ELSEVIER

International Journal of Pharmaceutics 196 (2000) 51–61

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Study on glycolic acid delivery by liposomes and microspheres

P. Perugini [a,*], I. Genta [a], F. Pavanetto [a], B. Conti [a], S. Scalia [b], A. Baruffini [a]

[a] *Department of Pharmaceutical Chemistry, University of Pavia, V.le Taramelli 12, 27100 Pavia, Italy*

[b] *Department of Pharmaceutical Sciences, University of Ferrara, Via Fossato da Mortara, 44100 Ferrara, Italy*

Received 2 July 1999; received in revised form 3 November 1999; accepted 24 November 1999

Abstract

Glycolic acid is used in many cosmetic products as exfoliant and moisturizer. Unfortunately, the greater glycolic acid cosmetic benefits the greater is the potential for skin irritation as far as burning. The aim of this work was to investigate the feasibility of topical controlled delivery systems loading glycolic acid in order to optimize the acid cosmetic properties lowering its side effects. For this purpose different types of microparticulate systems have been evaluated: liposomes, liposomes modified by chitosan addition and chitosan microspheres. Liposomes, composed of phosphatidylcholine and cholesterol (1:1 molar ratio) and with different glycolic acid/lipid molar ratio, were prepared by reverse phase evaporation method. Two types of interaction between liposomes and chitosan were investigated: chitosan addition into lipidic bilayer during liposome preparation and coating of already formed liposomes with chitosan. Glycolic acid loaded chitosan microspheres were prepared by the dry-in-oil emulsion method. The microparticulate systems were morphologically characterized by electron microscopy and particle size analysis. In vitro dissolution tests were performed to evaluate the feasibility of microparticulate systems in modulating glycolic acid release. The results obtained show that liposomes are always suitable to modulate glycolic acid release and that the best condition to achieve this control is obtained by the liposomal systems in which glycolic acid/lipid molar ratio is 5:1. Further significant release control is obtained by addition of chitosan into the liposomes, while chitosan microspheres are not able to control glycolic acid release even after crosslinking. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Glycolic acid; Liposomes; Chitosan; Cosmetics

1. Introduction

Owing to its multilayered structure, the skin represents a complex and relatively efficient barrier against penetration of toxic exogenous compounds, physical assaults, excessive loss of water and other essential compounds from the body.

Normal hydration in healthy skin results from a balance of several factors as water transport from the dermis, the composition of lipid-enriched intercorneocyte matrix and the environmental influ-

---

* Corresponding author. Tel.: +39-0382-507363; fax: +39-0382-422975.

E-mail address: pperugini@chifar.unipv.it (P. Perugini)

PII: S0378-5173(99)00439-1

ences. However, the normal structure of the skin may be altered producing an excessive skin dryness, or leading to keratinization (Morganti et al., 1994)

Alpha hydroxy acids and particularly glycolic acid (GA) are used in many cosmetic products as exfoliants and moisturizers (Scholz et al., 1994). Glycolic acid activity is mainly influenced by its absorption into the different layers of the skin (Kraeling and Bronaugh, 1997). It acts as a solvent for the intercorneocyte matrix reducing excessive epidermal keratinization; this soft peeling leads to thinning of stratum corneum useful in the renewal of epidermis, and to a visible reduction of facial lines (Campos et al., 1999).

Moreover, glycolic acid is efficacious in increasing skin elasticity; this action is probably due to the direct stimulation in production of collagen, elastin and mucopolysaccharides in the deeper layers of the skin (Ditre et al., 1996).

Unfortunately, highest glycolic acid concentrations are combined to high potential for skin irritation as far as burning is concerned (Smith, 1996). The main problem connected to glycolic acid in its cosmetic appliances, i.e. its irritative properties, generates the rationale for studies of cosmetic formulations with reduced adverse reactions.

The aim of this work was to investigate the feasibility of topical controlled delivery systems in order to optimize the cosmetic properties of glycolic acid and to lower its side effects.

For this purpose different types of microparticulate systems have been evaluated: liposomes, liposomes modified by chitosan and chitosan microspheres.

Liposomes have been chosen to be studied in this work because they seem to be a useful carrier for topical delivery: they are able to increase the concentration of the bioactive compounds both in epidermis and in the deeper layers of the skin. The mechanism of liposome action on transfer of drug through the skin is probably due to the composition of the vesicle bilayer similar to that of skin lipid thus leading to fusion of vesicles in the intercellular space of the skin (Egbaria and Weiner, 1990).

Chitosan meets the requirements of being a natural, bioadhesive, biocompatible and biodegradable polymer (Akbuga, 1995); it seems to be a good candidate to be combined to liposomes and to render them stable and bioadhesive (Takeuchi et al., 1996). Moreover, recent studies showed the efficacy of the cationic biopolymer in improving skin compatibility of cosmetic formulations and enhancing the penetration of bioactive cosmetic ingredients.

