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PRONIOSOMES – A SURROGATE FOR TRANSDERMAL DRUG DELIVERY

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Abstract

Nanotechnology is an advancing technology expected to bring revolutionary changes in the field of life sciences including drug delivery, diagnostics, nutraceuticals and biomedical for implants and prosthesis. The advance in nanotechnology helps in preparing newer formulations. Controlled release drug products are often formulated to permit the establishment and maintenance of drug concentration at target site for longer interval of time. One such technique of drug targeting is 'niosome'. In order to minimize the problems associated with niosome physical stability such as aggregation, fusion and leaking and to provide additional convenience in transportation, distribution, storage and dosing etc. a dry product can be prepared from niosome called proniosome. In all comparisons, proniosomes are as good as or better than conventional niosome. One of the advancement in nanotechnology is the preparation of proniosomes - derived niosomes. Proniosomes are solid colloidal particles which may be hydrated immediately before use to yield aqueous niosome dispersions similar to those produced by more cumbersome conventional methods. These 'proniosomes' minimize problems of niosome physical stability such as aggregation, fusion and leaking, and provide additional convenience in transportation, distribution, storage, and dosing. This article describes the preparation of dispersions of proniosome-derived niosomes; In addition proniosome-derived niosomes are described in terms of their morphology, particle size, particle size distribution, and drug release. In all parameters proniosome-derived niosomes are as good as or better than conventional niosomes.

INTRODUCTION

To pursue optimal drug action, functional molecules could be transported by a carrier to the site of action and released to perform the task. The concept of carrier to deliver drugs to target organ and modify drug disposition has been widely discussed and is well documented. The majority of such reports have concerned the use of phospholipids vesicle or liposome. These exhibit certain disadvantages, one of which relates to their chemical instability because of their predisposition to oxidative degradation, thus phospholipid must be stored and handled in a nitrogen atmosphere. The cost and variable purity of natural phospholipids might be other considerations militating against adoption of liposome as drug delivery vehicles. Alternative to phospholipids are thus of interest from the technical viewpoint and could also allow a wider study of the influence of chemical composition on the biological fate of vesicle. Colloidal particulate carriers such as liposomes or niosomes have been widely employed in drug delivery systems and producing them from proniosomes provides them a distinctive advantage. These carriers can act as drug reservoirs and the rate of drug release can be controlled by modification of their composition. These lipid vesicles can carry both hydrophilic drugs (by encapsulation) and hydrophobic drugs (in lipid domain). Due of their capability to carry a variety of drugs, these lipid vesicles have been extensively used in various drug delivery systems like drug targeting controlled release and permeation enhancement of drugs.[1,2] But there remains certain draw backs to be addressed and can be avoided if they are prepared in dry form. Proniosomes, prepared in dry form and hydrated by agitation in hot water to form niosomes provide an alternative with prospective for drug delivery via the transdermal route [3,4]

NIOSOMES

Niosomes are non-ionic surfactant vesicles that can entrap a solute in a manner analogous to liposomes. They are osmotically active, and are stable on their own, while also increasing the stability of the entrapped drugs. Handling and storage of surfactants require no special conditions. Niosomes possess an infrastructure consisting of hydrophilic and hydrophobic moieties together, and as a result, can

accommodate drug molecules with a wide range of solubilities [3]**.** Although niosomes as drug carriers have shown advantages such as being cheap and chemically stable, they are associated with problems related to physical stability such as fusion, aggregation, sedimentation and leakage on storage4**.** All methods traditionally used for preparation of niosomes are time consuming and many involve specialized equipments.

