Review Article

Natural and multifunctional colloidal carriers: A new prospective in drug delivery

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ABSTRACT

Oleosomes are oil comprising, intracellular, microcarriers of herbal origin which is constituted of oleosin proteins embedded in a monolayer of phospholipid and an internal core containing triacylglycerol (TAGs) that imitate their noteworthy benefits. These oil carriers are gaining momentum and receiving ample consideration in the arena of formulation development owing to their simple yet robust structure and their amenability to the biological system. They could be exploited to the development of proficient and explicit dosage forms with fewer ingredients and to deliver the specific drugs to the ultimate target. Their oleosin proteins are amphiphilic in nature and get self-emulsified even at very low temperature and hence lead to fewer ingredient such as emulsifiers and co-emulsifier into the system. Consequently, it offers emoliency, occlusivity, film forming action to the skin. They have ability to, load ingredients and to sustain/control their release for longer duration and hence may helpful to deliver drug, vitamin, fragrance, and many more through topical route. A review of patents revealed their massive applications in the development of variety of cosmetics, food products, personal care products, pharmaceutical products, industrial products, etc. Therefore, employing oleosomes in various formulation development procedures could be an applicable and cost-effective alternative to the synthetic ingredient based and controlled release formulations. The present review provides information about the nature and basic characteristics of oleosomes and focuses mainly on the key aspects of development of an effective formulation of natural origin.

Keywords: Drug delivery, drug loading, natural carriers, oleosomes, products

INTRODUCTION

Miniature oleosomes are unique type of native structures which are collectively called as lipid bodies, spherosomes, and oil-containing microspheres. These are basically intracellular store house of plant oils.[1,2] In other words, these are natural storehouses of energy used by the seeds until germination. A membrane of phospholipid embedded with oleosin proteins provides strength and protection to these oil bodies against physical and chemical exposures *i.e.*, from temperature fluctuation and in the presence of oxidative reagents. Oleosomes are remarkably stable in cells because of the electronegative repulsion provided by oleosins proteins on their surface.[2-4]

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Oleosomes are found in all oil-bearing seeds and have been isolated from soybean,[3,5,6] onion, cabbage, cottonseed,[7] sunflower,[8,9] almond,[10] maize germ,[11] etc. Currently, employed method to extract oleosomes involved hydrating intact seeds or cotyledons, grinding, filtration, and centrifugation. These stepts result into intact oleosomes with <45% yields of the total oil. Major contributing factors behind low yield of oleosomes extraction are inappropriate treatment which ruptures the cellular structure and oleosomes membranes up to some extent. Therefore, it is preferred to develop more efficient extraction/isolation procedures.[12] Investigation of the use of enzymes to increase oil body extractability is in trend. Kapchie *et al.*[12] reported

enzyme-assisted extraction procedure as a best yielding method after comparing conventional method of extraction of oleosomes involving hydrating, grinding, filtering, and centrifuging and enzyme-assisted extraction with some modifications in the process that would enable the release of oleosomes from soybeans.[1]

In recent decades, oleosome-based dosage form is attracting the interest of various researchers because they seem to be most promising systems as oil phase owing to their unique potentials such as their self-emulsifying nature, emolient, occlusive, and anti-oxidant properties. The presence of phospholipids and proteins together in their membrane which provides strength to the oil bodies are believed to prevent oleosomes from coalescing in to much larger oil droplets.[2,4,10,13] These capabilities are lost during commercial extraction of the oil from seeds because of removal of proteins and phospholipids along with cellulosic materials when the oleosomes are crushed. Therefore, taking special care is recommended to recover these miniature particles.[13] Apart from membrane stabilizing nature, oleosin proteins are found to show amphiphilic surfactants such as characteristics, namely self-emulsifying in nature. They provide totally different sets of benefits when used in a cosmetic application,[2,4,10] for example, their excellent cold process emulsification characteristic. Their utilization as a novel carrier systems for drug delivery may lead to sustain and control the release of the content for longer duration. Release of oil from oleosomes as well as any active ingredients loaded within it are the key features of their application in an emerging new categories of topical formulations.[2] Therefore, exclusive structure and natural origin of these multifunctional, unique and tiny carrier systems could be used to develop variety of cosmetics, food, personal care, pharmaceutical, and industrial products.

The current trend of investigations related with oleosomes is limited to their isolation only whereas their applications as self-emulsifier, emollient, novel carrier system, etc., have not yet been deliberated fruitfully. Till date, some of the researchers have reported their self-emulsifying nature[5,6,14-18] which reveals their utility to use in the development of formulations such as emulsions and ointment. As a natural emulsifier, it is a broad area of worth investigation because these systems can replace chemically derived emulsifiers for ease and better efficacy. Their biodegradable nature offers a number of advantages over non-biodegradable mineral oil-based products which are leading in the market and in direct contest.[13] The present review focuses mainly on the key formulations aspects of oleosomes, provides information about its nature and basic characteristics for the development of effective topical formulations of natural origin and considers them as all in one ingredient for future applications. Consequently, their loading capability, prolonged release, and targeting capability are also explored which imitate the optimum utilization of this technology for maximum efficacy.

