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EXPLORING POTENTIAL OF UFASOMES AS TOPICAL/ TRANSDERMAL DELIVERY SYSTEMS: REVIEWING DECADE OF RESEARCH

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ABSTRACT

Due to the lower risk of systemic side effects topical treatment of skin disease appears favourable, yet the stratum corneum counteracts the penetration of xenobiotics into viable skin. Particulate carrier systems may mean an option to improve dermal penetration. Since epidermal lipids are found in high amounts within the penetration barrier, lipid carriers attaching themselves to the skin surface and allowing lipid exchange between the outermost layers of the stratum corneum and the carrier appear promising. Besides liposomes and niosomes, ufasomes have been tested for their potential topical/transdermal delivery. Unsaturated fatty acid vesicles (ufasomes) are suspensions of closed lipid bi-layers 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. The advantage of ufasomes over liposomes is the ready availability and lower cost of fatty acid. In this review special focus is laid upon the interactions of active ingredients and the lipid matrix as well as the quantification of dermal penetration.

Key words: ufasome, topical delivery

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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 few fatty acid such as linoleic acid (cis, cis-9, 12-octadecadienoic acid) and oleic acid (cis-9-octadecenoic acid) formed vesicles that enabled the ufasomes to full-fill these criteria. Other fatty acids, palmitic acid is tolerated up to 33% and stearic acid up to 5% by weight in oleic acid vesicles. Small amounts of oleic, linoleic or stearic acid amides, used for charging of the membrane do not improve the preparations. Stability tests conducted for both the fatty acid showed that oleic acid remained uncontaminated by peroxides for at least 6 weeks while linoleic acid developed significant peroxide after 2-3 weeks[1].

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.*[1] compared leakage of model drug from oleic and linoleic acid ufasomes with leakage from spheres containing 17% of incorporated cholesterol by weight. They concluded from their results that leakage of model drug from vesicles containing 17% of incorporated cholesterol was higher than leakage from cholesterol free oleic and linoleic acid ufasomes.

pH

pH and concentration of fatty acid are important factor for self asemblance of fatty acid into vesicles. Narrow pH range (7-9) is essential for formation of vesicles. 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. 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[2]. 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[3, 4].

Selection of buffer

Selection of buffer for ufasomal formulation is largely dependent on both compositions of buffer as well as type of solute to be incorporated. As reported in literature, spheres also form in borate, glycine-hydroxide and bicarbonate solutions but widely accepted buffer which is used for ufasome preparation is *tris*-hydroxymethyl aminomethane.. 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[1].

Electrolyte

Literature is abounding regarding ufasomes formation inhibition by electrolytes. However vesicles can be exposed to solutions of phosphates or chlorides only when spheres are stabilized in appropriate buffer, and still retain occluded glucose[1].

Peroxidaton

Hicks *et al*, [5] studied the quantitative relationship between permeability and the degree of peroxidation in ufasome membranes. Introduction of a bulky hydrophilic group by peroxidation on the ufasome membranes produced disturbance in the normal bilayer arrangement of fatty acid molecules i.e. distort the hydrophobic membrane interior, allowing an easier passage of water-soluble molecules. 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.

Method of preparation can widely affect the extent of peroxidation of fatty acid. For e.g. no peroxidation occurred during the short periods required for hand vortexing but in the case of violent ultrasonic resuspension, linoleic acid oxidized at 0.1% per minute in air-saturated buffers when exposed to 30-W irradiations did not produce extensive oxidation of even oxidation sensitive linoleic acid[1]. In addition to method of preparation Hicks and Gebicki found that nitroxide radicals, butylated hydroxytoluene, and a-tocopherol can significantly inhibit peroxidation of linoleic acid membranes[6,7]. Lipid peroxidation (LPO) involves both enzymatic and nonenzymatic catalytic mechanism. Transition metal ions are important components of

nonenzymatic lipid peroxidation[8, 9, 10]. It has been shown that at low concentrations (~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 (~10^{-3}), inhibitory effect of Ca^{2+} was based on its interaction with superoxide anion radicals. 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[2]. *Cis* - 4, 7, 10, 13, 16, 19-docosahexaenoic acid (DHA) was reported to self-assemble into vesicles between pH 8.5 and 9[11]. 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*:* 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 co-addition of an equimolar amount of SDBS to decanoic acid, vesicles also formed below pH 6.8[12]. 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[2]. 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)[13]. 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[14, 15, 16].

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 head group dissociation, by formation of stable complexes between protonated and ionized carboxyl head groups 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 head group repulsion; in a film of closely packed head groups 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 head group 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[1].

Comparison with conventional liposome

It seems profitable to discuss ufasomes by comparing them with the thoroughly studied liposomes[1, 17]. 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. 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[2]. 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. 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. 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. A liposome is a micro vesicle 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. Conventional fatty acids are inexpensive, certainly cheaper than purified diacylglycero-phospholipids. Ufasomes are relatively less costly than liposomes.

