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Diclofenac nanosuspensions: Influence of preparation procedure and crystal form on drug dissolution behaviour

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ABSTRACT

The aim of this paper was to ascertain the role of drug crystalline form and preparation procedure in nanosuspension formulations in order to optimise dissolution properties of lipophilic, poorly soluble drugs, thus improving their oral bioavailability. The non-steroidal anti-inflammatory drug diclofenac acid (DCF), which is known to exist in different crystal forms, was chosen as a model drug. To this purpose, the influence of homogenization technique was studied by preparing several nanosuspensions with two different crystalline forms of the drug (DCF1 and DCF2). Particle size and size distribution, morphology, microstructure, and thermal behaviour of the different formulations were studied by photon correlation spectroscopy (PCS), scanning electron microscopy (SEM), X-ray powder diffraction (XRD) and differential scanning calorimetry (DSC). Solubility studies of the bulk drug crystalline forms and dissolution experiments of nanosuspensions in comparison with different controls (bulk drug, physical mixtures, coarse suspensions) were carried out in different media: distilled water, simulated gastric fluid (SGF) and simulated intestinal fluid (SIF).

Besides well known factors capable of affecting drug nanoparticle dissolution, results showed that drug dissolution rate in nanosuspensions is strongly affected by the drug solubility, which depends on the crystal form, and preparation procedure (high pressure homogenization process). Results demonstrated that this process partially transformed DCF2 in DCF1 while it did not have any effect on the DCF1 crystals.

1. Introduction

Oral bioavailability of poorly water-soluble drugs depends on their dissolution rate in the absorption site. In recent years it has been estimated that up to 40% of the new drugs discovered by the pharmaceutical industry are poorly soluble or lipophilic compounds (Merisko-Liversidge, 2002). Many procedures have been investigated to enhance dissolution properties and, thus, oral bioavailability of drugs with very low aqueous solubility. Conventional approaches include use of cosolvents, salt formation, pH adjustment, emulsions and micellar dispersions, micronisation, complexation with cyclodextrin (Lawrence and Rees, 2000; Nakano, 2000; Stella and Rajewski, 1997). An alternative to such methods is nanonization of drug particles. The reduced particle size within the nanometer range leads to an enhanced dissolution rate not only because of increased surface area but also because of increased saturation solubility as described by Freundlich–Ostwald equation (Kesisoglou et al., 2007). The saturation solubility is not only a compound specific constant depending on temperature but it increases when drug particle diameter is below 1 μm.

Influence of the particle size reduction on the drug dissolution rate has been studied for different drugs formulated as nanosuspension (Kocbek et al., 2006; Liversidge and Cundy, 1995; Moschwitzer and Muller, 2006; Trotta et al., 2003). Nanosuspensions are submicron colloidal dispersions of surfactant stabilised drug nanoparticles in an outer liquid phase, usually water, but also water miscible liquids or non-aqueous media. Nanosuspensions can be prepared by bottom up and top down technologies (Keck and Muller, 2006; Rabinow, 2004). In the bottom up technologies the low water soluble drugs are dissolved in a solvent and then precipitated in different ways in a surfactant solution (Kocbek et al., 2006; Trotta et al., 2003). The top down technologies are based on particle fragmentation to submicron units and include ball milling (Liversidge and Cundy, 1995; Merisko-Liversidge et al., 2003) and high pressure homogenization (Muller et al., 1999; Jacobs et al., 2000).

Since the fifties, it has been shown that high pressure homogenization is a simple technique, well established on large scale for the production of oil-in-water (o/w) parenteral emulsions and already available in pharmaceutical industry. High pres-

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sure homogenization is also an efficient technique that has been utilised to prepare stable nanosuspensions of several drugs such as carbazepin, bupravaquone, aphidicolin, cyclosporine, paclitaxel, prednisolone, etc. (Muller et al., 2003).

During homogenization, cavitation forces as well as collision and shear forces determine break down of the drug particles down to the nanometer range. Process conditions lead to an average particle size that remains constant as a result of continuous fragmentation and reaggregation processes. These high energetic forces can also induce a change of crystal structure and/or partial or total amorphization of the sample (Muller et al., 2003), which further enhances the solubility. For a long-term storage stability of the nanosuspension formulation, the crystal structure modification must be maintained over the storage time.

To our knowledge no study regarding the influence of crystal structure change on the dissolution rate of nanosuspension formulation has been published yet. Recently, Sigfridsson et al. compared pharmacokinetic parameters of nanosuspension formulations of amorphous and crystalline neurokinin NK receptor antagonist (AZ68) after oral and intravenous administration. However, in this study the amorphous and crystalline formulations differed for composition and preparation method (Sigfridsson et al., 2007).

Diclofenac (DCF), 2-[(2,6-dichlorophenyl)amino] phenylacetic acid, is a potent nonsteroidal anti-inflammatory drug (NSAID) with a very low aqueous solubility and gastrolesive actions. It is used in inflammatory and painful conditions of rheumatic and non-rheumatic origin (Martindale, 2002).

Three polymorphic forms of diclofenac acid are reported: two are monoclinic and are referred as HD1 (space group $P2_1/c$) and HD2 (space group $C2/c$). In both forms molecules are linked to each other through the carboxyl groups giving rise to centrosymmetric dimers (Castellani and Ottani, 1997). Third polymorph is an orthorombic form (HD3, space group *Pcan*) where no intermolecular hydrogen bond is present (Jaiboon et al., 2001). In all the forms a bifurcated intramolecular hydrogen bond involves N–H group.

In this work the production of nanosuspensions intended for oral use of DCF, using a high pressure homogenization technique, is reported. The aim of this investigation was to ascertain the role of crystal structure and molecular conformation of the drug, and preparation procedure in nanosuspension formulations in order to optimise dissolution properties of DCF, thus improving its oral bioavailability.

To this purpose several nanosuspensions were prepared starting from two different crystal forms of DCF obtained by precipitation from an aqueous solution of diclofenac sodium salt or by crystallization from chloroform. Poloxamer 188, a non-ionic sterically stabilizing surfactant suitable for oral administration was employed.

Characterization of nanosuspensions was carried out by different techniques: scanning electron microscopy (SEM), differential scanning calorimetry (DSC), X-ray powder diffractometry, photon correlation spectroscopy (PCS). Dissolution study of nanosuspension formulations was performed in distilled water (pH 5.5), simulated gastric fluid (SGF, pH 1.2), and simulated intestinal fluid (SIF, pH 7.5) and was compared to that of coarse suspensions, physical mixtures of the drug and stabiliser, and bulk DCF samples.

2. Materials and methods

2.1. Materials

Diclofenac sodium salt was purchased from Galeno (Comeana, Italy). Pluronic F68 (Poloxamer 188) was a gift from BASF AG (Ludwigshafen, Germany). High-performance liquid chromatography (HPLC)-grade methanol was purchased from Sigma–Aldrich (Milan, Italy). All the other compounds were of analytical grade and used as received from Sigma–Aldrich (Milan, Italy).

Table 1
Composition of diclofenac acid (DFC) nanosuspension formulations.

