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ORIGINAL ARTICLE

Nimesulide/cyclodextrin/PEG 6000 ternary complexes: physico-chemical characterization, dissolution studies and bioavailability in rats

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Abstract
Purpose Ternary solid dispersions were prepared in order to estimate the effect of a double hydrophilization by cyclodextrins and PEG 6000 on nimesulide apparent characteristics. Ternary solid dispersions of nimesulide, cyclodextrins and PEG 6000 were characterized using DSC, FT-IR, dissolution studies and evaluating the bioavailability in rats.
Methods Ternary solid dispersions were prepared either using native powders or using a preformed inclusion complex of nimesulide and cyclodextrin. Inclusion complexes and pure drug were used as references. Circulating nimesulide was measured out in rat plasma after orally administration of our different products (ternary solid dispersions, inclusion complexes and pure drug).
Results An improvement of the nimesulide dissolution rate was obtained with inclusion complexes and ternary solid dispersion. In rat plasma, inclusion complexes and ternary solid dispersion improved T_{max}.
Conclusions A second hydrophilization of inclusion complexes by PEG 6000 does not allow to achieve better results concerning nimesulide concentration in rat plasma or in dissolution studies than with inclusion complexes alone.

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Keywords Solid dispersion · PEG 6000 · Cyclodextrin · Nimesulide · Bioavailability

Introduction

Nimesulide (Nim), is a weakly acidic non-steroidal anti-inflammatory drug. It differs from other non-steroidal anti-inflammatory drugs (NSAIDs) in that its chemical structure contains a sulfonanilide moiety instead of carboxylic group. Nim presents high anti-inflammatory, antipyretic, and analgesic activities in addition to low toxicity, a moderate incidence of gastric side effects, and a high therapeutic index [1]. However, recent findings reported that Nim had a higher risk of hepatic toxicity when compared to other marketed NSAIDs [2, 3]. The poor aqueous solubility and wettability of Nim gives rise to difficulties in pharmaceutical formulations for oral or parenteral delivery. To overcome these drawbacks, cyclodextrins (CDs) are used to enhance aqueous solubility of lipophilic substances resulting in an increase of their bioavailability or a toxicity decrease [4]. However, the application of β-CD in the pharmaceutical field is limited by its rather low aqueous solubility, which led to a search for more soluble CDs derivatives. The fully per-*o*-methylated β-CD (PMβ-CD) and hydroxypropyl-β-CD (HPβ-CD), could be good candidates for oral delivery. Therefore, their ability in solubilizing Nim through complexation was evaluated, and compared to that of β-CD and γ-CD. Ternary solid dispersions were prepared to estimate the effect of CDs included in PEG (polyethyleneglycol)-6000 on Nim apparent solubility and dissolution rate. Additional information on the complexing ability of CDs towards Nim was

obtained by differential scanning calorimetry (DSC) and infrared spectroscopy (FT-IR). The second part of this study consisted in quantifying circulating Nim in rat plasma after oral administration of ternary solid dispersions, inclusion complexes and pure drug. All the rats were sacrificed at the end of experiments and their stomach observed to determine the possible ulcers.

Experimental

Materials

Nim was obtained from Helsinn Chemicals SA (Switzerland). β, γ, HPβ and PMβ-CDs were obtained from Wacker (Lyon, France), Roquette (Lestrem, France) and Orsan (Paris, France). PEG 6000 was obtained from BASF (Ludwigshafen, Germany). Male Wistar rats weighing 225–250 g were obtained from Charles River Laboratories (France). Other chemicals and solvents were of analytical and HPLC grade.

Preparation of solid inclusion complexes (IC) and corresponding physical mixtures (PM)

CDs were dissolved in distilled water. To these solutions Nim was added in a well-determined molar ratio (Nim/CDs) 1:1 or 2:1 and pH of the solution was adjusted at 9–10 with sodium hydroxide. Final solutions obtained, were freeze-dried or evaporated under reduced pressure, placed for 24 h at 40 °C and then ground in a mortar. Corresponding PM were obtained by thoroughly mixing the various components with a spatula.

