NANO – NIOSOMES FOR TARGETING INFECTIOUS BACTERIA - AN IN VITRO EVALUATION

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ABSTRACT

Over the past several years, treatment of infectious diseases and immunization has undergone a revolutionary shift. With the advancement of biotechnology and genetic engineering, not only a large number of disease-specific biological have been developed, but also emphasis has been made to effectively deliver these biological. Niosomesare vesicles composed of non-ionic surfactants, which are biodegradable, relativel nontoxic, more stable and inexpensive, an alternative to liposomes. This article reviews the current deepening and widening of interest of niosomes in many scientific disciplines and, particularly its application in medicine. This article also presents overview ofthe techniques of preparation of niosome, types of niosomes, characterization and their applications.

Key words: Bilayer, drug entrapment, lamellar, niosomes.

INTRODUCTION

Nanotechnology is defined as the technology of the use of materials at Nano scale which is generally one billionth of a metre. Nanotechnology is used to form materials, devices and systems with structures and components showing new and improvised physical, chemical and biological properties. The tailoring of materials at atomic, molecular and supra-molecular levels is been done to create new structures with completely new properties [1]. Successful growth in nanotechnology has led to the opening up of new and fundamental applications in material science and engineering including Nano biotechnology, bio nanotechnology, quantum dots, Surface Enhanced Raman Spectroscopy (SERS) and applied microbiology [2]. Moreover it is being now applied in many of the fields such as mechanics, optics, biomedical sciences, chemical industries, electronics, space industries, drug-gene delivery, energy science, catalysis, optoelectronic devices, photo electrochemical applications and nonlinear optical devices [3].
Multiple methods have been described in the literature for the preparation of Niosomes ether injection method, reverse-phase evaporation method, trans membrane pH gradient drug uptake process, bubble method, and micro-fluidization method [4]. Each method could result in the formation of niosomes of different sizes and size distributions. A brief overview of some of the methods that have been described for the preparation of niosomes is provided. A detailed description of the thin-film hydration technique that has been commonly described for the preparation of niosomes is demonstrated [5].

Nanotechnology and Drug Delivery they have discussed about the field of nanotechnology, its impact and also the wide application of nanotechnology in targeted drug delivery. The interdisciplinary nature of nanotechnology enables diversification and development in order to improve quality of life. Scientists in various fields such as engineering, material science, food, biomedical sciences, environmental sciences, agriculture, energy and information technology should be abreast with and employ nanotechnology, as appropriate, for the advancement of research and development. Furthermore, nanotechnology is a technology that every government

(including developing nations) should invest in to bring about improvement in sectors such as healthcare, water, agriculture, energy and environment [6-8].

Green synthesis of metal nanoparticles using plants discussed about the development of efficient green method of synthesis of metal nanoparticles. Some important methods for manufacturing nanoparticles have been discussed here. Mainly the plant extract mediated synthesis of nanoparticles is been analyzed. Various chemical, physical and biological synthetic methods have been used in production of metal nanoparticles. Most of these methods are still in the development stage and the problems experienced are stability and aggregation of nanoparticles, control of crystal growth, morphology, size and size distribution [9]. Moreover, separation of produced nanoparticles for further applications is still an important issue. It was shown that the metal nanoparticles produced by plants are more stable in comparison with those produced by other organisms. Plants (especially plant extracts) are able to reduce metal ions faster than fungi or bacteria. Furthermore, in order to use an easy and safe green method in scale-up and industrial production of well-dispersed metal nanoparticles, plant extracts are certainly better than plant biomass or living plants. Researchers have focused their attention on understanding the biological mechanisms and enzymatic processes of nanoparticle biosynthesis as well as detection and characterization of biomolecules involved in the synthesis of metallic nanoparticles. Many biomolecules in plants such as proteins/enzymes, amino acids, polysaccharides, alkaloids, alcoholic compounds, and vitamins could be involved in bio-reduction, formation and stabilization of metal nanoparticles. Reduction potential of ions and reducing capacity of plants which depend on the presence of polyphenols, enzymes, and other chelating agents present in plants have critical effects on the amounts of nanoparticle production. It should be mentioned that the future of investigations might move toward the optimization of reaction conditions and engineering the recombinant organisms for production of high amounts of proteins, enzymes, and biomolecules involved in biosynthesis and stabilization of nanoparticles. Understanding the biochemical processes/pathways involved in plant heavy metal detoxification, accumulation, and resistance will help to improve nanoparticle production. Genetic modification of plants with improved metal tolerance and accumulation capacities is the future approach to increase the productivity of these organisms in nanoparticle synthesis [10-12].

