site. [16-20]

**Materials and Methods**

The drug was found to be white to off white in color, odorless crystalline powder that was similar appearance (I.P. 2007). Melting point of OZ was near to the reported value as mentioned in Indian Pharmacopeia, 2007. FTIR spectrum of OZ confirmed the presence of different group. The various peaks obtained in the IR spectrum matched with IR spectrumgiven in the official. Sorbitan monostearate (Span 60) was purchased from Sigma-Aldrich (United States). Cholesterol was obtained from Fluka Chemie (GmbH, Japan). Chloroform, methanol, and ethanol were purchased from RCI Labscan (Thailand). All materials used in the study were of analytical grade.

**Preparation of OZ Niosomes:** Niosomes encapsulated OZ were prepared by a thin-film hydration technique [1,2]. The surfactant Span 60 (73 mg) and cholesterol (65 mg) were mixed into a 100 ml-round bottom flask and dissolved in 3 ml of solvent mixture of ethanol and chloroform (2:1 v/v). The organic solvent was then evaporated and the resulting thin film was performed under vacuum pressure at 70 °C for 1h, using a rotary evaporator (EYEL4, Eyela Tokyo Rikakikai, Japan) followed by air dry for 30 min. The resultant film was

hydrated with 1ml of 0.01 M phosphate-buffered saline (PBS), pH 7.4 containing OZ at 50 °C for 15 min and niosome suspension was stored at 4°C for further experiments [21-25].

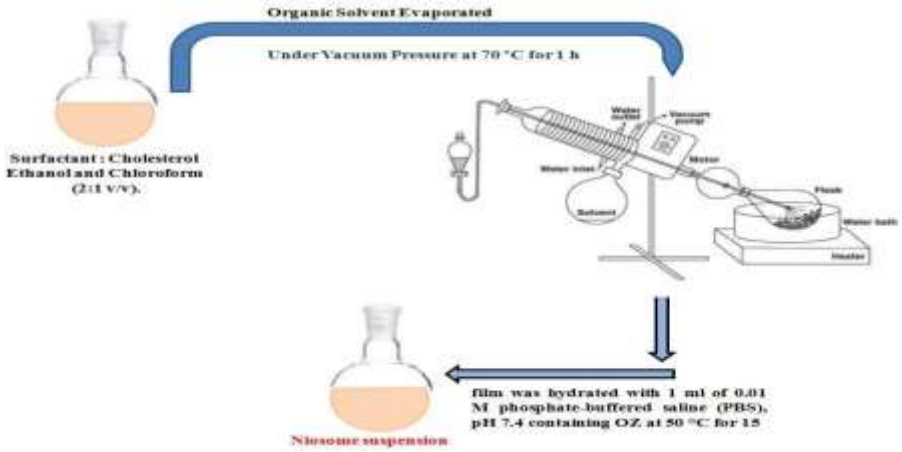


*Figure 2: Formulation of Niosome*

Solvent: chloroform methanol mixture (2:1v/v), Hydrationtime: 2 hour, Hydration media: Phosphate buffer saline pH 7.4 (5ml)

**Removal of unentrapped drug from niosomes**

The unentrapped drug from niosomal formulation was separated by centrifugation method. Theniosomal suspension was taken in centrifuge tube. The formulation was centrifuged at 15,000rpm for 30 min using cooling centrifuge and temperature was maintained at 5°C. The supernatant was separated. Supernatant contained unentrapped drug and pellet contained drug encapsulated

vesicles. The pellet was resuspended in phosphate buffer saline pH 7.4 to obtain a niosomal suspension free from unentrapped drug [26].

**Encapsulation efficiency**

The encapsulation efficiency (EE) of peptide-encapsulated niosomes was investigated, and the extraction condition of niosomes was modified from the previous study [27-30]. Briefly, 1ml of noisome suspension was centrifuged at 15,000 rpm for 2 h at 4°C. Niosome pellets were collected, and 10µl was added to 0.2 ml of methanol (60%v/v), followed by mixing vigorously. After centrifugation at 15,000 rpm for 10 min, 0.1 ml of supernatant was used to determine the OZ content by using an Eppendorf Bio Spectrometer (Eppendorf AG, Germany) at a wave length of 266 nm using phosphate buffer saline pH 7.4. The percentage of encapsulation efficiency (EE%) of the OZ-encapsulated niosomes was calculated by using the following equation:

$$\text{Encapsulation Efficiency (EE)\%} = (A/B) \times 100$$

Where A is the amount of OZ contents in niosomes and B is the amount of feeding OZ.

