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Effect of preparation technique on the properties of liposomes encapsulating ketoprofen–cyclodextrin complexes aimed for transdermal delivery

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Abstract

The combined approach of cyclodextrin complexation and entrapment in liposomes was investigated in order to develop an effective topical formulation of ketoprofen. Equimolar complex of drug and hydroxypropyl-β-cyclodextrin (HPβCyd) was added at different concentrations to the aqueous phase of liposomes consisting of phosphatidylcholine and cholesterol (60%/40%, w/w). Liposomes were prepared with different techniques, such as thin layer evaporation, freezing and thawing, extrusion through microporous membrane, and reverse phase evaporation method, obtaining, respectively, multi-lamellar vesicles (MLV), frozen and thawed MLV (FATMLV), small uni-lamellar vesicles (SUV) and large uni-lamellar vesicles (LUV). Size and morphology of the different types of liposomes were investigated by light scattering analysis, transmission electron microscopy, and confocal laser scanning microscopy, whereas drug entrapment efficiency was determined by dialysis experiments. Cyclodextrin complexation improved drug solubilization and allowed a strong improvement of its entrapment into the aqueous liposomal phase. Liposome preparation method and operating conditions clearly affected both liposome size and drug loading capacity. Encapsulation efficiency increased with increasing the complex concentration up to 10 mM, and was in the order MLV > LUV > SUV. An opposite behaviour was observed for FATMLV, probably due to the freezing phase required by such a preparation method, which reduced the complex solubility. Moreover, it was not possible to use higher complex concentrations, due to the destabilizing effect of cyclodextrins toward the liposomal membrane. Permeability studies of drug–HPβCyd complexes, directly in solution or incorporated in liposomes, performed across artificial membranes simulating the skin behaviour, highlighted, as expected, a prolonged release effect of liposomal formulations. Furthermore, the drug permeation rate depended on the vesicle characteristics and varied in the order: SUV > MLV = FATMLV > LUV. Therefore, the most suitable liposome preparation method can be suitably selected on the basis of drug encapsulation efficiency and/or desired drug release rate.

Keywords: Ketoprofen; Cyclodextrin; Liposome; Permeation studies

1. Introduction

Liposomal formulations are widely used in the pharmaceutical field as drug delivery systems due to their versatility and clinical efficacy (Torchilin and Weissing, 1990). They have been used to administer drugs by several routes such as the oral, parenteral, and topical (Takino et al., 1994; Moutardier et al., 2003; Verma et al., 2003a). Among these, topical delivery of drugs carried by liposomes exhibits interesting applications, not only for promoting dermal delivery of drugs which have to act topically, such as local anaesthetics, but also for enhancing transdermal delivery of drugs intended for systemic use, thus more effectively exploiting this non-invasive alternative route to oral administration (Manosroi et al., 2004a; Simões et al., 2005). In fact, liposomes have been widely used as safe vehicles for topical drug delivery systems due to their effectiveness in entrapping drugs and delivering them to the skin, therefore enhancing their clinical efficacy (Gregoriadis, 2000; Verma et al., 2003a, 2003b) in spite of possible vehicle-mediated phenomena of skin uptake (Alvarez-Román et al., 2004).

Ketoprofen is a poorly water-soluble non-steroidal anti-inflammatory drug, broadly used as analgesic and for the treatment of rheumatoid arthritis and osteoarthritis. Its oral administration is associated with a high risk of adverse gastro-intestinal effects; it is therefore considered a good candidate for transdermal administration (Cordero et al., 1997, 2001; Hadgraft et al.,

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2000). However it is well known that, owing to the strong barrier function of the skin, transdermal permeation of drugs has to be appropriately improved (Irion et al., 1995; Sinh et al., 1996; Cho and Choi, 1998).

Unfortunately, the carrier functions of liposomes across the skin cannot be effectively exploited by drugs, such as ketoprofen, that, due to their lipophilic nature, are commonly incorporated in the membrane bilayers, and, consequently, are quickly set free after administration (Takino et al., 1994). Moreover, accommodation of water-insoluble drugs in the lipid bilayers of liposomes can be detrimental to the bilayer formation and stability and requires the use of suitable organic solvents (McCormack and Gregoriadis, 1994). Entrapment into the aqueous liposomal phase of lipophilic drugs in the form of water-soluble cyclodextrin inclusion complexes has been recently investigated as a new potential strategy for overcoming such problems and merging the relative advantages of the two types of carriers into a single system, by obtaining drugs-in-cyclodextrins-in-liposomes formulations (McCormack and Gregoriadis, 1994). This approach can be useful to both increase drug solubility and stability (Loukas et al., 1995, 1998) and better control the in vivo fate of poorly soluble drugs, avoiding the rapid release observed after conventional incorporation into the liposome lipid phase (Takino et al., 1994; McCormack and Gregoriadis, 1996, 1998).

