INVITRO COMPARISON OF CONTROLLED AND TARGETED DRUG DELIVERY USING NIOSOMES AND LIPOSOMES

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ABSTRACT:

Niosomes and liposomes are noble drug delivery systems used for the targeted drug delivery in this research, the lipid Nanovesicles like Niosomes and liposomes were synthesised from cholesterol, a Phosphatidylcholines. The L-Tyrosine, an amino acid is taken as a model drug which has been used as a drug for entrapment and release at the targeted sites. The entrapment efficiency of the drug in their respective Nano vesicles is calculated. The release was done *invitro* by membrane dialysis method. The release was estimated and graphs were constructed. The release of the drugs from the different Nanovesicles was done at different pH to optimise the release and to target the site of release as an application. The optimisation is done by changing the factors like chemicals ratio, changing the pH to identify the areas with highest and lowest release. As a result the water soluble drug, L-tyrosine shows better entrapment in both Niosomes and liposomes but the entrapment efficiency was highest in the Niosomes of the chemical composition 1:1 i.e., Cholesterol and span-60 respectively. The drug release was much more effective again in niosomes than in other Nanovesicles as the drug release was constantly increasing but the 2:1 ratio sample of Niosomes shown controlled release. The pH optimisation shows that the drug release was maximum at alkaline pH of 11 and next in the acidic pH of 3. The release was least at the alkaline pH of 9. In overall comparison, Niosomes vesicles composed of non-ionic surfactants, which are biodegradable, relatively nontoxic, more stable and inexpensive, an alternative to liposomes in the targeted drug delivery systems and are superior to liposomes in this case.

Keywords: Bionanotechnology, niosomes, liposomes, drug delivery, targeted drug delivery, controlled drug delivery.

INTRODUCTION:

Paul Ehrlich, in 1909, initiated the era of development for targeted delivery when he envisaged a drug delivery mechanism that would target directly to diseased cell. Drug targeting can be defined as the ability to direct a therapeutic agent specifically to desired site of action with little or no interaction with non-target tissue. The concept of targeted drug delivery is designed for attempting to concentrate the drug in the tissues of interest while reducing the relative concentration of the medication in the remaining tissues. As a result, drug is localised on the targeted site. Hence, surrounding tissues are not affected by the drug. In addition, loss of drug does not happen due to localisation of drug, leading to get maximum efficacy of the medication.

NIOSOMES:

Niosomes are described as one of the best lipid nanovesicles for drug delivery. The self-assembly of non-ionic surfactants usually span-60 into vesicles was first reported in the 70s by researchers in the cosmetic industry. Niosomes (non-ionic surfactant vesicles) obtained on hydration are microscopic lamellar structures formed upon combining non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class with cholesterol. The non-ionic surfactants form a closed bilayer vesicle in aqueous media based on its amphiphilic nature using some energy for instance heat, physical agitation to form this structure. In the bilayer structure, hydrophobic parts are oriented away from the aqueous solvent, whereas the hydrophilic heads remain in contact with the

aqueous solvent. Due to presence of hydrophilic, amphiphilic and lipophilic moieties in the structure, these can accommodate drug molecules with a wide range of solubility. The structure of niosomes is shown in figure-1.

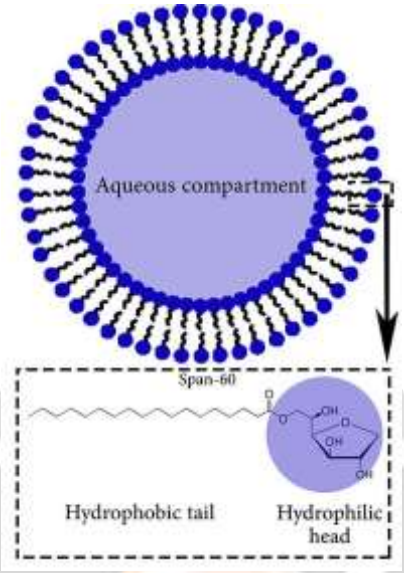

Fig 1 - niosomes

LIPOSOMES:

