Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis
35 (2004) 267–275
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Photostability studies on nicardipine–cyclodextrin complexes by capillary electrophoresis

R. Pomponio [a], R. Gotti [a], J. Fiori [a], V. Cavrini [a,*], P. Mura [b], M. Cirri [b], F. Maestrelli [b]

[a] *Dipartimento di Scienze Farmaceutiche, Università di Bologna, via Belmeloro 6, 40126 Bologna, Italy*
[b] *Dipartimento di Scienze Farmaceutiche, Università di Firenze, via G. Capponi 9, 50121 Firenze, Italy*

Received 1 August 2003; received in revised form 1 August 2003; accepted 23 September 2003

Abstract

Nicardipine (NC)–cyclodextrin solid systems were prepared in equimolar ratios and their photostability in aqueous solution under exposure to UV(A)–UV(B) radiations was evaluated. The photodegradation process was monitored by a capillary electrophoresis (CE) method able to provide the enantioresolution of the *rac*-nicardipine. Enantioresolution was achieved using the mixture 3.0% sulfate-β-cyclodextrin (SβCD) and 2.0% heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TMβCD) as chiral selector in 20 mM triethanolammonium phosphate solution (pH 3.0). The photostability studies were carried out on inclusion complexes of *rac*-nicardipine with α-cyclodextrin (αCD), β-cyclodextrin (βCD), γ-cyclodextrin (γCD), hydroxypropyl-α-cyclodextrin (HPαCD), hydroxypropyl-β-cyclodextrin (HPβCD), hydroxypropyl-γ-cyclodextrin (HPγCD), (2-hydroxyethyl)-β-cyclodextrin (HEβCD) and methyl-β-cyclodextrin (MβCD). A photoprotective effect was observed by βCD, HPαCD, HEβCD, whereas γCD, MβCD, HPβCD and HPγCD did not affect the nicardipine photostability. Conversely, αCD was found to favour the drug photodegradation.

Evidences for CD_s-mediated stereoselective photodegradation of *rac*-nicardipine were observed only for the β-CD complex. In this case, two distinct photodegradation profiles, with two different kinetic constants (*k*), were observed for the nicardipine enantiomers.

Keywords: Cyclodextrin complexes; Nicardipine; Capillary electrophoresis; Enantioresolution; Photostability

1. Introduction

Nicardipine (NC) hydrochloride is a calcium channel blocking agent effective in the management of hypertension, angina pectoris and cerebral disease [1,2]. The drug is administered as racemic mixture, even though (+)-nicardipine was found to be the most powerful of the two enantiomers, which, moreover, present different pharmacokinetic profiles [3]. Like other 1,4-dihyropyridines, NC presents problem of poor water solubility and fast photochemical decomposition [4] which can strongly reduce its bioavailability [1,5].

* Corresponding author. Tel.: +39-051-2099731; fax: +39-051-2099734.
E-mail address: vcavrini@alma.unibo.it (V. Cavrini).

0731-7085/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0731-7085(03)00532-6

Cyclodextrins are cyclic oligosaccharides with the ability to include, entirely or partially into their hydrophobic cavity, a variety of molecules with appropriate molecular size and polarity [6]. The inclusion phenomenon has be often successfully exploited for improving solubility, dissolution rate, bioavailability and chemical stability from oxidation, hydrolysis and photodegradation reaction of several drugs [7–10,15]. Complexation with cyclodextrins has been experimented as a possible approach to enhance the nicardipine solubility and/or stability [11–14]. However, whereas all the authors agreed on the cyclodextrin solubilizing effect towards the drug, conflicting results have been reported as regards their stabilizing effect against NC photodecomposition [11,12]. Moreover, after complexation, different photodegradation rates were observed for the two enantiomers, the effect being dependent on the cyclodextrin used for complexation [12]. On the other hand, it must be also considered that the cyclodextrin interaction with labile compounds can result in different outcomes: cyclodextrins in fact can retard degradation, or have no effect on reactivity or even accelerate drug degradation rate.

Therefore, in the present work we thought of interest to more in-depth investigate the effect of cyclodextrin complexation on the drug photochemical decomposition. Different kinds of cyclodextrins, both natural (α, β, γ) or chemically modified (hydroxypropyl, hydroxyethyl, methyl-cyclodextrin) have been selected, with the aim of evaluating the effect of a series of variables such as the cyclodextrin cavity size, the presence and type of a substituent on the complexation efficiency and NC photodegradation pathway. Drug–cyclodextrin solid complexes were prepared in equimolar ratios according to the results of previous phase solubility [11,12]and ^{1}H NMR [16] studies and characterized by differential scanning calorimetry (DSC), X-ray powder diffractometry and FTIR spectroscopy. Photodegradation studies on *rac*-nicardipine–cyclodextrin complexes in aqueous solution were carried out using a xenon arc lamp (solar simulator) as a UV(A) radiation source. Capillary electrophoresis (CE) under chiral conditions was used to monitor the photostability of the complexed drug and also to evaluate cyclodextrin-induced enantioselective effects in the photodegradation process.

