Freeze-dried crystalline dispersions: Solid-state, triboelectrification and simultaneous dissolution improvements

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Highlights

- Freeze-dried crystalline dispersion successfully made with piroxicam and glucosamine
- Drug content level dictated the electrostatics of the dispersions made
- Glucosamine improves the handling of piroxicam
- Freeze-drying process improved the dissolution of the piroxicam-glucosamine dispersion

Graphical abstract

Abstract:

The charging propensities of freeze-dried samples produced from combinations of Piroxicam (PXM) - a BCS Class II drug with D-glucosamine HCl (GLU) - a potential hydrophilic carrier were investigated in this study. Freeze-dried PXM:GLU solid dispersion samples were prepared at different PXM:GLU ratios and characterised using analytical techniques such as XRPD, DSC and SEM. In addition, the dissolution rate and triboelectric charging of the samples were investigated. DSC and XRD results showed that the freeze-drying process produced crystalline materials which were a mixture of PXM polymorphs that included the monohydrate form. The dissolution rate of PXM was improved in the solid dispersions due to the presence of GLU compared to pure PXM with the freeze-dried PXM:GLU solid dispersion at the ratio of 1:1(500) having the best improved dissolution of all the prepared samples. Electrostatics results showed PXM to have a higher tendency for charging in comparison to the carrier GLU (- 3.2 v 0.5 nC/g). Freeze-dried PXM:GLU showed an electropositive charging behaviour when the content of drug was ≤ 50 % (w/w), whereas the freeze-dried samples containing >50 % (w/w) of drug showed an opposite electronegative charging behaviour. The charge results also confirmed that GLU improved the handling of PXM through potential ordered mixture formations and shows how powder handling as well as dissolution can be manipulated to obtain a desired outcome.

Keywords: Solid dispersions; Piroxicam; D-glucosamine HCl; Dissolution; Electrostatics; Freeze-drying

1. Introduction

The Biopharmaceutical Classification System (BCS) classifies drugs into 4 classes (Class I-IV) based on important factors such as aqueous solubility, dissolution rate and the intestinal permeability. BCS Class II drugs usually have a low drug concentration gradient between the gut wall and the blood vessels thereby limiting drug transport into systemic circulation and oral bioavailability. Solubility enhancement for BCS class II drugs is important to attaining efficient therapeutic systemic concentrations due to the fact that dissolution rates are typically the rate-limiting step for oral bioavailability of poorly soluble drugs[1].

Particle size reduction is one of the most common methods used in the enhancement of the dissolution rate of poorly soluble drugs [2,3]. The reduction in particle size leads to an

increase in the effective surface area of the drug particles available for interaction with the solvent. This particle size reduction brings about an increase in solubility which has been reported to be seen in the range [4] below $1\mu\text{m}$ [5]. However, particle size reduction to this size range may lead to particle agglomeration which may have a negative impact on the solubility and bioavailability of the drug in the final product [6]. Researchers have used several methods in the enhancement of solubility of drugs and they include complexation with carriers such as cyclodextrins [7], liquisolid techniques [8–10], salt formation [11–13] and the solid dispersion approach. The solid dispersion method has been reported to be one of the most efficient methods of improving drug dissolution [14–20] and can be prepared using solvent evaporation [21], co-grinding [22], spray drying [23], freeze drying [24] and hot melt extrusion [25]. Several studies have demonstrated that the presence of glucosamine (GLU) in solid dispersions with drugs like piroxicam (PXM), ibuprofen (IBU), carbamazepine (CBZ) and indomethacin (IND) prepared using either the solvent evaporation or grinding method produced crystalline material that significantly increased their rates of dissolution. Other carriers that have been explored by researchers in improving the dissolution rate of PXM include gluconolactone [26], microcrystalline cellulose, crospovidone [27], PEG 4000 [28] and Pluronic F-98 [29].

