Hydrophilic and hydrophobic cyclodextrins in a new sustained release oral formulation of nicardipine: in vitro evaluation and bioavailability studies in rabbits

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Abstract

The feasibility of using complexes with cyclodextrins (CDs) in nicardipine (NC) controlled delivery has been examined, with a view to extending the pharmaceutical applications spectrum of these carriers. For a fast release fraction, a hydrophilic β-cyclodextrin derivative (hydroxypropyl-β-cyclodextrin) was employed to form a water-soluble complex. For the sustained-releasing portion, triacetyl-β-cyclodextrin (TAβCD) was used to provide complexes with appropriate hydrophobicity. An optimal formulation was designed by the combination of each fraction in different mixing ratios. The release behaviour of the complexes, as well as of their mixtures, was examined in simulated gastric (pH 1.2) and intestinal (pH 6.8) fluids. The formulations released the drug rapidly at the initial stage, followed by a slow release. The drug release rate was markedly retarded in the increasing order of the amount of NC/TAβCD complex. When NC was administered to rabbits, its absorption was very rapid with a short elimination half-life, while a prolonged maintenance of the plasma levels was obtained for the two selected formulations. The drug bioavailability was considerably improved especially after the administration of the mixture of hydrophilic and hydrophobic complexes, when compared with the NC/TAβCD complex. The results suggested that the critical combination of hydrophilic and hydrophobic CDs complexes, in appropriate ratios, could be a promising drug delivery system with a prolonged therapeutic effect coupled with a more balanced bioavailability.
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1. Introduction

In an attempt to optimise the efficacy of drug activity and control the drug release at the desired level, many drug carrier materials, such cyclodextrins (CDs) have been rationally designed and improved. CDs, cyclic oligosaccharides with a hydrophobic central cavity that provides a microenvironment for appropriate sized non-polar molecules, have been widely applied in the improvement of the

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physical, chemical and biological properties of several drug molecules, due to their characteristic inclusion ability [1–3]. In recent years, various kinds of chemically-derived CDs have been prepared to extend the physicochemical and inclusion properties and also the multi-functional pharmaceutical characteristics of the parent host molecules, their hydroxyl groups being available as starting points for structural modification, without eliminating the central cavity for guest accommodation [3]. Among the chemically modified CDs, the hydrophilic CD derivatives, such as hydroxypropyl-β-CD (HPβCD) and sulfobutyl ether-βCD, are useful to improve the solubility, dissolution rate and bioavailability of poorly water-soluble drugs [4–6], while hydrophobic CD derivatives, such as ethylated and acylated CDs, have potential as sustained-release carriers of drugs with short half-lives [7–9]. Furthermore, advanced controlled release can be achieved by a pertinent hybridising of complexes obtained with hydrophilic and hydrophobic CD derivatives.

We have recently reported [10] that HPβCD is particularly useful to improve the low solubility and dissolution rate of nicardipine (NC) in alkaline medium. TAβCD was also successfully used to achieve inclusion compounds with NC and a sustained drug release profile was observed from these hydrophobic complexes [11]. This led us to design a sustained release formulation of NC consisting of a combination of both types of complexes: the hydrophobic NC/TAβCD complex as the slow-release portion, to provide an appropriate hydrophobicity, and the highly hydrophilic NC/HPβCD complex as the fast-dissolving fraction.

NC, a calcium channel-blocking agent, is an effective drug in the management of mild to moderate hypertension, angina pectoris and cerebral disease. It was used as model drug since its bioavailability is very limited (15–40%) and like other dihydropyridine derivatives, its standard formulation undergoes rapid absorption and extensive biotransformation in the liver, with a short elimination half-life (about 1 h), which often results in significant fluctuations in plasma concentrations [12]. To attain a prolonged therapeutic effect and a reduced incidence of side-effects, sustained release formulations of NC have been developed to maintain a suitable plasma level for a long period of time with minimal

frequency of daily administration [13,14]. The incorporation of a rapid-dissolving fraction together with a slow-release portion could be advantageous, since the initial release of a certain amount of NC will give more balanced oral bioavailability, reducing the first-pass metabolism in the liver.

In the present study, the release behaviour of NC/HPβCD and NC/TAβCD complexes, as well the corresponding mixtures in different molar ratios, was evaluated in acidic (pH 1.2) and alkaline (pH 6.8) media. Furthermore, we have investigated the oral bioavailability of the selected formulations in rabbits.

