Characterization of ternary solid dispersions of nimesulide with Inutec SP1 and β-cyclodextrin and evaluation of anti-inflammatory efficiency in rats

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Objective
The objective of this investigation is to enhance the physicochemical properties of nimesulide (NS) and the stability of NS solid dispersions in order to improve the anti-inflammatory activity of the drug.

Background
NS – a NSAID – is sparingly soluble in water and this low aqueous solubility in addition to its poor wettability leads to variability in the bioavailability of the drug.

Materials and methods
In the present study, ternary dispersions of NS were investigated using a new polymeric carrier, Inutec SP1 (Inutec), in combination with β cyclodextrin (β-CD). The ternary dispersions were prepared using different ratios of NS and β-CD (2 : 1; 1 : 1; 1 : 2), to which a fixed amount of Inutec (20% w/w of total formula) was added using different methods of incorporation of the drug. Physical mixtures of equivalent compositions were prepared by physically mixing the ingredients. The optimal formulation obtained with a full factorial experimental design was used for the evaluation of anti-inflammatory activity.

Results
In the ternary dispersions, the dissolution behavior improved in comparison with the physical mixtures and was found to be dependent on the technique of incorporation of the drug, the method of preparation, and the molar ratio of drug to β-CD. Physical characterization of the ternary dispersions by infrared spectroscopy (FTIR), differential scanning calorimetry, and X-ray powder diffraction indicated a decrease in crystallinity because of partial inclusion in β-CD and the effect of Inutec, which promoted the formation of microcrystals or partial amorphization of the drug during the processing of the dispersions by kneading. Differential scanning calorimetry and X-ray powder diffraction curves of the dispersions prepared by the solvent method indicated the presence of a polymorphic form of NS with a lower melting point. The optimized ternary dispersion predicted by the full factorial design showed good physical stability following an accelerated stability test. The ternary dispersion of NS, Inutec, and β-CD was found to show better anti-inflammatory efficiency in rats compared with a commercial tablet of NS.

Conclusion
It can be concluded that the dissolution properties and the anti-inflammatory efficacy of the ternary dispersions of NS with β-CD and Inutec were enhanced because of a secondary solubilization of the inclusion by the polymeric surfactant.

Keywords:
accelerated stability, β-cyclodextrin, Inutec SP1, in-vivo evaluation in rats, nimesulide, ternary solid dispersion

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Introduction

Nimesulide (NS) is an important anti-inflammatory drug and shows selective COX-2 inhibition, which contributes toward its good gastrointestinal tolerability. Moreover, despite concerns over its potential hepatotoxicity, it remains approved for the market because of the beneficial action overweighing the risks associated with the drug [1]. However, the very poor aqueous solubility of NS is a huge hurdle in formulation development. Therefore, enhancement of water solubility has been an ongoing challenge for pharmaceutical researchers as it can lead to more efficient and safer formulations of this important medicament.

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Numerous studies have dealt with the use of different carriers for the preparation of solid dispersions (SD) of NS [2–8]. Considerable amount of research has been published on complexation of NS with β-cyclodextrin (β-CD). Nalluri and colleagues [9,10] studied binary systems in 1 : 1 and 1 : 2 molar ratios of drug and carrier. They reported that the increase in dissolution properties was because of the formation of a 1 : 1 complex in solution. Further increase in β-CD led to the formation of a 1 : 2 complex in the solid state. However, despite the true inclusion formed, the dissolution rate and efficiency values obtained were not as anticipated. The reason for this is the formation of crystalline inclusion complexes (IC) [9,10].

To overcome the drawback of the limited aqueous solubility of β-CD, Dutet *et al.* [6] examined the effect of double hydrophilization in ternary systems of NS and β-CD and PEG 6000, but found no improvement in the bioavailability of NS in rats.

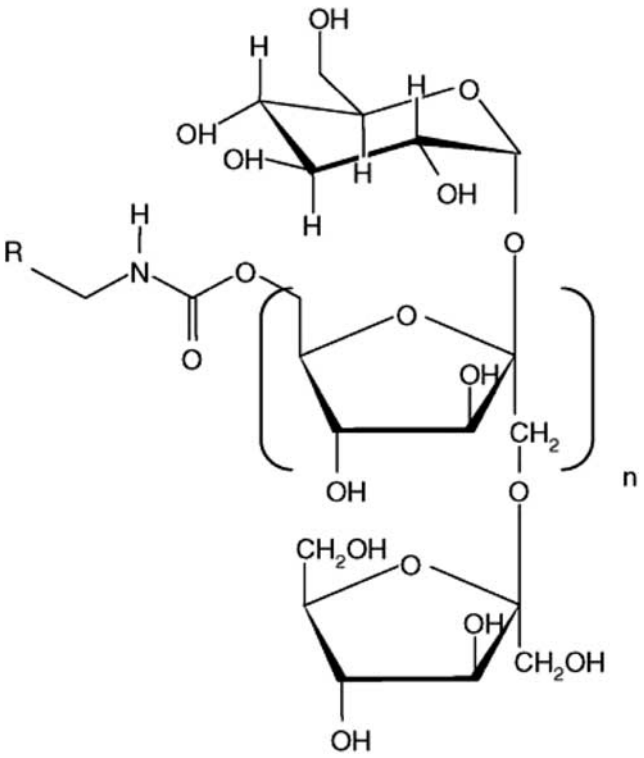
Generally, these and other attempts have failed to produce a stable marketable NS product, and thus there is a need for novel, more efficient carriers for NS. Recent advances in excipient technology have resulted in new surfactants such as Inutec SP1 (Inutec) of Orafti Non-Food. It is derived frominulin by grafting alkyl groups on a polyfructose backbone (Fig. 1).

In this way, a structure is obtained, with polyfructose loops providing steric stability. The polymeric nature of this surfactant has made Inutec very useful as an emulsifier in cosmetic and food industries [11]. Its application in pharmaceutical formulations has been reported by Van den Mooter *et al.* [12] as a carrier for SD of itraconazole, a drug with very low aqueous solubility. A 20/80 w/w SD of itraconazole and Inutec led to an improved dissolution rate. The dissolution efficiency (DE) depended more on the method of preparation than on the degree of amorphization. In a recent study, Janssens *et al.* [13] investigated further the effect of Inutec on itraconazole in ternary dispersions with polyvidone-vinylacetate 64 (PVPVA 64) and found that the improvement vis-à-vis the binary systems depended on the incorporation of a sufficient amount of PVPVA 64 required for the molecular dispersion of itraconazole. Ibrahim *et al.* [14] used Inutec and hydroxypropyl-β-CD for the preparation of chewable tablets of etodolac. The authors reported that the dissolution rate of etodolac at pH 1.2 and 6.8 was improved compared with a pure drug and physical mixture (PM) as a result of loss of crystallinity.