The possibility of using such a combined strategy aimed to simultaneously exploit the cyclodextrin solubilizing power towards the drug (Mura et al., 1998) and the liposome carrier function through the skin has recently been investigated for improving the transdermal delivery of ketoprofen (Maestrelli et al., 2005). The use of ketoprofen–cyclodextrin complexes, particularly with hydroxypropyl-β-cyclodextrin, allowed its entrapment in the aqueous core of multi-lamellar liposomal vesicles (MLV), thus providing a more effective and stable encapsulation and better control of drug release with respect to the drug alone (Maestrelli et al., 2005).

Therefore, we considered it worthy of interest to extend these previous studies and investigate in depth the role of the liposome preparation method, the vesicle membrane structure (uni- or multi-lamellar) and the liposome dimensions (Verma et al., 2003b) on their properties as drug carriers. The aim of this work was to find the most effective operative conditions to improve the effectiveness of the ketoprofen–cyclodextrin–liposome system in terms of both drug encapsulation efficiency and permeation properties. Toward this purpose liposomes of constant composition (i.e., phosphatidylcholine and cholesterol, 60%/40%, w/w) were prepared by using different preparation methods, namely thin layer evaporation, freezing and thawing, extrusion and reverse phase evaporation techniques, thus obtaining different kinds of vesicles, i.e., respectively multi-lamellar (MLV), frozen and thawed multi-lamellar (FATMLV), small uni-lamellar (SUV) and large uni-lamellar (LUV) ones. The ketoprofen–hydroxypropyl-β-cyclodextrin complex, previously selected as the most effective (Maestrelli et al., 2005), was in all cases added (at different concentrations) to the aqueous phase.

All liposomal systems were examined for drug encapsulation efficiency, particle size, morphology and structure, using, respectively, dialysis, light scattering, transmission electron microscopy and confocal laser scanning microscopy techniques. Permeation properties of the drug from these systems were evaluated in vitro by using artificial lipophilic membranes simulating the skin behaviour (Maestrelli et al., 2005).

2. Materials and methods

2.1. Materials

Ketoprofen (keto), l-α-phosphatidylcholine (PC) and cholesterol (CH) were provided by Sigma–Aldrich (Italy), hydroxypropyl-β-cyclodextrin (HPβCyd) DS 0.6 was a gift from Wacker (Italy). All other reagents were of analytical grade.

2.2. Preparation of drug–cyclodextrin complexes

Ketoprofen–HPβCyd complex was prepared by coevaporation of equimolar drug–Cyd ethanol–water (5:5, v/v) solutions in a rotary evaporator (Büchi R 200/205) at 55 °C. All products were sieved (Retsch, type Vibro) and the 75–150 μm sieve granulometric fraction was used for the following studies.

2.3. Liposome preparation

Liposomes consisting of mixtures of PC and CH (60%/40%, w/w) were prepared by different techniques. Keto–HPβCyd complex at different concentrations (5, 10, 15 and 20 mM) was added to the aqueous phase used to hydrate liposomes.

Multi-lamellar vesicles (MLV) were prepared according to the thin layer evaporation technique (Bangham et al., 1965). In brief, the lipid phase (consisting of a mixture of 60 mg PC and 40 mg CH) was dissolved in chloroform which was then removed under reduced pressure in a rotary evaporator (Büchi R 200/205) at 58 °C, thus obtaining a thin film of dry lipid on the flask wall. Evaporation was continued for 2 h after the dry residue appeared, to completely remove all the traces of the organic solvent. The film was then hydrated by adding 4 mL of distilled water (with or without the dissolved drug–Cyd complex) under vigorous stirring in order to favour the vesicle formation.

Frozen and thawed MLV (FATMLV) were obtained by freezing and thawing MLV (Mayer et al., 1985). Briefly, 4 mL of MLV suspension were placed in a pirex tube and dipped in a nitrogen bath for 30 s; the tubes were then removed and placed in a water bath at 58 °C for 30 s. The sequence was repeated nine times.

