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RESEARCH ARTICLE

STUDY OF WIDELY DIVERGENT DEVELOPMENT OF NIOSOMES AND ITS APPLIANCES

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Abstract:
Niosomes are vesicles composed of non-ionic surfactants, which are biodegradable, relatively nontoxic, more stable and inexpensive, an alternative to liposomes. They present a structure similar to liposome and hence they can represent alternative vesicular systems with respect to liposomes. The niosome ability to encapsulate different type of drugs within their multi environmental structure. Niosomes have been developed as an alternative to phospholipids based liposomes. The technology utilized in niosomes is still greatly in its infancy, and already it is showing promise in the fields of cancer and infectious disease treatments. Niosomes can also be utilized for targeting drugs to organs other than the RES. They are based on several families of synthetic non ionic amphiphilic molecule. This review article focuses on the structure of niosome, advantages of niosomes, methods of preparation, characterization of nisosomes, application of niosomes.

Keywords: Niosomes, Liposomes, Non ionic surfactants, Amphiphilic Molecule

Introduction

In the past few decades, considerable attention has been focused on the development of new drug delivery system (NDDS). The NDDS should ideally fulfill two prerequisites. Firstly, it should deliver the drug at a rate directed by the needs of the body, over the period of treatment. Secondly, it should channel the active entity to the site of action. Conventional dosage forms including prolonged release dosage forms are unable to meet none of these. At present, no available drug delivery system behaves ideally, but sincere attempts have been made to achieve them through various novel approaches in drug delivery[1].

Lipid vesicles were first described by Dr. Alec Bangham in 1965. He had observed that handshaken phospholipid dispersions in water form multilamellar spherical structures. These vesicles, soon named leptosomes, it consist of an aqueous cavity encapsulated by one or more lipid bilayer membranes.

Since these early investigations, more than 22 years have past till the first cosmetic products containing liposomes appeared on the market (NIOSOMES from Lancome and capture from Dior, 1986). However, only three years later, more than one hundred different niosomal formulations could be found.

Liposomes are used to carry and protect hydrophilic agents. Water soluble agents are enclosed into liposomes if they are present during the preparation. However, some part of the material always remains in the outer phase. In addition to water soluble substances, also amphiphilic and lipophilic substances can be loaded into liposomes to some extent. Amphiphilic molecules stick to the membrane whereas lipophilic substances can be incorporated into the hydrophobic part of the bilayer. Usually such molecules have a negative influence on the stability of the liposomes.

In contrast to liposomes, nanoparticles are the ideal carrier system to transport and protect lipophilic agents. Niosomes are nonionic surfactant vesicles that have potential applications in the delivery of hydrophobic or amphiphilic drugs.

Niosomes have been developed as an alternative to phospholipids based liposomes. They are based on several families of synthetic non ionic amphiphilic molecule. At present, there are rather limited experiences with niosomes as parentral delivery system[2].
Topical liposomes or niosomes may serve as solubilization matrix, as a local depot for sustained release of dermally active compounds, as penetration enhancers, or as rate-limiting membrane barrier for the modulation of systemic absorption of drugs[3].

2. STRUCTURE OF NOISOMES

Structurally, niosomes are similar to liposomes, in that they are also made up of a bilayer. However, the bilayer in the case of niosomes is made up of non-ionic surface active agents rather than phospholipids as seen in the case of liposomes. Most surface active agents when immersed in water yield micellar structures however some surfactants can yield bilayer vesicles which are niosomes. Niosomes may be unilamellar or multilamellar depending on the method used to prepare them.

The niosome is made of a surfactant bilayer with its hydrophilic ends exposed on the outside and inside of the vesicle, while the hydrophobic chains face each other within the bilayer. Hence, the vesicle holds hydrophilic drugs within the space enclosed in the vesicle, while hydrophobic drugs are embedded within the bilayer itself. The figure below will give a better idea of what a niosome looks like and where the drug is located within the vesicle[15].

A typical niosome vesicle would consist of a vesicle forming ampiphile i.e. a non-ionic surfactant such as Span-60, which is usually stabilized by the addition of cholesterol and a small amount of non ionic surfactant such as diacetyl phosphate, which also helps in stabilizing the vesicle[4].

3. NIOSOMES OR NON-IONIC SURFACTANT VESICLES[25]

A number of non-ionic surfactants have been used to prepare vesicles viz. polyglycerol alkylether, glucosyl dialkylethers, crown ethers, ester linked surfactants, polyoxyethylene alkyl ether[30] Brij[31] and a series of spans and tweens[32].

3.1 Alkyl Ethers

Alkyl glycerol ethers shown in Fig.3 synthesized at L'Oreal, France,were the first compounds reported to form niosomal vesicular dispersions. A wide variety of drug delivery applications for the L'Oreal non-ionic surfactants have been explored. Notable application of these compounds has been as vesicle dispersions in the field of experimental cancer chemotherapy where both methotrexate and doxorubicin have been encapsulated and the effect of delivery in niosomes studied. In the control of leishmaniasis, vesicles formed from the alkyl glycerol ethers have been employed in the delivery of sodium stibogluconate and direct comparisons made with liposomes. The encapsulation of haemoglobin into these non-ionic surfactant vesicles as well as antipyrine has been documented. Alkyl glycerol ethers have also been used to prepare non-ionic surfactant vesicles for cosmetic application.

3.2. Alkyl Esters

Alkyl esters, such as the sorbitan esters (Fig.4), widely used in foodstuffs, have been studied as the basis of non-ionic surfactant vesicles. A non-ionic surfactant vesicle formulation consisting of a mixture of alkyl ether and alkyl ester surfactants, namely polyoxyethylene-10-stearyl ether (C_{18}EO10): glyceryl laurate (C_{12}G1): cholesterol (27:15:57) has been used in the transdermal delivery of cyclosporin A.