We reported in a previous publication [15] on the effect of Inutec on the dissolution behavior of NS in binary dispersions of NS with increasing amounts of Inutec. The dissolution rate was enhanced proportionally with the increase in the Inutec concentration and a ratio of drug to Inutec of 1 : 3 led to a maximum of 87% DE after 180 min.

The aim of the present study is to evaluate the effect of Inutec at a low concentration (20% w/w) to act as a second hydrophilization factor in ternary dispersions based on NS–β-CD complexes. Another aim is to use an experimental design for optimization of the formula to conduct accelerated stability tests and consequently for its use in the evaluation of anti-inflammatory efficiency in rats.

Figure 1



Chemical structure of Inutec SP1.

Materials and methods

Materials

NS was obtained as a gift sample from Sigma (Monofia, Egypt). Inutec SP1 was generously provided by Orafti Non-Food (Tienen, Belgium). β-CD (MW 1135) was purchased from Sigma Chemical Company (St Louis, Missouri, USA). All other materials were of analytical grade.

Preparation of ternary systems from inclusion complex and Inutec (ICSD)

First, the IC of NS and β-CD were prepared in 2 : 1, 1 : 1, and 1 : 2 molar ratios using two methods: solvent and kneading methods.

Solvent method (IC/S)

The alcoholic solution of NS was added to an aqueous solution of β-CD. The resulting mixture was stirred for 30 min and evaporated under reduced pressure at a temperature of 60°C until dry. The dried mass was ground in a mortar and passed through a sieve (250 μm).

Kneading method (IC/K)

A mixture of NS and β-CD was wetted with water and kneaded thoroughly for 30 min in a glass mortar. The resulting paste was dried under vacuum for 24 h.

The dried mass was ground in a mortar and passed through a sieve (250 μm).

Second, the IC of NS–β-CD was mixed with a fixed amount of Inutec (20% w/w of the total formula), and then wetted together with water and kneaded as discussed in the kneading method.

These systems are distinguished by the preparation method of the binary IC.

It should be noted that by maintaining the amount of Inutec added to the ternary systems constant, the ratio of Inutec to NS increases with an increase in the molar ratio of β-CD to drug.

Preparation of ternary solid dispersions

NS, β-CD, and Inutec were dispersed together using either the solvent or the kneading method.

Solvent method (SD/S)

The aqueous solution of β-CD was added to an alcoholic solution of NS and Inutec. The solvents were evaporated using the rotavapor as discussed previously.

Kneading method (SD/K)

A mixture of NS, β-CD, and Inutec was wetted with water and kneaded as discussed previously.

Preparation of physical mixtures

The corresponding PM were obtained by mixing the various components together in a mortar by trituration for 5 min, followed by sieving (250 μm).

Determination of NS content in the prepared formulations

An accurately weighed amount of NS formulation was dissolved in phosphate buffer (pH = 7.4) and sonicated for 30 min to ensure complete extraction of the drug from the dispersion. The content of NS was determined spectrophotometrically at 392 nm using a UV spectrophotometer. Each preparation was tested in triplicate.

Wettability study

A powder sample (3 g) was placed in a sintered glass funnel (33 mm internal diameter). The funnel was plunged into a beaker containing water such that the surface of water in the beaker remained at the same level as the powder in the funnel. Methylene blue powder (100 mg) was layered uniformly on the surface of the powder in the funnel. The time required for wetting of the methylene blue powder was measured. The average of three observations was used for drawing the conclusions [3].

Phase solubility study

Solubility studies were carried out as described by Higuchi and Connors [16]. An excess amount of NS was added to screw-capped vials containing different concentrations of the carrier solution. The vials were shaken mechanically at 37 ± 0.5°C for 72 h until reaching equilibrium. Filtration of the suspension was carried out using 0.45 μm millipore filters. An aliquot portion of the filtrate was diluted with phosphate buffer (pH = 7.4) and analyzed for drug content by measuring its absorbance spectrophotometrically at 392 nm against a blank solution containing the same concentrations of the carrier. Each experiment was conducted in triplicate.

Solid-state characterization

Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectra were recorded using an FTIR-6100 type A spectrophotometer (Jasco, Tokyo, Japan) equipped with a deuterated triglycine sulfate detector. Samples were prepared in KBr disks using a hydrostatic press. The scanning range was between 4000 and 400 cm^{-1} at 4 cm^{-1} resolution.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed using a Pyris5 instrument (Perkin Elmer, Waltham, Massachusetts, USA) equipped with an intercooler. A dry purge of nitrogen gas was used at 20 ml/min. The instrument was calibrated with pure indium. Samples (2–3 mg) were analyzed in closed Al pans from 50 to 220°C at a heating rate of 10°C/min.

X-ray powder diffraction

X-ray powder diffraction (XRPD) patterns of the pure ingredients and all of the SD containing varying proportions of NS in the matrix were recorded using an X-ray diffractometer (Scintag Inc., Cupertino, California, USA) equipped with CuKα as the source of radiation. Measurements were carried out using 45 kV voltage and 9 mA current. The 2θ values and the intensities of the peaks were compared for pure ingredients, the PM, and the SD systems.

Solubility study

The solubility was determined in distilled water at 37°C. A sample equivalent to 25 mg of NS (excess amount of NS) was added to 10 ml of distilled water in a vial with a teflon-lined screw cap. The vials were shaken mechanically at 37 ± 0.5°C for 72 h until reaching equilibrium. Filtration of the suspension was carried out using 0.45 μm millipore filters. An aliquot portion of the filtrate was diluted with phosphate buffer (pH = 7.4) and analyzed for drug content by measuring its absorbance spectrophotometrically at 392 nm. Each experiment was conducted in triplicate.

In-vitro dissolution study

The dissolution rate was determined in the USP Dissolution Tester, Apparatus I, at 37 ± 0.5°C. The dissolution medium was 900 ml of phosphate buffer (pH = 7.4) at a rotation speed of 50 rpm. Powder samples containing 25 mg of NS or its equivalent of PM or SD were filled in transparent zero-sized hard gelatin capsules. Aliquots, each of 5 ml, from the dissolution medium were withdrawn at time intervals 15, 30, 45, 60, 90, 120, 150, and 180 min and replaced by an equal volume of fresh dissolution medium. The samples were filtered through a 0.45 μm millipore filter and assayed

spectrophotometrically for NS at 392 nm using fresh dissolution medium as a blank.

The DE was calculated according to Khan [17] and is defined as the area under the dissolution curve up to the time, *t*, expressed as a percentage of the area of the rectangle described by 100% dissolution at the same time. The DE can have a range of values depending on the time interval chosen. The DEs at 30, 60, and 180 min. were calculated from the dissolution profiles. The experiments were conducted in triplicate.

