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PREPARATION AND *IN VITRO* CHARACTERIZATION OF LIPOSPHERES AS A CARRIER FOR THE COSMETIC APPLICATION OF GLYCOLIC ACID

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Summary

Lipospheres for the cosmetic delivery of glycolic acid were prepared by the melt method using tristearin as the lipid phase and hydrogenated soybean phosphatidylcholine as the emulsifier. The most favourable conditions leading to the highest liposphere yield involved triglyceride/phospholipid ratios of 4:1 or 5:1 and a phospholipid concentration of at least 2%. The lipospheres, sized from 5 to 40 μm, contained a rather high glycolic acid loading level probably due to a partial polymorphic modification of the lipid and determined glycolic acid sustained release pattern.

Riassunto

Tramite la tecnica di fusione sono state preparate liposfere per il rilascio di acido glicolico ad uso cosmetico. A questo scopo è stata impiegata tristearina quale fase lipidica e fosfatidilcolina di soia idrogenata quale stabilizzante. Le condizioni operative migliori, in grado di determinare la resa più alta in liposfere, hanno previsto rapporti 4:1 o 5:1 tra trigliceride e fosfolipide e una concentrazione in fosfolipide di almeno il 2%. Le liposfere così ottenute, di dimensioni comprese tra i 5 e i 40 μm, presentavano elevati livelli di caricamento in acido glicolico, probabilmente attribuibili ad una parziale trasformazione polimorfa del lipide e profili di rilascio di tipo sostenuto.

INTRODUCTION

Lipospheres represent a recent fat-based microparticulate system developed for parenteral (1), oral (2) and topical (3) delivery of bioactive compounds. The recent focusing of the cosmetic technology on such a lipid carrier is due to its ability to modulate the cutaneous permeation by retaining or sustaining the release of active substances to the stratum corneum. This is an important feature, since an increase of the effectiveness of compounds involving or not skin absorption as well as a decrease of side local or systemic effects could be achieved. Moreover, lipospheres represent a microparticulate system utilizing naturally occurring lipids having chemical affinity with cutaneous components (triglycerides and phospholipids). Lipospheres consist of water dispersible, solid microparticles composed of a solid fat core, stabilized by one layer of phospholipid molecules at the surface. They have some advantages over other microparticulate systems, including liposomes and microspheres, for example, better physical stability, low cost of ingredients, ease of preparation and scale-up, high dispersibility in an aqueous medium, high entrapment of hydrophobic substances, controlled particle size and non-toxicity. Lipospheres having particular structure and names were developed for cosmetic companies, whereas lipid nanoparticles were registered under trade names as Lipopearls™ or SLN™ (4). A range of cosmetic ingredients like coenzyme Q10, vitamin E and its derivatives, retinol and sunscreen agents have been incorporated into lipid nanoparticles (5).

In this regard, the present study describes the preparation and the characterization of lipospheres for the cutaneous delivery of glycolic acid in order to reduce the skin damage and improve the effectiveness of this compound. In particular, lipospheres were obtained by the melt method using tristearin as the lipid phase and hydrogenated soybean phosphatidylcholine as the emulsifier. The formed lipospheres were evaluated for yield, morphology, size, thermal properties, entrapment capacity and glycolic acid release pattern.

MATERIALS AND METHODS

Materials

The following chemicals were obtained from commercial suppliers and used without further purification. Tristearin as the lipid phase and glycolic acid as the active substance were supplied by Fluka Chemie (Buchs, Switzerland), hydrogenated soybean phosphatidylcholine as the emulsifier was supplied by Lucas Meyer (Hamburg, Germany). All solvents and other products were analytical grade.

Methods

Unloaded liposphere preparation. Tristearin (1.6 g) was melted at 80°C and hot phosphate buffer solution at pH 7.4 containing hydrogenated soybean phosphatidylcholine was added in a ratio of lipid to phospholipid (lip/pho) ranging from 2:1 to 5:1. The ratio of lipidic phase to aqueous phase ranged from 1:25 to 1:10 (Method I) or it was kept constant at 1:25 (Method II) (Table I). The mixture was homogenized at 13,000 rpm by Ultra-Turrax (T25 Basic IKA-Werk, Labortechnik, Staufen, Germany). After 3 min the O/A emulsion was rapidly cooled under stirring to below 20°C. The formed lipospheres were washed with water, recovered by centrifugation at 4,000 rpm and freeze-dried.