3.3. Alkyl Amides

Alkyl galactosides and glucosides incorporating amino acid spacers have also been found to produce vesicles (Fig.5). While, as a general rule, the alkyl groups in all vesicles forming amphiphiles consist of fully or partially saturated C_{12} to C_{22} hydrocarbons, certain novel amide compounds bearing fluorocarbon chains

3.4 Fatty Acid and Amino Acid Compounds

In addition to alkyl glycosides, amino acid moieties, when made suitably amphiphilic by the addition of hydrophobic alkyl side chains, are vesicle formers (Fig.6). In the presence of a water soluble carbodimide condensing agent, these amino acid vesicles form peptide liposomes. Long chain fatty acids also form 'ufasomes', closed vesicles formed from fatty acid bilayers. The latter vesicles were prepared at pH 8 resulting in a degree of ionization and subsequent increase in the effective volume of the hydrophilic head group. The influence of the hydrophilic head group on vesicle formation is dealt with below.

A number of non-ionic surfactants have been used to prepare vesicles viz. polyglycerol alkyl ether, glucosyl dialkyl ethers, crown ethers, ester linked surfactants, polyoxyethylene alkyl ether,Brij[5],and a series of spans and tweens[6,7,8,9].

Niosomes improve the oral bioavailability of poorly absorbed drugs[10] and enhance skin penetration of drugs. They can be made to reach the site of action by oral absorption of niosomes is better as compared to

liposomes as replacement of phospholipids by nonionic surfactants has made niosomes less susceptible to the action of bile salts, parenteral, as well as topical routes[11]. Similar to liposomes, there are 3 major types of niosomes multilamellar vesicles (MLV, size >0.05 µm), small unilamellar vesicles (SUV, size -0.025-0.05 µm), large unilamellar vesicles (LUV, size >0.10 µm). MLVs vesicles exhibit increased trapped volume and equilibrium solute distribution, and require hand-shaking method. They show variations in lipid compositions. SUVs are commonly produced by sonication, and French Press procedures. Ultrasonic electrocapillary emulsification or solvent dilution techniques can be used to prepare SUVs. The injections of lipids solubilised in an organic solvent into an aqueous buffer, can result in spontaneous formation of LUV. But the better method of preparation of LUV is reverse phase evaporation, or by detergent solubilisation method[12].

Varshosaz *et al* prepared niosomes of sorbitan monoesters (span 20, 40, 60 and 80), using the film hydration method without sonication, and demonstrated that vesicles containing span 60 showed the highest protection of insulin against proteolytic enzymes[13]. A study of daunorubicinhydrochloride encapsulated in niosomes by Balasubramanium *et al*, suggests that the multilamellar vesicles obtained by the reverse evaporation process, resulted in vesicles that resisted the immediate lysis in the Kupffer cells, whereby a prolonged drug concentration was achieved which enhanced the cell lysis. Hao *et al*, prepared niosomes, which have high encapsulation capacity for soluble drugs, starting from span 60 and cholesterol, using evaporation-sonication method, and demonstrated that niosomes prepared in this way not only have high encapsulation capacity, but it is also expected that side effects of drugs may be reduced. Niosomes have also been used as carriers for iobitridol, a diagnostic agent used for X-ray imaging. Non-ionic surfactant vesicles (niosomes) were prepared and appended with a polysaccharide cap using hydrophobic anchors[12]. Hydrophobized polysaccharides, O-palmitoyl pullulan (OPPu) and cholesteroyl pullulan (CHPu), were anchored onto propranolol HCl containing preformed niosomes for oral drug delivery. The niosomal formulation displayed higher and sustained plasma drug level profile, compared to free drug solution, and hence act as promising carriers for 5-fluorouracil[14].

4. ADVANTAGES OF NIOSOMES

Use of niosomes in cosmetics was first done by L'Oreal 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.

4.1 Other advantages of niosomes are:

- They are osmotically active and stable.
- They increase the stability of the entrapped drug
- Handling and storage of surfactants do not require any special conditions
- Can increase the oral bioavailability of drugs
- Can enhance the skin penetration of drugs
- They can be used for oral, parenteral as well topical use
- The surfactants are biodegradable, biocompatible, and non-immunogenic
- Improve the therapeutic performance of the drug by protecting it from the biological environment and restricting effects to target cells, thereby reducing the clearance of the drug.
- The niosomal dispersions in an aqueous phase can be emulsified in a non-aqueous phase to control the release rate of the drug and administer normal vesicles in external non-aqueous phase.

5. COMPARISON OF NIOSOME V/S LIPOSOME

Niosomes are different from liposomes in that they offer certain advantages over liposomes. Liposomes face problems such as they are expensive, their ingredients like phospholipids are chemically unstable because of their predisposition to oxidative degradation, they require special storage and handling and purity of natural phospholipids is variable. Niosomes do not have any of these problems. Also since niosomes are made of uncharged single-chain surfactant molecules as compared to the liposomes which are made from neutral or charged double chained phospholipids, the structure of niosomes is different from that of liposomes.

6. METHOD OF PREPARATION OF NIOSOMES

Niosomes can be prepared by a number of methods which are as follows:

6.1 Ether injection method

In this method, a solution of the surfactant is made by dissolving it in diethyl ether. This solution is then introduced using an injection (14 gauge needle) into warm water or aqueous media containing the drug maintained at 60°C. Vaporization of the ether leads to the formation of single layered vesicles. The particle size of the niosomes formed depend on the conditions used, and can range anywhere between 50-1000 µm.

6.2 Hand shaking method (Thin film hydration technique)

In this method a mixture of the vesicle forming agents such as the surfactant and cholesterol are dissolved in a volatile organic solvent such as diethyl ether or

chloroform in a round bottom flask. The organic solvent is removed at room temperature using a rotary evaporator, which leaves a thin film of solid mixture deposited on the walls of the flask. This dried surfactant film can then be rehydrated with the aqueous phase, with gentle agitation to yield multilamellar niosomes. The multilamellar vesicles thus formed can further be processed to yield unilamellar niosomes or smaller niosomes using sonication, microfluidization or membrane extrusion techniques.