2. Material and methods

2.1. Materials

NC, HPβCD (Kleptose HPB®, MW~1300, DS 0.63) and TAβCD were purchased from Effechem SRL (Italy), Roquette (France) and Sigma–Aldrich (Germany), respectively. All other chemicals and solvents were of analytical reagent-grade (Merck and Sigma–Aldrich) and deionised water (Millipore Elix 5 system) was used throughout the study.

Since NC is light sensitive, almost all experiments were carried out in a darkroom under yellow light (Philips Powertone SON E27). When this photoprotection was impossible to achieve, all suspensions or samples containing NC were protected from light by wrapping the vials with aluminium foil.

2.2. Preparation of inclusion complexes

The 1:1 guest/host inclusion complexes were prepared by the spray-drying method as previously described [10,11]. Briefly, hydroalcoholic solutions containing equimolar quantities of NC and HPβCD or TAβCD were spray-dried (LabPlant SD-05), under the following conditions: air flow rate, 50 m^3/h; atomising air pressure, 1.0 bar; inlet temperature, 160 °C, outlet temperature, 85 °C and flow rate of the solution, 400 ml/h.

Physical mixtures of NC and both CDs, in a 1:1 molar ratio, were also prepared for reference.

The evidence of inclusion complexation between NC and HPβCD or TAβCD and the detailed

physicochemical characterization of the corresponding complexes were previously reported [10,11].

Several combinations of both complexes were prepared by mixing, in different molar ratios (1:1, 1:2; 1:4, 1:6 and 1:10), the corresponding amounts of NC/HPβCD and NC/TAβCD complexes, in a glass mortar.

2.3. In vitro dissolution studies

The dissolution profiles of NC from the CDs inclusion complexes, as well from the respective mixtures, were collected using a Vankel VK7000 apparatus, according to the USP rotating basket method. The dissolution media consisted of 1000 ml of enzyme-free simulated gastric (pH 1.2) and intestinal (pH 6.8) fluids (USPXXIV). The stirring speed was 100 ± 2 rpm and the temperature was maintained at 37 ± 0.2 °C. Powdered samples containing 30 mg of NC or its equivalent were used. At set time intervals for a period of 8 h, the amount of dissolved drug was automatically measured by UV spectroscopy at 357 nm. Dissolution runs were carried out six times for all samples.

2.4. Bioavailability studies in rabbits

The bioavailability studies were carried out using male *New zealand* albino rabbits with an average weight of 4 kg, housed individually in standard cages in a room with air, humidity and temperature control and on a 12-h light, 12-h dark cycle. The animals were kept on a standard diet.

At least 12 h prior to drug administration, the animals were fasted but had free access to water. NC and the two selected formulations were administered orally to six animals, at a dose of 15 mg/kg, with a wash-out period of 2 weeks between the different administrations. The formulations were previously filled in hard capsules in order to facilitate the oral administration.

Blood samples (1.5 ml) were withdrawn from a heparinized catheter placed in the marginal vein of the ear before administration and at predetermined times, using EDTA as anticoagulant. Plasma samples were immediately separated by centrifugation at 3000 rpm for 10 min and stored at -80 °C until analysis. Baseline plasma samples obtained prior to NC administration at time 0 served as the blank control for each animal.

The drug plasma levels were determined by a previously described high performance liquid chromatography (HPLC) method with UV detection [15]. Briefly, the method involved the addition of the internal standard (nimodipine) to the plasma samples with subsequent solid phase extraction using C_{18} cartridges. The HPLC system consisted of a Hewlett Packard (Waldbronn, Germany) model 1050 pump, equipped with a HP1050 multiple wavelength UV detector operated at 254 nm, an injector with a 20-μl loop and a HP3396A recording integrator. The stationary phase was a Licrospher® 100 RP-18 reversed-phase column (250 mm×4 mm i.d.; 5 μm mean particle size, Merck) equipped with a guard column similarly packed and maintained at 30 °C. A mixture of acetonitrile–0.02 M sodium phosphate buffer–methanol (45:40:15) with 0.2% triethylamine and at pH 6.1, delivered at a flow rate of 1.2 ml/min, was used as mobile phase.