The term liposomes cover a very large number of different structures, but it can be defined as a lipid bilayer structure or a membrane that encloses an internal aqueous volume. The structure of the membrane can vary significantly, making it possible to create a vast amount of different liposomes, each with their own characteristics and applications. When this system is used for drug delivery, both hydrophilic and lipophilic drugs can be transported therein (Torchilin, 2007). Liposomes were first described in 1964 by A.D. Bangham and his colleague R.W. Thorne after examining and analysing a dispersion of phospholipids in water under an electron microscope (Betageri et al., 1993). They found that the phospholipids automatically arranged themselves to form structures that they referred to as "bag-like". A close colleague, Gerald Weissman, suggested the structures be called liposomes, which he then defined as "microscopic vesicles composed of one or more lipid bilayers". This discovery led the way to a large field of research. The uses found for liposomes have been wide-spread and even include drug delivery systems for cosmetics (Deamer, 2010).

The main reason why research into liposomes advanced as it has can be largely attributed to the fact that liposomes can mimic biological cells. This also means that liposomes are highly biocompatible, making them an ideal candidate for a drug delivery system, with applications ranging from delivering enzymes, antibacterials, antiviral drugs, antiparasite drugs, fungicides, transdermal transporters, diagnostic tools and adjuvants for vaccines (Lasic, 1998).

MATERIALS AND METHODS:

Synthesis of liposome Nanovesicles:

The multilamellar vesicles of liposomes were synthesised by the hand shaking method technique, in the hand shaking method, surfactant and cholesterol are dissolved in a volatile organic solvent such as diethyl ether, chloroform or methanol in a rotary evaporator, leaving a thin layer of solid mixture deposited on the wall of the flask. The dried layer is hydrated with aqueous phase containing drug at normal temperature with gentle agitation. For the synthesis of liposomes, 20mg of cholesterol was weighed accurately and taken in a round bottom flask. To this, 5ml of chloroform was added (any non-polar solvent can be used) the mixture was mixed well to dissolve the cholesterol and the flask was kept in a shaker for 24 hours.

Synthesis of niosome Nanovesicles:

The niosomes were synthesised by the incorporation of a lipid usually a phospholipid and a non-ionic surfactant. The procedure is similar to that of the liposome synthesis. 10mg of cholesterol and 10 mg of sorbitan monostearate (span 60) was taken in a round bottom flask 5ml of the chloroform was added to it, dissolved it. It was kept in a shaker for 24 hours.

Preparation of tyrosine standard:

0.2, 0.4, 0.6, 0.8, 1.0, ml of stock solutions were taken in test tubes and the solution is made upto 1 ml with distilled water. From these different dilutions, pipette out 0.2 ml protein solution to different test tubes and add 2 ml of alkaline copper sulphate reagent (analytical reagent). Mix the solutions well. This solution is incubated at room temperature for 10 mins. Then add 0.2 ml of reagent Folin-Ciocalteau solution (reagent solutions) to each tube and incubate for 30 min. Zero the spectrometer with blank and take the optical density (measure the absorbance) at 660 nm. Plot the absorbance against protein concentration to get a standard calibration curve.

Drug entrapment in liposome and niosomes and separation:

Evaporated the chloroform by keeping in a water bath and formed a thin film on the surface of the flask. After complete evaporation of the chloroform, the drug is added dropwise 5ml of drug is added and was heated in water bath for about 10 minutes and cooled**.** Kept in the shaker for about 24 hours for the drug entrapment and the separation of the drug entrapped vesicles are done by centrifugation process. The mixture is transferred into centrifuge tubes the tubes are centrifuged at about 5000rpm for about 5 minutes after centrifugation process, both the supernatant and the pellet are separated and stored in different glass tubes the pellet re-suspended in the buffer and stored separately.

