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The development of a dense gas solvent exchange process for the impregnation of pharmaceuticals into porous chitosan

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ABSTRACT

The aim of this study was to prepare stable formulations of poorly water-soluble drugs in amorphous forms to enhance their dissolution rates, promote the bioavailability, minimize the dosage, thereby theoretically decreasing their side effects. A dense gas solvent exchange process was developed for the impregnation of poorly water-soluble drugs such as camptothecin and griseofulvin into a chitosan matrix. The amount of drug impregnated was measured by UV-spectrophotometery and gravimetric techniques. Pore characteristics and the crystallinity of the drugs in the impregnated chitosan were measured. Homogenous nano-sized pores with thin walls were formed in chitosan using the dense gas solvent exchange process. The method was efficient for the impregnation of a drug into chitosan. Results of XRD, Fourier transform infrared spectroscopy and differential scanning calorimetry demonstrated that as a result of interaction between chitosan and the drug, both camptothecin and griseofulvin were in amorphous forms after processing. The dissolution rate of processed griseofulvin was increased threefold due to the hydrophilic properties of chitosan and its interaction with the drug. A new approach was developed for promoting drug bioavailability that has the potential to decrease the required dose and side effects, particularly for chemotherapeutic drugs with narrow therapeutic index.

1. Introduction

Class II drugs have low bioavailability due to their poor solubility in aqueous media. Common strategies to increase the solubility and hence bioavailability of such drugs include reduction of particle size (micronization) and decreasing the degree of crystallinity (Gao, 2008; Overhoff et al., 2006). A smaller drug particle size allows for greater contact surface area between the drug and the dissolution media within the body (Calvino-Casilda et al., 2008). Amorphous drugs are at a higher energy state, less stable, and exhibit higher dissolution rates than those in crystalline forms (Thassu et al., 2007).

A potential approach for decreasing drug crystallinity is to impregnate them into a polymer matrix. The impregnation results in dissolution of the drug in the polymer at a molecular level and can stabilize it in an amorphous state. The entrapment of poorly water-soluble drugs in polymer matrices may improve the bioavailability of drugs (Kluge et al., 2009). The polymer selected for drug–polymer composites should be harmless, the degradation products should not cause any cytotoxic effects in the body, and drug–polymer interactions should not affect drug activity (Alexis et al., 2004). In general, polymers that can increase solubility or wettability of drugs are desirable.

Chitosan is a promising material for the development of drug delivery systems. Chitosan is a deacetylated derivative of chitin, commonly found in the exoskeleton of marine crustaceans and the cell walls of some fungi (Madihally and Matthew, 1999). Chitosan has many biomedical applications and has been widely used for drug encapsulation because of its properties such as pH sensitivity, biocompatibility with living tissue, mucoadhesive properties and low toxicity (VandeVord et al., 2002; Wang et al., 2007). Chitosan increased the dissolution rate of poorly water-soluble drugs such as prednisolone (Sawayanagi et al., 1983), phenytoin (I) (Nambu et al., 1983), naproxen (Maestrelli et al., 2004; Mura et al., 2003), valdecoxib (Gurusamy et al., 2006) and spironolactone (Acarturk et al., 1993).

The preparation of drug–polymer composites requires the use of a mobile phase such as an organic solvent to dissolve and carry the poorly water-soluble drug component into the polymeric matrix (Lopez-Periago et al., 2009). However, the traditional methods require another stage for the purification and removal of the organic solvent residues (Natu et al., 2008).

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1.1. Dense gas techniques for the impregnation

The fluid above critical temperature and pressure is known as supercritical fluid. Dense gases are fluids near or above their critical point, generally with a reduced temperature (T/T_c), and pressure (P/P_c) of between 0.9 and 1.2. These near-critical fluids have excellent processing properties including low viscosity, high diffusivity, low surface energy, and moderate density. The ability of dense gases such as CO_2 to diffuse into polymer matrices allows the development of dense gas techniques for the impregnation of active compounds (Busby et al., 2002; Jung et al., 2002). Dense gases, such as CO_2, can be removed from the system during the depressurization, hence eliminating the product purification stage of conventional methods for the removal of organic solvent.

The dense gas process was used for the impregnation of a compound with a considerable solubility in a dense gas, into an amorphous or semi-crystalline polymer matrix that CO_2 can diffuse easily (Duarte et al., 2006; Gong et al., 2006; Kazarian and Chan, 2003). Active compounds with low solubility in dense gas CO_2 such as proteins can also be impregnated into a polymer. In this case the compound can be dissolved in a solvent, the polymeric matrix is placed into the solution, and the dense gas is added to facilitate impregnation due to polymer swelling and increasing the free volume between polymer chains (LeClair Ellis et al., 2008). β-Galactosidase was impregnated on the surface of polystyrene using this method (LeClair Ellis et al., 2008).

1.2. Porosity and drug release

Dense gas CO_2 has negligible solubility in hydrophilic polymers; however, methods have been developed to generate porosity in these polymers. Shih et al. (2003) used a cosolvent such as ethanol, or diluted acid, to improve the diffusion of the dense gas into polymers and produce porous hydrogels. Cooper et al. developed a CO_2–water emulsion template technique to produce highly porous hydrogels in various hydrophilic polymers such as poly(vinyl acetate) (PVA), blended PVA/PEG and chitosan (Lee et al., 2007). Annabi et al. (2009) developed a dense gas technique for the preparation of porous elastin that involves the synthesis of cross-linked elastin in an aqueous solution and subsequent fabrication of porosity into the structure.

The primary objective of this study was to develop a dense gas solvent exchange technique to create porosity in a hydrophilic polymer such as chitosan and at the same time impregnate a poorly water-soluble drug into the hydrogel matrix. Carbon dioxide was used as a dense gas due to its moderate critical properties (P_c = 73 bar and T_c = 31 °C), low toxicity and non-flammability. The effects of operating conditions such as temperature and pressure on the drug loading efficiency and the physical properties of the drug were investigated. An advantage of dense gas solvent exchange is the ability to generate porous structure and at the same time impregnate a compound into polymer matrices. The presence of porosity increases the surface area between polymer–drug and the dissolution media, and improves both the dissolution rate of a drug and its bioavailability.

2. Materials and methods

2.1. Materials

Chitosan (medium molecular weight), griseofulvin (purity from ≥97%) and toluene (99.5+ %A.C.S. Reagent) were purchased from Sigma. Camptothecin was donated by Nanomaterials Pte. Ltd. (Singapore). Glacial acetic acid (Ajax FineChem) was used to dissolve chitosan in MilliQ water. Phosphate buffered saline (PBS) was prepared by dissolving one PBS tablet (Sigma) in 200 mL MilliQ water to have a pH between 7.2 and 7.4. Simulated gastric fluid (SGF) was made by the addition of 2 g NaCl (Silform Chemicals) and 7 mL HCl (Ajax FineChem) into 1 L MilliQ water to achieve pH 1.2. Fed state simulated intestinal fluid (FESSIF) was prepared by the addition of 4.04 g NaOH (Ajax FineChem), 8.65 g glacial acetic acid and 11.87 g NaCl into 1 L MilliQ water with a pH 5. Ethanol with 99.7% purity and mesitylene with 99% purity (Merck Pty., Ltd.), chloroform with 99.8% purity (Ajax FineChem), acetone with 99.5% purity (Analar) and food grade carbon dioxide with 99.99% purity (BOC) were used as received from suppliers.