RESEARCH DONE SO FAR

A Kinetic Study of the Growth of Fatty Acid Vesicles

A FRET-based assay for growth of preformed vesicles revealed that growth occurred in a single exponential phase for low micelle/vesicle ratios, but two phases were observed when the

micelle/vesicle ratio exceeded 0.4. The rates of both fast and slow phases were independent of initial oleate micelle or vesicle concentration, thus excluding a single-step direct micelle-vesicle fusion model. Apparently, some micelles are initially quickly consumed by a fast reaction (up to 40% growth), whereas the remaining micelles are ‘‘trapped’’ in a kinetically stable intermediate state which contributes to growth via a slower pathway. The fast phase of growth appeared to be first-order with respect to the concentration of vesicles, indicating that the rate-limiting step involves the bilayer. The fact that the fast phase of growth had a constant amplitude A, corresponding to a 40% increase in vesicle surface area (when the micelle/ vesicle ratio exceeded 0.4), indicated that the amount of micelle incorporation during the fast phase was stoichiometrically limited by the amount of preformed vesicles[18] (figure 1). More vesicles resulted in proportionally faster phase incorporation in total, but the amount incorporated per vesicle was the same[19, 20, 21]. Recent work indicates that correlations among counterion may decrease the magnitude of electrostatic repulsion between negatively charged surfaces, an effect which can lead to attractive interactions (for experimental studies[22, 23]; for theoretical studies[24, 25, 26, 27].

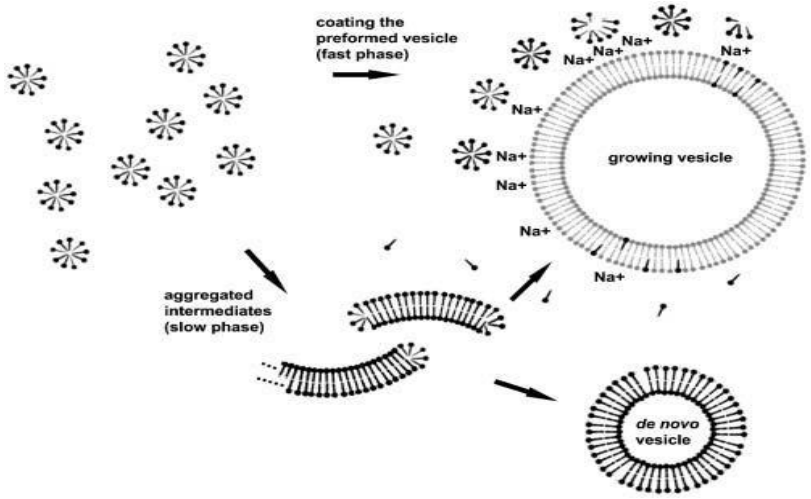

Figure 1 Proposed scheme of dynamic processes occurring during micelle addition to preformed fatty acid vesicles.

Interpretation of the data indicates that if preformed vesicles are present when micelles are added to a buffered solution, some micelles may be adsorbed and rapidly incorporated into the vesicles. The remainder, however, may be converted into large metastable structures. Some of these structures may eventually form new vesicles, rather than contribute to the growth of preformed vesicles. Therefore it can be inferred that slow addition of new fatty acid leads to optimal growth of preformed vesicles[18].

Potential of ufasomes as carrier for topical delivery

Fatty acids have been widely used as adjuvant, vehicles in drug delivery viz penetration enhancers in topical delivery and in polymeric micelles to provide sustained release. Sharma *et al*.[28] explored the potential of fatty acid vesicles for the topical delivery of 5-FU. Optimized vesicles possessed higher entrapment efficiency (64.0±4.2%) with optimum vesicle size and homogeneity in regard to size distribution (PDI = 0.234 ± 0.016) at 7:3 oleic acid-to-5-FU ratio. Increased diffusion of drug from the vesicles at low pH may have resulted due to decreased stability of the vesicles at lower pH. This further suggests that vesicles tend to fuse when they are exposed to low pH. This particularly holds for the pH that is lower than physiological pH .*Ex-vivo* skin permeation and Confocal microscopic studies suggested that oleic acid vesicles penetrate the stratum corneum and retain the drug accumulated in the epidermal part of the skin. The amount of 5-FU permeated from the plain carbopol gel was 19.83%. The drug penetration following application of an equivalent amount of drug in vesicular dispersion was significantly high, i.e. 35.8%. The permeation parameters were calculated by plotting a curve between cumulative amounts of drug permeated per unit area (μg/cm^2) vs. time. The flux was obtained from the slope of the linear portion of the graph. The transdermal permeation rate constants obtained were higher for oleic acid vesicle dispersions (21 ± 1.68μg/h/cm^2) than the plain drug gel (3.5 ± 0.6 μg/h/cm^2). It has also been observed that drug retained in the skin was more in the case of vesicular dispersion 25.94 ± 1.64 as compared to plain drug gel (4.06 ± 0.74%) as depicted in Table 1.However, it was clear from CLSM study as the thickness of skin increases, the fluorescent intensity tends to decrease. The oleic acid vesicles provided good skin permeation property as 36% of the 5-FU permeated across the skin. The DSC curve of 5-FU showed a single melting peak at 284.1°C and oleic acid thermogram displayed an endothermic peak at 309.6°C .For physical mixture the peak was detectable at the melting point of 5- FU. However, no characteristic peak of 5-FU was observed in DSC of drug-loaded oleic acid vesicles, but it showed that the small peak was at 196.9°C. This suggests that the drug was molecularly dispersed in the polymer matrix. There is no detectable endotherm if the drug is present in a molecular dispersion or solid solution state in the polymer systems loaded with drug[29]. The increase in size indicates inter-vesicular fusion. At ambient temperature (28°C), the phase transition temperature of oleic acid is exceeded, hence they tend to fuse[30,31]. The drug leakage studies carried out also suggested better stability of fatty vesicles at refrigerated conditions. On

