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Functional Properties of Amoxicillin Encapsulated in Niosomes or Liposomes

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ABSTRACT

The functional properties of amoxicillin encapsulated in drug delivery systems (anionic niosomes, cationic liposomes and anionic liposomes) were investigated. Each of the novel drug delivery systems were made in three runs using different molar ratios of anionic niosomes 100:100:10, 400:100:10 and 150:300:10 (Tween 20:cholesterol:dicetyl phosphate), anionic liposomes of 75:75:10, 75:150:10, 100:300:10 (cholesterol: phospholipids: dicetylphosphate), cationic liposomes of 100:100:10, 75:150:10, 100:300:10 (cholesterol: phosphatidylcholine: stearylamine). The susceptibility of three clinical isolates to the drug delivery systems encapsulating amoxicillin was carried out using agar diffusion method. Also, physicochemical studies such as morphology, particle size and stability studies were assessed. The results of the susceptibility of the clinical isolates showed a sustained release from the delivery systems. Morphology of niosomes revealed spherical shaped vesicles. For the particle size determination, all the batches gave mean particle size diameter below 3.8 µm. Stability studies carried out on each of the different unlyophilized drug formulations kept at 28 ˚C and at 40 ˚C after two weeks showed destabilization while those kept at 4˚C were very stable for two weeks. Differential scanning calorimetry was also performed.

KEYWORDS: Amoxicillin, Liposome, Niosome, Encapsulation

INTRODUCTION

Vesicular drug delivery systems like liposomes have been in existence since 1965 when Bangham discovered them accidentally in the laboratory. They are ever novel drug formulations that exhibit highly organized assemblies of one or more concentric lipid bilayers. They are formed upon hydration of amphiphiles which self-close into lipid bilayers like biomembranes. The core and the surface of the liposome are hydrophilic while the bilayer is lipoidal. Aqueous drugs are entrapped in the core and adsorbed to the surface of the liposome while lipophilic drugs are sandwiched in the bilayer. Both hydrophilic and lipophilic drugs are solubilized in these amphiphilic carriers and hence improve bioavailability [1-3]. Most niosomes and all liposomes fall within the nanoparticulate window opening a whole new world of possibilities at targeting to tissues and even certain impermeable sites of delivery. These carriers offer a great number of other advantages making them ideal drug delivery vehicles. The technique of preparation also contributes to the nature, size and chemistry enabling them to be highly potential for gene, protein and enzyme delivery. It also offers an attractive solution for transforming of biosystems and provides a broad platform in several areas of bioscience [4]. The surface properties of carriers can be modified for targeted drug delivery

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and this can reduce drug toxicity and provide more efficient drug distribution [5-8]. Liposomes are the microscopic vesicles composed of one or more concentric lipid bilayers, separated by water or aqueous buffer compartments with a diameter ranging from 10 nm - 100 µm. Depending on their size, liposomes can have small unilamellar vesicles (SUV) 10-100 nm or large unilamellar vesicles (LUV) 100 – 3000 nm). If more than one bilayer is present, they are referred to as multilamellar vesicles (MLV) which is as a result of method of preparation and type of phosphatidylcholine [9]. Niosomes are non-ionic surfactant vesicles and like liposomes form bilayered structures on interaction with water. Niosomes are cheap, have greater stability and are easier to store. They have lower risk of leakage of entrapped drugs unlike the liposomes. Niosomes are chemically stable and can entrap both lipophilic and hydrophilic drugs either in aqueous layer or in vesicular membrane and present low toxicity because of their non-ionic nature. Niosomes are biocompatible, biodegradable and act as adjuvants in vaccine delivery. Niosomes are present within a size range of 10-1000 nm. [10]. Dicetylphosphate, an anionic lipid was included in the preparation of noisome to impart a negative charge and create charge stabilization of the colloidal system. A Cationic lipid namely steraylamine was included in the preparation of liposome to impart a positive charge on their surface. A cationic lipid generally contains the three following structural domains: i) a hydrophilic head-group which is positively charged, usually via the protonation of one (monovalent lipid) or several (multivalent lipid) amino groups; ii) a hydrophobic portion composed of a steroid or of alkyl chains (saturated or unsaturated); and iii) a linker connecting the cationic head-group with the hydrophobic anchor) whose structure may impact on the stability and the biodegradability of the vector [11]. Cationic liposomes protect the cargo from the harsh environment of the gastrointestinal tract and target its delivery to specific sites.