Small uni-lamellar vesicles (SUV) were obtained by the extrusion technique (Berger et al., 2001). According to this method, 2 mL of FATMLV suspension were placed in a mini-extruder (Avanti Polar Lipids, Birmingham, AL) thermostated at 58 °C and manually extruded through a 0.2 μm polycarbonate membrane for 19 times.

Large uni-lamellar vesicles (LUV) were prepared by reverse phase evaporation method (Szoka, 1978, 1980; Vyas et al., 2005). In brief, the lipid phase (a mixture of 60 mg PC and 40 mg CH) was dissolved in diethylether, and then mixed with 4 mL of

the aqueous solution containing the drug–Cyd complex (organic phase:aqueous phase 3:1, v/v) in an ultrasound bath (Transonic 460 H, Singen) at 0 °C for 5 min at 355 W, to obtain a water-in-oil emulsion. After removal of the organic solvent with a rotary evaporator under reduced pressure, a gel was formed. Upon vigorous mechanical agitation using a vortex mixer, the gel evolved into a dispersion of large liposomes (Rongen et al., 1997).

All products were quickly sealed in glass containers and stored in the darkness at 4 °C.

2.4. Liposome characterization

2.4.1. Determination of encapsulation efficiency

Liposome encapsulation efficiency was determined using the dialysis technique for separating the non-entrapped drug from liposomes. The previously described technique (Maestrelli et al., 2005) consists of placing 2 mL of drug-loaded liposomal dispersion into a dialysis bag of cellulose acetate (Spectra/Por®, MW cut-off 12,000, Spectrum, Canada) which was immersed in 150 mL water and magnetically stirred at 30 rpm. Samples taken from the receiver solution at predetermined times were replaced with equal volumes of fresh water and spectrometrically assayed at 260 nm for drug content (Hitachi Mod. 2000 spectrophotometer). No interference was found for other components. The experiment was stopped when constant drug concentration values were obtained in subsequent withdrawals from the receiver phase (taking into account the progressive dilution of the medium). The percent of encapsulation efficiency (EE%) was then calculated according to the following equation:

$$\mathrm{EE\%} = \frac{[\text{total drug}] - [\text{diffused drug}]}{[\text{total drug}]} \times 100$$

2.4.2. Determination of liposomal size

The average diameter of the vesicles was determined by photocorrelation spectroscopy (PCS), using a Zetamaster (Malvern Instruments, Malvern, UK) at a temperature of 25 ± 0.1 °C. The intensity of the laser light scattered by the samples was detected at an angle of 90° with a photomultiplier. Liposome suspensions were suitably diluted with distilled water in order to avoid multi-scattering phenomena. At least three independent samples were taken, each of which was measured at least twice, up to four times. For each specimen, 10 autocorrelation functions were analysed using a cumulative analysis. From this analysis, the *z*-average value was obtained, which is an approximation of the diameter of the liposomes. Samples were analyzed 24 h after their preparation.

2.4.3. Transmission electron microscopy (TEM)

TEM analysis (Philips CM 10, Philips, USA) was used to examine the ultrastructure of liposomes. To prepare samples, copper grids were coated with a solution of collodion and then a drop of liposomal dispersion was applied and left in contact for 15 min. Finally, grids were picked up, blotted with filter paper, left to dry for 3 min and then analyzed with TEM (Manosroi et al., 2004b).

2.4.4. Confocal laser scanning microscopy (CLSM)

CLSM studies were performed to characterise liposome morphology and lamellarity directly in solution. Analysis was carried out on a drop of suitably diluted, freshly prepared liposomal dispersion using a Leica TCS SP II Laser Scanning Confocal Imaging System (Leica, Heidelberg, Germany); the apparatus was equipped with a Kr–Ar–He–Ne ion laser and a Leica DM IRE 2 microscope endowed with HC PL Fluotar Leica ×10 and ×20 dry objectives and HCX PLAN APO Leica ×40 multi-immersion objective on its oil position (numeric aperture 0.85). Samples were analyzed by using the transmitted light (López-Pinto et al., 2005).

