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Research paper

Development of a new delivery system consisting in "drug – in cyclodextrin – in nanostructured lipid carriers" for ketoprofen topical delivery

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ABSTRACT

A new delivery system based on drug cyclodextrin (Cd) complexation and loading into nanostructured lipid carriers (NLC) has been developed to improve ketoprofen therapeutic efficacy. The proposed strategy exploits both the solubilizing and stabilizing properties of Cds and the prolonged release, high tolerability and percutaneous absorption enhancer properties of NLC. Two different polymeric Cds, i.e. β-Cd-epichlorohydrin polymer (EPI-βCd) and carboxymethylathed-β-Cd-epichlorohydrin polymer (EPI-CMβCd) were tested and two different techniques to obtain solid ketoprofen-polymeric Cd complexes (i.e. co-grinding and co-lyophilization) were compared, to investigate the influence of the preparation method on the physicochemical properties of the end product. EPI-βCd was more effective than EPI-CMβCd in enhancing the solubility and dissolution properties of ketoprofen. Co-grinding in dry conditions was the best preparation technique of solid drug-Cd systems, allowing obtainment of homogeneous amorphous particles of nanometric range. NLC consisting in a mixture of Compritol® 888 ATO (glyceryl behenate) and Labrafac Lipophile were obtained by ultrasonication. Both empty and loaded NLC were suitably characterized for particle size, pH, entrapment efficiency and drug release behavior. The best (drug-Cd)-loaded NLC system, formulated into a xanthan hydrogel, exhibited drug permeation properties clearly better than those of the plain drug suspension or the plain drug-loaded NLC, in virtue of the simultaneous exploitation of the solubilizing effect of cyclodextrin and the penetration enhancer properties of NLC.

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1. Introduction

Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) have been introduced in the last decade as an interesting alternative to the traditional colloidal carriers, such as nanoemulsions, liposomes and polymeric nanoparticles, in virtue of the lower cost of raw materials, particularly compared to phospholipids, higher physical stability, easy of scale-up and manufacture, and great versatility [1–3]. Moreover, lipid nanoparticles combine the advantages of both polymeric nanoparticles, due to the presence of a solid matrix, which can protect chemically labile drugs and allow a control of drug release rate, and nanoemulsions and liposomes, being made of biocompatible, well-tolerated and regulatory accepted lipids [4]. In particular, NLC have been recently developed as the second generation of lipid nanoparticles, in order to overcome some drawbacks presented by SLN. Differently from SLN, which are made only by solid lipids, NLC consist of a solid lipid matrix entrapping a liquid lipid (oil) as nanocompartments. Such a particular structure leads to distinct advantages with respect to SLN, such as improved drug loading ability, increased physical stability, reduced risks of drug leakage during storage [5,6]. Several administration routes of lipid nanoparticles, including the parenteral, oral, and topical ones, have been investigated [7–9]. Among these, lipid nanoparticles showed to be particularly advantageous as carriers for topical administration of active pharmaceutical ingredients, allowing obtainment of a prolonged release and improved bioavailability of the drug at the site of action [10–12].

Ketoprofen is a non-steroidal anti-inflammatory drug commonly used in the treatment of musculoskeletal disorders, such as osteoarthritis and rheumatoid arthritis, whose bioavailability is limited by its very low water solubility. An improvement in ketoprofen bioavailability has been reported as directly related to its improved solubility and dissolution rate obtained by complexation with natural and alkylated cyclodextrins [13–15]. Topical administration of ketoprofen should be particularly beneficial for the treatment of arthritic conditions, since it would make it possible an increased presence of the drug locally and a reduced appearance of systemic side effects. Different approaches have been exploited in the attempt of improving the non-optimal characteristic of ketoprofen to be delivered through the skin, such as use of prodrugs [16,17], or loading into liposomes [18] or into lipid nanoparticles [12].

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Considering all these premises, in the present work, we thought it worthy of interest to investigate the feasibility of developing a new drug delivery system for ketoprofen topical administration based on a combined strategy which exploits both cyclodextrin complexation, to enhance drug solubility and dissolution properties, and loading of the complex into NLC, to improve drug delivery through the skin. In the framework of this project, as a further objective of our work, it seemed of interest to evaluate the performance of some water-soluble polymeric cyclodextrin derivatives for ketoprofen complexation, owing to the superior solubilizing and complexing abilities toward different drugs exhibited by these derivatives with respect to both parent cyclodextrins and non-polymeric derivatives [19,20]. With this aim, two different water-soluble polymeric cyclodextrins, i.e. β-cyclodextrin-epichlorohydrin and carboxymethylathed-β-cyclodextrin-epichlorohydrin polymers have been selected, and the effectiveness of two different techniques (co-grinding and co-lyophilization) to obtain solid ketoprofen-polymeric cyclodextrin complexes with improved drug dissolution properties has been investigated. The most effective drug-cyclodextrin systems were selected for loading into NLC obtained by ultrasonication. Both empty and loaded NLC were suitably characterized for particle size, pH, entrapment efficiency and drug release properties. The best complex-loaded NLC systems were finally formulated into a xanthan hydrogel and assessed for rheological behavior and *in vitro* permeation properties.

