Naproxen microparticulate systems prepared using in-situ crystallisation and freeze drying techniques

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Abstract

Poor drug solubility and dissolution rate remain to be one of the major problems facing pharmaceutical scientists, with approximately 40% of drugs in the industry categorised as practically insoluble or poorly water soluble. This in turn can lead to serious delivery challenges and poor bioavailability. The aim of this research was to investigate the effects of the surfactants, poloxamer 407 (P407) and caprol® PGE 860 (CAP) at various concentrations (0.1, 0.5, 1 and 3% w/v) on the enhancement of the dissolution properties of poorly water-soluble drug, naproxen using in-situ micronisation by solvent change method and freeze drying. The extent at which freeze drying influences the dissolution rate of naproxen microcrystals is investigated in this study by comparison with desiccant drying. All formulations were evaluated and characterised using particle size analysis and morphology, *in vitro* dissolution studies, differential scanning calorimetry (DSC) and Fourier transform infra-red (FT-IR) spectroscopy. An increase in poloxamer 407 concentration in freeze dried formulations led to enhancement of drug dissolution compared to desiccator dried formulations, naproxen/caprol® PGE 860 formulations and untreated drug. DSC and FT-IR results show no significant chemical interactions between drug and poloxamer 407, with only very small changes to drug crystallinity. On the other hand, caprol® PGE 860 showed some interactions with drug components, alterations to the crystal lattice of naproxen and poor dissolution profiles using both drying methods, making it a poor choice of excipient.

Key Words: In-situ Micronisation; Freeze drying; Dissolution enhancement; Naproxen; Poloxamer 407, Caprol® PGE 860.

Introduction

Many newly developed active drug molecules demonstrate considerable lipophilicity, hence low solubility, low dissolution rate, and poor bioavailability. This can be a major formulation challenge, especially for orally administered drugs (1-3), where more than 40% of new compounds need formulation optimisation to overcome poor oral absorption because they fall under Biopharmaceutical Classification System (BCS) class II (low solubility, high permeability) and/or class IV (low solubility, low permeability) (4-5).

Various techniques or approaches are available to improve the solubility and dissolution of poorly soluble drugs. These include, but are not limited to: 1) physical approaches such as particles size reduction, solid dispersion, use of surfactants; 2) chemical modification such as salt formation; 3) nanotechnology, such as nanoparticles (6); and 4) liquisolid technique (7-9).

Physical modifications often aim to increase surface area, solubility and wettability of drug particles and typically focus on particle size reduction. Particle size reduction is one of the oldest strategies for improving drug bioavailability (10), where it is well known from the Noyes-Whitney equation that the dissolution rate of an active pharmaceutical ingredient is proportional to the available surface area. Hence, a decrease in particle size and corresponding increase in the surface area of the particles, increases the dissolution rate (10-11).

One of the effective and increasingly used techniques for achieving particle size reduction is by micronisation (12-13). Micronisation is a term used to describe size reduction, where the resulting particle size distribution is less than 10 microns (14-15). A number of methods have been suggested to produce micron sized particles such as milling and grinding (16-17), supercritical fluid techniques and spray drying.

Milling processes do not always result in significant enhancement of drug dissolution rate (16). The milled particles have a tendency to agglomerate as a result of their hydrophobicity, thus reducing their available surface area (18). It is also difficult to obtain uniform fine particles and control the dose of the drug (17). In addition to this, micronisation using milling techniques are not efficient due to their high energy input, and disruptions in the crystal lattice can cause physical and chemical instability (19-20).

Supercritical fluid technology is environmentally friendly and suitable for mass production, however, requires specially designed equipment and is more expensive (21).

In-situ micronisation is a promising particle engineering technique, it is a one-step process where micron sized crystals are obtained by controlled crystallisation without the need for any further particle size

reduction (15, 22-23). This technique uses common equipment (refer to section 2.2), whereas other micronisation techniques like milling, supercritical fluid and spray drying are more complicated and require specialised containment facilities which could be expensive and labour intensive (15).

In recent years, in-situ micronisation using solvent change (anti-solvent) method has been found to be an effective way to produce micronised drug particles (13). This method allows the micronisation of a drug substance along with surface modification using hydrophilic polymers which will in turn enhance wetting properties and increase the stability of microcrystals (15). Rasenack and Muller (22) looked at dissolution rate enhancement by in-situ micronisation using a rapid solvent change process, the results showed markedly enhanced dissolution rate of the drug powders. They also found that the particle size is more uniformly distributed and the powder is less cohesive. In another study Varshosaz et al. (19) found that in-situ micronisation using solvent change method reduced the particle size of gliclazide by approximately 50 times and that the dissolution efficiency at 15 minutes was increased about 4 times. A study investigated the dissolution rate enhancement of tolbutamide by in-situ micronisation, and reported the dissolution rate efficiency of tolbutamide microcrystals to be increased approximately 8 times (13).

The aim of this study was to apply in-situ micronisation technique using the solvent change method to enhance the dissolution properties of poorly water-soluble drug naproxen. The effects of the polymers poloxamer 407 and caprol® PGE 860 used as stabilisers was investigated. In addition to this, a number of studies have shown freeze drying to enhance dissolution rate of poorly soluble drugs (24-26). Therefore, the extent to which freeze drying influenced the dissolution rate of naproxen was also studied.

2. MATERIALS AND METHODS

2.1. Materials

Naproxen (NAP; BN. 027K003, Roche-Syntex S.A. de C.V., Mexico) with mean particle size of 355µm and molecular weight (Mw) of 46.07 g/mol, was used as the model hydrophobic drug throughout the experiment. Poloxamer 407 (P407; Pluronic F127) (BN. 027K003, Sigma – Aldrich company Ltd, UK), and caprol® PGE 860 (CAP; BN. 061213-8, Abitec, USA, given as a gift) were selected as the surfactants for investigation in this study. The chemicals ethanol (Mw: 46.07 g/mol) and methanol (Mw: 32.04 g/mol) (Fisher Scientific, UK) were of analytical grade.