ISOLATION, STRUCTURE, AND COMPOSITION OF OLEOSOMES

The oil bodies could be isolated from any part of the plant namely stems, roots, or seeds, etc. The conventional steps for the isolation of oil bodies involve hydration, filtration, cell lysis, and centrifugation.[12] The oil bodies could be obtained by employing alkaline,[19-21] conventional,[22] or enzyme-assisted[12] extraction method (as summarized in Figure 1) followed by respective recovery methods namely acid coagulation,[11,20] detergent washing,[21] enzyme assisted,[12] repeated washing with alkaline sucrose solution,[11] and aqueous flotation centrifugation method.[23]

Oleosomes are spherical-shaped micelle-like structures which have size in the range of 0.2–10 μm.[5,6] Oleosomes consist of a phospholipid membrane embedded with proteins (oleosin) and an oil core (mainly constituted by TAGs) [Figure 2]. The oil contributed up to 99%, whereas the membrane constituted only 1% of the total oleosome unit. Proteomics analysis revealed the presence of three types of membrane-proteins such as oleosins, caleosins, and steroleosins. Proportion of individual protein is plant specific.[11,24] Among all types of proteins, oleosin is the most abundant one and usually termed as structural proteins.[25-27] The oleosin protein (molecular weight of between 15 and 26 kDa)[5,6] has an exclusive three-domain structure with amphipathic/charged N- and C-termini exposed on the outer surface and a central hydrophobic domain inserted into the phospholipid membrane[28] which serves as an anchor and the hydrophilic portion resides on the surface of the oleosomes. Therefore, oleosin proteins are also reported to serves as size regulator of oleosomes which strengthen the membrane of the oleosomes.[27] Oleosins are mainly expressed during seed development and maturation which stabilize the structure of the oil bodies. The amphiphilic nature of the protein coat gives the oleosomes their unique self-emulsifying/emulsifying capability. Phospholipid membrane is the main protective monolayer which serves to hold the oil (TAGs molecules) inside. TAGs are mainly constituted by glycerol moiety in which each of hydroxyl group is esterified to fatty acids[2,4-6] which usually determines the physical and chemical nature of the plant oil. The number of double bonds in the fatty acids chain (degree of unsaturation) affects the melting point of the oil while the chain length of the fatty acids influences viscosity, lubricity, and solubility. Tocopherol is a known anti-oxidant and occurs naturally within the oil part of oleosomes of some plants where its function is to prevent the rancidization of the unsaturated triglycerides stored within the natural microsphere.[4,18]

CHARACTERISTICS OF OLEOSOMES

Oleosomes are almost colorless and odorless oil carriers. These naturally occurring oil carriers[2,13] shows emollient action (due to the presence of TAGs,[5,6] self-emulsifying characteristic (due to the presence of amphiphilic protein), and antioxidant action (due to the presence of Tocopherol *i.e.* Vitamin E).[4-6,18] Their small diameter range (from 0.2 to 10.0 μm) is very comparable to novel drug delivery systems such as nanocarriers and they also have the ability to load other oil phase actives, such as fragrances, vitamins, and ultraviolet (UV) filter as novel drug delivery systems do for better cosmetic efficacies. Owing to their specific oleosin protein and phospholipid membrane, these particles are able to modify release of the loaded matters which could be very promising to deliver drug in sustained manner, even in the absence of nanotechnology approaches.[2,5,6] Marcoux *et al.* 2004 have reported an oil-in-water emulsion which had been designed using oleosomes as a carrier for sphingolipid and compared its efficacy with a classical water-in-oil emulsion in investigator-blind randomized way and resulted that, oleosomes emulsion was enabling penetration of lipophilic compounds to the epidermis through the sub-cutaneous route and thus able to improve dry skin condition.[29] In addition, oleosomes could also provide superior