6.3 Reverse Phase Evaporation Technique (REV)

This method involves the creation of a solution of cholesterol and surfactant (1:1 ratio) in a mixture of ether and chloroform. An aqueous phase containing the drug to be loaded is added to this, and the resulting two phases are sonicated at 4-5°C. A clear gel is formed which is further sonicated after the addition of phosphate buffered saline (PBS). After this the temperature is raised to 40°C and pressure is reduced to remove the organic phase. This results in a viscous niosome suspension which can be diluted with PBS and heated on a water bath at 60°C for 10 mins to yield niosomes.

6.4 Trans membrane pH gradient (inside acidic) Drug Uptake Process
(Remote Loading)

In this method, a solution of surfactant and cholesterol is made in chloroform. The solvent is then evaporated under reduced pressure to get a thin film on the wall of the round bottom flask, similar to the hand shaking method. This film is then hydrated using citric acid solution (300mM, pH 4.0) by vortex mixing. The resulting multilamellar vesicles are then treated to three freeze thaw cycles and sonicated. To the niosomal suspension, aqueous solution containing 10mg/ml of drug is added and vortexed. The pH of the sample is then raised to 7.0-7.2 using 1M disodium phosphate (this causes the drug which is outside the vesicle to become non-ionic and can then cross the niosomal membrane, and once inside it is again ionized thus not allowing it to exit the vesicle). The mixture is later heated at 60°C for 10 minutes to give niosomes.

6.5 The "Bubble" Method

It is a technique which has only recently been developed and which allows the preparation of niosomes without the use of organic solvents. The bubbling unit consists of a round bottom flask with three necks, and this is positioned in a water bath to control the temperature. Water-cooled reflux and thermometer is positioned in the first and second neck, while the third neck is used to supply nitrogen. Cholesterol and surfactant are dispersed together in a buffer (pH 7.4) at 70°C. This dispersion is mixed for a period of 15 seconds with high shear homogenizer and immediately afterwards, it is bubbled at 70°C using the nitrogen gas to yield niosomes.

6.6 Formation of niosomes from proniosomes

To create proniosomes, a water soluble carrier such as sorbitol is first coated with the surfactant. The coating is done by preparing a solution of the surfactant with cholesterol in a volatile organic solvent, which is sprayed onto the powder of sorbitol kept in a rotary evaporator. The evaporation of the organic solvent yields a thin coat on the sorbitol particles. The resulting coating is a dry formulation in which a water soluble particle is coated with a thin film of dry surfactant. This preparation is termed Proniosome.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[39].

6.7 Micro fluidization

Micro fluidization is a recent technique used to prepare unilamellar vesicles of defined size distribution. This method is based on submerged jet principle in which two fluidized streams interact at ultra high velocities, in precisely defined micro channels within the interaction chamber. The impingement of thin liquid she*et al*ong a common front is arranged such that the energy supplied to the system remains within the area of niosomes formation. The result is a greater uniformity, smaller size and better reproducibility of niosomes formed[20].

6.8 Multiple membrane extrusion method[16]

Mixture of surfactant, cholesterol and dicetyl phosphate in chloroform is made into thin film by evaporation. The film is hydrated with aqueous drug polycarbonate membranes solution and the resultant suspension extruded through which are placed in series for upto 8 passages. It is a good method for controlling niosome size.

6.9 Sonication

A typical method of production of the vesicles is by sonication of solution as described by Cable[32]. In this method an aliquot of drug solution in buffer is added to the surfactant/cholesterol mixture in a 1 0ml glass vial. The mixture is probe sonicated at 60°C for 3 minutes using a sonicator with a titanium probe to yield niosomes..

7. SEPARATION OF UNENTRAPPED DRUG
8.

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

7.1 Dialysis

The aqueous niosomal dispersion is dialyzed in a dialysis tubing against phosphate buffer or normal saline or glucose solution.

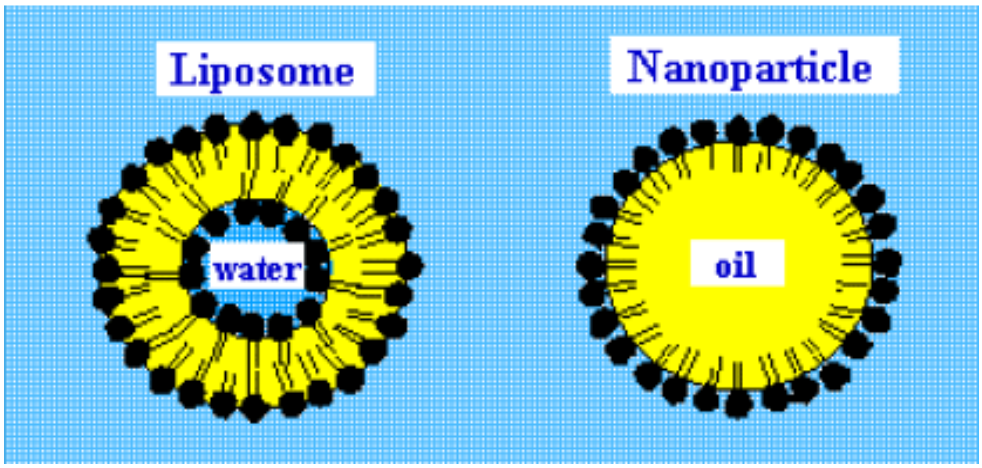

Fig. 1:- *Comparison of the structure of liposomes and nanoparticles formed by phospholipids.*

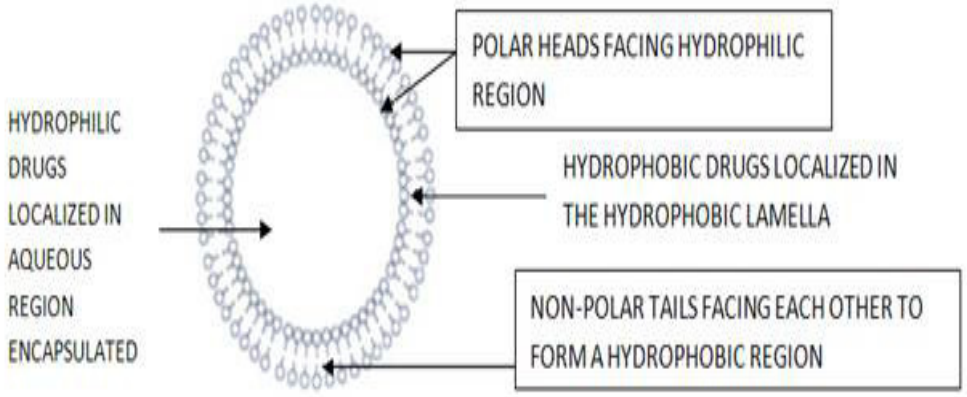

Fig.2:-Structure of Niosomes.