2.5. In vitro permeation studies

In vitro permeation studies of the drug complex, as such or entrapped in the different types of liposomes were performed for 24 h at 37 ± 1 °C using Franz diffusion cells (Vidrafoc, Barcelona, Spain), with an effective diffusion surface of 2.54 cm^2 and a receiver compartment of 14.5 mL volume (degassed pH 7.4 phosphate buffer solution). The donor compartment was filled with 3 mL of aqueous solution of drug–Cyd complex or liposomal suspension containing the drug–Cyd complex entrapped, all at a drug concentration of 5 or 10 mM. Cellulose nitrate membranes impregnated with lauryl alcohol (membrane weight increase 90–110%) as lipid phase were used as artificial diffusion membranes simulating the epidermal barrier (Mura et al., 1993). Previous comparative studies performed by using excised rat skin or such artificial lipophilic membranes made it possible to demonstrate the good correlation between permeation data obtained with the two methods (Maestrelli et al., 2005).

Care was taken to remove any bubbles between the underside of the diffusion membrane and the solution in the receiver compartment. At predetermined intervals, samples (0.55 mL) were withdrawn from the receptor compartment, replaced with an equal volume of fresh medium, and spectrometrically assayed for drug content at 260 nm. No interference in the drug assay was given by other components. Experiments were performed in sextuple. A correction was made for the cumulative dilution of the receptor medium. The mean cumulative amount of drug diffused through the artificial lipophilic membrane was calculated and plotted as percent of the initial amount in the donor compartment against time. The results obtained at various times (1, 2, 4, 6 and 24 h) were statistically analyzed by one-way analysis of variance (ANOVA) followed by the Student–Neuwman–Keuls multiple comparison post test (GraphPad Prism, version 3). The differences were considered statistically significant when $P < 0.01$.

3. Results and discussion

3.1. Characterization of liposomes

The encapsulation efficiency values (EE%), determined by the dialysis method, of MLV vesicles prepared in the presence of increasing concentrations of keto–HPβCyd complex in the aqueous phase used for liposomal film hydration, are summarised in

Table 1
Encapsulation efficiency (EE%) and concentration of drug entrapped into MLV vesicles prepared in the presence of increasing concentrations of ketoprofen–HPβCyd complex

Complex conc. in the aqueous hydration phase (mM)	MLV EE%	*c*(Keto) (mM)
5	42.4 ± 2.2	1.4 ± 0.4
10	75.1 ± 0.7	4.8 ± 0.1
15	57.3 ± 5.5	5.3 ± 0.5
20	59.9 ± 4.2	5.5 ± 0.4

Table 1. As shown, the EE% increased with increasing the complex concentration up to 10 mM, while it decreased at higher complex concentrations. A series of concomitant phenomena may lead to such a finding, including in particular the destabilizing action of Cyds towards liposomal membranes and the low aqueous drug solubility.

As for the first effect, it has been reported that Cyds can reduce vesicle stability by interacting with the membrane lipids (Fatouros et al., 2001). In particular, the liposome destabilizing effect due to the presence of Cyds has been found to depend on both the lipid and the Cyd type and concentration and it has been mainly attributed to a vesicle fluidization induced by Cyd as a consequence of cholesterol extraction from the phospholipid bilayer (Hartel et al., 1998; Bouldemarat et al., 2004; Piel et al., 2004).

On the other hand, as for the poor drug solubility, even if it markedly increased by Cyd complexation, it is however negatively influenced by temperature reductions. Probably, when the keto–Cyd complex is added at concentrations near to its maximum solubility at ambient temperature, it could partially precipitates during the liposome storage at 4 °C.

The results of TEM analyses of MLV vesicles prepared, respectively, in the presence of 5 and 10 mM complex concentrations are shown in Fig. 1A and B. Both these kinds of liposomes presented a homogeneous, regular, spherical shape; a slight increase in size was observed with increasing the drug loading, in agreement with the findings of light scattering measurements. Moreover, TEM analysis confirmed also the previous hypothesis of a possible drug precipitation phenomenon with increasing the complex concentration. Indeed vesicles prepared in the presence of 15 and 20 mM of complex concentration, whose images are reported respectively in Fig. 1C and D, showed the presence of drug crystals. The same phenomenon was previously observed in MLV vesicles loaded with keto–βCyd complex at 5 mM concentration (Maestrelli et al., 2005), and it has been attributed to drug precipitation, during the MLV preparation process, due to the poor stability of the drug complex with the native Cyd (Mura et al., 1998). In fact, drug crystals were not detectable in the presence of the same concentration of the more stable complex of keto with HPβCyd (Maestrelli et al., 2005).

Therefore, 5 and 10 mM concentration of keto–HpβCyd complex in the aqueous phase were selected as the optimal ones for liposome preparation and used to prepare other kinds of liposomes, i.e., FATMLV, LUV and SUV.