Preparation of nimesulide–CDs–polymer ternary systems

Two methods were used to prepare ternary solid dispersions. The first method consisted in mixing and heating Nim, CDs and PEG 6000 until melting. The second method consisted in mixing and heating an IC prepared by freeze-drying with PEG 6000 until melting. Then, when the blend was fluid and homogenous, each system was fastly cooled in an ice bath in order to make it solid to give respectively solid dispersion (SD), or inclusion complexes in solid dispersion (ICSD). The products obtained were then ground in a mortar. Each solid dispersion was prepared with 500 mg of Nim or an equivalent amount in IC for ICSD and final weight was fixed at 10 g. Corresponding PM were obtained by thoroughly mixing the various components with a spatula, in the same amount as in SD.

Physico-chemical characterizations

Thermal analysis was performed using a DSC-6 from Perkin-Elmer, between 30 and 300 °C at a 10 °C/min increasing rate.

A FT-IR (Perkin-Elmer) equipped with an ATR-Ge crystal from Pike Technologies was used for the analysis in the frequency range between 4,000 and 550 cm^{-1}, at a 4 cm^{-1} resolution.

Dissolution studies

In vitro dissolution studies of pure drug, SD, IC and ICSD were carried out, for 60 min, at pH 7.4 at 37.0 ± 0.5 °C, using the rotating paddle method (stirring: 100 rpm) with a USP XXII apparatus 2 (Erweka type DT6R, Germany), with a dissolution medium composed of phosphate buffer, 10 g/L polysorbate 80. The amount of Nim dissolved was evaluated on a spectrophotometer Beckman DU 640 B at 398 nm. All samples were analyzed in triplicate.

Nimesulide absorption after oral administration to rats

Male Wistar rats weighing 225–250 g were fasted prior to drug administration, water was allowed ad libitum. Each product was administered orally to a group of three rats after being dissolved in water or in a mixture of glycerol/polysorbate 80/water. The equivalent dose of Nim administered was 12.5 mg/rat. Blood samples (1 mL) were taken periodically at 1 h, 2 h, 3 h, 5.5 h, 7.5 h and 23 h, in intracardiac, and centrifuged at 5,000 rpm for 10 min. Plasma was taken and frozen at –15 °C until quantification.

Nimesulide quantification by HPLC

Nim concentration was evaluated by an HPLC method with a Varian® 9010 pump, equipped with an 9050

Table 1 Solubilization and apparent stability of nimesulide by cyclodextrins

Cyclodextrins at the maximum solubility (mM)	Maximum concentration of solubilized nimesulide (mM)	Stability constant K_C (mM^{-1})
Without cyclodextrins	≈ 0.03	
β (16.3)	0.3	0.473
γ (179)	0.47	0.0646
HPβ (300)	1.2	0.11
PMβ (250)	4.75	0.497

