Review Article

Ufasomes: A Vesicular Drug Delivery

Patel DM, Jani RH, Patel CN

Department of Pharmaceutics and Pharm Technology, Shri Sarvajanik Pharmacy College, Mehsana, Gujarat, India

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ABSTRACT

Unsaturated fatty acid vesicles (ufasomes) are suspensions of closed lipid bilayers that are composed of fatty acids, and their ionized species (soap) which are restricted to narrow pH range from 7 to 9. In ufasomes, fatty acid molecules are oriented in such a way that their hydrocarbon tails are directed toward the membrane interior and the carboxyl groups are in contact with water. Stable ufasome formulation critically depends on proper selection of fatty acid, amount of cholesterol, buffer, pH range, amount of lipoxygenase, and the presence of divalent cations. Recent innovations can provide opportunity to formulate ufasomes with tailorable features such as extension of pH range, insensitivity toward divalent cations, and enhancement of stability. This article describes method of ufasome preparation, key issues in ufasome manufacturing, recent innovations in ufasomes, dynamicity, stability, and microscopic characterization of ufasomes. Later part of this article deals with comparison of ufasomes with thoroughly studied liposomes.

Introduction

Fatty acid vesicles are colloidal suspensions of closed lipid bilayers that are composed of fatty acids and their ionized species (soap). They are observed in a small region within the fatty acid–soap–water ternary phase diagram above the chain melting temperature (Tm) of the corresponding fatty acid–soap mixture. Fatty acid vesicles always contain two types of amphiphiles, the nonionized neutral form and the ionized form (the negatively charged soap). The ratio of nonionized neutral form and the ionized form is critical for the vesicle stability. Fatty acid vesicles are actually mixed "fatty acid/soap vesicles," but for the sake of simplicity, we just call them fatty acid vesicles. The formation of fatty acid vesicles was first reported by Gebicki and Hicks in 1973 and the vesicles formed were initially named "ufasomes," "unsaturated fatty acid liposomes."[1,2] Later investigations have shown that fatty acid vesicles form not only from unsaturated fatty acids such as oleic acid, linoleic acid, but also from saturated fatty acid such as octanoic acid and decanoic acid. In liposome formulation, phospholipids are generally used. However, even natural phospholipids are chemically heterogeneous, and pure synthetic phospholipids are not yet available in reasonable quantities. The advantage of ufasomes over liposomes is the ready availability of fatty acids.[3,4]

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Correspondence:
Dr. Dasharath M. Patel; E-mail: drdmpatel1971@gmail.com

Method of preparation

Only unoxidized materials are preferred for preparation of ufasome. Stock solutions containing 10% of oleic and linoleic acids in chloroform are prepared and stored at 20°C. For typical preparations, 0.02 ml of the stock solution is evaporated in a test tube on a water pump and finally dried with a stream of nitrogen. The fatty acid film is then broken up completely in 0.2 ml of 0.1 M *tris*-hydroxymethyl aminomethane buffer, pH 8–9, by vigorous shaking on a vortex mixer. The resultant suspensions of ufasomes are stable for at least 24 h. In some experiments, an ultrasonic generator with a titanium microtip is used to prepare the particles. Air is first removed from the buffer by a stream of nitrogen and the suspension is blanketed with the gas during irradiation. Constant temperature is maintained by an ice water bath.[3]

Key issues in manufacturing of ufasomes

Selection of fatty acid

Analysis of natural membrane phospholipids and information from the pressure area measurements on fatty acid surface films suggest that the 12 to 22 carbon fatty acids would be suitable for formulation of stable ufasomes. In fact, most of the studies were confined to the C-18 acids because they showed the greatest promise in early trials. Only oleic acid (cis-9-octadecenoic acid) and linoleic acid (cis, cis-9,12-octadecadienoic acid) formed membranes that enabled the ufasomes to fulfill these criteria. Of other fatty acids, palmitic

acid is tolerated up to 33% and stearic acid up to 5% by weight in an oleic acid membrane. Charging of the membrane with small amounts of oleic, linoleic or stearic acid amides do not improve the preparations. Stability tests showed that oleic acid remained uncontaminated by peroxides for at least 6 weeks while linoleic acid developed significant peroxide after 2–3 weeks.[3]