2. Materials and methods

2.1. Chemicals

rac-Nicardipine hydrochloride, triethanolamine and natural cyclodextrins (αCD, βCD and γCD) were purchased from SIGMA (St. Louis, MO, USA); sulfated-β-cyclodextrin (SβCD) and heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TMβCD) were obtained from Aldrich (Milw., WI, USA); randomly chemically modified HPαCD, HPβCD, HPγCD, HEβCD and MβCD were from Wacker Chemie (Germany).

Phosphoric acid, sodium hydrogen phosphate, sodium dihydrogen phosphate and all the other chemicals were of analytical grade and were purchased from Carlo Erba Reagenti (Milan, Italy). The water used for preparation of the solutions and running buffers was purified by a Milli-RX apparatus (Millipore, Milford, MA, USA).

2.2. Preparation of inclusion complexes

Equimolar drug–CD inclusion complexes were prepared according to the coevaporation method: the drug was dissolved in ethanol and the cyclodextrin in water; the resulting hydro-alcoholic solution was evaporated under vacuum in a rotary evaporator at 70 °C. The residue was dried under vacuum at room temperature up to constant weight. Equimolar drug–CD physical mixtures were also prepared for reference. The choice of the 1:1 molar ratio was based on previous phase solubility studies [12,13] and ^{1}H NMR experiments [16].

2.3. Apparatus

Differential scanning calorimetric analysis was performed with a Mettler TA 4000 apparatus (Star^{e} system) equipped with a DSC 25 cell. Samples were weighed (Mettler M3 microbalance) in Al pans (5–10 mg) pierced with a perforated lid and scanned at 10 K × min^{-1} between 30 and 250 °C under static air. X-ray powder diffraction spectra were taken with a computer controlled Philips PWα 1830 apparatus over the 10–50° 2θ range (scan speed 1°/min), using a Cu Kα radiation monochromatized with a graphite crystal.

Infrared spectra were measured on KBr disks using a Perkin-Elmer mod. 1600 FTIR spectrophotometer.

A 150 W Xenon-arc lamp (Solar simulator, model 68805, Oriel Corporation, USA), was used as light source to perform UV radiation exposure tests. Electrophoretic analyses were carried out using a BIO-FOCUS 2000 system (Bio Rad, Hercules, CA, USA). An untreated, fused-silica capillary of total length 50 cm (effective length 43.5 cm), 50 μm i.d. was used for separation. Routine analyses for chiral separations were performed using 20 mM triethanolamine phosphate (pH 3.0) with 3.0% (w/v) SβCD and 2.0% (w/v) TMβCD; $V = 25$ kV and $T = 25\,^{\circ}$C.

2.4. Capillary electrophoresis

The background electrolyte (BGE) solutions were prepared in water. For the optimization of the chiral separation conditions, the following BGE components were evaluated: triethanolamine phosphate (5–200 mM), SβCD (1–7% (w/w)) and TMβCD (1–5% (w/w)). Routine analyses were performed using 20 mM triethanolamine phosphate (pH 3.0) with 3.0% (w/v) SβCD and 2.0% (w/v) TMβCD. Prior the use, the capillary was conditioned by flushing sequentially 1 M sodium hydroxide, 0.1 M sodium hydroxide and finally water (10 min each). When chiral conditions were applied, the highest reproducibility of the migration times was obtained by flushing the capillary between the runs for 2 min with BGE containing 3.0% SβCD and 2.0% TMβCD. Vials of BGE with cyclodextrins were replaced after every injection to avoid change due to the electrolysis of the solutions.

Calibration graphs were simultaneously obtained for the separated *rac*-nicardipine enantiomers. Solutions of *rac*-nicardipine over the concentration range of 0.020–0.100 mg/ml were analyzed by CE under the enantioselective conditions above described. The corrected peak area (area/migration time) for each enantiomer was plotted against the corresponding concentration to obtain the respective calibration graph.