Currently, there are no pharmacopoeial methods used in the characterization of particle charges [30]. However, recently there has been an increase in the research into the characterization of the electrostatic properties of powders [31]. The electrostatic charging observed in powder samples occur as a result of contacts and collisions between individual particles and particle-surface collisions in a gaseous environment [32]. In this study, a novel equipment developed in the Wolfson Centre, University of Greenwich [33] is used to assess the charge properties and charge distribution of freeze dried particulate materials. This specific equipment was chosen for this study due to its reported high level of sensitivity (charges on the particles equal or more than $30 \times 10^{-15} \text{ C}$ are detectable), fast turnaround times for measurements ($< 1 \text{ min}$) and the lack of particle flow disturbance [33–35]. The effect of static electrification on powder handling is not completely understood and hard to predict due to the inherent multivariate nature of electrostatic charging [32,36–38]. It is however acknowledged that electrostatic charge can hinder powder flow or at least increase the flow instability of powders due to the induced formation of agglomerates [39,40]. Particle sticking on the surfaces is another commonly observed practice in the powder handling which is caused by particle-surfaces image forces [41]. Typically, pharmaceutical powders acquire charges between 0.0 and $1.0 \times 10^{-12} \text{ C}$ of charges during the handling processes [37,42].

Electrostatic charge can lead to detrimental consequences on powder handling when it is sufficiently high, e.g. above 6×10^{-13} C [41].

Previous studies have explored the use of other drying methods such as spray drying on PXM-GLU solid dispersions [35]. The authors found the spray drying technique to improve the dissolution of piroxicam as well as the handling of the powder due to a reduction in the powders charge [35]. The freeze drying (lyophilization) method was used in this study as the manufacturing technique due its simplicity and the low/mild temperatures at which drying occurs which leads to minimal mechanical or thermal stress on the drug particles as compared to the spray-drying technique. Freeze drying has been explored in the preparation of solid dispersions by various researchers [24,43,44]. It is important to evaluate the impact of manufacturing processes on the effect of pharmaceutical products. Homayouni et al., 2014 [45] investigated the effect of an antisolvent crystallization technique by spray drying and freeze-drying. The authors reported significant changes in morphology and size from the micro- range to the nano range depending on the technique used. This also significantly impacted on the dissolution studies. Asare-Addo et al. 2019 also investigated a spray drying, freeze-drying and homogenizing-freeze drying technique on indomethacin soluplus amorphous solid dispersions. The authors also found the different manufacturing techniques to impact on the surface properties of compacts for intrinsic dissolution rate (IDR) measurements [46]. The impact of the freeze-drying processes on the dissolution rate of solid dispersions containing D-glucosamine HCl (GLU and PXM ((4-hydroxy-2-methyl-N-(2-piridyl) 2H-1,2-benzothiazine-3-carboxamide-1, 1-dioxide) and its impact of the charging properties were assessed in this study. This was also conducted to evaluate potential changes or similarities that may arise as a result of change in manufacturing process.

PXM is one of the potent non-steroidal anti-inflammatory drugs (NSAIDs) used in treatment of various musculoskeletal and joint disorders [47]. The use of GLU as a hydrophilic carrier was investigated in this study due to the fact that GLU is a likely nutritional supplement used by patients currently on PXM in the treatment of pain and to improve mobility in osteoarthritic joints [48,49]. The charging characteristics of the prepared crystalline samples were also investigated to evaluate the effect of the drying process on the handling of these solid dispersions. Information of the charging of these can inform a formulator on the best candidate samples to use that may avoid detrimental consequences further down the formulation process. To the best of the authors' knowledge, there is currently no reported study that has investigated the use of GLU in freeze-dried crystalline solid dispersions and the charge distributions from the resulting samples.

2. Materials and Methods

2.1. Materials

The model drug – PXM (Form I) and GLU were purchased from TCI Chemicals (Japan) and Sigma-Aldrich (UK), respectively. Analytical grade acetone was used as the solvent and was used as obtained from Fischer Scientific (UK). The dissolution medium (KCl:HCl buffer, pH 1.2) was prepared according to the USP 2003 method with KCl and concentrated HCl obtained from Sigma, (UK) and Fisher (UK), respectively.