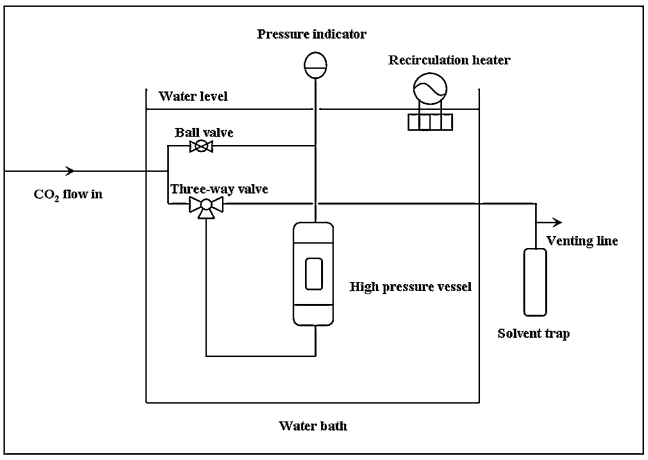

Fig. 1. Experimental set-up for investigating threshold pressure and drug impregnation using dense gas.

2.2. The fabrication of chitosan matrix by solvent exchange

Chitosan film was prepared by dissolving chitosan (1.7 g/100 mL) in a 2% acetic acid solution using a magnetic stirrer. Ethanol was added to the solution in a 1:2 volume ratio (chitosan solution:ethanol) to decrease the viscosity of the solution. The solution was filtered through a 5 μm nylon syringe filter to remove any non-dissolved residual chitosan. The filtered solution was cast on a Petri dish and the cast dried in a fume cupboard at room temperature to form a clear chitosan film with no bubbles. The cast film was cut into 1.7 cm diameter and soaked in 100% MilliQ water for 20 min. The process was repeated with a gradual decrease in MilliQ water (80%, 60%, 40%, 20%, 10% and 0%) with a corresponding increase in absolute ethanol until complete solvent exchange was achieved. Once the solvent exchange process was completed the cast film was left to soak in absolute ethanol for 24 h at ambient conditions to allow complete penetration of the solvent into the polymer matrix.

2.3. The determination of threshold pressure for a drug solution

Prior to the impregnation study the threshold pressure, *i.e.* the pressure at which the desired drug was precipitated from the drug solution, was determined using the apparatus as shown in Fig. 1. In this experiment, 10 mL of the drug solution (camptothecin in chloroform:ethanol (4:1, v/v) or griseofulvin in acetone) with a known concentration was injected from the top of a high pressure view cell (Jerguson sight gauge series 13 no. 32). At this stage, the fitting on the top of the vessel was opened and a syringe was used to inject the solution. A frit with 50 μm porosity was used at the bottom of the high pressure vessel to prevent liquid solution purging form the vessel, when the system was at ambient pressure; this

frit was also used during the pressurization of the vessel from the bottom to disperse CO_2 bubbles homogeneously into the solution and to collect any precipitate from solution during the last stage of purging the solution from the system and depressurization. After injecting the solution, the vessel was sealed and the system was maintained at constant temperature by submerging the vessel in a controlled temperature water bath using a recirculation heater (Ratek TH5-2KW). After the thermal equilibrium was attained at a desired temperature, the system was slowly pressurized from the bottom of the vessel in 5 bar increments using a syringe pump (ISCO, 260D). At each pressure the system was isolated for at least 10 min to achieve equilibrium and monitor the pressure at which precipitation commenced accurately. The point at which solid particles were precipitated was observed visually and reported as the threshold pressure. The inlet valve was closed and the system was then connected to the exit valve for purging the solution from the bottom of the vessel.

2.4. Impregnation of a drug in the hydrogel matrix by a dense gas process

The soaked chitosan sample was placed on a metal gauze shelf which was then located inside the same high pressure vessel. A three-way valve and ball valve were used to control the CO_2 flow direction and pressurization modes (*i.e.* pressurization from the top or the bottom of the vessel). The solution of the drug was injected into the vessel which was then sealed and pressurized after thermal equilibrium was attained at a desired temperature. At this stage CO_2 was fed into the system from the bottom of the vessel using a syringe pump (ISCO, 260D). The pressure was increased at a rate of 3 bar/min until the predetermined pressure was attained. The dense gas penetrated through the polymer, aiding both swelling of the polymer and the formation of pores while simultaneously resulting in the expansion of the organic solution. The system was then isolated, thereby allowing the CO_2 to penetrate into the chitosan sample for 1 h. After this stage, CO_2 was delivered at a flow rate of 10 mL/min from the top of the vessel for half an hour to remove residual solvent from the sample; the system was then rapidly depressurized. The amount of the impregnated drug was determined by a gravimetric method, using the residues collected in the solvent trap (as shown in Fig. 1). The impregnation at atmospheric conditions was carried out in a well-sealed glass bottle.

2.5. Characterization of drug-impregnated chitosan matrices

2.5.1. Porosity

The Brunauer–Emmet–Teller (BET) (Autosorb-1 Quanta Chrome) analysis was used to determine the surface area from the adsorption of nitrogen gas on a solid surface. A sample of chitosan processed by CO_2 was inserted into a tube for pre-treatment at 60 °C and the air was evacuated. Liquid nitrogen was then injected into the system and the BET measured the surface area of the pores.

2.5.2. Residual solvent

Gas chromatography (GC) was used to determine the residual amount of organic solvents such as ethanol, chloroform and acetone. In brief, the drug-impregnated chitosan matrix was immersed in toluene in an amber GC vial, and the vial was kept in a shaker (Roller trap) at a speed of 100 rpm for at least 3 days. The toluene was collected for GC analysis. A known amount of mesitylene was added as an internal standard into the vial prior to GC analysis using GC-17A (Shimadzu) with a flame ionization detector (FID), a SGE capillary column (BPX5, 30 m × 0.25 mm × 0.25 μm), auto sampler (AOC 20s) and an auto injector (AOC 20i). Filtered nitrogen was the carrier gas, and the analysis was preformed using an oven temperature of 40 °C with ramp of 20 °C/min until achieving 180 °C. Calibration data was collated for injecting known amount of each organic solvent (*i.e.* ethanol, chloroform and acetone) with the internal standard to the GC column. Consequently, the residual amount of organic solvents in drug-impregnated chitosan matrix was calculated.

2.5.3. Morphology

Scanning electron microscopy (SEM) (SEM XL30 Philips; S900 and S4500 Hitachi) was used to observe the morphology changes of both drugs and porous structure of chitosan. Dry samples were mounted on circular aluminium stubs, then gold coated prior to SEM analysis.