2.5. Photodegradation studies

Solutions of equimolar *rac*-nicardipine-CD complexes (0.050 mg/ml nicardipine in aqueous solutions) were exposed to UV(A)–UV(B) radiations (solar simulator). Solutions of *rac*-nicardipine (0.050 mg/ml) in aqueous solution, containing 15% of methanol to ensure the drug solubility, were simultaneously subjected to the photostability testing. When NC–HEβCD and NC–HPαCD complexes were subjected to the photostability testing, three different concentrations of nicardipine were employed: 0.025, 0.050 and 0.100 mg/ml. Briefly, the solutions were transferred into quartz cells (1 cm path length), closed with Teflon caps and exposed to UV(A)–UV(B) radiations (Xe-arc lamp) for increasing irradiation times, corresponding to increasing radiation doses (J/cm^2). After irradiation all samples were immediately protected from light by aluminum foil. Photoexposed solutions were then subjected to capillary electrophoresis analysis to monitor nicardipine photodegradation.

3. Results and discussion

The photodegradation studies on *rac*-nicardipine–CD inclusion complexes involved the following steps:

1. preparation and solid state characterization of the nicardipine–CD_s solid system;
2. development of CE methods suitable for the determination of *rac*-nicardipine in the presence of its degradation product and also able to provide enantioresolution of *rac*-nicardipine;
3. evaluation of the combined effects of light exposure and CD_s inclusion complexation on the stability of *rac*-nicardipine.

3.1. Solid state characterization of rac-nicardipine–cyclodextrin solid systems

The DSC curve of NC was typical of a crystalline anhydrous substance, exhibiting an initial flat profile followed by a sharp endothermic peak ($T_{onset} = 170.67\,^{\circ}$C, $T_{fus} = 174.00\,^{\circ}$C, $\Delta_{fus}H = 88.0$ J/g) due to the drug fusion process. The thermal profiles of the cyclodextrins in the examined temperature range were all characterized by the presence of very broad endothermic bands ranging from 50 to about 120–130 °C, due to the release of water molecules. The endothermic event corresponding to the drug melting

was always well detectable in the thermal curves of the physical mixtures with the different examined cyclodextrins, even if it appeared slightly broadened and reduced in enthalpy, particularly in combination with amorphous cyclodextrins. On the contrary, DSC analyses of all coevaporated products (COE) showed the total disappearance of the NC melting peak, independent of the cyclodextrin type, indicating the formation of an amorphous solid dispersion, or the inclusion complex formation, or both [13,16,17]. Representative thermal curves of drug binary systems with αCD and HPαCD are reported as an example in Fig. 1.

X-ray powder diffraction spectra confirmed the results of DSC analysis. The diffractograms of NC and natural cyclodextrins exhibited a series of intense peaks, which are indicative of their crystalline nature, whereas all the CD derivatives showed completely diffuse diffraction patterns, which revealed their amorphous state. The patterns of the physical mixtures were the simple superimposition of those of the pure components, whereas all the co-evaporated products showed typical amorphous profiles, independent of the crystalline (for the natural CDs) or amorphous (for the CD derivatives) nature of the carrier. Representative examples of X-ray diffraction spectra are shown in Fig. 2. It was observed that drug–CD solid state interactions were generally slightly more intense with the CD derivatives than with the corresponding natural ones, probably as a consequence of the amorphous nature of the chemically modified cyclodextrins. Moreover, the presence of substituents extends the CD cavity length, and therefore possibly favors the occurrence of hydrogen bonds with the guest molecule.

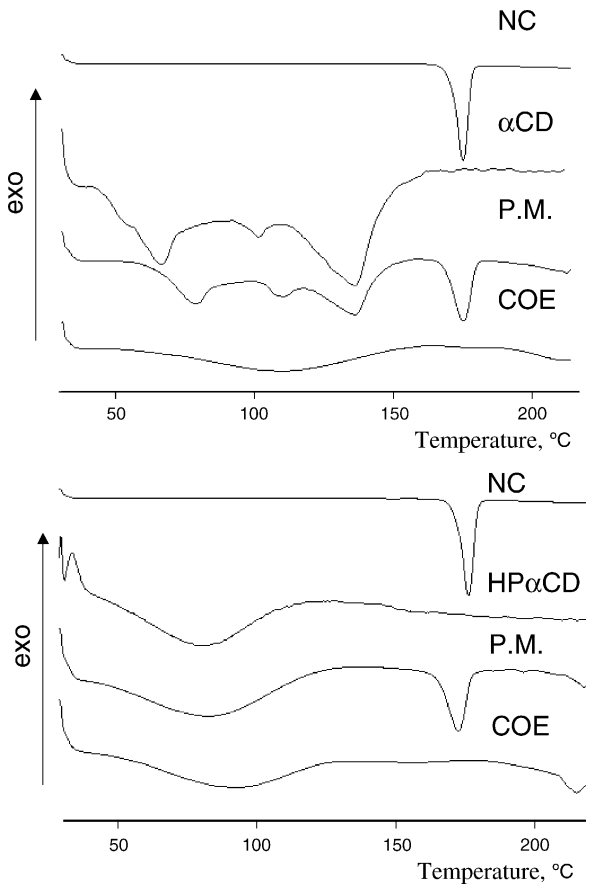

Fig. 1. DSC curves of pure nicardipine (NC), α-cyclodextrin (αCD), hydroxypropyl-α-cyclodextrin (HPαCD) and their respective equimolar drug–CD physical mixtures (PM) and coevaporated products (COE).