MATERIALS AND METHODS

Chemicals

Cholesterol (Sigma Grade, minimum purity 99 %, Sigma Aldrich Chemie, St. Louis, USA), dicetyl phosphate (Sigma Aldrich Chemie, St. Louis, USA), sorbitan monolaurate (Sigma Aldrich Inc., Germany), stearylamine (Sigma-Aldrich, USA), phospholipon 90H (GmbH Nattermannallee 1.D50829 Köln, Germany), methanol (extra pure Ph. Eur., NF, Scharlau Chemie S.A. EU.), chloroform (Sigma-Aldrich GmbH Germany).

Culture media

Nutrient broth and nutrient agar were the culture media employed in the study.

Test organisms

The organisms used were typed clinical isolate of gram positive organism Staphylococcus aureus, Escherichia coli and Klebsiella sp. obtained from the Department of Pharmaceutical Microbiology Laboratory, UNN.

Methods

Preparation of dry lipid films

Phospholipid, cholesterol and cationic/anionic additive were weighed as indicated in Table 1, 2 and 3 dissolved in 3 ml of chloroform/methanol system (2:1) in a round bottom flask. The solvent mixture was evaporated at room temperature and the flask rotated until a smooth, dry film on the wall of the flask was obtained. Furthermore, 0.4 % of amoxicillin was used to hydrate the films produced until a creamy colloidal dispersion was formed.

Table 1: Formulation composition of anionic noisome

Batches	Tween 20		Cholesterol		Dicetylphosphate	
	mM	mg	mM	mg	mM	mg
A_1	300	368.4	100	38	10	5.46
A_2	200	245.6	100	38	10	5.46
A_3	100	122.6	100	38	10	5.46

Morphological study

Approximately 0.01ml of the multilamellar vesicles was smeared on a microscope slide using a glass rod. The mixture was covered with a cover slip and viewed with a digital photomicroscope. Relevant images were recorded with a digital camera attached to the photomicroscope at a magnification of x 40.

Sensitivity studies of the clinical isolates

Molten nutrient agar (15 ml) was inoculated with 0.1ml of *Escherichia coli* broth culture. It was mixed thoroughly, poured into Petri dishes and rotated to ensure even distribution of the organism. The agar

plates were allowed to set and a sterile cork-borer (5mm diameter) was used to cut three cups in the agar medium plate. Liposomal and niosomal based samples were prepared and used as follows: 3 drops each of the samples were added respectively into the different cups in each of the plates using sterile Pasteur pipettes. The plates were allowed to stand at room temperature for 15 min to enable the samples diffuse into the medium before incubating at 37 °C for 24 h. The inhibition zone diameters were carefully measured and recorded. The procedure above was repeated for broth cultures of Klebsiella sp and Staphylococcus aureus.

Stability studies

Organoleptic properties such as colour, texture, odour, flocculation and sedimentation exhibited by the different formulations were observed after storage at 4 °C, 25°C, and 40 °C for two weeks.

Differential scanning calorimetry (DSC)

DSC thermograms were obtained using DSC 204F1 (Netzsch DSC 204F1, Germany). Samples were dried in a vacuum desiccator, 1.0 mg of the dried powder crimped in a standard aluminum pan and heated from 20°C at a heating rate of 10°C/minute under constant purging of nitrogen.

RESULT AND DISCUSSION

Photomicrographs of the vesicles

The technique used for the preparation of the vesicles was lipid film hydration technique (hand shaking method) which formed films on the wall of the flasks and on hydration with phosphate buffer solution (pH 7.4)

These multilamellar vesicles are formed when thin lipid films are hydrated and swell. The hydrated lipid sheets detach during agitation and self-close to form large, multilamellar vesicles [12]. The photomicrographs of the vesicles were studied by means of optical microscope attached to a moticam 2.0 MP camera at a magnification of x40. The vesicles were spherical and polydisperse with sizes ranging from 0.002-0.015 µm. The net surface charge of the liposomes was modified by the incorporation of positively charged stearylamine lipid and negatively charged dicetylphosphate lipid. The phosphatidylcholine which is a zwitter-ionic or non-ionic lipid was used as the basic lipid for the preparation of the cationic liposomes. The fluidity of the vesicles when usually made from a single lipid depends on the lipid phase transition temperature (Tc) [13]. Membrane permeability is highest at the phase transition temperature, and is lower in the gel phase than in the fluid phase. In the solid gel phase, the lipid hydrocarbons are ordered while in the fluid lipid phase, the lipid hydrocarbons are in a disordered state. Since the particle sizes of the cationic liposomes or the niosomes had an average size of < 500 nm, they will escape phagocytosis and circulate longer in the blood stream. When given by the oral route, liposomes have to survive the "detergent effect" of bile salts and phospholipase activity hence the addition of a cationic lipid to protect it.

