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Review Article

UFASOMES: UNSATURATED FATTY ACID BASED VESICULAR DRUG DELIVERY SYSTEM

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ABSTRACT

Various novel drug delivery systems have been developed encompassing several administration routes to deliver drugs at a rate decided as per the need of the body during the course of treatment and to achieve targeted therapy, also decreases undesirable side effects. Different types of vesicular drug delivery systems were developed, such as liposomes, niosomes, ufasomes etc. Ufasomes are unsaturated fatty acid vesicles which is a suspension of closed lipid bilayer formed from fatty acid and their ionized species having limited, narrow pH ranging from 7-9. Composition of fatty acid molecules is such that the hydrocarbon tails are pointed towards the inner core of the membrane and the carboxyl group are in touch with water. Stable ufasomes preparation mainly relies on appropriate choice of fatty acid, cholesterol quantity, range of the pH, buffer and lipoxygenase amount. Recent innovation provides very efficient features such as stability considerations, dynamic features and microscopic features of ufasomes. The article furthermore provides the difference between ufasomes with liposomes. For this review, the complete databases have been collected from various search engines such as researchgate, elsevier, pubmed, sciencedirect, google scholar, scopus etc., from the year 1965-2020 using the following keywords.

Keywords: Vesicular drug delivery system, Unsaturated fatty acid vesicles, Lipid peroxidation, Birefringence, Ufasomes, Liposomes

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INTRODUCTION

In the developing delivery system, a novel drug delivery system has proved to be the most fulfilling and approachable to accomplish the targeted and controlled drug delivery. Target drug delivery is a technique in which the therapeutic agents are delivered to the tissue of interest and relatively diminishing the concentration of the therapeutic agent in other tissues, which higher the concentration of drug at the target site and with less side effect [1, 2]. Paul Ehrlich in 1909, first produced the target drug delivery system, in which target drugs are directly delivered to the unhealthy cells. Afterward, the number of novel vesicular drug delivery system aims to convey the pharmaceutical compound in the body at a rate coordinated as per the need of the body during the treatment period and securely accomplish its desired therapeutic effect. In the previous few decades, the advancement of the new drug delivery system has gained extensive consideration [3]. Bingham first announced the biological origin of these vesicles in 1965 and has been given the name 'Bingham bodies' [4]. The novel drug delivery system is focused to satisfy two requirements that it ought to deliver drugs at a rate directed as per the need of the body over the treatment period and to convey the medication straightforwardly to the injured tissue, receptor or organ. For the targeted therapy number of carriers was utilized to deliver the drug, which includes immunoglobulins, serum proteins, synthetic polymers, particulate systems (fig. 1). Colloidal carrier system is the other name of the particulate system which includes microspheres, lipid particles-high density lipoprotein (HDL) and low-density lipoprotein (LDL), polymeric micelles, nanoparticles and vesicular carriers such as liposomes, niosomes, ufasomes, ethosomes and so forth [5, 6]. In which vesicular drug delivery system is the most noticeable among the carriers. In vesicular drug delivery, there is improved bioavailability of medication with reduced expense of therapy, mainly in the case of drug with less solubility. Also, both lipophilic and hydrophilic drugs can be incorporated [7, 8].

Different novel methodology for delivering drugs by the vesicular system were developed, which includes liposomes, aquasomes, ethosomes, sphingosomes, bilosomes, transferosomes, virosomes, niosomes, ufasomes [9].

Advantages of vesicular drug delivery system

- Delays elimination of quickly metabolizable drugs.
- Presence of drugs in systemic circulation can be prolonged.
- Certain medications with toxicity issues can be resolved.
- Both lipophilic and hydrophilic drugs can be incorporated easily.
- Improving bioavailability of drugs particularly in the case of poorly soluble drugs.
- Issues like drug insolubility, instability and rapid disintegration can be controlled.
- Lessens the cost of treatment [10].

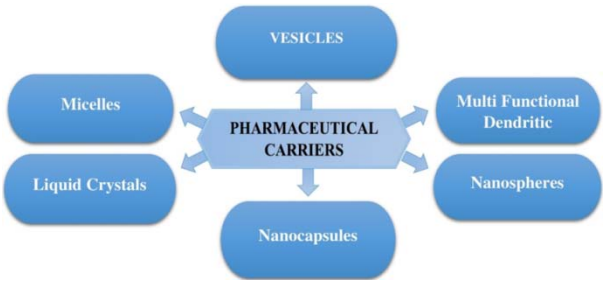

Fig. 1: Pharmaceutical carriers [11]

Classification of vesicular drug delivery system (VDDS)

The vesicles are grouped on the basis of their composition [12]

- Lipoidal Biocarriers
- Nonlipoidal Biocarriers.