Calculation of entrapment efficiency:

The drug in the supernatant was estimated by Folin-Ciocalteau method. The total unentrapped drug was calculated the entrapped drug is calculated by subtracting the unentrapped drug from the total drug.

Entrapped drug = Total drug – unentrapped drug

The entrapment frequency is calculated by the following formula,

Entrapment frequency = (entrapped drug / total drug) x 100

Activation of dialysis membrane:

The dialysis membrane is activated by following method, 0.01M EDTA and 0.1 M sodium carbonate was prepared. Equal volume of 0.01M EDTA and 0.1 M sodium carbonate were mixed together to form the buffer 1, 0.01M EDTA acts as buffer 2. The dialysis membrane is activated by first boiling the membrane in the buffer 1 solution for 15 minutes. Then the membrane is again boiled in the buffer 2 for another 15 minutes. Thus the membrane filter is activated.

Chemical stocks:

Buffer-1: 0.01M EDTA – 0.3722g of EDTA dissolved in 200 ml of distilled water adjusted the pH to 8.0 using NaOH to enable dissolving of EDTA.

Buffer-2: 0.1M Sodium Carbonate – 1.05 g of Na_2CO_3 in 100ml of distilled water.

Release and estimation of drug:

The release of the drug is tested in-vitro by dialysis tubing method. In this a dialysis membrane was activated and soaked in distilled water until use. The drug entrapped vesicle containing pellet was resuspended in ml of water. It was added to a dialysis membrane sac and was sealed tightly. The release is made by keeping it in a beaker containing 50ml of water kept in Taken 2ml of sample for every 30 minutes and replacing with new 2ml of distilled water. It was made for about 3 hours. The aliquots are estimated for the drug by Folin-Ciocalteau method.

OPTIMISATION:

Chemical optimisation:

The chemical optimisation of the Nanovesicles was done by changing the ratio of cholesterol and span-60 in the ratio of 1:2 and 2:1 respectively. They were dissolved in the chloroform and were shaken for 24 hours. The drug entrapment was done. They were centrifuged and the entrapment efficiencies were calculated. The drug release was tested for about 3 hours. The drug is then estimated with Folin-Ciocalteau method and graphs were drawn.

pH optimisation:

The pH optimisation is done by releasing the drug in different pH by changing the environment. The drug is released by dialysis in different pH ranging from very acidic pH to very basic pH and a range between 1to13. The drug in the release is estimated by Folin-Ciocalteau method and the graph was drawn.

RESULTS, INTERPRETATIONS AND CALCULATIONS:

Niosomes and liposomes are noble drug delivery systems used for the targeted drug delivery in this research, the lipid Nanovesicles like Niosomes and liposomes were synthesised from cholesterol, a Phosphatidylcholines. The L-Tyrosine, an amino acid is taken as a model drug which has been used as a drug for entrapment and release at the targeted sites. The entrapment efficiency of the drug in their respective Nano vesicles is calculated. The release was done *invitro* by membrane dialysis method. The release was estimated and graphs were constructed. The release of the drugs from the different Nanovesicles was done at different pH to optimise the release and to target the site of release as an application. The optimisation is done by changing the factors like chemicals ratio, changing the pH to identify the areas with highest and lowest release.

The results, graphs, interpretations and calculations of the research are as following,

As a result the water soluble drug, L-tyrosine shows better entrapment in both Niosomes and liposomes but the entrapment efficiency was highest in the Niosomes of the chemical composition 1:1 i.e., Cholesterol and span-60 respectively. The drug release was much more effective again in niosomes than in other Nanovesicles as the drug release was constantly increasing but the 2:1 ratio sample of Niosomes shown controlled release. The pH optimisation shows that the drug release was maximum at alkaline pH of 11 and next in the acidic pH of 3. The release was least at the alkaline pH of 9. In overall comparison, Niosomes vesicles composed of non-ionic surfactants, which are biodegradable, relatively nontoxic, more stable and inexpensive, an alternative to liposomes in the targeted drug delivery systems and are superior to liposomes in this case.