2.2. Preparation of freeze-dried solid dispersions of drug-carrier

The solid dispersions were prepared by making suspensions of PXM and GLU at different drug: carrier ratios as shown in Table 1. In the preparation of the 1:1 (500) ratio, PXM (0.5g) was dissolved in a beaker containing 50 mL of acetone. In a separate beaker, GLU (0.5g) was dissolved in deionised water (50 mL) under agitation at 400 rpm. The two solutions were then mixed together with the drug acetone solution added to the GLU solution under continuous agitation for 1 h. The sample was agitated and maintained at a temperature of 50 °C to evaporate the acetone after which the samples were flash-frozen in liquid nitrogen and freeze-dried using a Christ Alpha 2-4 LD freeze dryer maintained at an average temperature of -50 °C and pressure of 0.0010 mbar for 24 h. The solid dispersion samples obtained were then stored in a desiccator until required. For comparison, freeze dried PXM (FD-PXM) samples were also prepared using the same method described above without the glucosamine.

Table 1. Formulation table used in the experimentation process

Formulation code	Piroxicam (g)	Acetone (mL)	Glucosamine (g)	Deionised water (mL)
1:1 (250) FD	0.25	50	0.25	50
1:1 (500) FD	0.50	50	0.50	50
1:1 (750) FD	0.75	50	0.75	50
1:1 (1000) FD	1.00	50	1.00	50
1:2 FD	0.50	50	1.00	50
1:4 FD	0.50	50	2.00	50
2:1 FD	1.00	50	0.50	50
4:1 FD	2.00	50	0.50	50
FD-PXM	0.50	50	0.00	50

Note: FD means freeze-dried

2.3. Particle Size Analysis (PSD)

A Sympatec laser diffraction particle size analyser (Clausthal-Zellerfeld, Germany) was used in the determination of the particle size distribution of the sample formulations. The WINDOX software provided was used to automatically calculate the mean particle size ($D_{10\%}$, $D_{50\%}$ and $D_{90\%}$). The procedure used is outlined in previous studies [15,35].

2.4. Scanning electron microscopy (SEM)

Pure PXM, freeze-dried PXM (FD-PXM), pure GLU, freeze-dried GLU (FD-GLU) and all the freeze-dried solid dispersion formulations in their different ratios were mounted on a metal stub with double-sided adhesive tape. These samples were sputter-coated with gold using a Quorum SC7620 Sputter Coater under vacuum in an argon atmosphere prior to observation. Images at different magnifications were obtained using a scanning electron microscope (Jeol JSM-6060CV SEM) operating at 20 kV to facilitate the study of the morphology of the solid dispersions.

2.5. Differential scanning calorimetry (DSC)

Pure PXM, freeze-dried PXM (FD-PXM), pure GLU, freeze-dried GLU (FD-GLU) and all the freeze-dried solid dispersion formulations in their different ratios (3 - 6 mg) were placed in standard aluminium pans (40 μ L) with a vented lid. The sample pans were heated across a temperature range of 20 to 250 $^{\circ}$ C at a scanning rate of 10 $^{\circ}$ C/min using nitrogen gas as a purge gas in a DSC 1 (Mettler-Toledo, Switzerland). The enthalpies, onset temperatures, peak temperatures and melting points of the samples were determined using the STARe thermal analysis software.

2.6. X-ray powder diffraction (XRPD)

Pure PXM, freeze-dried PXM (FD-PXM), pure GLU, freeze-dried GLU (FD-GLU) and all the freeze-dried solid dispersion formulations in their different ratios were characterised by X-ray powder diffraction (XRPD) according to a previous study [50] using a D2 Phaser diffractometer (Bruker AXS GmbH, Karlsruhe, Germany), with a sealed microfocus generator operated at 30 kV and 10 mA, producing CuK α (λ X = 0.1542 nm) radiation and a Lynxeye 'silicon strip' multi-angle detector. The samples were scanned in scattering (Bragg, 2θ) angle range from 5 to 40 $^{\circ}$, in 0.02 $^{\circ}$ steps at 1.5 $^{\circ}$ min $^{-1}$.