The association of chitosan to liposomes is expected to affect also the release rate of glycolic acid from the vesicles by decreasing it; in fact this compound is very small and hydrophilic and for these reasons it is very problematic to control its diffusion rate from lipidic bilayers. The aim is to maintain efficacious concentrations of glycolic acid at the site of administration for at least 6 h.

In recent years chitosan has been widely studied also as polymer to obtain microspheres for the controlled release of drugs (Genta et al., 1997b). Owing to polymer properties (Akbuga, 1995) and to the previous studies concerning chitosan microspheres, also this microparticulate system seems to be suitable to be tested as carrier for topical administration of glycolic acid.

Both liposomes and microspheres were prepared in this work, and their ability to modify the release rate of glycolic acid were evaluated and compared.

2. Materials and methods

2.1. Materials

Glycolic acid (Sigma, Milano, Italy). Egg L,α, phosphatidylcholine (PC) type XI-E, 100 mg/ml chloroformic solution (Sigma); cholesterol (Col) supplied by Carlo Erba (Milano, Italy). Chitosan (LC), MW 70 000 deacetylation degree 87.4% (Fluka Chimica, Milano, Italy); chitosan (MC), MW 250 000 deacetylation degree 80% (Polysciences, Warrington, PA).

All other reagents and solvents were of analytical grade.

2.2. Liposome preparation

Liposomes containing glycolic acid were prepared by the reverse phase evaporation method (REV) (Weiner et al., 1989). Amounts of PC and Col (1:1 molar ratio) were dissolved in a 1:1 v/v chloroform: diethylether mixture. The glycolic acid aqueous solution (1.25-2.5-5% w/v) was dropped to the lipid solution to form a W/O emulsion. The volume ratio between the two phases was maintained 1:5.

The W/O emulsion was ultrasonicated for 1 min and then reduced to a semi-solid gel by evaporation under vacuum in a rotary evaporator (150 rpm) at 45°C for 1 h. The next step was to subject the gel to vigorous mechanical shaking with a vortex mixer, to give a free-flowing aqueous suspension of liposomes.

In order to obtain unilamellar liposomes with homogenous size, some batches of REV vesicles were submitted to extrusion process by an extruder device LiposoFast™-100 (Milsch Equipment, Laudenbach, Germany) equipped with a 100 nm pore size polycarbonate membrane.

Table 1
Composition of liposomes (PC:col 1:1 molar ratio) containing glycolic acid

Batches	µmolGA/µmol lipid theoretical	µg Chitosan/µmol lipid theoretical
LP1–LP3	20	–
LP4–LP6	10	–
LP7–LP9	5	–
Chitosan loaded liposomes		
LP13–LP15	20	30
LP16–LP18	10	30
LP28–LP30	10	6
LP31–LP33	10	15
LP34–LP36	10	60
Chitosan coated liposomes		
LP19–LP21	20	15
LP22–LP24	10	15
LP35–LP37	10	3
LP38–LP40	10	6
LP41–LP43	10	30
Extruded liposomes		
LP10–LP12	5	–
LP25 –LP27(^)	10	15

(^) Chitosan coated liposomes.

Unencapsulated drug was removed by ultracentrifuging at 75 000 × g for 1 h at 4°C in an ultracentrifuge model L7-65 with rotor type 60Ti (Beckman, Palo Alto, USA).

2.2.1. Chitosan coated liposomes

The already prepared liposomes were coated with chitosan by dropping 0.5 ml of vesicle suspension in 2 ml of aqueous MC chitosan solution (pH 5) under continuous magnetic stirring; afterwards they were incubated at room temperature for 2 h and ultracentrifuged. Different chitosan concentrations in the aqueous solution, ranging between 0.005 and 0.05% w/v were evaluated. The coating of liposomes with chitosan was performed also on extruded liposomes in order to understand the affinity between lipid bilayer and chitosan. The amount of chitosan reacted with liposomes was evaluated analysing the supernatant after centrifugation by colorimetric assay with the anionic reactive dye Cibacron Brilliant Red (Muzzarelli, 1998). The calibration curve was drawn for chitosan concentrations in the range 7–40 µg/ml. Absorbance was measured at 575 nm with a Beckman spectrophotometer model DU 7500 (Beckman Instr., USA).

2.2.2. Chitosan loaded liposomes

MC Chitosan was encapsulated into the liposomes during vesicle preparation process as follows: the W_1/O emulsion, obtained by emulsifying a glycolic acid aqueous solution (1.25% p/v) into PC:Col (1:1 mol:mol) lipidic mixture, was dropped into a pH 5 aqueous chitosan solution (W_2) containing the same concentration of glycolic acid (volume ratio among the three phases 1:6:1.5) and a $W_1/O/W_2$ emulsion was obtained by sonication. The subsequent steps to produce the vesicles were the same as described above for liposome preparation. Different chitosan concentrations, in W_2 phase, were evaluated ranging between 0.05 and 0.33% w/v. The amount of chitosan reacted with liposomes was determined in the supernatant, as described above.