Drug release in liposomes and niosomes:

The drug release in the liposomes and the niosomes are in the fig- 2. Niosomes show better release than that of the liposomes.

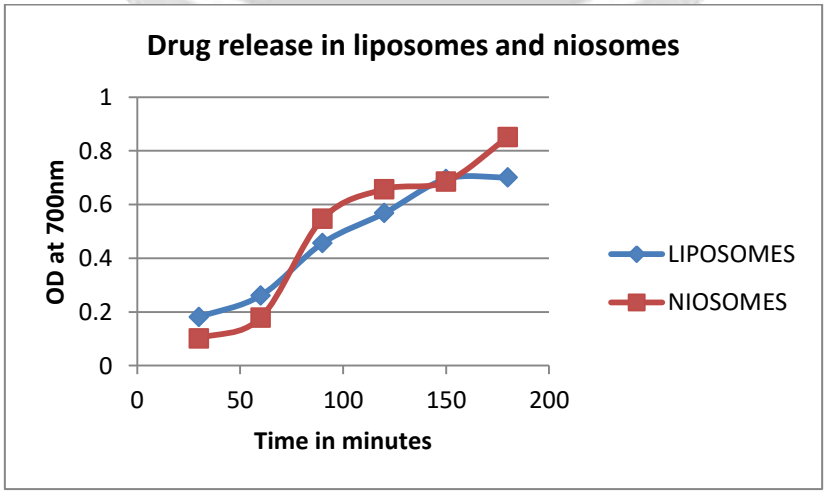

Fig-2: Drug release in liposomes and niosomes

Entrapment efficiency:

The entrapment frequency is calculated by the following formula,

Entrapment frequency = (entrapped drug / total drug) x 100

The entrapment frequency is calculated for, Liposomes, Niosomes, 2:1 ratio niosomes and 1:2 ratio niosomes. The results are shown in the fig-3. The entrapment efficiency was highest in the niosomes with 94.13% whereas, liposomes show the least entrapment efficiency of 89.74%.

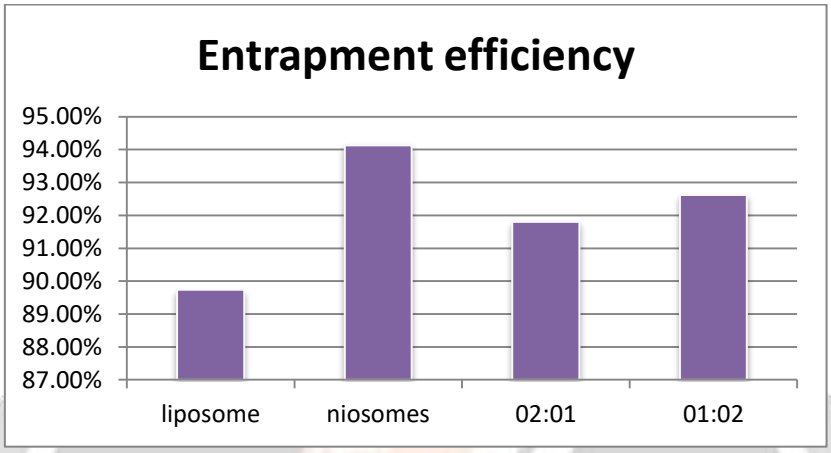

Fig-3: Entrapment efficiency

Chemical optimisation:

The ratio of cholesterol and span-60 were optimised into 1:2 and 2:1 ratio of cholesterol and span-60 respectively. The results are shown in the figure-4. There was a constant gradual increase in the release until 150th minute, and then the release gradually decreases in the 1:2 niosomes. Whereas in the 2:1 niosomes the release was quite increasing at first till the 90th minute and was almost stationary with a controlled release until 150th minute then the release was decreasing.

