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Note

Marinosomes®, marine lipid-based liposomes: physical characterization and potential application in cosmetics

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Abstract

Marinosomes® are liposomes based on a natural marine lipid extract containing a high polyunsaturated fatty acid (PUFA) ratio. They were prepared and characterized in conditions that mimic that of topical application in terms of pH, temperature and calcium. Marinosomes® were stable in storage conditions for 1 month. At low pH (pH 4) or in presence of high calcium concentrations (9 mM), complex structural rearrangements, such as aggregation and size reduction, occurred which were kinetically dependant.

Keywords: Liposome; Polyunsaturated fatty acid; Physical stability; pH; Calcium

For several years now, liposomes have proved to be good vehicles for cosmetic applications. They help to dissolve and formulate water-insoluble ingredients, can encapsulate water-soluble drugs and moisturized, and form a viscous support matrix (Lasic, 1993). In parallel, eicosapentaenoic acid (EPA, $20{:}5n-3$) and docosahexaenoic acid (DHA, $22{:}6n-3$), are two major polyunsaturated fatty acids (PUFA). They are not present in normal skin epidermis. However, they are metabolised by skin epidermal enzymes into anti-inflammatory and antiproliferative metabolites that are associated with a variety of benefits regarding inflammatory skin disorders (Ziboh et al., 2000). In this context, marinosomes®, i.e. liposomes made of a natural marine lipid extract, were envisaged for the prevention and treatment of skin diseases.

The natural lipid mixture was extracted from a marine organism and provided by Phosphotech (France). It mainly contained phosphatidylcholine (68 wt.%) and phosphatidylethanolamine (23 wt.%) (Nacka et al., 2001a). Fifty-six percent of total phospholipid fatty acids were PUFA among which EPA and DHA represented 30 and 59%, respectively. α-Tocopherol (Sigma) (5 wt.%) was added to the marine lipid extract to prevent PUFA oxidation (Nacka et al., 2001b). Marinosomes® were prepared in 10 mM HEPES (Sigma) buffer (pH 7.4), after a single extrusion

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through polycarbonate filters (5-μm pore size, Millipore) of a lipid suspension (20 mg ml^{-1}) (Nacka et al., 2001a). Structural characterization of the vesicle suspensions was performed using turbidity measurements (optical density (OD) at 400 nm, Lambda Bio 20 spectrophotometer, Perkin Elmer), granulometry measurements (Mastersizer 2000, Malvern) and microscopy techniques. Marinosomes® suspensions were directly observed using an optical microscopy setup composed of an Olympus CX 40 microscope equipped with a phase-contrast device, an $\times 40/0.65$ objective (Olympus) and a Sony DKC-CM30 video camera. Freeze-fracture transmission electron microscopy (TEM) was carried out as already described (Arnold et al., 2002). Platinum–carbon replicas were imaged using a Jeol 2000 FX TEM operating in conventional transmission mode at 200 kV.

Marinosomes® initial preparations examined by phase-contrast microscope consisted of a mixture of optically more or less contrasted spherical structures (Fig. 1). These latter corresponded to vesicles characterized by a variable number of lamellae. Indeed, freeze-fracture TEM mainly revealed the presence of oligolamellar and multilamellar liposomes (Fig. 2a,b). Optical and electronic microscopy observations suggested that the vesicle size largely varied. This was confirmed by granulometry measurements indicating that the filtration preparation method led to marinosomes® with diameters ranging from 300 nm to 20 μm. Turbidity is a simple method to test the morphological stability of liposomes. Turbidity measurements performed on marinosomes® sus-

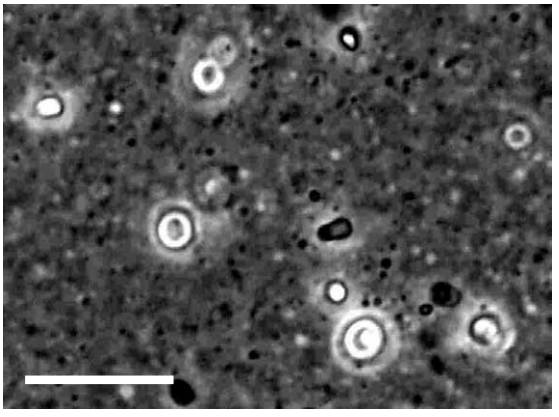

Fig. 1. Video image of marinosomes® observed by phase-contrast microscopy. Marinosomes® were prepared by filtration through polycarbonate filters (5-μm pore size) of a 20 mg ml^{-1} lipid suspension. The bar corresponds to 10 μm.