**Scanning electron microscopy**

SEM scanning was used to analyse the surface morphology of theniosomes (Figure 3). 1 cc of niosome suspension was centrifuged for 10 minutes at 12,000 rpm. Niosomal pellets were centrifuged once more after being rinsed twice with distilled water. Re-suspending the pellets in distilled water (0.4 ml). On a covered glass slide, the sample (2–5 l) was dropped, and it was then allowed to air dry. A Quanta 450 scanning electron microscope (FEI, Eindhoven, the Netherlands) was used to observe the particle characteristics after coating the sample with gold [31-34].

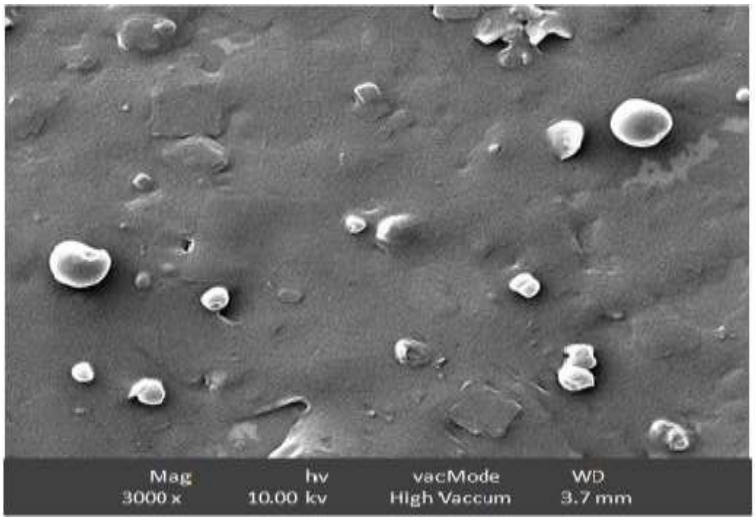


*Figure 3: Scanning electron microscopy (SEM)*
*Singlebar=40µm*

**Results and Discussion**

**Development of OZ niosomes**

In this study, non-ionic surfactants like span 20 and cholesterol were used to create OZ-loaded niosomes utilising the thin-film hydration method. As a solvent, a 2:1 v/v mixture of chloroform and ethanol was utilised. Thin film was created once the solvent from the formulation evaporated. Phosphate buffered saline pH 7.4 moistened and removed the thin film. By sonicating the formulation in the Probe sonicator, the size of the vesicles formulation was decreased.

**Encapsulation efficiency**

The niosomal vesicles were prepared with cholesterol and Span 60 at the molar ratio of 1:1 viathin film hydration technique. According to some previous studies, the formulation of noisome vesicles was successful by using the combination of cholesterol and Span 60 [6, 7]. Span 60 isnon-ionic surfactant, whereas cholesterol acts as an additive agentwhich can promote self-assembly into niosomes [35]. In addition, cholesterol can be used to provide rigidity and propershape and good physical stability of niosome vesicles. Drug entrapped vesicles were separatedfrom unentrapped drug by centrifugation method. 0.5 ml of OZ loaded niosome preparation wasadded with 0.5 ml of 10% triton X 100 and mixed well then incubated for 1 hour. The triton X100 was added to lyse the vesicles in order to release the encapsulated OZ. The solution was diluted with phosphate buffer saline pH 7.4 and filtered through whatmann filter paper. The filtrate was measured spectrophotometrically at 266 nm using phosphate buffer saline pH 7.4 and triton X 100 mixture as blank. The percentage of encapsulation efficiency (%EE) of niosomes was 86.3± 2.3.