Experimental design

An experimental design was generated to estimate the effects on the dissolution properties of the following experimental variables: method of preparation at two levels (solvent, kneading) and the NS : β-CD : Inutec ratio at three levels (*2* : 1 : 20%, 1 : 1 : 20%, and 1 : *2* : 20%).

Accelerated stability study

An accelerated stability study was carried out by subjecting the SD to stressed conditions at 40°C and 75% relative humidity (maintained using a saturated solution of NaCl) for a period of 3 months. The effect of the stressed conditions was determined by measuring in-vitro dissolution and by DSC and XRPD studies.

Evaluation of anti-inflammatory activity of selected nimesulide solid dispersions

Twenty-four adult female albino rats 150 ± 20 g were used. The rats were randomly allocated into four groups, each including six animals. Carrageenan was used to induce rat's paw edema. This effect was determined according to the method described previously in the literature [18,19]. The animals were kept on a standard laboratory diet. The rats were kept fasted for 16 h before the experiment, but were allowed free access to water. The samples were administered orally as a suspension to the respective animal groups at a dose of 50 mg/kg [20,21].

One hour after administration, edema was induced by an injection of 0.1 ml of 1% (w/v) carrageenan solution in distilled water into the planter aponeurosis of rats' right hind paws. The volume of the injected paw was measured immediately after carrageenan injection and after 1, 2, 3, 4, and 5 h using a plethsymometer. The percentage increase in paw volume was calculated according to the equation given by Delporte *et al.* [22].

$$\%\,\text{increase in paw volume} = (V_f - V_i)/V_i \times 100,$$

where V_f and V_i are the final and the initial paw volume of an animal, respectively.

In addition, the percentage inhibition of edema volume for each time was calculated from the mean effect in control and in treated animals according to the following equation [23].

$$\%\,\text{inhibition of edema volume} = (1 - V_t/V_c) \times 100,$$

where V_t and V_c are the mean increase in the volume of the carrageenan-injected paw of the treated group and the control group, respectively. The one-way analysis of variance test was carried out on the area under percentage increase in edema volume versus time curve.

Statistical analysis of data

All data were analyzed statistically using the analysis of variance test for a *P* value of 0.05 using the social package for statistical study Software (SPSS Company, IBM Corporation, New York, USA). Differences were considered statistically significant at a value of *P* less than 0.05.

Results and discussion

The composition and method of preparation of all the systems studied are listed in Table 1.

Nimesulide content in the prepared formulations

The drug content and the percentage recovery were determined in all prepared formulations in order to confirm that there was no drug loss during preparation and that the SD showed good content uniformity.

Wettability study

The mean wetting times of representative PM and dispersions are shown in Fig. 2. It can be seen that the wetting time for pure NS (8 h) was significantly reduced in the PM. The wettability was further improved in the dispersions and the best results were obtained for the dispersions prepared using the SD technique [SD/S (1 : *2* : 20%) and SD/K (1 : *2* : 20%) in Fig. 2]. It is also evident from Fig. *2* that the wetting times decreased significantly ($P < 0.05$) on adding Inutec to the binary complexes (compare ternary ICSD/S; ICSD/K to binary IC/S; IC/K in Fig. 2). This confirms the secondary hydrophilization effect of Inutec in the ternary systems.

Phase solubility study

The phase solubility diagrams of NS and β-CD with and without 0.5% Inutec can be classified as A_L-type according to Higuchi and Connors [16] as shown in Fig. 3. The aqueous solubility of NS increased linearly ($R^2 = 0.9952$ and 0.9971 in the absence and in the presence of 0.5% of Inutec, respectively) as a function of the β-CD concentration. The phase solubility of NS in aqueous solutions of β-CD increased in the presence of 0.5% of Inutec as reflected by the small increase in the stability constant from $K_c = 354$ to $K_c = 430$ mol/l. This is in agreement with the increased wettability of the PM (Fig. 2).

Solid-state characterization

Studies were carried out to determine the nature of the products obtained.

Fourier transform infrared spectroscopy

FTIR data were obtained to determine whether chemical interactions occurred during the preparation of the SD.

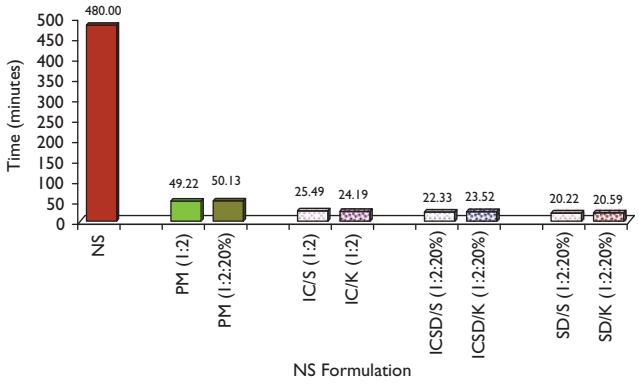
Figure 4 shows the FTIR spectra of the individual components, their PM, and the different dispersions. The FTIR spectra of the PM showed the patterns of each component. In the FTIR spectra of the ICSDs and the SDs, the peak of the N–H function at 3290 cm^{-1} was slightly pronounced or invisible. Otherwise, no other new bonds

Table 1 Composition of physical mixtures and solid dispersions prepared using different methods

NS formulation	NS : β-CD (mol/mol)	Inutec % (w/w)	Method of preparation[a]
PM (2 : 1)	2 : 1	0	Physical mixing
PM (1 : 1)	1 : 1	0	Physical mixing
PM (1 : 2)	1 : 2	0	Physical mixing
PM (2 : 1 : 20%)	2 : 1	20	Physical mixing
PM (1 : 1 : 20%)	1 : 1	20	Physical mixing
PM (1 : 2 : 20%)	1 : 2	20	Physical mixing
IC/S (2 : 1)	2 : 1	0	IC/solvent (50 ml)
IC/S (1 : 1)	1 : 1	0	IC/solvent (50 ml)
IC/S (1 : 2)	1 : 2	0	IC/solvent (50 ml)
IC/K (2 : 1)	2 : 1	0	IC/kneading
IC/K (1 : 1)	1 : 1	0	IC/kneading
IC/K (1 : 2)	1 : 2	0	IC/kneading
ICSD/S (2 : 1 : 20%)	2 : 1	20	ICSD/solvent (50 ml)
ICSD/S (1 : 1 : 20%)	1 : 1	20	ICSD/solvent (50 ml)
ICSD/S (1 : 2 : 20%)	1 : 2	20	ICSD/solvent (50 ml)
ICSD/K (2 : 1 : 20%)	2 : 1	20	ICSD/kneading
ICSD/K (1 : 1 : 20%)	1 : 1	20	ICSD/kneading
ICSD/K (1 : 2 : 20%)	1 : 2	20	ICSD/kneading
SD/S (2 : 1 : 20%)	2 : 1	20	SD/solvent (70 ml)
SD/S (1 : 1 : 20%)	1 : 1	20	SD/solvent (100 ml)
SD/S (1 : 2 : 20%)	1 : 2	20	SD/solvent (150 ml)
SD/K (2 : 1 : 20%)	2 : 1	20	SD/kneading
SD/K (1 : 1 : 20%)	1 : 1	20	SD/kneading
SD/K (1 : 2 : 20%)	1 : 2	20	SD/kneading