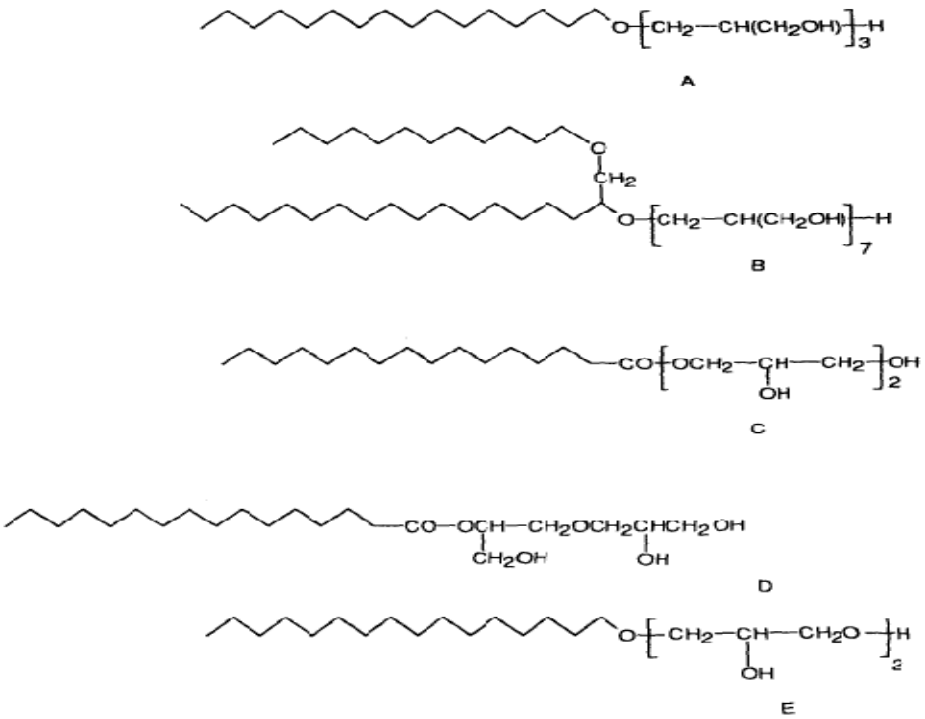

Fig.3:- L'Oreal vesicle forming surfactants, A = $C_{16}G$ 3, B=C_{12}C1~G7, C: D=Surfactant III, E = C16G 2

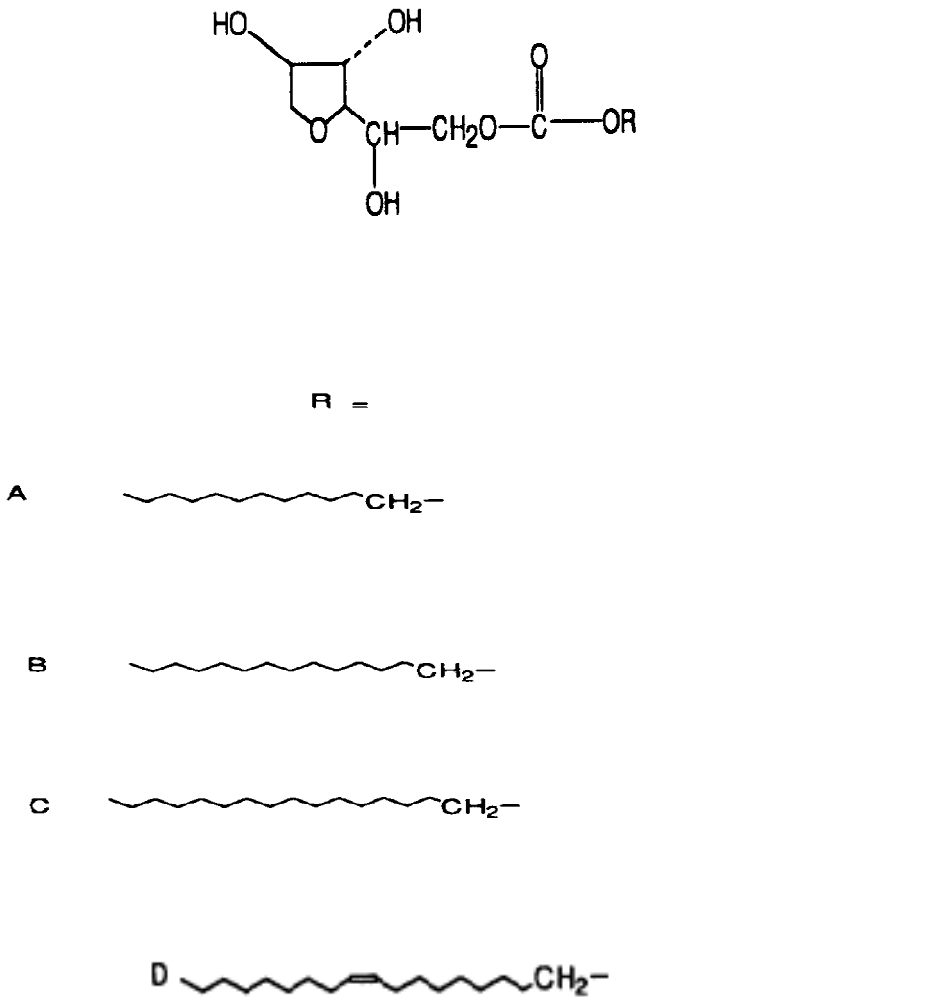

Fig4:- Sorbitan monoester vesicle forming surfactants. A = sorbitan monolaurate (Span20), B = sorbitan monopalmitate (Span 40), C = sorbitan monostearate (Span 60), D =sorbitan monooleate (Span 80).