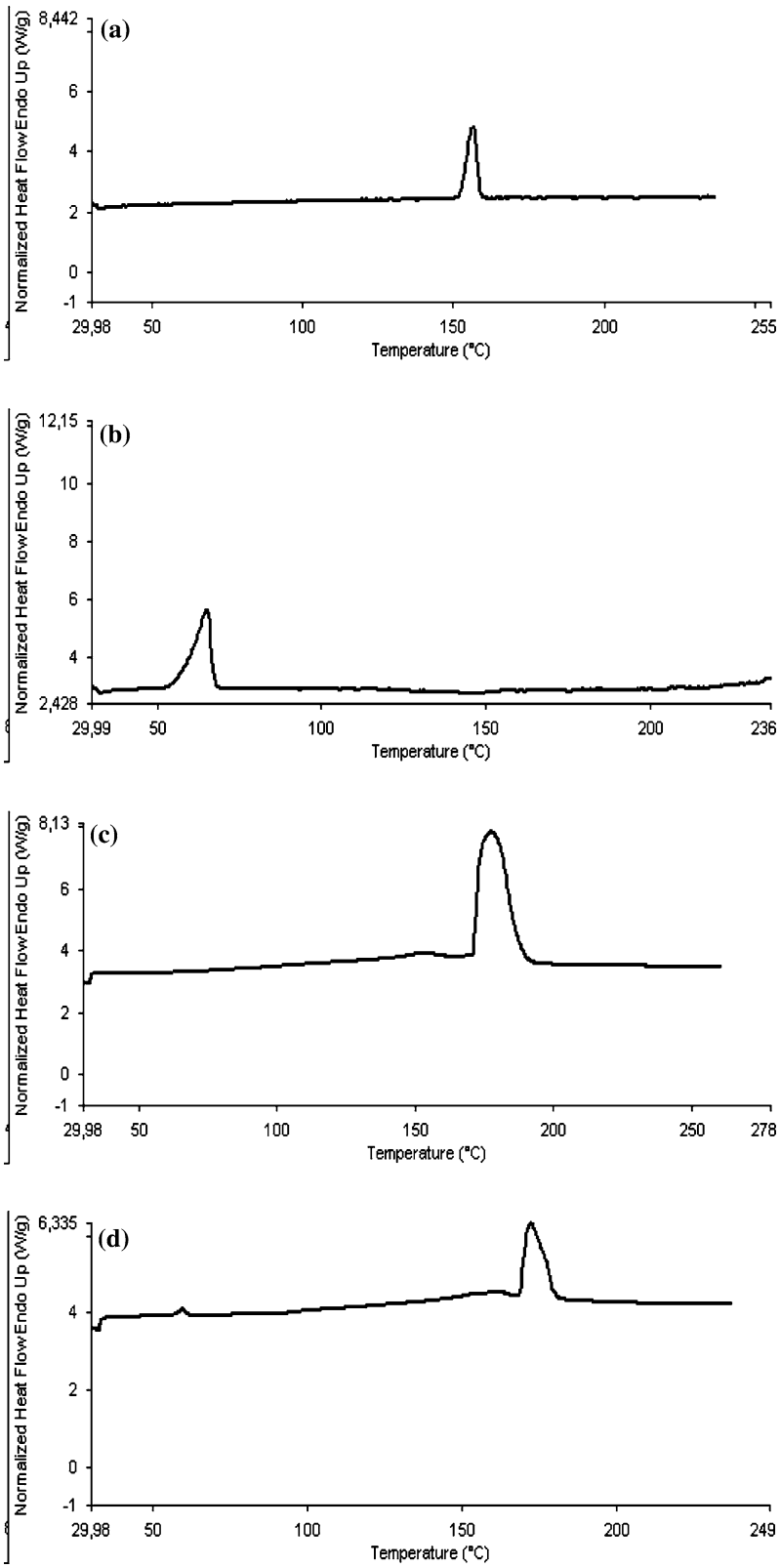

Fig. 1 DSC spectra of nimesulide (**a**), PEG 6000 (**b**), β-CD (**c**), HPβ-CD (**d**)

injector, 9100 UV detector, a Varian Star® software (1990, version C), an Interchrom Nucleosil® C18 (5 μm,15 cm) reverse phase column. Mobile phase consisted in a mixture of acetonitrile, methanol and potassium dihydrogenophosphate buffer 15 mM, pH 7.3, 30:5:65 (v/v). Flow rate was fixed to 1 mL/min, injection volume to 10 μL and detection wavelength to 404 nm. In that case, retention time was between 5 and

7 min. Before injection samples to analyze were prepared by the following method: 250 μL of plasma were added to 1 mL of methanol and vortexed, centrifuged at 3,500 rpm for 5 min, then 500 μL of the supernatant were taken and added to 500 μL of dihydrogenophosphate buffer [5].

