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Supercritical fluid assisted impregnation of indomethacin into chitosan thermosets for controlled release applications

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Abstract

Supercritical carbon dioxide (sc-CO_2) was used to impregnate indomethacin (a non-steroidal anti-inflammatory drug) into chitosan thermosets for the preparation of controlled release formulations. The products were analyzed by a range of methods including powder X-ray diffraction (XRD) and scanning electron microscopy (SEM). The effects of the experimental temperature and pressure of the sc-CO_2 on the thermal behavior of chitosan–indomethacin drug composites (DCs) was investigated via differential scanning calorimeter (DSC). The interaction of chitosan and indomethacin after impregnation was then studied by Fourier transform infrared (FTIR) and Raman spectroscopy, respectively. Our results suggest that the supercritical fluid impregnation process results in indomethacin being amorphously dispersed within the chitosan matrix. FTIR data suggest that the aliphatic carbonyl group of indomethacin interacts with the NH_2 group of the chitosan backbone. In vitro dissolution studies (via UV–vis spectroscopy) reveal that the dissolution rate of indomethacin substantially increases after processing in sc-CO_2, particularly, under the experimental conditions 20.7 MPa and 70 °C.

Keywords: Chitosan; Indomethacin; sc-CO_2; Supercritical fluid; Amorphous; Impregnation; Drug

1. Introduction

The manufacture of active pharmaceutical products often involves processes such as spray-drying, crystallization, milling, and grinding. Some of these techniques generally do not produce completely solvent-free products (Rodrigues et al., 2004); therefore, cleaner technologies, such as those involving supercritical fluids (SCFs) have attracted attention over recent years. Supercritical CO_2 (sc-CO_2), which is by far the most widely used SCF, is relatively inexpensive, non-toxic, and non-flammable (Kazarian, 2000). One of the important properties of sc-CO_2 is its ability to plasticize certain polymers resulting in a decrease of the polymer glass transition temperature (Kikic and Vecchione, 2003).

Several researchers have successfully synthesized intimately mixed drug (or therapeutic agent)–polymer composites or significantly reduced the particle size of the drugs using SCF based technologies. For example, Chattopadhyay and Gupta (2001) produced antibiotic nanoparticles using supercritical CO_2 antisolvent methods. Elvassore et al. (2001), reported that protein-loaded poly(lactic acid) could be prepared by adding a sc-CO_2 antisolvent to an organic solvent solution of protein and polymer. Supercritical assisted atomization methods have been used by Reverchon and Porta (2003) to produce tetracycline and rifampicin microparticles with particle characteristics suitable for aerosolizable drug delivery applications. More recently, molecular dispersions of ibuprofen and poly(vinylpyrrolidone) were prepared via sc-CO_2-assisted impregnation of the drug into the polymer (Kazarian and Martirosyan, 2002). Once the drug was solubilized, the hydroxyl group of the drug became hydrogen-bonded to the carbonyl group (C=O) of the polymer. Hence, the drug was stabilized as a molecular dispersion.

Chitosan is a derivative of chitin, a natural polysaccharide found in the shells of crustaceans such as crabs and shrimps (Illum, 1998). Chitosan exhibits a variety of physicochemical and biological properties; therefore, it has found numerous applications such as in waste and water treatment, in agriculture, as a fabric, in cosmetics, as a nutritional enhancement, and in

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food processing (Senel et al., 2000). In addition to its lack of toxicity and allergenicity, its good biocompatibility, biodegradability, and bioactivity, make it very attractive substance for use in biomaterials and pharmaceutical science (Singla and Chawla, 2001; Senel and McClure, 2004). Chitosan can be formulated in a variety of forms including powders, gels and films (Kas, 2000; Schneider et al., 2004). Kim et al. (2001) explored the application of the chitosan as a DNA delivery carrier after deoxycholic acid-modification. The potential of chitosan nanoparticles for ocular drug delivery was assessed by De Campos et al. (2004) who investigated the interaction of chitosan with the ocular mucosa in vivo and in vitro. Dufes et al. (2004) reported that the anticancer drug, doxorubicin, could be loaded into chemically modified (glycolated) chitosan vesicles.