Loaded liposphere preparation. A 4:1 lip/pho ratio according to the Method I was selected for the loaded lipospheres. Practically, glycolic acid (1.2 g) was mixed with tristearin (1.6 g), melted and homogenized in presence of 20 ml of 2 % hydrogenated phosphatidylcholine pH 7.4 phosphate buffer solution, as described above.

Table I

Liposphere formulation parameters

Lipidic/aqueous phase ratio	Lip/pho ratio	Phospholipid (%)
1:25	2:1	2
1:17	3:1	2
1:12	4:1	2
1:10	5:1	2
Method I		

Lipidic/aqueous phase ratio	Lip/pho ratio	Phospholipid (%)
1:25	2:1	2
1:25	3:1	1.3
1:25	4:1	1
1:25	5:1	0.8
Method II		

Liposphere yield. The yield in lipospheres was calculated on the weight of the supernatant fraction recovered by centrifugation compared with the whole sample.

Morphological and particle size analysis. Liposphere morphological structure was examined by both optical microscope and Scanning Electron Microscope (SEM, XL-40, Philips, Eindhoven, The Netherland). The particle size was determined by computerized image analysis (IMG-VIEW, CIGS, University of Modena and Reggio Emilia) of at least 200 lipospheres on SEM micrographs.

Determination of tristearin/water apparent partition coefficient of glycolic acid. A known volume of glycolic acid water solution was added to a known amount of melted tristearin. The mixture was maintained at 80°C under stirring until equilibrium was reached. Glycolic acid concentration was determined in the aqueous phase by spectrophotometrical analysis at a wavelength of 220 nm (model Lambda 3B, Perkin-Elmer, Norwalk, CT, USA).

Entrapment capacity. Glycolic acid content was determined by placing a weighted amount of loaded lipospheres in water. After 48 h glycolic acid concentrations in the filtered solutions were assayed by spectrophotometry.

Thermal analysis. Thermograms of commercial tristearin, commercial glycolic acid, (10:1) tristearin/glycolic acid physical mixture and loaded lipospheres were recorded on a differential scanning calorimeter (DSC-4, Perkin-Elmer, Norwalk, CT, USA) coupled with a computerized data station (Perkin-Elmer). Samples (about 10 mg) were heated in crimped aluminum pans at a scanning rate of 10°C/min using dry nitrogen flow (30 ml/min).

Glycolic acid dissolution and release. Drug dissolution and release from the loaded lipospheres were examined by using a column-type apparatus (Apparatus 4, USP XXIV) (Dissotest CE-1, Sotax, Basel, Switzerland) in 100 ml of pH 7.4 phosphate buffer at a flow rate of 25 ml/min and a temperature of 37±0.2°C. All experiments were carried out under sink conditions by determining the amount of glycolic acid released spectrophotometrically at fixed time intervals. All the data were averaged on three determinations.

RESULTS AND DISCUSSION

Lipospheres can be produced by melt or solvent technique. A melt process was preferred to avoid the risk of organic solvent residuals. Various lipid/phospholipid (lip/pho) ratios ranging from 2:1 to 6:1 were used by other authors and the formation of different phospholipid structures such as liposomes is described for higher phospholipid contents. Moreover, the determination of the surface phospholipid showed that 79-90% of the phospholipid polar heads were on the surface of the lipospheres prepared from lip/pho at a 2:1 to 4:1 w/w ratio (3).

To examine the effect of such a parameter on liposphere yield, morphology and size, lip/pho ratios ranging from 2:1 to 5:1 were obtained by keeping constant both the phospholipid concentration (2%, w/v) (Method I) and the internal/external phase ratio (1:25) (Method II). According to the Method I, lipospheres having spherical shape, smooth surface and without aggregates were recovered in the supernatant by centrifugation (Fig. n. 1). On the contrary, the lower fraction as well as the whole samples obtained by the Method II resulted in unshaped aggregates (Fig. n. 2).

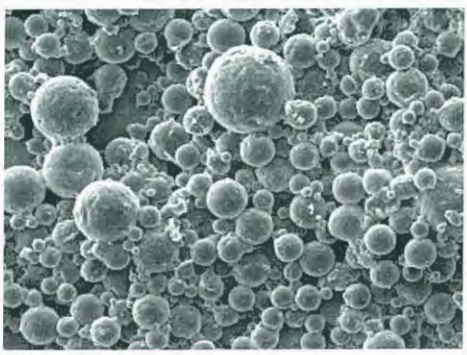

Fig. 1 SEM micrograph of lipospheres recovered in the supernatant by the Method I.