Most of these methods allow only for a predetermined lot size so material is often wasted if smaller quantities are required for particular dose application [5] .The size of niosomes are microscopic and lies in nanometric scale. The particle size ranges from 10nm-100nm.[3, 4]

DISADVANTAGES OF NIOSOMES

1. Physical instability
2. Aggregation
3. Fusion
4. Leaking of entrapped drug
5. Hydrolysis of encapsulated drugs which limiting the shelf life of the dispersion

PRONIOSOMES:

Proniosomes are dry formulations of surfactant-coated carrier, which can be measured out as needed and rehydrated by brief agitation in hot water. These "proniosomes" minimize problems of niosomes physical stability such as aggregation, fusion and leaking and provided additional convenience in transportation, distribution, storage and dosing. Proniosome-derived niosomes are superior to conventional niosomes in convenience of storage, transport and dosing. Stability of dry proniosomes is expected to be more stable than a pre-manufactured niosomal formulation. In release studies proniosomes appear to be equivalent to conventional niosomes. Size distributions of proniosome-derived niosomes are somewhat better that those of conventional niosomes so the release performance in more critical cases turns out to be superior [5, 6, 7].

Proniosomes are dry powder, which makes further processing and packaging possible. The powder form provides optimal flexibility, unit dosing, in which the proniosome powder is provided in capsule could be beneficial. A proniosome formulation based on maltodextrin was recently developed that has potential applications in deliver of hydrophobic or

amphiphilic drugs. The better of these formulations used a hollow particle with exceptionally high surface area. The principal advantage with this formulation was the amount of carrier required to support the surfactant could be easily adjusted and proniosomes with very high mass ratios of surfactant to carrier could be prepared. Because of the ease of production of proniosomes using the maltodextrin by slurry method, hydration of surfactant from proniosomes of a wide range of compositions can be studied [8, 10].

ADVANTAGES OF PRONIOSOMES OVER THE NIOSOMES [7, 10]

- Avoiding problem of physical stability like aggregation, fusion, leaking.
- Avoiding hydrolysis of encapsulated drugs which limiting the shelf life of the dispersion.

MATERIALS AND METHODS OF PRONIOSOMES PREPARATIONS:

SURFACTANTS:

Selection of surfactant should be done on the basis of HLB value. As Hydrophilic Lipophilic Balance (HLB) is a good indicator of the vesicle forming ability of any surfactant, HLB number in between 4 and 8 was found to be compatible with vesicle formation. It is also reported that the hydrophilic surfactant owing to high aqueous solubility on hydration do not reach a state of concentrated systems in order to allow free hydrated units to exist aggregates and coalesced to form lamellar structure. The water soluble detergent polysorbate 20 also forms niosomes in the presence of cholesterol. This is despite the fact that the HLB number of this compound is 16.7 Degree of entrapment is affected by the HLB of a surfactant. Transition temperature of surfactants also affects the entrapment of drug in vesicles. Spans with highest phase transition temperature provide the highest entrapment for the drug and vice versa. Span 40 and Span 60 produces vesicles of larger size with higher entrapment of drug. The drug leaching from the vesicles is reduced due to high phase transition temperature and low permeability. High HLB value of Span 40 and 60 results reduction in surface free energy which allows forming vesicles of larger size hence large area exposed to the dissolution medium and skin. No significant difference is observed in the skin permeation profile of formulation containing Span 60 and Span 40

due to their higher phase transition temperature that is responsible for their lower permeability. The encapsulation efficiency of Tween is relatively low as compared to Span. The geometry of vesicle to be formed from surfactants is affected by its structure, which is related to critical packing parameters. On the basis of critical packing parameters of Surfactants can predicate geometry of vesicle to be formed. Critical packing parameters can be defined using following equation, CPP 0.5 micelles form

$$CPP = \frac{v}{lc \times ao}$$

CPP $\leq$0.5 micelles form

CPP= 0.5 – 1 spherical vesicles form

CPP = 1 inverted vesicles form

V – Hydrophobic group volume

lc = the critical hydrophobic group length,,

a0= the area of hydrophilic head group.

Span 60 is the good surfactant because it has CPP value between 0.5 and 1

Stabilizers: Cholesterol is essential component of vesicles. Incorporation of cholesterol influence vesicle stability and permeability. Concentration of cholesterol plays an important role in entrapment of drug in vesicles. There are reports that entrapment efficiency increase with increasing cholesterol content and by the usage of span 60 which has higher transition temperature. It was also observed that very high cholesterol content had a lowering effect on drug entrapment to the vesicles. This could be due to the fact that cholesterol beyond a certain level starts disrupting the regular bi-layered structure leading to loss of drug entrapment. Lecithin also provides stability but to a lesser extent compared to cholesterol.