Indomethacin, is a non-steroidal anti-inflammatory drug (NSAID) that is effective in the management of rheumatoid arthritis, ankylosing spondylitis, osteoarthritis, and acute gout (Rusu et al., 1998). Indomethacin is generally a highly crystalline and poorly water-soluble drug (Longuemard et al., 1998; Sakari et al., 2002); therefore, there is much interest in being able to improve its solubility by either increasing surface area or stabilizing the amorphous form of the drug within a polymeric matrix.

2. Materials and methods

2.1. Materials and equipment

Chitosan (75–85% deacetylated, medium $M_w \approx 50{,}000$) and indomethacin (99%) were purchased from Sigma–Aldrich Company Ltd. (Dorset, UK) and used as-received. A liquid withdrawal CO_2 cylinder at 5.0 MPa pressure was supplied by BOC gases. The CO_2 was chilled to −6 °C before being delivered via an Isco model 260D syringe pump with a chilled piston barrel (from a copper cooling coil). A custom made 220 ml stirred high pressure autoclave (approximate $d = 340$ mm, length = 45 mm) with a viewing window and paddle type stirrer was built by Vince Ford of the materials department at QMUL.

2.2. Preparation of homogenous drug formulations in sc-CO_2

The 4.00 g chitosan and 1.00 g indomethacin were accurately weighed and gently mixed by hand with mortar and pestle for 10 min, before being transferred into the 220 ml high pressure windowed autoclave. The autoclave was sealed and liquid CO_2 was withdrawn from the CO_2 cylinder at 5 MPa added to the autoclave and then pressurized slightly using the Isco pump. The autoclave was then held at 20.7 MPa (3000 psi)/70 °C under stirring (310 rpm) for 2 h. At the end of the experiment, the controller was switched off and CO_2 was released over a period of 15 min. The color of final product was bright yellow compared to the original sample (very light yellow), especially in the mixture prepared at 20.7 MPa/70 °C, indicating that the drug was in an amorphous state (Okano et al., 1998).

2.3. Characterization

X-ray powder diffraction (XRD) data were collected for the powdered sample using a Siemens D5000 diffractometer, using Cu Kα radiation ($k\alpha_1 = 1.5406$ Å). Data were collected over the 2θ range 7–30° with a step size of 0.02° and step time of 1.0 s.

Differential scanning calorimetry (Perkin-Elmer DSC 7) was employed to study the thermal behavior of the chitosan–indomethacin mixtures before, and after supercritical processing. The DSC was calibrated using pure samples of indium and zinc, respectively. From our experiments, samples containing indomethacin equivalent to 6 mg were carefully weighed in aluminium pans, and covered with an aluminium lid incorporating a pinhole. DSC curves of each sample were obtained from the first heating run at a rate of 10 °C/min under dry nitrogen atmosphere from 30 to 200 °C. Each sample was run in triplicate.

FTIR spectra were carried out using a Digilab Excalibur 4000 FTIR spectrometer with microscope accessory (UMA 600) and focal plane array (FPA) detector. Spectra were obtained using 4 cm^{-1} resolution, averaging for 32 scans.

Raman spectra were run using a dispersive Raman spectrometer (Nicolet Almega XR with 785 nm laser). Spectra were obtained for 10 scans at 10 s exposure time.

Scanning electron microscopy (SEM) was carried out on the products using a JEOL 6300^{TM} (accelerating voltage 10 kV). Prior to examination, samples were mounted onto 5 cm diameter circular aluminium stubs using double-sided adhesive tape and then coated with a thin layer of gold by using a sputter coater (Emitech K550) to render them electrically conductive.