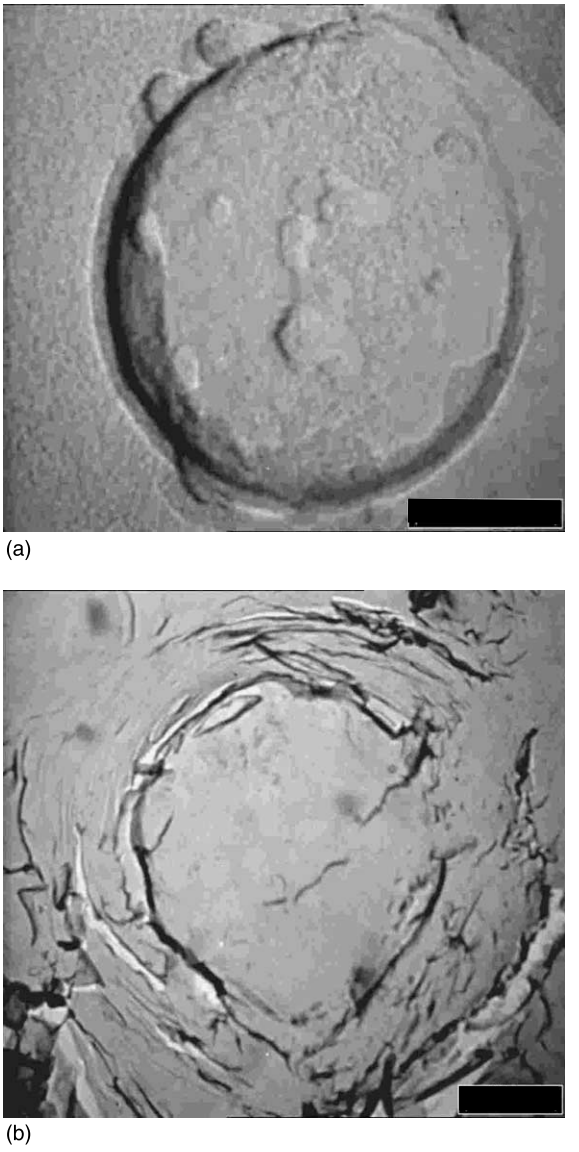

Fig. 2. Freeze-fracture TEM micrographs of marinosomes® obtained by filtration through polycarbonate filters (5-μm pore size): (a) a small oligolamellar liposome (the bar corresponds to 0.1 μm), (b) a multilamellar liposome (the bar corresponds to 2 μm).

pensions stored at room temperature for 40 days indicated that turbidity remained stable for one month (results not shown). Thus, physical liposome stability was ensured during this period of time. Then, a turbidity increase was observed corresponding to the appearance of large lipid structures with 15 μm mean diameter as measured by granulometry. Optical microscopy observations indicated the presence of aggregated vesicles. As a whole, marinosomes® physical stability was ensured for 20 days when the vesicles were stored at room temperature.

Marinosomes® physical stability was assessed in conditions that mimic skin parameters, i.e. low pH and high calcium concentrations. Fig. 3 shows marinosomes® turbidity variations when pH was decreased down to pH 4. For pH ranging from 7 to 4, suspension turbidity did not significantly vary just after medium acidification as well as after 3 h incubation (results not shown). However, optical microscopy observation of marinosomes® exposed to pH 4 evidenced complex structural rearrangements (Fig. 4). Indeed, aggregates consisting of four to five liposomes surrounded by small dense lipid structures were observed. The pictures obtained could be interpreted in terms of a liposome aggregation phenomenon occurring simultaneously with a vesicle size reduction. The

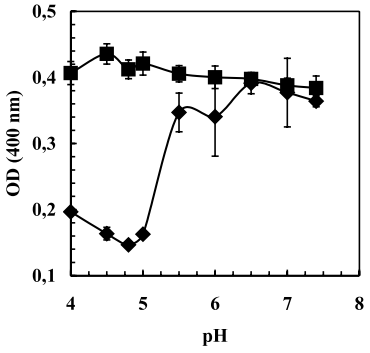

Fig. 3. Variations of optical density (OD) at 400 nm as a function of pH for marinosomes® suspensions just after medium acidification (–■–) and after 24-h incubation time (–◆–). Marinosomes® were prepared by the filtration technique using 5-μm pore diameter filters ([lip]tot = 0.2 mg ml^{-1}). Acidification was performed diluting marinosomes® suspensions with different acid solutions at the required pH. Storage temperature was 37 °C.

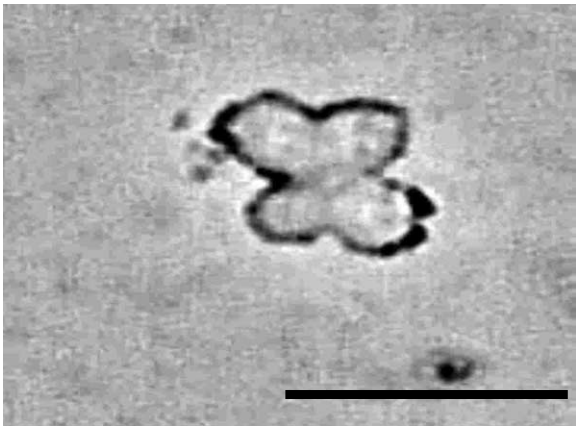

Fig. 4. Video image of marinosomes® observed by phase-contrast microscopy. Marinosomes® were prepared by filtration through polycarbonate filters (5-μm pore size) of a 20 mg ml^{-1} lipid suspension and incubated less than 1 h in acid medium at pH 4. The bar corresponds to 10 μm.