Addition of cholesterol

Cholesterol serves a unique purpose of modulating membrane fluidity, elasticity, and permeability in vesicle prepared from lipid. It literally fills in the gaps created by imperfect packing of other lipid species. There is a rapid decrease in the ability to hold solute by vesicle in the presence of higher proportions of cholesterol. Also, there is no enhancement of membrane impermeability at any cholesterol concentration. Hicks *et al*.[3] compared leakage of glucose from oleic and linoleic acid ufasomes with leakage from spheres containing 17% of incorporated cholesterol by weight. It was concluded from their results that leakage of glucose from vesicles containing 17% of incorporated cholesterol was higher than leakage from cholesterol free oleic and linoleic acid ufasomes. Leakage of glucose from ufasome of different composition is depicted in Figure 1.

pH

The formation of fatty acid vesicles is restricted to a rather narrow pH range (7–9), where approximately half of the carboxylic groups are ionized. Below this range the fatty acids only form unstructured precipitates, while above, they are too soluble. A titration curve of the oleic acid/oleate system determined at a total concentration of 80 mM can differentiate three regions for formation of micelles, vesicles, and oil droplet that is shown in Figure 2. Micelles are the dominant aggregation species at higher pH (higher ratio of ionized to protonated molecules), whereas oil droplets form in the low pH region. It is also better to understand fatty acid vesicle systems at concentrations just above the concentration at which vesicle formation is observed, often called "critical vesiculation concentration," CVC.[1] At the critical vesiculation concentration, monomers and nonvesicular aggregates assemble into a bilayer structure and form colloidal suspensions of vesicles. It is also interesting to know that dilution of a fatty acid micellar solution at basic pH toward neutrality results in spontaneous formation of vesicles with a broad size distribution.[5,6]

Selection of buffer

The widely accepted buffer for ufasome preparation is *tris*-hydroxymethyl aminomethane. However, spheres also form in borate, glycine-hydroxide and bicarbonate solutions. Selection of buffer is largely dependent on the type of solute to be incorporated, i.e., in the case of glucose entrapment in vesicle; ufasomes prepared in bicarbonate did not hold glucose, while the borate preparations could not be tested for retention because of formation of glucose-buffer complex. With *tris,* the optimum weight of buffer has to equal the weight of fatty acid used to form membranes; thus, 0.1 ml of 0.1 M *tris* at pH 8 is needed to form ufasomes from 1 mg of fatty acid.[3]

Electrolyte

Most electrolytes inhibit formation of ufasomes. However, once the spheres are stabilized in appropriate buffer, they can be exposed to solutions of phosphates or chlorides and still retain occluded glucose.[3]

Peroxidaton

The main effect of peroxidation on the ufasome membranes is to produce disturbance of the normal bilayer arrangement of fatty acid molecules. Introduction of a bulky hydrophilic group by peroxidation would distort the hydrophobic membrane interior, allowing an easier passage of water-soluble molecules.

Hicks *et al*.,[7] studied the quantitative relationship between permeability and the degree of peroxidation in ufasome membranes. They used soya bean lipoxygenase to induce release of sequestered glucose from vesicles made from linoleic acid. Lipoxygenase induce release of glucose from fatty acid membrane by formation of linoleate peroxides. The maximum rate of glucose efflux from linoleic acid ufasomes was proportional to the concentration of lipoxygenase [Figure 3]. It is interesting to note that the enzyme was unable to induce leakage from oleic acid ufasomes. It means lipoxygenase fails to peroxidase monoenoic fatty acids. The kinetics