2. Materials and methods

2.1. Materials

Ketoprofen (Keto) and xanthan gum were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and glycerol from Galeno s.r.l. (Prato, Italy). β-Cyclodextrin-epichlorohydrin polymer (EPI-βCd, average MW 3500) and carboxymethylathed-β-cyclodextrin-epichlorohydrin polymer (EPI-CMβCd, average MW 5300) were kindly supplied by CycloLab, Hungary. Compritol® 888 ATO (glyceryl behenate), a mixture of mono-, di-, and tri-glycerides of behenic acid (C22), and Labrafac Lipophile (caprylic/capric tri-glycerides) were kindly donated by Gattefossè (Milan, Italy). Lutrol® F68 was a gift of BASF Chemical Trade GmbH (Burgbernheim, Germany). All other materials and solvents were of analytical reagent grade.

2.2. Preparation and characterization of Keto-Cd solid systems

Physical mixtures (P.M.) of Keto with EPI-βCd and EPI-CMβCd at 10/90 (w/w) drug/carrier ratios were prepared by simple blending of the powders in a turbula mixer for 15 min. Co-ground products (GR) were obtained by grinding the physical mixture in a high-energy vibrational micromill (Mixer Mill Type MM 200, Retsch, GmbH, Düsseldorf, Germany) at 24 Hz for different times (10, 20, 30 or 60 min), in dry conditions or in the presence of 10% moisture content. Co-lyophilized products (COL) were prepared by dissolving the physical mixtures in an aqueous solution containing the minimum volume of ethanol. The resulting solutions were frozen at −40 °C and then lyophilized (Lyovac GT2, Leybold–Heraeus, SRK System Technik GmbH, Germany).

The physicochemical and morphological properties of pure components and their solid combinations were investigated by differential scanning calorimetry (DSC), Fourier transform-infrared (FT-IR) and dynamic light scattering (DLS).

DSC curves were recorded using a Mettler TA 4000 Star^e system apparatus equipped with a DSC 25 cell (Mettler Toledo, Milano, Italy). Accurately weighed samples (M3 microbalance, Mettler Toledo) were scanned in pierced Al pans (5–10 mg) at 10 °C min^{-1} over a temperature range of 30–200 °C, under static air. Melting point and fusion enthalpy of indium were used for temperature and heat capacity calibration. The relative degree of drug crystallinity (*%RDC*) in the samples was calculated according to the following equation:

$$\%RDC = \frac{\Delta H_{sample}}{\Delta H_{drug}} \times 100 \quad (1)$$

where ΔH_{sample} and ΔH_{drug} are the measured heat of fusion of the sample and of pure drug, respectively, normalized to the drug content in the sample.

FT-IR spectra were recorded on a Perkin-Elmer Model 1600 apparatus (Wellesley, USA) using KBr discs in the range of 4000–400 cm^{-1}.

DLS analyses were carried out using a Brookhaven Instrument apparatus (Brookhaven Instruments Corporation, New York, USA) equipped with a BI 9000AT correlator card and BI 200 SM goniometer. Samples were suspended in bidistilled water, sonicated for 10 min and suitably diluted. Analyses have been performed at 25 °C on 0.5 mL samples transferred into cylindrical Hellma scattering cells. The intensity of the laser light scattered by the samples was detected by an EMI 9863B/350 photomultiplier at an angle of 90°corresponding to a scattering vector $q = 4\pi n/\lambda \sin(\theta/2) = 2.22e^{-03}$, where n is the refractive index of the medium, equal to 1.33. At least three independent samples were taken, each of which was measured four times. The analysis of the line width distribution was carried out by the constrained regularization method (CONTIN). The particle size distribution was characterized by means of the polydispersity index (P.I.).

2.3. NLC preparation and characterization

NLC were prepared according to the procedure of Puglia et al. [21], which was slightly modified. Briefly, Labrafac Lipophile (1.52 g) was added to the Compritol® 888 ATO (4 g) melted at 70 °C; the lipid phase was then dispersed in the hot (70 °C) surfactant solution (Lutrol® F68, 1.35% w/v) by a high-speed stirrer (Ultra Turrax T25, IKA-Werke GmbH&Co. KG, Staufen, Germany) at 8000 rpm for 1 min. The obtained pre-emulsion was ultrasonified 5 min with a UP 400 S (Ultraschall-prozessor, Dr. Hielscher GmbH, Germany) at constant temperature (75 °C). Finally, the dispersion was cooled to solidify the lipid matrix and produce NLC. Loading of NLC with pure drug (80 mg) or drug-Cd systems (equivalent to 80 mg Keto) was performed, respectively, by adding plain Keto to the melted lipid mixture or the Keto-Cd system to the surfactant solution, so that the drug concentration in the final dispersion was 0.4 mg/mL.

Mean particle size of the lipid dispersions was measured by DLS analysis as described above. NLC dispersions were suitably diluted with bidistilled water in order to avoid multiscattering phenomena. The pH of the lipid dispersions was measured using a Crison 20 pHmeter (Crison Instruments, S.A., Barcelona, Spain). Standard buffer solutions (pH 4.0 and 7.0) were used to calibrate the instrument before use at 20 °C.