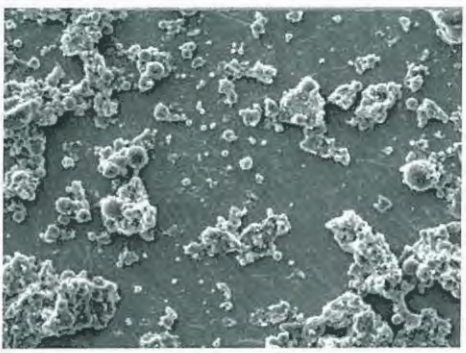

Fig. 2 SEM micrograph of lipospheres obtained by the Method II.

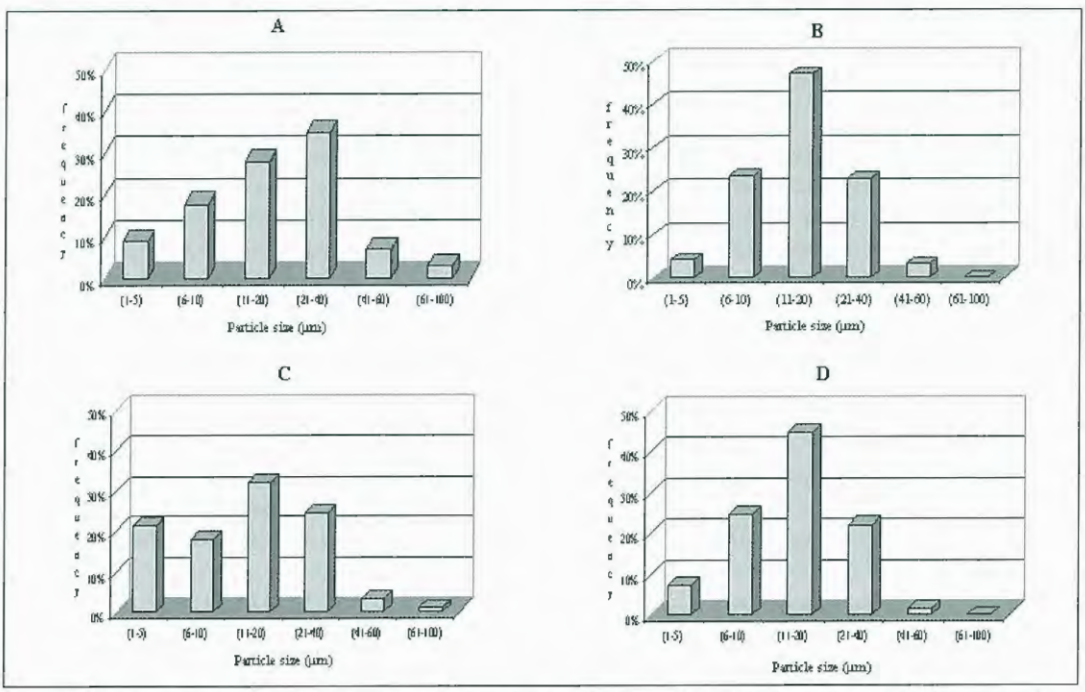

Fig. 3 Particle size distribution of lipospheres obtained by the Method I according to the lip/pho ratio: A) 2:1; B) 3:1; C) 4:1, D) 5:1.

The lip/pho ratio did not appear to affect significantly both average and distribution size of the lipospheres (Table II, Fig. n. 3). Lipospheres sized between 1 and 100 μm, the most population (80-90%) being in the size range of 5-40 μm, considered useful for topical applications (3).
The yield in lipospheres, calculated by considering the percentage of supernatant on the whole samples obtained by the Method I, increased with increasing the lip/pho ratio until 4:1 ratio (Table II). Therefore, the most favourable lip/pho ratios were found to be 4:1 or 5:1.

Table II

Yield and particle size lipospheres obtained according to the Method I

Lip/pho ratio	Particle size (μm)	Yield (%)
2:1	20.0±14.3	44.1±2.2
3:1	16.1±9.3	64.1±3.1
4:1	15.9±13.3	82.0±4.1
5:1	15.2±8.5	86.7±4.3