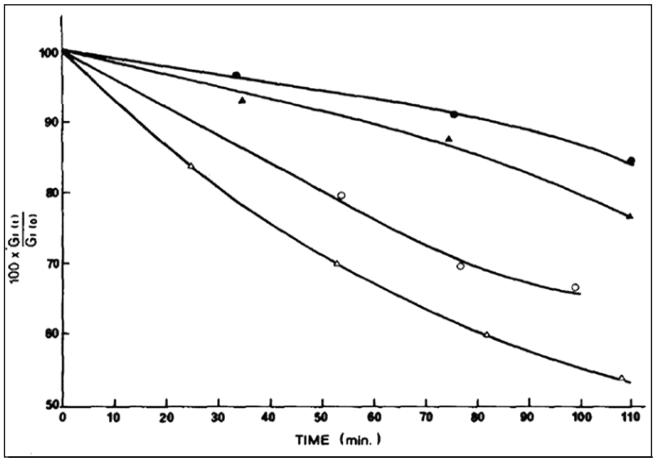

Figure 1: Leakage of glucose from ufasomes of different composition

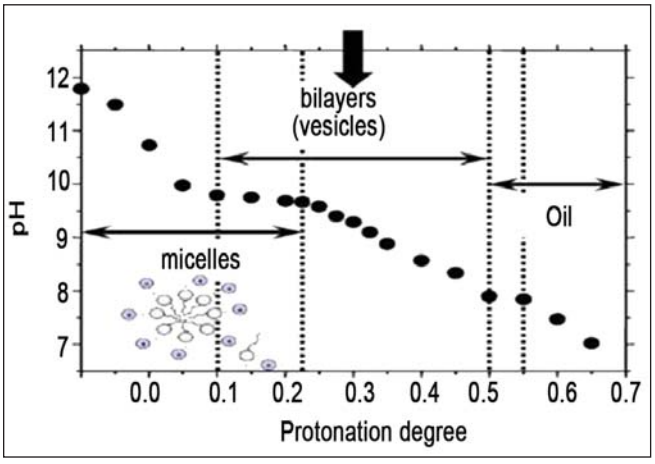

Figure 2: Titration curve for 80m Moleic acid/sodium oleate

of glucose release from the vesicles under different conditions is shown in Figure 4. Curve A shows glucose release after addition of 0.1 ml of 10% triton X-100 that solubilized the fatty acid membranes and releasing all trapped glucose. Curve B and C show the kinetics of glucose release after addition of 170 and 56 units of lipoxygenase /ml assay, respectively. However, even the highest concentration of lipoxygenase used did not release all of the trapped glucose until completion of peroxidation of linoleic acid by the enzyme. Small amounts of peroxide (up to 4–5%) did not produce any significant efflux of glucose, but peroxidation above this level led to a rapid increase in the leakage rate and reaching a maximum after about 10% peroxidation that is shown in Figure 5. Therefore, it is clear that increase in membrane permeability were strictly dependent on the peroxidation of constituent fatty acid.

Method of preparation can widely affect the extent of peroxidation of fatty acid. No peroxidation occurred during the short periods required for hand vortexing. Under the more violent ultrasonic resuspension, linoleic acid oxidized at 0.1% per minute in air-saturated buffers when exposed to 30-W irradiations. Since 3 min was the longest exposure used, this method did not produce extensive oxidation of even oxidation sensitive linoleic acid.[3] However, Hicks and Gebicki found that nitroxide radicals, butylated hydroxytoluene, and α-tocopherol can significantly inhibit peroxidation of linoleic acid membranes.[8,9]

Divalent cations

Lipid peroxidation (LPO) involves both enzymatic and nonenzymatic catalytic mechanism. Transition metal ions are important components of nonenzymatic lipid peroxidation.[10-12] Relatively few metals that undergo a change in valency involving a single electron transfer can catalyze a rapid rate of peroxidation in unsaturated lipids. Nonvariable valence state metals such as calcium, magnesium, and zinc which cannot take part in redox-coupled homolysis have also been shown to influence lipid peroxidation.[13-15] Calcium ion has biphasic effect on LPO means not only can they stimulate LPO, but they may also exhibit an inhibitory effect. Babizhayev[16] studied this biphasic activity of calcium in liposome (from egg yolk lecithin) and ufasomes (from linoleic acid and methyl linolenate). LPO in liposome and ufasome was induced in the presence of ascorbate or cumol hydroperoxide and also Fe^{2+}.