2.4. Determination of NLC entrapment efficiency

The percentage of drug entrapped into NLC was determined indirectly after centrifugation for 12 min at 4000*g* (HERMLE Labortechnik, mod. Z200A, Wehingen, Germany) of 1.5 mL of NLC dispersion placed in the upper chamber of a membrane concentrator (Vivaspin 2 MWCO 10,000, Vivascience AG, Hannover, Germany). The drug concentration in the filtrate collected in the lower chamber was determined by UV spectroscopy (Shimadzu UV-1601) at 260 nm. The percent of entrapment efficiency (% EE) was calculated according to the following equation:

$$\% \text{ EE} = \frac{[\text{total drug}] - [\text{diffused drug}]}{[\text{total drug}]} \times 100 \quad (2)$$

where "total drug" is the amount of the initial drug added during NLC preparation and the "diffused drug" is the amount of free drug detected in the filtrate.

It was verified that the presence of lipids did not interfere with the UV spectrophotometric assay of the drug. Each result is the mean of five separate experiments.

2.5. Drug release studies

Drug release studies from NLC were performed according to the dialysis technique [18]. Experiments were carried out under sink conditions. Briefly, 1.5 mL of NLC dispersion was dropped into a cellulose acetate dialysis bag (Spectra/Por®, MWCO 12,500, Spectrum, Canada) immersed in 150 mL of a pH 7.4 phosphate buffer solution magnetically stirred at 37 °C (Keto solubility at pH 7.4 > 1.4 mg/mL). Samples were taken at predetermined intervals from the receiver solution, replaced with equal volumes of fresh solvent, and spectrometrically assayed for drug concentration at 260 nm. The correction for the cumulative dilution was calculated. The release studies were performed in triplicate (C.V. < 3%).

2.6. Preparation and characterization gel formulations

NLC were formulated into hydrogels based on xanthan gum as gel-forming agent and glycerol as plasticizer [21]. Gel formulations were prepared by adding to Keto-loaded or Keto-EPI-βCd-loaded NLC suspensions (93%, w/w), glycerol (5% w/w) and xanthan gum (2% w/w), and maintaining under stirring at 1000 rpm for 5 min, up to gelification. Control formulations were produced in the same way, by using plain Keto or Keto-EPI-βCd aqueous suspensions instead of NLC suspensions (Table 1). The gel formulations were stored at 4 °C before use.

Rheological properties of gel formulations were assessed at ambient temperature (25 °C) using a rotational viscosimeter (Contraves Rheomat 108, Contraves Industrial Products Ltd., Ruislip, U.K) equipped with a rotor N1, 30 mm diameter. The gel (30 g) was placed in a 50-mL beaker. The spindle speed was increased up to 355 s^{-1} and then lowered up to 0. The shear stress (τ) and viscosity (η) values obtained allowed construction of the flow and viscosity curves for each gel. The consistency index (K) and flow index (n) were calculated from the following equation [22]:

$$\tau = KD^n \quad (3)$$

where τ is the shear stress and D the shear rate. The slope of the plot obtained by plotting $\log \tau$ versus $\log D$ represents the flow index and the antilog of the y-intercept the consistency index.

The gel spreadability was determined by the following technique: 0.1 g gel was placed within a 1-cm-diameter circle premarked on a glass plate over which a second glass plate was placed. A weight of 500 g was allowed to rest on the upper glass plate for 5 min. The increase in the diameter due to gel spreading was noted.

The pH of the gels was measured with a Crison 20 pH-meter (Crison Instruments, S.A., Barcelona, Spain).

Table 1
Composition (% w/w) of gel formulations.

Constituents	Formulation code				
	A[a]	B[a]	C	D	E
Keto suspension	93				
Keto-EPI-βCd co-ground suspension		93			
Keto-loaded NLC suspension			93		
Keto-EPI-βCd phys. mix.-loaded NLC suspension				93	
Keto-EPI-βCd co-ground-loaded NLC suspension					93
Glycerol	5	5	5	5	5
Xanthan gum	2	2	2	2	2

[a] Keto amount was correspondent to Keto content in formulations C–E.

2.7. In vitro permeation studies

In vitro permeation studies of Keto from gel formulations were performed at 37 °C using Franz-type diffusion cells provided by Vidrafoc (Barcelona, Spain). A cellulose nitrate membrane (effective permeation area: 2.54 cm^2) impregnated with lauryl alcohol as lipid phase (membrane weight increase 90–110%) was used as artificial lipophilic membrane simulating the epidermal barrier [18,23]. The receiving compartment (7.2 mL) consisted of a degassed pH 7.4 phosphate buffer solution. The donor compartment was filled with 0.100 g of gel formulations. Care was taken to remove any bubbles between the underside of the diffusion membrane and the solution in the receiving compartment. Experiments were performed in triplicate for 6 h. At predetermined time intervals, samples (0.5 mL) were withdrawn from the receptor compartment, spectrometrically assayed, as described above, for drug content and replaced with an equal volume of fresh medium. A correction for the cumulative dilution was calculated. The cumulative amount of drug transferred into the receptor side was calculated, and the results were averaged (C.V. < 3%).