Formulations		Components (%, w/w)
1	DCF	10
	Pluronic F68®	1
	Water	89
2	DCF	10
	Pluronic F68®	2
	Water	88
3	DCF	10
	Pluronic F68®	3
	Water	87
4	DCF	10
	Pluronic F68®	4
	Water	86
5	DCF	10
	Pluronic F68®	5
	Water	85

2.2. Preparation of DCF acid crystal forms

A saturated aqueous solution of diclofenac sodium salt was acidified with diluted HCl until a white precipitate of diclofenac acid was observed. The precipitate was filtered, washed with bidistilled water and air dried (DCF1 crystal form). A portion of DCF1 was crystallised from hot chloroform, filtered, washed with bidistilled water and air dried (DCF2 crystal form).

2.3. Preparation of Poloxamer 188/DCF physical mixture

Physical mixtures were prepared by blending Poloxamer 188/DCF (DCF1 or DCF2) in an agata mortar until a homogeneous mixture was obtained. Physical mixtures were prepared using 2:1 drug/surfactant ratio (w/w).

2.4. Preparation of coarse suspensions

Coarse suspensions of the drug were prepared dispersing DCF1 or DCF2 in a Poloxamer 188 bidistilled water solution using an Ultra Turrax T25 basic (IKA, Werke) for 1 min at 8000 rpm. Coarse suspensions were prepared using 2:1 drug/surfactant ratio (w/w).

2.5. Preparation of nanosuspensions

All formulations were prepared using both crystal forms of diclofenac acid (DCF1 and DCF2). DCF1 or DCF2 were dispersed in a Poloxamer 188 bidistilled water solution using an Ultra Turrax T25 basic for 1 min at 8000 rpm. The obtained coarse suspensions were then homogenized at high pressure (4 cycles at 200 bar, 4 cycles at 500 bar and 50 cycles at 1500 bar) using an Emulsiflex C5 apparatus (Avestin Ottawa, Canada). Composition of the prepared formulations is shown in Table 1.

2.6. Lyophilization of nanosuspensions and coarse suspensions

DCF1 and DCF2 coarse and nanosuspensions were frozen at −15 °C/−20 °C and then freeze dried for 24 h at −70 °C and 60 mmHg, using a Freeze-Dryer Criotecnica (MMCOTA, Rome, Italy).

2.7. Solubility studies

The solubility of DCF1 and DCF2 was determined in water, simulated intestinal fluid, and simulated gastric fluid. SGF and SIF without pancreatin were prepared according to United States Pharmacopeia (USP 23). In particular, SIF was prepared by dissolving 6.8 g of potassium dihydrogen phosphate in 250 mL of water, and adding 190 mL of 0.2N sodium hydroxide solution. Finally, water was added up to 1000 mL. For preparation of SGF, 2 g of sodium chloride were dissolved in 70 mL of 1N HCl, finally water was added up to 1000 mL.

An excess of drug was added to the medium in screw capped tubes (10 mL) and stirred at 25 °C for 48 h. Each sample was centrifuged and 0.2 mL of the clear supernatant were diluted with methanol and analyzed by high-performance liquid chromatography.

2.8. In vitro dissolution studies

In vitro dissolution studies were performed using transparent gelatine capsules containing an amount of the formulation (lyophilised coarse suspension, lyophilised nanosuspension or physical mixture) equivalent to 25 mg of DCF. Tests were performed in water, SGF and SIF according to the United States Pharmacopeia (USP) using rotating basket method (Erweka apparatus). The experiments were performed on 500 mL samples at 37 °C ± 0.1 °C at a rotation speed of 100 ± 2 rpm. At preselected time intervals, 1 mL samples were withdrawn, filtered through polycarbonate membranes (0.45 μm, Millipore), and replaced with 1 mL of pre-thermostated fresh dissolution medium. Quantitative determination of DCF was performed by HPLC according to the method described below. Dissolution tests were performed in triplicate. Dissolution profiles were evaluated on the basis of dissolution efficiency (DE) and percentage of drug dissolved (DP) at 10 min and 60 min.

2.9. HPLC analysis

Quantitative determination of DCF was performed by an HPLC system consisting of a liquid chromatograph Alliance 2690 (Waters Corp, Milford, MA) equipped with a photodiode array detector and a computer integrating apparatus (Millennium 32, Waters). Analyses were performed at 227 nm with a Nova Pack C18 column (60 Å, μm, Waters). The mobile phase, a mixture of 60% methanol and 40% water (v/v), was delivered at a flow rate of 1.2 mL/min. Samples (20 μL) were injected using an auto sampler. The stock standard solution of diclofenac (1 mg/mL) was prepared by dissolving the drug in methanol and storing at 4 °C. A standard calibration curve (peak area of DCF vs. known drug concentration) was built up by using standard solutions (50–0.135 μg/mL) prepared by dilution of the stock standard solution with the mobile phase. Calibration graphs were plotted according to the linear regression analysis, which gave a correlation coefficient value (R^2) of 0.999. Sample preparation and analyses were performed at room temperature.

2.10. Particle size analysis

The average diameter (Z-AVE) and polydispersity index (PI) of DCF nanosuspensions were determined by PCS (Zetasizer 4, Malvern Instruments, UK) at 25 °C. The aqueous or lyophilised nanosuspensions were diluted with distilled water before analysis. Samples were scattered (633 nm) at a fixed angle of 90°. Data were fitted by the method of inverse "Laplace transformation" and Contin. Each value is the average of 10 measurements.

2.11. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed with PerkinElmer DSC7 instrument, under a pure argon flux and with a heating rate of 10 °C/min in the temperature range from 30 °C to 200 °C. Each sample was accurately weighted (~1–2 mg) in an aluminium pan, crimped and sealed. Temperature calibration was obtained using palmitic acid and indium. Enthalpies were calibrated using indium.

2.12. X-ray powder diffractometry

X-ray diffraction (XRD) patterns were collected with a Seifert X3000 diffractometer operating at 35 mA and 50 kV using Cu Kα radiation and equipped with a graphite monochromator on the diffracted beam. XRD patterns were recorded in step scan mode in the range $3° \leq 2\theta \leq 30°$ with step size 0.05°, collecting at least 1000 counts for each step. The divergence and receiving slits were chosen in order to ensure a high resolution mode for the crystalline phases. Attention was paid in the sample preparation in order to minimize preferred orientation. Average crystalline size was estimated by Scherrer equation where the integral breadth was corrected for instrumental broadening. The instrumental profile broadening was derived from the fitting of XRD data obtained from standard samples.

2.13. Scanning electron microscopy (SEM)

Morphology of pure materials, of lyophilised, coarse and nanosuspension, and of the physical mixture were examined by scanning electron microscope (E-SEM Quanta 200 - FEI Company TM). The samples were fixed on a brass stub using carbon double-sided tape. Pictures were then taken at an excitation voltage of 20 kV.

2.14. Statistical analysis

Data analysis was carried out with the software package Microsoft Excel version 2003. Results were expressed as a mean ± standard deviation. Statistically significant difference was determined using the analysis of variance (ANOVA) with $P = 0.05$ as a minimal level of significance.

3. Results and discussion

Reduction of particle size diameter down to the submicron range has known to increase dissolution rate and saturation solubility of poorly soluble drugs. However, other factors can affect performance of nanosized drugs such as their crystalline form and molecular conformation, and preparation procedure. In order to study the influence of these factors on the dissolution property of diclofenac acid, during this work five nanosuspensions were prepared by high pressure homogenization technique using two different DCF crystal forms (i.e. DCF1 and DCF2).