2.2. Microcrystallisation of naproxen: In-situ micronisation

In-situ micronisation was used following the solvent change method (22). This process involved rapidly mixing the prepared naproxen solution with a specific concentration of surfactant solutions, being either poloxamer 407 (P407) or caprol® PGE 860 (CAP) (stabilising agents). Initially 1% w/v pure naproxen

crystals were dissolved in a required volume of ethanol (as solvent), in 250 ml (for P407 study), and in 500 ml (for CAP study). The stabilising agents were dissolved in a required amount of distilled water (as non-solvent) (up to 100 ml for P407, and up to 200 ml for CAP). Four different concentrations (0.1, 0.5, 1 and 3% w/v) of the two surfactants were chosen (Table 1). Both naproxen and surfactant solutions were then sonicated for approximately 30 minutes using an Ultrasonic Cleaner (Hilsonic, UK). Using a 10ml syringe (B-D Plastipak, UK), a total volume of 25 ml of 1% w/v naproxen was added as 5ml (x5) aliquots every 2 minutes to each concentration of both surfactants (P407 and CAP). Additionally, two samples of naproxen (F1 and D1) were tested without the addition of either P407 or CAP, and were added to 100 ml of fresh distilled water in the same manner. The newly formed suspensions were continuously stirred for 10 minutes using a magnetic mixer (Ikamag RCT, UK). The preparations were then centrifuged for 10 minutes at a speed of 20,000 rpm and supernatants were decanted before being dried by either freeze drying via VirTis Benchtop Freeze Dryer (Biopharma, USA), or desiccator drying using desiccators with silica gel.

Insert Table 1

2.3. Evaluation of naproxen microcrystals

2.3.1. Microscopic examination of naproxen microcrystals

Morphological assessment of the 19 samples prepared from the in-situ micronisation technique was undertaken using an Olympus - BH2 polarized light microscope (Optivision Ltd. Japan) fitted with a digital camera (AxioCam MRc-Zeiss, UK), and an image analyser (AxioVision, vs4.4., Carl Zeiss). The samples were observed with a 20x objective lens and a 10x eyepiece lens.

2.3.2. Particle size analysis

The size distribution of the microcrystals produced was measured using a Swift M4000D optical microscope (Swift optical instruments, San Jose, California, America), where more than 100 particles were used. Also, the microscope used a calibrated British standard eye piece graticule.

2.3.3. *In vitro* dissolution testing

The dissolution rate of all naproxen microcrystals was carried out under sink conditions using the United State Pharmacopoeia paddle apparatus (Erweka DT 6, Heusenstamm, Germany). Samples of 30mg were placed into 1L of distilled water maintained at 37 ± 0.5^{o}C, and the stirring speed employed was 100rpm. Samples of 10ml were withdrawn periodically (5, 10, 15, 20, 30, 40, 50 and 60 minutes) and replaced with the same volume of the fresh dissolution medium. The amount of drug released was determined by

initially measuring the absorbance at 271 nm using a UV/VIS spectrophotometer (Campsec M501 Single Beam Scanning, Cambridge, UK) and applying the calibration equation. The results are the mean and standard deviation of three replicates.

2.3.4. Differential scanning calorimetry (DSC)

Thermal analysis of all naproxen microcrystals was performed using a differential scanning calorimeter (DSC Q1000, TA instruments, England) equipped with a DSC refrigerated system using liquid nitrogen. Approximately 3-6 mg samples were individually crimped into hermetically sealed aluminium pans (TA instruments, England). Samples were scanned at a ramp rate of 10^{o}C/ minute and heated between 0.00^{o}C – 200.00^{o}C.

2.3.5. Fourier-transform infrared spectroscopy (FT-IR)

Fourier transform infrared spectra for all naproxen microcrystals were obtained using IR spectrophotometer (PerkinElmer FT-IR System, Spectrum BX, PerkinElmer, UK). The spectra were recorded over a scanning range of 550 to 4000 cm^{-1}, a resolution of 4.0 cm^{-1} was maintained and the number of scans used was 10.

2.4. Statistical analysis

One-way analysis of variance (ANOVA) was carried out to analyse the dissolution test results using SPSS vs16.0 software package for windows (SPSS Inc. Chicago, USA). Comparison between the two means was determined following either a post-hoc Scheffè test (for freeze dried naproxen/poloxamer 407 samples) or the Games-Howell test (for freeze dried naproxen/caprol® PGE 860 samples). Additionally, an independent T-test was performed to verify the significance between the two methods of drying (freeze drying and desiccator drying) at all concentrations. All data were produced at a 95% confidence interval and differences were considered as significant when probability (ρ) was < 0.05.

3. RESULTS AND DISCUSSION

3.1. Particle size and morphology

The commercial drug (U1) appeared as smooth white crystals, representing a high degree of crystallinity (Fig. 1), this was confirmed using DSC. Particle sizes were broadly distributed across ranges from 7.5 µm to 41.37 µm. This was expected as U1 did not undergo in-situ micronisation, which would have yielded a tighter and more symmetric particle size distribution (22). For freeze dried pure naproxen (F1), in-situ

micronisation generated smaller, more homogenous microcrystals, showing areas of both white crystals and dark amorphous particles. Approximately 54% of F1 microcrystals were 7.5 µm in size; a 4.5- fold decrease in size from U1, which showed only 12% of microcrystals having 7.5 µm in size.

Freeze dried samples containing P407 as the surfactant, showed a decrease in particle size with an increase in P407 concentration. The upper quartile for F3 (naproxen/ 0.5% w/v P407) microcrystals was 12.6 µm and was considerably lower than that observed for F1 (33.4 µm). For F4 (naproxen/ 1% w/v P407), 100% of microcrystals were 7.5µm in size.

A similar pattern was seen for desiccator dried samples containing P407, where an increase in P407 concentration lead to a decrease in particle size, and an increase in homogeneity. However, the reduction in the size of the crystals was not as pronounced for desiccator dried samples as it was for freeze dried samples. Rasenack et al. (27) stated that the strength at which the surfactant is adsorbed onto the surface of the crystal is determined by the method of drying. It can therefore be understood that certain processes involved in freeze drying, such as rapid sublimation, results in a more intense adsorption of P407 on the surface of crystals ensuring the stability of particles (28). Additionally, by preventing aggregation and ensuring homogeneity, freeze drying is responsible for delivering particles of a small size distribution if this drying process used to dry initially controlled sized particles. This was in contrast to the results seem by Talari et al. (18), where in-situ micronisation was used through a pH change method, and found no significant change in particle size distribution of microcrystals before and after freeze drying. This suggests that it is in fact a combination of solvent change method and freeze drying that influence naproxen crystals' properties. Varshosaz et al. (5) found that the highest dissolution rate and smallest particle size of piroxicam microcrystals were observed using the solvent change method when compared to pH shift method.