the basis of sustained release behaviour and skin retention it can be inferred that oleic acid vesicles can serve as a potential carrier for the topical localized delivery of bioactives[28].

Table 1: Skin permeation and retention of 5-FU from various formulations

Formulation	Percentage drug retained in skin	Transdermal flux Jss (µg/h/cm2)
Optimized vesicular preparation (pH 7.4)	25.95 ± 1.64	21 ± 1.68
Plain drug gel (pH 7.4)	4.06±0.74	3.5±0.6

Table 2. Composition and Characterization of Different Formulations

Form. Code	Composition (OA:AZT)	Vesicle(nm)	Entrapment efficiency (%)	Zeta potential(mV)	Turbidity (N.T.U)
OA-1	9:1	126 ± 12	37.6 ± 2.3	-1.8 ± 0.3	102 ± 10
OA -2	8:2	133 ± 13	47.6 ± 1.5	-12.8 ± 0.4	112 ± 13
OA -3	7:3	146 ± 11	55.6 ± 2.8	-2.8 ± 0.8	132 ± 14
OA -4	6:4	129 ± 15	39.6 ± 3.5	-9.8 ± 0.6	92 ± 10
OA-5	5:5	106 ± 9	24.6 ± 4.5	-11.8 ± 0.9	72 ± 10

Table 3. Permeation profile (Across the Rat Skin) of Different Formulations of AZT (after 12 hr)

Form. code	J*ss ($mg/cm^2/hr$)	LT^{2}* (h))	ER^{3}*
OA-1	15.2 ± 0.8	3.6 ±0 .2	7.6
OA -2	44.2 ± 0.8	2.1 ± 0.3	6.6
OA -3	56.2 ± 0.9	1.6 ± 0.1	17.2
OA -4	35.2 ± 0.8	2.5±0.3	12.6
OA-5	25.2 ± 0.7	3.6 ±.0 9	11.8
LIPO	15.2 ± 0.5	4.5±0.3	12.4
PD	4.2 ± 0.8	12.5±0.3	-

Sharma *et al.*[32] also investigated fatty acid vesicles for the transdermal delivery of zidovudine. The vesicles possessed higher entrapment efficiency (54.11%) with optimum vesicle size and homogeneity in regard to size distribution (PDI = 0.234 ± 0.016) at 7:3 oleic acid-to-AZT ratio. In vitro drug release study suggested sustained release of drug from the vesicles. The vesicles were fairly stable at refrigerated conditions. Vesicle-skin interaction study showed that oleic acid vesicles influenced the ultra structure of stratum corneum. Distinct regions with lamellar stacks derived from vesicles were observed in intercellular spaces of the stratum corneum. These stacks disrupted the organization of skin bi-layers leading to increased skin permeability, whereas no changes were observed in the underlying viable epidermis and dermis. Ex-vivo skin permeation and Confocal microscopic studies suggested that oleic acid vesicles penetrate the stratum corneum and retain the drug accumulated in the epidermal part of the skin. The results of the present study demonstrated that drug entrapped in oleic acid vesicles increased the transdermal flux, prolonged the release, and represented an attractive strategy for sustained delivery of AZT.

Entrapment in oleic acid vesicle has also been found to be suitable for reduction of drug leakage and hemolytic toxicity.

Foziyah *et al.*[33] developed fatty acid vesicles for the topical delivery of fluconazole. Vesicles were prepared by film hydration method using oleic acid as a fatty acid principal component. Developed vesicles were characterized for size, size distribution, shape, in vitro release, pH dependent and storage stability, skin irritation study, and ex-vivo skin permeation. Penetration behaviour of vesicles was further evaluated and elucidated using confocal microscopic study. Optical microscopy and TEM studies confirmed vesicular dispersion of fatty acid. The vesicles possessed higher entrapment efficiency (44.11%) with optimum vesicle size and homogeneity in regard to size distribution (PDI = 0.234 ± 0.016) at 7:3 oleic acid-to-fluconazole ratio. In vitro drug release study suggested sustained release of drug from the vesicles. The kinetics release pattern followed Higuchi model. The vesicles were fairly stable at refrigerated conditions. Ex-vivo skin permeation and confocal microscopic studies suggested that oleic acid vesicles penetrate the stratum corneum and retain the drug accumulated in the epidermal part of the skin. On the basis of sustained release behaviour and skin retention it can be inferred that oleic acid vesicles can serve as a potential carrier for the topical localized delivery of bioactives[33.]

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