Results and discussion

Each phase solubility diagram of Nim in aqueous solutions of CDs were of A_L type diagram. According to Higuchi and Connors theory [6], this may be attributed to the formation of soluble 1:1 Nim–CD IC. The

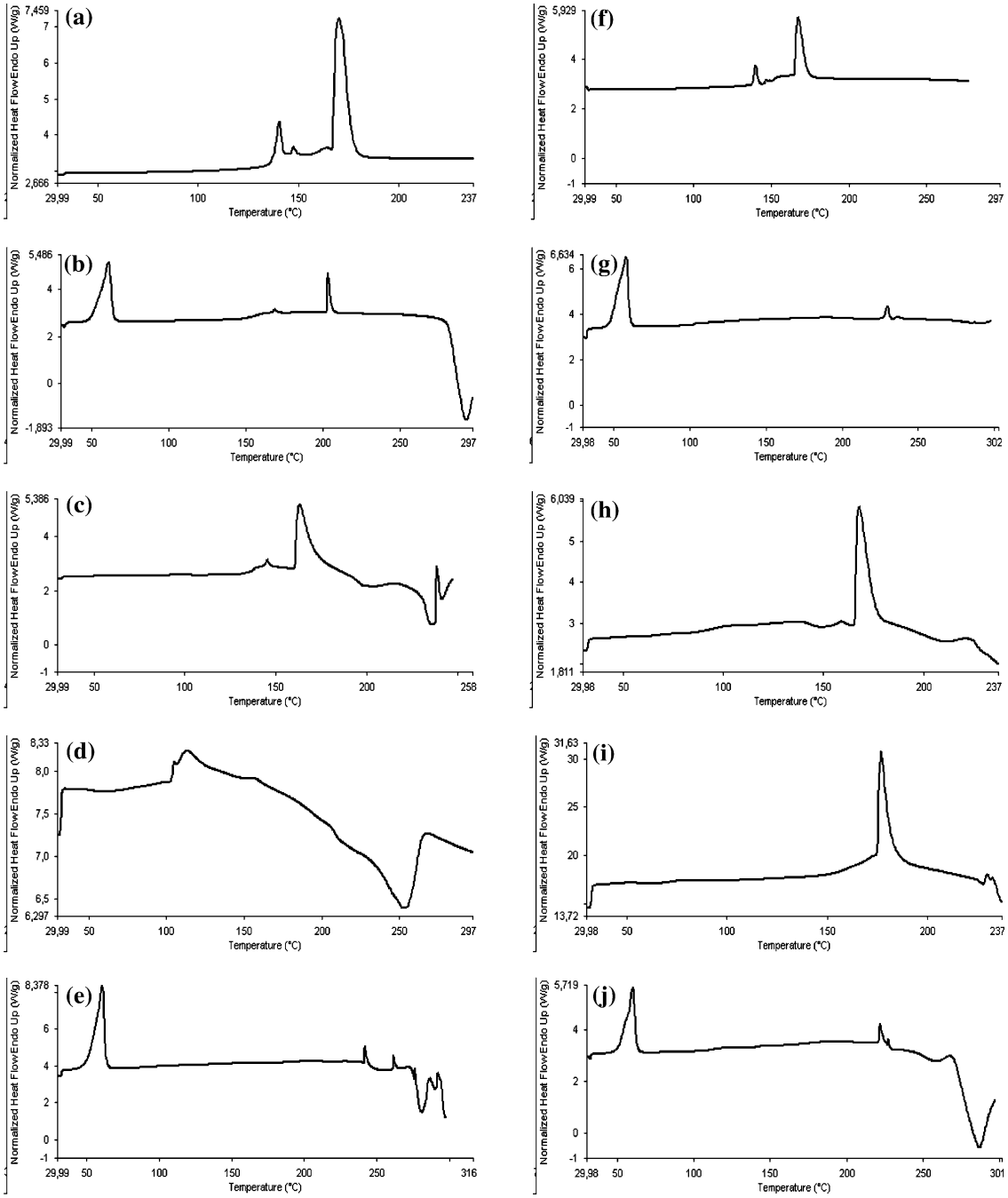

Fig. 2 DSC spectra of PM of nimesulide and β-CD (**a**), SD of nimesulide and β-CD (**b**), IC of nimesulide and β-CD (evaporation) (**c**), IC of nimesulide and β-CD (freeze-drying) (**d**), ICSD of nimesulide and β-CD (**e**), PM of nimesulide and HPβ-CD (**f**), SD of nimesulide and HPβ-CD (**g**), IC of nimesulide and HPβ-CD (evaporation) (**h**), IC of nimesulide and HPβ-CD (freeze-drying) (**i**), ICSD of nimesulide and HPβ-CD (**j**)

best improvement of aqueous solubilization and stability constant, was obtained with PMβ-CD (Table 1). DSC and FTIR characterizations were conducted only on products containing β- and HPβ-CD with a molar ratio of 1:1 (Nim:CDs) because β-CD IC presented a high stability constant and HPβ-CD IC had an interesting improvement in Nim aqueous solubilization.