3. Results and discussion

3.1. Characterization of Keto-Cd solid systems

The structural formulas of Keto and the two types of polymeric Cds are shown in Fig. 1. Equimolar solid systems of the drug with both polymeric Cds were prepared by two different techniques, i.e. co-grinding in a high-energy vibrational micro-mill and co-lyophilization, in order to investigate the influence of the preparation method on the physico-chemical properties of the end product and select the most effective system. In particular, since it has been reported that both the time of treatment [24] and the moisture presence during co-grinding [25–27] can markedly affect the drug-Cd interaction and the formation of nanoparticles, the co-grinding process was performed for different times and under dry or controlled moisture conditions. The obtained products were characterized by DSC, FT-IR and DLS analyses, and compared with the corresponding physical mixtures.

Table 2 summarizes the thermal parameters, in terms of onset and fusion temperature, fusion enthalpy and relative degree of crystallinity of Keto alone and in its binary systems with EPI-βCd and EPI-CMβCd obtained by the different techniques and under different experimental conditions. As shown, the co-lyophilization process was able to induce a complete loss of drug crystallinity, irrespective of the cyclodextrin used. In the case of co-grinding treatment under dry conditions, for both series of products a progressive lowering of drug onset and melting peak temperature, accompanied by a concomitant reduction in the related enthalpy value, was observed with increasing the co-grinding time, indicative of a progressive drug amorphization process and/or inclusion complexation, that was completely achieved after 60 min in the systems with EPI-βCd and only after 30 min in those with EPI-CMβCd. A different behavior was instead observed for co-ground products obtained in the presence of 10% moisture content. In fact, in the case of Keto combinations with EPI-βCd, the moisture presence promoted the drug-carrier interaction, facilitating the drug amorphization process, which was achieved after only 30 min of treatment. An opposite effect was instead observed for Keto combinations with EPI-CMβCd, where a residual crystallinity degree

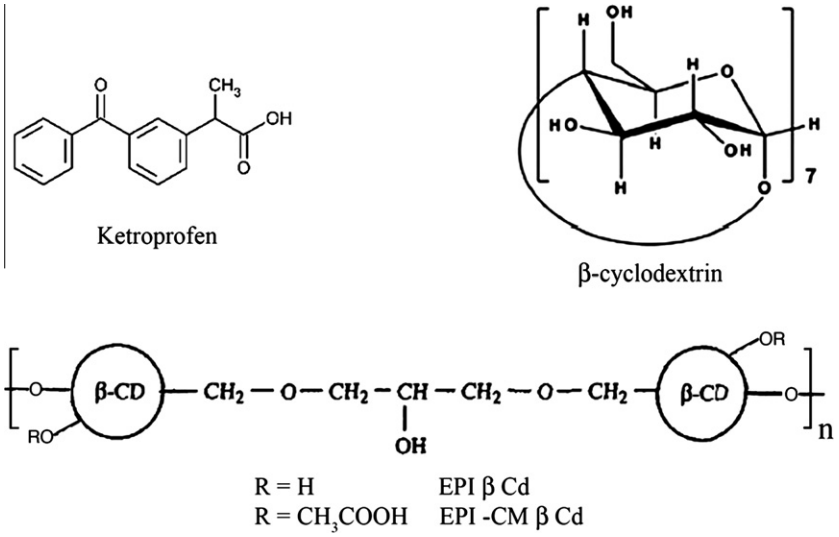

Fig. 1. Chemical structure of ketoprofen, β-cyclodextrin-epichlorohydrin polymer (EPI-βCd) and carboxymethylathed-β-cyclodextrin-epichlorohydrin polymer (EPI-CMβCd).

Table 2
Thermal parameters and relative degree of crystallinity (RDC%) of binary systems of Ketoprofen (Keto) with βCd- and carboxymethylβCd-epichloroydrin polymers (EPI-βCd and EPI-CMβCd) obtained by physical mixing (P.M.), co-lyophilization (COL) and co-grinding (GR) for different times under dry or controlled humidity conditions.

Sample	T_{Onset} (°C)	T_{peak} (°C)	ΔH_{fus} (J/g)	(RDC%)
Keto	94.6	96.5	102.2	100
Keto:EPI-βCd P.M.	91.6	94.0	102.0	99.8
Keto:EPI-CMβCd P.M.	84.8	88.3	93.5	91.5
Keto:EPI-βCd COL	–	–	–	–
Keto:EPI-CMβCd COL	–	–	–	–
GR under dry conditions				
Keto:EPI-βCdEPI GR10′	84.2	89.1	37.1	36.3
Keto:EPI-βCdEPI GR20′	81.5	87.0	17.2	16.8
Keto:EPI-βCdEPI GR30′	80.4	86.3	3.4	3.3
Keto:EPI-βCdEPI GR60′	–	–	–	–
Keto:EPI-CMβCd GR10′	80.0	84.6	36.5	35.7
Keto:EPI-CMβCd GR20′	78.5	82.5	5.7	5.6
Keto:EPI-CMβCd GR30′	–	–	–	–
GR in the presence of 10% humidity				
Keto:EPI-βCdEPI GR10′	81.1	86.4	30.6	29.9
Keto:EPI-βCdEPI GR20′	80.7	87.3	20.9	20.4
Keto:EPI-βCdEPI GR30′	–	–	–	–
Keto:EPI-CMβCd GR10′	85.8	90.2	79.1	77.4
Keto:EPI-CMβCd GR20′	85.1	89.2	67.1	65.7
Keto:EPI-CMβCd GR30′	84.8	89.1	62.9	61.5
Keto:EPI-CMβCd GR60′	84.9	89.8	54.3	53.1
Keto:EPI-CMβCd GR90′	79.2	83.4	26.5	25.8
Keto:EPI-CMβCd GR120′	–	–	–	–