Such an effect was also evident from the FTIR analysis results. In fact, as it can be observed in Fig. 3 for the series of NC–αCD and NC–HPαCD binary systems, the disappearance of the characteristic carbonyl stretching vibration of NC at 1702 cm^{-1}, which was observed in all drug–CD coevaporated products, appeared, in the case of NC combinations with CD derivatives, also in the simple physical mixtures. Similar results have been reported by other authors and attributed to the formation, during inclusion complexation, of hydrogen bonds between the carbonyl groups of NC and the hydroxyl groups of the host cavity [13].

In conclusion, solid state studies pointed out that the coevaporation technique was always effective in preparing amorphous cyclodextrin complexes with NC, independent of the nature of the cyclodextrin, i.e. its cavity size or its amorphous or crystalline character.

3.2. Enantioresolution of rac-nicardipine by CE

A rapid CE method was developed able to monitor the fast photodegradation of nicardipine and to enantioresolve the remaining nicardipine. Such a method was necessary in order to ascertain a possible alteration of the enantiomeric ratio of the drug when its racemate is photoexposed in a chiral medium (CD inclusion complex).

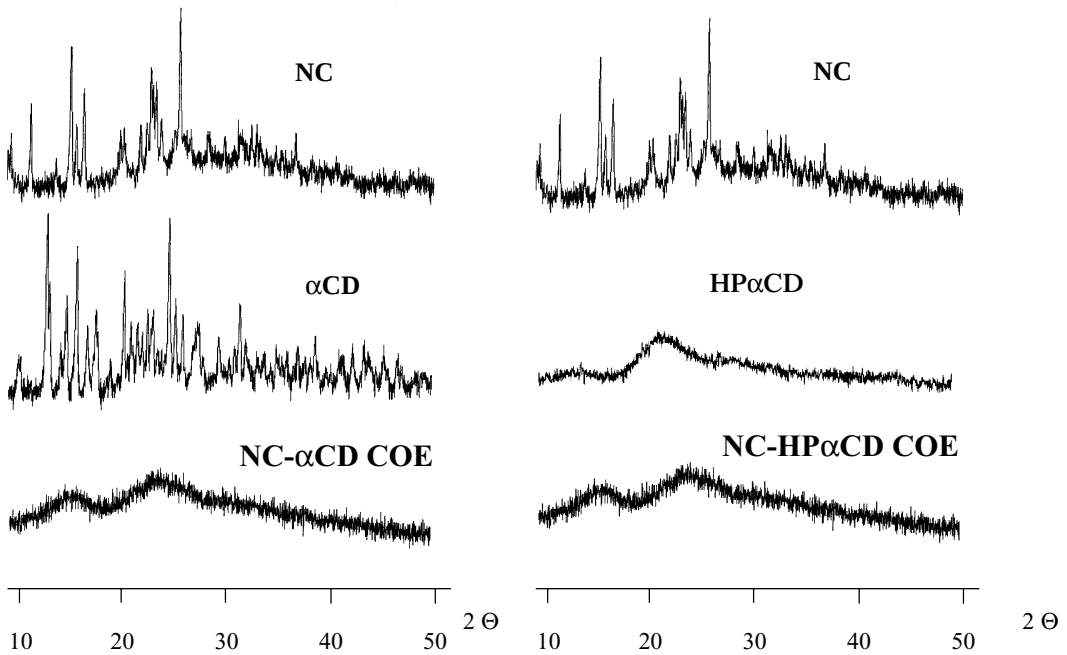

Fig. 2. Powder X-ray diffraction patterns of pure nicardipine (NC), α-cyclodextrin (αCD), hydroxypropyl-α-cyclodextrin (HPαCD) and their respective equimolar coevaporated products (COE).