DSC spectra of Nim showed an endothermic peak around 150 °C, PEG 6000 around 60 °C and both CDs (β and HPβ-CD) around 170 °C (Fig. 1). PM analysis showed changes in endothermic peaks compared to products alone (Fig. 2). According to these differences found between products alone and PM, IC, SD and ICSD comparison was done with corresponding PM rather than with products alone. IC spectrum prepared by evaporation presented no differences with PM spectrum ((c) and (h) versus (a)) whereas ICSD spectrum (Fig. 2, (e) and (j) versus (a)), showed Nim endothermic peak disappearance. These HPβ-CD (Fig. 2) with the disappearance of Nim endothermic peak. This is the proof of interactions between components. Our results concerning the Nim inclusion in β-CD prepared by freeze-drying were the same as those obtained by Nalluri et al. (2003) [7]: these authors prepared by coevaporation or kneading method a partial inclusion of Nim in β-CD.

Table 2 FT-IR peaks of nimesulide

Bonds	N–H	Aromatic C=C		C–O	
Position (cm^{-1})	3280	1600	1580	1520	1690

FT-IR analyses proved that PM spectra were the superposition of each product alone, suggesting, that there was no interactions. IC (Fig. 3), whatever the CD and the preparation way used, showed the same spectrum: disappearance of Nim peaks at 3,280 and 1,520 cm^{-1}. ICSD (Table 2, Fig. 3) were the result of