Lipoidal biocarriers as VDDS

Liposomes

Liposomes are concentric bilayered vesicle which is tiny (unilamellar or multilamellar), where the fluid compartment is entirely encased by a bilayered membrane, made out of natural or

synthetic lipids. In liposomal drug delivery system, phosphatidylcholine and cholesterol are the essential components [13]. Liposomes have the capacity to entrap compounds of different solubility due to their alternating hydrophilic and hydrophobic structure.

Liposomes are capable of entrapping compounds with varying solubilities because of their alternating structure [14] and therefore can entrap hydrophilic and lipophilic drugs can be entrapped into liposomes. In targeted delivery of drugs, Liposomes are considered as a potential drug carrier. Liposomes is an efficient drug delivery system with enhanced biodegradability, high cell or tissue specificity and in intranasal liposomal delivery, drug degradation due to enzymes are well protected and allow the drug to pass through blood-brain barrier [15]. Some of the constraints of liposomes are high expense, spillage and fusion of the encapsulated drug. Short half-life, low solvency and stability.

Emulsomes

Emulsomes is a lipoidal drug delivery system. Principally intended for parenteral delivery of drug having a poor water solubility. Fats and triglycerides comprise the main part of the interior core and are stabilized in the form of oil in water emulsion by the addition of a high concentration of lecithin. It has the quality of both liposomes and emulsion. Medicaments can be delivered through several routes such as oral, parenteral, topical, rectal ocular and intranasal as emulsomes. Advantages of emulsomes include improved biodegradability, biocompatibility, sustained release of drugs, increased entrapment efficiency, gastrointestinal tract stability [16].

Ethosomes

Ethosomes have been intended for delivering drugs having high penetration power through the skin as well as systemic circulation. Ethosomes are made up of phospholipids (phosphatidylcholine, phosphatidylserine, phosphatitidic acid), alcohol (ethanol, isopropyl alcohol) in relatively high concentration and water. The high ethanol concentration makes the ethosomes capable of better penetration through the skin [17]. Ethosomes are utilized for the delivery of huge and diverse group of medicaments like antifungal agents, antiviral agents, nonsteroidal anti-inflammatory drugs, antibiotics and various other drugs like propanolol, testosterone, etc. One of the main advantage of ethosomes include the targeted delivery of drugs through the skin for transdermal and dermal delivery [2].

Transferosomes

Transferosomes have high vesicle deformability which is its unique property and gives better penetration of intact vesicle and have both hydrophilic and hydrophobic properties. They are stress-responsive and possess a complex vesicle with a complex lipid bilayer with an aqueous core [18] for the controlled and targeted delivery of drugs like peptides and hormones, transferases are predominantly used as carrier. Due to the brutal condition of the gastrointestinal tract and due to rapid degradation and instability of insulin and interferon in the stomach, oral delivery of peptides is not possible. In this manner, biogenic molecules such as vaccines, insulin can be administered through transferosomes without any degradation.

Sphingosomes

Sphingosomes have been introduced to overcome certain stability problems related with liposomes, such as oxidation, hydrolysis, degradation, leaching, sedimentation, drug aggregation. This prompted to the development of sphingosomes [19]. Here sphingolipids are present rather than phospholipids in liposomes, in this way the name sphingosomes. Sphingosomes are colloidal, concentric bilayered vesicle having a lipid bilayered film which encloses the aqueous volume in it and is mainly made up of natural or synthetic sphingolipids. Points of interest of sphingosomes include: better drug retention is the main point of interest in the case of sphingosomes. There are several routes by which they can be administered, such as oral, intravenous, intra-arterial, subcutaneous, intramuscular and so on [20]. Therapeutic index and efficacy of the encapsulated drugs are expanded and the danger of encapsulated drugs is also decreased.

Pharmacosomes

Pharmacosomes means drug carrier as 'pharmacon' signifies drug and 'soma' signifies carrier. Pharmacosomes are novel vesicular drug delivery system and have remarkable properties over other drug delivery system. Pharmacosomes are amphiphilic lipid vesicular system formed by the colloidal dispersion of drugs and for increasing the bioavailability of poorly water-soluble drug and poorly lipophilic drug, it is covalently bound to lipids [21]. Drug, solvent and lipids are the three components required for the preparation of pharmacosomes.

Spillage of the drug doesn't occur, as the drugs are covalently conjugated with lipids and consequently provides maximum entrapment efficiency.

Enzymosomes

It is a supramolecular vesicular drug delivery system. Numerous approaches like polymeric carriers can be adopted for the delivery of therapeutic proteins such as enzymes. Enzymosomes are generated by complexing enzymes with lipids. A prominent response was shown in the antibody release on the target site, by coupling covalently on the surface of liposomes. Enzymosomes shows increased stability and encapsulation. This increased therapeutic effect and lower side effect by carrying the therapeutic agent to the destined tissue receptor, while maintaining enzymatic activity in intact form [22].