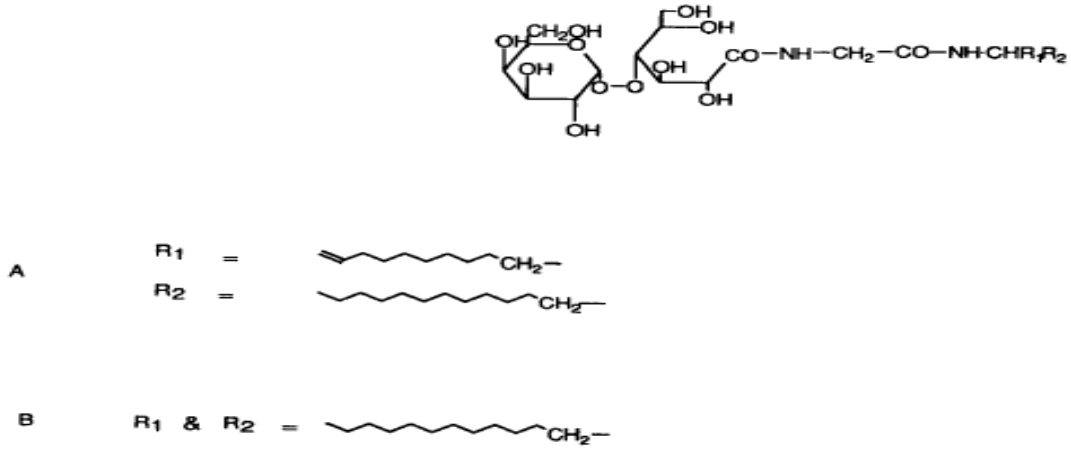

Fig.5:- Alkyl glycosides incorporating amino acid spacers that are vesicle forming Surfactants

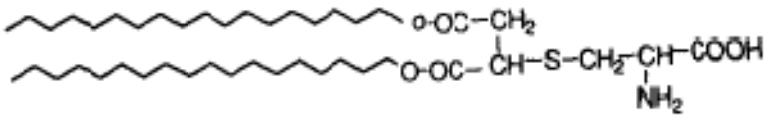

Fig.6:- Amphiphilic vesicle forming amino acids

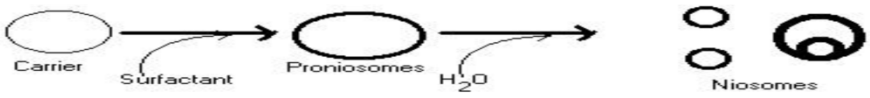

Fig.7:- Formation of Niosomes from Proniosomes.

Fig.8:- Ultra Sonification

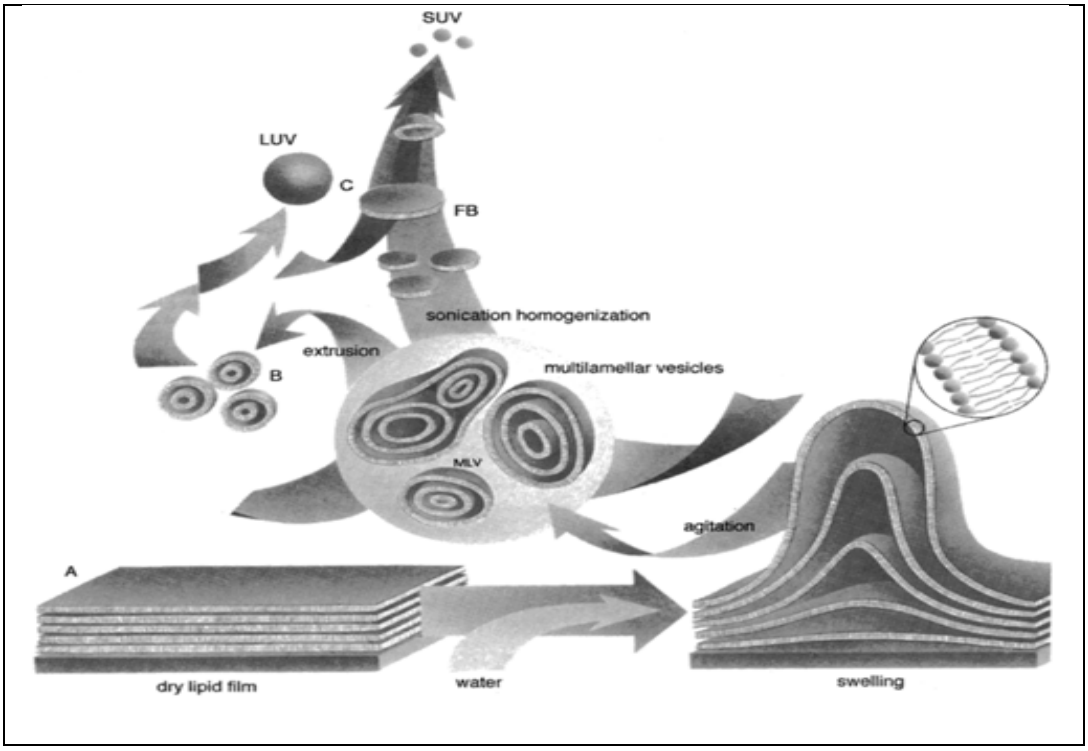

Fig.9:- Preparation Method of Niosomes.