In terms of morphology. The dark amorphous regions seen for F1 are also observed for desiccator dried samples (D3 and D5, Fig. 1), and become more pronounced with the addition of P407 and with an increase in its concentration, as represented by the photomicrographs of D3 and D5. However, for freeze dried samples, the addition of increased amounts of P407, as seen for F5, results in a return to a more crystalline state that can explain the lower drug released from this formula, refer to Fig. 2a.

Microcrystals containing caprol® PGE 860 (CAP) show small particle size, increased homogeneity and very narrow particle size distribution. For 0.1 and 0.5% w/v CAP, identical particle sizes and homogeneity for both freeze dried and desiccator dried samples were seen (Inter Quartile Range = 1.5µm). Photomicrographs for D8 and F9 (Fig. 1) show the existence of a polymorphic crystal form with

needle-shaped appearance. Rasenack et al., (1) reported that these crystals are thermodynamically unstable and galenically unacceptable due to their felted nature.

Insert Fig. 1

3.2. *In vitro* dissolution testing

Dissolution profiles of freeze dried (F2 – F9) and desiccator dried (D2 – D9) naproxen/surfactant microcrystals, prepared using in-situ micronisation, are illustrated in Fig. 2a – 2d and are represented as percentage drug release versus time. Dissolution profiles of pure naproxen microcrystals (F1 and D1) (crystallised using in-situ micronisation in the absence of surfactants) and untreated naproxen (U1) (did not undergo in-situ micronisation) are also included these figures.

As shown in Fig. 2a, the data revealed that untreated naproxen (U1) had poor dissolution profiles compared to freeze dried pure naproxen microcrystals (F1) and naproxen/P407 microcrystals (F2 – F4), with only 18±8.85% drug released after 5 minutes and 70±3.77% drug release after 60 minutes for U1, compared with a burst release of 81.07±1.92% and 77.45±7.03% after 5 minutes for F1 and F3 respectively, achieving over 90% drug release within 15 minutes. The highest drug release was seen for F4, with 99±2.77% after 5 minutes (5.5 times greater than U1). Similar observations were detected for desiccator dried samples (Fig. 2b), with an enhanced dissolution rate of naproxen/ P407 microcrystals (D2 – D5) when compared to untreated naproxen, with the dissolution of the microcrystals after 5 minutes being 35±7.00% (D2) and 84.76±4.68% (D5), which is 1.9 to 4.7 times greater than that of untreated naproxen (U1). However, this was not as pronounced as those reported for the freeze dried samples and generally showed higher standard deviations over the course of 60 minutes.

From Fig. 2a and 2b, it is also evident that the higher concentrations of P407 (both freeze dried and desiccator dried) yielded an improved dissolution rate over the course of 60 minutes. For example, at 30 minutes, the drug release increased from 79±0.57% to about 100% (F2 – F4) and from 64±1.11% to 95±5.63% (D2 – D5). The low percentage drug release detected at lower poloxamer concentrations show that microcrystals have not been sufficiently wetted by the surfactant.

In general, when comparing the two drying methods used, it is evident that all freeze dried formulations showed improved drug dissolution than that of desiccator dried formulations. The total drug release for freeze dried formulations was observed with F4 after only 10 minutes. However, the same formulation desiccator dried (D4) showed a considerably lower drug release of 67±7.72% ($\rho < 0.05$). The highest percentage drug release for desiccator dried formulations was 96.72±6.19% for D5, achieved after 60 minutes of dissolution. The increased dissolution rate with increased P407 concentration for freeze dried

microcrystals, could partly be explained by the presence of amorphous regions (29) as shown in Fig.1. The decreased dissolution rate seen for F5, showing only 65% ± 1.59 after 60 minutes is reflected by the photomicrograph seen in Fig. 1, with increased crystalline regions.

Insert Fig. 2

The effect of CAP on the dissolution rate of naproxen shows less encouraging characteristics for its potential use as a dissolution enhancer. Fig. 2c and d show that, in contrast to P407, an increase in caprol® PGE 860 concentration results in a decrease in the dissolution rate of naproxen microcrystals. For example, drug release of freeze dried microcrystals after 10 minutes decreased as follows: 57±3.68%, 15±1.64%, 10.27±1.08% and 7.18±0.04% for F6, F7, F8 and F9, respectively. This can be attributed to the highly viscous physical nature of CAP, whereby upon addition to an aqueous medium, a viscous diffusion layer is formed causing the extremely low passage of drug into solution (30). Ultimately, the naproxen microcrystals are not adequately wetted and the dissolution rate constant is lowered even though the solubility may have been higher (31). A study by Akinlade et al. (32) described CAP to be a poor choice of excipient, and was attributed to its highly viscous nature.

The dissolution rate of naproxen/CAP is significantly lower ($\rho<0.05$) than that of pure naproxen microcrystals (F1) and naproxen/P407 microcrystals for both freeze dried and desiccator dried samples. For example, drug release for F3 (0.5% w/v poloxamer 407 freeze dried) after 10 minutes was 87±6.35%, showing sufficient wetting and rapid dissolution of the microcrystals and drug release was 81% for F1 (pure naproxen microcrystals freeze dried). In comparison to this, only 16±1.65% of drug released after 10 minutes for F7 (0.5% w/v CAP) ($\rho<0.05$).

The results imply that naproxen/P407 microcrystals and freeze drying show enhanced dissolution properties than that of naproxen/CAP microcrystals and desiccator drying. The highest percentage drug release for naproxen/poloxamer microcrystals was for F4 and was achieved after 10 minutes. However, the highest percentage drug release for naproxen/CAP microcrystals was 91±2.00% (F6) and was achieved after 60 minutes' dissolution. The results could also indicate that P407 preparations had a greater presence on the surface of naproxen microcrystals than CAP., which would result in greater reduction of the surface tension between the hydrophobic naproxen microcrystals and the aqueous medium. Consequently, the energy within the system is reduced allowing individual naproxen particles to become dispersed in the aqueous solution. Hence, the dissolution of these microcrystals is more rapid due to the increase in specific surface area of naproxen microcrystals. In a study by Steckel et al (33), the physico-chemical properties of in-situ micronised fluticasone-17-propionate (FP) and jet milled FP were compared, and found the milled drug to possess amorphous areas on its surface due to the energy input of

the milling process. However, in-situ micronised drug was found to be less susceptible to chemical degradation or alternation in physical properties with improved followability and dispersibility.