The dissolution rates of crystalline indomethacin, chitosan–indomethacin (4:1, w/w) drug composites (DCs) prepared at 15.2 MPa/40 °C and 20.7 MPa/70 °C, respectively, were measured using UV–vis spectroscopy dissolution tests (Nicolet Evolution 500 UV–vis spectrophotometer). Each sample contained an amount equivalent to 50 mg indomethacin. The dissolution study was undertaken according to the USPXXI dissolution test method (Mi et al., 2001). The dissolution medium consisted of 1000 ml of phosphate buffer (pH 7.2), maintained at temperature of 37 ± 0.5 °C. A paddle rotation speed of 20 rpm was employed. A 10.0 ml aliquot was collected at 10, 20, 30, 45, 60 min, 2, 4, 8 and 12, and 24 h, respectively, with an equal volume of fresh deionized water supplemented to the dissolution flask immediately after sampling. The peak intensities at 320 nm of were recorded using the UV–vis spectrophotometer and the concentration of drug in solution was calculated using the Bouguer–Lambert–Beer law (Perkampus, 1992).

3. Result and discussion

X-ray powder diffraction was used to analyze the chitosan–indomethacin physical mixtures (PMs) and the chitosan–indomethacin DCs (4:1, w/w) processed in sc-CO_2 at 15.2 MPa/40 °C and 20.7 MPa/70 °C, respectively (Fig. 1). XRD data clearly show a notable decrease in crystallinity of the drug in the mixture after processing in sc-CO_2 (15.2 MPa/40 °C, Fig. 1c)

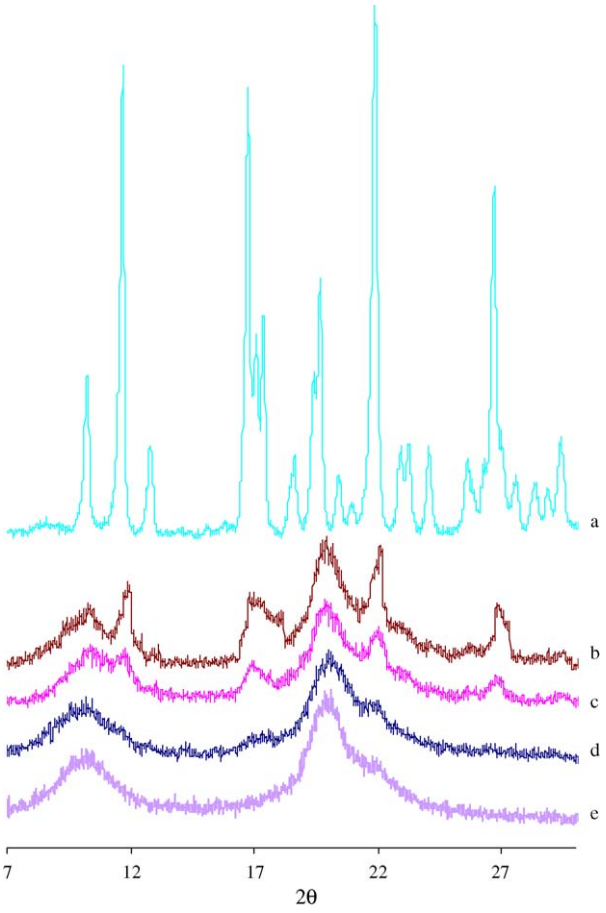

Fig. 1. X-ray diffraction pattern: (a) pure indomethacin, (b) chitosan–indomethacin (4:1, w/w) physical mixture, (c) chitosan–indomethacin (4:1, w/w) drug composites (DCs) in sc-CO_2 at 15.2 MPa/40 °C, (d) chitosan–indomethacin DCs in sc-CO_2 at 20.7 MPa/70 °C, and (e) virgin chitosan.

as compared to the physical mixture (Fig. 1b). However, broad peaks due to crystalline indomethacin can still be observed in the XRD data. After processing the physical mixture of chitosan and indomethacin at 20.7 MPa and 70 °C, the characteristic peak in the XRD data due to crystalline indomethacin crystals were very weak (Fig. 1d). The XRD pattern of this product is similar to that for the virgin chitosan, suggesting that the drug is most likely in an amorphous state in the chitosan matrix.