The method proved to be linear in the range of 5–100 ng/ml with a regression coefficient of 0.9993. The relative standard deviations of intra- and inter-day analysis for NC in plasma were 3.26–6.52% ($n=5$) and 4.71–9.38% ($n=5$), respectively. The differences in the mean value measured from the concentration prepared, expressed as percentages (% bias), were only -5.2, 0.4 and 0.8% at NC concentrations of 5, 25 and 50 ng/ml, which confirmed the accuracy of the method.

The pharmacokinetic parameters extracted from the plasma data were calculated using noncompartmental analysis (WinNonlin®, Version 1.1) and included the maximum plasma concentration (C_{max}), the time to reach the maximum plasma concentration (T_{max}), the area under the drug plasma concentration–time curve up to 24 h post-administration ($AUC_{0\text{-}24\text{ h}}$), the mean residence time (MRT) and the elimination half-life ($T_{1/2}$).

The statistical analysis of the pharmacokinetic parameters was carried out using one-way analysis of variance (ANOVA) with Bonferroni post test for multiple comparisons. A value of $P < 0.05$ defined the statistical significance.
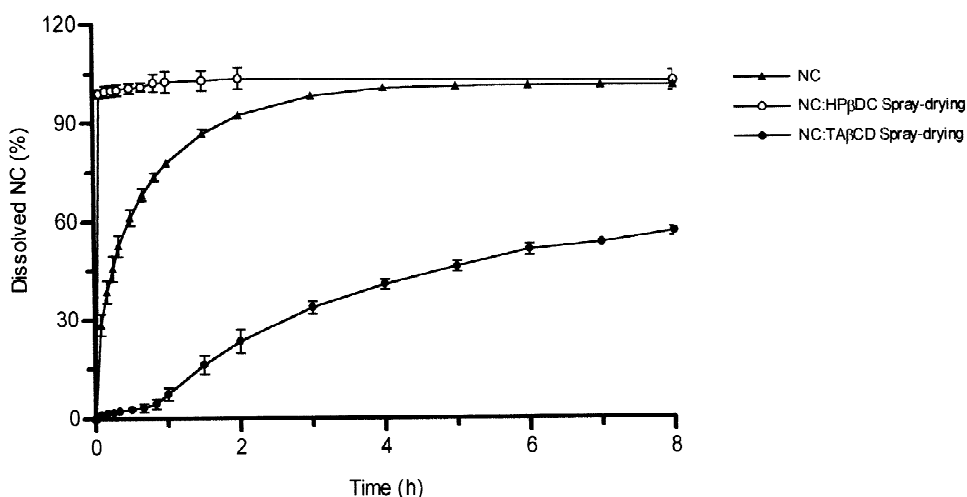
Fig. 1. Dissolution profiles of NC, NC/HPβCD and NC/TAβCD inclusion complexes in simulated gastric fluid (pH 1.2). Data are presented as mean±S.D., $n=6$.

3. Results and discussion

3.1. In vitro dissolution studies

The dissolution profiles of NC and of the NC/CDs spray-dried inclusion complexes in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 6.8) are presented in Figs. 1 and 2, respectively. The dissolution of the plain drug, in the acidic medium, was completed only within ca. 3 h. As expected,

since NC is a weak basic drug ($pK_a=7.2$), the amount of dissolved drug in the intestinal fluid was lower than that observed in the gastric fluid. The NC/HPβCD complex displayed better dissolution properties with respect to NC alone, being immediately dispersed and completely dissolved within 15 min. The improvement of NC dissolution characteristics upon complexation with HPβCD was more evident in the simulated intestinal fluid, which is in accordance with the higher stability constants for the
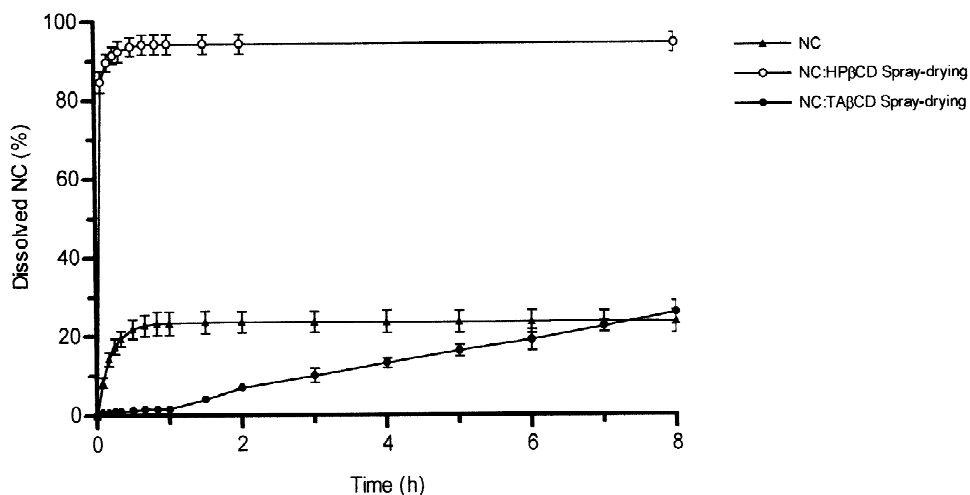