2.7. HPLC analysis

HPLC was performed on a Shimadzu system equipped with a SPD-20 AV Prominence UV/VIS detector set at 350nm, an LC 20 AT pump, and SIL-20A Prominence auto-sampler. The acquisition of data was carried out on a LC solution software integrator with separation performed using XTerra® 3.5 µm RP18 column (150 x 4.6 µm) (Waters) equipped with an XTerra® guard column. Mobile phase was a mixture of disodium hydrogen phosphate (5mM, pH 3): acetonitrile: methanol: glacial acetic acid (27:52:20:1)[51]. The analysis was performed at a flow rate of 1 ml/min determined at 30 °C with an injection volume of 5µl with an average run time of 6 minutes. The HPLC method was validated to determine the linearity, limit of detection (LOD), limit of quantification (LOQ) and inter and intraday variations. The calibration curve was determined over a concentration range of 0.5 to 20 µg/ml.

2.8. Dissolution studies

The dissolution studies of pure PXM and all the freeze-dried solid dispersions were carried out in a USP dissolution apparatus I (DT700, ERWEKA, Heusenstamm, Germany). Sample formulations used for the dissolution process contained amounts equivalent to 20 mg PXM regardless of the carrier (GLU) quantity. The powder samples were weighed and carefully introduced into dissolution baskets that have modified by having their bases covered with a flat circular paper discs. This was to prevent the powder samples falling through the bottom pores of the basket. The dissolution medium (KCl:HCl buffer: pH 1.2, 900 mL) was equilibrated to 37 °C ± 0.5 °C with the baskets rotated at 50 rpm. Samples were withdrawn at selected time intervals between 5 to 120 min and analysed by HPLC with the concentration of PXM in the samples calculated from the standard PXM calibration curve as detailed in section 2.7. All dissolution tests were carried out in triplicate.

2.9. Dissolution parameters

The mean percentage of PXM dissolved in the first 10 min (Q_{10min}) and 30 min (Q_{30min}) were used as an independent metric to assess the dissolution rate from various preparations. The dissolution efficiency (DE) of a pharmaceutical dosage form is the area under the

dissolution curve up to the time, t , expressed as the percentage of the area of the rectangle [31] as detailed in previous studies [32,33].

$$DE (\%) = \frac{\int_0^t y \times dt}{y_{100} \times t} \times 100, \quad (1)$$

where, y is the percentage of drug dissolved at time t .

In order to obtain a parameter that describes the dissolution rate another approach that can be explored is the determination of the mean dissolution time (MDT). MDT is the most likely time taken for a molecule to be dissolved from a solid dosage form. Therefore, MDT is the mean time for the drug to dissolve under in vitro dissolution conditions and is calculated using the following equation:

$$MDT (\text{min}) = \frac{\sum_{j=1}^n t_j \Delta M_j}{\sum_{j=1}^n \Delta M_j}, \quad (2)$$

where, j is the sample number, t_j is the midpoint of the j th time period (calculated with $((t + t-1)/2)$ and ΔM_j is the additional amount of drug dissolved between t_j and $t-1$. Based on this, the mean dissolution rate (MDR) can be calculated using the following equations:

$$MDR (\% \text{min}^{-1}) = \frac{\sum_{j=1}^n \Delta M_j / \Delta t}{n}, \quad (3)$$

where, n is the number of dissolution sample times, Δt is the time at the midpoint between t and $t-1$ (easily calculated with $[t + (t-1)/2]$).