Differential scanning calorimetry (DSC) was conducted on chitosan–indomethacin PM and DCs to investigate the thermal behavior of the materials. As shown in Fig. 2a, the melting peak of crystalline indomethacin in the physical mixture was located at 159 °C, which is consistent with that for γ-indomethacin (Singla and Wadhwa, 1995). However, after impregnation of indomethacin into chitosan at 15.2 MPa/40 °C in sc-CO_2 (Fig. 2b), the intensity of the melting peak decreased dramatically, suggesting the amount of crystalline indomethacin had been significantly reduced. When the operative pressure and temperature were 20.7 MPa/70 °C, respectively, the DSC data for the products showed only a small peak at 156 °C (Fig. 2c), which corresponds to the melting point of α-indomethacin (Matsumoto and Zografi, 1999). This suggests that γ-indomethacin has essentially disappeared, and some of

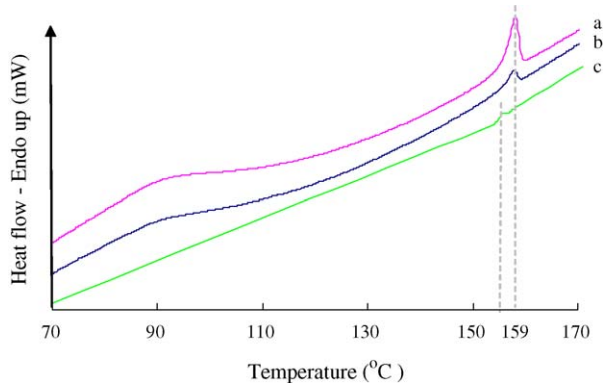

Fig. 2. DSC data for: (a) chitosan–indomethacin (4:1, w/w) physical mixture, (b) chitosan–indomethacin (4:1, w/w) drug composites (DCs) in sc-CO_2 at 15.2 MPa/40 °C, and (c) chitosan–indomethacin (4:1, w/w) DCs in sc-CO_2 at 20.7 MPa/70 °C.

it has been converted to α-indomethacin (a metastable state). It was also observed that the peak due to water loss (range ca. 80–130 °C) was not observed in the DSC data for the products prepared at 20.7 MPa/70 °C.

FTIR spectroscopy was used to study the interaction between the chitosan and indomethacin in the mixtures prepared in sc-CO_2. Bands due to υ(C=O) bond stretches were studied to infer the physical state of the drug. FTIR data in υ(C=O) region (1800–1500 cm^{-1}) were compared for pure indomethacin and indomethacin impregnated into chitosan (Fig. 3). As expected, two main characteristic C=O bands (Fig. 3a) were observed for crystalline indomethacin at 1729 and 1691 cm^{-1}, assigned to the aliphatic and aromatic carbonyl stretching bands, respectively (Rusu et al., 1998). Virgin chitosan also shows a distinct amide I and amide II band at 1662 and 1554 cm^{-1} (Fig. 3b), respectively (Kolhe and Kannan, 2002). After processing the DCs in sc-CO_2 at 15.2 MPa/40 °C, the FTIR band of indomethacin at 1729 cm^{-1} appeared to shift to a lower wavenumber of 1725 cm^{-1} (Fig. 3c). This may be explained by the interaction of this carbonyl with the amino group of chitosan (as shown in Fig. 4). The FTIR