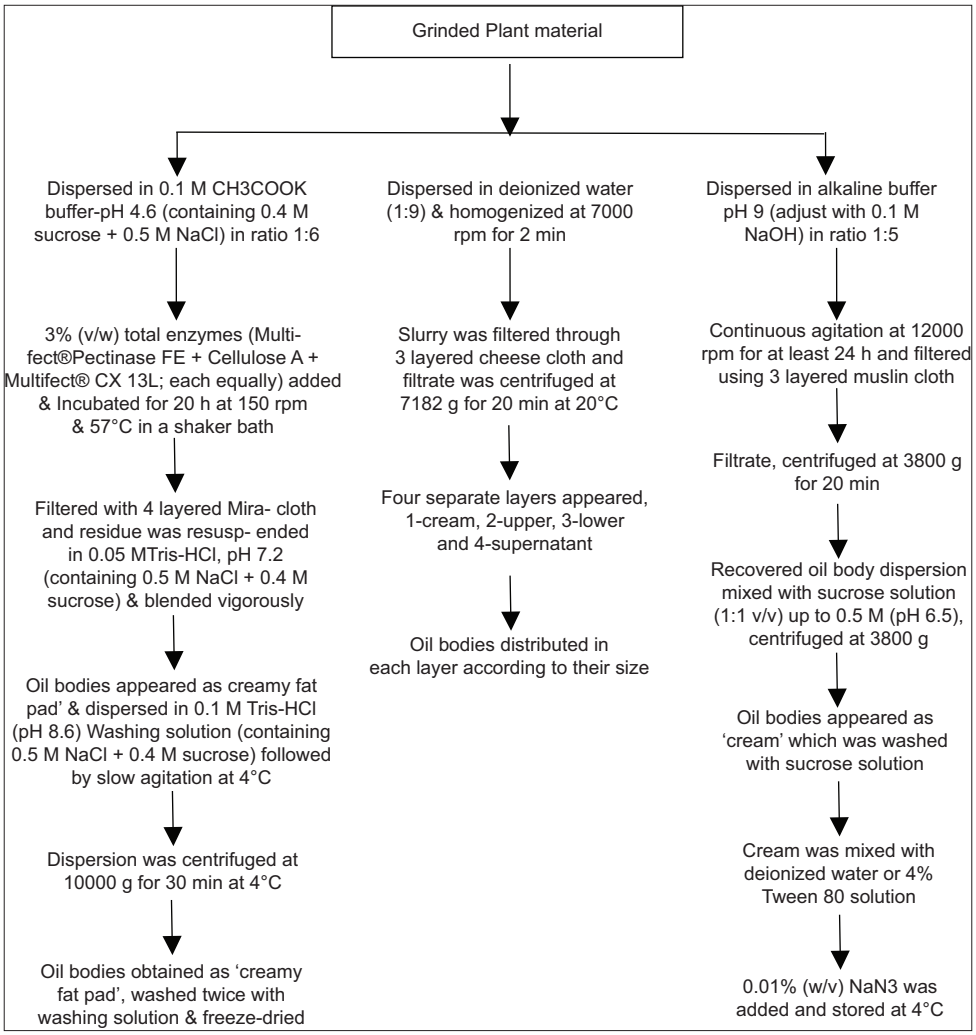

Figure 1: Summary of oleosomes (oil body) extraction methods. (a) enzyme-assisted extraction method (b) conventional extraction method (c) extensive alkaline extraction method

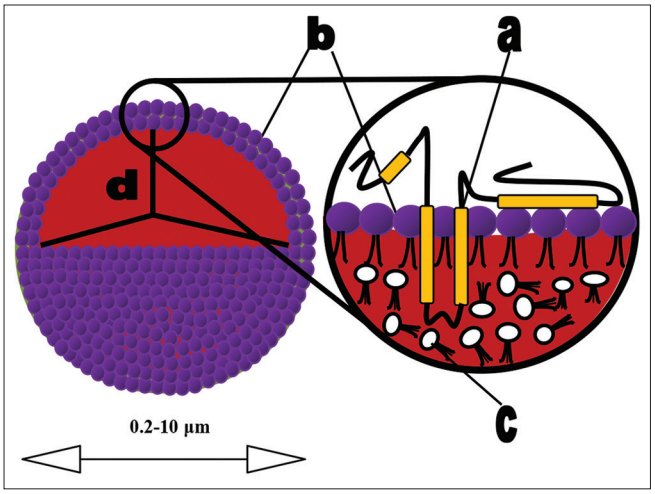

Figure 2: Pictorial representation of oleosome and its morphology

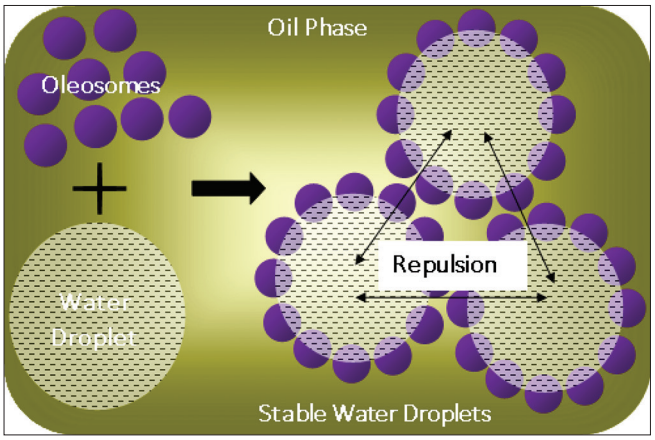

Figure 3: Graphical representation of generalized mechanism of emulsification by means of oleosomes used as an emulsifier

route to cold process formulation with all the benefits of low energy costs, reductions in inventory, increased manufacturing throughput, and could very suitable to heal labile ingredient/substances.[4,5,18]

The oleosomes could be characterized for their macro- and microscopic properties, using physical and chemical analysis. Some of them are discuss here: Proximate analysis is carried out to determine the total oil content present in oleosomes using Goldfisch apparatus followed by its quantification