It was shown that at low concentrations ($\sim 10^{-6}$-10^{-5}), Ca^{2+} stimulated LPO in lipid by its ability to interact with negatively charged groups of lipid (phosphate groups of lecithin, carboxyl groups of linolenic acid), thereby displacing the bound Fe^{2+} ions so increasing the concentration of free Fe^{2+} ions, which participate directly in LPO catalysis. At high concentrations ($\sim 10^{-3}$), inhibitory effect of Ca^{2+} was based on its interaction with superoxide anion radicals.

Incidentally, not only Ca^{2+} ions may have such a biphasic action on LPO; other cations with high charge density are also capable of releasing Fe^{2+} ions bound with negatively charged groups of lipids and of interacting with superoxide free radicals. It was found that in the absence of Ca^{2+} ions, addition of La^{3+} ions to linolenic acid ufasomes in a concentration corresponding to that of the Fe^{2+} ions stimulated LPO. An effect of inhibition of peroxidation of linolenic acid was observed on the combined action of equimolar concentrations of Ca^{2+} and La^{3+} (when their total concentration exceeded that of Fe^{3+}.

Recent innovations in conventional ufasomes

Applications of fatty acid vesicles in the fields of food additives and drug delivery are largely unexplored, which is at least partially due to concerns regarding the colloidal stability of fatty acid vesicles (pH- and divalent cation-sensitivity). However, there are some recent studies, using either new types of fatty acids or mixed systems with other surfactants, which may change the situation in future.[1]

New type of fatty acids in ufasome preparation

Cis- 4, 7, 10, 13, 16, 19-docosahexaenoic acid (DHA) was reported to self-assemble into vesicles between pH 8.5 and 9.[17]

Extension of the pH range

The pH range suitable for the formation of fatty acid vesicles are generally narrow due to the requirement that approximately half of the carboxylic acid must be ionized. The pH range can, however, be extended by using the following novel approaches.