Carriers: Maltodextrin is a polysaccharide. It has minimal solubility in organic solvent. Thus it is possible to coat maltodextrin particles by simply adding surfactant in organic solvent. The use of maltodextrin as carrier in Proniosomes preparation permitted flexibility in the ratio of surfactant and other components which can be incorporated. Coating sorbitol results in solid cake like mass

METHODOLOGY

Preparation of proniosomes:

The proniosomes can be prepared by

1. Spraying method.

2. Slurry method.

Spraying method [7]:

The proniosomes were prepared by spraying the surfactant in organic solvent onto sorbitol powder and then evaporating the solvent. Because the sorbitol carrier is soluble in the organic solvent, it is necessary to repeat the process until the desired surfactant load has been achieved. The surfactant coating on the carrier comes out to be very thin and hydration of this coating allows multilamellar vesicles to form.

Slurry method [8, 9, 10, 11]

The slurry method was developed to produce proniosomes using maltodextrin as a carrier. The time required to produce proniosome by this is independent of the ratio of surfactant solution to carrier material. In slurry method, the entire volume of surfactant solution is added to maltodextrin powder in a rotary evaporator and vacuum applied until the powder appears to be dry and free flowing. Drug containing proniosome-derived niosomes can be prepared in manner analogous to that used for the conventional niosomes, by adding drug to the surfactant mixture prior to spraying the solution onto the carrier (sorbitol, maltodextrin) or by addition of drug to the aqueous solution used to dissolve hydrate the proniosomes.

Formation of Niosomes from Proniosomes [8, 11]

The niosomes can be prepared from the proniosomes by adding the aqueous phase with the drug to the proniosomes with brief agitation at a temperature greater than the mean transition phase temperature of the surfactant.

$T > Tm$

Where,

T = Temperature

Tm = mean phase transition temperature

The formulation of niosomes from maltodextrin based proniosomes. This provides rapid reconstitution of niosomes with minimal residual carrier. Slurry of maltodextrin and surfactant was dried to form a free flowing powder, which could be rehydrated by addition of warm water.

SEPARATION OF UNENTRAPPED DRUG:

The removal of unentrapped solute from the vesicles can be accomplished by various techniques, which include: -

1. **Dialysis [11, 12, 15]:**

The aqueous niosomal dispersion is dialyzed in dialysis tubing against suitable dissolution medium at room temperature. The samples are withdrawn from the medium at suitable time intervals, centrifuged and analyzed for drug content using suitable method (U.V. spectroscopy, HPLC etc).

2. **Gel Filtration [13, 14]:**

The unentrapped drug is removed by gel filtration of niosomal dispersion through a Sephadex- G-50 column and eluted with suitable mobile phase and analyzed with suitable analytical techniques.

3. **Centrifugation [6,7,11]:**

The proniosome derived niosomal suspension is centrifuged and the supernatant is separated. The pellet is washed and then resuspended to obtain a niosomal suspension free from unentrapped drug.

CHARACTERIZATION OF PRONIOSOMES:

Proniosomes are characterized for vesicle size, size distribution, shape and surface morphological studies.

Particle size analysis and Surface Morphology: Particle size of Proniosomes is very important characteristic. The surface morphology (roundness, smoothness, and formation of aggregates) and the size distribution of Proniosomes were studied by Scanning Electron Microscopy (SEM).

Microscopy: The vesicle formation by the particular procedure was confirmed by optical microscopy in 400x resolution. The niosome suspension placed over a glass slide and fixed over by drying at room temperature, the dry thin film of niosome suspension observed for the formation of vesicles. The photomicrograph of the preparation also obtained from the microscope by using a digital SLR camera.

Measurement of angle of repose:

The angle of repose of dry proniosomes powder and maltodextrin powder was measured by a funnel method. The maltodextrin powder or proniosomes powder was poured into a funnel which was fixed at a position so that the 13mm outlet orifice of the funnel is 5cm above a level black surface. The powder flows down from the funnel to form a cone on the surface and the angle of repose was then calculated by measuring the height of the cone and the diameter of its base.

