

Electron-microscopical Detection of Liposomes in a Skin Treatment Gel

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Introduction

Liposomes are increasingly used at present in cosmetic formulations. Of particular interest in this respect are the skin care gels, since it is expected that any incompatibilities between the ingredients of these formulations are less probable than in the case of skin care creams, for instance.

If liposomes from vegetable raw materials (e.g. soya phospholipids) are used for production of these skin care gels, it will be useful to add further natural ingredients and auxiliaries such as gel forming agents to the formulation.

A very suitable gel forming agent in this respect is xanthan (xanthan gum), a complex polysaccharide composed of glucose, mannose, glucuronic acid and pyruvic acid units (1). Xanthan is registered for use in cosmetics, pharmaceuticals and foodstuffs. A good supplement in xanthan formulations may be aloe-vera concentrates (2), due to their chemical composition, in liquid or powder form.

Methods for detection and characterization of liposomes in purely aqueous dispersion have already been described (3, 4). The analytical effort for proper quality control of liposome gel as final product is increased by the fact that the development of a new method may become necessary, depending on the formulation ingredients used. In the following report we present an example of the electron-microscopical detection of liposomes in a skin care gel by means of the freeze fracturing technique. For comparison, an aqueous liposome dispersion was analogously prepared.

Composition of the Liposomal Skin Care Gel

The skin care gel is made of commercial raw materials and is composed as follows:

1.0% liposome raw material (dry substance) based on soya phospholipids in the form of liposomes (10% concentrate), which are loaded, especially for this formulation, with a water-soluble NMF factor (5),
1.0% xanthan gum (6),
0.2% aloe vera (powder) (7),
0.4% preservative,
0.08% natural perfumes,
water ad 100%.

For comparison, a 1% purely aqueous liposome dispersion (1% liposome raw material based on soya phospholipids of the same composition as above) is used.

Material and Methods

Preliminary note: In order to image the expected three-dimensional reticular structure of a gel, the relevant samples have to be stabilized, fractured, dehydrated and contrasted. Freeze-etching (8, 9, 10) permits all these preparation steps: The use of a Cryo-jet permits cryo-fixation of up to 20 µm-thick liposome dispersions (11) and, in combination with the freeze-fracturing technique, allows faithful reproduction of aqueous gels (12).

Preparation: A sample of the skin care gel and the comparative sample were taken by means of a small gold net used as a »spacer« (diameter: 3 mm, thickness: 20 µm) and were placed between two copper platelets (thickness: 0.1 mm). These »sandwiches« were frozen in the two-sided Cryo-jet (QFD 020, Balzers) by shooting with liquid propane, inserted in a double replica holder under liquid nitrogen and transferred into a freeze etcher (BAF 400, Balzers). In the high vacuum ($p < 10^{-6}$ mbar), at an object temperature of -105°C , the preparation sandwiches were

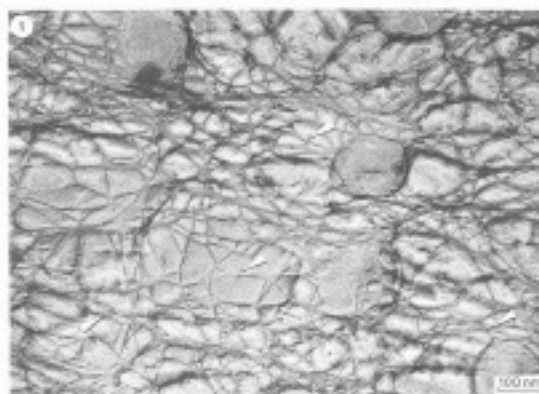


Fig. 1 Liposomal skin care gel after freeze-fracturing and a sublimation time of 300 seconds



Fig. 2 Aqueous liposome dispersion (comparison) after freeze-fracturing and a sublimation time of 30 seconds

fractured and exposed at the same temperature during 300 or 30 seconds in the high vacuum, in order to make object water sublime away. In this process the filaments of the skin care gel sample were laid open in addition to the fracture surfaces of the phospholipid membranes. Contrasting was carried out by rotary shadowing (13) under an angle of 45°. The calculated layer thickness of the platinum-carbon mixture was 2 nm. The replicas thus achieved of the preparations were cleaned over-night on a 14% sodium hypochloride solution, transferred several times on distilled water and dried. The replicas adhered to the small gold nets were taken with them into the transmission electron microscope (TEM). The apparatus used was of the Zeiss type EM 109 with anticontamination device (cooled by liquid nitrogen), accelerating voltage: 80 kV. The magnification was calibrated with test preparations.

Results

a) Skin care gel after a sublimation time of 300 seconds (Fig. 1; positive contrast: the vapourization material appears dark):

The liposomes are situated statistically distributed, but individually closely embedded in the three-dimensional network of the gel. The filamentous gel structure is laid open by the sublimation of the water; the pattern of the fracture surface in the gel can be recognized by upright, torn filaments (arrows in Fig. 1). At these points the filament diameter appears in full size (light point, enveloped by dark-looking vapourization material) and amounts to about 3 nm. The network is well-preserved by the shock-freezing, the finest meshes have a width of about 10 nm.

b) Aqueous liposome dispersion (comparison) after a sublimation time of 30 seconds (Fig. 2; positive contrast: the vapourization material appears dark): The liposomes appear statistically distributed. They are mainly unilamellar liposomes, which can be recognized by the only fracture point of the phospholipid membrane (arrows in Fig. 2). The fracture point is visible because the surface of the ice

mass surrounding the liposomes was slightly lowered by the sublimation. Occasionally there are non-spherically-shaped or multilamellar liposomes.

Summary

This study is an example for the quality control of a liposomal skin care gel and shows the compatibility of liposomes based on soya phospholipids with a gel forming agent of the xanthan type. The analysis described allows imaging of liposomes in their surroundings and demonstrates that the preparation is correct as far as formulation is concerned.

References

- (1) G. A. Nowak, Die kosmetischen Präparate, Band 2, Verlag für chem. Industrie H. Zolkowsky KG, Augsburg 1984, Seite 392
- (2) E. Thomascheck, SÖFW 112 (1), 6 (1986)
- (3) H. Lautenschläger, J. Röding und M. Ghyzcy, SÖFW 114 (14), 531 (1988)
- (4) E. Zellmann und J. Röding, Zeiss Anwendungsblatt EM 902 (1989)
- (5) Produkt der Nattermann Phospholipid GmbH
- (6) Rhodigel 200 (Rhône-Poulenc)
- (7) UP 200 (Worlée)
- (8) R. L. Steere, J. Biophys. Biochim. Cytol. 3, 45 (1957)
- (9) H. Moor, K. Mühlethaler, H. Waldner und A. Frey-Wyssling, J. Biophys. Biochim. Cytol. 10, 1 (1961)
- (10) A. W. Robards und U. B. Sleytr, Low temperature methods in biological electron microscopy in: A. M. Glauert, Practical methods in electron microscopy, Elsevier Press 1985
- (11) M. Müller, N. Meister und H. Moor, Mikroskopie (Wien) 36, 129 (1980)
- (12) T. Müller, H. Hakert und Th. Eckert, Colloid and Polymer Science, in press
- (13) T. Müller, Proceedings 4th Asia-Pacific Conference on Electron Microscopy, Bangkok, pp 361-366 (1988)

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