6.10 Drugs incorporated into niosomes by various methods

Method of preparation	Drug incorporated
1. Ether Injection	Sodium stibogluconate Doxorubicin[16]
2. Hand Shaking	Methotrexate Doxorubicin[16]
3. Sonication	9-desglycinamide 8-arginine Vasopressin Oestradiol[5]
4. Encapsulation	Riefampicine[22] Adriamycin[40]
5. Film hydration method.	Chloramphenicol sodium succinate (CMP)[26] Methotrexate (MTX)[39] Tretinoin (TRA)[27] Nimesulide[36] Clotrimazole[37]
6. pH Gradient Method	Salbutamol sulphate[29]
7. Reverse Phase Evaporation	Timolol[38]

7.2 Gel Filtration

The unentrapped drug is removed by gel filtration of niosomal dispersion through a Sephadex-G-50 column and elution with phosphate buffered saline or normal saline.

7.3 Centrifugation

The 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.

9. CHARACTERIZATION OF NIOSOMES

8.1 Size, Shape and Morphology

Structure of surfactant based vesicles has been visualized and established using freeze fracture microscopy while photon correlation spectroscopy used to determine mean diameter of the vesicles. Electron microscopy used for morphological studies of vesicles while laser beam is generally used to determine size distribution, mean surface diameter and mass distribution of niosomes.

8.2 Freeze Fractured Microscopy

Vesicles are freeze thawed and visualized using freeze fracture electron microscopy. The vesicular suspension is cryofixed in liquid propane. Some cryoprotectant like glycerol can be used. The cryofixed vesicles are fractured at an angle between 90-150º while low pressure is maintain (10^{-2}pa). The surface obtained following fracturing is then shadowed using platinum or carbon vapours at an angel of 45ºC. Coating with carbon further strengthens to form replica and examine using transmission electron microscope. The niosomes have been observed 80 nm to 800 nm as larger ULVs and they can be more 1 µm in size as MLVs. Freeze thawing (keeping vesicles suspension at –20°C for 24 hrs and then heating to ambient temperature) of niosomes increases the vesicle diameter, which might be attributed to fusion of vesicles during the cycle.

8.3 Vesicle diameter

Niosomes, similar to liposomes, assume spherical shape and so their diameter can be determined using light microscopy, photon correlation microscopy and freeze fracture electron microscopy.

9. EFFECTS OF ENTRAPPED SOLUTES ON SIZE OF THE VESICLES

Hexadecyldiglyceryl ether based vesicles demonstrated the effects of incorporated solution on the size of vesicles. It was observed on drug entrapment generally the size of niosomes increases. The effect is attributed to the interaction of solute molecules with the head group of surfactant. The net increased in resultant charge and

force of repulsion increases size of vesicles. The mean size of extruded vesicles prepared by extrusion method was recorded to be 136 nm where as on incorporation of solute (stilbogluconate and calcein) it tended to increase to about 200 nm and more than 300 nm respectively.

It was thought to be an effect of complex formation between polar head of surfactant and solute molecule or presumably the consequence of salting out of surfactant polar heads due to complex formation, thus leading to local high energy built. The latter may cause thermodynamic instability, which in turn could promote vesicles to aggregate and to form large vesicles.

10. ENTRAPMENT EFFICIENCY

After preparing niosomal dispersion, unentrapped drug is separated by dialysis, centrifugation, or gel filtration as described above and the drug remained entrapped in niosomes is determined by complete vesicle disruption using 50% n-propanol or 0.1% Triton X-100 and analysing the resultant solution by appropriate assay method for the drug. Where,

Entrapment efficiency (EF) = (Total amount entrapped) x 100

Entrapment efficiency determined by following method[20]

Entrapment efficiency may be determined by using carboxyfluorescein (CF) as a marker and relative effect of method of preparation on entrapment could be compare. It is generally assumed that the concentration, i.e. 200 mM of CF used for hydration should be the concentration within vesicles (before disruption). The carboxyfluorescein at this concentration in vesicles is self quenched and give no fluorescence. Vesicles disruption using hydrophilic surfactant solulan C_2, Triton X- 100 (octyl phenol ethoxylate), 0.2% desoxycholate or 50% propanol could be affected which increases the fluorescence by factor 100 owing to the releases of entrapped CF. The ratio of CF before and after disruption of vesicles is calculated to determine entrapment efficiency and expressed as ml of CF per mole of surfactant. Entrapment efficiency depends mainly on the preparation method. Non ionic surfactant prepared by ether injection method demonstrated higher entrapment efficiency as compared to those prepared by hand shaking method. The difference critically related to the vesicle type and size which in turn were found to be related to the method of preparation. Ether injection method produces conventional unilamellar vesicles more uniform in size resulting into higher drug entrapment compared to MLVs.

11. FACTORS AFFECTING VESICLES SIZE, ENTRAPMENT EFFICIENCY AND RELEASE CHARACTERISTICS

a) Drug

Entrapment of drug in niosomes increases vesicle size, probably by interaction of solute with surfactant head groups, increasing the charge and mutual repulsion of the surfactant bilayers, thereby increasing vesicle size. In polyoxyethylene glycol (PEG) coated vesicles, some drug is entrapped in the long PEG chains, thus reducing the tendency to increase the size[24]. The hydrophilic lipophilic balance of the drug affects degree of entrapment.

b) Amount and type of surfactant

The mean size of niosomes increases proportionally with increase in the HLB of surfactants like Span 85 (HLB 1.8) to Span 20 (HLB 8.6) because the surface free energy decreases with an increase in hydrophobicity of surfactant.

The bilayers of the vesicles are either in the so-called liquid state or in gel state, depending on the temperature, the type of lipid or surfactant and the presence of other components such as cholesterol. In the gel state, alkyl chains are present in a well-ordered structure, and in the liquid state, the structure of the bilayers is more disordered. The surfactants and lipids are characterized by the gel-liquid phase transition temperature (TC). Phase transition temperature (TC) of surfactant also affects entrapment efficiency i.e. Span 60 having higher TC, provides better entrapment.

c) Cholesterol content and charge

Inclusion of cholesterol in niosomes increases its hydrodynamic diameter and entrapment efficiency. In general, the action of cholesterol is two folds; on one hand, cholesterol increases the chain order of liquid-state bilayers and on the other, cholesterol decreases the chain order of gel state bilayers. At a high cholesterol concentration, the gel state is transformed to a liquid-ordered phase.