Separation of Free Un-entrapped Drug: The encapsulation efficiency of proniosomes is determined after separation of the un-entrapped drug using these techniques:

- Dialysis
- Gel filtration.
- Centrifugation.

Dialysis: The aqueous niosomal dispersion is dialyzed tubing against suitable dissolution medium at room temperature. Then samples are withdrawn from the medium at suitable time interval centrifuged and analyzed for drug content using UV spectroscopy.

Gel Filtration: The free drug is removed by gel filtration of niosomal dispersion through a sephadex G50 column and separated with suitable mobile phase and analyzed with suitable analytical techniques.

Centrifugation: The niosomal suspension is centrifuged and the surfactant is separated. The pellet is washed and then re suspended to obtain a niosoaml suspension free from un-entrapped drug.

Entrapment efficiency: Niosome entrapped drug was estimated by dialysis method. The calculated amount of prepared niosomes was placed in the dialysis bag 50 (pre-soaked for 24 hrs). Free drug was dialyzed for 30 minutes each time in 100 ml of phosphate buffer saline pH 6.8. The dialysis of free drug completed after 12-15 changes, when no drug was detectable in the recipient solution. The dialyzed drug was determined by finding out the concentration of bulk of solution by UV spectrophotometer at 275 nm. The samples from the bulk of solution diluted appropriately before going for absorbance measurement. The free drug in the bulk of solution gives us the total amount of un-entrapped drug.

The vesicles obtained after removal of un-entrapped drug by dialysis is then re suspended in 30% v/v of PEG 200 and 1 ml of 0.1%v/v triton x-100 solution is added to solubilise vesicles the resulted clear solution is then filtered and analyzed for drug content. The percentage of drug entrapped is calculated by using the following formula.

EE% = total drug - diffused drug ÷ total drug×100.

***In vitro* Drug Release from Proniosomal Vesicles:-**

In vitro drug release and skin permeation studies for proniosome scan be determined by different techniques like

- Franz diffusion cell
- Dialysis tubing
- Reverse dialysis

Franz Diffusion Cell: This Franz diffusion cell has a donor chamber fitted with a cellophane membrane. The Proniosomes are placed in it and dialyzed against a suitable dissolution medium at room temperature. The drug content is analyzed using suitable method (UV spectroscopy, HPLC) maintenance of sink conditions is essential.

Dialysis Tubing

This apparatus has prewashed dialysis tubing which can be hermetically sealed. The Proniosomes are placed in it and then dialyzed against a suitable dissolution medium at a room temperature. *In vitro* release pattern of niosomal suspension was carried out in dialysis bag method. 10 mg equivalent of drug niosomal suspension was taken in dialysis bag (Hi media) and the bag was placed in a beaker containing 75 ml of 0.1 N HCl. The beaker was placed over magnetic stirrer having stirring speed of 100 rpm and the temperature was maintained at 37+10C. 5 ml samples were withdrawn periodically and were replaced by fresh buffer. After two hours, 25 ml of 0.2 M tribasic sodium phosphate was added to change the pH of test medium to 6.8, and the test was continued for a further 22 hours. The sink condition was maintained throughout the experiment. The withdrawn samples were appropriately diluted and analyzed for drug content using U.V. spectrophotometer keeping phosphate buffer pH 6.8 as blank. All the determinations were made in triplicate.

Reverse Dialysis: In this apparatus a number of small dialysis tubes containing 1 ml of dissolution medium are placed. Proniosomes is then displaced into the dissolution medium. The direct dilution of the proniosomes is possible with this method. But the rapid release cannot be quantified using this method.[15]

Drug Release Kinetic Data Analysis: The release data obtained from various formulations were studied further for their fitness of data in different kinetic models like Zero order, Higuchi's and peppa's.