3.1. Bulk DCF characterization

Physico-chemical characterization of the two diclofenac acid forms (i.e. DCF1 and DCF2), obtained as described in the experimental section, was carried out by XRD, DSC and in vitro solubility tests.

XRD spectra of poloxamer, DCF1, and DCF2 are reported in Fig. 1.

Diffraction pattern of poloxamer is typical of crystalline polymeric substances and it is characterized by a peak around 19° 2θ and a second one, larger, centred around 23° 2θ (Fig. 1a).

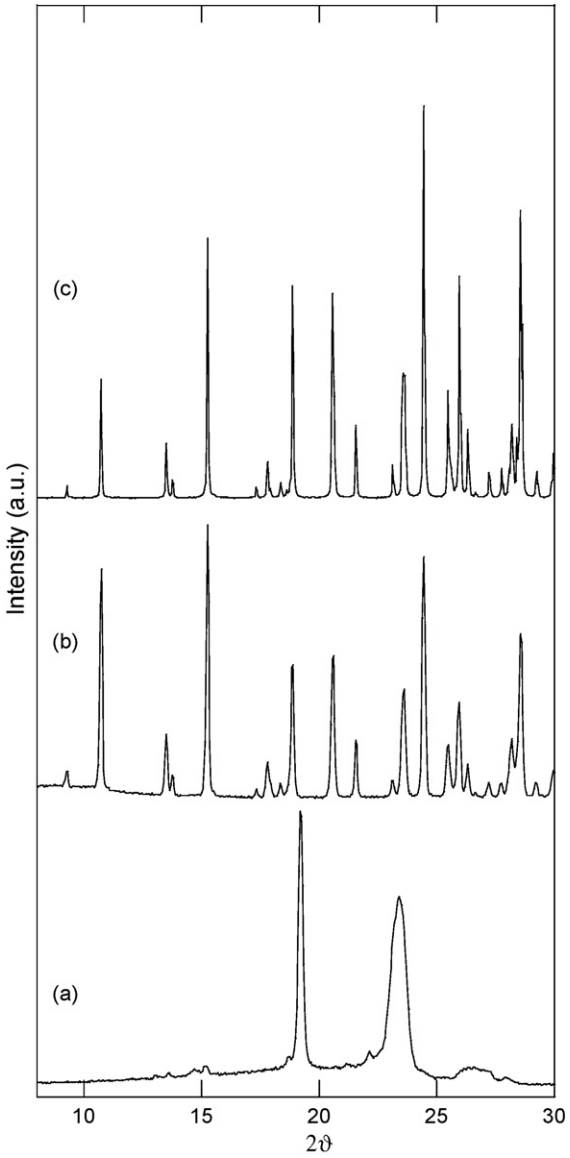

Fig. 1. Experimental XRD spectra of poloxamer (a), DCF1 (b) and DCF2 (c) respectively.

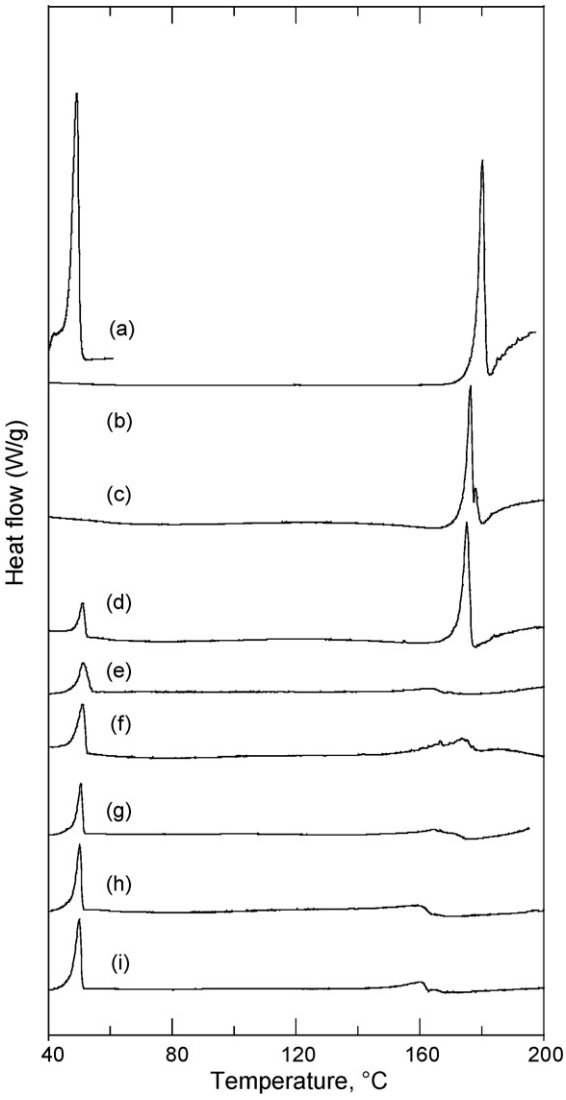

Fig. 2. DSC thermograms of poloxamer (a), bulk DCF2 (b), bulk DCF1 (c), DCF2-poloxamer physical mixture (d), DCF1-poloxamer physical mixture (e), DCF2 coarse suspension (f), DCF1 coarse suspension (g), nanosuspension prepared with DCF2 (h) and DCF2 (i) respectively.

X-ray powder diffraction pattern of bulk DCF2, the sample obtained by crystallization from chloroform, is that expected for a sample with HD2 structure (Fig. 1c).

Diffraction pattern of DCF1 sample, obtained by precipitation with diluted HCl from saturated sodium salt aqueous solutions, exhibits diffraction peaks still at 2θ angles expected for HD2 but with significant differences in their relative intensities (Fig. 1b). This effect could be the result of slight changes in molecular conformation, as previously reported in the structure of diclofenac embedded in a chitosan matrix (Muangsin et al., 2004). Such an effect does not affect parameters and symmetry of the unit cell. Peaks are slightly wider than in DCF2, indicating narrower crystalline size and/or presence of disorder. In both spectra no peaks due to HD1 or HD3 polymorphic forms are detectable.

XRD analysis of DCF1 and DCF2 samples leads to the conclusion that they are both HD2 polymorphs of diclofenac acid but with differences in molecular conformation, average crystal size or disorder. Such differences are confirmed by DSC thermograms of DCF2 and DCF1 forms. The first one is characterized by a sharp endothermic peak around 181 °C followed by sample decomposition (Fig. 2b) (Giordano et al., 2003) while DCF1 thermogram exhibits a large endothermic peak around 176 °C and a smaller one at 180 °C, followed by sample decomposition (Fig. 2c).

DSC of poloxamer (Fig. 2a) shows the expected endothermic melting peak at 50 °C.

Characterization of bulk diclofenac was completed by the solubility studies. As expected, solubility of both DCF1 and DCF2 increased with pH. However, as shown in Table 2, DCF1 showed a higher solubility than DCF2 in all tested media; in particular the solubility of DCF1 increased of 60% in SGF and of 50% in unbuffered distilled water with respect to that of DCF2. Higher solubility of DCF1 form can be due to variation in molecular conformation and/or disorder as shown by XRD and DSC analyses of the two samples.

Table 2
Solubility of DCF1 and DCF2 in different medium.