An increase in cholesterol content of the bilayers resulted in a decrease in the release rate of encapsulated material and therefore an increase of the rigidity of the bilayers obtained. Presence of charge tends to increase the interlamellar distance between successive bilayers in multilamellar vesicle structure and leads to greater overall entrapped volume.

d) Methods of preparation

Methods of preparation of niosomes such as hand shaking, ether injection and sonication have been reviewed by Khandare *et al.* Hand shaking method forms vesicles with greater diameter (0.35-13nm) compared to ether injection method (50-1000nm) [(17)]. Small sized niosomes produced by Reverse Phase Evaporation (REV) method[(15, 29)] Microfluidizationmethod[17] gives greater uniformity and small size vesicles. Parthasarthi *et al* prepared niosomes by trans membrane pH gradient (inside acidic) drug uptake process[20]. Niosomes obtained

by this method showed greater entrapment efficiency and better retention of drug.

e) Resistance to osmotic stress

Addition of a hypertonic salt solution to a suspension of niosomes brings about reduction in diameter. In hypotonic salt solution, there is initial slow release with slight swelling of vesicles probably due to inhibition of eluting fluid from vesicles, followed by faster release, which may be due to mechanical loosening of vesicles structure under osmotic stress.

12. ENCAPSULATION EFFICIENCY AND SOLUTE RELEASE RATES[20]

Encapsulation efficiency can be said as a product of the stability of the dispersion. That is, the encapsulated solute and the solute retention capability of encapsulating membrane, together with the stability of both surfactant and the vesicle structure, all contribute to the stability of formulation. Encapsulation efficiency is governed by the method of loading, nature of solute and hydration temperature. Vesicles loaded by transmembrane ion gradient show higher entrapment efficiency than those loaded during the hydration steps. As a rule, larger niosomes show higher entrapment efficiencies than smaller vesicles. Encapsulation of water soluble solutes results in increased vesicles size.

12.1 Nature of hydrophilic head group

One another factor governing the stability of niosomes is the nature of hydrophilic head group. The entrapment efficiency of a series of alkyl glycosides based vesicles gave different entrapment efficiencies, C_{16}-glucosides and C_{26}- mannosides gave entrapment of above 7% where C_{16}-galactosides show only 5%. This is attributed to the sugar moieties present in the glycosides, which differ in their orientation due to 4-hydroxyl group. Non-ionic surfactant vesicles prepared using surfactant with identical alkyl groups but different hydrophilic group (ethoxy v/s glycerol moieties) revealed that the glycerol moieties had lower head group area measurements compare to the ethoxy group. This gives an increased mobility to the polyethoxylated alkyl chains which in turns increases the fluidity of the corresponding bilayer and ultimately leads to leaky membranes as compare to alkyl glycerol derivatives.

12.2 Effect of cholesterol on NSVs

Encapsulation efficiency is a measure of the solute retention and cholesterol has been shown to assist solute retention. Cholesterol plays an important role in absolute Encapsulation efficiency of a formulation and in turn the stability of niosomes. The synthetic alkyl glycosides are capable of forming vesicles without the inclusion of cholesterol. Threefold increase in encapsulation efficiency of C_{16}-glucosides vesicles was observed after incorporation of 29 mole % cholesterol. Similarly, 50-100% increase in encapsulation efficiency was observed with vesicles formed by synthetic polyhydroxyl lipids with 20% mole cholesterol content and up to 50 mole % was reported with the series of sorbitan monoesters.

The amount of cholesterol also decides the release rate and extent of solutes from the vesicles. For examples, release of doxorubicin from vesicles containing surfactant and stearylamine with 8.5 mole % cholesterol was around 2% over a time period of 6 h. However, when the cholesterol content was increased to 47.5 mole % in the formulation only 0.1% release was recorded over the same time period.

12.3 Nature of the alkyl side chain

It is evident from below table that the longer the alkyl chain glycolipid better is the encapsulation efficiency; and the alkyl chain length cut off point for vesicles formation may be a 14 carbon chain. Also the dialysis procedure used to separate the encapsulated material from unencapsulated material would have Niosomes resulted in the loss of C_8, C_{10} and C_{12} compounds. However vesicles purification using gel filtration technique (using sephadex G-50) is said to prevent the destabilization of vesicles prepared from low molecular weight sorbitan lauryl esters (C_{12}). It was reported that a gradual decrease in size (3.4-0.9 μm) as the alkyl chain length increases is observed with 5(6) carboxyfluorescein entrapped niosomes.

12.4 Nature of encapsulated solutes

The effect of method of loading on retention of encapsulated doxorubicin within sorbitan monostearate vesicles was studied. It is found that when vesicles are loaded using ordinary pH gradients, approximately 50% of the drug is release over 24 hr at 37ºC. However, only 20% of the drug was released over the same period of time from vesicles loaded using ammonium sulphate gradients. This is attributed to the nature of the counter ion and ultimately the stability of the ionic association Niosomes produced by the cationic drug ions and the intravesicular sulphate counter ion, which have a major influence on solute retention. From this it can be understood that leakage of encapsulated solute due to membrane permeability can be overcome using a suitable intravesicular ionic trap. The release rate of a solute is affected by the nature of solutes, even when similar counter ion presents.

13. NIOSOMES AS DRUG CARRIERS

A number of workers have reported the preparation, characterization and use of niosomes as drug carriers. Niosomes containing anti-cancer drugs, if suitably designed, will be expected to accumulate within tumors in a similar manner to liposomes. The niosomal encapsulation of methotrexate and doxorubicin increases drug delivery to the tumor and tumoricidal activity of the

drug. Doxorubicin niosomes possessing muramic acid and triglycerol surfaces were not taken up significantly by liver. The triglycerol niosomes accumulated in the tumor and muramic acid vesicles accumulated in the spleen. Those vesicles with polyoxyethylene surface were rapidly taken up by the liver and accumulated to a lesser extent in tumor. *Baillie et al* investigated the encapsulation and retention of entrapped solute 5, 6-carboxy fluorescence (CF) in niosomes[18].