Stability Studies on Proniosomes: Physical stability study was carried out to investigate

the degradation of drug from proniosome during storage. Stability studies carried out by storing the prepared proniosomes at various temperature conditions like refrigeration on (2-8˚c), room temperature (25˚±0.5˚c) and elevated temperature (45˚c±0.5˚c) from a period of one month to 3 months. Drug content and variation in the average vesicle diameter are periodically monitored. ICH guidelines suggests stability studies for dry proniosomes powder meant for reconstitution should be studied for accelerated stability at 75% relative humidity as per international climatic zones and climatic conditions.[16]

Zeta potential analysis: Zeta potential analysis is done for determining the colloidal properties of the prepared formulations. The suitably diluted proniosome derived noisome dispersion is determined using zeta potential analyzer based on electrophoretic light scattering and laser Doppler velocimetry method. The temperature is set at 25°C. Charge on vesicles and their mean zeta potential values with standard deviation of 5 measurements are obtained directly from the measurement

Niosomes as drug carriers

Several studies have described the properties of niosomes as drug carriers. Niosomes behave similarly to liposomes in vivo by prolonging circulation time of the encapsulated drug and altering chemical distribution within the body[16, 17]. However, niosomes have advantages over liposomes as drug carriers, including greater chemical stability, lower cost, easier storage and handling, and are less likely than liposomes to become toxic. Niosomal encapsulation reduces toxicity of drugs in many different applications and therapies. Niosomal drug delivery has been studied using various methods of administration[9], including intramuscular, intravenous peroral and transdermal Nebulized surfactants entrapping all-trans-retinoic acid (ATRA) were delivered as an inhaled aerosol reducing the drug toxicity and altering the pharmacokinetics. In addition, as drug delivery vesicles, niosomes have been shown to enhance absorption of some drugs across cell membranes to localize in targeted organs and tissues and to elude the reticulo-endothelial system (RES) .Cellular uptake of niosomes can be via endocytosis; however they have been

shown to bind and fuse with cell plasma membranes via cellular receptors when vesicle surface charge is sufficiently negative[18].

Current trends in niosomes:

Use of niosomes in cosmetics was first done by as they offered the following advantages:

- The vesicle suspension being water based offers greater patient compliance over oil based systems, since the structure of the niosome offers place to accommodate hydrophilic, lipophilic as well as ampiphilic drug moieties, they can be used for a variety of drugs.
- The characteristics such as size, lamellarity etc. of the vesicle can be varied depending on the requirement. The vesicles can act as a depot to release the drug slowly and offer a controlled release.

APPLICATIONS OF NIOSOMES:

The application of niosomal technology is widely varied and can be used to treat a number of diseases. The following are the few uses of niosomes which are either proven or under research

Drug Targeting:

One of the most useful aspects of niosomes is their ability to target drugs. Niosomes can be used to target drugs to the reticulo-endothelial system. The reticulo-endothelial system [43] (RES) preferentially takes up niosome vesicles. The uptake of niosomes is controlled by circulating serum factors called opsonins. These opsonins mark the niosome for clearance. Such localization of drugs is utilized to treat tumors in animals known to metastasize to the liver and spleen. This localization of drugs can also be used for treating parasitic infections of the liver. Niosomes can also be utilized for targeting drugs to organs other than the RES. A carrier system (such as antibodies) can be attached to niosomes (as immunoglobulin bind readily to the lipid surface of the niosome) to target them to specific organs[44]. Many cells also possess the intrinsic ability recognize and bind specific carbohydrate determinants, and this can be exploited by niosomes to direct carrier system to particular cells.[19]

Anti-neoplastic Treatment [20, 21]:

Most antineoplastic drugs cause severe side effects. Niosomes can alter the metabolism; prolong circulation and half life of the drug, thus decreasing the side effects of the

drugs. Niosomal entrapment of Doxorubicin and Methotrexate (in two separate studies) showed beneficial effects over the unentrapped drugs, such as decreased rate of proliferation of the tumor and higher plasma levels accompanied by slower elimination[20].

Leishmaniasis [21]:

Leishmaniasis is a disease in which a parasite of the genus Leishmania invades the cells of the liver and spleen. Commonly prescribed drugs for the treatment are derivatives of antimony (antimonials), which in higher concentrations can cause cardiac, liver and kidney damage. Use of niosomes in tests conducted showed that it was possible to administer higher levels of the drug without the triggering of the side effects, and thus allowed greater efficacy in treatment.