Niosomes, non-ionic-based vesicles, have drawn much interest in various biomedical applications, owing to their unique characteristics and their ability to encapsulate both hydrophilic and lipophilic cargoes. Niosomes share structural similarity with liposomes while overcoming limitations associated with stability, sterilization, and large-scale production of liposomes. Different methods for preparation of niosomes have been described in the literature, each having its own merits and a great impact on the sizes and characteristics of the formed niosomes (13-15). In this article, procedures involved in the thin-film hydration method, a commonly used method for the preparation of niosomes, are described in detail, while highlighting precautions that should be considered for consistent and reproducible construction of niosomes.

Methodology

Preparation of plant extract: Sun drying is a traditional drying method for reducing the moisture content of paddy by spreading the grains under the sun. The solar radiation heats up the grains as well as the surrounding air and thus increases the rate of water evaporating from the grains. It is the most common drying method in Asia because of its low cost compared to mechanical drying. It requires little investment and is environmentally friendly since it uses the sun as the heat source and therefore produces no CO2. However, sun drying tends to be labor-intensive

and has limited capacity. Temperature control is also difficult in this method and grains can easily be overheating causing cracked grains which leads to low milling quality. It is also not possible to sun dry at night or during rain. Drying is reducing moisture content by the solar radiation heats .It takes time nearly 5-6 days for drying. The Plant are shrimp there size by the absorbing of heat at finally the plant leaves are dried out. Researcher took plant leaves of *Acalypha indica* (Kuppaimeni) a medicinal plant separated by a plant wise. Grinding (milling) is used for the size reduction of solid dry material. It may also improve the eating quality and/or suitability of the material for further processing.is a unit operation widely used in the food industry and designed to reduce the size of materials to give a usable form or to separate their components. In developing countries, it is the common method for food powder processing. Grinding the separating fruit peel into smaller particle collect down it, packing into zip lock covers (13-15).

Dilution is the process of "lowering the concentration of a solute in a solution by simply adding more solvent to the solution, such as water." Diluting a solution entails adding more solvent without adding more solute add 1 g of prepared Plant Extract powder into 30 ml of water. diluted the Plant Extract powder by adding water. Filter papers from cellulose are the most used filters for all kinds of applications in Filtration is a simple technique used to separate solid particles from suspension in a liquid solution. There are many filtration methods available, but all are based on the same general principle: a heterogenous mixture is poured over a filter membrane. The filter membrane has pores of a particular size. Filtration, the technique used to separate solids from liquids, is the act of pouring a mixture onto a membrane (filter paper) that allows the passage of liquid (the filtrate) and results in the collection of the solid. Two filtration techniques are generally used in chemical separations in general chemistry lab: "gravity" filtration and "vacuum" filtration. Gravity Filtration Gravity filtration uses a polyethylene or glass funnel with a stem and filter paper. Filter paper can have pore sizes ranging from small to large to permit slow to fast filtering. The paper is folded in half then folded in quarters, and the conical flask fixed with the filter paper a Now place the funnel into a beaker and wet the filter paper completely with the dominate solvent or solvents in the mixture to be filtered. This step adheres the filter paper to the funnel walls preventing solid from escaping. Before filtering, allow most of the solid in the mixture to settle. Now pour the supernatant liquid through the filter first. This will allow the initial part of the filtration to proceed faster and may prevent clogging of the filter by the solid. To prevent splattering pour the liquid down a glass rod.