Table 1 lists all the batches of liposomes produced.

2.3. Chitosan microsphere preparation

Chitosan microspheres were prepared according to the 'dry-in-oil emulsion method' (Genta et al., 1997a). Briefly, glycolic acid (2% w/v) was dissolved in solutions of chitosan (2% w/v) in CH_3COOH (2%) containing 20% methanol. Two chitosans with 70 000 and 250 000 Mw were used. The polymeric solutions were emulsified in the continuous phase prepared from light mineral oil containing 2% Span 20 (dispersed to continuous phase ratio 1:15). Emulsification was performed at 38°C under continuous stirring at 9500 rpm with an Ultraturrax (Ika Labortechnik, Staufen, D) model T25 homogenizer. Subsequent solvent evaporation took place at 55°C, under reduced pressure for 10 h, while stirring at 60 vibration/s with a vibromixer (Vibromixer E1, Chemap, Volketswil, CH). The microspheres obtained (batches *n.* LCGA1–LCGA3, MCGA7–MCGA9) were rinsed twice with petroleum ether, centrifuged and filtered through a 2 μm stainless steel filter.

When chemically cross-linked chitosan microspheres were produced (batches *n.* LCGA4–LCGA6, MCGA10–MCGA12), glutaraldehyde aqueous solution was used as crosslinking agent; it was dropped into the W/O emulsion (glutaraldehyde solution:aqueous phase ratio 1:5 v/v) before starting solvent evaporation.

2.4. Characterization

2.4.1. Morphology

2.4.1.1. Optical Microscopy. Microparticulate systems were observed by optical microscopy, using a standard microscope (Carl Zeiss, Oberkochen, Germany) at 400 × magnification equipped with camera (Reichert, Wien, Austria).

2.4.1.2. Transmission electron microscopy (TEM). Liposomes were analysed on negative stain electron microscopy using a JEM 1200 EXII electron microscope (Jeol, Tokyo, Japan).

A drop of liposome suspension (5 μmol/ml) was applied to carbon-coated grids and, after 2 min, the excess was drawn off with filter paper; a satured uranyl acetate aqueous solution was used as a staining agent. The excess was eliminated with distilled water and the sample was analysed by TEM at 80 kV.

2.4.1.3. Scanning electron microscopy (SEM). The microspheres were morphologically characterized by electron microscopy using a microscope Jeol JX 840-A (Jeol LTD, Tokyo, Japan). Samples for SEM observation were prepared by sputter-coating under argon atmosphere with a thin layer of Au/Pd the dried microspheres, suitably arranged on a sample holder.

2.4.2. Particle size analysis

Granulometric analyses of microparticulate systems were performed by a light blockage method which consists in the determination of particle size by obscuration of a white light beam. The particles are measured as a function of the two-dimensional cross-section area. The samples of microspheres were suspended in isopropanolic solution while the samples of liposomes were suspended in bidistilled filtered water. They were analysed by an HIAC/ROYCO apparatus (model 3000), equipped with HC60HR detector, in a size range between 2 and 50 μm (AM Instruments, Desio, I). The results are the average of three analyses for each sample.

2.5. Glycolic acid content

The actual amount of glycolic acid entrapped in the microparticulate systems was determined by HPLC. The chitosan microspheres (24–25 mg) loaded with glycolic acid were dissolved in 5 ml of HCl (0.1 N) under sonication.

The HPLC analyses were performed on a Hypersil ODS column eluted at 0.3 ml/min flow-rate, with 2% methanol in phosphate buffer (0.025 M, pH 6.2) containing 0.002M tetrabutylammonium dihydrogen phosphate as mobile phase. UV detection was performed at 210 nm.

Glycolic acid content in the liposomes was calculated as difference between the total amount of

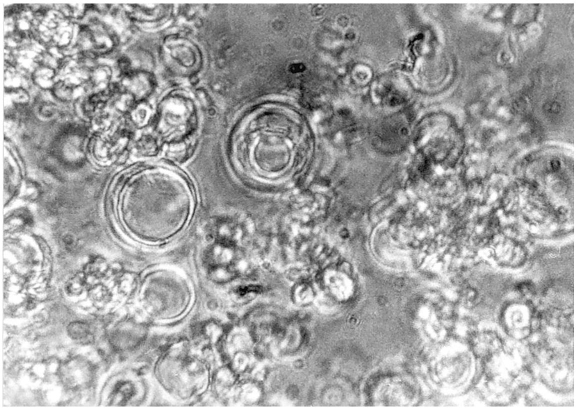


Fig. 1. Optical micrograph of glycolic acid loaded liposomes (batch LP4) (1 cm bar = 1 μm).

the acid added to the preparation and the amount of extraliposomal GA. Extraliposomal GA was determined by HPLC analysis performed as described above for GA content determination, on the supernatant, recovered after liposome centrifugation.