3.2. Differential scanning calorimetry (DSC)

The prepared samples (untreated naproxen, pure naproxen microcrystals, naproxen/P407 and naproxen/CAP microcrystals) were examined using DSC, the melting point (T_m) and enthalpy of fusion are presented in Table 2, and the thermograms produced are shown in Fig. 3A - G

Insert Table 2

According to the British Pharmacopeia (34), the melting point of pure untreated naproxen ranges between 154 to 158^{o}C. From Table 2 and Fig. A and B, pure naproxen showed a single sharp melting peak (T_m) at about 157.70^{o}C and ΔH of 134.3 J/g for freeze dried microcrystals (F1), this was slightly lower than the value obtained for desiccator dried microcrystals (D1) with a T_m of 158.51^{o}C and ΔH of 145.7 J/g. The sharp peak is characteristic of naproxen's crystalline nature, and is similar to that obtained by Dixit et al. (35) and Akbari et al. (36) with a sharp T_m at about 154^{o}C, and by Elkordy et al. (37) with the endothermic peak at 158.9^{o}C for pure untreated naproxen. The melting point of P407 as stated in the British Pharmacopeia is about 50^{o}C (34). Therefore, the short peaks observed at the beginning of the heating procedure (Fig. 3C and D) are related to the T_m of P407, with a value of 50.82^{o}C for 0.5% w/v P407 and 51.20^{o}C for 1% w/v P407. The increased intensity seen in Figure 3D is due to the increased poloxamer concentration.

The characteristic endothermic peak for pure naproxen was present in all profiles. However, a slight reduction in T_m was seen with the addition of surfactant and with an increase in surfactant concentration. For example, the addition of 0.5% w/v P407 reduced the T_m from 157.70^{o}C for pure freeze dried naproxen microcrystals, to 156.99^{o}C. The enthalpy of fusion was also reduced from 134.3 J/g to 120.1 J/g. A further increase in P407 concentration to 1% w/v lead to further reduction in T_m and enthalpy of fusion to 155.64^{o}C and 88.51 J/g, respectively. As mentioned previously, in-situ micronisation leads to the reduction in surface tension and enhanced wetting properties of poorly water soluble drugs as a result of the stabilising agent (in this case P407) being adsorbed on to the drug particles (5, 15). This would therefore explain the reduced T_m seen with increased poloxamer concentration and goes hand in hand

with the dissolution results obtained in this study, and with the morphology photomicrographs (Fig.1) mentioned previously.

Insert Fig. 3

Even though the T_m of naproxen decreases with an increase in P407 concentration, this slight reduction remains consistent with literature values mentioned previously, and the thermograms for P407 containing samples show characteristic features of both the naproxen and P407, this could therefore indicate the absence of significant chemical interactions between the drug and polymer. Similar observations were found in a number of studies for different drugs/polymers and was also explained as there being no interaction between the two (5, 9, 13, 38).

In addition, the intense reduction in enthalpy of fusion has been considered by a number of studies to be related to the loss of drug crystallinity (39-40). It has also been reported that the reduction in microcrystal particle size and the small amount of stabiliser may affect the reduction in enthalpy and that the precipitated drug is sterically stabilised against crystal growth by adsorbed polymer. Hence, the surface energy and consequently the enthalpy of the system is lowered (5, 19). Dixit et al. (40) looked at enhancing the solubility and dissolution of indomethacin by freeze drying, they found that the dissolution of freeze dried crystals was increased and was thought to be linked to the better wettability and reduction in particle size with increasing surface area of freeze dried crystals. Therefore, it can be stipulated that the improved dissolution properties documented in this study for naproxen/ P407 may not entirely be due to changes in drug crystallinity, but could be due to the improved wetting properties and reduction in particle size as a result of the combined effect of in-situ micronisation, freeze drying and the type of surfactant used.

The DSC thermogram produced for caprol® PGE 860 (Fig. 3E and F) contained the recognisable naproxen melting peak (T_m) at 153.52^{o}C, with enthalpy of fusion of 98.83 J/g for F6, and a T_m at 153.29^{o}C for D6, with a much lower enthalpy of 83.05 J/g. Interestingly, these values were not consistent with literature values for naproxen T_m, and the enthalpy of fusion was even lower than that obtained using P407. Additionally, this may be due to different arrangement of drug molecules in the crystal lattice.

Increasing the concentration of CAP further to 1% w/v, resulted in an alteration in the thermogram profile, with a marked broadening and reduction in the intensity of the naproxen endotherm, which was shifted to a T_m of 147.95^{o}C, and the enthalpy of fusion was considerably reduced to 5.028 J/g, which is a 96.5% reduction in enthalpy compared to desiccator dried pure naproxen microcrystals (D1).

This would imply the possibility of interactions between the components (41-42) and/or polymorph formation. This coincides with the dissolution results that show marked reduction in dissolution rate with an increase in CAP concentration. This could also be due to the highly viscous nature of caprol® PGE 860 (32), whereby upon addition to an aqueous medium, a viscous diffusion layer is formed causing the extremely slow passage of drug into solution (30). Ultimately, the naproxen microcrystals are not adequately wetted and the dissolution rate constant is lowered even though the solubility may have been higher (31).

3.3. Fourier-transform infrared spectroscopy (FT-IR)

FT-IR spectroscopy was used to study any possible interactions between the model drug naproxen and stabiliser (being either poloxamer 407 or caprol® PGE 860), and to analyse any possible changes in chemical structure. The spectra of all samples are shown in Fig. 4.