of about 50% was observed even after 60 min co-grinding, and the complete sample amorphization was achieved only after 120 min of treatment. Different moisture levels in drug-Cd ground mixtures can be responsible for different solid-state interactions between the components [25–27]. However, in this case, the different role played by water molecules in the two series of samples cannot be ascribed to a different content of the total water present, since both polymeric Cds had the same content of hydration water, around 2–3%. Therefore, the reason for this unexpected result remains to be investigated further.

Table 3 shows the most representative parameters of FT-IR spectra of Keto, alone and in its different systems with EPI-βCd and EPI-CMβCd. The two characteristic absorption bands of Keto assigned, respectively, to the carboxyl and ketonic carbonyl stretching vibrations [28] are located at 1697 and 1654 cm^{-1}. These bands appeared almost unchanged in the spectra of its physical mixtures with both polymeric Cds, which corresponded to the superposition of the spectra of pure components. On the contrary, a shift to higher frequency of the carboxylic band was registered in the case of all Keto:Cd co-ground and co-lyophilized systems, indicative of the presence of solid-state interactions between drug and carrier brought about by the sample treatment. An analogous shift of this same band was previously observed in co-lyophilized systems of Keto with parent and alkylated β-cyclodextrins [14] and attributed to the breakdown of intermolecular hydrogen bonds between drug molecules [29] and the formation of a monomeric dispersion of drug as a consequence of the interaction with Cds [14].

The different drug-Cd samples were subjected to particle size measurements by DLS analysis, in order to evaluate the effectiveness of the different examined preparation methods in obtaining nano-sized particles. The results obtained, in terms of mean particle size and polydispersity index, are collected in Table 4. Co-grinding under dry conditions and co-lyophilization seemed the most powerful techniques to obtain particles in a nanometer scale range (from 300 to 500 nm). Interestingly, differently from that reported by other authors in the case of natural Cds [25,26], moisture conditions during co-grinding not only did not favor, but, on the contrary, did not allow nanoparticle formation, giving rise to micron-sized particles with both the polymeric Cds used. Evidently, the effect of the moisture content on fine particle formation is depending not only on the type of drug [26], but also on the type of Cd involved in the co-grinding process. In particular, in the case of polymeric Cds, moisture conditions instead of promoting nanoparticle formation, facilitated formation of micro-aggregate of particles. Then, based on the results of DLS analysis, Keto-Cd systems obtained by co-grinding in dry conditions and co-lyophilization were selected for loading into NLC.

3.2. Characterization of drug-loaded NLC

The prepared NLC batches, reported in Table 5, were characterized for particle size, polydispersity index, pH, and entrapment efficiency. The average particle size of empty NLC was estimated to be 460 nm. Loading of drug, alone or in combination with both polymeric Cds, only slightly modified the NLC particle size, which always remained in the nanometric range. Nanoparticles were found to be more homogenously distributed in the case of NLC containing the co-ground products. On the contrary, a wider particle

Table 3
Carbonyl stretching bands (cm^{-1}) of ketoprofen (Keto) alone or as physical mixture (P.M.), coground (GR) and co-lyophilized products (COL) with βCd- and carboxymethylβCd- epichloroydrin polymers (EPI-βCd and EPI-CMβCd).

Sample	Carboxyl C=O stretching (cm^{-1})	Ketonic C=O stretching (cm^{-1})
Keto	1697	1654
Keto:EPI-βCd P.M.	1696	1654
Keto:EPI-CMβCd P.M.	1696	1654
Keto:EPI-βCd GR60′, dry conditions	1745	1656
Keto:EPI-CMβCd GR30′, dry conditions	1735	1654
Keto:EPI-βCd GR30′, 10% humidity	1725	1656
Keto:EPI-CMβCd GR60′, 10% humidity	1701	1654
Keto:EPI-βCd COL	1725	1656
Keto:EPI-CMβCd COL	1735	1654

Table 4
Mean particle size ($n = 4$) and polydispersity index (P.I.) of amorphous systems of ketoprofen (Keto) with EPI-βCd and EPI-CMβCd obtained by co-lyophilization (COL) and co-grinding (GR) in different conditions.

Sample	Particle size (nm) (±SD)	P.I.
Keto: EPI-βCd GR60′, dry conditions	436 ± 26	0.26
Keto:EPI-CMβCd GR30′, dry conditions	300 ± 16	0.13
Keto:EPI-βCd GR30′, 10% humidity	1275 ± 129	0.23
Keto:EPI-CMβCd GR60′, 10% humidity	1581 ± 178	0.13
Keto:EPI-βCd COL	298 ± 18	0.20
Keto:EPI-CMβCd COL	494 ± 32	0.07

size distribution was found for NLC loaded with the co-lyophilized products, as indicated by the polydispersity index values, which were ranged between 0.4 and 0.6. The pH values of all formulations ranged between 3.87 and 4.33, according to the acidic nature of the drug (pH of Keto aqueous suspension = 3.78).