Gravity Filtration Scrape the solid onto the filter with a rubber spatula. Rinse the spatula, glass rod and beaker and pour the washings into the filter funnel. If the remaining solid residue is to be washed, rinse with three small portions of an appropriate solvent. If the solid is to be saved, remove the filter paper carefully and place it on a watch glass to dry. A water bath is laboratory equipment made from a container filled with heated water. It is used to incubate samples in water at a constant temperature over a long period of time. Most water baths have a digital or analogue interface to allow users to set a desired temperature, but some water baths have their temperature controlled by a current passing through a reader. Water bath at 50°c for 15 minutes the samples are heating over at a constant temperature .the temperature is check by a temperature indicator. The mercury is inside the temperature indicator it indicates the temperature after reaching the degree of celcious the samples are loaded into water bath after the 15 minutes process ends.

UV-Vis spectroscopy is an analytical technique that measures the amount of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample in comparison to a reference or blank sample. Basically, spectroscopy is related to the interaction of light with matter. As light is absorbed by matter, the result is an increase in the energy

content of the atoms or molecules. When ultraviolet radiations are absorbed, this results in the excitation of the electrons from the ground state towards a higher energy state. Molecules containing π-electrons or nonbonding electrons (n-electrons) can absorb energy in the form of ultraviolet light to excite these electrons to higher anti-bonding molecular orbitals. The more easily excited the electrons, the longer the wavelength of light they can absorb. There are four possible types of transitions. The absorption of ultraviolet light by a chemical compound will produce a distinct spectrum that aids in the identification of the compound.

The optimal properties of Niosomes are observed using UV vis spectrometer after the addition of plant extract to nanoparticle spectra were taken. Add a blank (distilled water) was used as a reference for baseline correction. The results were recorded. The UV was taking by day by day to checking the stability of niosomes day one take control and test values. After set blank the extract is poured into cuveatee load into spectroscopy read the solution. The absorbance and wavelength are observed note it the graph is shown peak line of agnps is noted. The readings are noted 1-5 day, 15th day, 30th day are observed.

Preparation of conjugate niosomes and PE: PE– 10 Ml and Niosomes; 10 Ml. Put hot air oven in the beaker to remove the wetness for 15 minutes. Take 10 ml of PE add into beaker pour 10 ml of synthesized Niosomes kept into the magnetic stirrer for 4 hours .it stirr completely the process is stops. take UV- via spec and observed the readings. Agar- well diffusion method: na media, prepare 150 ml- Peptone-0.75g, Nacl-0.45 g, Yeast extract-0.45 g, Beef extract-0.45 g and Agar – agar-3 g+ 0.5 Take 150 ml of distilled water in a beaker put into peptone, Nacl, yeast extract, beef extract shake the beaker for mixing add agar – agar to the beaker after adding agar- agar do not shake the beaker close with the help of cotton plug .Take petri plates washed it kept into hot air oven for 15 minutes .after that take beaker and petri plates autoclave for 20 minutes .the pressure is released take it from into laminar flow.. Cool down it after shake the beaker for 2 minutes pour into petri-plates .switch on the blower the media get solidy it take 10-15 minutes. Bacterial strain *E. coli, P. auregenosa, B. subtilis, S. auereus*. these strain are used for antibacterial activity