2.6. Lipid content

Lipid content was evaluated by a FT-IR spectrophotometer Perkin Elmer model 1600 (Perkin Elmer, Deaconsfield, UK) equipped with a cell with sodium chloride windows, and 5 mm aperture (Pidgeon et al., 1989).

A calibration curve was developed using chloroformic solutions of lipids at concentrations ranging between 2 and 25 mg/ml.

The liposome samples solubilized in $CHCl_3$, sonicated, filtered and analyzed at 1730.4 1/cm and 2932 1/cm wavelengths.

2.7. 'In vitro' glycolic acid release

A dialysis method was used to perform the 'in vitro' release test of all batches of glycolic acid loaded microparticulate systems. Amounts of liposome or microsphere suspensions were placed in the dialysis sacks (Spectra/Por membranes Mwco 12–14 000), suspended in 10 ml phosphate buffer (pH 5.5) at 30°C and agitated at 50 rpm.

At scheduled time intervals, agitation was stopped, the dissolution medium was collected, filtered and HPLC analyzed for glycolic acid content. The dissolution medium was then replaced with fresh medium. All dissolution tests were run in triplicate and mean values are reported.

3. Results and discussion

Reverse phase evaporation method permitted to obtain well-formed glycolic acid loaded vescicles as shown, as an example, in Fig. 1.

In order to obtain vesicles added with chitosan, two methods were compared: the first method consists of the addition of liposome suspensions to chitosan solutions in order to obtain chitosan adsorption to liposome bilayer (chitosan coated liposomes); in the second process tested the chitosan is added to vesicles during their preparation method and it is supposed to be mainly entrapped inside the liposomes (chitosan loaded liposomes).

In the coating of liposomes we follow a method reported in the literature by Henriksen (Henriksen et al., 1994). These authors found that it is favourable to add the liposome suspension to chitosan solution (and not the opposite order of addition) in order to obtain coating of vesicles; in fact, in this way, the excess of polymer solution volume can react instantaneously with liposome bilayer while the concentration of chitosan it-self should not be very high in order to avoid aggregation and polymer filaments. Moreover they reported that chitosan should not have very low molecular weight. Accordingly chitosan with molecular weight of about 250 000 D was used.

When chitosan is included into vesicles during their preparation, its concentration should not be very low, since the viscosity of polymer solution has to be high enough to stabilize the W/O/W emulsion before solvent evaporation takes place.

The liposomes modified by chitosan addition (loaded and coated liposomes) were characterized by optical microscopy, transmission electron microscopy and quantification of the amount of chitosan entrapped or adsorbed to liposomes. Results of optical microscopy observation and chitosan reacted determination are listed in Table 2.

Optical microscopy gave an indication about morphological characteristics of the microparticulate systems: good results are referred as appearance of well separated liposomes showing evidence of chitosan coating or chitosan entrapment. In fact the presence of chitosan gives opalescent aspect to liposomes, and hides their lamellar structure. This is the case of batches LP16–LP18, LP22–LP24, LP25–LP27.

The results of colorimetric determination of polymer in chitosan loaded liposomes (Table 2) show that the highest amount of polymer was recovered in batches LP31–LP33 and LP16–LP18. These batches are obtained starting from different amounts of chitosan, this result demonstrated that liposome intrinsic capability plays an important role in determining the amount of compound entrapped.

The case of chitosan coated liposomes is quite different because the amount of polymer interacted with the liposomes increases together with the starting amount of chitosan. This result gives evidence of chitosan deposition to liposome surface. Moreover, extruded liposomes (batches LP25–LP27) show double amount of adsorbed chitosan with respect to batches LP22–LP24 that are equivalent for theoretical chitosan concentration: this happens because extruded liposomes are smaller and they expose higher surface area to polymer adsorption. These data confirm the surface phenomenon of chitosan adsorption on liposomes.

Table 2
Characterization of the liposomes modified by chitosan addition

Batch no.	Optical microscopy	μg Chitosan/μmol lipid actual
Chitosan loaded liposomes		
LP28–LP30	*	0
LP31–LP33	*	3.4
LP16–LP18	***	3.0
LP34–LP36	****	–
Chitosan coated liposomes		
LP35–LP37	*	0
LP38–LP40	*	2.0
LP22–LP24	***	5.6
LP41–LP43	****	6.3
Chitosan coated extruded liposomes		
LP25–LP27	***	12

* Unchanged liposomes.

*** Evidence of chitosan adsorption/loading.

**** Aggregates and polymer filaments.

Negative stain electron micrographs were performed on chitosan loaded and coated liposomes to highlight their morphologic differences (Fig. 2). The chitosan coated liposomes have more solid appearance (Fig. 2a) with respect to the chitosan loaded liposomes (Fig. 2b). Moreover, the existence of polymer layers surrounding the liposomes is well visualized by filament structures on the surfaces of liposomes (Fig. 2a). The results of TEM analysis confirmed the data obtained by optical microscopy and by chitosan determination.