2.10. Triboelectric assessment of freeze- dried solid dispersions

A triboelectric device electrostatic inductive sensor (developed at the in the Wolfson Centre at the University of Greenwich) was used to investigate the triboelectrification of the PXM, GLU and freeze-dried formulation [33]. For a detailed account of the procedure and information on this instrumentation, authors are referred to Adebisi et al., 2016 [34]. The net charge is the sum of positive charges and negative charges. The charge-to-mass ratio (CMR or charge density) was defined as the charge (negative charge for N-CMR, positive charge for P-CMR, net charge for net-CMR) per unit mass, in nC/g. Each sample was analysed an

average of six times to confirm good reproducibility (humidity and temperature controlled laboratory: 50 % RH, 22 °C).

3. Results and discussion

3.1. Solid state characterization and electrostatic charging

DSC analysis showed PXM had an average melting point of 201.63 ± 0.05 °C ($n=3$) signifying the PXM Form I polymorph (Figure 1a) [56]. PXM is a polymorphic drug with two well characterised polymorphic forms – Form I and Form II and the monohydrate (yellow) form. Form III and IV which are the amorphous or metastable forms respectively have also been reported [57]. Form I (Figure 3a) has a prism-like shape while Form II is needle-like in shape [58]. GLU showed a single endothermic peak on the DSC trace (Figure 1b) with a melting point at 208.97 ± 0.07 °C. After freeze-drying, the DSC scan of all the samples exhibited three prominent peaks; the first ranging from 127°C to 147 °C. This is attributed to the monohydrate form of piroxicam. This corroborates the change in the colour of the PXM sample from white to yellow as the monohydrate form of piroxicam has a distinct yellow colour [59]. The rate of the crystallization of the monohydrate form is what influences the shape of the dehydration peak as seen in Figure 2 (a-h) [60]. With the exception of figure 2h, the second peak for all the freeze-dried samples ranged from 195.81 – 199.91 °C. This is a lower melting point compared to the original PXM (Form I) used and suggests that the freeze-drying process led to the re-crystallization of some PXM molecules into the Form II polymorph [60]. There were some needle-shaped PXM particles that were characteristic of Form II PXM observed in some of the SEM images in Figure 3 [35,61]. The second melt in figure 2 h of 201.43 °C suggests the form I of PXM. The third peak for all the freeze-dried samples ranged from 205.65 – 213.15 °C. This is the peak for the GLU and it seems that the freeze-drying process may have altered the characteristics of the GLU. It was also interesting to note from figures 2 e-h that an increase in the PXM content in the formulations increased the intensity of the monohydrate peak in the DSC thermograms. The freeze-dried PXM:GLU formulations developed an intense yellow colour. This has been observed by our research group before as well as other authors and has been attributed to the presence of amorphous PXM due to zwitterionic PXM molecules which are formed by an inter-molecular proton transfer in the amorphous form of the drug [35,62]. The XRPD diffractograms of pure PXM (Figure 4a) showed characteristic peaks at 8.64°, 14.51°, 17.70°, 21.71 and 27.72° typical of peak positions observed in the cubic Form I polymorph of PXM while that of GLU showed

characteristic peaks at 16.73°, 17.42°, 27.19° and 31.94° on the 2θ scale [17,35,61].The freeze-dried PXM:GLU samples (Figure 4b) showed characteristic peak at 26.11° representative of the monohydrate form of PXM and peaks from the carrier GLU and this was in agreement with published literature [60,63–65].

Figure 1. DSC thermograms of piroxicam (a) glucosamine (b)

Figure 2. DSC thermograms of freeze-dried solid dispersions of PXM:GLU in ratios 1:1 (250) (a), 1:1 (500) (b), 1:1 (750) (c), 1:1 (1000) (d), 1:2 (500) (e), 2:1 (500) (f), 1:4 (500) (g), 4:1 (500) (h).