β-CD, β cyclodextrin; IC/K, inclusion complex prepared using the kneading method; IC/S, inclusion complex prepared using the solvent method; ICSD/K, inclusion complex in solid dispersion prepared using the kneading method; ICSD/S, inclusion complex in solid dispersion prepared using the solvent method; NS, nimesulide; PM, physical mixture; SD/K, solid dispersion prepared using the kneading method; SD/S, solid dispersion prepared using the solvent method.
[a]Values in parentheses represent the amount of methanol used in the preparation (for 1 mol NS).

Figure 2



Wettability of selected NS–β-CD and NS–β-CD–Inutec SP1 formulations: comparison of different methods of preparation.

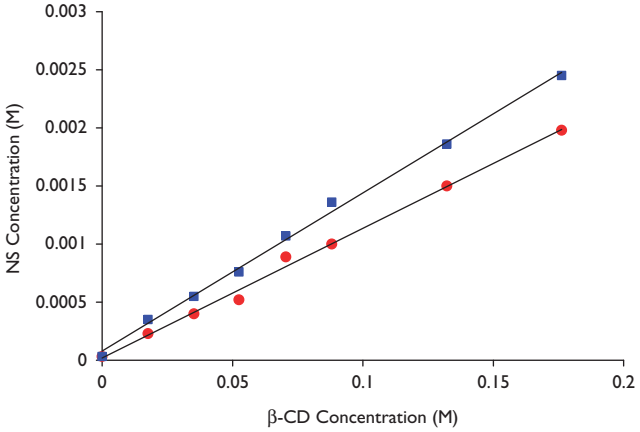
were observed, which indicates that there was no interaction between NS and the carriers at the molecular level.

Differential scanning calorimetry

DSC thermograms were generated to test for the possibility of the inclusion of NS in β-CD.

Figure 5 shows the DSC thermograms of the individual components and their ternary systems at a 1 : 2 molar ratio prepared by the solvent (S) and kneading (K) methods.

Figure 3



Phase solubility diagrams of NS in different concentrations of β-CD with and without 0.5% Inutec SP1 at 37°C: ●, NS–β-CD: $y=0.0112x+2E-05$, $R^2=0.9952$; ■, NS–β-CD–Inutec: $y=0.0136x+8E-05$, $R^2=0.9971$. β-CD, β cyclodextrin; NS, nimesulide.

The thermogram of NS showed a single endothermic peak with onset at 148.8°C and a peak at 150°C corresponding to its melting point. These results were also reported by Chowdary and Nalluri [24] and by Abdelkader *et al.* [5]. The DSC thermogram of β-CD showed a broad endothermic effect with a peak at 97°C. Inutec showed a small endothermic peak at 103.5°C at the tail of the water evaporation endotherm at 60°C and a glass transition signal at 143°C as also reported by Van den Mooter *et al.* [12].

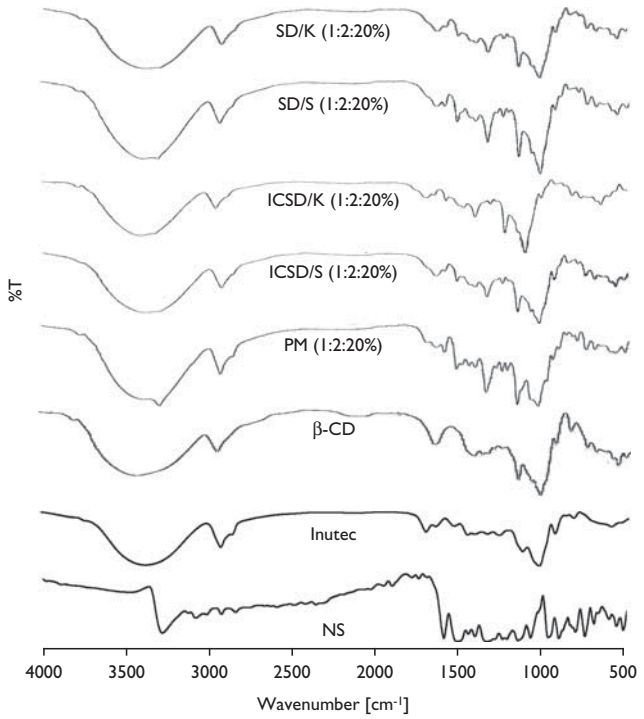
The thermogram of the PM is a combination of the DSC curves of the individual components without changes in the melting peaks. The ternary ICSDs prepared by both methods (S, K) and the ternary SD prepared by the kneading method (K) showed a marked reduction in the intensity of the NS endotherm when compared with that of the PM, indicating progressive partial inclusion of NS within the β-CD cavity. The thermogram of the ternary SD prepared by the solvent method was characterized by a split endotherm indicating that the NS showed polymorphism because of the use of an organic solvent (methanol) in the preparation of the dispersions. As we reported earlier, the use of a solvent induced a different polymorphic form of NS that melts at a slightly lower temperature [15]. Bergese *et al.* [25] also reported such a polymorphic form of the drug and Di Martino *et al.* [26] obtained a split endotherm because of the use of an organic solvent in their study.

The ICSD (solvent) system, in contrast, did not show the double-peaked endotherm. This might be attributed to the use of a smaller amount of alcoholic solvent used in its preparation (Table 1).

X-ray powder diffraction

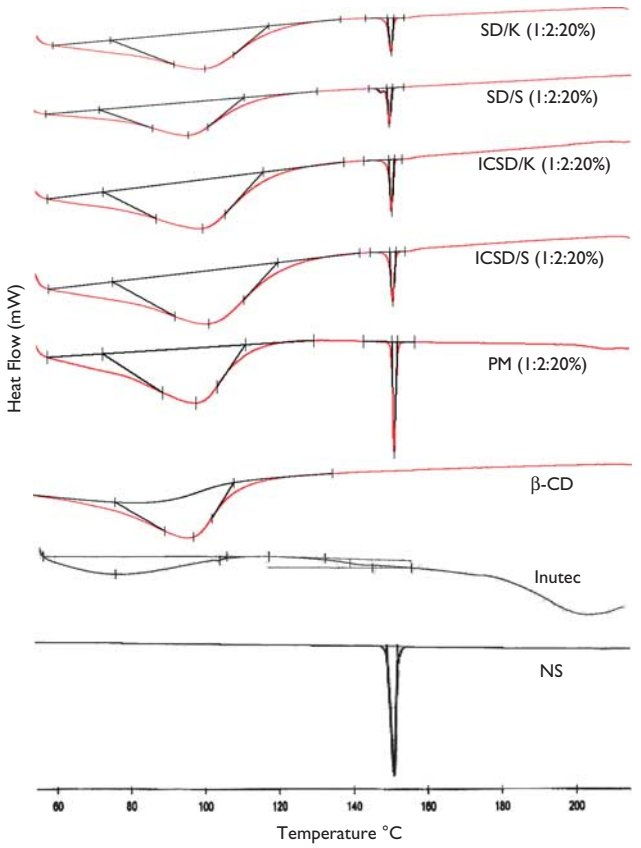
XRPD patterns were obtained to determine the crystallinity of the products obtained and to confirm the results of the DCS study.