The crystal structure of naproxen exists as a trimolecular catemer (44). Therefore, the spectra for pure untreated naproxen, U1, (Fig. 4A) shows the typical quartet of bands stretching between 1723 cm^{-1} and 1602 cm^{-1}, which is characteristic of carbonyl (-C=O) stretching vibrations (37, 42-43). Peaks at 1723 cm^{-1} and 1681 cm^{-1} attributed to non-hydrogen bonded carbonyl (-C=O) stretching and hydrogen bonded carbonyl stretching of the catemer respectively (44). The intensity of the vibrational bands at 1723 cm^{-1} is more dominant because most of the naproxen molecules are not engaged in hydrogen bonding (44-45). The spectra for both F1 and D1 show a small shift at the non-hydrogen bonded carbonyl group -C=O from 1723 cm^{-1} to 1726 cm^{-1}, which could indicate changes in hydrogen bond characteristics due to the removal of moisture exerted by either freeze drying or desiccator drying (46). Similar shift of the carbonyl stretch to higher energy was found by Islam et al. (46) and was attributed to the evaporation of solvent water.

Insert Fig. 4

From Fig. 4A, B and C, it can be concluded that the typical quartet of bands of naproxen carbonyl stretching were present in all naproxen/P407 spectra, with only small shifts in wavenumber with the addition of P407, for example, the addition of 0.1% w/v P407 lead to bathochromic shift at the hydrogen bonded carbonyl stretching band from 1680 cm^{-1} (pure untreated naproxen) to 1683 cm^{-1}, and from 1723 cm^{-1} to 1725 cm^{-1} at the non-hydrogen bonded carbonyl stretching band. Interestingly, further increases in P407 concentration to 1 and 3% w/v lead to the return of wavenumber back to its original value of 1680 cm^{-1} and 1723 cm^{-1}. These findings indicate that there may be some degree of hydrogen bonding interactions between the drug and polymer (39). The bonding is thought to occur between the carboxylic

acid functional group of naproxen and the polyoxyethylene chain of P407 (47-48). It has been reported by Anderson et al. (49) that hydrogen bonding leads to a decrease in carbonyl stretching frequency, this would explain the small hypsochromic shift seen in this study with an increased P407 concentration and the hydrogen bonding is thought to be intermolecular since it is concentration dependent. These findings go hand in hand with the dissolution data which shows a decrease in dissolution rate at a poloxamer 407 concentration of 3% w/v.

In General, the IR spectrum does not show any additional peaks, and only small shifts in wavenumber were seen. This indicates the absence of any significant chemical interactions between naproxen and P407, and no or very small alteration to naproxen crystallinity or internal structures (5, 50). It can therefore be reported that it is mainly the physical interaction of drug with polymer that is responsible for the enhanced dissolution properties recorded (50).

Analysis of the IR spectra for caprol® PGE 860 microcrystals shows an apparent change in profile noted with an increase in CAP concentration. Samples of high CAP concentration e.g. F8 (naproxen/ 1% w/v CAP; Fig. 4D) show a broad and less defined -C=O stretching band intensity suggesting a weakening of hydrogen bonding and a possible change in chemical structure.

Albuquerque et al. (51) noted that CAP yields a characteristic stretching vibration band at 1744cm^{-1} representing -C=O of the carboxylic groups. This band can be seen developing as the concentration of CAP is increased (Fig. 4D). Ultimately, these results combined with literature review suggest that the presence of CAP molecules cause a change in the crystal lattice arrangement in naproxen molecules (as can be observed as well in Fig. 1 for naproxen microcrystals with CAP, needle crystals), as the intra-hydrogen bonds between the drug molecules are reduced. This change proves that caprol® PGE 860 is not a good choice of excipient to enhance naproxen dissolution.

4. CONCLUSION

This study demonstrated that in-situ micronisation using the solvent change method had a positive effect on dissolution rate enhancement of the poorly water soluble drug, naproxen. The implementation of freeze drying showed improvement in the dissolution rate of samples by creating crystals that were smaller with a more homogenous size range in comparison to desiccator dried samples. The use of poloxamer 407 proved to be a good choice of excipient, serving as a surface active agent, enhancing the wetting properties of the microcrystals, yet with only very small changes in drug crystallinity. Caprol® PGE 860 on the other hand was found to significantly reduce the dissolution rate of naproxen, showing

some interactions with drug components and alterations to the drug's crystal lattice arrangement. It can therefore be documented that the enhanced dissolution rate seen in this study is due to the combined effect of in-situ micronisation using solvent change method, freeze drying and the choice of the polymer.

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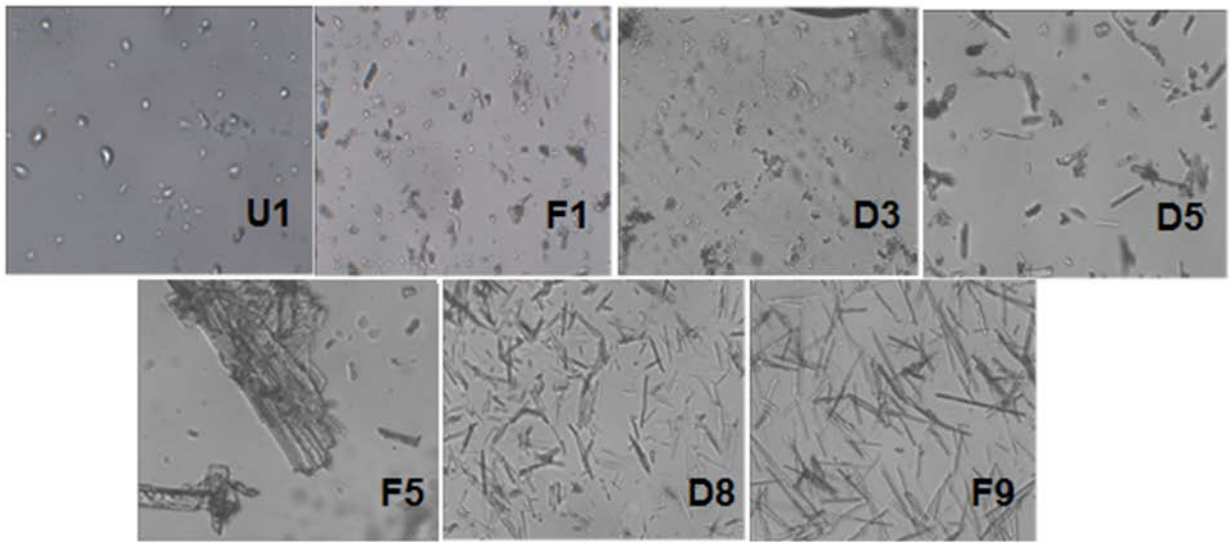