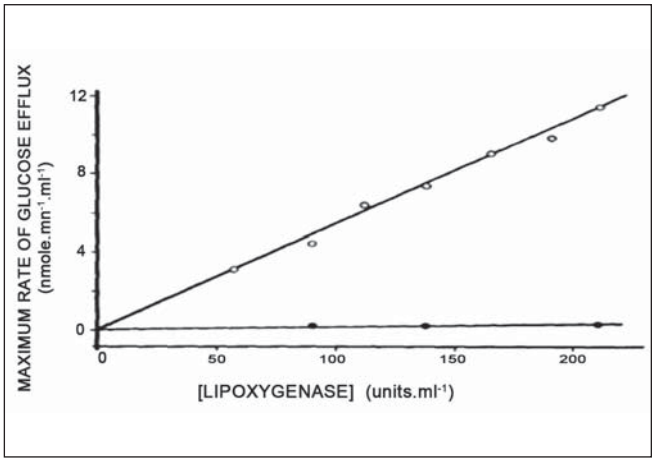

Figure 3: Rate of peroxidation as a function of lipoxygenase concentration

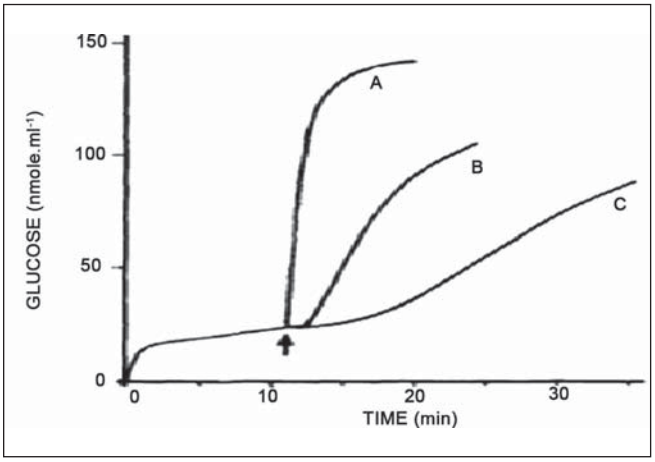

Figure 4: Kinetics of glucose release with addition of reagents

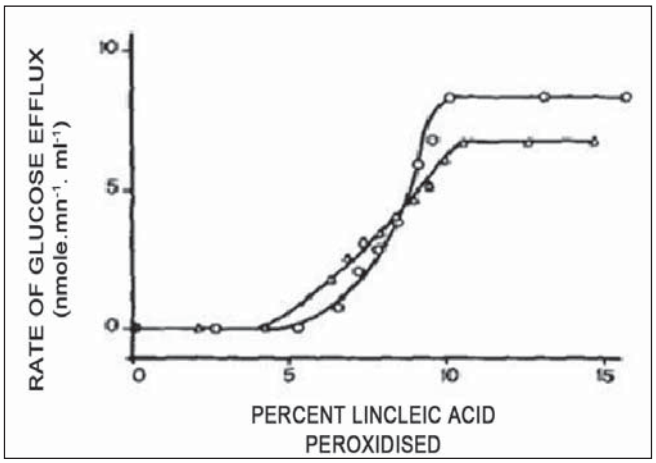

Figure 5: Effect of lipoxygenase concentration on maximum rate of glucose release from ufasomes

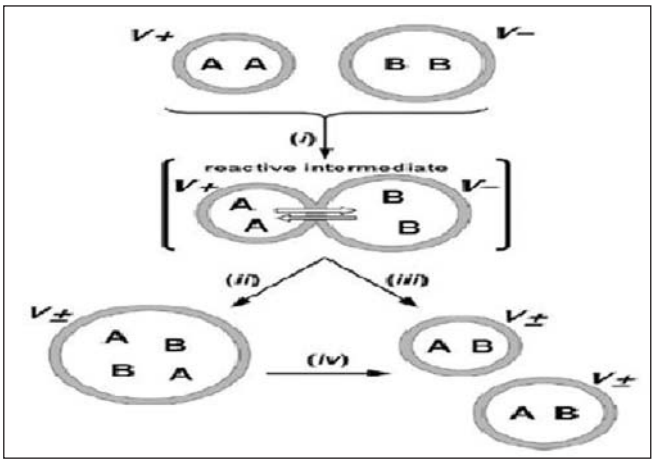

Figure 6: Mechanism of fusion of cationic vesicles and ufasomes

a) Addition of amphiphilic additives such as linear alcohols or a surfactant with a sulfate or the sulfonate head group:

for example, mixtures of decanoic acid and decanoate form vesicles between pH 6.4 and pH 7.8, but the pH for vesicle formation can be lowered to at least 4.3 by adding sodium dodecylbenzenesulfonate (SDBS). By coaddition of an equimolar amount of SDBS to decanoic acid, vesicles also formed below pH 6.8.[18]

b) Synthetically modify the size of the hydrophilic head group of fatty acids:

enhanced stability of vesicles at lower pH was reported by using a fatty acid with an oligo (ethylene oxide) unit intercalated between the hydrocarbon chain and the carboxylate head group. The very bulky polar group has two effects, a lowering of the phase transition temperature (close to the Kraft point) and a lowering of the pH region for vesicle formation.[1]

Insensitivity toward divalent cation

Divalent cations such as Mg^{2+}, Ca^{2+} cause precipitation of vesicles even at low concentrations. Addition of fatty acid glycerol esters was found to stabilize the fatty acid vesicles in the presence of ionic solutes. Cryogenic transmission electron microscopy studies of the ternary monoolein–sodium oleate–water system have also shown that uni- and multilamellar vesicles formed from mixtures of monoolein and sodium oleate and the vesicles remained stable for a prolonged period of time (over 1 year).[19]

Enhancement of stability by crosslinking fatty acid molecules by chemical bonds

One example is the formation of vesicles from anionic gemini surfactants with the carboxylic head group. Another example is the usage of a fatty acid (soap) with a polymerizable moiety (e.g., sodium 11-acrylamidoundecanoate: SAU). Both monomeric and polymerized SAU were reported to self-assemble into vesicular aggregates and vesicles from polymeric SAU were stable at elevated temperatures.[20-22]