Figure 4



FTIR spectra of NS, Inutec SP1, β-CD, their PM and their ternary ICSD, and SD at 1 : 2 molar ratio prepared by solvent (S) and kneading (K) methods. β-CD, β cyclodextrin; FTIR, Fourier transform infrared spectroscopy; ICSD, inclusion complex in solid dispersion; NS, nimesulide; PM, physical mixture; SD, solid dispersions.

Figure 5



DSC thermograms of NS, Inutec SP1, β-CD, their PM, and their ternary ICSD and SD at a 1 : 2 molar ratio prepared by solvent (S) and kneading (K) methods at a heating rate of 10°C/min. β-CD, β cyclodextrin; DSC, differential scanning calorimetry; ICSD, inclusion complex in solid dispersion; NS, nimesulide; PM, physical mixture; SD, solid dispersions.

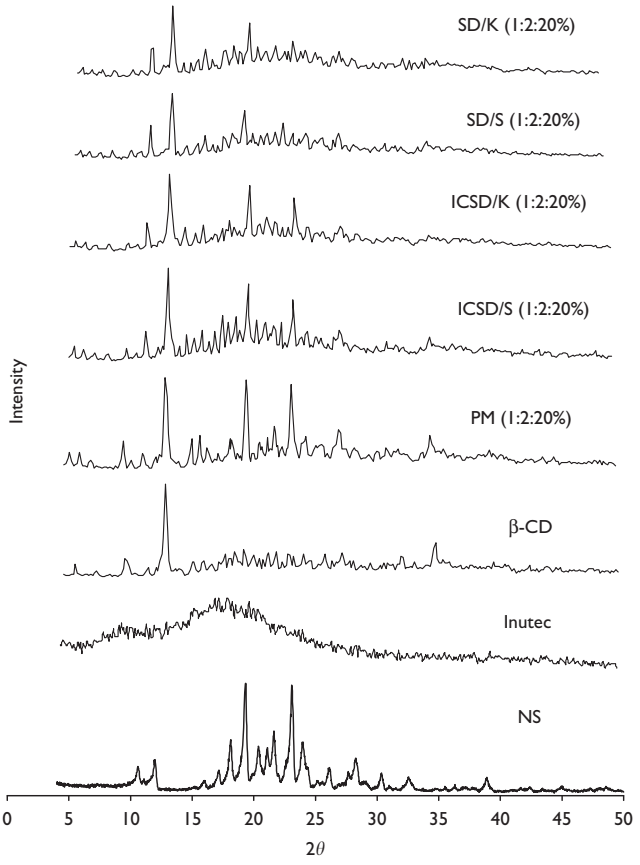
Figure 6 shows the diffractograms of NS, β-CD, Inutec, their ternary PM, and the different SD (ICSD and SD) at 1 : 2 molar ratios with β-CD, prepared using the solvent and the kneading methods.

NS showed the characteristic diffraction pattern with numerous distinctive peaks, indicating the highly crystalline nature of the drug. The most abundant peaks were observed at 2θ values of 19.3 and 23.1°. The diffraction pattern of β-CD showed numerous peaks, with a major peak at $2\theta = 12.48°$, whereas the diffraction pattern of Inutec was characterized by very small peaks protruding from the halo around $2\theta = 16–20°$.

The XRPD pattern of the PM represents a combination of the individual patterns of the drug and the carriers and the intensities of the peaks reflect the fraction of the drug in the mixture. The diffractograms of ICSD prepared using the solvent and kneading methods and SD prepared using the kneading method showed a notable reduction in the intensity of the characteristic peaks of the drug in comparison with the PM. This reduction in peak intensity is a result of loss of crystallinity of the drug in the preparation, indicating partial inclusion of NS within the β-CD cavity. The XRPD patterns of the ternary SD prepared using the solvent method showed a diffraction peak at $2\theta = 18.9°$, which was not observed in the XRPD pattern of the pure NS. This indicates the presence of polymorphism because of the use of an organic solvent (methanol).

The diffractograms of the binary and ternary systems were compared quantitatively with the diffractogram of the PM. For this purpose, the values of the relative intensity (I/I_o) were used, which were calculated from the intensity (I) of a selected peak ($2\theta = 23.1°$) and the intensity (I_o) of the major peak ($2\theta = 19.3°$). The relative intensity values decreased to $I/I_o = 85$ and 88% in the ICs and further to 80 and 82% in the ternary ICSDs depending on the method of preparation (solvent vs. kneading). The highest decrease in the relative intensity was observed in the ternary systems prepared using the SD technique, using the kneading method ($I/I_o =$ 69.8%), indicating the highest degree of amorphization compared with the PM and the other binary and ternary formulations. However, the I/I_o value of ternary dispersion prepared using the SD technique with methanol was not calculated because of the appearance of a new peak indicative of a different polymorphic form of NS. Janssens *et al.* [13] investigated the diffractograms of ternary systems of itraconazole with PVPVA 64 and Inutec SP1 in different ratios of polymer to Inutec. They reported that all the systems showed XRPD amorphous behavior, except for the one with the lowest ratio. They therefore concluded that itraconazole was molecularly dispersed in the PVPVA, whereas Inutec did not interact with any

Figure 6



X-ray powder diffractograms of NS, Inutec SP1, β-CD, their PM, and their ternary ICSD and SD at a 1 : 2 molar ratio prepared by solvent (S) and kneading (K) methods. β-CD, β cyclodextrin; ICSD, inclusion complex in solid dispersion; NS, nimesulide; PM, physical mixture; SD, solid dispersions.

of the components on a molecular level. This is in agreement with the present study that is NS interacts with the β-CD by means of partial inclusion. Further decrease in crystallinity in ternary systems compared with binary IC may be attributed to Inutec, which promoted the formation of microcrystals in the ternary systems. Consequently, the addition of this polymeric surfactant increased the saturation solubility and this could lead to better dissolution rates of the ternary dispersions vis-à-vis the binary systems. The XRPD findings are in full agreement with the DSC results (Fig. 5).