The mechanism of coating neutral PC liposomes by chitosan probably involves hydrogen bonding between the polysaccharide and the phospholipid head groups.

Granulometric analysis by light blockage method as performed to obtain information on liposome particle size (Fig. 3). The light blockage method is not conventionally used to determine liposome particle size distribution. However, in this case the change of liposome morphologic characteristics, as a result of the addition of chitosan, made the modified liposomes suitable to light blockage analysis. Results show that particle size distribution of chitosan coated liposomes (batch LP22) ranges between 2 and 5 μm while chitosan loaded liposomes (batch LP16) have broader size distribution ranging between 2 and 10 μm. In both cases addition of chitosan to liposomes seems to increase liposome size.

Microsphere morphology was investigated by SEM. SEM analyses of glycolic acid loaded chitosan microspheres showed microparticles of spherical shape whose surface was a little wrinkled (Fig. 4a).

Crosslinked microspheres resulted to have smoother surface, as shown in the Fig. 4b.

The results related to particle size distribution of all batches of microspheres are reported in Fig. 5. Particle size distribution of crosslinked microspheres (batch MCGA10) is quite broad as it ranges between 2 and 15 μm while batch MCGA7

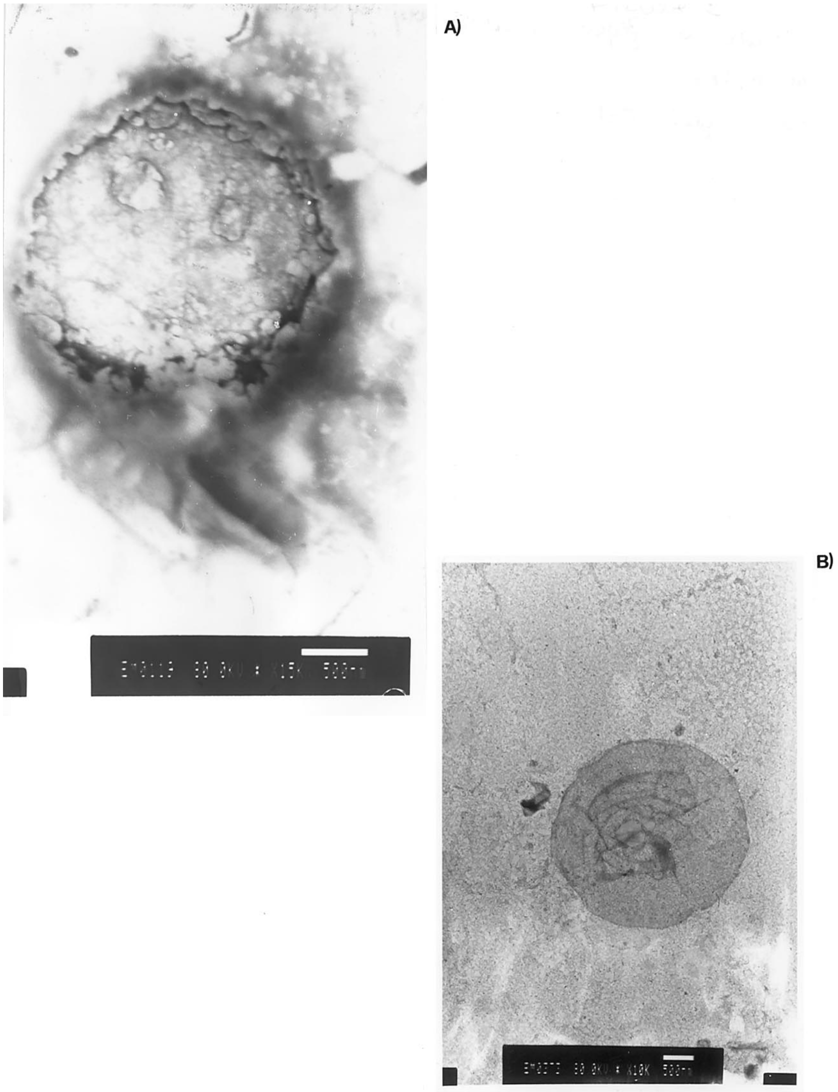


Fig. 2. Trasmission electron micrographs of modified liposomes: (a) chitosan coated liposomes (batch LP22); (b) chitosan loaded liposomes (batch LP16).

has narrow size distribution, the highest percentage of particles being about 10 μm. Glutaraldehyde addition leads to decrease of microsphere sizes, as previously reported in our work (Genta et al., 1997a). Similar results were obtained for microspheres made of low molecular weight chitosan (data not reported).

Table 3 reports glycolic acid content in liposomal systems. Encapsulation efficiency of glycolic acid into liposomes increased with decreasing drug/lipid molar ratio. It is interesting to point out that the actual value of μmol of GA with respect to μmol of lipid for all unmodified liposomes (batches LP1-LP9) is the same because this value represents the intrinsic capability of lipid to entrap the compound. It is possible to increase this capability by chitosan addition (batches LP16, LP22). Chitosan loaded liposomes show the highest loading of glycolic acid per unit of lipid with respect to unmodified vesicles (batches

LP16–LP18, LP4–LP6). High increase in glycolic acid content was obtained by coating with chitosan extruded liposomes (batches LP25–LP27): this result is probably due to the increased liposome exposed surface.