Non-lipoidal biocarriers as VDDS

Aquasomes

The Nanobiotechnology, i.e., the blend of biotechnology and nanotechnology has proposed another methodology as aquasomes as a solution to overcome the serious side effects associated with the bioengineered molecules such as peptide, hormone, protein. Aquasome is a three-layered self-assembled nanostructure compositions with ceramic carbon nanocrystalline particulate core covered with oligomeric film (made up of carbohydrates) on which biochemically active molecules are adsorbed by copolymerization, diffusion or absorption with alteration or not [23]. The structural stability is maintained by a solid core and the carbohydrate coating protects from dehydration and stimulates the biochemically active molecules.

Niosomes

Niosomes is a bilayered vesicle composed of non-ionic surface active agents. It is formed by the hydration of non-ionic surfactants for eg. Polygycerolalkyl ether, polyoxyethylenealkyl ether, glucosyldialkyl ether, etc. is added to cholesterol in aqueous media. Niosomes are mostly unilamellar or multilamellar vesicles and are small and microscopic in structure and size ranges from 10-1000 nm [24]. Entrapment efficacy of niosome is increased by the addition of non-ionic surfactant that also increases the size of the vesicle and also gives a charge to the vesicle. Non-ionic surfactants are used as it cause less irritation. Niosomes have a structure similar to that of liposomes and niosomes can entrap both hydrophilic and hydrophobic drug which can be delivered through different routes such as oral, parenteral, transdermal etc [25]. Also, niosomes are used as an alternative to liposomes for targeted drug delivery. Recently nisosomes have shown better efficacy in transdermal delivery of drugs.

Bilosomes

Bilosomes is a novel drug delivery carrier that consists of a membrane of niosomes incorporated with deoxycholic acid. In the gastrointestinal tract, dissolution and enzymatic degradation occurs in the case of conventional vesicles like liposomes and niosomes but in niosomal formulation when bile salt is incorporated could stabilize the membrane from the harmful effect of bile acid in gastrointestinal tract [26]. Bilosomes are therefore known as bile salt stabilized vesicle. Since they are highly stable in the gastrointestinal tract, they have increased biocompatibility and improved therapeutic efficacy of the drug. Bilosomes are found to have increased bioavailability as they are readily absorbed from the small intestine and are carried to the portal circulation. Through the hepatocirculation, they reach the liver and release the drug and is found to be effective in liver targeted drug delivery.

Ufasomes-as vesicular drug carrier

Ufasomes are unsaturated fatty acid vesicles that are closed lipid bilayered suspension, which are made from unsaturated fats and their ionized species (soap), where pH is limited and ranging from 7 to 9. Fatty acid vesicles generally have two types of amphiphiles, non-ionic neutral form and the other is ionized form (negatively charged soap) (fig. 2). The basic for vesicle stability is determined by non-ionic neutral and ionic form ratio. Gebicki and Hicks in 1973 first reported fatty acid vesicles formation and also oleic and linoleic acid in the following years, and the vesicles formed was initially named as ufasomes [27, 28]. But later investigation shown that not only unsaturated fatty acid forms fatty acid vesicles, but also octanoic and decanoic acid-like saturated fatty acid can form fatty acid vesicle. One important feature of ufasomes are that the fatty acids are readily available and they are composed of single-chain amphiphiles [29, 30]. Whereas phospholipids are used in the formulation of liposomes, but reasonable quantity of pure synthetic phospholipids are not available and also natural phospholipids are chemically heterogeneous.

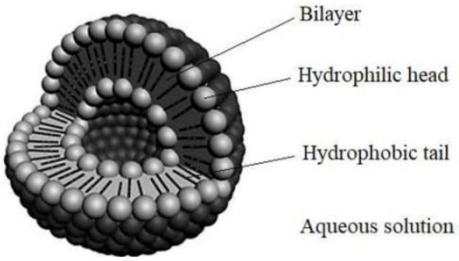

Fig. 2: Structure of ufasomes [31]

Advantages

- Ufasomes are stable than liposomes.
- Ufasomes have better entrapment efficiency for hydrophilic and hydrophobic drugs.
- Ufasomes are cheaper than liposomes.
- Easy to prepare.
- Therapeutic viability.
- Ready availability of fatty acid for ufasomal composition.
- Ufasomes are capable as carriers for oral intake of less absorbable drugs.
- Easy penetration of the drug in case of topical application.
- Ufasomes have better drug retention along with sustained release in the deeper layer of skin for long-term effect in the case of topical application.
- Potential carrier for anti-inflammatory for anti-inflammatory drug delivery.

Disadvantages

- Ufasomes are readily oxidized leading to stability problems in fatty drug products.
- Some products produced during oxidation may be toxic.
- Colloidal instability of ufasomes affects their application in food additives and drug delivery.
- Risk of atherosclerosis [32].