Table 2: Formulation composition of anionic liposome

Batches	Cholesterol		Phospholipids		Dicetylphosphate	
	mM	mg	mM	mg	mM	mg
B_1	75	29	75	57	10	5.46
B_2	75	29	150	114.0	10	5.46
B_3	100	38.6	100	76	10	5.46

Table 3: Formulation Composition of Cationic Liposome

Batches	Cholesterol		Phospholipids		Stearylamine
	mM	mg	mM	mg	mg
C_1	100	38.6	100	76	5
C_2	75	29	100	114	5
c_3	100	38.6	100	228	5

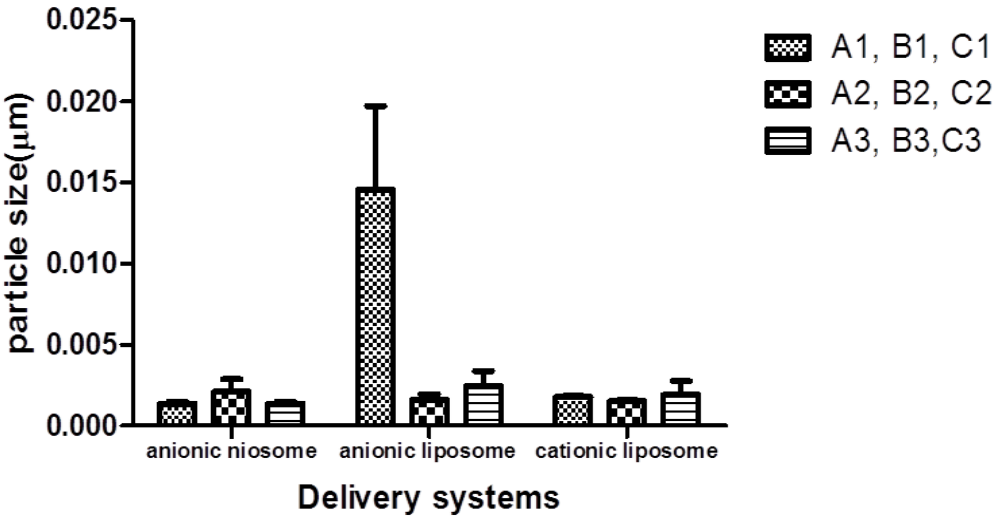

Figure 2: Graph showing the mean particle sizes of the formulations

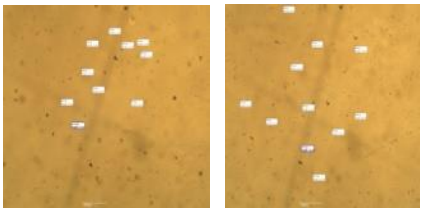

Figure 1a and 1b: Photomicrographs of the Niosomes

Susceptibilities studies

Table 4 shows the susceptibility of *E. coli, Klebsiella sp* and *Staphylococcus aureus* respectively to amoxicillin entrapped in the anionic niosomes, anionic liposomes and cationic liposomes. In liposomes and niosomes which are vesicular structures, the aqueous of non-aqueous drug is entrapped within the core or bilayer from which it is leaked gradually to the exterior in a pulsatile manner while making contact with the target tissue. The cationic liposomes having a positive surface would necessarily fuse with the negatively charged cell membranes of the test organisms thus delivering the liposomal content gradually. The result

of the sensitivity test showed a similarity to this well-known behavior of vesicular structures. The effect of the antibiotic in the three drug delivery systems did not have any appreciable effect on the organisms. The inhibition of the clinical isolates was insignificant. This makes the delivery systems suitable for sustained release. The amoxicillin tablet showed the highest inhibition zone diameter (IZD) for all the isolates probably because of a burst effect which releases the drug in an instant thereby causing a wider zone of inhibition.