In our previous studies [12], a negatively charged derivatised β-CD such as sulfated-β-CD, proved to be a suitable chiral selector for the enantioresolution of nicardipine. In fact, negatively charged cyclodextrins are expected to improve enantioresolution of basic compounds at low pH values, due to the opposite electrophoretic mobility of the complex with cyclodextrin compared to the free drug. Actually, under these conditions good enantioresolution of nicardipine was achieved; further, due to the strong analyte–cyclodextrin interactions and the slow electroosmotic flow, anodic migration of the resolved enantiomers was obtained. To reduce the migration times, the co-ion sodium of BGE was substituted with triethanolammonium, able to give an anodic EOF [18]. In the present work, the enantioresolution of *rac*-nicardipine was improved using a mixed CD_s system. This approach, useful to improve the selectivity, has been recently reviewed [19]. For the enantioresolution of *rac*-nicardipine, SβCD (negatively charged) and TMβCD (neutral) cyclodextrins, have been selected; after preliminary experiments, the ratio 3:2 (SβCD:TMβCD) proved to be suitable to achieve optimum enantioresolution. The obtained results are shown in Fig. 4.

The concentration of BGE (20 mM) was chosen on the basis of the effect on resolution (Rs) and migration time; a 20 mM concentration constitutes a compromise suitable for obtaining reproducible resolution and rapid analysis. In addition to the enantioresolution of the *rac*-nicardipine, these conditions improved the separation of the drug from its photoproducts having shorter migration times.

In order to validate the developed CE method and to confirm its suitability for detecting small photoinduced alterations of the enantiomeric ratio of nicardipine, the detection linearity was verified for the two enantiomers ($\lambda = 220$ nm). The regression data for the first and the second migrating enantiomer were, respectively: $Y_1 = (276.62 \pm 1.34) \times C + (0.9765 \pm 28.30)$; $r = 0.9996$; $n = 6$ and $Y_2 = (277.14 \pm 1.25) \times C + (1.9224 \pm 23.06)$; $r = 0.9997$; $n = 6$, where Y was the corrected peak area (area/migration time) and C was the drug concentration (0.020–0.100 mg/ml). According

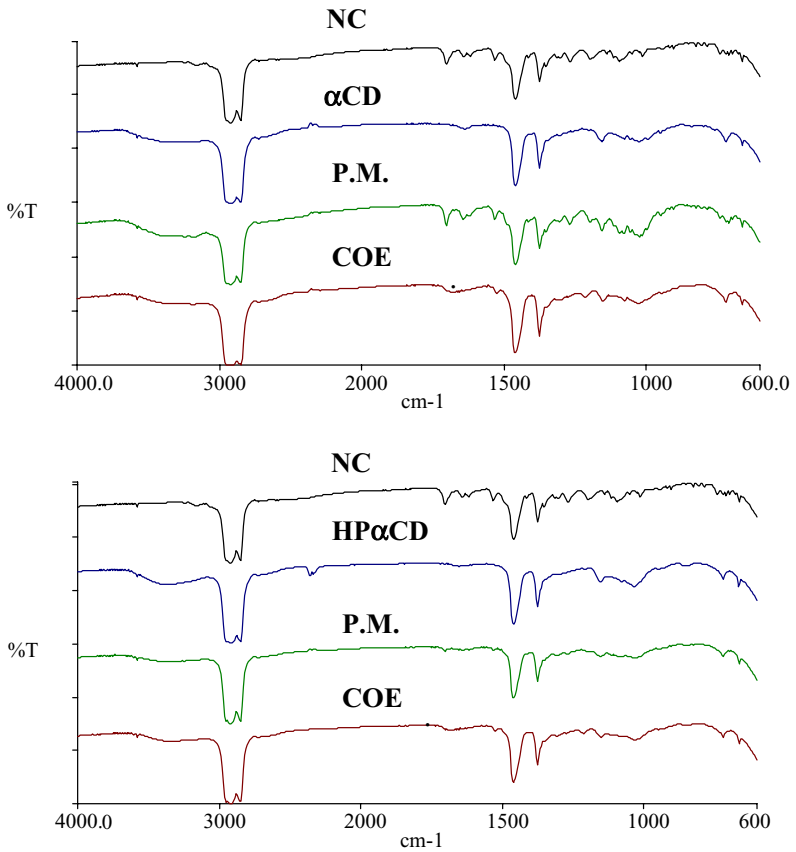

Fig. 3. FT-IR spectra of pure nicardipine (NC), α-cyclodextrin (αCD), hydroxypropyl-α-cyclodextrin (HPαCD) and their respective equimolar drug–CD physical mixtures (PM) and coevaporated products (COE).

to these data, the calibration graphs of the two enantiomers can be considered essentially identical; therefore, alterations observed in their corrected peak area reflect actual concentration variations. The intra-day and inter-day precision of the corrected peak area was also evaluated. Aqueous solution containing *rac*-nicardipine (0.050 mg/ml) photoexposed for 2 h was injected five times within 24 h (intra-day). The mean values of corrected peak areas for the enantiomers were: 23,467 (R.S.D.% = 3.02) and 23,571 (R.S.D.% = 2.97). On two consecutive days (inter-day) the obtained values (n = 10) were 22,136 (R.S.D.% = 3.10) and 22,843 (R.S.D.% = 3.00). These data support the adequate precision of the developed enantioselective CE method.