**Scanning electron microscopy**

***In-vitro* release study**

At phosphate buffered saline (pH 7.4), the *in-vitro* release of the medication from the formulation was assessed. For the release study, a dialysis membrane (MWCO, 2000 Da, Himedia, India) was chosen. Five mg of the formulation were added to the dialysis sac, sealed with tape, and then suspended in the aqueous receptor medium right away. The *in-vitro* drug release experiment was conducted in the receptor compartment at 37° C. under sink conditions with continuous stirring. With swirling at 100 rpm on a magnetic stirrer, the drug loaded with noise corresponding to 10 mg of drug was taken in a dialysis bag and dialyzed at room temperature against 100 ml of release medium (Remi, India). At each predetermined time interval, one ml of the sample was removed and replaced with an equal volume of fresh medium [36-40]. The medication was measured spectrophotometrically (UV/Vis Shimadzu 1601, Japan) at 266 nm after the proper dilutions.

**Table 1:** *In vitro* drug release of OZ loaded Niosome

| Time (hr) | Amount of Drug Release (mg) | Cumulative amount of drug release(mg) | Cumulative % drug release (%) |
|---|---|---|---|
| 1 | 0.2 | 0.211 | 2.11 |
| 2 | 0.4 | 0.471 | 4.71 |
| 3 | 1.1 | 1.136 | 11.36 |
| 4 | 1.5 | 1.524 | 15.24 |
| 5 | 2.1 | 2.144 | 21.44 |
| 6 | 2.7 | 2.673 | 26.73 |
| 7 | 3.1 | 3.085 | 30.85 |
| 8 | 3.6 | 3.587 | 35.87 |
| 9 | 3.9 | 3.921 | 39.21 |
| 10 | 4.2 | 4.213 | 42.13 |
| 11 | 4.7 | 4.741 | 47.41 |
| 12 | 4.9 | 4.923 | 49.23 |
| 13 | 5.2 | 5.231 | 52.31 |
| 14 | 5.7 | 5.742 | 57.42 |
| 15 | 6.1 | 6.124 | 61.24 |
| 16 | 6.5 | 6.486 | 64.86 |
| 17 | 6.8 | 6.752 | 67.52 |
| 18 | 7.1 | 7.142 | 71.42 |
| 19 | 7.3 | 7.313 | 73.13 |
| 20 | 7.5 | 7.534 | 75.34 |
| 21 | 7.8 | 7.698 | 76.98 |
| 22 | 8.1 | 8.134 | 81.34 |
| 23 | 8.4 | 8.441 | 84.41 |
| 24 | 8.7 | 8.676 | 86.76 |

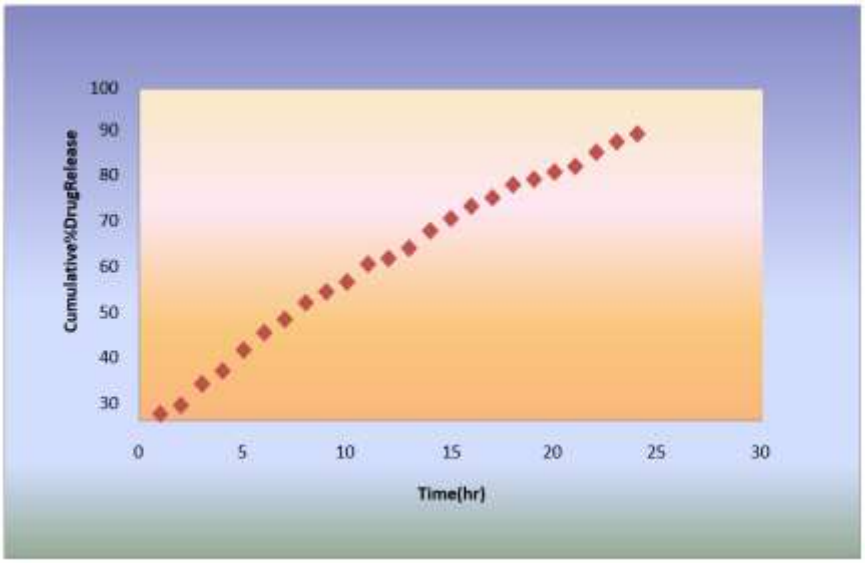


*Figure 1: Cumulative % drug release from OZ loaded Niosome (n=3)*

**Sterility testing**

The prepared and optimised OZ niosomal formulation's sterility was assessed using the sterility testasperIP. The testing method chosen is Method I, Membrane Filtration Method [1].