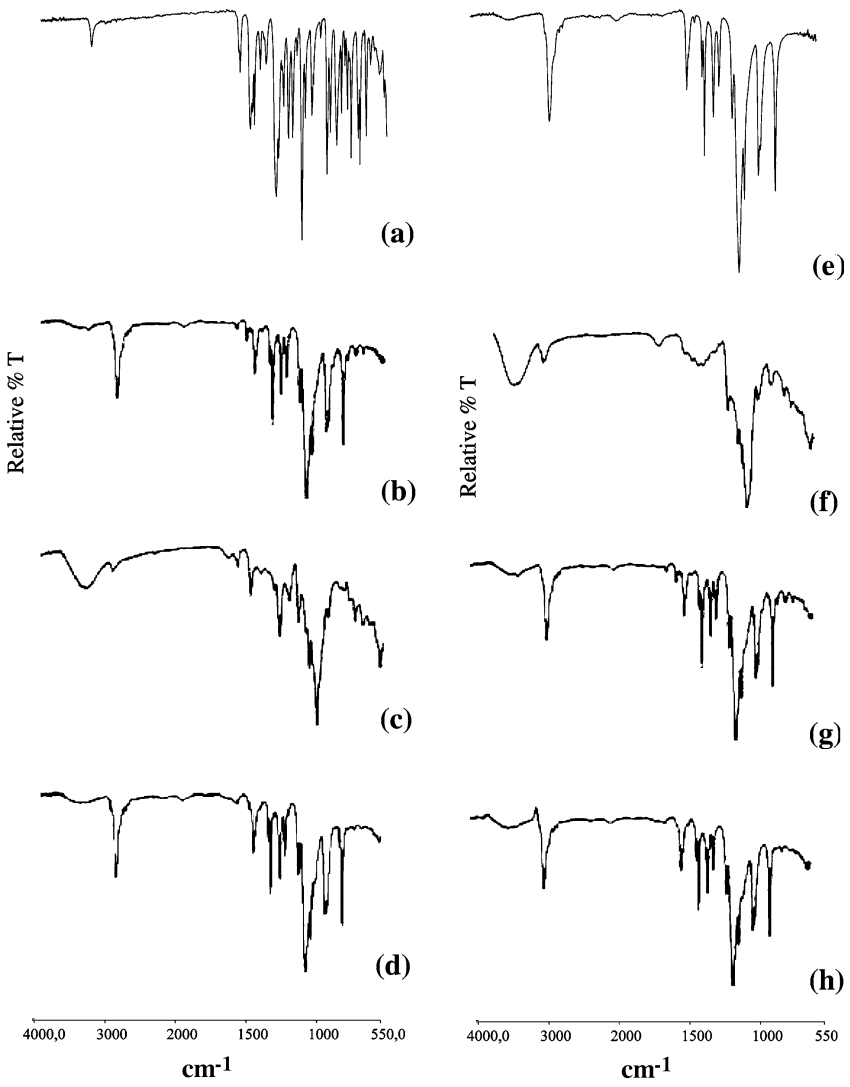

Fig. 3 FT-IR spectrum of nimesulide (**a**), SD of nimesulide and β-CD (**b**), IC of nimesulide and β-CD (**c**), ICSD of nimesulide and β-CD (**d**), PEG 6000 (**e**), HPβ-CD (**f**), SD of nimesulide and HPβ-CD (**g**), ICSD of nimesulide and HPβ-CD (**h**)

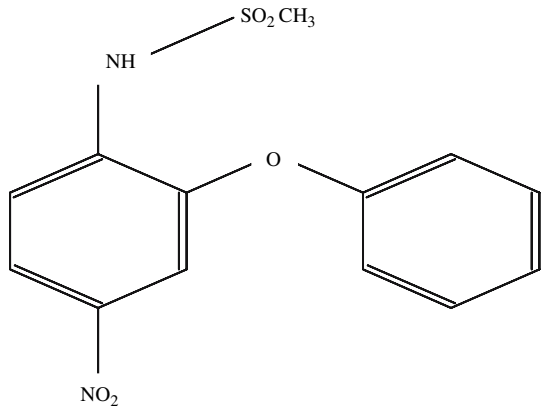

Fig. 4 Chemical structure of nimesulide

SD and IC spectra but 1,490, 3,280 and 1,520 cm^{-1} peaks disappeared. These results suggest that, in IC, the N-H function of Nim (Fig. 4) was included in CDs cavity with a partial inclusion of the oxygen atom. The SD spectra did not show the presence of IC of Nim-CDs (Fig. 3). Moreover, considering interactions between CDs and PEG 6,000, many peaks of HPβ-CD disappeared during melting with PEG 6000 (Fig. 3), this might sign a stronger interaction between PEG 6000 and HPβ-CD than between Nim and HPβ-CD. These interactions did not exist between PEG 6000 and β-CD. According to these results, we noticed a Nim inclusion in IC whatever the CDs and the preparation way used. Inclusions were kept in ICSD whereas in SD, interactions between CDs and PEG 6000 were stronger than interactions between CDs and Nim which were inexistant.

Fastest dissolution was obtained with IC prepared by evaporation may be due to nimesulide hydrophilization in presence of HPβ-CD (Fig. 5). For IC, whatever the CD used, the dissolution was achieved in 15 min whereas in case of SD and ICSD dissolution was maximum at 30 min. However, PM and Nim were not entirely dissolved one hour later. Consequently, IC, SD and ICSD have shown an improvement of the dissolution rate compared to PM and Nim. But addition of PEG 6000 did not improve the dissolution rate: those of SD and ICSD were lower than that of IC. PEG 6000 would be a limiting factor due to its lower hydrosolubility than that of IC alone.