Table 1: Oleosomes versus chemical emulsifier

Parameters	Oleosomes	Other emulsifiers	Refs.
Source	Natural, chemical free	Synthetic/synthetically derived, petrolatum	
Appearance	Tiny, spherical shaped, 0.2–10.0 μm in diameter, similar to liposomes and niosomes	Opaque yellow gel	[4]
Melting point	40–75°C	40–60°C	
Odor	Very light	None	
Release	After surface modification, they can be able to sustain the release of the medicament loaded into it	Surface modification cannot be possible so they are not able to sustain the release.	[2,40]
Properties	Stabilize emulsion Emollient (triacylglycerol core) Occlusive and anti-oxidant effects (Vitamin E) Can load fragrance/vitamins/retinol inside it	Provide stable emulsion with no or very less emollient and occlusive properties	[40]
Operational hydrophilic-lipophilic balance range	5–15, Requires no additional emulsifiers	Narrow hydrophilic-lipophilic balance value, so 2-3 different ones are needed leads to higher inventory cost	
Emulsification procedure	Help in cold process emulsification, suitable for heat labile substances, helps reduce process time	Requires co-emulsifier and heating for 2–3 h for proper emulsification, not suitable for heat sensitive materials	[7]
Stability	Unstable in the presence of monohydric alcohols, so they are used with poly-hydric alcohols stable in pH range of 3.5–5 and 6–8, glycerin (10%) and at low shear rate	Stable with all types of alcohols	[38]
Uses and applications	Food, cosmetics, personal care, pharmaceutical, and industrial products	Limited use in food products, some cosmetics and personal care products	[7]
Intrinsic water resistance	They stop emulsifying when they collapse on the skin, leaving the pigment on the skin for maximum water-resistance	Waterproofing polymers are required	[39]

through UV-VIS spectrophotometer.[1] Transmission electron microscopy is used to compare the isolated intact oleosomes to those *in situ* and to observe ultrastructure of residual material, initial plant material, oleosomes extracts and the remaining cell residual material.[1] Gas chromatography-flame ionization detector (GC-FID) and Fourier transform infra-red spectroscopy could be used to quantify the fatty acid content present in oleosomes and to study the difference in spectra of free oil and encapsulated oil (oleosomes), respectively.[30] UV-VIS spectrophotometer could be used determine the concentration of phenolics, cartenoids, and chlorophyll.[31] Light microscope is used to cheque the purity of oil bodies after staining the sample by Sudan Red 111.[9,31] Zeta-sizer system determines the size of oleosomes by first measuring the Brownian motion of the particles in emulsion/suspension using dynamic light scattering. Zeta potential analyzer is used to measure the surface charge of oleosomes. Biuret test could be carried out to analyze the presence of protein (oleosins, caleosins, and steroleosins) in oleosomes by detecting the number of peptide bonds present inside them. Confocal laser scanning microscopy could be used to elucidate the loading of drugs/actives inside oleosomes unit by utilizing florescence tagging approach.[32,33] High-performance liquid chromatography or GC-FID could be used to estimate the total amount of Tocopherol present in oleosomes.[30] Brewster angle microscopy (BAM) could be performed to unveil their adsorption, rupture, and structural changes over time at different subphase conditions (pH, ionic strength). These investigations are important in food applications but are also useful for the understanding of the general behavior of proteins and phospholipids at interfaces.[6] Some oleosins contain lipophilic allergens (IgE-binding allergens) present in peanut, hazelnut, and sesame oil bodies.[34]

STABILITY ISSUES AND STORAGE CONDITIONS FOR OLEOSOMES

Stability of oleosomes depends on phospholipids-protein membrane chiefly which protects the oil core from very stressful physical and chemical environmental conditions such as moisture, temperature, and pressure.[11,24,25] Oleosomes are found to be stable at the temperature range of 40–75°C but higher stirring rate and temperature fluctuations as occur in the case of formulation development (heating and cooling steps, or the addition of chemical reagent), can destroy their integrity. For practical applications, oleosomes preferably does not undergo desirable physicochemical changes in various conditions but during prolonged storage oleosomes may undergo some morphological changes due to rancidity and oxidation of oil stored inside them.[2,4] Nikiforidis *et al.* 2012 have investigated the stability of emulsions against oxidation after employing oleosomes and chemically modified surfactants separately and reported that emulsion stabilized by oleosomes showed noteworthy reduction in the hydroperoxide production as compared to such emulsions stabilized by chemically modified surfactants. Furthermore, the physical and chemical stability (during aggregation and coalescence) of oleosomes dispersion systems is primarily depends on the applied methods which were used to recover the oleosomes, presence or absence of the exogenous proteins, and their pH value.[11] Solvent systems could modify the integrity of the oleosomes. In the presence of monohydric alcohols (such as ethanol and propanol) oleosomes structure get weekend which could be results in to leakage of the oil from them. This nature of the oleosomes could result in to its destabilization in the formulations containing monohydric alcohols. Multihydric alcohols with two or more hydroxyl group such as non-aromatic diols, triols, and polyols is recommended with these oil bodies. Oleosomes formulations containing glycerol (polyhydric alcohol) could be more promising as far as their stability is concerned.[35] As reported by Guth *et al.* 2010, these systems are heat stable in the temperature range of 55–60°C over the period of 60 days, unaffected by nonionic and weakly anionic emulsifiers/surfactants and are compatible with most of the ingredients found in the formulator's repertoire.

Kapchie *et al.* in 2013 were determine how the oxidative stability of oil in oleosomes is affected by the amount of transition metal ions,

i.e. ferric ion, under different environmental stresses, as measured by the formation of primary and secondary oxidation products, during 12 days of storage at 60°C. In addition, they also examined the influence of freezing, freeze-drying, and reconstitution and heating on the oxidative stability of oil in isolated oleosomes. They reported that freezing, freeze-drying or heating of oleosomes have an insignificant impact on the oxidative stability of oil in isolated soybean oleosomes, therefore, manufacturers should be cautious when adding oleosomes as ingredients in food systems containing transition metal ions.[36] Furthermore, these oil bodies are stable in the pH range of 3.5–5 and 6–8, at 5% concentration of ethanol and 10% glycerin as well as low shear rate.[4] To encapsulate positively charged (pH-4) oleosome, pectin (negatively charged) can be used.