2.5.4. FTIR, DSC and XRD

The drug–polymer interactions were determined by Fourier transform infrared (FTIR) spectroscopy (Varian 660-IR and Nicolet Impact 410) using 4 cm^{-1} resolutions, averaging for 32 scans; the differential scanning calorimetry (DSC) (DSC TA instrument USA) was used to investigate the thermal behaviour of the product. Briefly, 3–8 mg of dry samples containing similar drug weight were carefully weighed in aluminium pans, and covered with aluminium lids. DSC profiles of each sample were acquired from heating runs at a rate of 10 °C/min under dry nitrogen atmosphere from 30 to 300 °C; the crystallinity of the samples was examined by X-ray diffraction (XRD) (Siemens D-5000 diffractometer) using CuKα radiation (λ = 1.54056 Å). Samples after at least 4 months storage at ambient temperature were placed in aluminium sample holders and were scanned from 10° to 50° at a scanning rate of 2 min^{-1}.

2.5.5. Solubility

The solubility of camptothecin in FESSIF buffer solution was determined by the method developed by Dong et al. In brief, excessive camptothecin was mixed with FESSIF buffer solution in a well-sealed glass bottle wrapped with aluminium foil. The suspension was placed in a shaker at 100 rpm at 25 °C for 1 week to approach equilibrium; 2 mL of this saturated solution was then filtered to remove any insoluble residue, and the solvent (FESSIF) was removed by using a rotary evaporator. Afterwards, 2 mL of chloroform–ethanol (4:1, v/v) solution was added to dissolve dry camptothecin powder. Subsequently, a certain amount of clear camptothecin solution in chloroform–ethanol was diluted carefully in a 10-mL volumetric flask. The amount of camptothecin was measured by UV-spectrophotometry (UV Cary 50) at $\lambda_{370\,nm}$ according to a predetermined standard curve of camptothecin to calculate the solubility (Dong et al., 2008). The studies were conducted in triplicate to obtain the solubility of camptothecin before and after dense gas impregnation.

2.5.6. Dissolution rate

The dissolution rate of the griseofulvin was measured under sink conditions using a varian dissolution equipment (Vankel 7000 Dissolution apparatus) with autosampler and Cary UV 50 spectrophotometer. All testings took place using the USP paddle method at 37.2 °C in 1-L vessels with paddle speed of 50 rpm. The dissolution media were USP 26 SGF. The dissolution vessel contained 900 mL of SGF and the absorbance was measured at a wavelength of 295 nm. Experimental data was the average of at least nine replicates.

The results of a preliminary experiment confirmed that the presence of chitosan in the dissolution media (SGF or FESSIF) had no effect on the UV absorbance of griseofulvin and camptothecin, respectively.

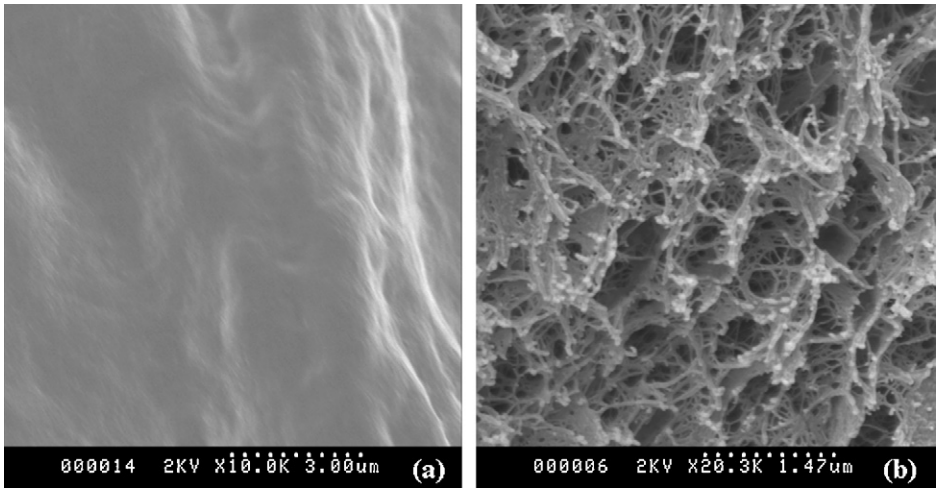

Fig. 2. SEM images of cross-section of drug-free chitosan film (a) exposed to CO_2 + 20 mol% ethanol at 200 bar and 60 °C, and (b) after dense gas solvent exchange process at 150 bar and 40 °C.

3. Results and discussion

3.1. Fabrication of porous chitosan matrices by a dense gas CO_2 technique

Increasing the degree of porosity enhances the surface area and minimizes the total amount of polymer required for a desired application such as drug delivery. A preliminary investigation was undertaken to assess the feasibility of fabricating porosity in chitosan films using neat CO_2 as a gas foaming agent. No porosity was observed in the cross-section of the chitosan films that were exposed to pure CO_2 and CO_2 modified with 20 mol% ethanol at 200 bar and 60 °C (Fig. 2a).

The limited solubility of CO_2 in the chitosan film was attributed to its polarity and the crystalline properties of this polymer (Joung et al., 2001; Quirk et al., 2005). An amorphous fraction in a polymer matrix plays a key role in gas foaming techniques (Quirk et al., 2005). The intrinsic viscosity of the polymer also has a significant impact on pore size in the gas foaming process (Quirk et al., 2005). Longer polymer chains are ultimately more entangled, and increase resistance to expansion during gas nucleation in the polymer matrix and subsequently result in a smaller pore size (Quirk et al., 2005).

A homogenous, highly interconnected, submicron diameter pore structure was formed using the dense gas solvent exchange process developed in this study. As illustrated in Fig. 3, transparent swollen chitosan in water turned translucent after solvent exchange with ethanol, and eventually became opaque after dense gas operation due to the generation of porosity. The pressure and temperature exhibited negligible effects on the pore characteristics of chitosan matrices at the conditions examined (*i.e.* 25–60 °C and 25–150 bar).

Phase separation is critical for the fabrication of porosity. The pores are fabricated when a homogenous multi-component system is separated into a multi-phase system to decrease the system free energy (Annabi et al., 2009; Ma, 2008). Two phases are generally separated out; a polymer-rich phase (e.g. a high polymer concentration phase) and a polymer-lean phase (e.g. a low polymer concentration phase) (Annabi et al., 2009; Ma, 2008). The porous structure is then formed when the polymer-lean phase is removed. The physical structure of the pores is governed by the kinetics of the phase separation in each system. It is possible to form powders, closed pore foams or open-pore foams with thick or thin wall structures depending on the rate of the phase separation (Ma, 2008). Generally, conventional methods such as freeze-drying and phase inversion generate non-homogenous pores with thick walls (Kang et al., 1999), thereby resulting in poor interconnectivity and low porosity (Annabi et al., 2009).