The satisfying values of entrapment efficiency obtained in all cases, never below 40%, can be attributed to the particular inner structure of NLC, where the combined use of a solid lipid matrix with a liquid lipid gives rise to a less compact structure with respect to SLN, able to accommodate greater amounts of drug [5,30]. However, as it appears from data in Table 4, the drug entrapment efficiency was higher for all the binary drug-Cd products than for the plain Keto-loaded NLC, arriving up to a maximum of 77% in the case of Keto-EPIβCd co-ground product. An analogous result was obtained in the case of solid lipid nanoparticles loaded with hydrocortisone, where the use of drug-Cd complexes rather than the drug alone allowed an improvement of about 70% of the incorporated drug amount [31]. Based on these findings, the NLC containing co-ground systems were selected for drug release studies.

3.3. Drug release studies from NLC

The drug release curves obtained by the dialysis method from NLC containing the co-ground products are shown in Fig. 2 together with those of the simple drug suspension and of NLC loaded with free drug or with the drug physical mixture with each

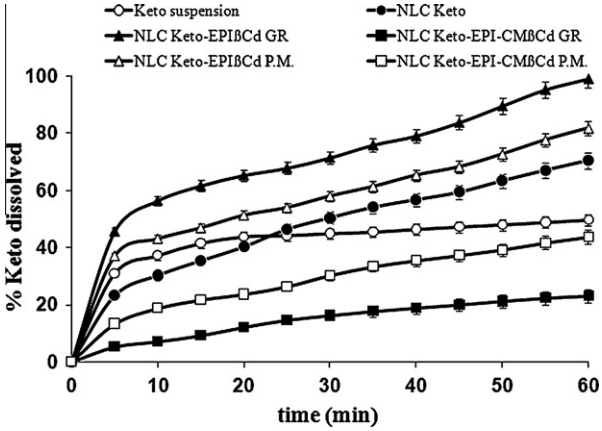

Fig. 2. Ketoprofen release profiles from drug aqueous suspension or from NLC loaded with drug alone or as physical mixture (P.M.) or co-ground product (GR) with EPI-βCd and EPI-CMβCd.

polymeric cyclodextrin for comparison purposes. The drug suspension showed an initial fast release rate, since the fraction of drug in solution was immediately ready to diffuse, but rapidly reached a pseudo-plateau phase. The lower initial release rate observed in the case of Keto-loaded NLC was instead attributable to the time required for drug partition from the lipid matrix. However, being the drug completely dissolved in the lipid nanoparticles, a more regular profile was observed, with a continuous progressive rise in Keto released amount, up to obtain a 1.4-fold increase at 60 min. The drug release from NLC loaded with co-ground systems with the examined polymeric cyclodextrins revealed a very different behavior. In fact, a marked increase in the drug release rate with respect to both drug suspension and plain drug-loaded NLC was observed in the case of Keto-EPI-βCd co-ground systems. An opposite effect was instead seen in the case of co-ground products with EPI-CMβCd. Such a finding seems to be directly related to the different nature of the two carriers as such and not to different drug-Cd interactions brought about by the co-grinding process; in fact, an analogous result has been obtained for NLC loaded with the corresponding physical mixtures. The improvement in the drug release rate registered for combinations with EPI-βCd could be explained with the marked wetting ability of such a cyclodextrin toward the drug, as well as to a solubilizing effect, as a consequence of drug complexation, which was clearly more evident in co-ground products than in simple physical mixtures. On the contrary, the slowing down in drug release rate observed in the case of its combinations with EPI-CMβCd could be attributed to the less water solubility and higher molecular weight of this Cd-derivative with respect to EPI-βCd. Therefore, only NLC loaded with the binary Keto:EPI-βCd co-ground system were selected to be formulated into a hydrogel based on xanthan gum as gel-forming polymer and glycerol as plasticizer in order to obtain topical formulations with the desired semisolid consistency, suitable for skin administration [2,21,32].

Table 5
Mean particle size ($n = 4$), polydispersity index (P.I.), entrapment efficiency (% EE) and pH values of empty NLC dispersions, and NLC loaded with ketoprofen (Keto) alone or as co-ground (GR) or co-lyophilized (COL) product with EPI-βCd and EPI-CMβCd.

Sample	Particle size (nm ± SD)	P.I. (±SD)	% EE (±SD)	pH
Empty NLC	460 ± 14	0.30 ± 0.04	–	
Keto-loaded NLC	494 ± 47	0.12 ± 0.02	43.0 ± 0.1	3.87
Keto:EPI-βCd GR-loaded NLC	500 ± 21	0.25 ± 0.03	77.0 ± 0.5	3.99
Keto:EPI-CMβCd GR-loaded NLC	415 ± 35	0.25 ± 0.03	68.7 ± 0.1	4.10
Keto:EPI-βCd COL-loaded NLC	393 ± 61	0.44 ± 0.04	58.4 ± 1.0	4.33
Keto:EPI-CMβCd COL-loaded NLC	491 ± 80	0.61 ± 0.04	49.6 ± 1.4	4.22