Method of preparation of ufasomes

Practically for the preparation of either type of vesicles almost identical techniques can be used.

But for the preparation of ufasomes only unoxidized materials are preferred. Stock solution is prepared which contains 10 % oleic acid and linoleic acid in chloroform and stored under 20 °C. In a typical preparation, test tube containing 0.02 ml of the stock solution is taken and the stock solution is evaporated in a water pump and with the nitrogen steam it is finally dried. The film fatty acid is totally broken in 0.2 ml of 0.1 M tri-hydroxymethyl aminomethane buffer at 8 to 9 pH range, on a vortex mixer by vigorous shaking. Thus, the ufasome suspension formed as a result of mixing are stable for minimum 24 h. In some experiments, particles are prepared by an ultrasonic generator with a microtip. By steam of nitrogen, air is first removed from the buffer and the suspension is overlaid with the gas during irradiation. Using ice bath, consistent temperature is kept up [30, 33]. The additives used in the preparation of ufasomes are depicted (table 1).

Table 1: Additives used in ufasome preparation

S. No.	Class	Example	Uses	Reference
1.	Fatty Acids	10 % oleic and linoleic acid	Vesicle forming component	[34]
2.	Solvents	1. Chloroform	1. For maintaining membrane permeability	
		2. Stream of nitrogen	2. Drying of preparation	
3.	Buffering agents	Tris-hydroxymethyl aminomethane buffer (pH 8-9)	As hydrating medium	

Key issues in manufacturing ufasomes

Selection of fatty acid

Fatty acid surface film-based analysis and information studies suggest that for the formulation of stable ufasomes, 12 to 22 carbon fatty acid will be appropriate. Studies were very limited since C-18 fatty acids demonstrated the best guarantee in the early tests. Only membrane made from oleic unsaturated fatty acid (cis-9-octadecenoic acid) and linoleic unsaturated fatty acid (cis, cis-9, 12-octadecadienoic acid) are only preferable in the preparation of ufasomes. There is no improvement in ufasome preparation on charging the membrane with little quantity of oleic, linoleic and stearic acid. Oleic acid remains uncontaminated for a minimum of six weeks, whereas significant peroxides were developed after two to three weeks in the case of peroxides and were interpreted by stability tests [30].

Addition of cholesterol

Cholesterol serves a unique property in the case of vesicles prepared from lipids, such that cholesterol modulates membrane fluidity, flexibility and permeability like purpose. Cholesterol is used for filling gaps formed due to defective packing of other lipid species. But there is a fast decrease in the capacity of the vesicle to hold solute in the presence of higher extent of cholesterol. Also, at any cholesterol concentrations there is no upgrade in membrane impermeability. Hicks *et al.*, compared the leakage of glucose from ufasomes made of oleic acid and linoleic acid with spheres incorporated with 17 % of cholesterol by weight. From the result it was understood that the leakage of glucose from spheres incorporated with 17 % of cholesterol by weight showed higher leakage than the oleic and linoleic acid ufasomes [30, 33].

pH range

Generally, for the formulation of fatty acid vesicle pH is restricted to a very narrow range 7 to 9, half of the carboxylic acids are ionized. Fatty acid under the pH range 7 is called unsaturated fatty acid. Also, below this particular range it frames only unstructured precipitates, whereas above this range they are excessively soluble. At higher pH, dominant aggregation species are called micelles and at lower pH

region oil droplets forms. 'Critical vesiculation concentration' (CVS) is the fatty acid vesicle systems at concentrations slightly over the concentration where the formation of vesicles takes place. Colloidal suspension of vesicles is formed at CVS where non-vesicular aggregates and monomers gather to form bilayer structure [35, 36].

Selection of buffer

In the preparation of ufasomes the widely preferred buffer is tris-hydroxymethyl aminomethane. Spheres can also be formed in solutions like bicarbonate, borate, glycine-hydroxide. Based on the solute needed to be incorporated, buffers are selected. In the case of vesicular glucose entrapment-ufasomes prepared in bicarbonates did not hold glucose and borate preparation due to the formation of glucose-buffer complex, it could not be examined for retention [30].

Electrolyte

Formation of ufasomes are mostly inhibited by electrolytes. The spheres are exposed to chloride or phosphate buffer solution once they are completely stabilized in appropriate buffers and they still hold on the occluded glucose [30].

Peroxidation

By peroxidation, when bulky hydrophilic group is introduced would cause distortion of the interior of the hydrophobic membrane, and thus allowing an easy passageway for water-soluble molecule. Disturbance of the normal bilayer arrangement of the fatty acid molecule is the main effect of peroxidation on the ufasomes membrane.