ADVANTAGES OF OLEOSOMES

Oleosomes have certified by Natural and Organic Certifications from USDA and could be isolated/produced by a chemical free process. These oil bodies have several advantages over other chemically processed ingredients [Table 1]. The presence of an amphiphilic structural protein (oleosin) provide chance to develop a stable emulsions which could be used for various applications. Due to the presence of oil inside, these systems could be used as a part of oil phase for the formation of emulsion. Their additional properties such as emollient, occlusive, self-emulsifiers, and antioxidant could replace a variety of ingredients used to impart such properties. Topical application of oleosome products could results into breaking of some oil bodies on skin surface, whereas some may leads to stay intact for prolonged release of their contents which finally gets absorbed. Slow release of the content from the oleosomes can be due to their partitioning efficiency with sebum. It is quite possible to load oil soluble ingredients such as fragrance/vitamins (retinol) inside the intact oleosome to get their long term release on the skin by avoiding frequent use of the preparations.[4,7,13,37,38] They could also be useful to boost sun protection factor values in daily synthetic skin products and can reduce matte appearance/whitening effect of some ingredients as frequently occur in the case of emulsions produced using stearic acid, steryl alcohol, cetyl alcohol, etc.[32]

Oleosomes have many properties (anti-oxidant, moisturizing, anti-inflammatory, emulsifier, emollient, soothing, and anti-aging) to offer due to the nutrients present in them like Vitamin E, K, triglycerides, iso-flavons, oleic acid, linolenic acid, tocopherol, genistein, and Mn. All these nutrients are very much required to maintain the health of the skin. Once oleosomes come in the contact with the skin, they begin to dry out and release contents to stratum corneum of the skin. Oleosomes comprise the entire nonactive part of the oil phase in a typical cosmetic formulations which could also be used as active part and could impart some extra characteristics as motioned bellow:

Their wide range of hydrophilic-lipophilic balance (HLB) from 5 to 15 allow the formulation scientist to replace most of the other emulsifiers including PEGs, Polysorbates (and many more which could not be possible in the case of chemically modified synthetic emulsifiers which have a very narrow HLB range and require a combination of emulsifiers to stabilize emulsions). Owing to their biodegradable nature, they could provide safe and effective formulations over the products developed incorporating chemically modified ingredients. Their formulations provided the maximal moisture retention capability and do not damage the skin while traditional emulsifiers do not retain moisture up to such extent and irritates the skin. They are found compatible with all surfactants except sodium lauryl sulfate (SLS) (which could be a point of consideration as far as development

Table 2: Reported investigations on oleosomes stabilized emulsions, composition and their role as a natural emulsifier

Objectives	Material/method used	Major outcomes	Author	Year	Refs.
Physical and oxidative stability	Aqueous extraction	Heat treatment (90°C, 30 min) to the prepared oil body suspensions after extraction improved storage stability whereas oxidative stability could not be affected	Chen *et al.*	2012	[15]
Stabilization of soya oleosomes emulsions	ι-carrageenan coating	Particle electrical charge, particle size distribution, creaming index have revealed that i-carrageenan coated oleosomes emulsions with improved stability than uncoated oleosomes emulsions	Wu *et al.*	2012	[16]
Self-emulsifying behavior of soy oleosomes	Surface pressure and Brewster angle microscopy	The study revealed behavior air–water interface of trypsin digested oleosomes and reported its emulsifying capability for the formation of a stable emulsions	Waschatko *et al.*	2012	[7]
Oleosomes Behavior at the Air−Water Interface	Brewster angle microscopy	Combination of surface pressure measurements and simultaneous Brewster angle microscopy revealed the oleosome behavior at the air−water interface which is a crucial factor in determining the driving forces in their surface active behavior and the key to using this in practical applications, e.g., food processing	Waschatko *et al.*	2012	[45]
Stabilization of soybean oil body emulsions	κ, ι, λ-carrageenan coating	Particle electrical charge, particle size distribution, creaming stability index revealed that at pH 7 emulsions stabilized with ι-carrageenan were found to be more stable to creaming stability index than stabilized with κ or λ-carrageenan due to depletion flocculation.	Wu *et al.*	2011	[46]
Isolation and study of their associated phytochemicals	Method of Tzen and others (1997)	Among all isoforms of tocopherol, δ-tocopherol was found to be greatly associated with soybeans oleosomes whereas isoflavones do not show a remarkable physical association.	Fisk and Gray	2011	[47]
To determine fatty acid composition	Gas chromatographic analysis	Nodules and seed oleosomes of *Sesbania rostrata* indicate the presence of unsaturated fatty acids ($C_{18:1}$ and $C_{18:2}$).	Denduluri and Bal	1995	[48]