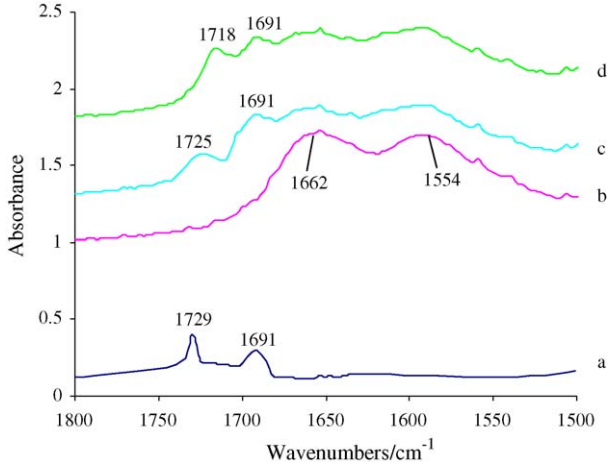

Fig. 3. FTIR spectra in the υ(C=O) spectral region (1800–1500 cm^{-1}): (a) pure indomethacin, (b) virgin chitosan, (c) chitosan–indomethacin (4:1, w/w) drug composites (DCs) processed in sc-CO_2 at 15.2 MPa/40 °C, and (d) chitosan–indomethacin (4:1, w/w) DCs processed in sc-CO_2 at 20.7 MPa/70 °C.

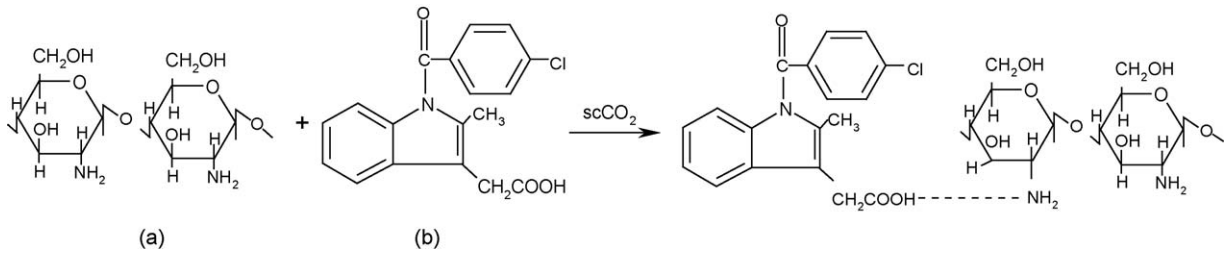

Fig. 4. Figure showing the interaction of chitosan (a) with indomethacin (b).

spectrum for the DC processed at 20.7 MPa/70 °C showed the peak at 1718 cm^{-1} (Fig. 3d), that is more shifted compared to the previous DC case.

Raman spectra of chitosan–indomethacin drug composites were measured and compared with that obtained for the chitosan–indomethacin physical mixture (Fig. 5). The Raman data for the physical mixture (Fig. 5a) revealed a carbonyl stretching vibration for the crystalline indomethacin at 1700 cm^{-1} (Schmidt et al., 2003), whilst the Raman spectrum for the DC prepared at 15.2 MPa/40 °C revealed two peaks at 1700 and 1680 cm^{-1}, respectively. The higher wavenumber peak was reduced in intensity compared to the peak observed for the PM. In contrast, the Raman spectrum for the DC prepared at 20.7 MPa/70 °C showed only a very broad peak at 1680 cm^{-1}. This can be compared to the C=O Raman peak for amorphous indomethacin at 1680 cm^{-1} as reported by Schmidt et al. (2003).

Scanning electron microscopy was conducted on PMs and processed indomethacin (with/without chitosan matrix) in sc-CO_2 to investigate the morphology changes of the drug and matrix after supercritical processing. The drug crystals can