	Solubility ± S.D (μg/mL)		
	SGF	Water	SIF
DCF1	3.4 ± 0.26	16.45 ± 0.72	1356.15 ± 32.39
DCF2	2.14 ± 0.34	11.08 ± 0.68	1276.33 ± 45.73

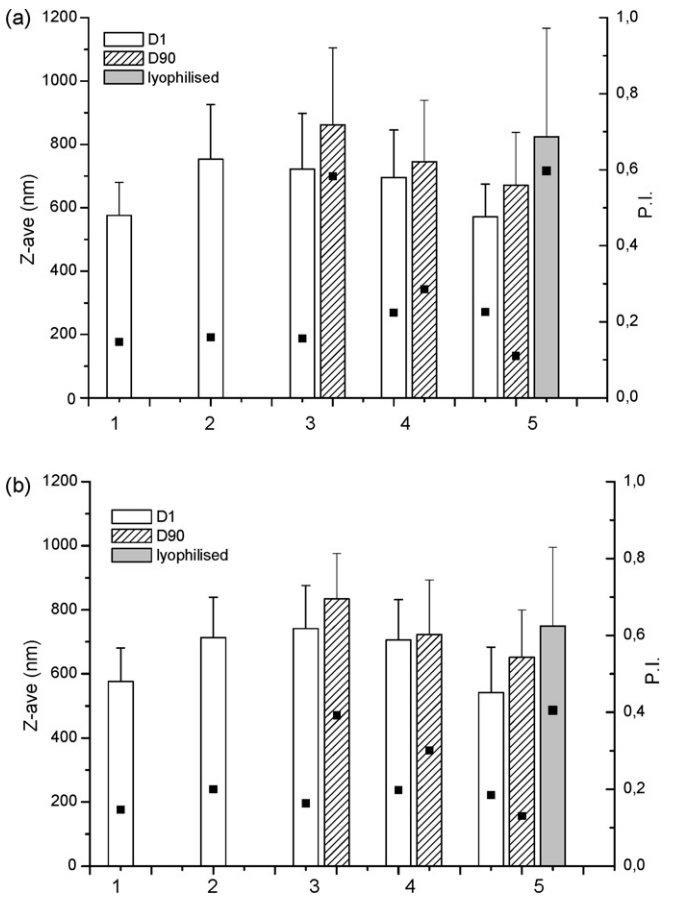

Fig. 3. PCS average diameter (Z-AVE) and polydispersity index (PI) of DCF nanosuspension 1 day (D1) and 90 days (D90) after production and after hydration of lyophilised nanosuspension (lyophilised) prepared using DCF1 (a) or DCF2 (b). Error bars represent standard deviation ($n = 10$).

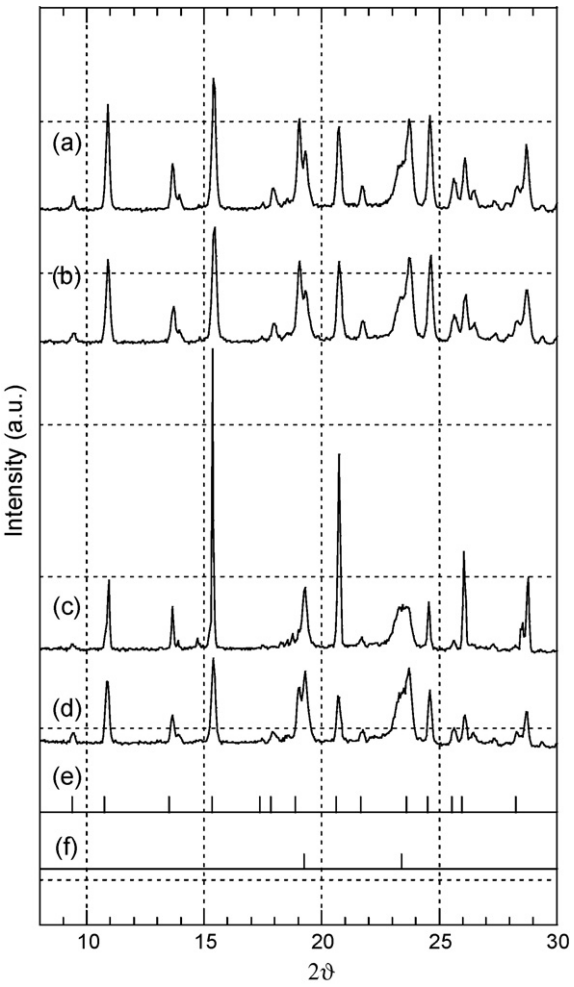

Fig. 4. Experimental XRD spectra of nanosuspensions prepared with DCF2 (a) and DCF1 (b), DCF2 coarse suspension (c) and DCF1 coarse suspension (d) respectively. Vertical markers correspond to calculated pattern of HD2 (e) and poloxamer (f).

3.2. Nanosuspension and coarse suspension preparation and characterization

The most important parameters for the production of nanosuspensions with the high pressure homogenization technique are homogenization pressure (power density of the homogenizer), number of homogenization cycles, and hardness of the drug. With the aim of obtaining nanosuspensions with mean particle size down to the sub-micron range and high monodispersity, preliminary tests were carried out in order to determine the appropriate operative conditions (data not shown). Homogenization of DCF1 and DCF2 at 1000 bar pressure led to average particle size larger than 3 μm independently of the number of cycles (less than 70 cycles). On the contrary, operating at 1500 bar, particle smaller than 1 μm were obtained. For this reason an operational homogenization pressure of 1500 bar was selected. Under this condition a reduction of the average particle size and/or the polydispersity index was observed increasing the number of cycles up to 50. No size change was observed operating at 2000 bar or by increasing the number of cycles over 50. Selected operational conditions for all the nanosuspension formulations were therefore 50 homogenization cycles at 1500 bar which were preceded by 4 cycles at 200 bar and by 4 cycles at 500 bar as a kind of pre-milling. Final nanosuspension formulations were then stored at 4 °C or lyophilised.

Two sets of five nanosuspensions were prepared each with a constant concentration (i.e. 10%, w/w) of one of the two DCF1 or DCF2 forms (Table 1). In addition, increasing amount of surfactant (1–5%, w/w) were used to find the most stable nanosuspensions. Indeed, it is well known that surfactant concentration affects aggregate prevention and stability of the nanosuspension formulations (Keck and Muller, 2006).

The PCS diameter (Z-AVE) and polydispersity index (PI) of DCF1 and DCF2 nanosuspensions are reported in Fig. 3. No significant differences were found on particle average diameter in formulations prepared with the two forms, indicating that this factor does not influence the particle dimensions of the pertinent nanosuspension.

One day after preparation, all formulations had a particle size below 800 nm and a PI lower than 0.25. Formulations 1 and 5 showed the lowest PCS average diameters, which were 580 nm (0.151 PI) and 571 nm (0.226 PI) for DCF1 formulations, and 576 nm (0.150 PI) and 541 (0.185 PI) for DCF2 ones. No direct relation between surfactant concentration and particle diameter was hence found.