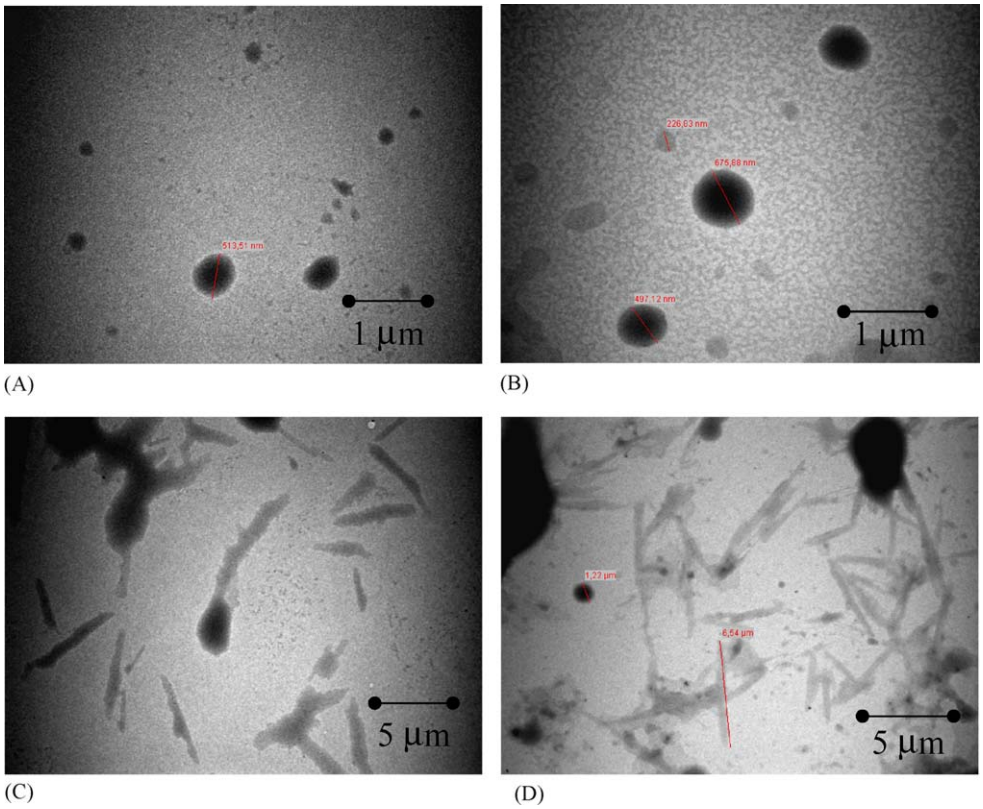

Fig. 1. TEM micrographs of MLV vesicles prepared in the presence of increasing concentrations of ketoprofen–HPβCD complexes: 5 (A), 10 (B), 15 (C) and 20 mM (D).