The mechanism of creating porosity using the method developed in this study is governed by facilitating the penetration of dense gas CO_2 into the chitosan matrices. Due to the limited solubility of water in dense gas CO_2 and vice versa, water was exchanged with ethanol. The solubility of CO_2 in the chitosan phase was, therefore, substantially enhanced. Ethanol and CO_2 are completely miscible at pressures above 90 bar and temperatures between 25 and 60 °C (Joung et al., 2001). It is, therefore, viable to remove

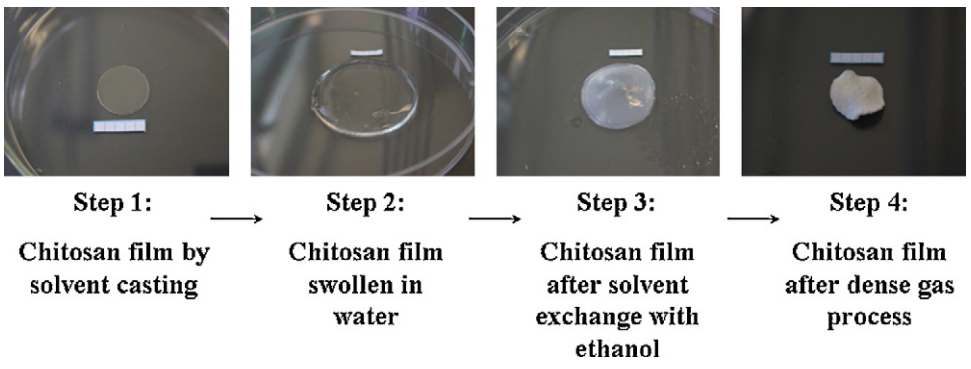

Fig. 3. Schematic diagram of various steps of dense gas solvent exchange process for the impregnation of active ingredient into hydrogel.

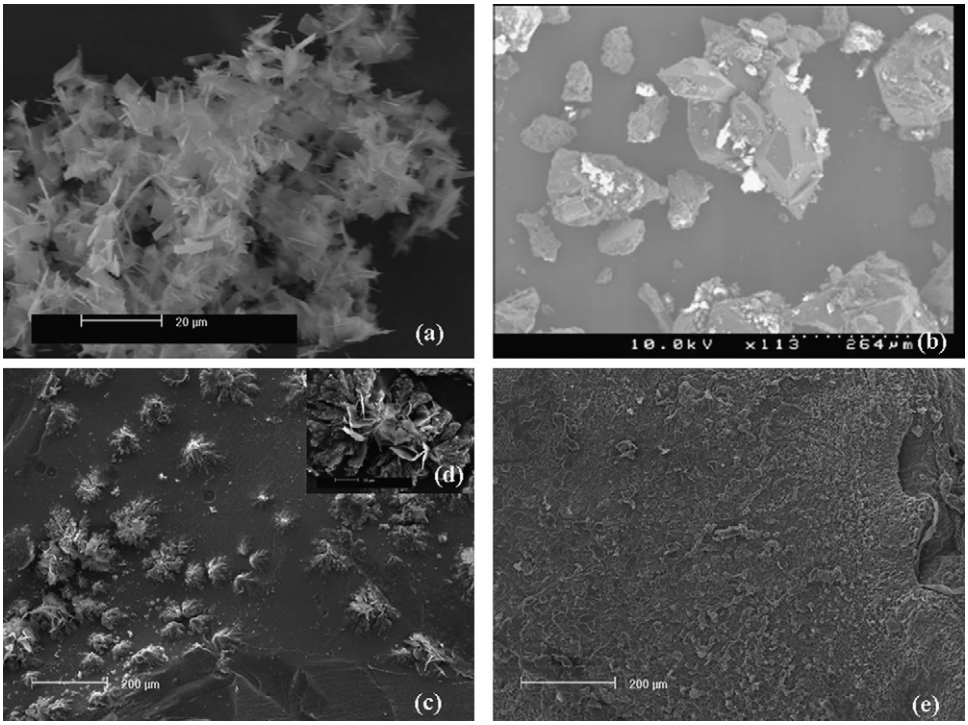

Fig. 4. SEM images of (a) camptothecin and (b) griseofulvin precipitated by the expansion of the solutions *via* dense gas CO_2; the surface of chitosan film impregnated with camptothecin at (c) and (d) atmospheric condition; (e) 25 bar and 40 °C.

ethanol at operating conditions from the matrices by washing with neat CO_2. Highly interconnected pores were created in the chitosan matrices upon depressurization, evaporation of CO_2 and the ultimate release of CO_2 and ethanol. The depressurization took place in a very short time (about 1 min) causing rapid evaporation of CO_2, ethanol and residual water (a polymer-lean phase) in the hydrogel matrices (a polymer-rich phase), thus creating a homogenous – highly interconnected – nanostructure pores with thin walls as shown in Fig. 2b. This image is a typical example of the pore characteristics obtained at various conditions and cross-sections of the samples. The pressure and temperature exhibited negligible effect on pore characteristics of chitosan. The surface area of pores in chitosan samples processed by dense gas solvent exchange was between 124 and 144 m^2/g, when using rapid depressurization rate (<5 min). The surface area of pores was dramatically decreased to 25 m^2/g, when using slow rate of depressurization (>30 min). Therefore in all conditions examined for drug impregnation rapid depressurization rate was used to generate high degree of porosity in the sample. The simultaneous creation of porous chitosan matrices and the extraction of solvent are also advantages compared with conventional methods where solvent evaporation can take up to a few days.

The surface of processed chitosan hydrogel had limited porosity. Skin formation (lack of pore on surface) is a common phenomenon in many pore formation processes (Cooper, 2001). The sudden reduction in pressure leads to the generation of nuclei in the matrix due to supersaturation, subsequent formation of a cellular structure upon nuclei growth and vitrification (McKelvey and Koros, 1996). However, the rapid diffusion of the gas from the surface may alleviate the nuclei formation substantially and lead to skin formation. The issue of skin formation can be addressed by addition of a salt or other porogen in the polymer matrix (Quirk et al., 2005). The skin layer may not greatly limit the diffusion of an aqueous solution into a hydrophilic polymer as water can be absorbed from the top surface and diffuse into the structure.

3.2. Determination of threshold pressure for the drug solution

The threshold pressure for camptothecin in chloroform–ethanol (4:1, v/v) solution (1 mg/mL) was 30 and 53 bar at 25 and 40 °C, respectively. Therefore, in this study, the operating pressure for the impregnation by dense gas CO_2 could not exceed these conditions. Higher concentrations of camptothecin solution (>1 mg/mL) were not used in this study, as it would cause precipitation at lower pressures; lower concentrations (<1 mg/mL) were also not used due to the lower impregnation efficiency. In the case of griseofulvin for 33 mg/mL concentration in acetone, the threshold pressure was 100 bar at 40 °C. The maximum operating condition of 90 bar and 40 °C was, therefore, used for the impregnation of griseofulvin.

Above threshold pressure, both camptothecin and griseofulvin were precipitated as crystalline powders, as shown in Fig. 4a and b, respectively. Our data demonstrate that the amounts of griseofulvin precipitated from 33 mg/mL solution by dense gas solvent expansion were below 16% at 50 bar and 40 °C; likewise, when using 1 mg/mL camptothecin solution at 100 bar and 40 °C.