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

Mixtures of tetradecyltrimethylammonium hydroxide (TTAOH) and fatty acids were investigated as a model system of mixed vesicles. Unilamellar and multilamellar vesicles were reported to form, if approximately the same concentration of TTAOH and fatty acid were mixed.[23] Caschera *et al*.,[24] reported significant fusion (23%) of oppositely charged vesicles based on cationic didodecyl dimethyl ammonium bromide (DDAB) and anionic fatty acid vesicles. A reactive intermediate, formed by the association of the oppositely charged vesicles can evolve into a single larger vesicle that contains both the solutes present in the initially separated vesicles and a mixed membrane (composed by cationic and anionic lipids). This giant vesicle that further reacts and gives rise to more vesicles, each of them containing the solutes initially separated in two vesicle populations. This can be also seen as a mechanism of "solute exchange." The mechanism of fusion is shown in Figure 6.

Dynamic nature of ufasomes

One important feature is the dynamic nature of fatty acid vesicles owing to the fact that they are composed of single chain amphiphiles. Dynamic features that place fatty acid vesicles in between conventional vesicles formed from double-chain amphiphiles and micelles formed from single-chain surfactants. The fact that a range of fatty acid aggregates are formed just by changing the protonation/ionization ratio of the terminal carboxylic acid. Chen *et al*.,[25] studied the formation kinetics of ufasome. The formation kinetics of micelles and vesicles from a saturated fatty acid/soap monomer solution was compared by dialyzing the fatty acid/soap monomers through a cellulose acetate membrane. Starting from an asymmetric distribution of the fatty acid/soap molecules between two chambers separated by the dialysis membrane, one chamber containing aggregates (micelles or vesicles), and the other containing the buffer solution only, the rate of attainment of equilibrium was monitored. An equilibrium state was readily obtained in the case of the micellar system (micelles formed in the diffusate chamber and the fatty acid/soap concentrations in both chambers became the same). In the case of vesicles, however, the attainment of an equilibrium state was severely hindered (the concentration in the diffusate increased very slowly after the solution was saturated with monomers). Vesicles are generally composed of a much greater number of amphiphiles than micelles. The results obtained from the dialysis experiments with fatty acid vesicles suggest that the formation of fatty acid vesicles poses a much higher energy barrier compared to the formation of fatty acid

(soap) micelles. A convenient way of fatty acid vesicle preparation is the addition of an alkaline soap solution to a buffer solution of intermediate pH. For example, a concentrated solution of sodium oleate micelles is added to a buffered solution at pH 8.5, and oleic acid/sodium oleate vesicles form spontaneously as a result of a partial protonation of the oleate molecules, caused by the drop in pH from about 10.5 to 8.5. Vesicles thus formed are polydisperse in size and lamellarity. In short, fatty acid vesicles grow spontaneously when alkaline micelles are added to buffered vesicles.[1,25]

Stability consideration in ufasome formulation

The long-term stability of ufasome membranes is highly dependent on decrease in free energy of the fatty acid-water system. The membrane formation is not spontaneous, because the acids form a separate phase at pH 8. However, even mild mechanical agitation is sufficient to induce bilayer formation under the right conditions. Clearly, much of the energy liberated in this process comes from the increased entropy of water that accompanies the hydrophobic interactions of the oriented hydrocarbon chains. The attractive interaction is opposed in the bilayer by mutual repulsions of the ionized carboxyl head groups. Electrolytic dissociation decreases fatty acid film stability and may cause its disruption. Charge repulsion can be lessened by a decrease of the degree of headgroup dissociation, by formation of stable complexes between protonated and ionized carboxyl headgroups or by the presence of screening counter ions. All these processes may operate in stabilization of ufasome membranes. Fortunately for the stability of membranes, lateral charge repulsions are decreased by the lowering of pH that occurs at particle surface. Decrease in ionization enhances the membrane stability in several ways. First, the protonated molecules are virtually insoluble in water by comparison to the anions. Secondly, there is a reduction in lateral headgroup repulsion; in a film of closely packed headgroups the average distance between charges increases by about 40% on the removal of every second charge, resulting in a halving of coulombic repulsions. Thirdly, protonated acid molecules (AH) and anions (A-) form series of strongly bound complexes, with a 1 : 1 complex the predominant species. The energy for binding is made up of three contributions: free energy changes arising from hydrophobic interactions, entropy of demixing associated with formation of dimers and a free energy lowering brought about by the formation of hydrogen bonds between the protonated and ionized carboxyl groups. Studies of interactions in dicarboxylic acids have shown that exceptionally strong hydrogen bonds form between -COOH and $–COO^-$ groups due to the presence of a negative charge close to the hydrogen involved in bonding.