3.3. Photodegradation of NC–CD_s complexes in aqueous medium

Aqueous solutions containing *rac*-nicardipine and 15% (v/v) methanol (reference solution), and aqueous solutions containing the drug–CD inclusion complex, all at the same nicardipine concentration, were simultaneously exposed to UV(A)–UV(B) radiations. At selected times the irradiated solutions were subjected to chiral CE analysis and the corrected peak area of the separated enantiomers were determined. The obtained results are illustrated in Fig. 5 and can be summarized as follows:

1. *NC–αCD complex*: solutions of the *rac*-nicardipine were found to be slightly more photostable than

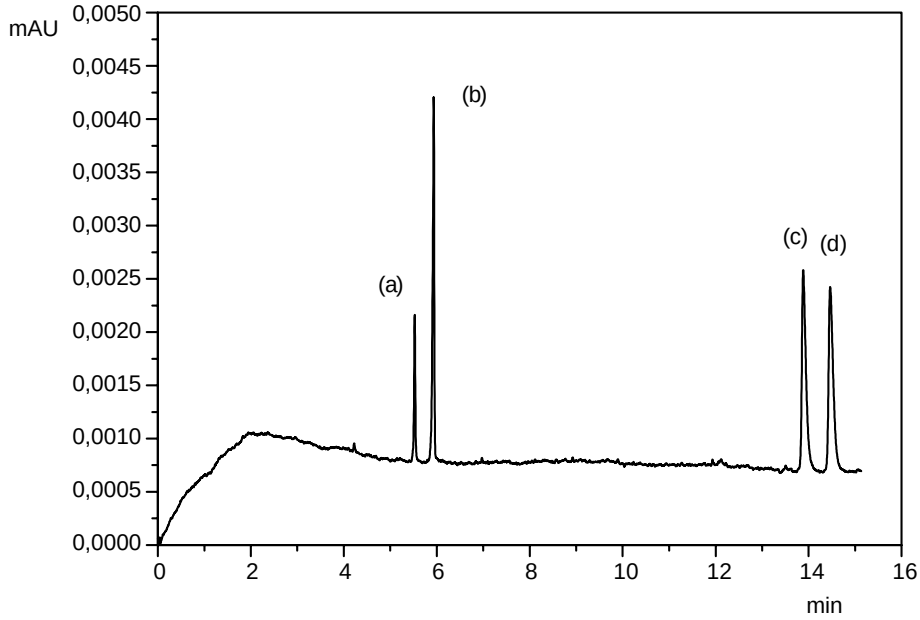

Fig. 4. Electropherograms obtained from photoexposed solutions of *rac*-nicardipine. Peaks: (a) and (b) are photoproducts; (c) and (d) are first and second migrating enantiomers of nicardipine, respectively. Conditions: buffer, 20 mM phosphoric acid/triethanolamine (pH 3.0) with 3.0% of SβCD and 2.0% of TMβCD. Fused-silica capillary, total length 50 cm (effective length 43.5 cm), 50 μm i.d., injection 5 psi for 2 s, voltage 25 kV, temperature 25 °C, detection wavelength 220 nm.

equimolar solutions containing the drug as inclusion complex. Therefore, αCD, appear to favor the drug photodegradation (Fig. 5a).

2. *NC–βCD complex*: solution containing *rac*-nicardipine-βCD complex proved to be more photostable, confirming previous reports on photoprotective effect of βCD [12]. Moreover, two slightly separate photodegradation profiles were obtained for the two enantiomers (Fig. 5b).
3. *NC–γCD, NC–HPβCD, NC–HPγCD and NC–MβCD*: the CD complexation did not modify the drug photostability (Fig. 5c).
4. *NC–HPαCD and NC–HEβCD*: solutions of the drug–CD inclusion complexes were found to be more photostable than solutions containing the *rac*-nicardipine, showing a photoprotective effect by the used CD_s (Fig 5d). This effect was confirmed at three different concentration level (0.025, 0.050 and 0.100 mg/ml) of the drug.