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317 Figure 3. SEM images of piroxicam (a), glucosamine (b), freeze-dried solid dispersions of
 318 PXM:GLU in ratios 1:1 (250) (c), 1:1 (500) (d), 1:1 (750) (e), 1:1 (1000) (f), 1:2 (500) (g),
 319 2:1(500) (h), 1:4 (500) (i), 4:1 (500) (j)

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Figure 4. XRPD patterns of Glucosamine (GLU), Piroxicam (PXM), freeze-dried solid dispersions of PXM:GLU in ratios 1:1 (250), 1:1 (500), 1:1 (750), 1:1 (1000) (a) and Glucosamine (GLU), Piroxicam (PXM), freeze-dried solid dispersions of PXM:GLU in ratios 1:1 (500), 1:2(500), 2:1(500), 1:4 (500) and 4:1 (500) (b)

Commercial PXM and commercial GLU showed opposite charging polarities. While commercial PXM showed an overall electronegative charge (-3.2 ± 0.9 nC/g), commercial GLU showed an overall electropositive charge (0.5 ± 0.4 nC/g) which was in agreement to previously published literature [35]. It was reported in previous studies that fine particulates generally charge negatively, whilst large particles tend to charge positively [66]. Such charging as experienced by the PXM may result in problems such as particle agglomeration, segregation and/or adhesion to the processing equipment [35]. The considerably higher absolute charge of PXM relative to GLU may be due to the differences in the inherent charging properties between PXM and GLU as well as the small size of PXM particles in comparison to GLU particles [34,66]. Interestingly, freeze-dried PXM:GLU mixtures showed mainly an electropositive charging behaviour when the content of drug was ≤ 50 % (w/w), whereas freeze-dried mixtures containing >50 % (w/w) of drug showed mainly and an opposite electronegative charging behaviour. This could be explained as the original charging polarities of commercial drug and excipient particles were inherited in co-processed drug:excipient mixtures. This suggested that PXM and GLU could potentially form an ordered mixture, in which the adherence of drug particles to the surface of excipient particles is encouraged by electrostatic forces. Comparing the charge values to common drugs and excipients reported previously showed PXM to have lower charge than common drugs whereas GLU carried a very low level of charge [67]. The highest level of absolute charge was observed from the freeze-dried PXM:GLU mixture containing the highest content of drug (PXM:GLU, 1:4 w:w). The results thus show that the selection of PXM:GLU ratio can influence the charging behaviour of the powder mixture and thereby could improve powder handling and dissolution properties. The authors believe that there is no apparent inverse relationship between powder charging and dissolution. High levels of powder charging can affect both drug-drug and drug-excipient adhesion forces in pharmaceutical formulations. While high levels of charging can cause unwanted particle agglomeration/segregation and thus reduce the effective surface area available for dissolution, it has been shown that the total adhesion forces between drug and excipient particles decrease with electrostatic charge decay during storage [68].

Figure 5. Net-charge-to-mass ratio (Net-CMR), positive-charge-to-mass ratio (P-CMR) and negative-charge-to-mass ratio (N-CMR) of freeze-dried piroxicam:glucosamine HCl (PXM:GHCl) mixtures in relation to PXM content. Note: GHCl is GLU

3.2. HPLC assay

PXM was detected at 350 nm with a retention time of 3.5 min. The linearity (R^2) of the calibration curve was 0.99 while the limit of detection (LOD) and limit of quantification (LOQ) were 0.024 ± 0.001 $\mu\text{g/ml}$ and 0.072 ± 0.003 $\mu\text{g/ml}$, respectively. The intraday and interday relative SD (RSD) were all less than $<5\%$.