Solubility study

The aqueous solubility in distilled water of pure NS and the ternary ICSDs and SDs is shown in Fig. 7. The solubilities obtained for the PMs and the binary ICs are also shown for comparison. The solubility obtained for pure drug was 14.3 μg/ml. In the PM, the solubility increased as a result of the IC formed in the solution. The addition of Inutec led to a slight increase in solubility (compare ternary PMs with binary PMs in Fig. 7). This is in agreement with the results from the phase solubility study.

In the dispersions, the solubility increased compared with the PMs, indicating further interaction between NS and the carriers in the solid state at all drug to β-CD molar ratios. It can be noted that the addition of Inutec to the preformed IC of NS and β-CD did not induce a change in saturation solubility in any significant way (compare ICs with ICSDs). However, direct dispersion of the components enhanced the solubility significantly ($P<0.05$). Of the two methods of dispersion, the use of solvent yielded better results (compare ternary SD/S with ternary SD/K in Fig. 7).

In-vitro dissolution study

The dissolution rate (*%D*) and the DEs at 30, 60, and 180 min of all the systems studied are summarized in Table 2. Maximum values were obtained after 180 min of dissolution testing. It should be noted that although the maximum values obtained did not reach 100% after 3 h of dissolution testing, conducting the tests for a longer period was not considered practical.

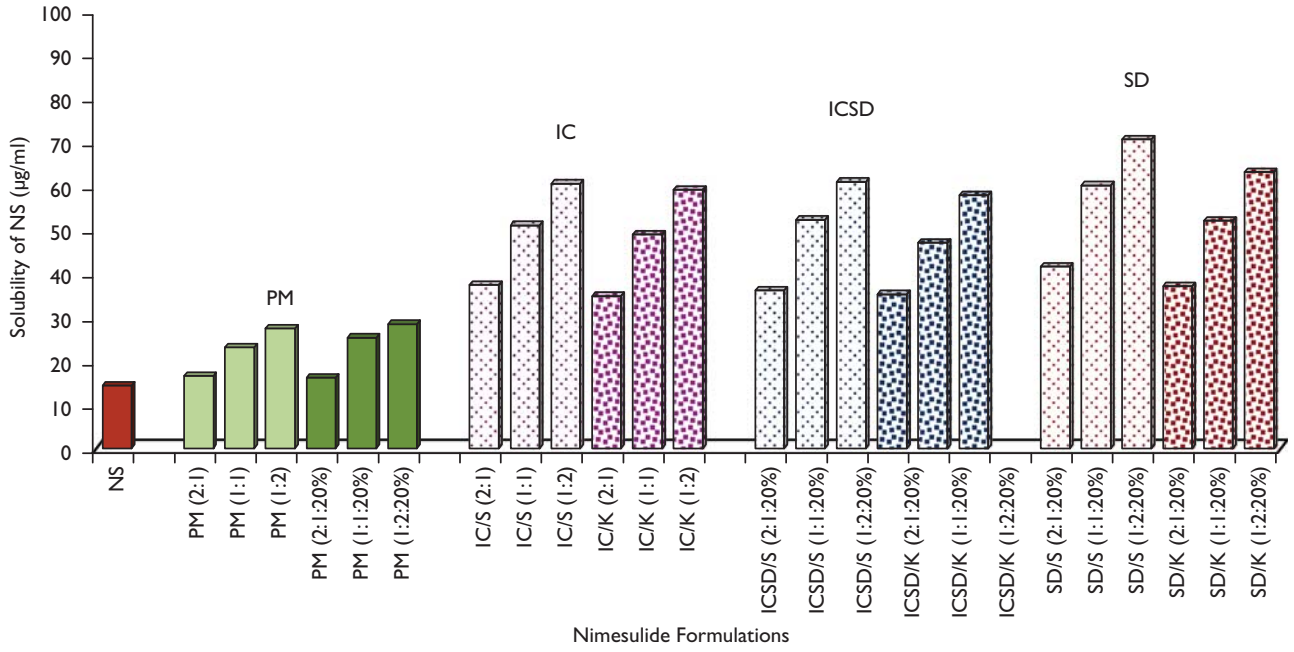
The dissolution enhancement in the PM was 2.2-fold compared with pure drug, which is in agreement with the improved wettability and complexation of the drug with β-CD in solution. The difference between the dissolution rates of the ternary PM and the binary PM was not statistically significant ($P>0.05$).

The dissolution profiles of all the dispersions showed a significant improvement ($P<0.05$) compared with the PM because of the progressive inclusion in the β-CD and/or the secondary hydrophilization action of Inutec. It can be seen from Table 2 that the increase in dissolution could be related to both the method of preparation and the presence of Inutec in the systems.

The dissolution profiles of the IC also increased compared with the PM but to a lesser degree than when compared with the dispersions. Similar to the saturation solubility, the dissolution rate is also the highest for solvent SD.

The improvement in the dissolution rate and the DE of the PM in comparison with the pure drug can be attributed to the formation of a soluble 1 : 1 complex of NS with β-CD as confirmed by the phase solubility studies and the results of the solubility tests. The addition of Inutec did not affect the dissolution of the ternary PM significantly ($P>0.05$), although this would have been anticipated from the improved wettability because of the solubilizing action of this polymeric surfactant. In the dispersion systems, however, the improved wettability resulting from the addition of Inutec significantly increased the dissolution of the ternary dispersions in comparison with the binary dispersions ($P<0.05$). This is because by physical mixing, the polymer is only deposited on the drug, whereas during the kneading process, there is deeper entrapment of the drug in the polymer network. Because of the unique action of Inutec to adsorb onto hydrophobic substrates with its alkyl chains, more hydrophobic particles become occupied by Inutec with its hydrophilic fructose loops in the solution. This leads to an increase in

Figure 7



Solubility (µg/ml) of physical mixtures and dispersions of NS with β-CD and Inutec SP1.

wettability and consequently to better dissolution properties of the ternary systems.

Generally, all dispersions showed better solubility and dissolution rate when compared with the PM. This can be attributed to the changes in the solid states as shown by the results of the physical characterization of the SD. In the kneaded dispersions, the predominant factor is the reduced crystallinity of the drug because of partial inclusion in the β-CD or the formation of microcrystals dispersed in the polymer network or the formation of some amorphous drug during the processing of the formulations. This was evidenced by the decrease in the melting endotherm on the DSC curves and by the reduced relative intensity of the characteristic peaks on the X-ray diffractograms. In addition to this, the dispersions prepared using the solvent method showed polymorphism as indicated by the split endotherm in the DSC curves and by the presence of a new peak on the X-ray diffractograms. Polymorphism of NS has been reported in the literature [25, 26] and the enhanced dissolution of the solvent dispersions found in the present study could be because of the polymorphic form with a lower melting point.