3.3. The impregnation of drugs into chitosan

The impregnation of a drug into a polymeric structure can be enhanced using a dense gas. Kazarian et al. (1998) used *in situ* FTIR and UV–vis spectroscopy to measure the partitioning of solutes between poly(methyl methacrylate) (PMMA) and supercritical CO_2 and supercritical CO_2–cosolvent systems, respectively. A partitioning coefficient of 10^4 was acquired for Dispersant Red 1 (DR1) impregnated in PMMA using supercritical CO_2. The dye (DR1) has a solubility of about 10^{-6} M in supercritical CO_2 at 40 °C and 90 bar; despite the low solubility of the dye, dense gas such as supercritical CO_2 can still be used as an effective transport media because of the high partition coefficient. This result demonstrates that a drug with a low solubility can also be impregnated into a polymeric matrix due to the significant enhancement of partition coefficient. It

Table 1
Residual amount of organic solvents in drug-impregnated chitosan matrix.

Samples	Organic solvents residual level (ppm, mg organic solvent/kg chitosan)		
	Acetone	Chloroform	Ethanol
Camptothecin-impregnated chitosan matrix-washed with CO_2 for 30 min	–	48	106
Griseofulvin-impregnated chitosan matrix washed with CO_2 for 30 min	<10	–	<10
USP acceptable level (USP-NF)	5000	60	5000

was demonstrated that for solvents such as methanol, isopropanol and acetone the partition coefficient was decreased by an order of magnitude when CO_2 pressure was increased from 50 to 175 bar (Kazarian et al., 1998).

The drugs selected in this study, camptothecin and griseofulvin, both had very low solubilities in dense gas CO_2. In the dense gas solvent exchange process, these drugs were dissolved in solvents such as chloroform, ethanol, and acetone. Swollen chitosan in water was solvent exchanged with either of these solvents, and then submerged in these solutions. Solutions were expanded by CO_2 to below the threshold pressure to assist impregnation and pore formation.

In our solvent exchange process, due to the presence of organic phase in chitosan matrices the partition coefficients were significantly enhanced. We believe that the presence of a large partition coefficient between the polymer and drug resulted in the drug remaining in the 'stationary' polymer phase rather than being removed from the system with the 'mobile' expanded solvent phase.

As shown in Table 1, the residual of organic solvents maintained in the processed samples was approached a low level after washing the samples with CO_2 for 30 min. The residues of chloroform and ethanol in camptothecin-impregnated chitosan matrices were 48 and 106 ppm (mg organic solvent/kg chitosan), respectively. In griseofulvin system, the residue of acetone and ethanol were less than 10 ppm. The residues were below the USP acceptable level (USP-NF), corroborating the data acquired from DSC and FTIR analysis that no peak for solvent was detected. The organic solvent residues could decrease more by using larger amount of CO_2 for washing stage. The impregnated drug in chitosan produced by solvent exchange is potentially harmless for pharmaceutical formulations.

3.3.1. Impregnation of camptothecin

The feasibility of impregnating camptothecin into chitosan was evaluated. Camptothecin, which is isolated from extracts of *Camptotheca acuminate* (Hertzberg et al., 1989), has very low solubility in water and a narrow therapeutic index (Yurkovetskiy et al., 2004). Camptothecin shows a relatively high water solubility and lower bioactivity in its carboxylate form than in the lactone form (Saetern et al., 2005). It is, therefore, critical to avoid processing conditions that affect the lactone form of camptothecin with a therapeutic activity.

Camptothecin was dissolved in a mixture of 4:1 volume ratio of chloroform and ethanol. Chitosan film was swollen in water and solvent exchanged, then submerged in the camptothecin solution and the drug was impregnated by the dense gas process developed in this study. After depressurization and drying of the sample, the collected chitosan matrix was opaque with porosity, and had a brittle structure in the dried form. When chitosan was immersed into acidic buffer solutions such as FESSIF and SGF, it began to swell due to the presence of NH_3^+ (Ladet et al., 2008), but dissolution of chitosan also occurred under acidic condition, and the whole matrix dissolved within 4 h. The results suggest that this matrix is suitable for oral administration, as the drug component can be released from the swollen chitosan matrix in the gastrointestinal tract, and chitosan will eventually be dissolved and excreted without any toxic residue. A number of studies have reported that chitosan or chitosan composites have been used as a carrier for transdermal delivery of active compounds (Puttipipatkhachorn et al., 2001; Thacharodi and Panduranga Rao, 1996a,b; Thacharodi and Rao, 1995), DNA (Huang et al., 2009; Lee et al., 2008), and hormone (He et al., 2008). The drug-impregnated chitosan produced in this study may have potential for use in transdermal drug delivery.

The results show that increasing temperature promoted the impregnation efficiency. However, pressure had negligible effect on the impregnation efficiency within the range examined. At atmospheric conditions, less than 5% of the camptothecin could be impregnated in the chitosan after 8 h, whereas 19% impregnation was achieved using the dense gas solvent exchange technique at 25 bar and 40 °C within 1 h.

Camptothecin crystals were found on the surface of chitosan after impregnation at atmospheric conditions, because of solvent evaporation and precipitation of non-impregnated solute (Fig. 4c and d). In the dense gas solvent exchange process, camptothecin that was not impregnated was removed from the system, therefore, no camptothecin crystals were observed on the surface of chitosan (Fig. 4e).

FTIR spectroscopy was used to study the interaction between camptothecin and chitosan after impregnation. Bands due to double bond stretches were studied to infer the physical state of camptothecin. FTIR data in the region of 1500–1800 cm^{-1} were compared for blank chitosan films and camptothecin-impregnated chitosan. As expected, blank chitosan films showed a peak at 1592 cm^{-1}, which corresponds to either the N–H band for primary amines or amide II, and another small peak was detected at 1645 cm^{-1}, corresponding to the C=O stretch for amide I, which indicated that chitosan was not completely deacetylated (Fig. 5d) (Osman and Arof, 2003). Four main characteristic double bond bands were observed for crystalline camptothecin at 1740, 1650, 1600 and 1579 cm^{-1}, respectively (Fig. 5a), which were assigned to the carbonyl band for lactone ring (1740 cm^{-1}) and for ketone

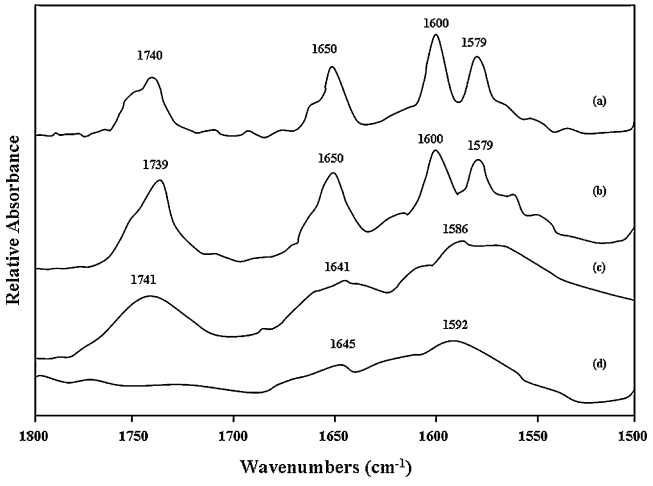

Fig. 5. FTIR spectra for (a) camptothecin powder; camptothecin-impregnated chitosan film at (b) atmospheric condition; (c) at 25 bar and 40 °C by the dense gas solvent exchange; and (d) neat chitosan obtained by dense gas solvent exchange.