RESULT AND DISCUSSION

Eschereshia coli, Pseudomonas auregenosa are gram – negative bacteria. *Bacillus subtilis, Staplococcus auereus* are gram – positive bacteria these strain are used to analysis antibacterial activity. Nutrient agar media is poured into the petriplate brings it for solidification petri plates are divided into for drug loading. Swap the bacterial strain before that give a mild heat by a lamp Upon solidification, wells were made using a sterile corn borer (6 mm in diameter) into agar plates containing inoculum extract solution at desired concentration is introduced into the water. after petri plates are incubated at 50 °c for 24 hrs. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested. A Zone of Inhibition Test, also called a Kirby-Bauer Test, is a qualitative method used clinically to measure antibiotic resistance and industrially to test the ability of solids and textiles to inhibit microbial growth. Researchers who develop antimicrobial textiles, surfaces, and liquids use this test as a quick and easy way to measure and compare levels of inhibitory activity.

3T3 (normal fibroblast cell line) was obtained from the National Centre for Cell Sciences (NCCS), Pune, India. Cells were maintained in the logarithmic phase of growth in Dulbecco's modified eagle medium (DMEM) supplemented with 10% (v/v) heat inactivated fetal bovine

serum (FBS), 100 U/mL penicillin, 100 µg/mL streptomycin. They were maintained at 37°C with 5% CO2 in 95% air humidified incubator. The cytotoxic effect of the sample was tested against 3T3 cell line by MTT (3-(4, 5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (Mossman, 1983). The cells were seeded in 96-well microplates (1 x 10^6 cells/well) and incubated at 37°C for 48 h in 5% CO2 incubator and allowed to grow 70-80% confluence. Then the medium was replaced and the cells were treated with different concentrations of sample and incubated for 24 h. The morphological changes of untreated (control) and the treated cells were observed under digital inverted microscope (20X magnification) after 24 h and photographed. The cells were then washed with phosphate-buffer saline (PBS, pH-7.4) and 20 µL of (MTT) solution (5 mg/mL in PBS) was added to each well. The plates were then stand at 37ºC in the dark for 2 h The formazan crystals were dissolved in 100 µL DMSO and the absorbance was read spectrometrically at 570 nm. Percentage of cell viability was calculated using the formula, Cell viability (%) = (Absorbance of sample/Absorbance of control) X 100. Graph was plotted using the cell viability (%) at Y-axis and concentration of the sample in X-axis. This experiment was performed according to the previously reported and standardized protocol (Liang et al., 2007). 3T3 cells were seeded in 6-well plates (8 × 105 cells/well) and grown until reached a confluence of 90-95%, in the optimum culture conditions. In the middle of cell monolayer, a scratch was made by a P10 pipette tip, to mimic a wound, and cell debris were removed by washing with fresh medium. The wound was exposed with mouthwash sample (50µg/ml) for 24-48 h at 37°C in a humidified atmosphere of 5 % CO2. The negative control cells were maintained without any treatment. Scratch wound closure was analyzed in two modalities: i) Under the digital inverted microscope, by acquiring digital images at time 0 (T0), 24 hrs (T2) and 48 hrs (T3) (static imaging). The closure of the scratch was quantified by measuring the difference between the wound widths at T0 and T1/T2, using the ImageJ processing software. Scratch closure rate (SCR) was calculated as described (16).

Thus, PE+NioNp represent a potential alternative for controlling multiple-drug resistant microorganisms understand whether the toxicity responses to Niosomes nanoparticle exposure are derived from their small size, the particle 3, or a combination of these factors. Increasing of multi drug resistant bacteria researches are going to inhibit it. conjugate Niosomes and Plant Extract more inhibition to bacteria And it have medical application. Niosomes and conjugated Niosomes and PE they are act bacteridal agent. **Figure 1** represents the conjugation of nioNPs with PEG. Based on these results, it is evident that the colloidal PE – Niosomes nanoparticle – fluoride nanocomposites exhibited antimicrobial activities against the growth of Gram-positive and Gram-negative bacteria Thus, PE+NiNp represent a potential alternative for controlling multiple-drug-resistant microorganisms. Substantial risk for use in living organisms or harm to human health. However, because an increased release of ions is expected from smaller particles

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