Delivery of Peptide Drugs [22]:

Oral peptide drug delivery has long been faced with a challenge of bypassing the enzymes which would breakdown the peptide. Use of niosomes to successfully protect the peptides from gastrointestinal peptide breakdown is being investigated [23]. In an *in vitro* study conducted by oral delivery of a vasopressin derivative entrapped in niosomes showed that entrapment of the drug significantly increased the stability of the peptide.

Uses in Studying Immune Response:

Niosomes are used in studying immune response due to their immunological selectivity, low toxicity and greater stability. Niosomes are being used to study the nature of the immune response provoked by antigens.[23]

Niosomes as Carriers for Hemoglobin:

The niosomes can be used as carriers for hemoglobin within the blood. The niosomal vesicle is permeable to oxygen and hence can act as a carrier for hemoglobin in anaemic patients.

Transdermal Drug Delivery Systems Utilizing Niosomes [24]:

One of the most useful aspects of niosomes is that they greatly enhance the uptake of drugs through the skin. Transdermal drug delivery utilizing niosomal technology is widely used in cosmetics; In fact, it was one of the first uses of the niosomes [25]. Topical use of niosome entrapped antibiotics to treat acne is done. The penetration of the drugs through the skin is greatly increased

as compared to un-entrapped drug. Recently, transdermal vaccines utilizing niosomal technology is also being researched.

OTHER APPLICATIONS:

a) Sustained Release:

The role of liver as a depot for methotrexate after niosomes are taken up by the liver cells. Sustained release action of niosomes can be applied to drugs with low therapeutic index and low water solubility since those could be maintained in the circulation via niosomal encapsulation[27].

b) Localized Drug Action:

Drug delivery through niosomes is one of the approaches to achieve localized drug action, since their size and low penetrability through epithelium and connective tissue keeps the drug localized at the site of administration. Localized drug action results in enhancement of efficacy of potency of the drug and at the same time reduces its systemic toxic effects e.g. Antimonials encapsulated within niosomes are taken up by mononuclear cells resulting in localization of drug, increase in potency and hence decrease both in dose and toxicity. The evolution of niosomal drug delivery technology is still at an infancy stage, but this type of drug delivery system has shown promise in cancer chemotherapy and anti-leishmanial therapy.

CONCLUSION

From the above article it is concluded that the concept of incorporating the drug into niosomes for a better targeting of the drug at appropriate tissue destination is widely accepted by researchers and academicians. Proniosomes derived niosomes represent a promising drug delivery module. They represent a structure similar to liposome and hence they can represent alternative vesicular systems with respect to liposomes, due to the niosome ability to encapsulate different type of drugs within their multi environmental structure. Proniosomes based niosomes are thoughts to be better candidates drug delivery as compared to liposomes due to various factors like cost, stability etc. Various types of drug deliveries can be possible using proniosomes based niosomes like targeting, ophthalmic, topical, Parentral, peroral vaccine etc. More researches have to be made in this field to come out with all the potential in this novel drug delivery system.