dense lipid structures would result from a water release from the liposome internal volume due to the variation in osmolarities on both sides of the liposome membrane. These two simultaneous phenomena (aggregation and vesicle size reduction) have opposite effects on turbidity variations and could explain that, in spite of the observed structural rearrangements, no turbidity variations were measured. After 24-h incubation of marinosomes® in acid conditions, turbidity dramatically decreased for pH lower than 6. No marinosomes® could be observed by optical microscopy. On the basis on the resolution power of the optical set-up used, the lipid structures formed upon acidification would have diameters lower than 0.4 μm. This result suggested that the size reduction that begun as soon as liposomes were placed in acid medium extended with time. It was shown that marine lipid-based liposomes could undergo various structural rearrangements depending on the temperature, the number of lamella and/or the membrane composition (Nacka et al., 2001a,b). The results obtained indicated that the structural rearrangements were also influenced by the medium ionic strength. Indeed, marinosomes® behavior upon pH variation was different whether the liposomes were prepared in NaCl free-medium (this work) or in a saline

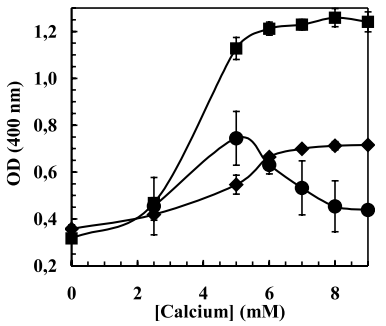

Fig. 5. Variations of optical density (OD) at 400 nm as a function of calcium concentration for marinosomes® suspensions just after $CaCl_2$ solution addition (–◆–); after 1-h incubation time (–■–) and after 27-h incubation time (–●–). Marinosomes® were prepared by the filtration technique using 5-μm pore diameter filters ([lip]tot = 0.2 mg ml^{-1}). Final calcium concentrations were obtained by dilution of marinosomes® suspensions with different $CaCl_2$ solutions at the required concentration. Storage temperature was 37 °C.

buffer (Nacka et al., 2001a,b). Turbidity variations of marinosomes® suspensions with respect to calcium concentrations are shown Fig. 5. Up to 6 mM, marinosomes® turbidity increased with increasing calcium concentrations, then it hold stable. This pattern was drastically amplified after 1-h incubation time. The turbidity increase was correlated with an aggregation phenomenon as visualized by optical microscopy (Fig. 6). This behavior agrees with the fact that phospholipid membranes could undergo aggregation and fusion phenomena through the formation of dehydrated intermembrane complexes with divalent cations and/or the existence of structural defects emerging on the boundaries between fluid and crystallized phospholipid domains (Duzgunes et al., 1981; Averbackh and Lobyshev, 2000). As incubation time was extended, turbidity values drastically decreased especially at high calcium concentrations. This behavior may be correlated with a vesicle size reduction because of water leakage and partial liposome desaggregation. This latter phenomenon was actually observed by optical microscopy.

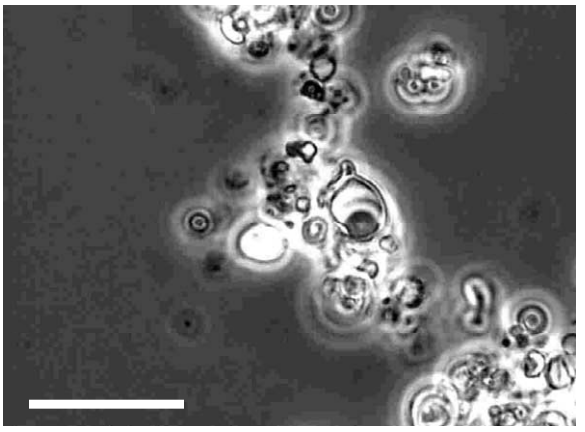

Fig. 6. Video image of marinosomes® observed by phase-contrast microscopy. Marinosomes® were prepared by filtration through polycarbonate filters (5-μm pore size) of a 20 mg ml^{-1} lipid suspension and incubated 1 h in a medium containing 9 mM calcium. The bar corresponds to 10 μm.

As a whole, marinosomes® were stable in storage conditions over 1 month. When placed in conditions that mimic topical application, complex structural rearrangements occurred. Preliminary freeze-fracture TEM observations concerning marinosomes® formulation in oil in water emulsions showed that the membrane structures were mostly preserved even in the presence of surfactant. In parallel, the first toxicology file indicated a good skin and eye tolerance toward marinosomes®. All these results allowed considering marinosomes® as potential candidates for topical application in view of the prevention and treatment of skin diseases.

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