The average diameter and PI variation was monitored for 3 months. Formulations 1 and 2 of both DFC1 and DFC2 showed aggregation phenomena after only 3 days from preparation. Since the size of these aggregates was in the range of 7–15 μm their average diameter could not be determined by PCS and was therefore evaluated by Light Microscopy (Zeiss Axiostar Plus). The mean particle size of formulations 3, 4, and 5 of both DFC1 and DCF2 forms increased slightly after 3 months of storage at 4 °C, indicating a high physical stability at this storage temperature. However, formulations 3 and 4 showed aggregation phenomena after lyophilization and re-hydration with bidistilled water. Size of these aggre-

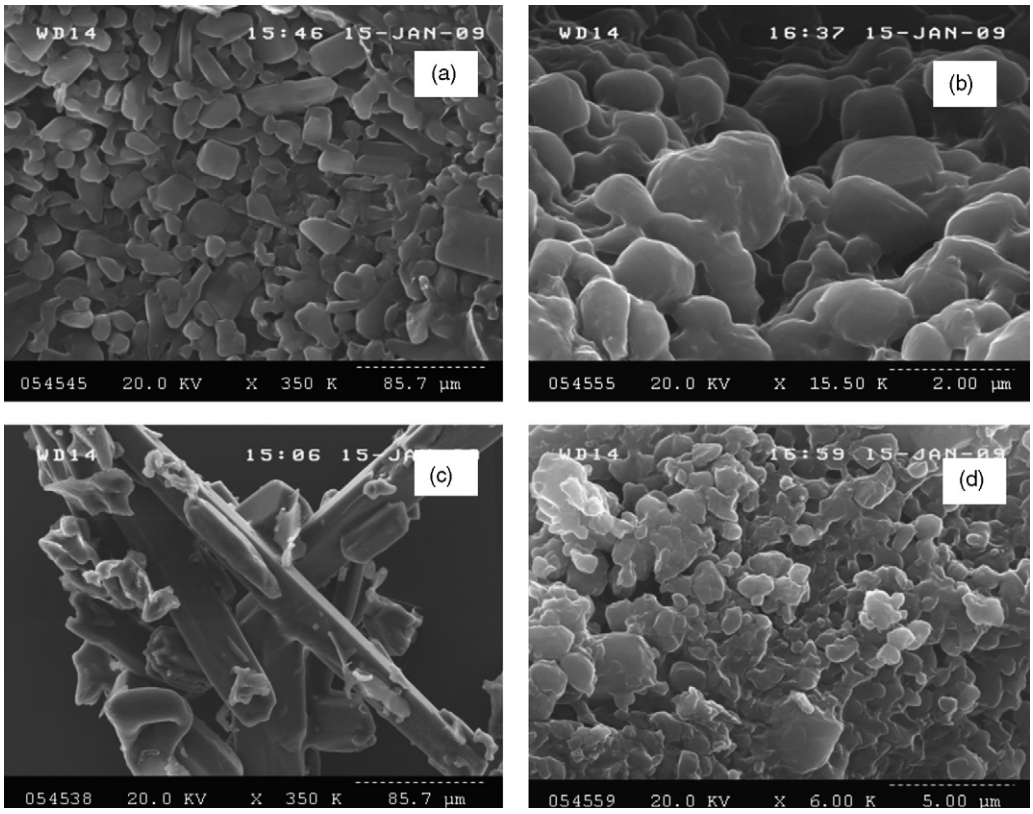

Fig. 5. Scanning electron microscopy images of DCF1 coarse suspension (a), DCF1 nanosuspension (b), DCF2 coarse suspension (c) and DCF2 nanosuspension (d).

gates was in the range of 10–20 μm as determined by Light Microscopy.

On the contrary, formulation 5 showed a small increase of the PCS average diameter and polydispersity index after lyophilisation, showing after re-hydration 824 nm (0.60 PI) and 749 nm (0.41 PI) for DCF1 and DCF2 forms, respectively. Consequently, only formulation 5 showed a good stability during the 3 months of storage and after rehydration of the lyophilised product, thus demonstrating that 5% of poloxamer were needed to avoid aggregation phenomena. Accordingly with these results, the study was continued using only nanosuspensions 5.

Light Microscopy of DCF1 and DCF2 coarse suspensions showed a bimodal particle size distribution with maxima centred around 15 μm and 70 μm.

Fig. 4 reports the XRD spectra of coarse- and nano-suspension formulations 5. Once again the main evidence is that DCF peak positions still correspond to those expected for HD2 monoclinic *C*2/*c* crystal structure, therefore excluding any influence of chemical and physical actions on the molecular packing in the solid. A close look evidences the similarity of the spectrum of DCF1 coarse suspension (Fig. 4d) with those of DCF1 and DCF2 nanosupension (Fig. 4b and a respectively). In all these spectra the relative peak intensities have the trend of that of bulk DCF1 (Fig. 1b). Peaks are broader as a result of lower particle size, whose average values calculated according to Scherrer equation result in the range of 50–80 nm. A partial contribution to peak broadening due to structural disorder cannot be excluded.

On the other hand XRD spectrum of DCF2 coarse suspension is intermediate between bulk DCF1 and DCF2 spectra (Fig. 1b and c respectively) suggesting that during the homogenization a change in the crystalline structure occurred, which is probably due to DCF2 solubilisation followed by its partial recrystallisation in the DCF1 form as a consequence of the temperature and pressure reached in the homogenizer. This is a well known and documented phenomenon (Keck and Muller, 2006; Muller et al., 2001).

The differences of the two DCF forms were evident also in morphological scanning electron microscopy studies. The SEM pictures of coarse- and nano-suspensions obtained from DCF1 and DCF2 are reported in Fig. 5. Micrographs prove a great morphological difference between DCF1 and DCF2 crystals. DCF2 coarse crystals (Fig. 5c) show a regular elongate shape, while coarse crystals of DCF1 (Fig. 5a) are more irregular and more rounded. The homogenization of the coarse crystals led to a change of DCF2 morphology. Indeed, the DCF1 and DCF2 nanosuspension have a crystal morphology very similar to that of the most irregular DCF1 coarse crystals but even more rounded. SEM analyses confirmed a change of the DCF2 crystal structure during the homogenization process.

In the thermograms of coarse- (Fig. 2f–g) and nano-suspensions (Fig. 2h–i) the endothermic peak around 50 °C, due to poloxamer melting, is always present. At temperatures higher than 120 °C all thermograms show a peculiar trend, which is similar to that of the physical mixture of DCF1 and poloxamer shown in Fig. 2e. On the contrary, the DSC of DCF2-poloxamer physical mixture shows the endothermic peak around 180 °C due to diclofenac melting (Fig. 2d). This result is expected for a physical binary mixture.