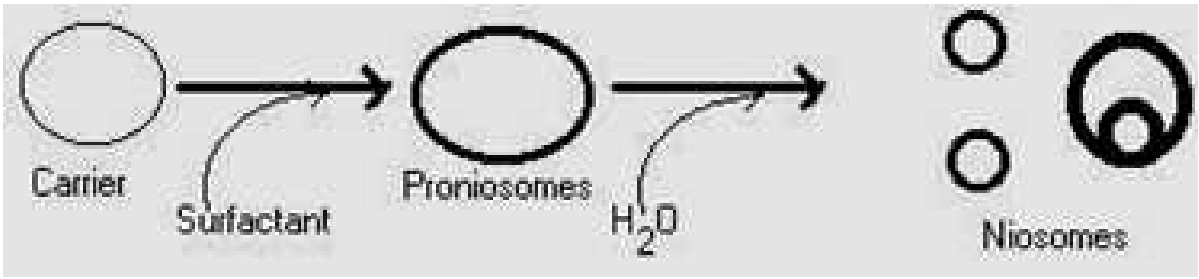

Figure 1 Formation of Niosomes from Proniosomes

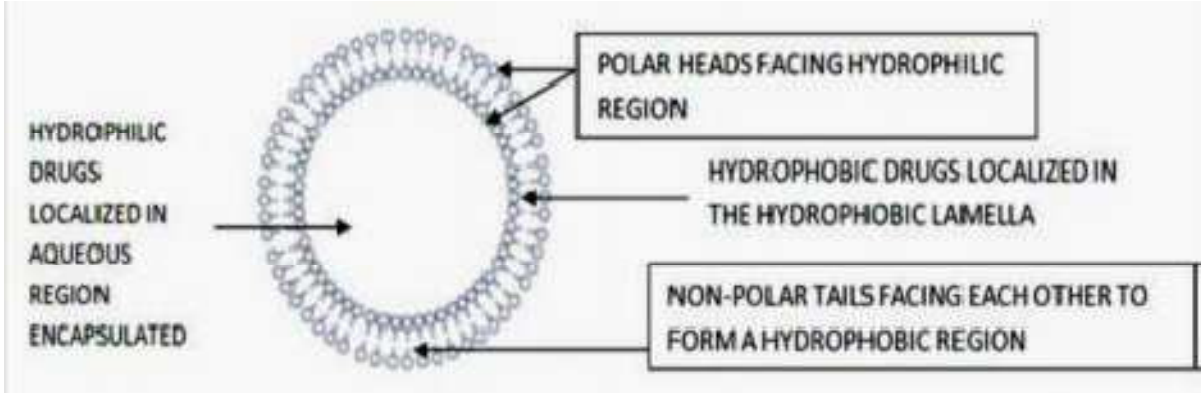

Figure 2 Representations of Niosomes

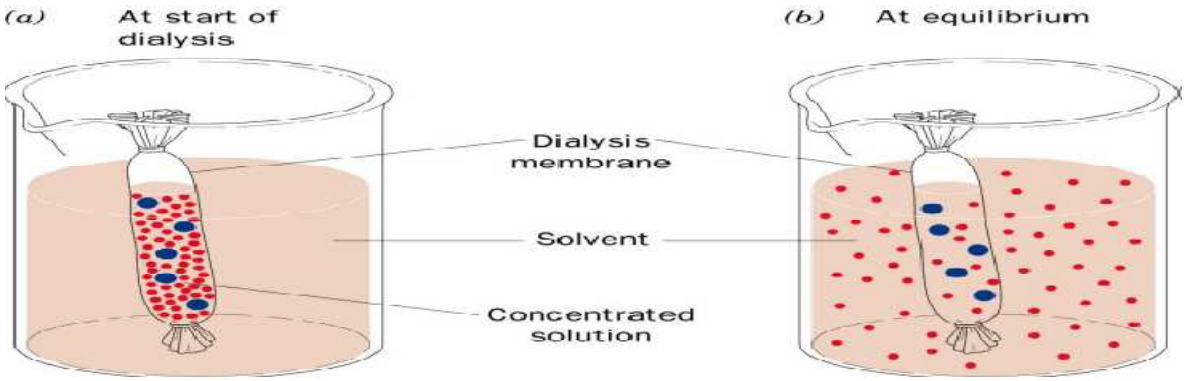

Figure 3 Dialysis Bag Method

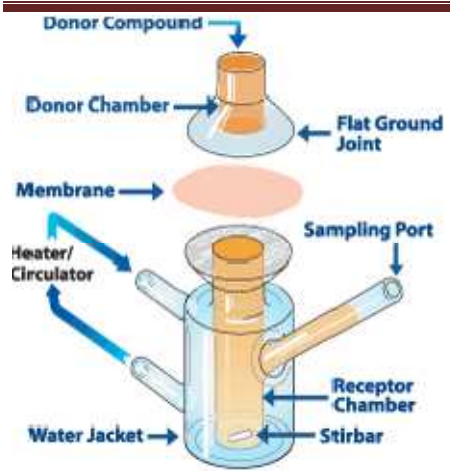

Figure 4 Franz Diffusion Cell

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