Fig. 2. Dissolution profiles of NC, NC/HPβCD and NC/TAβCD inclusion complexes in simulated intestinal fluid (pH 6.8). Data are presented as mean±S.D., $n=6$.

complexation between NC and this CD in alkaline media, as previously reported [10]. The significant enhancement of the low dissolution properties of NC from the NC/HPβCD spray-dried complex has been attributed to: (1) surfactant-like properties of the carrier, which can reduce the interfacial tension between water-insoluble drugs and the dissolution medium; (2) an increase in NC solubility upon complexation in the solid state; (3) the high energetic amorphous state following complexation, as confirmed by previous X-ray diffractometry studies [5,16,17].

On the other hand, it was clearly observed that the dissolution rate of NC, in both dissolution media, was markedly retarded by complexation with the hydrophobic TAβCD, mainly due to the poor water solubility of this complex. Indeed, a total release of NC at pH 1.2 was observed after ca. 3 h from the plain drug, whereas this state was not reached after 8 h from the NC/TAβCD spray-dried complex. The complex formation with TAβCD also resulted in a significant reduction in the release rate of NC in the simulated intestinal fluid: a ca. 25% drug release percentage from this hydrophobic complex was only achieved after 8 h.

NC should be released according to zero-order kinetics for a long period of time, because of such therapeutic advantages as the duration of its pharmacological effect and the lowering of the drug side-

effects. However, in the slow-release-type preparations there is usually a lag time for reaching an effective blood level of drug. For this reason, an initial rapid release would be necessary to avoid this lag time and offer a more balanced bioavailability. To gain a rapid appearance of NC in the blood and to maintain a constant drug level for a long period of time, we decided to hybridise a fast-releasing fraction with the slow-releasing NC/TAβCD complex. Because of the poor aqueous solubility and wettability of NC, we employed the highly water-soluble NC/HPβCD complex as the immediate-release compound, in the expectation of an enhanced initial release rate of NC.

Thus, in the next step, an optimisation of the release profile of NC was attempted by the combination of the HPβCD and TAβCD spray-dried complexes, in different molar ratios (1:1, 1:2, 1:4, 1:6 and 1:10). The effect of the varying molar ratios on the release behaviour of the formulations composed of NC/HPβCD and NC/TAβCD spray-dried complexes in the simulated gastric (pH 1.2) and simulated intestinal fluid (pH 6.8) is shown in Figs. 3 and 4, respectively.

The release patterns from the different formulations reflected that of each component, i.e. the NC corresponding to the fast-releasing fraction was rapidly released, and then the residual amount of drug was gradually released from the slow-releasing
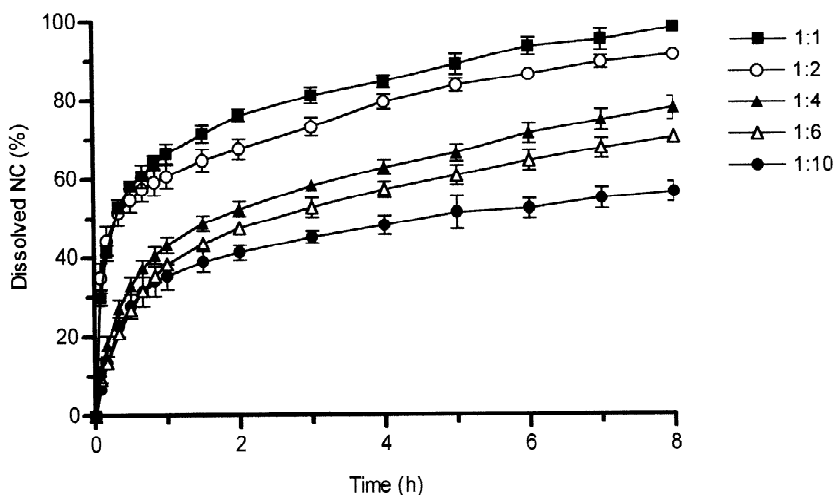