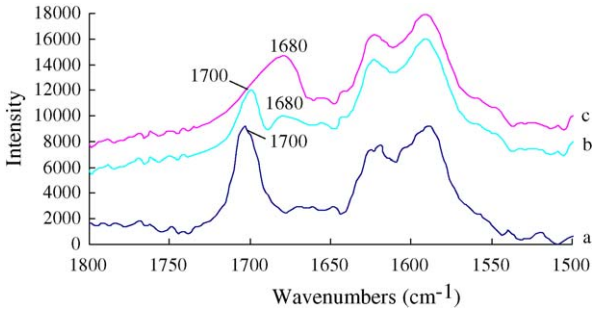

Fig. 5. Raman spectra: (a) chitosan–indomethacin physical mixture, (b) chitosan–indomethacin (4:1, w/w) drug composites (DCs) processed in sc-CO_2 at 15.2 MPa/40 °C, and (c) chitosan–indomethacin (4:1, w/w) DCs processed in sc-CO_2 at 20.7 MPa/70 °C.

clearly be seen in the chitosan–indomethacin physical mixture (Fig. 6a) compared to virgin chitosan (Fig. 6b). As a control experiment, some indomethacin was processed in sc-CO_2 (without a chitosan matrix, Fig. 6c). This study revealed a slight change in powder appearance compared to the PM (Fig. 6a). It

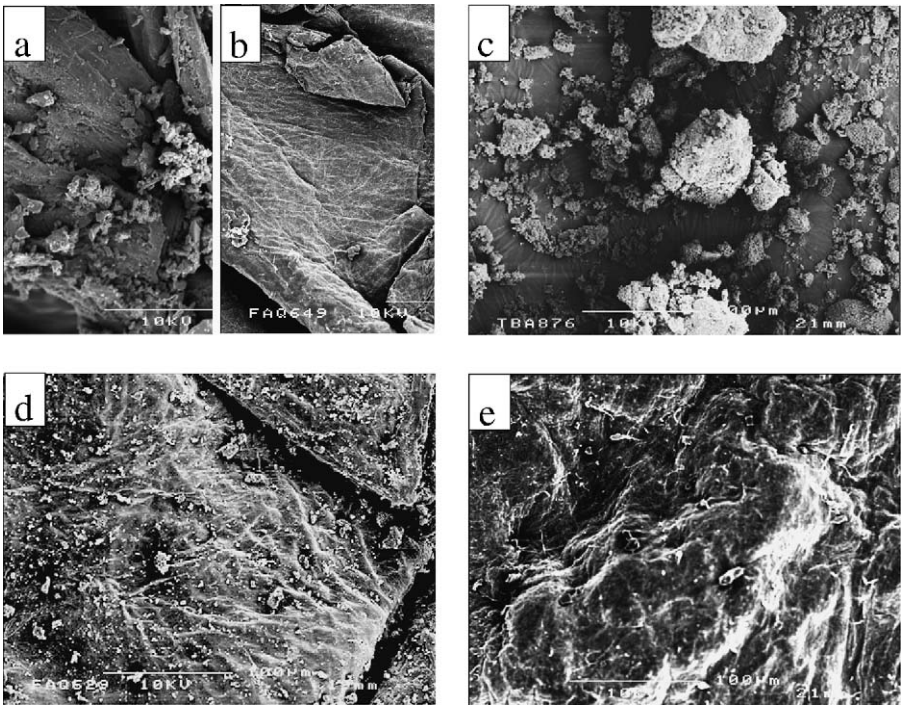

Fig. 6. SEM images: (a) chitosan–indomethacin (4:1, w/w) physical mixture, (b) virgin chitosan, (c) indomethacin dissolved and then precipitated in CO_2 at 15.2 MPa/40 °C, (d) chitosan–indomethacin (4:1, w/w) drug composites (DCs) in sc-CO_2 at 15.2 MPa/40 °C, and (e) chitosan–indomethacin (4:1, w/w) DCs in sc-CO_2 at 20.7 MPa/70 °C.