of a formulation of natural origin is concerned, so that they have been used successfully in a number of cleansers by natural players). Due to their small particle size they have ability to get absorbed inside the dermis of the skin. They have very good film forming ability also. Developing emulsion using oleosomes has the ability to hold and stabilize other oil soluble ingredients in the existing system and able to prepare finished formulations with low shear mixing at low temperature. When Oleosomes collapse on the skin, they lose their self-emulsifying ability. When the topical formulation is applied to the skin, an emulsifier free film with the pigments is left, which cannot be easily removed by water and act as a waterproof film on skin. An emulsion containing oleosomes could possibly be formulated by a low temperature emulsification method (an energy saving procedure). This procedure results in a rapid and safe production in order to minimize the decomposition of heat sensitive materials, *i.e.* <30 min (which is 3–4 h for hot process). Less costly mixing kettles and few ingredients (emulsifiers along with co-emulsifiers) are required to accomplish the procedure. Using fewer ingredients may be a good practice for development of emulsions which may lead to reduced skin irritation potential of the product along with reduced inventory costs.

MECHANISM OF STABILIZATION OF EMULSION BY MEANS OF OLEOSOMES

Principle mechanisms involved in the formulation of emulsions are surface tension theory, repulsion theory (emulsifying agent creates film barrier over a droplet that separate it from getting collapsed with other) and viscosity modification (using emulgents like acacia and tragacanth).[17] It is reported that oleosomes have ability to act as self-emulsifier and work on repulsion theory owing to the existence of mutual effect of hydrophobic/hydrophilic part of oleosin protein.[40,41] Therefore, repulsion theory is the mechanism of interest and the rest of the others are out of scope of this review [Figure 3]. To meet the terms Waschatko *et al.* 2012[5,6] has marked the possible scenario of oleosin arrangement at air-water interface. Oleosomes are investigated at the air−water interface of a film balance. This procedure allowed to study the stability and the behavior of their three different constituents, i.e. the oleosins, the phospholipids and the TAGs. This method offered two basic types of measurements. First, kinetics are recorded, which showed the instability and the "destruction" of the oleosomes at the air−water interface and second, the behavior of the oleosomes and their constituents under two-dimensional pressure is studied. They affirmed that after injecting oleosomes into the aqueous sub phase, they diffused immediately to the air−water interface due to their amphiphilic nature and buoyancy which results in to increase in surface pressure. At this stage, oleosomes are presumably intact. At lower concentrations of oleosomes (≤0.4 mg/L) the surface pressure increase sharply, whereas at high concentrations (≥0.8 mg/L) a constant rise in surface pressure with time is observed. Depending on the amount of oleosomes at the surface, bursting of the round structures occurs within different time scales. This rupture resulted in an additional increase in surface pressure and a decreasing amount of oleosomes visible by BAM. This rupture was associated with the oil bodies packing at the interface. Depending on their concentration and charge state on the surface, oleosins can aggregate or assemble in different conformations and arrangements with the surrounding lipids on the surface. The strong hydrophobicity suggests an aggregation to micelles, which would be stabilized in the sub phase by the two hydrophilic tails of each oleosin sticking in hairpin-like structure (N terminal of one oleosin with C terminal of other) with the hydrophilic parts remaining outside the oleosomes.[42] This decrease in surface pressure was seen after rupturing oleosomes which can be explained by the aggregation and descent of free oleosins and the subsequent formation of domains of free TAG and phospholipids. The charges on phospholipids cause repulsion between oleosomes and hence reduce flocculation and oleosin works synergistically with phospholipids to reduce oleosome coalescence. In concluding remarks it is clear that these micelles like structures are stable which could easily be suspended in oil sub-phase in order to get a stable emulsion. Emulsion formed in such way does not require further addition of other chemical emulsifiers.[5] This method have several advantages like low shear stresses, lower degree of required energy, uniform droplet size and ease in designing without using large amount of surfactants. These properties allow the formulator to prepare emulsions over a range of cosmetic applications simply by adding whatever oil or water phase ingredients are desired to the existing emulsion and then adding sufficient thickener to stabilize the finished product.[43] Works done so far on oleosomes composition, their stabilization and role as a natural emulsifier is listed in the Table 2. The uncoated emulsion has a stability effect on addition of Nacl which depends on the pH environment. The uncoated emulsions are stable on addition of Nacl (0–150 mM, pH-3) and incase of 50 mM of Nacl the aggregation occurs at a pH of 7. If oil bodies are coated with i-carrageenan then in both pH (3 and 7) and in both Nacl concentration (50–150 mM) remains stable. If we use cryoprotectant like sucrose, lactose that will prevent the leakage of oil from core of oleosome.[15] Except SLS, different polymeric surfactants are used as steric stabilizers for o/w emulsion and even silicone surfactants also play a role for the stabilization of oleosome.[40] To improve stability of the formulation lyophilisation can be performed and use of lyo-protectant to suppress charge fusion.[44]