On the basis of the data obtained, it can be concluded that several factors contributed toward the enhanced dissolution rate and DE of the ternary dispersions of NS, β-CD, and Inutec, that is increased wettability because of a second solubilization of the inclusion by the polymeric surfactant Inutec, decreased crystallinity because of partial inclusion of NS in β-CD, and increased solubility because of polymorphism.

The use of Inutec may offer advantages not found with the more commercial carriers used for the preparation of SD.

Evaluation of the results of an experimental design

Three characteristic points on the dissolution curves (responses) were used for the evaluation of the effects of the experimental variables (factors). They are D_{30}, D_{60}, and DE_{180}, representing the percentage drug dissolved at 30, 60 min and the DE at 180 min, respectively (Table 2). A $2 \times 2 \times 3$ experimental design was generated for the three factors (technique, method, and ratio) at the selected levels.

The least square model was used in order to predict the optimal values of the responses within the ranges of the factors used in the experimental design. The main effects represent the values of the estimates of the parameters calculated from the model that was used to fit the data. The largest effect on the DE_{180} (7.4) was because of the ratio. The second highest effect (3.2) was because of the method of preparation of the ternary system, whereas the effect of the technique (i.e. SD or ICSD) exerted the smallest effect, with a value of the estimate of 2.8.

The optimal formulation was predicted to be at the following levels of the experimental variables: technique = SD; method = solvent; ratio = 1 : 2 : 20%.

This formula was used for further studies (accelerated stability and in-vivo evaluation of anti-inflammatory activity).

Accelerated stability study

The physical stability was investigated by comparing the dissolution profiles as well as the solid-state characteristics of the freshly prepared samples and of samples aged 1, 2, and 3 months. For this study, the formula optimized by the factorial design was used, that is the ternary SD

Table 2 Dissolution rate (%*D*) and dissolution efficiency of different formulations

	%*D* (min)			DE (%) (min)		
NS formulation	30	60	180	30	60	180
PM (2 : 1)	22.35 ± 2.1	30.6 ± 1.5	40.6 ± 3.9	12.7 ± 0.1	19.7 ± 0.2	29.9 ± 1.4
PM (1 : 1)	27.25 ± 1.6	35.0 ± 3.4	47.3 ± 2.2	16.3 ± 1.1	23.9 ± 1.6	36.3 ± 0.3
PM (1 : 2)	33.78 ± 2.5	42.1 ± 2.6	54.6 ± 1.9	19.0 ± 1.9	27.0 ± 2.4	40.1 ± 3.3
PM (2 : 1 : 20%)	23.74 ± 2.2	31.7 ± 1.5	41.5 ± 4.0	13.7 ± 0.2	20.3 ± 0.2	30.9 ± 1.5
PM (1 : 1 : 20%)	28.62 ± 1.7	35.6 ± 3.4	49.6 ± 2.3	17.1 ± 1.1	24.9 ± 1.7	37.5 ± 0.3
PM (1 : 2 : 20%)	36.51 ± 2.7	43.9 ± 2.8	55.2 ± 1.9	21.7 ± 0.5	31.3 ± 1.5	44.6 ± 1.8
IC/S (2 : 1)	43.62 ± 2.2	62.4 ± 2.3	77.2 ± 1.0	25.4 ± 1.0	40.2 ± 1.2	61.3 ± 0.9
IC/S (1 : 1)	51.66 ± 2.8	68.9 ± 2.6	80.8 ± 1.8	28.0 ± 2.4	45.3 ± 2.2	65.5 ± 2.2
IC/S (1 : 2)	64.71 ± 3.8	78.3 ± 2.5	84.9 ± 2.5	36.4 ± 2.1	54.5 ± 2.5	73.0 ± 2.8
IC/K (2 : 1)	39.90 ± 3.6	54.9 ± 3.5	75.1 ± 4.2	20.5 ± 2.3	34.5 ± 2.3	56.5 ± 2.9
IC/K (1 : 1)	44.77 ± 3.4	61.1 ± 2.9	78.3 ± 4.2	24.7 ± 2.4	39.8 ± 3.1	60.9 ± 3.5
IC/K (1 : 2)	50.71 ± 2.5	72.4 ± 3.0	83.6 ± 2.9	26.2 ± 2.4	45.6 ± 2.7	66.8 ± 2.4
ICSD/S (2 : 1 : 20%)	47.81 ± 4.5	66.0 ± 3.4	80.0 ± 3.7	26.0 ± 1.7	41.7 ± 1.0	62.5 ± 0.6
ICSD/S (1 : 1 : 20%)	48.94 ± 2.6	65.6 ± 4.3	81.9 ± 3.1	23.0 ± 2.2	41.3 ± 2.1	64.9 ± 2.5
ICSD/S (1 : 2 : 20%)	59.18 ± 2.1	79.4 ± 3.2	88.9 ± 2.1	32.5 ± 1.6	51.8 ± 2.1	73.8 ± 2.1
ICSD/K (2 : 1 : 20%)	47.05 ± 3.3	58.6 ± 4.8	70.5 ± 3.2	26.0 ± 2.4	39.5 ± 2.3	57.4 ± 1.6
ICSD/K (1 : 1 : 20%)	47.68 ± 3.3	65.8 ± 3.3	81.7 ± 3.8	25.0 ± 2.6	41.2 ± 2.9	63.1 ± 0.7
ICSD/K (1 : 2 : 20%)	53.88 ± 3.3	75.2 ± 3.5	85.2 ± 3.3	31.7 ± 2.3	48.3 ± 2.6	69.6 ± 2.5
SD/S (2 : 1 : 20%)	56.08 ± 2.9	69.4 ± 2.9	82.3 ± 3.6	35.9 ± 2.0	50.4 ± 0.8	67.5 ± 1.7
SD/S (1 : 1 : 20%)	65.51 ± 3.8	77.6 ± 2.3	86.5 ± 3.3	40.7 ± 2.5	56.7 ± 2.5	73.6 ± 2.7
SD/S (1 : 2 : 20%)	75.68 ± 3.2	86.7 ± 4.3	98.6 ± 2.8	48.5 ± 1.9	65.1 ± 2.8	84.5 ± 3.1
SD/K (2 : 1 : 20%)	46.34 ± 2.7	59.9 ± 2.3	73.8 ± 2.3	25.3 ± 2.2	39.3 ± 2.7	59.0 ± 2.7
SD/K (1 : 1 : 20%)	49.39 ± 4.4	69.3 ± 2.9	83.7 ± 1.2	28.7 ± 2.4	44.6 ± 2.4	66.5 ± 1.9
SD/K (1 : 2 : 20%)	61.68 ± 2.3	77.5 ± 3.7	89.1 ± 3.9	33.2 ± 2.4	52.1 ± 1.6	74. 0 ± 2.5

DE, dissolution efficiency; IC/K, inclusion complex prepared using the kneading method; IC/S, inclusion complex prepared using the solvent method; ICSD/K, inclusion complex in solid dispersion prepared using the kneading method; ICSD/S, inclusion complex in solid dispersion prepared using the solvent method; NS, nimesulide; PM, physical mixture; SD/K, solid dispersion prepared using the kneading method; SD/S, solid dispersion prepared using the solvent method.

obtained using the solvent method, SD/S (1 : 2 : 20%). In addition, the ternary SD obtained using the kneading method, SD/K (1 : 2 : 20%), was also investigated.