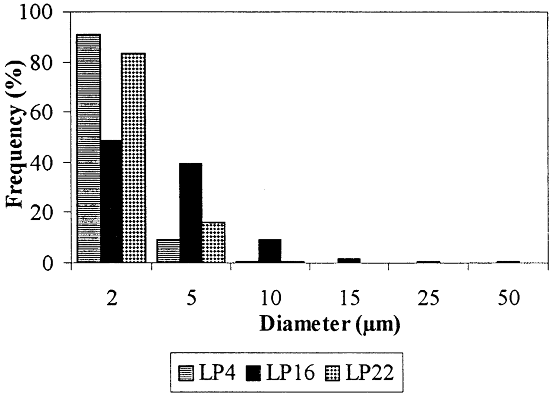


Fig. 3. Particle size distribution of glycolic acid loaded liposomal systems.

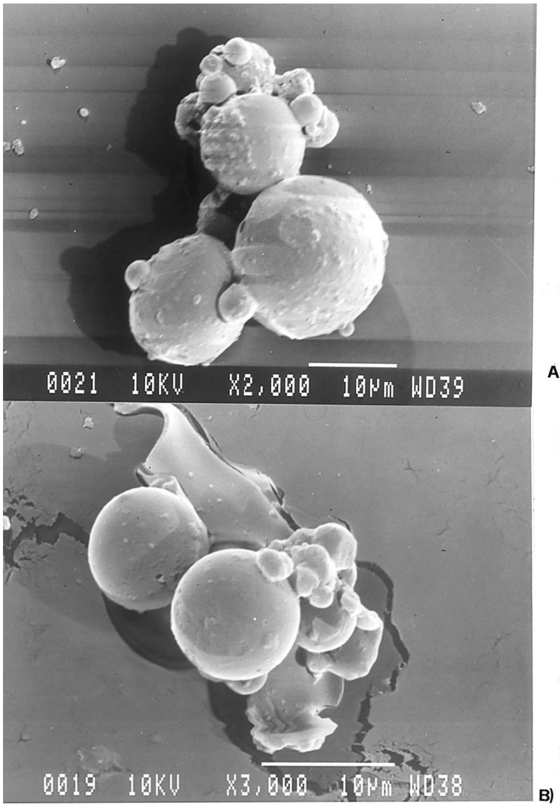


Fig. 4. Scanning electron micrographs of glycolic acid loaded chitosan microspheres: (a) uncrosslinked microspheres; (b) glutaraldheyde crosslinked microspheres.

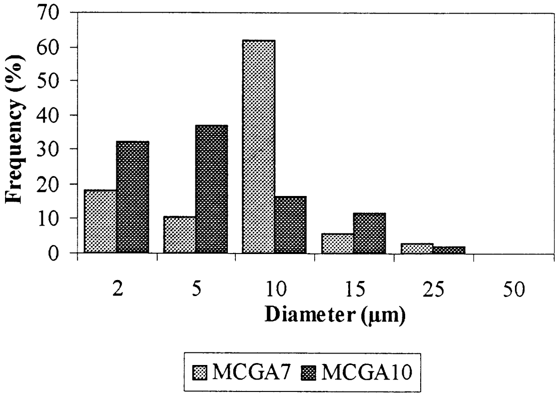


Fig. 5. Particle size distribution of glycolic acid loaded chitosan microspheres.

Table 4 reports the results concerning glycolic acid encapsulation yields into microspheres. Good actual glycolic acid content was obtained for both chitosans; moreover the production yields were always satisfactory, between 60 and 80%.

'In vitro' glycolic acid release curves from liposomes are shown in Figs. 6 and 7.

All batches of liposomes exerted controlled release of glycolic acid. First of all the effect of drug/lipid molar ratio on glycolic acid release rate has been evaluated as shown in Fig. 6a. The smaller drug/lipid ratio the slower drug release (63% of the drug released after 4 h). Extruded liposomes present faster drug release compared to REV vesicles, as shown in Fig. 6b. This result agrees with the different membrane structures between the two types of liposomes: reverse phase evaporation vesicles present higher bilayer number resulting in a more efficacious barrier to hydrophilic drug release. Moreover the higher specific surface area of extruded liposomes probably co-operates in increasing release rate of glycolic acid.