In order to shed some light on this effect, the physical mixture of polymer and DCF1 was heated under an argon flux in an electric furnace up to 120 °C, with an heating rate of 5 °C/min, left at this temperature for 1 h and then cooled to room temperature. XRD patterns of the mixture before and after thermal treatment are shown in Fig. 6. As can be seen, they are very similar and show peaks due to poloxamer and DCF1 form. This result would confirm the hypothesis that at temperatures higher than 120 °C and close to the acid melting point, DCF1 reacts with the poloxamer, which is already in the liquid state (Ferrari and Inoue, 1972). Moreover, the reaction is favoured in nanosuspensions as suggested by the smoother DSC

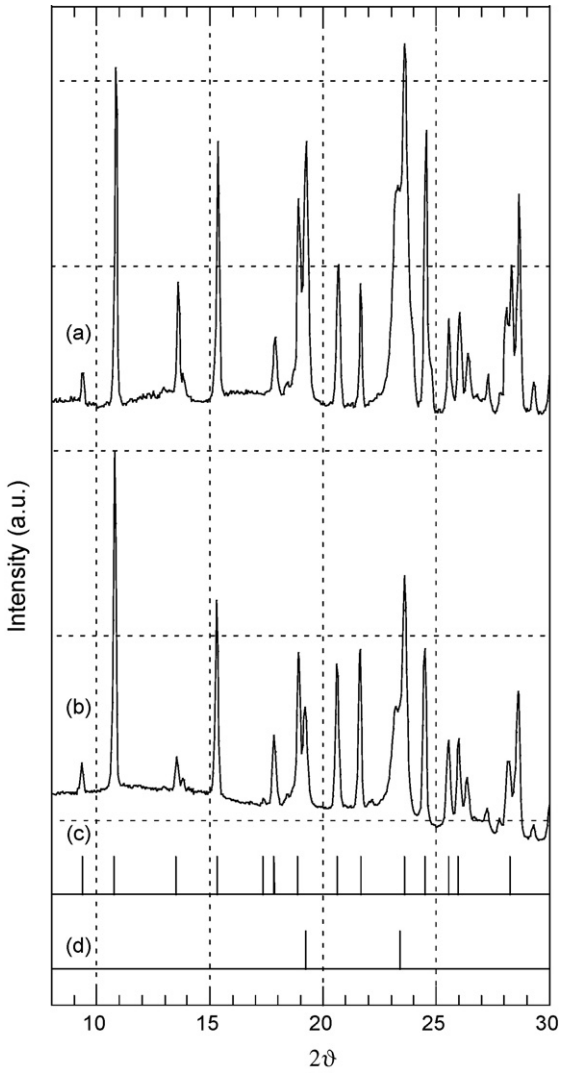

Fig. 6. XRD spectra of the DCF1 physical mixture (a) and after thermal treatment at 120 °C (b). Vertical markers correspond to calculated pattern of HD2 (c) and poloxamer (d).

thermograms. This reaction does not occur for DCF2, confirming a significant different behaviour of the two forms.

3.3. *In vitro dissolution studies*

DCF is a weak organic acid ($pK_a = 4.18$ at 25 °C) with a very low aqueous solubility that increases with pH. As written above, since nanosuspensions 5 had shown the highest stability against aggregation, dissolution behaviour was investigated only for these formulations. Coarse suspensions and physical mixtures were also prepared using the same drug/surfactant ratio (i.e. 2:1, w/w) of the nanosuspensions 5, as proper comparison to avoid introduction of any other variable that could affect results.

Figs. 7 and 8 report dissolution profiles of DCF1 and DCF2 formulations. In particular, Fig. 7a–c shows dissolution behaviour of DCF1 from freeze dried coarse suspension (CS), freeze-dried nanosuspension (NN), physical mixture (PM), and bulk drug (DCF1) in SGF, unbuffered distilled water, and SIF. In Fig. 8a–c dissolution profiles of the corresponding DCF2 samples are shown.

Figs. 7 and 8 clearly show that the drug dissolution rate increased with pH of the dissolution medium in all formulations for both DCF1 and DCF2, as expected because of higher DCF ionisation. In particular, after 60 min dissolved DCF from bulk DCF1 is 0.78 mg in SGF,

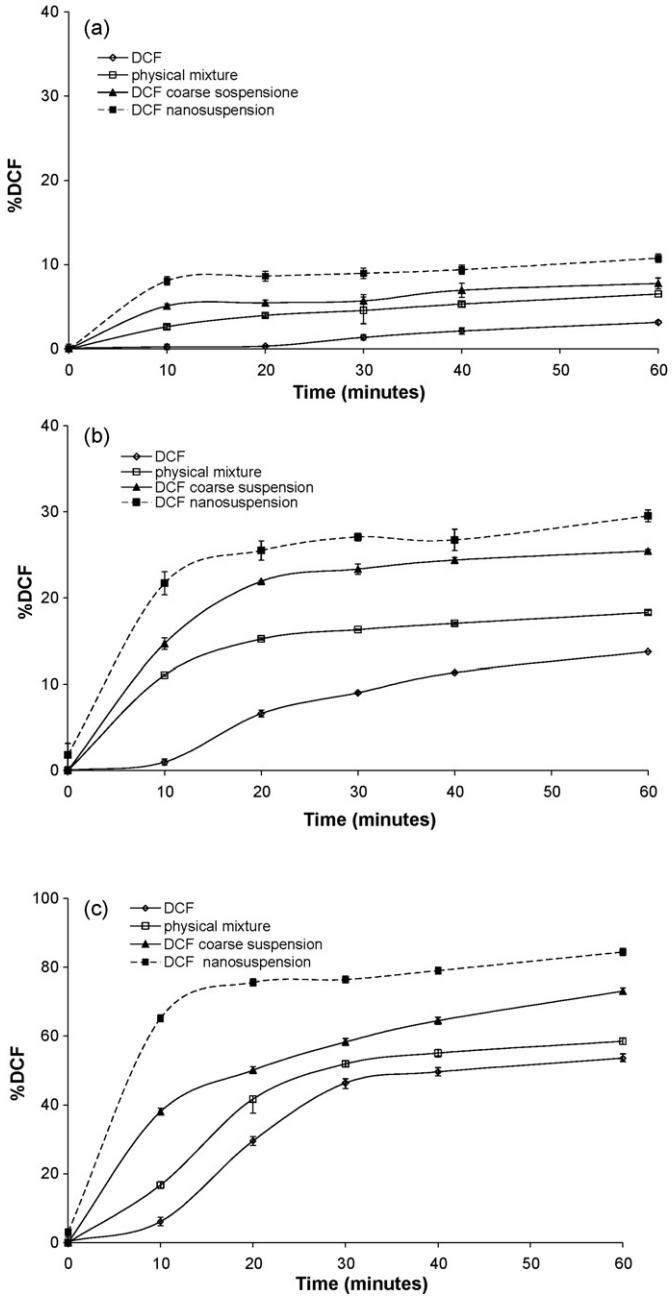

Fig. 7. Dissolution profiles of DCF1 formulations (bulk DCF, physical mixture, lyophilised coarse suspension, lyophilised nanosuspension): (a) in SGF (pH 1.2); (b) in water (pH 5.5); (c) in SIF (pH 7.5). All dissolution experiments were carried out at 37 °C. Error bars represent standard deviation of three independent experiments.

3.44 mg in unbuffered distilled water and 13.4 mg in SIF. These values decreased to 0.62 mg (SGF), 1.4 mg (H_2O) and 11.65 mg (SIF) for DCF2 form.