Fig. 3. Dissolution profiles of (NC/HPβCD complex)/(NC/TAβCD complex) in different molar ratios in simulated gastric fluid (pH 1.2). Data are presented as mean±S.D., *n*=6.
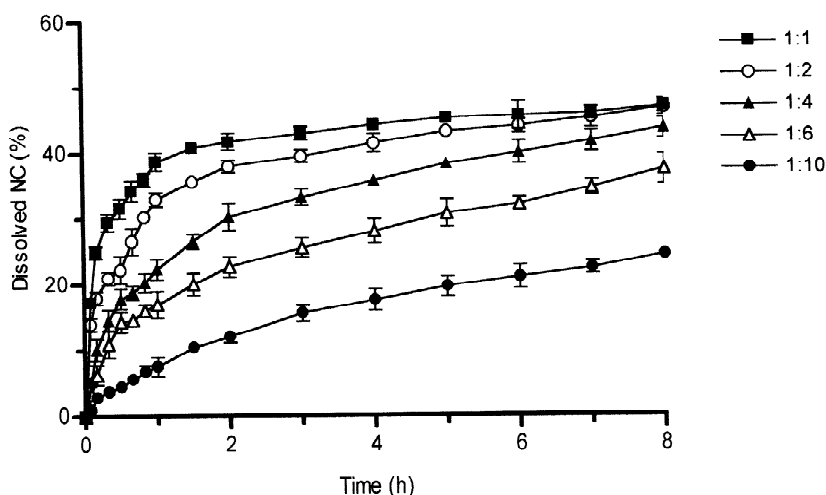
Fig. 4. Dissolution profiles of (NC/HPβCD complex)/(NC/TAβCD complex) in different molar ratios in simulated intestinal fluid (pH 6.8). Data are presented as mean±S.D., $n=6$.

fraction, according to zero-order kinetics. Indeed, the drug release occurred in two stages: faster release in the initial stage (up to 1 h) and slower release in the second stage. Compared to NC alone, the drug release rate from all these formulations was significantly suppressed, a result of the retarding effect of the hydrophobic complex in the mixture. In contrast, the increase in the initial dissolution rate appeared to be proportional to the molar ratio of the fast-dissolving faction, i.e. NC/HPβCD complex, in the formulations (1:10<1:6<1:4<1:2<1:1).

It was evident from these results that the NC release rate could be critically modified by changing the mixing ratio of the hydrophilic and hydrophobic CD inclusion complexes.

From inspection of the dissolution profiles, among the various combinations, the 1:4 mixture of both complexes, namely formulation I, seemed to be suitable for our purpose with regard to the release pattern and release time. This formulation provided a sufficiently slow release of the drug for a long period of time following an initial rapid dissolution (about 40% drug release at stomach pH). A sufficient release of drug in the early stage would be necessary to offer a more balanced bioavailability because the stomach is an important absorption site. We also selected the NC/TAβCD complex (formulation II) for further in vivo studies.

3.2. Bioavailability studies in rabbits

The mean plasma concentrations of NC following a single oral dose of the plain drug, formulation I (mixture of HPβCD and TAβCD complexes in a molar ratio of 1:4) or formulation II (TAβCD complex), equivalent to 15 mg/kg, are shown in Fig. 5. The pharmacokinetic parameters calculated for the six animals are summarized in Table 1.
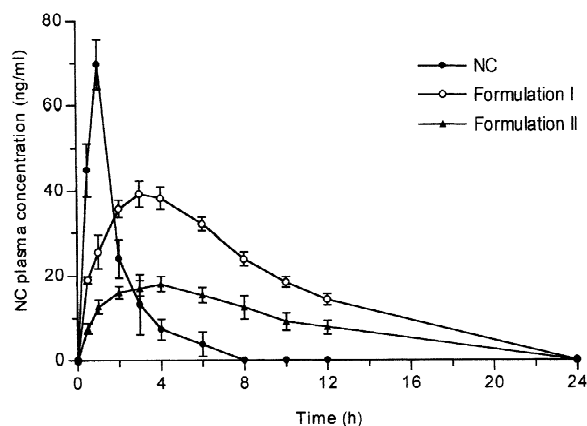

Fig. 5. NC plasma concentration–time profiles after oral administration of NC, 1:4 mixture of NC/HPβCD and TAβCD complexes (formulation I) and NC/TAβCD complex (formulation II), at a dose of 15 mg/kg, to rabbits. Each point represents the mean±S.E., $n=6$.