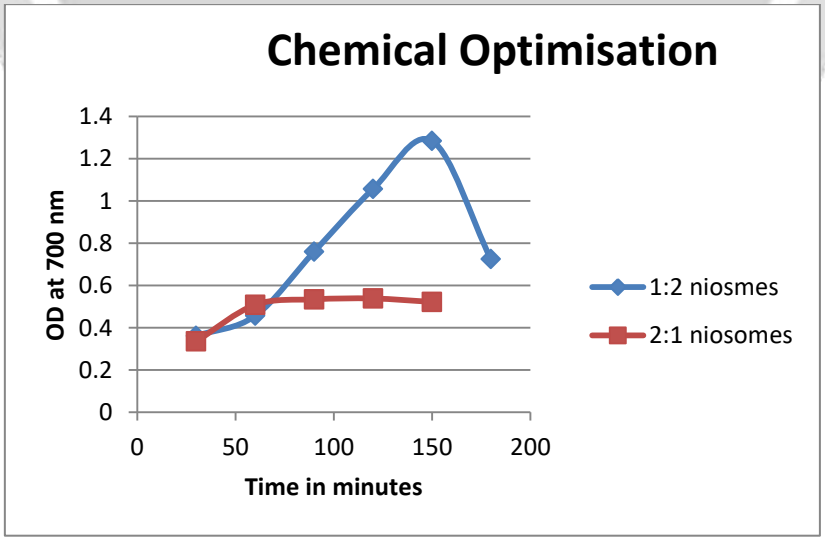

Fig-4: Chemical optimisation

pH optimisation:

The drug release was tested in two different environments, acidic and alkaline respectively. The results are shown in the figure 5. The drug release at the alkaline pH of 11 was the highest while the pH 3 also shown a high release. The alkaline pH 9 shows the least release. This shows that the drug can be effectively released in both acidic and basic pH.

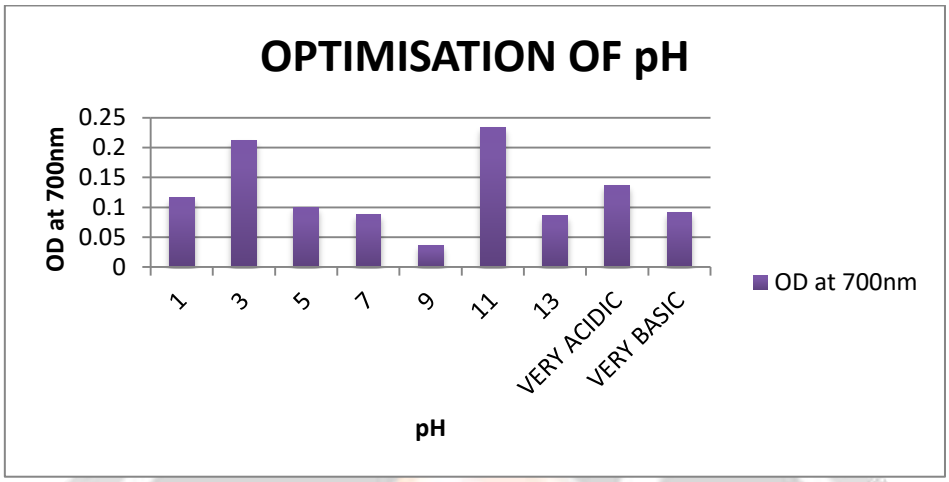

Fig-5: pH optimisation

CONCLUSION:

Niosomal drug delivery system is one of the examples of great evolution in drug delivery technologies. Niosomes have great drug delivery potential for targeted delivery of anti-cancer, anti-infective agents. Drug delivery potential of niosome can enhance by using novel concepts like proniosomes. Niosomes also serve better aid in diagnostic imaging and as a vaccine adjuvant. The concept of incorporating the drug into niosomes for a better targeting of the drug at appropriate tissue destination is widely accepted by researchers. Niosomes represent a promising drug delivery module.

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