3.4. Rheological properties of gel formulations

Preliminary studies indicated that 2% (w/w) of xanthan gum and 5% (w/w) of glycerol were the most suitable amounts to obtain a NLC-containing hydrogel with the desired rheological properties. The evaluation of the rheological behavior is very important in the development of any new drug delivery system for topical use. In fact, possible interactions between the gel-forming polymer and the formulation components may occur, which can affect the semi-solid consistency of the topical formulation. Unloaded gel and gels containing NLC loaded with pure Keto or Keto-EPI-βCd physical mixtures were also prepared for comparison purposes, in order to highlight any possible effect of the formulation variation on the gel rheological behavior. The flow curves of the gels containing NLC loaded with Keto, alone or as physical mixture or co-ground product with EPI-βCd, are presented in Fig 3, together with that of the blank gel, reported as a reference. The incorporation of lipid nanoparticles into the xanthan gum hydrogel changed its rheological behavior with respect to the blank gel, giving rise to flow curves typical of a pseudo-plastic material, instead of the plastic behavior with yield-point shown by the blank gel. This effect was observed for all gels containing NLC-loaded vesicles, irrespective of the presence or not of the polymeric Cd. The descending flow curves overlapped to the ascending ones, thus allowing to rule out any thixotropic effect. Moreover, for a same value of applied shear stress, the shear rate of NLC-based gels was much lower than that of the blank gel, indicating an increase in gel viscosity and consistency due to the incorporation of lipid nanoparticles, as also confirmed by the increase in both consistency and flow index values, which are reported in Table 6 together with other important characterization parameters of the gel formulation, such as spreadability and pH.

Spreadability is an essential property of topical formulations from the point of view of the patient compliance. In fact, gel application on inflamed or diseased skin would be more comfortable if it can be spread easily. The therapeutic efficacy of a topical formulation can also depend upon its spreadability value. As shown, a slight reduction in this parameter was observed for all the NLC-based gels with respect to the blank one, further confirming the increased gel consistency after incorporation of lipid nanoparticles. However, the obtained values indicated a good spreadability of all the obtained gel preparations [33]. Values of pH were around 4.6 for all NLC-loaded gels, which were within the acceptable range for topical formulations and compatible with the pH of the skin.

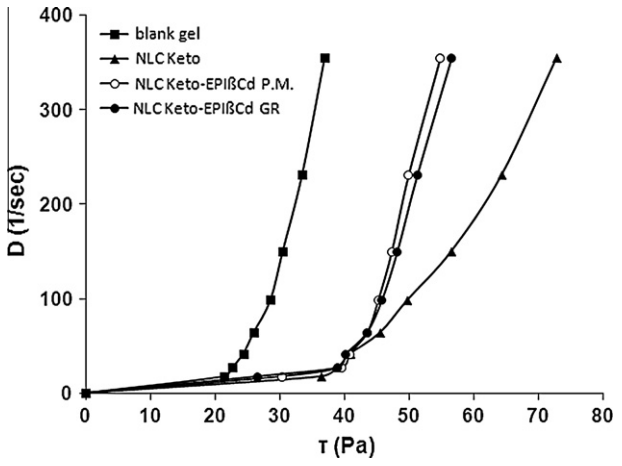

Fig. 3. Flow curves of blank hydrogel or hydrogel loaded with NLC-containing ketoprofen alone or as physical mixture (P.M.) or co-ground product (GR) with EPI-βCd.

Table 6
Physical–chemical and rheological properties of blank hydrogel or containing NLC loaded with ketoprofen (Keto) alone or as physical mixture (P.M.) or co-ground product (GR) with EPI-βCd.

Sample	Consistency index (*K*)	Flow index (*n*)	Spreadability (cm)	pH
Blank gel	1.1	0.054	7.0	4.8
Keto-loaded NLC-based gel	16.3	0.249	6.5	4.6
Keto:EPI-βCd P.M.-loaded NLC-based gel	25.2	0.129	6.2	4.6
Keto:EPI-βCd GR-loaded NLC-based gel	24.9	0.130	6.0	4.6

3.5. Drug permeation studies from gel formulations

Artificial membranes have been proposed as models for skin permeation studies to reduce the use of natural membranes, which presents problems of limited availability of excised skin, longer times and higher cost of experiments, high permeation variability related to the race, age, and anatomical site of the donor [34–36]. A previous study aimed at developing an artificial membrane simulating the skin, indicated lauryl alcohol as the most suitable lipophilic agent to use as representative of skin lipids for synthetic membrane impregnation [23]. The developed artificial membrane allowed for obtaining an optimal correlation with permeation experiments performed with both excised rabbit ear skin [23] and excised abdominal rat skin [18].

However, it must be taken into account that synthetic membranes provide the most useful information about the permeation process when the transport is mainly controlled by a passive transport mechanism, and the formulation does not contain a permeability enhancer. This is the case of NLC formulations, whose positive influence on topical drug availability has mainly attributed to an increased drug accumulation in the skin horny layer, with consequent promotion of its permeation by passive transport, as a function of drug concentration gradient, while any penetration enhancer effect has been excluded [8,12,21].

Therefore, on the basis of all the above considerations, we thought it adequate to perform permeation experiments for a preliminary screening among the different gel formulations by using the formerly developed artificial membrane. This was also in agreement with the general recommendation of reducing the use of experimental animals in biomedical research. Furthermore, the use of the same artificial membrane allowed a precise comparison with permeation experiments with the previously studied liposomal formulations of Keto-Cd complexes [18,37].