Under the same experimental conditions of light exposure and drug concentration (0.050 mg/ml) in water, the nicardipine photodegradation process was found to obey the apparent first-order kinetics, according to the equation:

$$\ln A = \ln A_0 - kt$$

where A is the % remaining peak area, k is slope (rate constant) and t is time (min). The kinetic constants (k) were obtained from the linear equation regressions, for the different photo-exposure conditions. For each condition, both: k_{CD} (kinetic constant in presence of CD complex) and k (kinetic constant without CD) were obtained from simultaneous experiments. To evaluate the effect of CD complexation on the nicardipine photostability, the ratios of k_{CD} to k have been calculated. The following data were obtained:

NC–αCD complex: $k_{CD}/k = 1.140$; NC–βCD complex: $k_1 = k_{CD}/k = 0.855$ and $k_2 = k_{CD}/k = 0.893$ for the first and the second migrating enantiomers, respectively; NC–γCD complex: $k_{CD}/k = 0.996$; NC–HPαCD complex: $k_{CD}/k = 0.821$; NC–HPβCD complex: $k_{CD}/k = 0.993$; NC–HPγCD complex: $k_{CD}/k = 1.000$; NC–HEβCD complex: $k_{CD}/k = 0.890$; NC–MβCD complex: $k_{CD}/k = 0.998$.