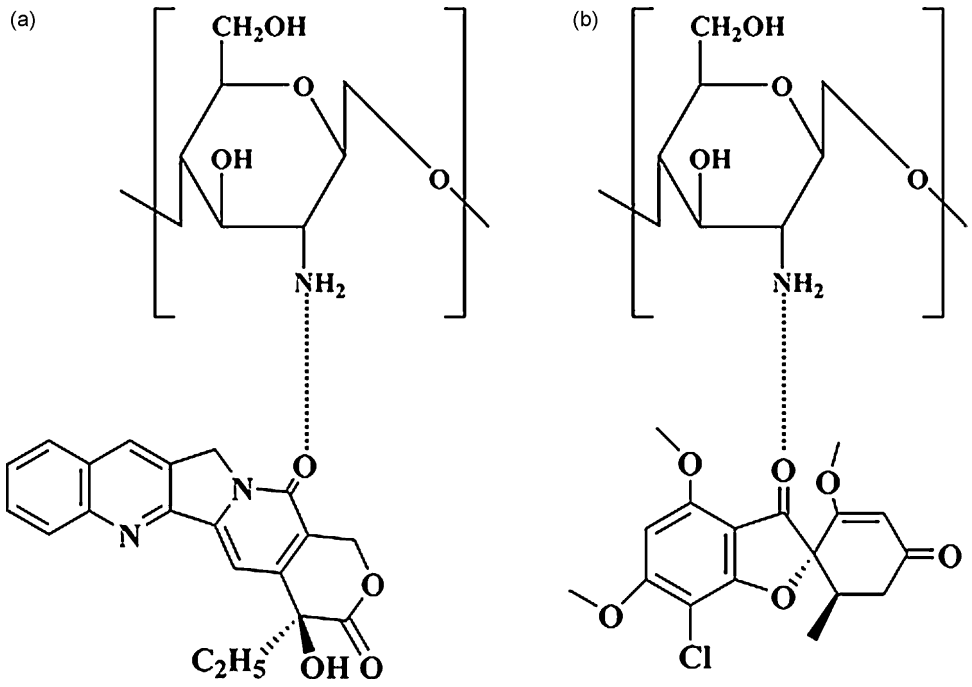

Fig. 6. Molecular interaction between chitosan and (a) camptothecin and (b) griseofulvin.

group (1650 cm^{-1}); and aromatic rings (1600 and 1579 cm^{-1}), respectively (Liu et al., 2008). The sample produced at atmospheric conditions had similar FTIR spectrum (Fig. 5b) as crystalline unprocessed camptothecin, indicating that free camptothecin particles were present in the sample. After dense gas solvent exchange and drug impregnation, a peak shift was found for ketone group (1650–1641 cm^{-1}), this shift indicated that the molecular interaction between camptothecin and chitosan took place between the carbonyl band for ketone group and the amino group from chitosan (Fig. 6a). The presence of a peak at 1741 cm^{-1} for the carbonyl bond of lactone rings in Fig. 5c, corroborates the presence of camptothecin. This peak also underlined that camptothecin was still in its active lactone form.

DSC analysis was conducted on camptothecin-impregnated samples produced by solvent exchange methods. Crystalline camptothecin powder had a first endothermic melting peak at 265 °C, followed by a second endothermic melting peak at 273 °C (Craig and Reading, 2007). Meanwhile, an exothermic peak was located at 277 °C due to the degradation of camptothecin (Fig. 7a). The DSC profile of camptothecin-impregnated chitosan processed at atmospheric conditions showed a broad endothermic peak at 137 °C (Fig. 7b), which referred to the melting temperature of chitosan (Fig. 7d). Another endothermic peak at 274 °C was also observed, which indicated camptothecin was still in the crystalline form after impregnation at atmospheric condition. However, the melting peak almost disappeared from the DSC profile of the sample impregnated by the dense gas solvent exchange process (Fig. 7c). This result suggests that camptothecin was in amorphous state after impregnation by the dense gas solvent exchange process.

The crystallinity has a significant impact on the therapeutic activity of drugs; the amorphous form often more readily dissolves than the crystalline, thus the time for the onset of therapeutic action decreases. The results of XRD analysis corroborated this conclusion that the crystallinity of impregnated camptothecin (Fig. 8c) was decreased substantially compared with pure camptothecin (Fig. 8a) and physical mixture of camptothecin and chitosan (Fig. 8b). The results of DSC and XRD analysis demonstrate that the crystallinity of camptothecin impregnated in chitosan was not changed after storage at ambient condition for a period of 4 months (Figs. 7 and 8).

The ultimate solubility of camptothecin in FESSIF buffer solution was determined. Camptothecin had a very low solubility in this buffer solution (0.85 ± 0.24 μg/mL), while after impregnation at high pressure, the solubility was increased to 1.51 ± 0.38 μg/mL. While the solubility of impregnated camptothecin in water-base buffer was still low, such an improvement can reduce the required dosage at least 40%.

3.3.2. Impregnation of griseofulvin

Griseofulvin, an orally administered antifungal drug (Thomas, 2001), was impregnated into chitosan film using the dense gas solvent exchange technique. Porosity was observed in the cross-

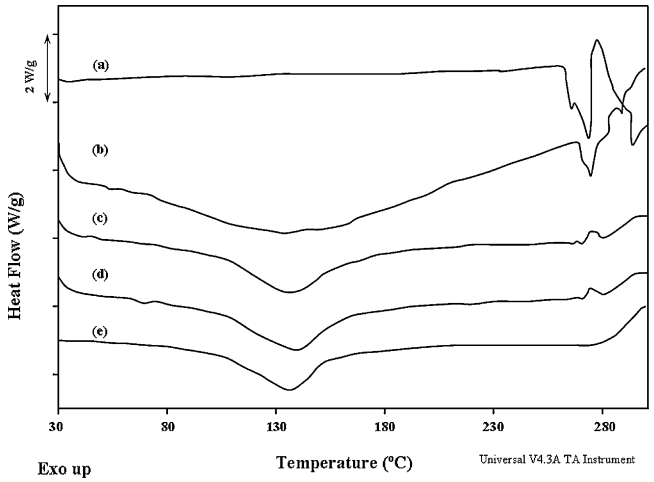

Fig. 7. DSC profiles of (a) pure camptothecin powder; (b) camptothecin-impregnated chitosan prepared at atmospheric condition; (c) and (d) camptothecin-impregnated chitosan prepared by dense gas solvent exchange at 25 bar and 40 °C directly after process and after 4 months storage at ambient condition, respectively and (e) pure chitosan film.