Fig. 7 shows the effect of chitosan addition to liposomes on glycolic acid release rate: the presence of chitosan inside the liposomes or surrounding them is always positive in modulating glycolic acid release from liposomal systems; the slowest

Table 3
Glycolic acid loaded liposomes (values are the mean of three batches)

Batches	μmolGA/μmol lipid theoretical	μmolGA/μmol lipid actual	Encapsulation efficiency (%)
LP1–LP3	20	2.03	10.15
LP4–LP6	10	2.05	20.5
LP7–LP9	5	2.16	43.2
LP10–LP12(*)	5	2.15	43
LP13–LP15(°)	20	3.57	12.05
LP16–LP18(°)	10	4.82	60.2
LP19–LP21(^)	20	2.88	14.4
LP22–LP24(^)	10	3.58	35.8
LP–25–LP27(*),(^)	10	6.19	61.9

(*) Liposomes extruded.
(°) Chitosan loaded liposomes.
(^) Chitosan coated liposomes.

glycolic acid release rate is achieved by chitosan loaded liposomes (Fig. 7a). This result suggests that the presence of polymer within the liposome is more efficacious in retaining the compound.

In Fig. 7b the 'in vitro' dissolution profiles of glycolic acid from chitosan coated liposomes and chitosan coated extruded liposomes are compared. Drug release rate from chitosan coated extruded liposomes is faster than from chitosan coated liposomes and it is almost superimposable to dissolution profile of batch LP4 (uncoated liposomes). This result is in keeping with the morphologic characteristics mentioned for extruded liposomes.

Chitosan microspheres made of both polymers tested did not control glycolic acid release (about 87% of drug dissolved from the microspheres after 15 min). Even chemical crosslinking with glutaraldehyde, that proved to be very efficacious in modulating the release of lipophilic drug loaded into chitosan microspheres (Genta et al., 1997a), was not able to slow down dissolution rate of a small and hydrophilic compound such as the glycolic acid.

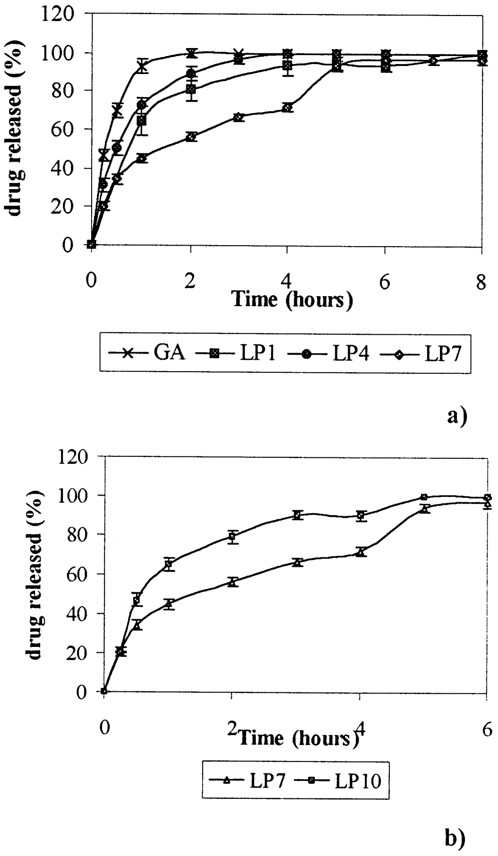
4. Conclusions

The loading of glycolic acid into liposomes, chitosan coated liposomes, chitosan loaded liposomes and chitosan microspheres was performed in this work with the aim of optimizing the effect of glycolic acid by modulating its release rate from the microparticulate systems.

The results obtained show that: liposomes are able to slow down the release of glycolic acid; the molar ratio GA/lipid 5:1 represents the best condition to achieve control of glycolic acid release;

Table 4
Glycolic acid loaded chitosan microspheres (values are the mean of three batches)

Batch	Theoretical GA content (w/w%)	Actual GA content (w/w%)	Encapsulation efficiency (%)
LCGA1–LCGA3	50.0	24.0	48.0
LCGA4–LCGA6	50.0	18.6	37.2
MCGA7–MCGA9	50.0	22.8	45.6
MCGA10–MCGA12	50.0	17.4	34.8



a)

b)

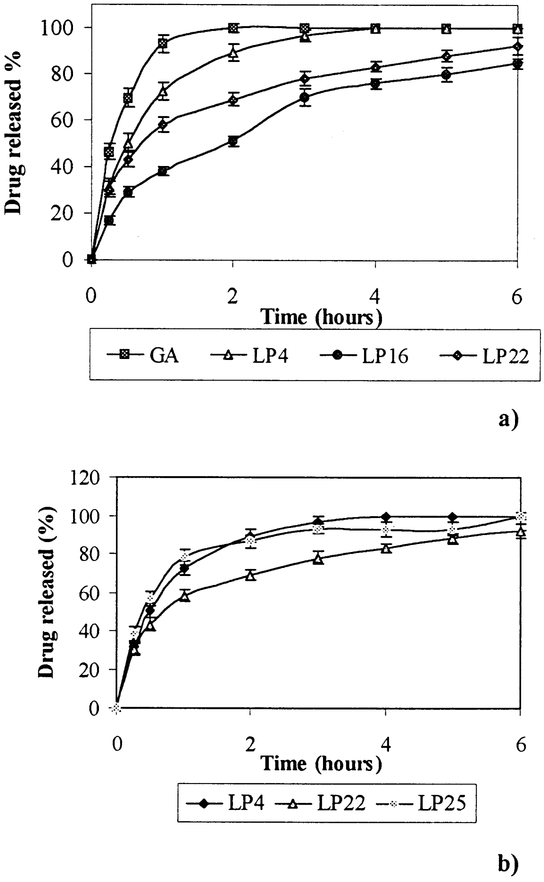
Fig. 6. Dissolution profiles of glycolic acid from liposomal systems: (a) effect of the drug/lipid molar ratio on the glycolic acid release rate (GA free glycolic acid; LP1 molar ratio 20:1, LP4 molar ratio 10:1, LP7 molar ratio 5:1, (b) effect of the extrusion process on the glycolic acid release rate (LP7 REV liposomes, LP10 extruded REV liposomes).