FACTS ABOUT FORMULATION OF OLEOSOMES EMULSIONS

Oleosomes are preferably used in the concentration range of 2–15 weight percent based on the total weight of cosmetic or topical dermatological product. If oleosomes are used as a primary emulsifier then incorporation of thickeners is required to ensure the optimum viscosity (over 9000 cps [spindle 2]) and stability of the finished dosage form. Stirring speed should limit to <500 rpm while adding oil phase ingredients to concentrate Oleosomes. After addition of entire water phase the homogenization speed limit could be taken up to 3000 rpm. Temperatures up to 55°C are acceptable for all oleosomes formulations. Oleosomes are freeze/thaw stable in most formulations; however, the raw material should not be frozen. Oleosomes emulsion can be formulated in the range of pH 3.5–9. Only polyhydric alcohols with two or more hydroxyl groups such as non-aromatic diols, triols and polyols is recommended to use to stabilize the structure. Most surfactant systems are compatible with oleosomes except SLS. Most preservatives are compatible, however; some of them should be avoided such as protein cross-linkers e.g. DMDM hydantoin. Oleosomes have a wide

operational HLB range of 5–15 unlike chemical emulsifier HLB which have a very narrow range. In case of a native oleosome a 9 nm film as a shell protects the oil core which may tolerate up to 90°C.[2,4,18,23,40]

APPLICATION OF OLEOSOMES EMULSION

Oleosomes were launched in to the cosmetics world several years ago with many early adopters taking advantage of their natural source, sustainability, moisturizing benefits and emulsification capabilities. In recent years, oleosomes application in product developments have been researched, demonstrated and introduced immensely for example, loading/release characteristics of oleosomes containing preparations such as sunscreens have recently been fully explored and documented. Oleosomes are known to be useful in cosmetics and other emulsion applications[1,2,4,49] of particular current interest and practical application is the development and introduction to the cosmetics market of a new generation of more robust, "stabilized" oleosomes. Stabilization allows the oleosomes to be used in a wider range of more challenging formulation matrices, including high label of alcohol.[7,10,13,29,45,49] Magnetic oleosomes with anticancer drugs are used to treat breast cancer. A green fluorescent protein LG is fused with oleosin and an specific antibody is fixed with protein to the target surface. The oleosome is composed of magnetic zinc-doped iron oxide particle. Cancer cells are treated with magnetic drug loaded oil bodies under magnetic field to interact between affected cell surface and functionalized oleosome.[50] Oleosomes emulsions may use to formulate ice-creams, milkshakes, or other food grade material with improved degree of freezing properties on account of their special characteristics like inhibiting/preventing ice crystal growth. Their film forming characteristic is used to formulate an emulsion system which could be developed for the controlled and sustained release of medicament for longer duration and their duration of release will depend upon the degree of thickness and dryness. An emulsion system formulated by employing oleosomes may also be used to develop spray and aerosol. Stable emulsion formulated using oleosomes could be able to withstand higher pressure as in the case of spray and aerosol containers. Han *et al.* 2012 stated in their investigation that higher degree of oleosome stability warrants their application as a useful scaffold for protein immobilization and display.[45]Waschatko *et al.* 2012 have reported that oleosins protein can be cleaved by trypsin enzyme therefore they can be safely used in food industries.[7] Several food and feed products such as nondairy cheese, yoghurt, vinaigrettes, candy, salad dressing, baking product, flavor carriers, texturing agent, chewing gum and many more could be formulated utilizing the concept of oleosomes stabilized emulsion. Some personal care products like soap, cosmetic, skin creams, facial cream, tooth paste, lipstick perfumes, makeup, foundation, sunscreen lotion, hair conditioner and hair coloring could also be develop using the concept of stable oleosomes emulsion system. Pharmaceutically it can be very useful in the combination with a variety of active pharmaceutical ingredients which could be either peptides/proteins or could be some synthetic molecules with therapeutic and diagnostic values. From the industrial point of view paint, coating, lubricants, films, gels, road construction materials, ink, dyes, waxes and many more could also be developed. To improve the degradation of organophosphate nerve agents, organophosphorus hydrolase is genetically fused with oleosin protein to to make oleosome based biocatalyst which enhanced 5 fold degradation of nerve agents.[51]

Novel approaches of oleosomes in drug delivery: Based on structure and composition, Oleosomes are reflected as an emergent novel carrier for hydrophobic drug delivery particularly at specific site. These natural carrier are very efficacious and safe for drug delivery owing to its biodegradable nature and are able to encapsulate pharmaceutically important oil soluble active (traditionally unstable) ingredients and avoid contact with one another (which change the native structure of actives *i.e.* harmful upon contact). These are also able to load fragrance/vitamins inside it for delayed release on the skin. After application of these finished products to the skin or ingestion in to the body, disruption of oleosomes structure occurs with the time. Use of a release modifier can modulate the release of an API loaded inside the oleosomes by controlling water evaporation from its surface.[5,24] Release controlling agents can alter the boiling point of water and hence affect the rate of evaporation. Monohydric alcohols like methyl, ethyl and isopropyl alcohol increase the vapor pressure, thereby speeding the release, whereas polyhydric alcohols such as glycerin, ethylene glycol and propylene glycol decrease vapor pressure and results in to retardation of release.[2,13,49,52]