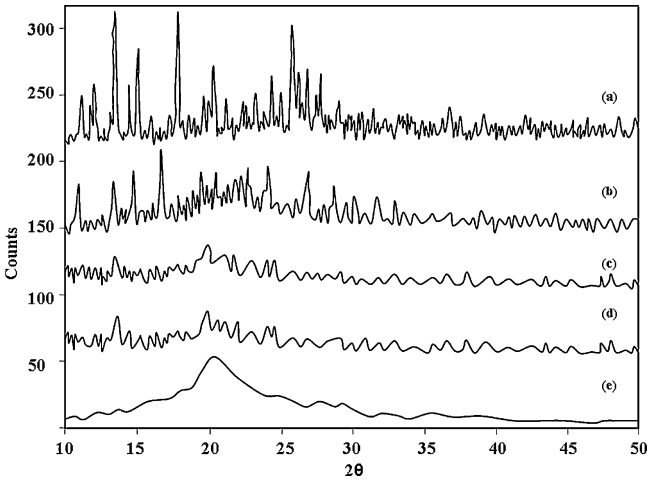

Fig. 8. XRD patterns of (a) pure camptothecin; (b) physical mixture of camptothecin and chitosan at atmospheric condition; (c) and (d) camptothecin-impregnated chitosan produced by dense gas solvent exchange process at 25 bar and 40 °C directly after process and after 4 months storage at ambient condition, respectively and (e) pure chitosan.

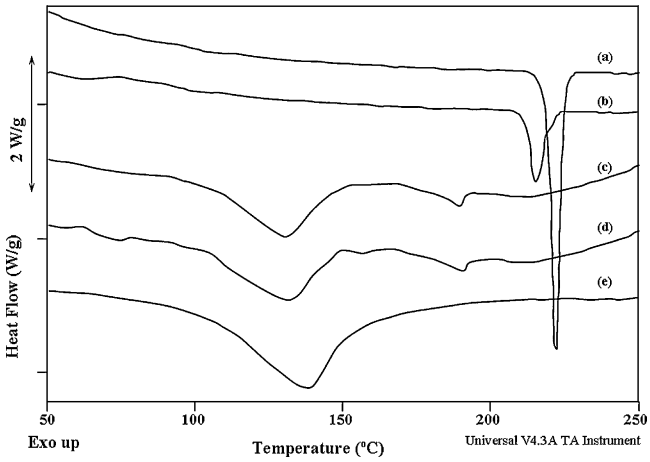

Fig. 10. DSC profiles of (a) pure griseofulvin; griseofulvin-impregnated chitosan produced at (b) atmospheric condition; (c) and (d) griseofulvin-impregnated chitosan produced by dense gas solvent exchange process 90 bar and 40 °C directly after process and after 6 months storage at ambient condition, respectively; (e) pure chitosan.

section of impregnated sample similar to the one produced when using pure chitosan. Impregnation efficiency of 95% was achieved for griseofulvin at the experimental conditions examined within 1 h, while only 56% impregnation efficiency was obtained at atmospheric condition for 8 h.

The affinity of chitosan for griseofulvin was examined by placing the swollen chitosan film (after completion of solvent exchange and prior to exposure to dense gas CO_2) into a closed vessel containing a 3.3 wt% solution of griseofulvin dissolved in acetone. The liquid samples were taken over a period of 8 h and tested using UV-spectroscopy. After 8 h 56% of the griseofulvin was adsorbed into the chitosan, confirming an affinity between chitosan and griseofulvin. During the dense gas solvent expansion process, CO_2 plays a critical role in swelling a polymer matrix and providing a transport medium for griseofulvin. As the partition coefficient of griseofulvin between the CO_2-swollen chitosan and dense gas CO_2 phase appears to be higher than between griseofulvin and CO_2–acetone phase, griseofulvin remains in chitosan matrix.

FTIR spectroscopy was conducted on pure griseofulvin, pure chitosan and griseofulvin-impregnated chitosan. According to Fig. 9, the peak of the non-sterically hindered carbonyl located on the five-member ring in griseofulvin structure was at 1708 cm^{-1} (Fig. 9a). The spectrum of the sample processed at atmospheric conditions showed a similar pattern to that of the unprocessed crystalline griseofulvin (Fig. 9b). However, after dense gas process, peak shift occurred from 1708 to 1691 cm^{-1} (Fig. 9c), indicating that interaction took place between the non-sterically hindered carbonyl group located on the five-member ring from griseofulvin and the amino group from chitosan (Fig. 6b). Therefore, it is believed that the impregnation of griseofulvin resulted in molecular interaction between two compounds. The higher drug loading efficiency for griseofulvin compared with camptothecin may result from a higher interaction between chitosan and griseofulvin, as confirmed by the FTIR analysis and a large shift in the peak.

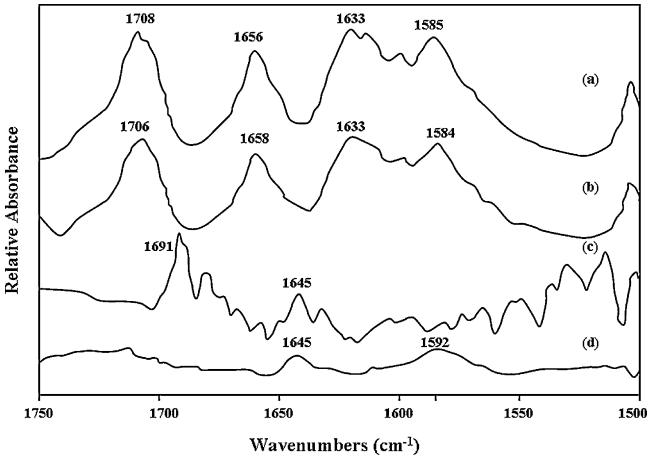

Fig. 9. FTIR spectra in the carbonyl spectral region for (a) pure griseofulvin; (b) griseofulvin-impregnated chitosan produced at atmospheric condition; (c) neat chitosan obtained by dense gas solvent exchange; (d) griseofulvin-impregnated chitosan produced at 90 bar and 40 °C by the dense gas solvent exchange method.

The DSC results confirmed that griseofulvin was in the amorphous phase when it was impregnated with the dense gas solvent exchange technique. As shown in Fig. 10, a well defined endothermic peak appeared at 218 °C (Fig. 10a) for crystalline griseofulvin. After impregnation under atmospheric condition, the sample still had an obvious melting peak (Fig. 10b). However, after dense gas impregnation, the intensity of the melting peak of griseofulvin decreased dramatically (Fig. 10c) suggesting that the amount of crystalline griseofulvin was significantly decreased. An obvious melting peak shift (from 218 to 188 °C) was observed on the impregnated griseofulvin. This result was expected because of the impregnation of griseofulvin into chitosan matrix (Bergese et al., 2004; Carli et al., 1986; Lovrecich, 1995). The degree of crystallinity can be calculated as the ratio of the fusion enthalpy of the impregnated and unprocessed drugs acquired from DSC results. It was found that the crystallinity of impregnated camptothecin and griseofulvin was dramatically decreased to less than 2% compared with unprocessed drugs.

The results of XRD analysis in Fig. 11, also suggest that the crystallinity of impregnated griseofulvin was dramatically decreased. Amorphous form of a drug is not generally stable because of the affinity to convert to a crystalline state with a lower energy. Previous studies have shown that partially amorphous griseofulvin was not stable and it converted to a crystalline form within 6 h (Ahmed et al., 1998). The results of DSC and XRD analysis demonstrate that the griseofulvin formulated with chitosan using the dense gas solvent exchange process maintained in amorphous state for at least 6 months (Figs. 10 and 11).