Table 1
Pharmacokinetic parameters of NC after oral administration of plain NC, 1:4 mixture of NC/HPβCD and TAβCD complexes (formulation I) and NC/TAβCD complex (formulation II), at a dose of 15 mg/kg, to rabbits ($n=6$)

	C_{max} (ng/ml)	T_{max} (h)	$AUC_{0-24\ h}$ (ng h/ml)	MRT (h)	$T_{1/2}$ (h)
NC	69.64 ± 8.57	1	129.10 ± 9.44	1.60 ± 0.14	1.60 ± 0.38
Formulation I	39.65 ± 1.11	3	411.84 ± 7.23	5.41 ± 0.02	5.49 ± 0.17
Formulation II	18.25 ± 0.74	4	202.82 ± 9.07	5.55 ± 0.11	7.38 ± 1.61

Each value represents the mean $\pm$ S.E., with the exception of the T_{max} parameter, which is represented by the median.

The absorption of NC in rabbits was very rapid for the plain drug, with a time to reach the maximum plasma level (69.64 ng/ml) of 1 h. This drug showed a short elimination half-life (1.60 h), reflecting its rapid disappearance in the plasma of the animals and the concentration could not be detected after 8 h, being below the minimum detection limit of 5 ng/ml. The plasma concentrations of NC after oral administration of both formulations did not show a sharp peak and the corresponding times to reach the maximum plasma level were significantly prolonged, 3 and 4 h for formulations I and II, respectively. Also, the plasma concentrations were maintained at a relatively constant level for a long period when the drug was administered as the two formulations, presenting a somewhat sustained-release pattern. However, the plateau obtained in the drug plasma concentrations was more evident after the administration of formulation II. In addition, the MRT values displayed by formulations I and II were about 3.5-fold larger than that of NC alone, confirming the sustained-release behaviour of these preparations in the rabbit model. However, no significant difference in the MRT values was observed between the two formulations.

It was interesting to note that there was a 3.2- and 1.6-fold increase in the $AUC_{0-24\ h}$ value for formulations I and II, respectively, corresponding to a greater extent of oral absorption when compared with the drug alone. This increase in the $AUC_{0-24\ h}$ may be due to a longer residence time of these formulations in the gastrointestinal tract, as was suggested by their larger MRT and T_{max} values. The difference in the $AUC_{0-24\ h}$ between formulations I and II was also found to be statistically significant ($P<0.05$). The drug plasma levels in the initial stage after the administration of formulation I were markedly higher than the levels obtained for formulation II, which could explain the sparing increase in the $AUC_{0-24\ h}$ of the later. This behaviour was probably due to the extremely slow dissolution rate of the NC/TAβCD complex.

From the inspection of these results, we could verify that the in vitro release behaviours were partially reflected in the plasma levels after the administration of the corresponding formulations. For example, NC gave an initial rapid increase in plasma drug levels, followed by a rapid decrease. The sustained plasma levels of the drug observed for a long period when formulations I and II were administered reflected their slow in vitro release pattern. Formulation I gave an initial rapid increase in the drug plasma level, followed by its maintenance at a relatively constant level, which could be well correlated with the two stages of the in vitro drug release mentioned above. Compared to the hydrophobic complex alone, formulation I produced a faster initial increase in the NC plasma level, which could reflect the presence of the hydrophilic complex, in a perfect correlation with the in vitro release profiles.

The attenuation of the initial high peak of the drug plasma concentrations, after the administration of the selected formulations, suggested that the frequency and severity of NC side-effects could also be reduced. Further, the formulation constituted by the combination of HPβCD and TAβCD complexes produced retarding effects with superior oral bioavailability compared with NC and the hydrophobic TAβCD complex alone.

4. Conclusions

The NC release rate could be controlled by adjusting the mixing ratio of its hydrophilic

(HPβCD) and hydrophobic (TAβCD) complexes, depending on the required sustained period. Formulations composed of hydrophilic and hydrophobic CDs inclusion complexes could be useful in oral administration of NC to achieve prolonged action, improved bioavailability and reduced side-effects.

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