Nim in rat plasma was quantified by the method given in experimental part. In case of the different complexes formed in the molar ratio 1:1 (β and HPβ-CDs, IC, SD and ICSD), C_{max} was comprised between 4.9 ± 1.1 and 13.2 ± 0.0 μg/mL (Table 3). C_{max} for Nim was 8.8 ± 1.5 μg/mL and only SD containing β-CD and IC containing HPβ-CD had an higher C_{max} than Nim, 9.1 ± 1.6 and 13.2 ± 0.0 μg/mL respectively. T_{max} was comprised between 1.0 ± 0.0 and 4.3 ± 1.1 h. Nim T_{max} was 2.7 ± 0.4 h. T_{max} lower than Nim alone were obtained with IC, ICSD and SD containing HPβ-CD (between 1.0 ± 0.0 h and 2.3 ± 0.4 h). The highest T_{max} was obtained with SD containing β-CD (4.3 ± 1.1 h). Therefore, IC containing HPβ-CD had a higher C_{max} and a lower T_{max} than Nim. It seems that IC and ICSD improved T_{max}: these formulations could allow to achieve a pain-relief faster than Nim alone. When a 12.5 mg/rat equivalent dose of Nim was used, no ulcers were observed with β-CD (IC, SD, ICSD or PM) or Nim (alone, SD or PM) whatever the form. On contrary, PMβ-CD in IC and SD, presented ulcers in 2 rats/3. Ulcers were more numerous with PMβ-CD formulations than with β-CD and Nim, even when considering only PMβ-CD PM.

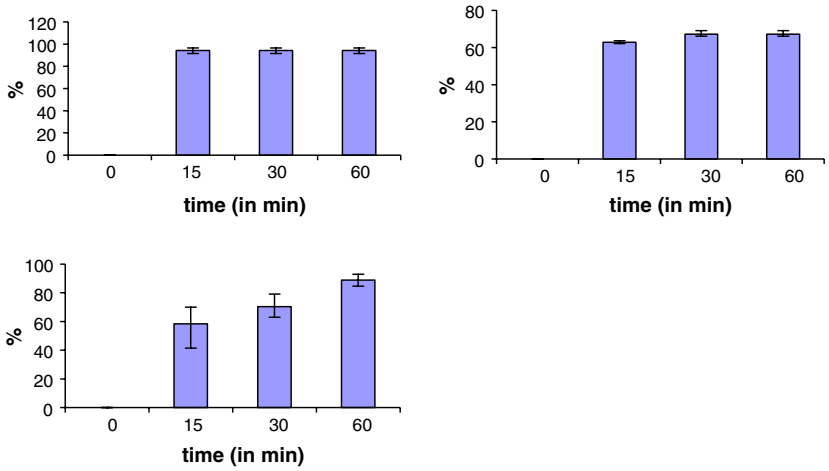

Fig. 5 Dissolution profile of IC of nimesulide and HPβ-CD (**a**), ICSD of nimesulide and β-CD (**b**), nimesulide (**c**)

Table 3 Mean C_{max} and mean T_{max} observed with different compounds in a molar ratio 1:1

Compounds	C_{max} (μg/mL)	T_{max} (h)	Correspondant PM	C_{max} (μg/mL)	T_{max} (h)
Nimesulide	8.8 ± 1.5	2.7 ± 0.4			
SD	4.9 ± 1.1	3.8 ± 0.9	PM N-PEG	4.6 ± 0.8	3.2 ± 1.1
IC β 1/1	8.5 ± 0.6	1.7 ± 0.4	PM N-β 1/1	6.5 ± 0.1	4.7 ± 0.9
IC HPβ 1/1	13.2 ± 0.0	2.0 ± 0.0	PM N-HPβ 1/1	5.6 ± 1.4	6.5 ± 1.0
SD β 1/1	9.1 ± 1.6	4.3 ± 1.1			
ICSD β 1/1	5.4 ± 0.1	1.0 ± 0.0			
SD HPβ 1/1	8.6 ± 0.0	2.3 ± 0.4			
ICSD HPβ 1/1	7.5 ± 0.7	1.7 ± 0.4			

PM: Physical mixture; N: nimesulide; SD: solid dispersion; IC: inclusion complex

ICSD: Inclusion complex in solid dispersion

Each value represents the means of three experiments

Conclusion

This study showed that IC, SD and ICSD improved the dissolution rate compared to that of PM and Nim. PEG 6000 would be a limiting factor due to its lower hydrosolubility than that of IC alone. Moreover, PMβ-CD formulations should be used with caution as it induced ulcers in rats.

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