Therefore, 4:1 lip/pho ratio was selected to obtain lipospheres loaded with glycolic acid. The loaded lipospheres were examined for morphology, size distribution, water dispersibility, entrapment capacity, thermal behaviour and glycolic acid release pattern.
No significant differences in morphology and size were found in comparison with the unloaded microparticles. The loaded lipospheres showed a high dispersibility in water (Fig. n. 4). Since topical products don't support high amount of solid components owing to applicability and feel of use failure, a relatively high entrapment capacity should be required to achieve the necessary dose. Several factors can affect the entrapment capacity such as active substance partition coefficient and lipid polymorphic form and crystallinity degree (5-6). Though glycolic acid was characterized by a high solubility in melt tristearin (apparent partition coefficient between melt tristearin and water at 80°C resulted 0.08), the entrapment capacity of lipospheres was found rather high (34.06 ± 6.44 % w/w). This can be related to both the emulsification short period and the re-partitioning of the substance into the lipid phase with decreasing temperature of the water phase during the emulsion cooling. When recrystallization temperature of the lipid was reached, a solid lipid will entrap the substance which is present at this temperature (5). In addition, the crystallization of tristearin in lipospheres led to partial lipid polymorphic modifications. In fact, as thermograms show (Fig. n. 5), the stable ß-form (m.p. 57.55 ± 0.50°C, enthalpy of melting 91.92 ± 4.48 cal/g)

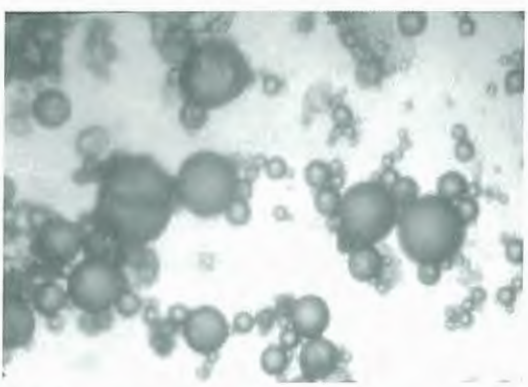

Fig. 4 Optical micrograph of an aqueous suspension of loaded lipospheres (X 400).

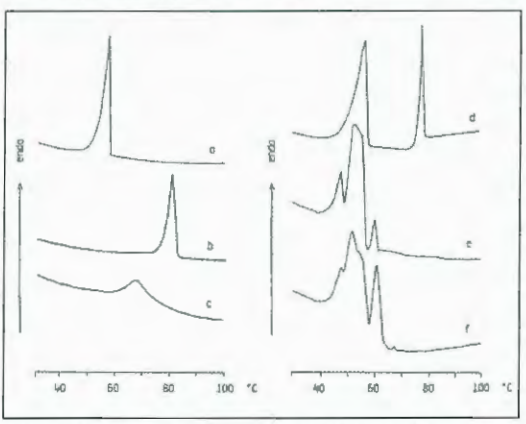

Fig. 5 DSC thermograms: A) tristearin; B) glycolic acid; C) hydrogenated phosphatidylcholine; D) (10:1) tristearin/glycolic acid physical mixture; E) loaded lipospheres.

transform partially in the unstable α (m.p. 48.92 ± 0.20°C, enthalpy of melting approximately between 6 and 30 cal/g) and ß' (m.p. 51.37 ± 1.59°C) forms. The unstable α–form is characterized by a lattice with disordered chains and imperfections that mean increased possibility to include and retain molecules (5). Moreover, the coexisting ß-form presents a decreased cristallinity degree (enthalpy of melting 22.41 ± 3.42 cal/g) with imperfections offering further space to accommodate the active substance.

Thermograms of lipospheres did not show the characteristic melting endotherm of glycolic acid, which is well evident in a 10:1 physical mixture, suggesting a complete molecular dispersion of the substance inside the lipospheres.

The lipid matrix so obtained provided prolonged release profiles (t_{50} = 30-90 min) compared with glycolic acid dissolution rate (t_{50} = 2 min) (Fig. n. 6). No "burst effect" was observed that could means the absence of glycolic acid at the liposphere surface.

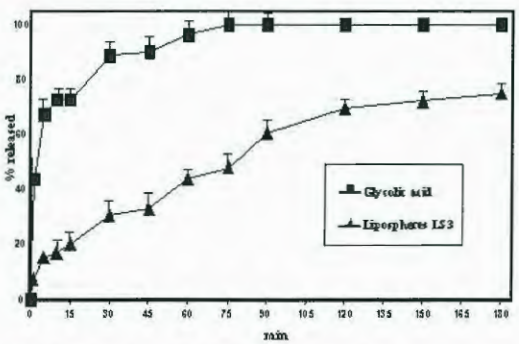

Fig. 6 Glycolic acid dissolution and release profile from lipospheres.

CONCLUSIONS

The present in vitro study involving the preparation and the characterization of lipospheres loaded with glycolic acid revealed lipid/phospholipid ratio and lipid crystalline structure as parameters to be considered in achieving appropriate liposphere yield and active substance incorporation, the latest being of particular interest for hydrophilic compound entrapment.

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