The extent of peroxidation of fatty acid can be affected by the method of preparation. During the short time periods needed for hand vortexing, no peroxidation occurred. Linoleic acid when exposed to 30-W irradiation, it is oxidized in air-saturated buffers at 0.1 % per m, when treated under more violent ultrasonic resuspension. Since 3 ms was the longest exposure period used, even the oxidation-sensitive linoleic acid didn't produce extensive oxidation by this method. However, Hicks and Gebicki found that peroxidation of linoleic acid membrane can be significantly inhibited by nitroxide radicals, butylated hydroxytoluene and α-tocopherol. Lipoxygenase fails to peroxidase monoenoic fatty acid since enzyme was not able to induce spillage from oleic acid made ufasomes [37, 38].

Both enzymatic and non-enzymatic catalytic mechanism are involved in Lipid peroxidation (LPO). In non-enzymatic lipid peroxidation transition metal ions are the important components [39, 40]. In unsaturated lipids, fast rate of peroxidation can be catalyzed by only few metal which involves change in valency and also which undergoes a single electron transfer. Metals like zinc, calcium, magnesium having non-variable valency cannot take part in redox-coupled homolysis also influences lipid peroxidation [41].

At low concentration ($\sim 10^{-6}$–10^{-5}) it was manifested that, Ca^{2+}triggered Lipid peroxidation in lipid due to its ability to interact with negatively charged group of lipids (such as in lecithin-phosphate group and linolenic acid-carboxyl group), therefore, increasing the free Fe^{2+}ion concentration by displacing the bound Fe^{2+}ion, that directly take part in Lipid peroxidation catalysis. At high concentration (10^{-3}), inhibitory effect of $Ca^{2+}$is dependent on its interaction with superoxide anion radicals. Incidentally, biphasic action on Lipid peroxidation is not only exerted by Ca^{2+}ions, but other cations having higher charge density are also effective in releasing Fe^{2+}ion attached to the negatively charged group of lipids and furthermore interacting with superoxide free radicals. In the absence of Ca^{2+}, it was found that the addition of La^{3+}ions to linolenic acid ufasomes in a concentration equivalent to that of Fe^{2+}ions stimulated Lipid peroxidation. On the combined action of $Ca^{2+}$and La^{3+}in equimolar concentrations (when the total concentration surpasses Fe3+), effect of inhibition of peroxidation was observed in linolenic acid [42].

Recent innovation in conventional ufasomes

New type of fatty acid in ufasome preparation

Fatty acid cis-4,7,10,13,16,19-docosahexaenoic acid that was known to form vesicle by self-assembling around a pH of 8.5 to 9 [45].

Extension of pH range

Usually, narrow range of pH is appropriate for the development of vesicles of fatty acid due to the necessity that ionization of only about a half of the carboxylic acid is required. Extension of pH range can be done using some new approaches as following:

a) Adding a surfactant or linear alcohol like amphiphilic additives with sulfate or sulfonate head group: For instance, constituents of decanoic acid, also decanoate make vesicles at a pH range across 6.4 and 7.8, by the addition of sodium dodecylbenzene sulfonate (SDBS), the pH for the formation of vesicles can be brought down slightly at a pH of 4.3. Vesicle are also framed underneath pH 6.8 when equimolar concentration of dodecylbenzenesulfonate is co-added to decanoic acid [27].

b) Modifying synthetically hydrophilic head group size of fatty acid: It was reported that at lower pH vesicles showed improved stability by employing fatty acid along an oligo unit (ethylene oxide) embedded between carboxyl head group and hydrocarbon chain. For the formation of vesicles, lowering the pH range and decreasing phase transition temperature (near to Kraft point) is the two effects shown by the very polar bulky group [33].

Insensitivity toward divalent cation

$Mg^{2+}$and Ca^{2+}like divalent cations even at reduced concentrations, result in the precipitation of vesicles. Within the presence of ionic solute, stabilization of vesicles of fatty acid when glycerol esters of fatty acid is added. Study of ternary monoolein mixed sodium oleate water system based on cryogenic transmission electron microscopy manifested also that unilamellar and multilamellar vesicles developed from monoolein and sodium oleate mixture, in addition the stable vesicle was mainted over a long period (above one year) [44].

Enhancement of stability by crosslinking fatty acid molecules by chemical bond

From anionic gemini surfactant, the development of vesicles with carboxyl head group is one example. One more example is fatty acid (soap) employed along the sodium-11-acrylamidoundecanoate (SAU) which is a polimerizable moiety. It was reported that monomeric as well as polymerized sodium-11-acrylamidoundecanoate form vesicular aggregates by self-assembling and at high-temperature vesicles from polymeric SAU stay stable [45, 46].