Table 4: Susceptibility of the clinical isolates to the drug delivery systems

	Inhibition zone diameter in millimeter									
Microorganism	**A1**	**A2**	**A3**	**B1**	**B2**	**B3**	**C1**	**C2**	**C3**	**Amoxicillin tablet**
Escherichia coli	0	0	0	0	0	0	0	0	0	22
Klebsiella sp.	4.3	2.7	2.7	0	0	0	2.7	1.3	2	17
Staphylococcus aureus	4	2	5.7	0	0	0	2.7	3.3	2	24

Stability studies

The drug delivery systems were stored for four weeks at 4 °C, 28 °C and 40 °C. The cationic and anionic liposomes or niosomes looked physically altered when observed after one month of storage at 28 °C and 40 °C. Very often these preparations are metastable. This means that the state of its free enthalpy is not in equilibrium with the environment [14]. As a result, the vesicles change their lamellarity, size, size distribution and shape with time. For example, small vesicles tend to form larger ones and large vesicles smaller ones. As a result of permeabilization of the membrane, there could be a leakage of the encapsulated drug [15]. Maintaining the physical properties of the vesicles can be difficult without lyophilisation and cryoprotectants such as mannitol, glucose and trehalose. Cryoprotectants increases stability from hydrolysis. Adding antioxidants during preparation can also maintain stability. For the liposomes, there may have been a gradual degradation of the lipid membrane by the presence of membrane destabilizing components, presumably lysolipid and free fatty acid generated by hydrolysis of the lipid [16]. The permeability properties of these amphiphilic carriers determine how well the drug is retained in the liposome or niosome interior. The electrical potential at the membrane surface will affect stability. Also the more ordered and hence tightly packed the membrane, the less permeable [17]. For future work, lyophilization would be included in the stability studies to increase the stability and the shelf life of the finished product by preserving it in a relatively more stable dry state, especially if the drug is not stable in the aqueous suspension [18-19].

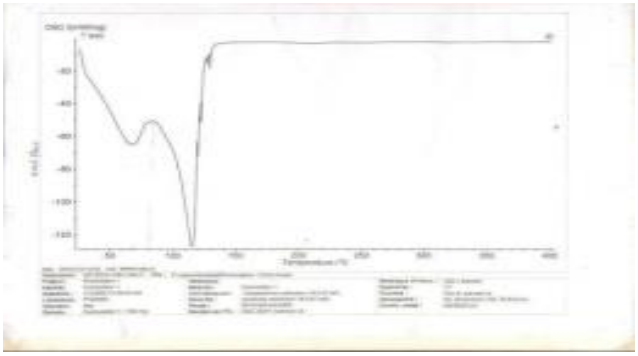

Figure 3: DSC thermographs of the niosome encapsulating amoxicillin

Differential Scanning Calorimetry (DSC)

Amoxicillin showed an endothermic peak at 97.4 °C while amoxicillin niosomes gave a minimum endothermic peak at 67 °C, 115 °C and 128.2 °C. Exothermic peaks of amoxicillin are 185°C and 220.4 °C while that of the amoxillin niosomes are 125.5 °C

and 127 °C. Endothermic peaks were correlated with loss of water associated to hydrophilic groups of polymers while exothermic peaks result from degradation of polyelectrolytes due to dehydration and depolymerisation reactions most probably to the partial decarboxylation of the protonated carboxylic groups and oxidation reactions of the polyelectrolytes.

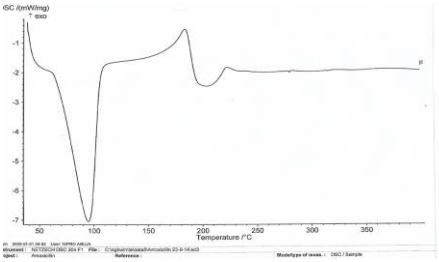

Figure 4: DSC thermographs of amoxicillin

CONCLUSION

From the results, the formulated niosomal or liposomal vesicles could be used as for the sustained release delivery of amoxicillin. The lipid film hydration technique produced vesicles below 500 nm. From the DSC, there was a slight deviation in the peaks of the pure amoxicillin and the formulated noisome which could be as a result of impurities rather than loss of integrity.

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