further significant control of GA release can be obtained by adding chitosan within the liposomes; chitosan microspheres do not control glycolic acid release even after crosslinking.

In conclusion liposomes represent a good delivery system to modulate release rate of an hydrophilic compound such as glycolic acid; moreover chitosan joined to the lipophilic matrix of liposomes can improve their modulating activity.

Acknowledgements

The authors thank Dr P. Riccardi and Dr L. Asti (Centro Grandi Strumenti-University of Pavia-Italy) for the SEM and the TEM analyses. This work was supported by a grant from FAR (Fondo Ateneo Ricerche).



a)

b)

Fig. 7. Dissolution profiles of glycolic acid from liposomal systems: (a) influence of chitosan addition to liposomes on drug release (GA free glycolic acid, LP4 unmodified liposomes; LP16 chitosan loaded liposomes, LP22 chitosan coated liposomes); (b) comparison between unmodified liposomes (LP4), chitosan coated liposomes (LP 22) and chitosan coated extruded liposomes (LP25).

References

Akbuga, J., 1995. A biopolymer: chitosan. Int. J. Pharm. Advances. 1, 3–18.

Campos, P.M.B.G.M., Ricci, G., Semprini, M., Lopes, R.A., 1999. Histopathological, morphometric, and stereologic studies of dermocosmetic skin formulations containing vitamin A and/or glycolic acid. J. Cosmet. Sci. 50, 159–170.

Ditre, C.M., Griffin, T.D., Murphy, G.F., 1996. Effects of alpha hydroxy acids on photoaged skin: a pilot clinical, histological and ultrastructural study. J. Am. Acad. Dermatol. 34, 187–195.

Egbaria, K., Weiner, N., 1990. Liposomes as a topical drug delivery system. Adv. Drug. Deliv. Rev. 5, 287–300.

Genta, I., Conti, B., Perugini, P., Pavanetto, F., Spadaro, A., Puglisi, G., 1997a. Bioadhesive microspheres for ophthalmic administration of acyclovir. J. Pharm. Pharmacol. 49, 737–742.

Genta, I., Giunchedi, P., Pavanetto, F., Conti, B., Perugini, P., Conte, U., 1997. Preparation of chitosan microparticulate ATEC-Groûammare (AP) drug delivery systems. In: Muzzarelli, R.A.A., Peters, M.G., (Eds) Chitin Handbook, pp. 391–396.

Henriksen, I., Smistad, G., Karlsen, J., 1994. Interactions between liposomes and chitosan. Int. J. Pharm. 101, 227–236.

Kraeling, M.E.K., Bronaugh, R.L., 1997. In vitro percutaneous absorption of alpha hydroxy acids in human skin. J. Soc. Cosmet. Chem. 48, 187–197.

Morganti, P., Persechino, S., Bruno, C., 1994. Effects of topical alpha-hydroxy acids on skin xerosis. J. Appl. Cosmetol. 12, 85–90.

Muzzarelli, R.A.A., 1998. The colorimetric determination of chitosan. Anal. Biochem. 260, 255–257.

Pidgeon, C., Apostol, G., Markovich, R., 1989. Fourier transform infrared assay of liposomal lipids. Am. Biochemistry. 181, 28–32.

Scholz, D., Brooks, G.J., Parish, D.F., Burmeister, F., 1994. Fruit acid extracts, a fresh approach to skin renewal. Int. J. Cosm. Sci. 16, 265–272.

Smith, W.P., 1996. Comparative effectiveness of a-hydroxy acids on skin properties. Int. J. Cosm. Sci. 18, 75–83.

Takeuchi, H., Yamamoto, H., Niwa, T., Hino, T., Kawashima, Y., 1996. Enteral absorption of insulin in rats from mucoadhesive chitosan-coated liposomes. Pharm. Res. 13, 896–901.

Weiner, N., Martin, F., Riaz, M., 1989. Liposomes as a drug delivery system. Drug. Dev. Ind. Pharm. 15, 1523–1554.