The drug permeation profiles from the different gels (formulations A–E, Table 1) are shown in Fig. 4. As expected, the gel with the drug-Cd product (formulation B) showed an improvement in Keto permeation rate in comparison with that with the free drug suspension (formulation A), according to the results obtained from aqueous suspensions of plain Keto alone or as complex with natural βCd or hydroxypropyl-βCd [18]. In fact, being the drug in a concentration above its saturation solubility, the Cd solubilizing effect can accelerate its release by enhancing the proportion of the diffusible species [38]. Gels containing Keto loaded-NLC (formulation C) gave rise to an increase in drug permeation rate with respect to those with the plain drug or its EPI-βCd co-ground product suspensions, exhibiting a positive effect of the lipid nanocarriers on drug permeation. The simultaneous presence of NLC and EPI-βCd, both as physical mixture (formulation D) and even more as co-ground product with Keto (formulation E), allowed a further improvement in the drug permeation rate. Such an effect could be attributed to the combination of the wetting and solubilizing abilities of EPI-βCd with the properties of lipid nanoparticles. In particular, the gel containing NLC loaded with the Keto-EPI-β Cd co-ground

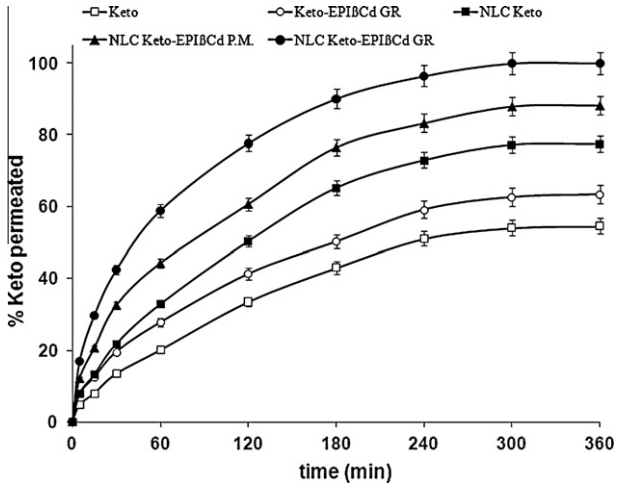

Fig. 4. Ketoprofen permeation profiles from hydrogels loaded with aqueous suspension of drug alone (Keto) or its co-ground product with EPI-βCd (Keto-EPI-βCd GR), or with NLC containing the drug alone (NLC Keto) or as physical mixture (P.M.) or co-ground product (GR) with EPI-βCd.

system gave rise to about a 2-fold increase in the drug amount permeated after 360 min with respect to the gel with the simple drug suspension and 1.3-fold increase with respect to the gel with NLC loaded with plain Keto. A synergistic effect between NLC and EPI-βCd in promoting the drug permeation rate was then pointed out. An opposite result was instead observed in the case of Keto liposomal formulations: the entrapment of Keto into liposomal vesicles caused a reduction in drug permeation rate with respect to the drug aqueous suspensions, and this effect became even more marked in the presence of Keto-Cd complexes [18,37]. The different behavior observed can be explained considering that in the case of liposomal formulations, Keto alone, incorporated in the external membrane bilayer, was more quickly released than the complexed drug, entrapped in the internal aqueous core, which had first to overcome the lipidic barrier of the vesicle. On the contrary, in the case of NLC formulations, the drug-Cd complex, more hydrophilic, is faster released from the lipidic matrix than plain Keto. At the same time, the nano-dimensions and the lipidic nature of NLC ensured a close contact with the lipophilic artificial membrane simulating the stratum corneum, thus allowing a better drug permeation with respect to the simple aqueous suspension.

4. Conclusions

The present work demonstrated the usefulness of the proposed approach, based on drug complexation with polymeric-β-cyclodextrin and loading into NLC, for improving topical delivery of Keto. The newly developed delivery system strengthened the benefits of both the utilized carriers, i.e. Cd and NLC, with regard to their potential for improving the drug therapeutic efficacy and safety, allowing an improvement in both the dissolution and the skin permeation properties of Keto.

The importance of the careful selection of the most effective Cd derivative and of the most suitable preparation method for obtaining effective solid drug-Cd systems has been shown. In particular, EPI-βCd exhibited higher solubilizing properties than EPI-CMβCd, and co-grinding under dry conditions was the best complex preparation technique, giving rise to homogeneous amorphous particles of nanometric range, which enabled a greater entrapment efficiency into NLC with respect to the product obtained by co-lyophilization.

Permeation experiments through the artificial lipophilic membrane previously developed to mimic the skin barrier properties [18,23] enabled a rapid screening among the different gel formulations, and pointed out the best permeation properties of the formulation containing the Keto-EPI-βCd co-ground product loaded into NLC, which gave rise to a 50% and a 23% increase in drug permeation rate with respect to gels containing, respectively, the simple Keto suspension or the plain Keto-loaded NLC.

Future in vivo studies on human volunteers have been planned, in order to verify the superior therapeutic effectiveness of the NLC-Keto-EPI-βCd-loaded gel formulation.

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