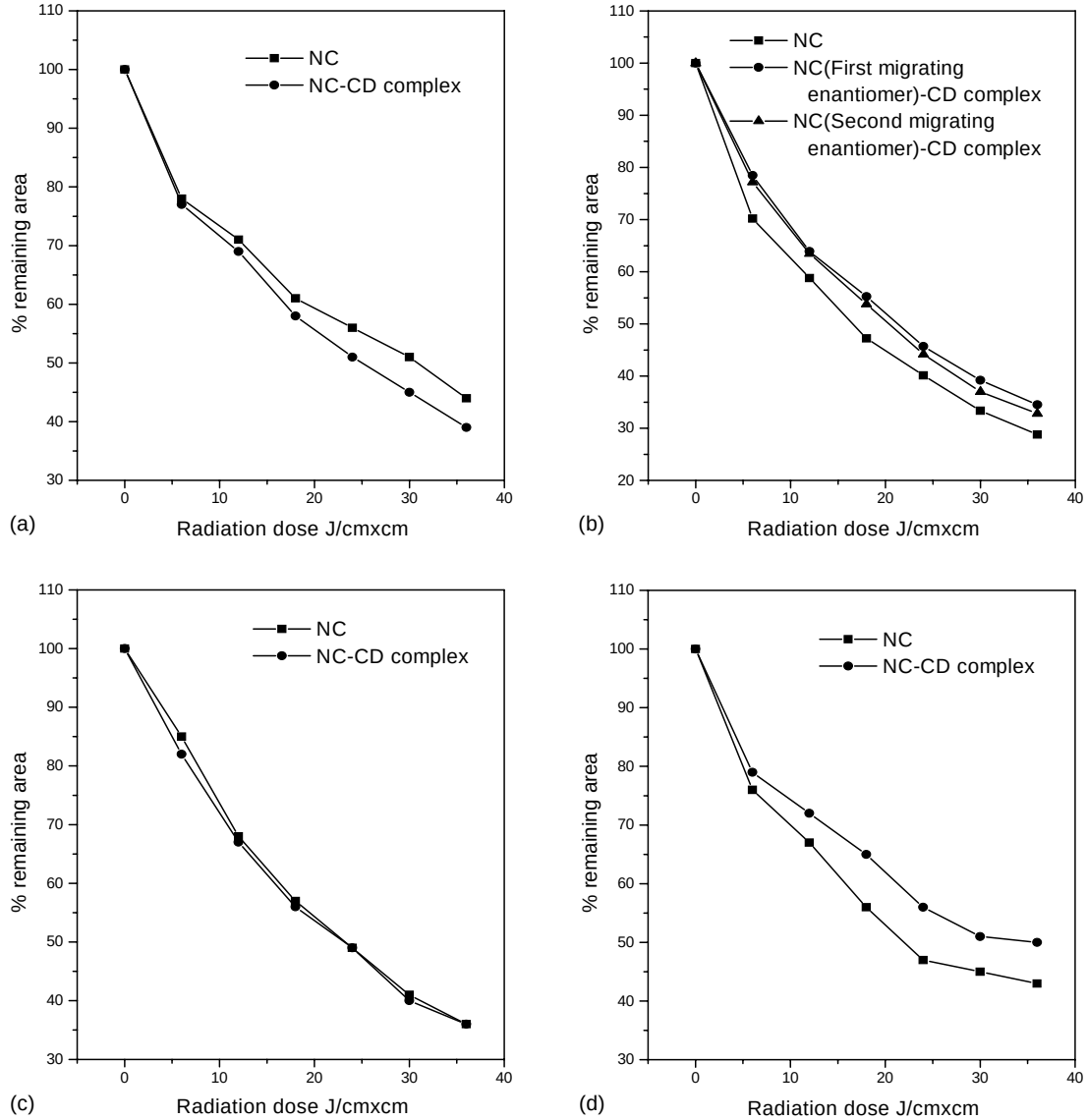

Fig. 5. Photodegradation profiles of the *rac*-nicardipine under photoexposure to UV(A)–UV(B) radiations of an aqueous solution containing *rac*-nicardipine (0.05 mg/ml) and nicardipine (0.05 mg/ml) complexed with: αCD (a), βCD (b), γCD (c) and HPαCD (d).

4. Conclusion

Capillary electrophoresis (CE) was found to be a reliable and versatile analytical tool suitable for photostability studies of *rac*-nicardipine. Under chiral conditions, the developed method enabled also to monitor the individual photodegradation of the drug enantiomers. Photostability experiments carried out on equimolar nicardipine–CD complex systems, in aqueous solutions, showed a photoprotective effect by βCD, HPαCD and HEβCD and a photodegradative effect by αCD. The other used CD_s did not significantly modify the photoreactivity of nicardipine. When, the drug–βCD inclusion complex was subjected to photoexposure to UV(A)–UV(B) radiation, a different photodegradation rate was observed for the two

enantiomers. These results can be considered of practical interest for nicardipine and for other chiral, photolable drugs, whose solubility could be enhanced by complexation with CD_s. In fact, different outcomes can results using different CD_s; in addition, the possibility of different photodegradation profiles for the enantiomers of a chiral photoreactive drug should be considered.

References

[1] X. Martindale, in: J.F.F. Reynolds (Eds.), The Extra Pharmacopoeia, 31st ed., Royal Pharmaceutical Society, London, 1996, pp. 914–915.
[2] E. Sorkin, S. Clissold, Drugs 33 (1987) 296–345.
[3] N. Inotsume, T. Iwaoka, M. Honda, M. Nakano, Y. Okamoto, K. Tomita, T. Teramura, S. Higuchi, Eur. J. Clin. Pharmacol. 52 (1997) 289–292.
[4] M.C. Bonferoni, G. Mellerio, P. Giunchedi, C. Caramella, U. Conte, Int. J. Pharm. 80 (1992) 109–117.
[5] J.S. Grundy, R. Kherany, R.T. Foster, J. Pharm. Biomed. Anal. 12 (1995) 1529–1532.
[6] J. Szejtli, Cyclodextrin technology, Kluwer Academic Publisher, Dordrecht, 1988.
[7] K.H. Fromming, J. Szejtli, Cyclodextrins in Pharmacy, Kluwer Academic Publisher, Dordrecht, 1994.
[8] K. Uekama, F. Hirayama, T. Irie, Chem. Rev. 98 (1998) 2045–2076.
[9] T. Loftsson, Drug Stability 1 (1995) 22–33.
[10] K. Thoma, Photodecomposition and stabilization of compounds in dosage forms, in: H.H: Tonnensen (Eds.), The Photostability of Drugs and Drugs Formulations, Taylor and Francis, London, 1996, pp. 111–140.
[11] J. Mielcarek, Pharmazie 51 (1996) 477–479.
[12] R. Pomponio, R. Gotti, C. Bertucci, V. Cavrini, Electrophoresis 22 (2001) 3243–3250.
[13] C.M. Fernandes, M.T. Vieira, F.J.B. Veiga, Eur. J. Pharm. Sci. 15 (2002) 79–88.
[14] C.M. Fernandes, F.J.B. Veiga, Chem. Pharm. Bull. 50 (2002) 1597–1602.
[15] T. Loftsson, M.A. Brewster, J. Pharm. Sci. 85 (1996) 1017–1025.
[16] C.M. Fernandes, R.A. Carvalho, S. Pereira da Costa, F.J.B. Veiga, Eur. J. Pharm. Sci. 18 (2003) 285–296.
[17] P. Mura, E. Adragna, A. Rabasco, J. Moyano, J. Pérez-Martinez, M. Arias, J. Ginés, Drug Dev. Ind. Pharm. 25 (1999) 279–287.
[18] M. Fillet, I. Bechet, P. Chiap, Ph. Hubert, J. Crommen, J. Chromatogr. 717 (1995) 203–209.
[19] M. Fillet, Ph. Hubert, J. Crommen, J. Chromatogr. A 875 (2000) 123–134.