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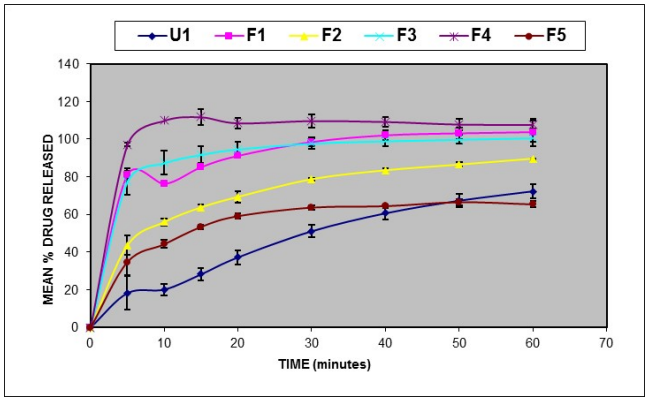
Fig. 1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F2
F3
F4
F5
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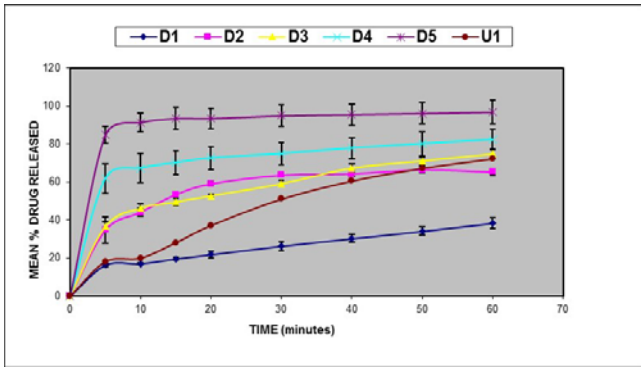
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b