Polymeric nanoparticles and lipid based liposomal systems are particularly used to target many drugs that potentially improve the pharmacokinetic properties and therapeutic indices of the drugs. In spite of encouraging results these systems are often associated with some complications such as the need of optimization of many biophysicochemical parameters for drug formulations, particularly for hydrophobic drugs. Low bioavailability and high local concentration of drugs at the site of the aggregate deposition, hydrolytic instability and hence adverse drug interaction are some other associated problems. Therefore, the major key issues were how to target/deliver hydrophobic agents via oral or intravenous administration effectively. Recently Chiang *et al.* 2011[53] have addressed the above issue by developing nano-scale oil bodies (as a potential drug delivery carrier) for targeted delivery of a hydrophobic drug *i.e.* camptothecin to HER2/neupositive tumor cells. In particular, these oil bodies could be functionalized simply by fusion of any bioactive motifs of interest with oleosin protein. Oleosin protein also confers on oil bodies a negative surface charge, thereby contributing to the negative zeta potential. This feature is helpful to prevent undesired interaction of oil bodies with non-target cells. These camptothecin loaded oil bodies were found to be stable and exhibited strong cytotoxic effect on HER2/neupositive tumor cells both *in vitro* and *in vivo*. Based on this finding authors are introducing the probable schematic diagram of targeting via oil bodies which is represented in Figure 4.

FUTURE SCOPES

Oleosomes could be an emerging option next to chemical emulsifiers which are being used today in pharmaceutical, nutraceutical and beverage industries at very large scale. As far as the use of oleosomes as potential emulsifier is concern their compatibility issues with the human digestive system must be explored before starting its use in food and nutraceutical industries because of allergic nature of the proteins. Digestive enzymes unable to digest a fragment of protein could result in an allergic reaction which triggers an immune response to that fragment of protein. Therefore, it is to be assured that the digestion of oleosin protein inside the body is possible or not. Their HLB value must be determined before

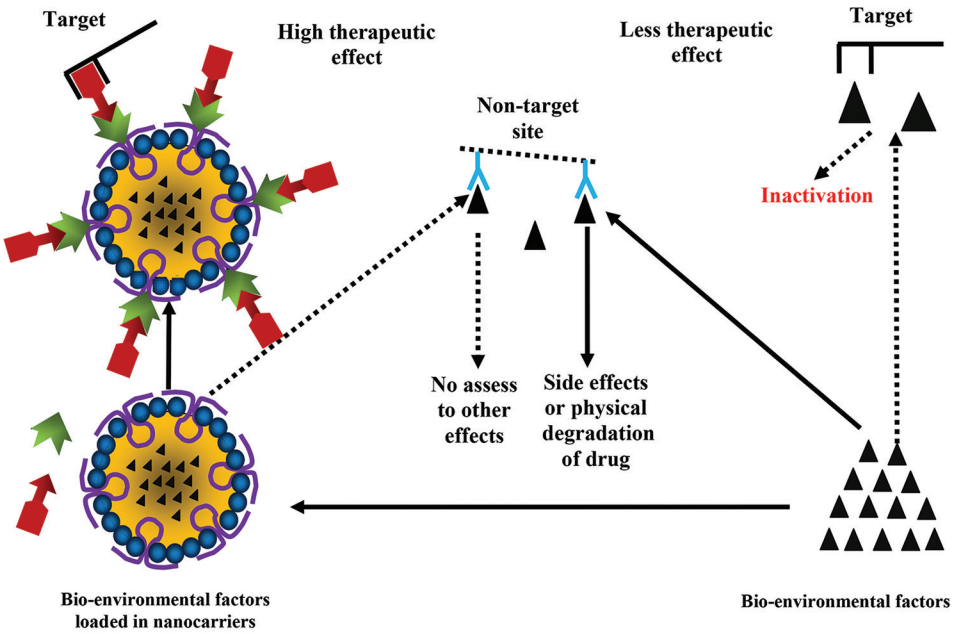

Figure 4: Probable mechanism of drug targeting by oleosomes

using them in the formulation of an emulsion therefore, their use as an emulsifier in oil phase could be more promising. Oleosomes are the natural and multifunctional carrier system having a large variety of advantages over different synthetic carrier in terms of their low toxicity, bio- degradable nature and drug loading ability in the core. In this lipoidal carrier, lipophilic drug vastly entrapped due to its peculiar structure. Oleosome carriers can play major role in the case of pharmaceutical and neutriceutrical delivery. Nowadays it is also used as a self-emulsifying agent. However, overcoming stability and leakage related limitations of these oil bodies could help them reach to the pharmaceutical industry. Oleosomes are nowadays playing a vital role in case of food industry and cosmeceuticals. The wide application of these carrier systems can open new vistas in the field of pharmaceutical product development.

CONCLUSION

Oil containing microspheres (oleosomes) is naturally occurring, multipurpose, delivery systems which could have the properties like emolliency, occlusivity, antioxidant effects and could be worked as a self-emulsifying systems due to the presence of oleosin protein and phospholipid containing which covers the oil core. These systems are called as oil containing microspheres due to their ability to load fragrance, vitamins, active medicament etc. Its self-emulsifying characteristics can lead to stabilize emulsions and could be used as an alternative to a variety of chemically modified emulsifiers. They are the best systems used to formulate an emulsion for topical purposes because single system is working as an emulsifier, providing occlusivity, emoliency at the application site along with its vitamin nourishment. One of the most important merits of this system is their ability to stabilize the emulsion even in a cold process, formulation which cost very low, reduced inventory and increased manufacturing output. Therefore, oil bodies could be very promising in the huge market of products employing chemically modified ingredients and could eliminate possible limitations/toxicity related to them by reducing their number.

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