Dissolution profiles of physical mixtures prepared by simply blending Poloxamer 188 and DCF1 or DCF2 samples show a slight increase of dissolution rate for both forms compared to the bulk DCF1 or DCF2 samples. This behaviour is probably due to the solubilization power of the surfactant. Coarse suspensions of both DCF1 and DCF2 showed a dissolution rate higher than the corresponding physical mixtures in all tested media. In particular, after 60 min dissolved DCF1 increased of about 25% starting from 1.62 mg (SGF), 4.57 mg (H_2O), 14.62 mg (SIF) for physical mixtures and rising to 1.94 mg (SGF), 5.98 mg (H_2O) and 18.26 mg (SIF) for coarse suspension. A similar trend in dissolution rate from the physical mixture to

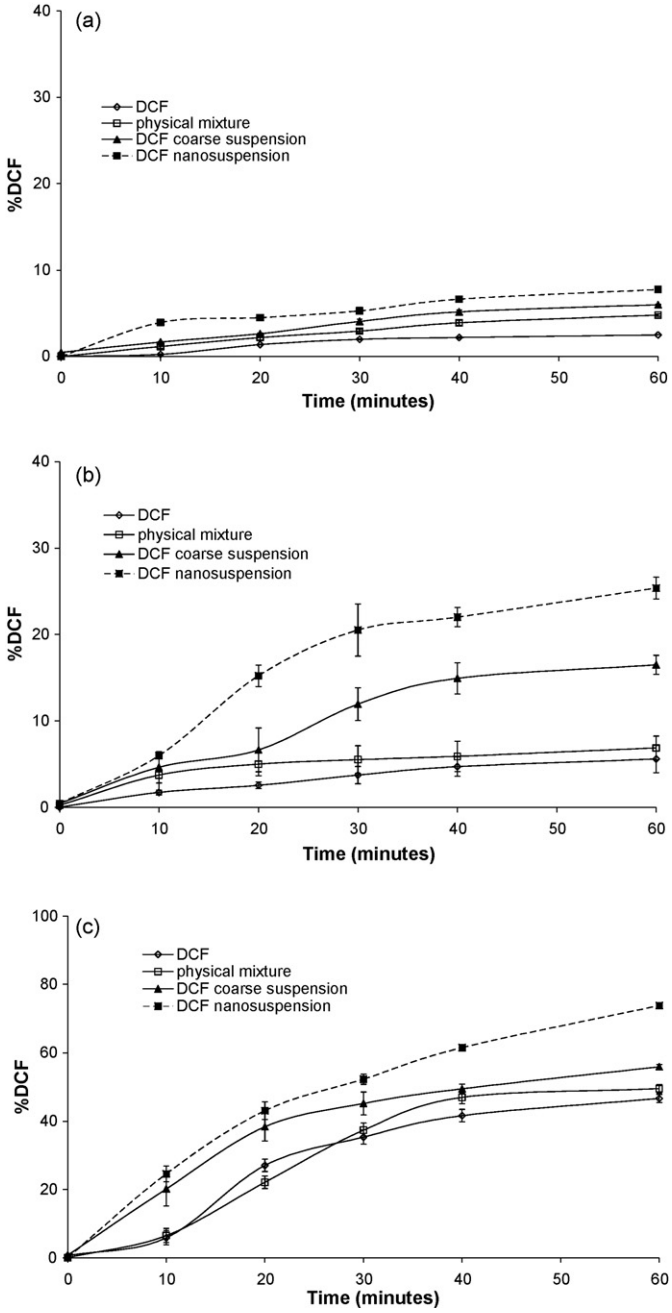

Fig. 8. Dissolution profiles of DCF2 formulations (bulk DCF, physical mixture, lyophilised coarse suspension, lyophilised nanosuspension): (a) in SGF (pH 1.2); (b) in water (pH 5.5); (c) in SIF (pH 7.5). All dissolution experiments were carried out at 37 °C. Error bars represent standard deviation of three independent experiments.

coarse suspension was also found for DCF2. The higher dissolution rate of the coarse suspensions can be explained considering that they had been lyophilised to be encapsulated into gelatine capsules. It is well known that, in comparison to other drying techniques, lyophilization produces more rapidly soluble dried products as a consequence of their highly porous and friable structure (Aulton, 2002).

A further dissolution enhancement was observed in nanosuspension formulations for both DCF1 and DCF2 in all dissolution media. According to the Noyes–Whitney equation (Noyes and Whitney, 1897), the increase of surface area of the exposed drug in the nanosuspensions determines a dissolution rate enhancement. Moreover, the decrease of particle size below 1 μm increases saturation solubility as described by literature (Kesisoglou et al., 2007).

Table 3
Dissolution efficacy (DE) and dissolution percentage (DP) values at 10 and 60 min and time to dissolve 50% of drug ($t_{50\%}$) for DCF1 formulations.

	Bulk DCF1	Physical mixture	Coarse suspension	Nanosuspension
Water				
DE_{10}	1.87	6.25	8.12	12.18
DP_{10}	0.99	11.07	17.98	19.14
DE_{60}	8.25	14.75	20.80	25.01
DP_{60}	13.77	18.30	23.94	30.76
$t_{50\%}$	>60	>60	>60	>60
SGF				
DE_{10}	1.42	1.99	3.41	5.41
DP_{10}	0.23	2.61	5.09	8.07
DE_{60}	1.63	4.74	6.37	9.17
DP_{60}	3.15	6.50	7.77	10.75
$t_{50\%}$	>60	>60	>60	>60
SIF				
DE_{10}	3.16	8.05	25.02	31.03
DP_{10}	6.07	16.72	38.02	55.48
DE_{60}	35.58	42.80	61.37	67.28
DP_{60}	53.60	58.48	77.60	84.8
$t_{50\%}$	41	30	12	8

After 60 min, in DCF1 nanosuspension dissolved DCF increased of about 25% in H_2O and SGF and 10% in SIF compared to relative coarse suspension. For DCF2 nanosuspension after 60 min dissolved DCF increased of about 50% in H_2O and SGF and 35% in SIF compared to relative coarse suspension. Therefore, dissolution tests showed a higher improvement of drug dissolution rate from CS to nanosuspension when DCF2 was used.

These results further support a partial conversion of DCF2 to the more soluble DCF1 during the homogenization process, as suggested by the analysis of XRD spectra in Fig. 4.

In Tables 3 and 4 dissolution efficiency (DE), dissolution percentage (DP) at 10 min and 60 min are reported together with the time needed to dissolve 50% of drug (t_{50}) in the different dissolution media. As can be seen, for each DFC formulation both dissolution efficiency and dissolution percentage values increased in the following order: bulk DCF < physical mixture < coarse suspension < nanosuspension; while the time needed to dissolve 50% of drug decreased in the same order. Moreover, all dissolution parameters demonstrated that DCF1 products had better dissolution properties than DCF2. In particular, after 10 min in SIF, the

Table 4
Dissolution efficacy (DE) and dissolution percentage (DP)values at 10 and 60 min and time to dissolve 50% of drug ($t_{50\%}$) for DCF2 formulations.