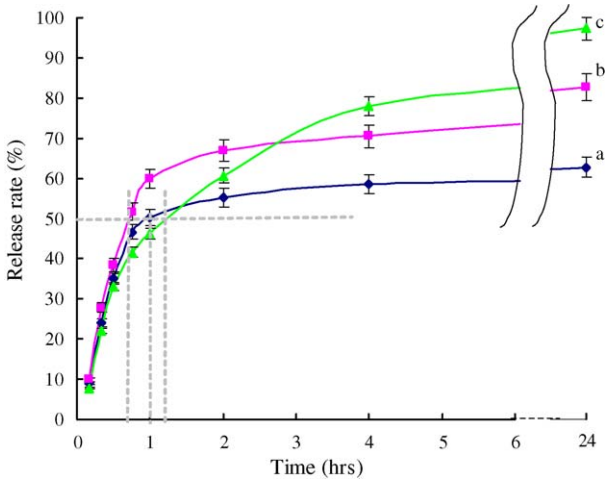

Fig. 7. Dissolution profiles during 24 h: (a) physical mixture of chitosan and indomethacin, (b) chitosan–indomethacin (4:1, w/w) drug composites (DCs) processed in sc-CO_2 at 15.2 MPa/40 °C, and (c) chitosan–indomethacin (4:1, w/w) DCs processed in sc-CO_2 at 20.7 MPa/70 °C.

did not suggest that all the indomethacin had dissolved though, as some large crystals were still observed, that were reminiscent of the original crystals. The number of crystals for the DC processed in sc-CO_2 were substantially reduced after processing at 15.2 MPa/40 °C (Fig. 6d). Under the conditions of 20.7 MPa/70 °C, indomethacin drug particles were almost totally absent from the surface of the chitosan (Fig. 6e). The physical appearance of DCs under this condition is similar with that for the virgin chitosan (Fig. 6b). Overall, this suggests that the most of the drug was dispersed into the chitosan matrix in an amorphous form.

Drug dissolution studies were carried out on the PM and chitosan–indomethacin DCs processed in sc-CO_2, respectively (Fig. 7). After processing in sc-CO_2, the indomethacin dissolution rate and solubility (in the experimental timeframe) were increased dramatically compared with crystalline indomethacin alone. The drug composite prepared at 20.7 MPa/70 °C achieved $97 \pm 3\%$ dissolution at 24 h. In contrast, the DCs processed at 15.2 MPa/40 °C only achieved $83 \pm 3\%$ dissolution at 24 h. It was observed that chitosan–indomethacin DCs processed at 15.2 MPa/40 °C had the fastest initial dissolution rate ($T_{50} \approx 40$ min) (Fig. 7b) whilst that processed at 20.7 MPa/70 °C has the lowest initial dissolution rate ($T_{50} \approx 72$ min), even slower than the physical mixture initially ($T_{50} \approx 60$ min) (see Fig. 7a and c, respectively). This can be explained as follows: the drug is more intimately mixed into the chitosan matrix (for the higher temperature/pressure SCF processed mixture) and it is released only after ingress of water in the chitosan matrix followed by subsequent dissolution of the drug. The initial dissolution rate for crystalline indomethacin is therefore faster than this, as dissolution of the drug occurs at the surface of the crystals.

4. Conclusions

The generally poorly water-soluble drug indomethacin, was impregnated into a natural thermoset polymer (chitosan) using supercritical carbon dioxide assisted infusion. XRD, DSC, and SEM observations suggest that, under the certain experimental conditions, the drug is dispersed into the chitosan polymer in an amorphous form. FTIR and Raman spectroscopy were used to study the nature of the drug–matrix interactions; and suggested an interaction between the amine group of chitosan and the aliphatic carbonyl group of indomethacin after SCF processing. This processing leads to a slower initial dissolution rate; however, the overall solubility of the drug is considerably enhanced over a 24 h period. In contrast, we have shown that when a highly water-soluble polymer rather than a non-soluble one is used with this processing methodology, the drug solubility and dissolution rate can both be increased (Gong et al., 2005). Our current research is focused on utilizing this methodology in the formation of homogenous polymer–drug composites in sc-CO_2 for a range of drugs with thermoplastic/thermoset polymers, towards: (i) improving the ultimate solubility of drugs, (ii) improving the stability of drug forms and (iii) controlling drug dissolution kinetics.

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