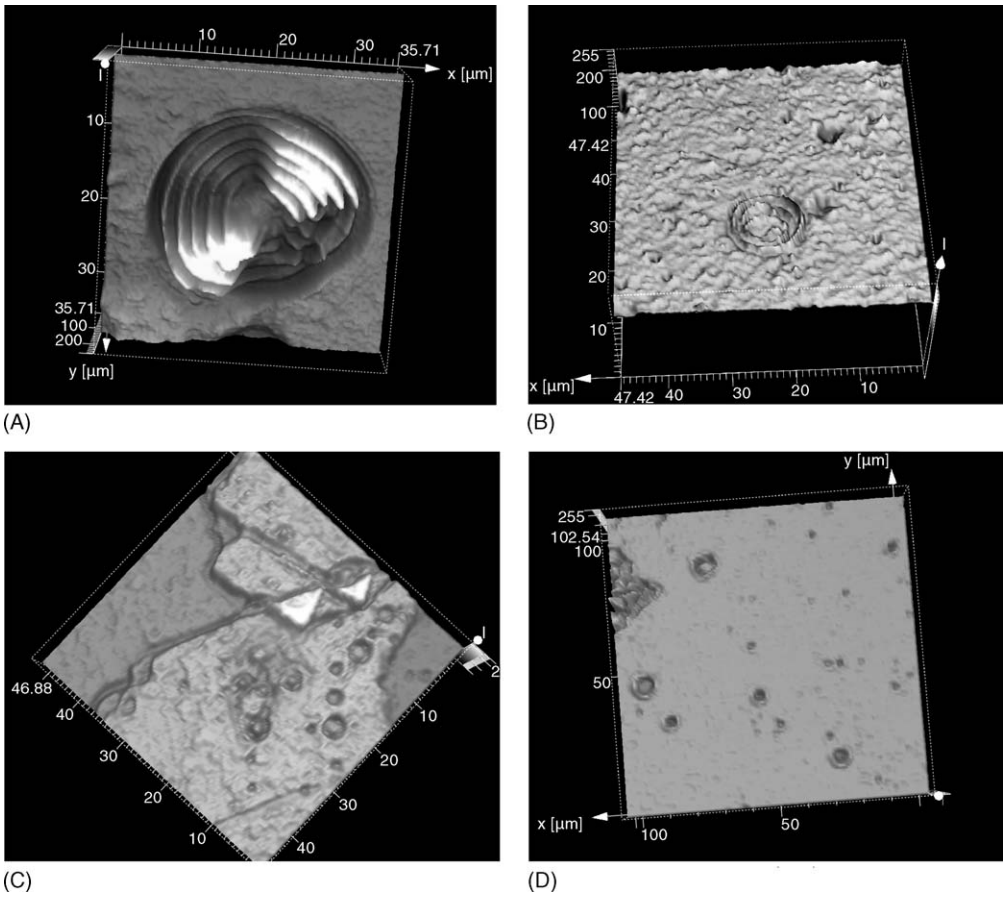

Fig. 2. Transmission tridimensional CLSM images of different kinds of liposomes all prepared in the presence of 5 mM ketoprofen–HPβCyd complex: MLV (A), FATMLV (B), LUV (C) and SUV (D).

CLSM images of vesicles prepared with different methods, always in the presence of 5 mM complex concentration, are shown in Fig. 2. As observed in Fig. 2A, MLV vesicles present a typical concentric lamellar structure, while FATMLV ones (Fig. 2B) show a less regular morphology due to the "traumatic" preparation method, as reported also by other authors (Mayer et al., 1985). In fact the cycles of freezing and thawing give rise to the rupture of phospholipid bilayers that can re-constitute in an untidy lamellar structure. LUV and SUV vesicles (Fig. 2C and D, respectively) are instead unilamellar and have a smaller particle size than MLV and FATMLV ones (Szoka, 1978, 1980; Pidgeon et al., 1986; Berger et al., 2001). No morphologic or structural differences were observed in comparison with the corresponding types of vesicles obtained in the absence of Cyd; therefore, it can be assumed that the presence of Cyd does not affect the liposome lamellar structure.

The mean particle size and the EE% of vesicles obtained with the different procedures examined, both empty or loaded with the keto–HpβCyd complex (5–10 mM), are summarised in Table 2. Encapsulation of the complex resulted in an appreciable size increase for MLV, FATMLV and LUV vesicles, in agreement with previous findings (Maestrelli et al., 2005). However, literature data about the influence of Cyd presence on the liposome dimensions are controversial. In accordance with our results, some authors observed significant size increases for liposomes containing the drug–Cyd complexes in comparison with empty liposomes or liposomes containing drug alone (Becirevic-Lacan and Skalko, 1997; Oommen et al., 1999); on the contrary, other authors reported the opposite (Skalko et al., 1996; Fatouros et al., 2001). Probably, these conflicting results are due the different

Table 2
Encapsulation efficiency (EE%) and mean particle size of liposomes obtained with different methods, in the presence of different concentrations (0, 5 and 10 mM) of ketoprofen–HPβCyd complex

Liposome type	Complex conc. in the aqueous hydration phase (mM)	EE%	Particle size (μm)
MLV	0	/	1.58 ± 0.08
	5	42.4 ± 2.2	3.16 ± 0.10
	10	75.1 ± 0.7	3.71 ± 0.01
FATMLV	0	/	4.52 ± 0.52
	5	68.6 ± 3.0	5.32 ± 0.69
	10	50.2 ± 10.2	5.45 ± 0.71
LUV	0	/	0.73 ± 0.02
	5	43.7 ± 4.7	1.16 ± 0.05
	10	61.6 ± 0.8	1.85 ± 0.04
SUV	0	/	0.22 ± 0.01
	5	38.0 ± 0.3	0.25 ± 0.02
	10	54.8 ± 1.1	0.26 ± 0.01