	Bulk DCF2	Physical mixture	Coarse suspension	Nanosuspension
Water				
DE_{10}	1.74	2.48	3.72	4.71
DP_{10}	1.7	3.75	4.64	5.98
DE_{60}	3.50	5.17	10.72	17.28
DP_{60}	5.61	6.88	16.49	25.36
$t_{50\%}$	>60	>60	>60	>60
SGF				
DE_{10}	0.53	1.87	2.67	4.01
DP_{10}	0.23	1.14	1.66	2.48
DE_{60}	1.49	3.16	4.05	6.15
DP_{60}	2.48	4.79	5.98	8.22
$t_{50\%}$	>60	>60	>60	>60
SIF				
DE_{10}	3.14	4.01	11.71	14.02
DP_{10}	5.89	6.55	20.17	24.61
DE_{60}	29.58	31.42	39.29	48.28
DP_{60}	46.63	49.50	55.91	73.84
$t_{50\%}$	>60	>60	52	>28

percentage of dissolved drug was more than 6% for the pure DCF1, approximately 17% for PM, 38% for CS and above 55% for the nanosuspension. In the case of DCF2 sample in SIF, the percentage of dissolved drug increased from almost 6% (bulk) to 6.55% (PM) to more than 20% (CS) to above 24.5%. Therefore, in the first 10 min of the dissolution study in SIF there was a 2.65-fold improvement in DCF dissolution when the DCF1 nanosuspension is compared to the DCF2 nanosuspension.

In addition, the time to dissolve 50% of the drug at 60 min $t_{50\%}$ was strongly reduced in SIF only when DCF1 formulations were tested. As can be seen from the tables $t_{50\%}$ for DCF1 coarse suspension was even lower than that of DCF2 nanosuspension. Furthermore, DCF1 coarse suspension showed a 1.5-fold higher dissolution rate than DFC2 nanosuspension.

All these results confirm that particle size is an important parameter in enhancing drug dissolution rate and therefore drug bioavailability, but maybe even more important it is to know the crystalline structure of the studied drug: coarse DCF1 suspension showed a higher dissolution rate than the DCF2 nanosuspension (where a partial transformation to DCF1 crystals occurred during homogenization) as a consequence of the intrinsic higher solubility of the DCF1 crystals. Therefore, the larger but more soluble DCF1 coarse crystals have a dissolution rate higher than the smaller but less soluble DCF2 nanoparticles.

4. Conclusion

Results of this study have clearly demonstrated that dissolution behaviour of nanosuspension is a rather complex issue since several factors can participate in affecting drug dissolution rate of the nanosized drug. In particular, this study showed that DCF dissolution rate is strongly affected by the drug solubility that depends on the crystalline form. Moreover, this study has also shown the role of the high pressure homogenization process.

In conclusion, when different polymorphs of a drug exist the choice of the crystal form should be done after evaluation of its stability during the preparation procedure. In fact, this study has demonstrated that the homogenization process partially transformed DCF2 in DCF1 while it did not have any effect on the DCF1 crystals.

References

Aulton, M.E., 2002. Pharmaceutics: The Science of Dosage Form Design, 2nd ed. Churchill Livingstone, London.

Castellani, C., Ottani, S., 1997. Two monoclinic forms of diclofenac acid. Acta Crystallogr. 53, 794–797.

Ferrari, H.J., Inoue, M., 1972. Pharmaceuticals Differential Thermal Analisys, vol. 2. Academic Press, London, New York.

Giordano, F., Rossi, A., Pasquali, I., Bettini, R., Frigo, E., Gazzaniga, A., Sangalli, M.E., Mileo, V., Catinella, S., 2003. Thermal degradation and melting point determination of diclofenac. J. Thermal Anal. Calorim. 73, 509–518.

Jacobs, C., Kayser, O., Muller, R.H., 2000. Nanosuspensions as a new approach for the formulation for the poorly soluble drug tarazepide. Int. J. Pharm. 196, 161–164.

Jaiboon, N., Yos-In, K., Ruangchaithaweesuk, S., Chaichit, N., Thutivoranath, R., Siritaedmukul, K., Hannongbua, S., 2001. New orthorhombic form of 2-[(2,6 dichlorophenyl)amino]benzeneacetic acid (diclofenac acid). Anal. Sci. 17, 1465–1466.

Keck, C.M., Muller, R.H., 2006. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenization. Eur. J. Pharm. Biopharm. 62, 3–16.

Kesisoglou, F., Panmai, S., Wu, Y., 2007. Nanosizing—oral formulation development and biopharmaceutical evaluation. Adv. Drug Deliv. Rev. 59, 631–644.

Kocbek, P., Baumgartner, S., Kristl, J., 2006. Preparation and evaluation of nanosuspensions for enhancing the dissolution of poorly soluble drugs. Int. J. Pharm. 312, 179–186.

Lawrence, M.J., Rees, G.D., 2000. Microemulsion-based media as novel drug delivery systems. Adv. Drug Deliv. Rev. 45, 89–121.

Liversidge, G.G., Cundy, K., 1995. Particle size reduction for improvement of oral bioavailability of hydrophobic drugs: I. Absolute oral bioavailability of nanocrystalline danazol in beagle dogs. Int. J. Pharm. 125, 91–97.

Martindale-The complete drug reference, 2002. 33rd ed. Sweetman, C.S. (Ed.), Pharmaceutical Press, London, pp. 30–32.

Merisko-Liversidge, E., 2002. Nanocrystals: resolving pharmaceutical formulation issues associated with poorly water-soluble compounds. In: Marty, J.J. (Ed.), Particles. Marcel Dekker, Orlando.

Merisko-Liversidge, E., Liversidge, G.G., Cooper, E.R., 2003. Nanosizing: a formulation approach for poorly-water-soluble compounds. Eur. J. Pharm. Sci. 18, 113–120.

Moschwitzer, J., Muller, R.H., 2006. Spray coated pellets as carrier system for mucoadhesive drug nanocrystals. Eur. J. Pharm. Biopharm. 62, 282–287.

Muangsin, N., Prajuabsook, M., Chimsook, P., Chantarasiri, N., Siraleartmukul, K., Chaichit, N., Hannongbua, S., 2004. Structure determination of diclofenac in a diclofenac-containing chitosan matrix using conventional X-ray powder diffraction data. J. Appl. Crystallogr. 37, 288–294.

Muller, R.H., Becker, R., Kruss, B., Peters, K., 1999. Pharmaceutical nanosuspensions for medicament administration as systems with increased saturation solubility and rate of solution. United States Patent 5,858,410, USA.

Muller, R.H., Jacobs, C., Kayser, O., 2001. Nanosuspensions as particulate drug formulations in therapy. Rationale for development and what we can expect for the future. Adv. Drug Deliv. Rev. 47, 3–19.

Muller, R.H., Jacobs, C., Kayser, O., 2003. In: Rathbone, M.J., Hadgraft, J., Roberts, M.S. (Eds.), Modified-Release Drug Delivery Technology. Marcel Dekker, New York, pp. 135–149.

Nakano, M., 2000. Places of emulsions in drug delivery. Adv. Drug Deliv. Rev. 45, 1–4.

Noyes, A., Whitney, W., 1897. The rate of solution of solid substances in their own solutions. J. Am. Chem. Soc. 19, 930–934.

Rabinow, B.E., 2004. Nanosuspensions in drug delivery. Nature 3, 785–793.

Sigfridsson, K., Forssén, S., Hollander, P., Skantze, U., de Verdier, J., 2007. A formulation comparison, using a solution and different nanosuspensions of a poorly soluble compound. Eur. J. Pharm. Biophys. 67, 540–547.

Stella, V.J., Rajewski, R.A., 1997. Cyclodextrins: their future in drug formulation and delivery. Pharm. Res. 14, 556–567.

Trotta, M., Gallarate, M., Carlotti, M.E., Morel, S., 2003. Preparation of griseofulvin nanoparticles from water-dilutable microemulsions. Int. J. Pharm. 254, 235–242.