Mixture of fatty acid/soap vesicle and cationic surfactant-based vesicles

As a mixed vesicles model system, tetradecyltrimethylammonium (TTAOH) mixtures and fatty acids were studied. It was discovered that when roughly equal concentration of fatty acids and TTAOH was mixed, unilamellar as well as multilamellar vesicles were formed [47]. Depending upon anionic fatty acid vesicles and didodecyldimethyl ammonium bromide (DDAB), major association (23 %) of vesicles with opposite charge was reported by Caschera *et al.* [35]. By the association of vesicles of opposite charges evolved a reactive intermediate which gradually develops into a bigger single vesicle that consists of both the solutes that were sited initially in disassociated vesicles and a mixed membrane (comprising of lipids of cations as well as anions). By further reaction of the huge vesicle results in additional vesicles, in which each consist of the solute that is separated initially into two populations of vesicle. This is also observed as 'solute exchange' mechanism.

Dynamic nature of ufasomes

Dynamic characteristics of unsaturated fat vesicles are due to the reason that it is made up of amphiphiles having a single chain. Dynamic nature that set the unsaturated fat acid vesicles in the middle of double-chained amphiphiles that form the conventional vesicle and single chained surfactants that form the micelles. The fact by simply altering the ionization or protonation ratio of the terminal carboxylic acid forms a range of aggregates of fatty acid. Chen *et al.*, examined in ufasomes on kinetic formation [48]. The formation kinetics of vesicles and micelles from a soap monomer

solution or saturated fat was analysed by dialyzing the soap monomers or saturated fatty acid across a cellulose acetate membrane. Beginning with asymmetric distribution of soap monomers or fatty acid in the middle of two chambers splits apart by the dialysis membrane, where one of the chambers consist of aggregated such as vesicles or micelles and the other chamber having only buffer solution, the rate at which equilibrium attained is observed. On account of micellar system an equilibrium state was promptly acquired (micelles attained in the diffusate chamber and the concentration of soap monomer or fatty acid in either of chambers became equivalent). But on account of vesicles, accomplishment of equilibrium state was seriously impeded (the diffusate chamber concentration raised gradually following the saturation of solution with monomers). Compared to micelles, basically vesicles are made up of a much larger number of amphiphiles. Through the dialysis experiment, the obtained result interprets that a much greater energy barrier is possessed in the development of fatty acid vesicle than the fatty acid monomer (soap) generation. A suitable method for the preparation of fatty acid vesicle is by taking a buffer solution at intermediate pH and adding alkaline soap solution to it. To give an example, a buffer solution of pH 8.5 is taken and a concentrated sodium oleate micelles solution is added to it, and instinctively due to partial protonation of molecules of oleic acid because of fall in pH over 10.5-8.5. Therefore, the vesicles produced are polydisperse in lamellarity and also size [27, 48].

Stability consideration in ufasome formulation

Ufasome membrane connected with the long-term stability is highly reliant on the decline in free energy associated with the fatty acid-water system. The formation of ufasome membrane is not spontaneous as a separate phase is formed by acids at pH 8. Under the appropriate condition, even gentle mechanical agitation is adequate in the bilayer development. Definitely considerable amount of energy released during this process emerges from the increased entropy of water that goes along with the hydrophobic interactions associated with oriented hydrocarbon chains. In the bilayer, the attractive interaction is denied by the ionized carboxyl head group that possesses mutual repulsion. Electrostatic separation diminish the film stability of fatty acid and may result in disruption by diminishing the level of head group separation and by the development of stable complexes across the ionized and protonated carboxyl head group or by screening of counter ions, the charge repulsion can be minimized. Every one of these procedures may work in the adjustment in the ufasome membrane stability. In the membrane stability favorably, lateral charge repulsions are diminished by bringing down the pH that happens at the particle surface. Decline in ionization upgrades the stability of membrane by various methods. Initially, protonated molecules are basically not soluble in water that is in correlation with anions. Secondly, there is a decrease in later head group repulsion; in a membrane of firmly filled head groups the normal separation of charges increased around 40 percentage on the expulsion of every subsequent charge bringing about bisect of coulombic repulsions. Then, a firmly bound succession of complexes is formed by anions (A-) and protonated acid molecules (AH), in which a ratio of 1:1 complex predominant species. Three contributions constitutes the energy required for binding-change in energy emerging from hydrophobic interaction, the entropy of demixing related to the development of dimers, also decrease in free energy achieved due to the development of hydrogen bond within the protonated group as well as ionized carboxyl groups. Studies based on interaction in carboxylic acid indicated that unusually strong H bond develops within–COOH and–COO groups due to the existence of negative charge from nearby the hydrogen engaged in bonding. Stabilization of ufasome membrane is done by bonding hydrogen of the head group with water, formation of the complex among the ionized molecule and neutral acid molecules and also as the dissociated carboxyl groups get hydrated. Besides, the hydrocarbon part of the fatty acids is held together by exactly similar dispersion, also the hydrophobic interaction which stabilizes the micelles as well as the inner area of the membrane [29, 34].