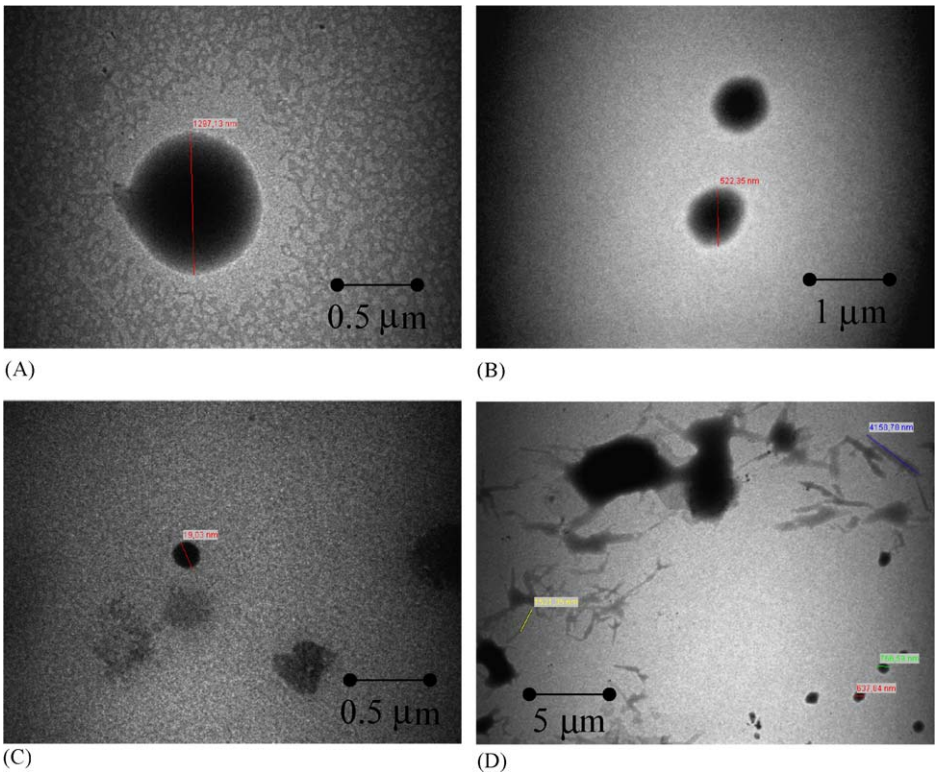

Fig. 3. TEM micrographs of liposomes prepared in the presence of ketoprofen–HPβCyd complex 5 mM: FATMLV (A), LUV (B), SUV (C) and FATMLV prepared in the presence of 10 mM complex (D).

experimental conditions and/or techniques utilised to prepare and/or analyze empty and loaded liposomes.

To support this possible explanation, in the case of SUV vesicles, no significant size modification was observed in the presence of Cyd, not even when the complex concentration was increased. These findings can be explained by taking into account that the particle size of this kind of liposomes is mechanically determined by the pore size of the membrane used for their extrusion and, consequently, the presence of Cyd cannot significantly affect the liposome dimension.

Moreover, SUV vesicles showed the lowest EE% compared with the other types of liposomes. The reduced volume of the aqueous compartment, with respect to the other formulations, due to the smaller dimensions of these uni-lamellar vesicles can be considered the main factor responsible for this result.

As for the other types of liposomes examined, MLV and LUV vesicles showed an increase of drug loading with increasing the keto–Cyd complex concentration, while the opposite effect was observed for FATMLV ones. These results can be explained by the different liposome preparation methods. In fact, in the case of FATMLV vesicles, the decreased EE% at higher complex concentration was due to the drug precipitation arising during the freezing cycle, as demonstrated also from TEM images presented in Fig. 3. This picture shows the micrographs of FATMLV, LUV and SUV vesicles prepared in the presence of 5 mM of complex (Fig. 3A–C) and FATMLV ones prepared in the presence of 10 mM of complex (Fig. 3D). In this last image the presence of typical drug crystals, probably precipitated in the aqueous phase during the freezing process, is evident.

3.2. In vitro permeation studies

A preliminary study, performed to evaluate the effectiveness of the drug-in-cyclodextrin-in liposome approach for the development of a transdermal formulation of keto, highlighted a favourable effect of such a formulation type on the drug permeation rate but did not evidence any skin-permeation enhancer properties of liposomes (Maestrelli et al., 2005). This was in agreement with most reports which generally exclude a liposome transport process through the skin and attribute their positive influence on drug skin delivery to a localising effect whereby they accumulate drugs in surface or upper layers of stratuum corneum, thus favouring their diffusion (Barry, 2001). In this study a good correspondence was found between drug permeation data from excised rat skin and an artificial lipophilic membrane (Maestrelli et al., 2005). Therefore, being the composition of the liposomes used in the present work the same (as in the previous study), we considered it adequate to perform permeation experiments using such an artificial membrane, thus overcoming problems of longer times, higher costs and lower reproducibility related to the use of animal membranes.