Niosomes can be used as stable carriers for tretinoin, when incorporated in P90 or span vesicles. The results presented a half-life shorter, or very close to that of the free drug. Niosomes have also been used as carriers for iobitridol, a diagnostic agent used for X-ray imaging[23]. Carter *et al* reported that multiple dosing with sodium stibogluconate loaded niosomes was found to be effective against parasites in the liver, spleen and bone marrow as compared to simple solution of sodium stibogluconate. Azmin *et al* reported the preparation and oral as well as intravenous administration of methotrexate loaded niosomes in mice. They observed significant prolongation of plasma levels and high uptake of methotrexate in liver from niosomes as compared to free drug solution[10]. Chandraprakash *et al* reported the formation and pharmacokinetic evaluation of Methotrexate niosomes in tumor bearing mice[8]. Cable *et al* modified the surface of niosomes by incorporating polyethylene alkyl ether in the bilayered structure. They compared the release pattern and plasma level of doxorubicin in niosomes and Doxorubucin mixed with empty niosomes and observed a sustained and higher plasma level of doxorubicin from niosomes in mice. D' Souza *et al* studied absorption of ciprofloxacin and norfloxacin when administered as niosome encapsulated inclusion complexes. Namdeo *et al* reported the formulation and evaluation of indomethacin loaded niosomes and showed that therapeutic effectiveness increased and simultaneously toxic side effect reduced as compared with free Indomethacin in pawoedema bearing rats. Parthasarthi *et al* prepared niosomes of vincristine sulfate which had lesser toxicity and improved anticancer activity. Jagtap and Inamdar prepared niosomes of Pentoxifylline and studied the *in-vivo* bronchodilatory activity in guinea pigs. The entrapment efficiency was found to be 9.26 ± 1.93% giving a sustained release of drug over a period of 24 hrs. Raja Naresh *et al* reported the anti-inflammatory activity of niosome encapsulated diclofenac sodium in arthritic rats. It was found that the niosomal formulation prepared by employing a 1:1 combination of Tween 85 elicited a better consistent anti-inflammatory activity for more that 72 hrs after administration of single dose[19].complexation of methotrexate could increase the entrapment of the drug in non ionic surfactant vesicles and could improve the anticancer activity[24].

A critical analysis of (trans) dermal delivery of substances encapsulated within liposomes and niosomes is presented. Topical liposomes or niosomes may serve as solubilization matrix, as a local depot for sustained release of dermally active compounds, as penetration enhancers, or as rate-limiting membrane barrier for the modulation of systemic absorption of drugs[26].

14. SOLUTE RELEASE PROFILE FROM NIOSOMAL FORMULATION

In regard to release of entrapped solute from niosomal system, cholesterol was noted to have distinct effect on release profile as well as on vesicles stability. In a study the effect of cholesterol incorporation on $C_{16}G3$ based niosomes has been well correlated with mixed monolayer consist of surfactant and cholesterol. Cholesterol was found to retard rate of solute and stabilize the system against degradation. Non ionic surfactant vesicles demonstrated temperature dependency on release of solute, which could successfully utilized in therapeutics using local hyperthermia.

15. PHARMACOSOMES

These are defined as colloidal dispersions of drugs covalently bound to lipids, and may exist as ultrafine vesicular, micellar, or hexagonal aggregates, depending on the chemical structure of drug-lipid complex. Pharmacosomes bearing an unique advantage over liposomes and niosomes vesicles have come up as potential alternative to conventional vesicles. Like other vesicular drug delivery systems, pharmacosomes, on storage, undergo fusion and aggregation, as well as chemical hydrolysis. Similar to other vesicular system pharmacosomes still play an important role in the selective targeting, and the controlled delivery of various drugs. Current research trends are generally based on using different approaches like pegylation, biotinyzation etc. for cellular targeting.

16. DISCOSOME

Discosome have disc like structure based on non ionic surfactants discosome in addition to their many advantage seem to have a special advantage for ocular route, where as their large size may present their drainage in to systemic pool as well as the disc shape could provide for better fit in the cul-de-sac of eye.

17. APPLICATIONS OF NIOSOMES[15]

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

Drug Targetting:

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 (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's bind readily to the lipid surface of the niosome) to target them to specific organs.

Anti-neoplastic Treatment[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 tumor and higher plasma levels accompanied by slower elimination [(8)].

Leishmaniasis: [20]
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[18].

Delivery of Peptide Drugs:
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. In an invitro study conducted by Yoshida et al, oral delivery of a vasopressin derivative entrapped in niosomes showed that entrapment of the drug significantly increased the stability of the peptide[20].

Use 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. Non-ionic surfactant vesicles have clearly demonstrated their ability to function as adjuvants following parenteral administration with a number of different antigens and peptides[34].

Transdermal Drug Delivery Systems Utilizing Niosomes: [20]
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. 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. A study conducted by P. N. Gupta *et al* has shown that niosomes (along with liposomes and transfersomes) can be utilized for topical immunization using tetanus toxoid. However, the current technology in niosomes allows only a weak immune response, and thus more research needs to be done in this field.

Other Applications:
a) Sustained Release
Azmin *et al* suggested 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[10].

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. Niosomal system also used for delivery of rifampicin to lymphatics. 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[18,40].

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.

18. MARKETED PRODUCTS[44].

19. CONCLUSION
Over the years, there has been a great evolution in drug delivery technologies. They present 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 multienvironmental structure. The

Fig.11:- Marketed Products

technology utilized in niosomes is still greatly in its infancy, and already it is showing promise in the fields of cancer and infectious disease treatments. The system is already in use for various cosmetic products. Niosomes represent a promising drug delivery technology various type of drug deliveries can be possible using niosomes like targeting, ophthalmic, topical, parentral, etc. and much research has to be inspired in this to juice out all the potential in this novel drug delivery system.

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