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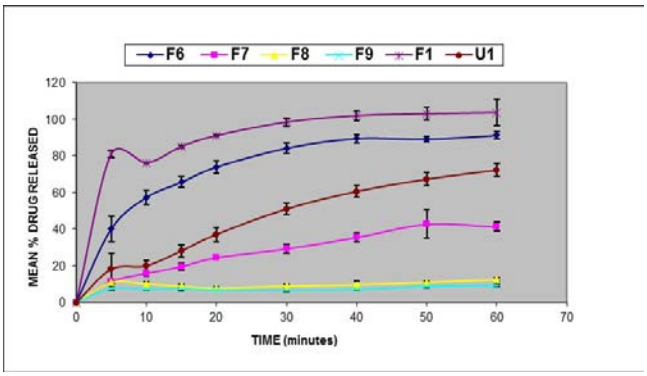
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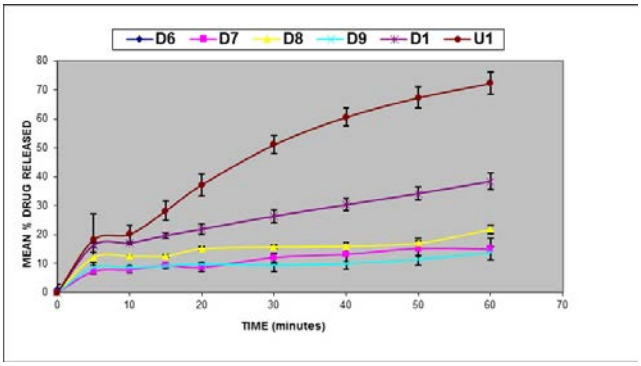
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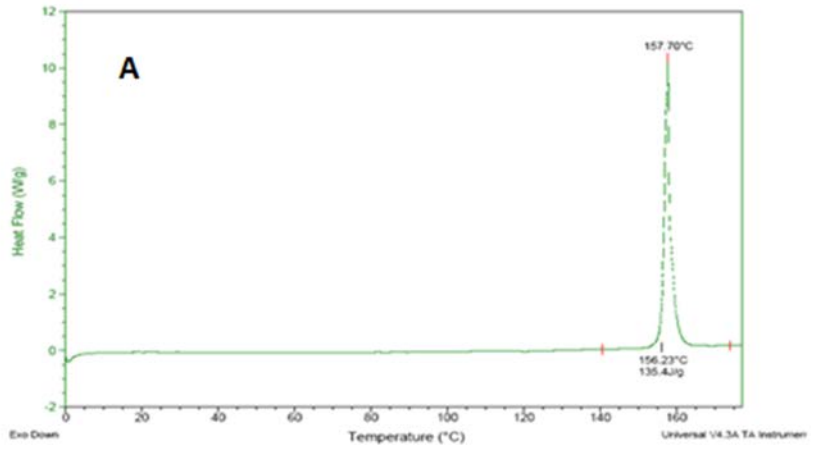
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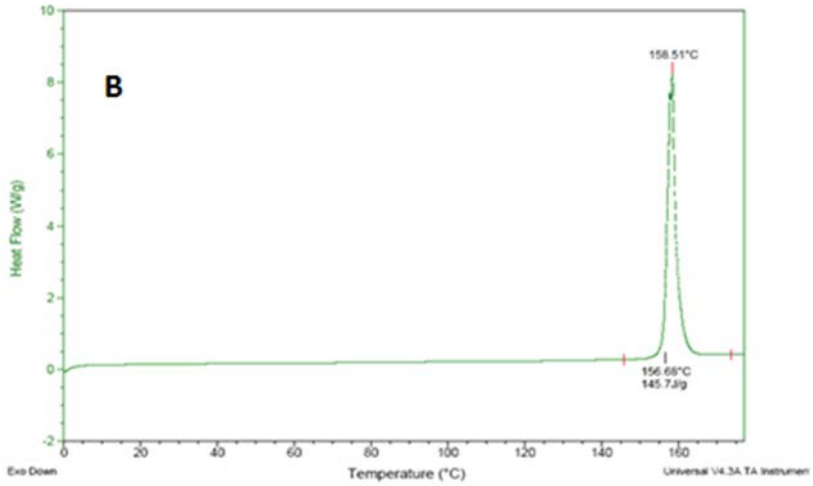
Exo Down
Universal V4.3A TA Instrumen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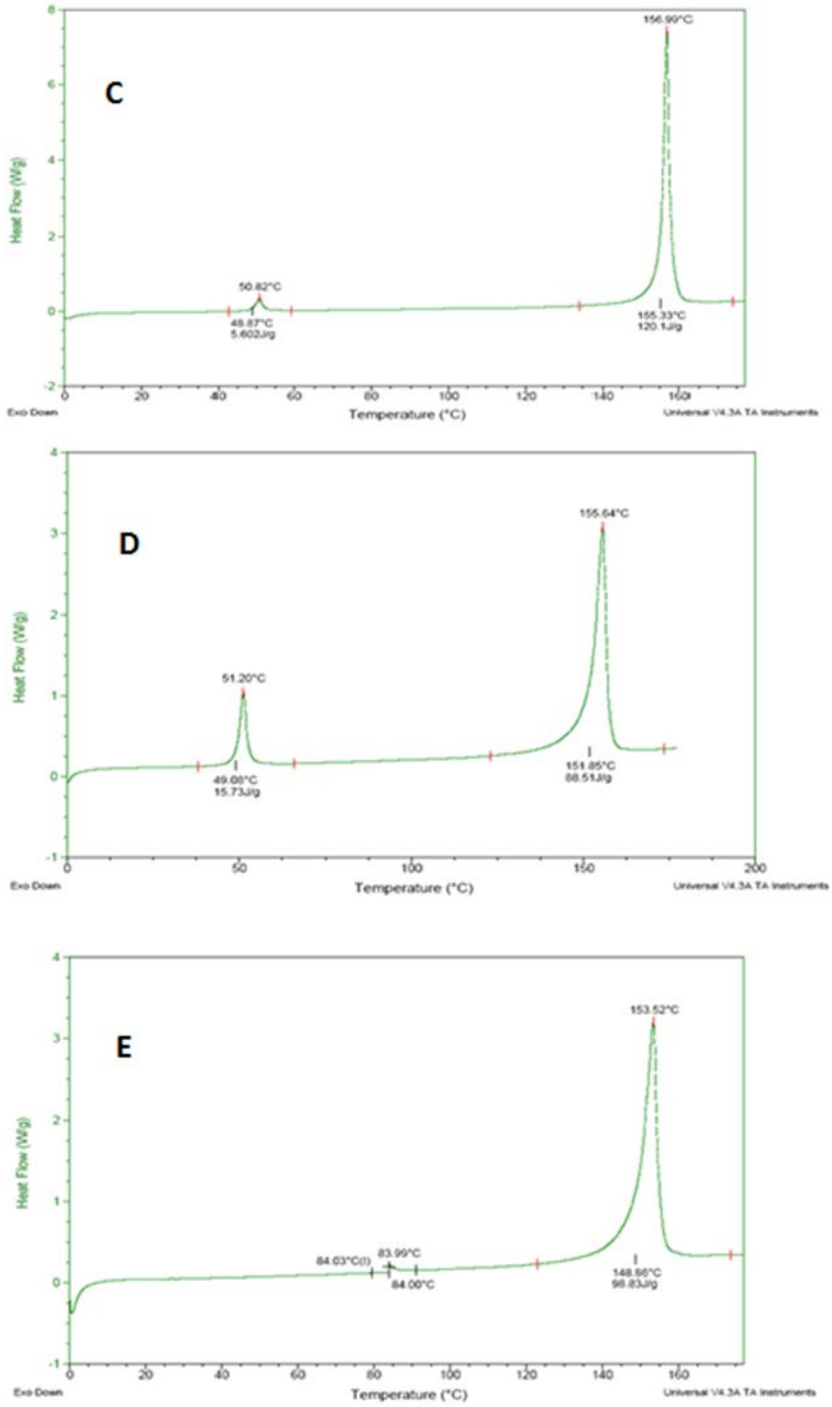
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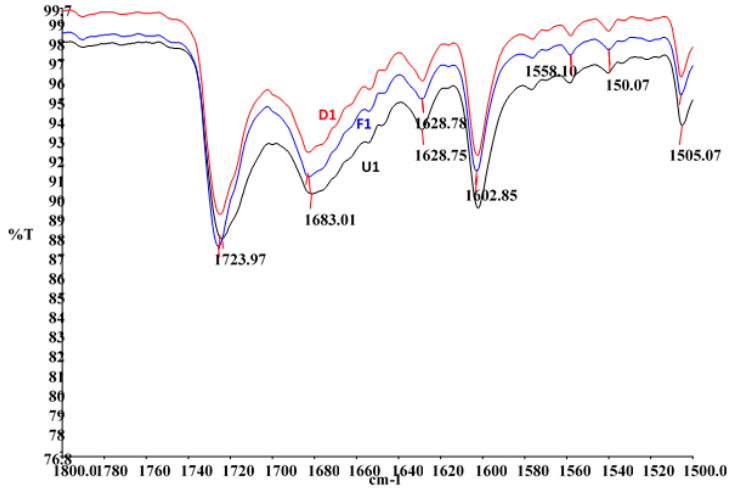
Fig. 3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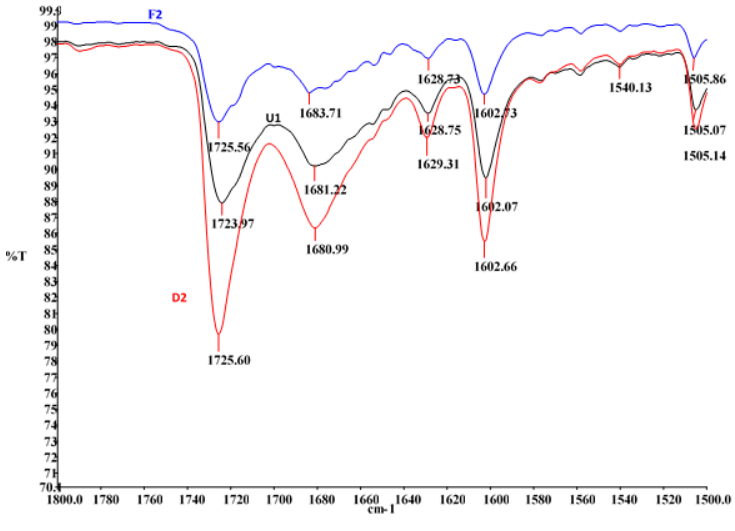
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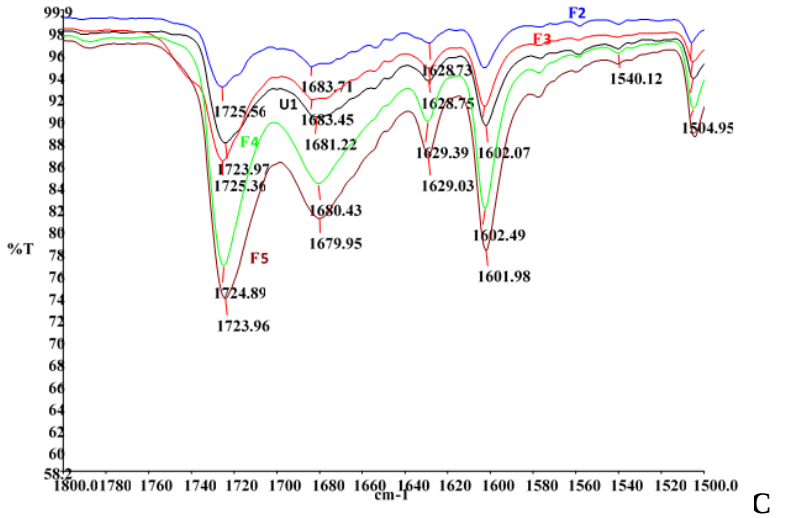
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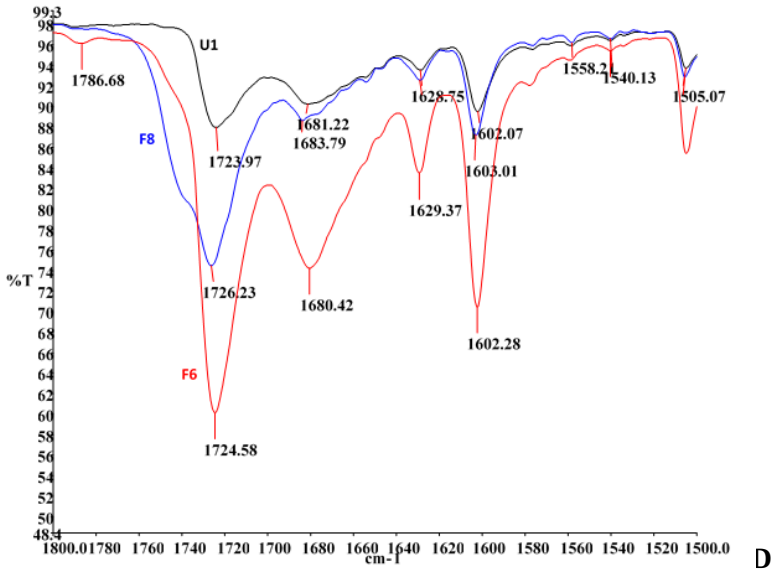
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F6
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%T
cm-1
D