**Preparation of Soyabean Casein Digest medium (SCDM)**

25 gm of dehydrated media was dissolved in 1000 ml of distilled water. The solution was boiled for 10 min. This solution was used as medium. The medium was cooled to room temperature and pH adjusted to 7.3±0.2. The medium was dispensed in suitable container and sterilized at 121°C for 15 min.

**Preparation of Fluid Thioglycollate medium (FTM)**

Dehydrated media 26 gm was dissolved in distilled water to get 1000 ml of the medium. It was boiled for 10 min. The medium was cooled to room temperature and pH adjusted to 7.2±0.2. The medium was sterilized at a temperature of 121°C for 15min. The sterilised media should not have more than upper one-third of the medium in pink colour.

**Procedure**

The vials containing OZ niosomes were opened while being kept in a hygienic environment using a laminar airflow work station. To prevent contamination by the procedure or the analyzer, all necessary precautions and preventive measures were implemented. Then, a sterile membrane that was attached to a membrane holder assembly was passed through with the medication solution. The membrane was cleaned three times with 100 cc of sterile peptone after passing through the solution (Fluid A). Using sterile scissors, the membrane was divided in half. The SCDM container received one half of the filter paper, and the FTM container received the other half. SCDM containers were then incubated at 22.5±2.5°C and containers at 32.5±2.5°C. For 14 days, the containers were checked for turbidity or signs of microbial development. To validate the sterility testing process, positive control and negative control tests were conducted.

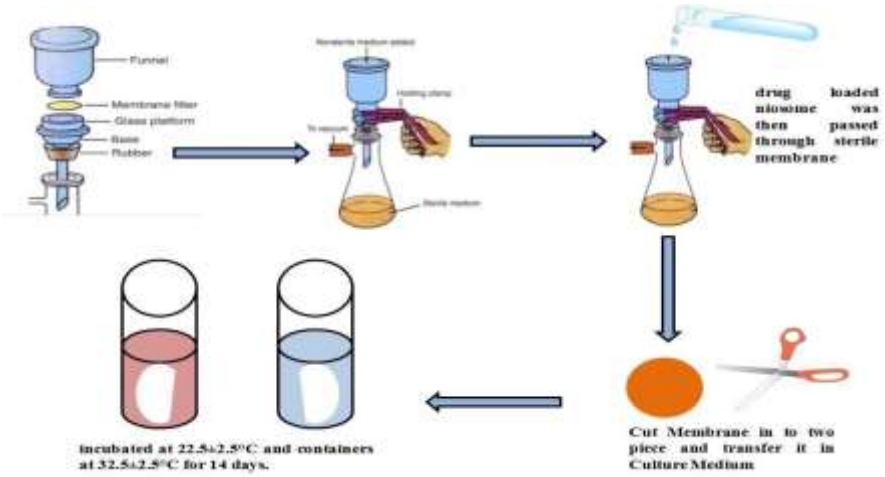


*Figure 2: Sterility testing Procedure*

**Stability study of OZ niosomes**

The optimized OZ niosome formulation was examined for stability study. The formulations were taken in a 20 ml sealed glass vial and stored in three different environments such as 4°C, room temperature and 45°c/75% RH for a period of five weak [41-42].

**Table 2:** Stability study of OZ loaded Niosome for mulation

| Parameter | After Five weak | | | | | |
|---|---|---|---|---|---|---|
| | Dark | | | Light | | |
| | 4°C | R.T. | 45°C | 4°C | R.T. | 45°C |
| **Turbidity** | - | - | - | - | - | + |
| **Precipitation** | - | - | - | - | - | - |
| **Color Change** | - | - | + | - | - | - |
| **Change in Consistency** | - | - | + | - | - | - |

(-)=No Change (+)=Small Change

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