Drug permeability profiles across artificial lipophilic membranes from liposomal suspensions or simple solutions containing the keto–Cyd complex are reported in Fig. 4.

A rapid drug permeation rate was observed in the case of solutions containing the drug complex, where more than 60% of drug permeated within the first 4 h. As expected, liposome formulations gave rise to a slower and more prolonged drug permeation in comparison to simple drug solutions

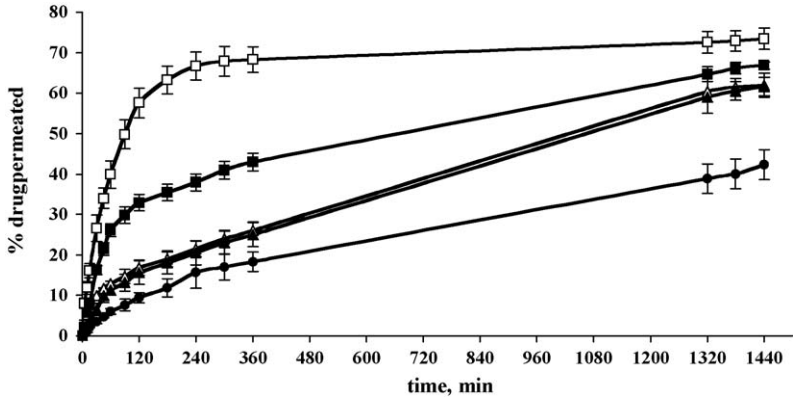

Fig. 4. Ketoprofen permeation profiles across artificial lipophilic membrane from solutions (□) or from liposomes MLV (▲), FATMLV (△), LUV (●) and SUV (■) all containing 5 mM ketoprofen–HPβCyd complex.

(Gregoriadis, 1988). Interestingly, different permeation profiles were observed, depending on the vesicle characteristics. In particular, LUV vesicles, prepared by the reverse phase evaporation technique, showed the slowest drug release with respect to the other liposomal formulations at all the examined times ($P < 0.01$), reaching only about 40% of permeated drug after 24 h. This could be a consequence of the greater density and viscosity of such a liposomal dispersion, due to the higher phospholipid concentration arising from the particular preparation method used (Pidgeon et al., 1986). On the contrary, the faster drug release ($P < 0.01$) obtained from SUV liposomes, particularly evident in the initial phase (almost 40% drug permeated within 4 h) is attributable to their smaller size (Verma et al., 2003b). An intermediate behaviour (about 40% drug permeated within 12 h) was observed for MLV and FATMLV vesicles which showed substantially similar drug release rates ($P > 0.05$). Such a result can be explained by the substantially analogous multilamellar structure of these liposomes.

4. Conclusion

The influence of liposome preparation method on the encapsulation efficiency and release properties of keto, entrapped in the vesicle aqueous phase as complex with HPβCyd, has been investigated in the present work.

Entrapment of keto–HPβCyd complex was successfully obtained with all the examined preparation techniques. The EE% depended on both the liposome preparation method and the complex concentration in the aqueous phase used for liposome preparation, and was in the order MLV > LUV > SUV. The only exception to this trend was given by FATMLV vesicles, where a reduced EE% was observed when increasing the complex concentration from 5 to 10 mM, due to the drug precipitation during the freezing cycles required by the method. On the other hand, because of the destabilizing effect of Cyd toward liposomes, due to its complexing capacity toward the components of the membrane bilayers, it was not possible to use complex concentration higher than 10 mM.

Interestingly, a different drug permeation rate was observed from the different liposomal formulations (i.e., solutions > SUV > FATMLV = MLV > LUV). These findings could be usefully exploited to suitably modulate and control keto release rate, depending on the need for a rapid drug release, as in the case of acute treatments, or a slower and prolonged drug release, as for long term therapies, with the final aim of improving its clinical effectiveness.

In particular, MLV vesicles exhibited the highest EE% value, thus they represent the best formulation to optimize both drug entrapment efficiency and permeation rate when a prolonged drug release is desirable.

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