The dissolution rates of fresh and aged samples using the solvent method and those using the kneading method are shown in Fig. 8a and b, respectively. Statistical analysis showed that under the conditions of the accelerated test, no significant changes occurred in the dissolution behavior of the ternary dispersions of NS, β-CD, and Inutec ($P > 0.05$).

The thermal (by DSC) and crystalline (by XRPD) characteristics of the aged samples after 3 months of accelerated stability testing did not show any significant changes compared with those of the fresh samples, indicating good stability in the solid state.

Evaluation of the anti-inflammatory activity in rats

The presence of edema is one of the prime signs of inflammation [27]. It has been documented that carrageenan-induced rat paw edema is a suitable in-vivo model to predict the efficacy of anti-inflammatory agents, which act by inhibiting the mediators of acute inflammation [28]. The efficiency of NS in the inhibition of the edema volume was determined using the method described in the experimental part.

The samples used in the study included the optimized ternary SD obtained using the solvent method [SD/S (1 : 2 : 20%)] and the commercially available NS tablet (designated as market tablet) as well as the control. The anti-inflammatory effect was monitored during 5 h following carrageenan injection. The results are presented in Fig. 9.

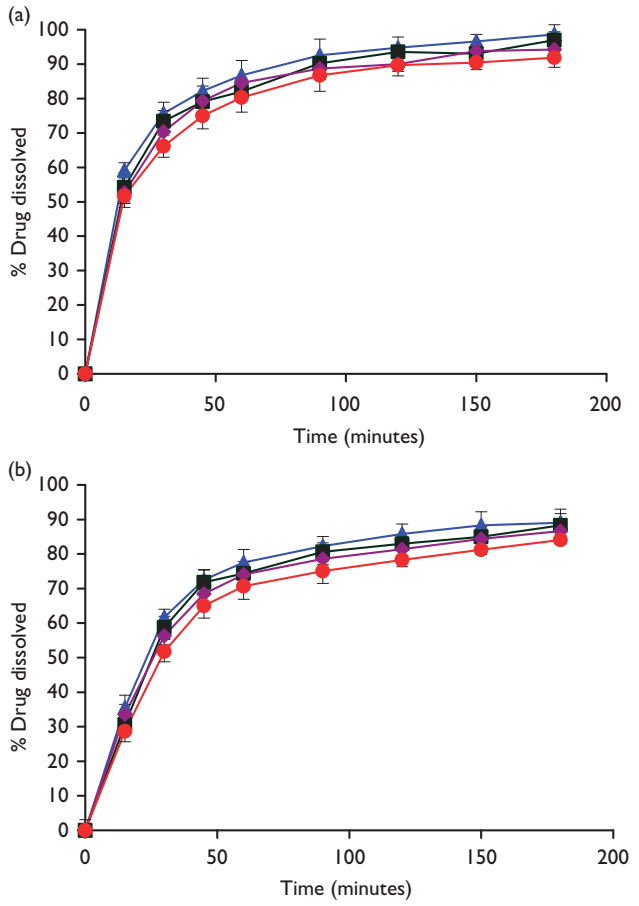
The results shown in Fig. 9 indicate that there is a marked increase ($P < 0.05$) in the mean percentage inhibition of edema volume with the SD when compared with the commercially available tablet.

With respect to the pharmacodynamic parameters, it can be seen that the maximum percentage inhibition of edema volume for all samples occurred 1 h after dosing. Also, it can be seen in Fig. 9 that the dispersion inhibited the increase in paw volume during the early phase of inflammation (1–3 h after carrageenan injection) and also showed a good inhibitory effect at a later phase (up to 5 h). This is in agreement with the studies of Garcia-Pastor *et al.* [29], who suggested a biphasic model in carrageenan-induced edema. The first phase begins immediately after injection and decreases within 1–1.5 h. The second phase remains through 3 h. The delayed phase is considered to result from the effect of prostaglandins on mediator release.

Conclusion

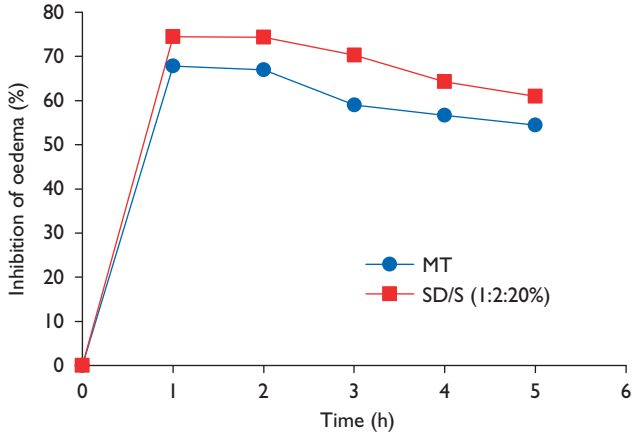
The effects of the carriers investigated in this study that resulted in the enhancement of the dissolution properties and the anti-inflammatory activity of the water-insoluble drug NS represent potential incentive toward the development of a stable formulation that can lead to a reduction in the dose without the need to modify the basic molecule of the drug. The addition of a hydrophilic polymeric surfactant (Inutec) in a small concentration markedly enhanced the dissolution rate of NS compared with the binary IC with β-CD.

Figure 8



Dissolution profiles of fresh and aged solid dispersions prepared using the solvent method [(a) SD/S (1 : 2 : 20%)] and the kneading method [(b) SD/K (1 : 2 : 20%)]:▲, fresh sample; ■, after 1 month; ♦, after 2 months; ●, after 3 months. Data represent mean ± SD (n = 3).

Figure 9



Mean percentage inhibition of edema volume after administration of the selected NS formulations in Carrageenan-induced paw edema in rats: ●, market tablet; ■, solid dispersion prepared using the solvent method [SD/S (1 : 2 : 20%)]. NS, nimesulide.

The polymeric surface-active agent Inutec, which was shown to improve the anti-inflammatory activity of NS but that has not as yet been fully investigated or reported in the literature, might have huge potential as a carrier for other water-insoluble drugs.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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