Ufasome membranes are stabilized by headgroup hydrogen bonding with water, complex formation between ionized, and neutral acid molecules and by hydration of the dissociated carboxyl groups. In addition, the hydrocarbon regions of the fatty acids are held together by precisely the same dispersion and hydrophobic interactions that stabilize micelles and the interior regions of membranes.[3]

Microscopic studies

The arrangement of biological membrane components such as fatty acid, phospholipid was derived from the electron microscopy of sectioned vesicular structures. It was usually recognized, however, that the necessary fixing and staining requires harsh chemicals that can produce distortion of these delicate structures, with consequent loss of definition and formation of artifacts. Such problems can sometimes be reduced by the use of less harsh techniques. One of the most successful methods applied to natural components is the freeze fracture technique. An even less harsh method is the detection of birefringence. As attempts to study the ufasome structure by electron microscopy of negatively stained specimens showed that they did not survive the preparatory steps. All attempts to stain ufasomes with neutralized potassium phosphotungstate for electron microscopy failed to produce specimens with any internal structure.[26]

Freeze fracturing and eaching

First of all, ufasome suspension is equilibrated with 17% glycerol for 10 min before freezing. The ufasome suspensions are then rapidly frozen on to copper helmets with Freon and then stored in liquid nitrogen. Fracturing is carried out in a Balzers microtome at 110°C and at 2×10^{-6} Torr pressure. For etching, the temperature is increased to 100°C for 1 min. After cutting, a film of platinum and carbon is deposited on the fracture face to a thickness of 3 nm at an angle of 45°. The most successful technique used to clean replica is to float them off the metal helmet on to water, to which methanol is gradually added, until the solution is 80% alcohol. It took 30 min to remove all traces of fatty acid. The replicas are then examined in a Hitachi HS8 electron microscope.[27,28] Hicks and Gebicki found that there was no difference in the appearance of ufasomes made from oleic or from linoleic acids.[26] Since ufasome preparations contained a large proportion of water, much of the freeze fractured face consisted of ice, which often had a very irregular surface. Etching of the surface, especially if the ufasomes were pre-equilibrated in glycerol, produced a marked difference in appearance between ice and the particle surface. The exposed outer and inner fatty acid surfaces are smooth, while the surrounding ice is typically granular. The space between the membranes is also rough, suggesting that it was filled with water.[29-31]

Birefringence

The difference in the frequency of birefringent particles can be explained by the wide variability of inter-membrane distances commonly observed in the ufasomes. The different type of birefringence observed in multilamellar particles is made up of an intrinsic component, which is usually positive and negative in sign. The positive sign component arises from the perpendicular orientation of lipid molecules to the membrane surface, and a negative "form" component which is due to the parallel arrangement of adjacent membranes. As distance between neighboring membranes increases, the intensity of birefringence decreases. Freeze-etched ufasome preparations showed clearly that the irregular multimembrane particles or large water-filled spheres are much more common than the symmetrical particles which would be expected to give rise to strong birefringence.[26,32]

Comparision of ufasome and liposome

It seems profitable to discuss ufasomes by comparing them with the thoroughly studied liposomes.[3,33]

Method of preparation

Virtually identical techniques can be used for either type of vesicle. The one interesting difference is that intensive sonication of fatty

acid dispersions does not lead to uniformly-sized particles. Instead, there is some evidence to suggest that oleic and linoleic acids can be forced into the solution to produce a clear supersaturated system that becomes turbid after standing for a few minutes. Ufasomes prepared by sonication retain less solute per unit weight of fatty acid. This is probably due to the much smaller size of spheres prepared by the more drastic treatment.

pH sensitivity

Compared to liposomes, ufasomes are much more sensitive to pH and ionic strength of medium. While the phospholipid vesicles tolerate the range of conditions, fatty acid membranes fail to form, except at slightly alkaline pH and at low ionic strengths.[1]

Light scattering property

Comparison of the light scattering properties of ufasomes and liposomes shows that the phospholipid vesicles are stronger scatterers per mole of material. It is not easy to make an exact comparison; roughly, a 10^{-3} molar liposome suspension has absorbance of 0.7, while a similar preparation of ufasomes reads about 0.2. Part of this difference may lie in the relatively large cross-sectional area of phospholipids.