Microscopic studies

The arrangement of constituents of biological membrane-like phospholipid, fatty acid was attained from the electron microscopy of sectioned vesicular structures. Generally, it was acknowledged, although, that essential fixing and staining needs strong chemicals that can deform those delicate structure, with subsequent loss of definition and development of artifacts. These kinds of issues can be diminished by utilizing less brutal strategies. One of the best successful technique called freeze-fracture technique, is exerted to natural components. Detection of birefringence is relatively less harsh technique. Electron microscopy of specimens that are negatively stained, as an experiment to study the structure of ufasome, indicated that they did not make it through the preparatory steps. All efforts taken in staining ufasomes for electron microscopy using neutralized potassium phosphotungstate became unsuccessful to generate specimen having any interior structure [49].

Freeze fracturing and etching

Before anything else the suspension containing ufasomes is equilibrated for ten ms with 17 % glycerol prior to freezing. Then the suspension of ufasomes is then frozen quickly on to copper helmet containing Freon, which later reserved inside liquid nitrogen. In Balzers microtome, fracturing is done at pressure 2×10^{-6} torr and temperature 110 °C. Temperature is raised to 100 °C for etching for about 1 m. After etching, a film of carbon and platinum is accumulated over the fracture face up to a thickness of 3 nanometers and at an angle 45 °. The best strategy employed to clean replica is by floating them away from the metal helmet towards the water, followed by gently adding methanol until the solution become 80 % alcohol. For the removal of all the traces associated with fatty acid, it took 30 ms. In electron microscope Hitachi HS8, the replicas are then analyzed [50, 51]. Hicks and Gebicki reported that the ufasomes developed from oleic acid or ufasomes formed from linoleic acid have no dissimilarity in their appearance [49]. Since ufasome composition hold large quantity of water, ice constitutes most of the freeze fractured face that usually possessed an extremely improper surface. In the case of ufasomes during etching of surface, especially when ufasomes are pre-equilibrated in glycerol and in appearance developed a noticeable variation amidst ice as well as the particle surface. The outer and inner surface of fatty acid that is exposed are smooth, whereas it is typically granular in the case of surrounding ice. Also, the space between the membrane is also rough, denoting that it comprised of water [52-54].

Birefringence

The frequency difference of birefringent particles can be elucidated by the large instability of inter-membrane distances frequently seen in ufasomes. Various forms of birefringence seen in multilamellar particles are formed of an intrinsic component that in sign form is generally positive and negative.

From the perpendicularly arranged lipid molecule to the surface of the membrane emerges the component containing positive sign and negative component owing to parallel orientation of adjacent membranes. Reduction in birefringence intensity occurs as distance between the neighboring membranes elevates. Freeze-etched preparation of ufasome clearly showed that water-filled spheres or improper multi-membrane particles are a lot more typical than that of symmetrical particles, which would be relied upon to offer strong birefringence [55, 49].

Recent studies on ufasome for targeted delivery

Recent studies performed in oleic acid-based ufasomes are summarized briefly in a tabulated form (table 2).

Table 2: Recent studies done in oleic acid ufasomal preparation

Drug category	Drug name	Route of administration	Key findings	Reference
Antifungal	Clotrimazole	Topical	The study revealed that a greater amount of clotrimazole loaded ufasomes was accumulated in the skin than the marketed formulation.	[56]
	Clotrimazole	Topical	*In vivo* study showed prolonged release of the drug and concluded that it may be good for treatment of topical infection like candidiasis.	[57]
	Fluconazole	Topical	Various studies showed that oleic acid vesicles penetrate stratum corneum and retain the drug accumulated and inferred that ufasome is a potential carrier topical targeted delivery.	[58]
Vasodilator	Minoxidil	Follicular	Study suggests that the vesicular minoxidil gel was 10 fold greater than the control and are effective in targeted skin and follicular drug delivery.	[59]
Calcium channel blocker	Cinnarizine	Nasal	The studies that lyophilized ufasomes gel can penetrate deep nasal mucosa layer and cinnarizine loaded ufasome vesicle is possible for intranasal delivery.	[60]
Antiosteoarthritic	Glucose amine sulphate	Topical	The drug loaded oleic acid vesicle showed 51 % remission at the end of the study and concluded that it can be used as an alternative for topical delivery of antiosteoarthritic drug.	[61]

Comparison of ufasome and liposome

Comparison of ufasomes with the thoroughly studied vesicular drug delivery system liposomes are done based on: [42, 28]

a) Method of preparation of ufasomes versus liposomes

Practically almost similar methods can be employed in the preparation of ufasomes and liposomes. One interesting dissimilarity is that particles of uniform size are not formed by intensive sonication of fatty acid dispersion. Rather, there are some evidence which indicates that, a clear supersaturated system is formed when oleic acid as well as linoleic acid is forced into the solution, and on standing for some time the solution turns turbid. Ufasomes retain less solute per unit weight of fatty acid that is prepared by sonication. This may be because as the sphere has much smaller size which is produced by much drastic technique [48].