3.3. Dissolution and dissolution parameters

Untreated PXM had a very low DE of 14 % over the 120 min interval (Table 2). This confirms the reported poor solubility of PXM. This poor solubility can in turn affect its bioavailability. The poor dissolution characteristics of PXM (Figure 6a, Table 2) may be due to poor wettability as well as agglomeration of the drug particles [19]. When PXM was freeze-dried with GLU in the ratios 1:1 but at the various concentrations (1:1 (250) FD; 1:1 (500) FD; 1:1 (750) FD and 1:1 (1000) FD), the 1:1 (500) FD samples had the best DE of 44 % as compared

to the others (28 – 37 %). This was also evident with their higher Q_{10min} and Q_{30min} values. Their relatively smaller particle sizes (Table 2, $d_{90} = 64.4 \mu m$) may have contributed to this phenomenon also. Table 2 and Figure 6a again shows a decrease DE to occur past the 1:1 (500) FD formulation. As the dissolution profiles did not improve past the 1:1 (500) FD formulation, this then became the concentration used for the 1:2, 2:1, 1:4 and 4:1 ratios. Figure 6b shows the dissolution profiles of the 1:1 (500) FD formulation in comparison with the other ratios (1:2, 2:1, 1:4 and 4:1). The results show the 1:1 ratio to have the best dissolution profile of all the samples tested with a DE value 44 %., representing a 2.3 fold increase in DE relative to the untreated DE. It was interesting to note that an increase in the concentration of GLU brought about an increase in the DE values of the other ratios studied (Table 2, comparing ratios 1:2 to 2:1 and ratios 1:4 to 4:1). This is also evident with relatively smaller amount of fines in ratios 1:2 and 1:4 ($d_{10} = 6.55$ and $6.69 \mu m$ respectively) compared to ratios 2:1 and 4:1 ($d_{10} = 9.58$ and $11.3 \mu m$ respectively). This formation of small particle sizes leads to increased surface area and potentially reduced inter-particle cohesive forces could in turn account for their relative enhancement in dissolution.

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Table 2. Effects of the freeze-drying process and carrier ratios on the dissolution parameters and particle size of the formulations

Piroxicam									
(PXM): (GLU)	Carrier	DE _{120min} (%)	MDT (min)	MDR (% min ⁻¹)	Q _{10min} (%)	Q _{30min} (%)	d _{10%} (μm)	d _{50%} (μm)	d _{90%} (μm)
Untreated PXM		13.52	10.33	0.16	4.68	8.17	3.14	8.71	20.90
1:1 (250) FD		36.56	10.14	0.44	14.62	29.23	5.45	23.60	72.80
1:1 (500) FD		44.46	11.15	0.59	16.53	39.09	6.87	22.10	64.40
1:1 (750) FD		28.17	12.60	0.38	9.44	20.00	6.30	33.90	107.00
1:1 (1000) FD		32.10	18.24	0.39	8.50	21.35	6.96	29.35	77.65
1:2 FD		33.71	14.48	0.48	12.29	21.44	6.55	25.70	101.90
2:1 FD		17.49	10.52	0.20	5.49	13.53	9.58	30.13	89.93
1:4 FD		25.33	14.52	0.19	6.94	18.10	6.69	22.47	91.87
4:1 FD		18.61	13.59	0.43	10.26	12.18	11.30	29.13	70.53

Note: FD is freeze-dried

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401 Figure 6. Dissolution profiles of untreated PXM, freeze dried PXM and freeze-dried solid
 402 dispersions of PXM:GLU in ratios 1:1 (250), 1:1 (500), 1:1 (750), 1:1 (1000) (a), piroxicam,
 403 1:1 (500), 1:2, 2:1, 1:4 and 4:1

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405 4. Conclusions

406 This study showed that freeze-drying PXM with GLU enhanced the dissolution of PXM.
 407 This demonstrated the effectiveness of glucosamine as a hydrophilic carrier for this process.

The PXM underwent some polymorphic changes during the freeze-drying process and this was confirmed by the DSC traces and XRPD diffraction patterns. Freeze-dried PXM:GLU showed an electropositive charging behaviour when the content of drug was ≤ 50 % (w/w), whereas the freeze-dried samples containing >50 % (w/w) of drug showed an opposite electronegative charging behaviour. The charge results also proved GLU to improve the handling of PXM through potential ordered mixture formations and shows how powder handling as well as dissolution can be manipulated to obtain a desired outcome.

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