Cross-sectional area

Reasonable cross-sectional areas at 10–20 dyne cm^{-1} are 0.8 nm^2 for lecithin and 0.4 for oleic and linoleic acids. It appears likely, therefore, a mole of lecithin forms a membrane twice as large as that formed from a mole of either of these acids.

Osmotic response

Measurements of the effects of osmotic pressure changes on the amount of light scattered by ufasomes and liposomes are made with an Aminco–Morrow stopped-flow apparatus operated in conjunction with an Aminco DW-2 spectrophotometer. For osmotic shrinking measurements, a 2 ml suspension of vesicles is diluted to 20 ml with 0.1 M *tris* pH 8. This is placed in one storage reservoir of the stopped-flow machine. The other reservoir is filled with a similarly buffered sucrose solution. Because of the geometry of the apparatus, the ufasomes are exposed on mixing to sucrose concentration equal to a half of that in the storage reservoir. For swelling experiments, the ufasomes are prepared in buffered sucrose and diluted 1 : 1 with buffer in the mixing chamber during flow. From the results of Bangham and Hicks,[3] it is clear that unlike liposomes, the ufasomes are permeable to sucrose. The swelling curve shows that although over 90% of the volume change occurs in the first 2 min following mixing, the process continues at a decreasing rate. Bangham *et al*.,[3] showed that addition of KCL to suspensions undergoing swelling resulted in contraction of both spheres. The opposite effect, reswelling of liposomes shrunk by sucrose, was reported by Rendi.[34,35] Hicks *et al*.,[3] found that swelling of ufasomes cannot be reversed by addition of sucrose.

Solute entrapment capacity

Ufasomes and liposomes have a similar capacity to entrap glucose. Liposomes made up from lecithin with added cholesterol and dicetyl phosphate held about 1200 nM glucose per μM lipid. When lecithin is replaced by sphingomyelin, this amount was nearly doubled. Compared to this, ufasomes entrap about 450 nM of glucose per μM of fatty acid. This may again be due to a smaller number of spheres forming per mole of fatty acid.

Internal arrangement

A liposome is a microvesicle composed of a bilayer of phospholipid molecules enclosing an aqueous compartment. In ufasomes, the membrane fatty acids are oriented in a bilayer form with their hydrocarbon tails toward the membrane interior and the carboxyl groups in contact with water.

Cost

Conventional fatty acids are inexpensive, certainly cheaper than purified diacylglycero-phospholipids. Ufasomes are relatively less costly than liposomes.

Intestinal absorption

In rats, orally delivered insulin, encapsulated into liposomes, proved to exert a considerably smaller hypoglycemic response than i.p. delivered free or encapsulated insulin as reported by Patel and Ryman.[36] Entrapment into egg phosphatidylcholine-cholesterol liposomes strongly reduced carboxyfluorescein absorption from the rat everted jejunum and only marginally increased absorption of fluorescein isothiocyanate-conjugated dextran. Patel *et al*.,[36] reported an irreproducible increase of plasma immunoreactive insulin on administration of liposomal insulin in the dog duodenum. As the subsequent fall in plasma glucose was negligible, it was concluded that a very small amount of insulin of not more than 1% was absorbed intact.[36,37] Because of the unfavorable results of the majority of studies, it was concluded that liposomes do not appear to have any absorption promoting properties of practical importance. Murakami *et al*.,[37] reported that carboxyfluorescein absorption proved to be enhanced by entrapment into ufasomes. Results indicate that the fusogenic lipid, liberated on intestinal degradation of the ufasomes, promotes drug absorption. Future research will be necessary to decide on the applicability of this type of lipid vesicle as an intestinal absorption enhancer.[38-40]

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