b) pH sensitivity

Ufasomes when compared to liposomes are relatively more sensitive to ionic strength and also the pH of the medium. Whereas, the range of conditions that fail in the formation of fatty acid lipid membrane are well tolerated by phospholipid vesicles, aside from low ionic strength and alkaline pH [27].

c) Light scattering property

Phospholipid vesicles resulted as stronger scatterers per mol material, as a result of comparing light scattering property of liposomes and ufasomes. It is difficult to produce an equivalent comparison; approximately 10-3 molar liposomal suspension possess an absorbance of 0.7, whereas similar suspension of ufasomes gives an absorbance around 0.2 and the part of the difference in comparison may lie in the phospholipid's relatively large cross-sectional area.

d) Cross-sectional area

At 10 to 20 dyne cm-1, the reasonable cross-sectional areas for lecithin is 0.8 nm2and for linoleic acid and oleic acid is 0.4 nm2. Therefore, it seems such that lecithin forms a membrane double as that of the membrane framed from the mol of either oleic acid or linoleic acid.

e) Solute entrapment capacity

Both liposomes and ufasomes have same almost the same glucose entrapment capacity. Liposomes produced from lecithin with dicetyl phosphate and added cholesterol held around 1200 nm glucose/µm lipid. The amount was nearly doubled when lecithin is replaced by sphingomyelin. In the case of ufasomes, compared to this ufasomes can entrap around 450 nm glucose/µm fatty acid. Again, this may be because of smaller spheres per mol.

f) Internal arrangement

Liposomes are concentric bi-layered microvesicle where the fluid compartment is completely encased by a bi-layered membrane made up of phospholipids. Ufasomes are closed lipid bilayered vesicle composed of fatty acid with their hydrocarbon tails oriented along the inside of the membrane and carboxyl groups towards the water.

g) Cost

Purified diacylglycerol-phospholipids are much expensive than a conventional fatty acid. Liposomes are costlier than ufasomes [34].

h) Internal absorption

It has been reported by Patel and Ryman that, in rats, considerably lower hypoglycemic effect was shown by orally taken insulin that is encapsulated to liposomes than intraperitoneally delivered insulin that is encapsulated or free [62]. Entrapment into egg phosphatidylcholine-cholesterol liposomes firmly decreased the everted jejunum of the rat from carboxyfluorecein absorption and slightly raised fluorescein isothiocynate-conjugated dextran absorption. Patel *et al.* also reported that in the dog duodenum on administration of liposomal insulin there was an unreproducible rise in plasma immunoreactive insulin level. Majority of the studies showed unfavorable results and therefore concluded that liposomes in practical significance do not hold any absorption promoting effect. Murakami *et al.* reported that when carboxyfluorescein is entrapped into ufasomes showed enhanced absorption. Results manifest that intestinal degradation of ufasomes due to liberation of fusogenic lipid advances drug absorption [62-66].

Some of the major characteristics difference between ufasomes and liposomes are represented (fig. 3).

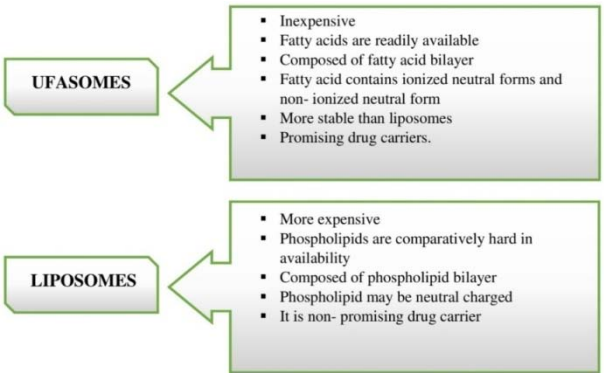

Fig. 3: Characteristic difference between ufasomes and liposomes [34]

CONCLUSION

Vesicular drug delivery systems are gaining popularity in the current field because of its site specifically targeted drug delivery

and other advantages. Ufasome is a vesicular system as a novel drug delivery. They are oleic acid vesicles which is a suspension of closed lipid bilayer preparation of stable ufasomes mainly relies on appropriate choice of fatty acid, cholesterol quantity, range of the pH, buffer and lipoxygenase amount. Recent innovation provides opportunities to produce ufasomes with very efficient features such as stability considerations, dynamic features and microscopic features of ufasomes. Also, they are capable as carriers for oral intake of less absorbable drugs and the horizontal transfer of genes from plants. The article furthermore provides the difference between ufasomes with liposomes.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors report that there are no conflicts of interest to disclose.

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