Fig. 4

Figure legend

Figure 1. Photomicrographs of untreated naproxen (U1), freeze dried pure naproxen (F1), desiccator dried NAP/ 0.1 and 0.5% w/v P407 (D3 and D5), freeze dried NAP/ 3% w/v P407 (F5), desiccator dried NAP/ 1% w/v CAP (D8) and freeze dried NAP/ 3% w/v CAP (F9). All images were taken using the same magnification (section 2.3.1).

Figure 2. Dissolution profiles of naproxen/ P407 microcrystals (a) freeze dried (b) desiccator dried, and of naproxen/ CAP microcrystals (c) freeze dried (d) desiccator dried. Untreated naproxen (U1) and pure naproxen microcrystals (F1 and D1) are included in the profiles. For formulation composition refer to Table 1.

Figure 3. DSC thermograms of: **A)** pure naproxen (F1, freeze dried), **B)** pure naproxen (D1, desiccator dried), **C)** naproxen/0.5% w/v P407 (F3), **D)** naproxen/ 1% w/v P407 (F4), **E)** naproxen/ 0.1% w/v CAP (F6), **F)** naproxen/ 0.1% w/v CAP (D6), **G)** naproxen/ 1% w/v CAP (D8).

Figure 4. FT-IR spectra for naproxen formulations: **A)** Untreated NAP & NAP microcrystals (freeze dried and desiccator dried), **B)** Untreated NAP and NAP/ 0.1% w/v P407 (freeze dried), **C)** Untreated NAP and all NAP/ P407 formulations (freeze dried), **D)** untreated NAP and NAP/ CAP (0.1 and 1% w/v, freeze dried).

List of Tables

Table 1. Concentrations and drying method of naproxen/surfactant microcrystals produced following in-situ micronisation

Drying method	Sample Code	Concentration of Naproxen (%w/v)	Concentration of Poloxamer 407 (%w/v)	Concentration of Caprol® PGE 860
Freeze Drying	F1	1	-	-
	F2	1	0.1	-
	F3	1	0.5	-
	F4	1	1	-
	F5	1	3	-
	F6	1	-	0.1
	F7	1	-	0.5
	F8	1	-	1
	F9	1	-	3
Desiccator Drying	D1	1	-	-
	D2	1	0.1	-
	D3	1	0.5	-
	D4	1	1	-
	D5	1	3	-
	D6	1	-	0.1
	D7	1	-	0.5
	D8	1	-	1
	D9	1	-	3
Untreated Naproxen	U1	1	-	-

Table 2. Results of thermal analysis for naproxen formulations

Formulation	Drying Method	Melting Point, T_m peak (^oC)	Enthalpy of Fusion, ΔH (J/g)
F1 (Pure naproxen)	Freeze dried	157.70	134.3
D1 (Pure naproxen)	Desiccator dried	158.51	145.7
F3 (Naproxen/ 0.5% P407)	Freeze dried	156.99	120.1
F4 (Naproxen/ 1% P407)	Freeze dried	155.64	88.51
F6 (Naproxen/ 0.1% CAP)	Freeze dried	153.52	98.83
D6 (Naproxen/ 0.1% CAP)	Desiccator dried	153.29	83.05
D8 (Naproxen/ 1% CAP)	Desiccator dried	147.95	5.028