In vitro dissolution test was carried out on pure griseofulvin, grisovin, a commercial product containing griseofulvin, and

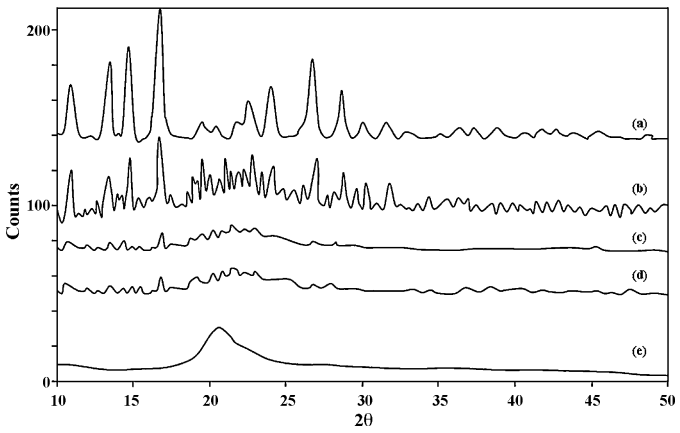

Fig. 11. XRD patterns of (a) pure griseofulvin; (b) physical mixture of griseofulvin and chitosan at atmospheric condition; (c) and (d) griseofulvin-impregnated chitosan produced by dense gas solvent exchange process at 90 bar and 40 °C directly after process and after 6 months storage at ambient condition, respectively; (e) pure chitosan.

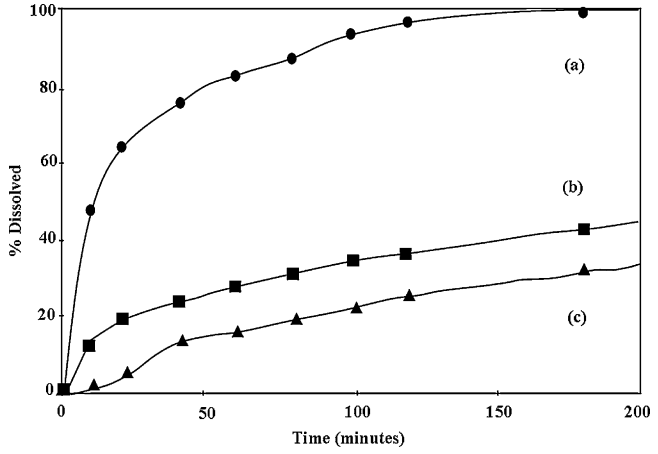

Fig. 12. *In vitro* dissolution profiles of (a) griseofulvin-impregnated chitosan; (b) grisovin; (c) pure griseofulvin.

griseofulvin-impregnated chitosan, respectively (Fig. 12). The dissolution rate of griseofulvin impregnated increased greatly; 50% of the drug content was dissolved in the medium within 15 min, and 100% dissolution could be achieved within 180 min (Fig. 12a). However, unprocessed griseofulvin showed a very slow dissolution rate, and only 30% of the compound was dissolved after 200 min (Fig. 12c). At the same time interval, only 50% of the grisovin was dissolved (Fig. 12b). These results demonstrate that impregnation by the solvent exchange technique was efficient in enhancing the dissolution rate of poorly water-soluble drugs.

4. Conclusions

A dense gas solvent exchange process (DGSEP) was developed for the impregnation of a drug into a hydrophilic polymer matrix. Camptothecin and griseofulvin with low solubility in CO_2 were impregnated into porous chitosan using this technique. Both drugs were maintained in amorphous from because of the interaction between each drug and chitosan. The dissolution rate of griseofulvin from porous chitosan structure was substantially higher than current formulation in the market. The process developed can be used for the production of drug formulations such as those suitable for oral administration and transdermal delivery. The increased potency, coupled with the mucoadhesive properties of chitosan make this formulation potentially suitable for sublingual or buccal dosage forms. Use of these forms would enable the issues associated with "first pass" absorption to be overcome, thereby enabling the drug to be delivered systemically *via* the oral mucosa and into the blood stream.

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References

Acarturk, F., Sencan, A., Celebi, N., 1993. Enhancement of the dissolution of spironolactone with chitosan and low-molecular weight gelatin. S.T.P. Pharm. Sci. 3, 369–373.
Ahmed, H., Buckton, G., Rawlins, D., 1998. Crystallization of partially amorphous griseofulvin in water vapor: determination of kinetic parameters using isothermal heat conduction microcalorimetry. Int. J. Pharm. 167, 139–145.
Alexis, F., Venkatraman, S.S., Rath, S.K., Boey, F., 2004. *In vitro* study of release mechanisms of paclitaxel and rapamycin from drug-incorporated biodegradable stent matrixes. J. Control. Release 98, 67–74.
Annabi, N., Mithieux, S.M., Weiss, A.S., Dehghani, F., 2009. The fabrication of elastin-based hydrogels using high pressure CO_2. Biomaterials 30, 1–7.
Bergese, P., Colombo, I., Gervasoni, D., Depero, L., 2004. Melting of nanostructured drugs embedded into a polymeric matrix. J. Phys. Chem. B 108, 15488–15493.
Busby, A.J., Morley, K.S., Roberts, C.J., Watson, M.S., Webb, P.B., Wong, B., Zhang, J., Kokturk, G., Howdle, S.M., 2002. Polymers, biomaterilas and supercritical fluids. In: Proceedings of the 8th Meeting on Supercritical Fluids, International Society for the Advancement of Supercritical Fluids (ISASF), Bordeaux, France.
Calvino-Casilda, V., Lopez-Peinado, A.J., Vaganova, E., Yitzchaik, S., Pacios, I.E., Pierola, I.F., 2008. Porosity inherent to chemically crosslinked polymers. Poly(N-vinylimidazole) hydrogels. J. Phys. Chem. B 112, 2809–2817.
Carli, F., Colombo, I., Magarotto, L., 1986. In: Chaudy, I. (Ed.), Proceedings of the 13th International Symposium on Controlled Release of Bioactive Materials. Nortfolk. The Controlled Release Society, Minneapolis, MN, p. 193.
Cooper, A.I., 2001. Recent developments in materials synthesis and processing using supercritical CO_2. Adv. Mater. Weinheim 13, 1111–1114.
Craig, D.Q.M., Reading, M., 2007. Thermal analysis of pharmaceuticals. In: Craig, D.Q.M. (Ed.), Pharmaceutical Application of DSC. CRC Press, Boca Raton, p. 416.
Dong, N., Xue, S.-F., Zhu, Q.-J., Tao, Z., Zhao, Y., Yang, L.-X., 2008. Cucurbit[n]urils (n = 7, 8) binding of camptothecin and the effects on solubility and reactivity of the anticancer drug. Supramol. Chem. 20, 659–665.
Duarte, A.R.C., Costa, M.S., Simplicio, A.L., Cardoso, M.M., Duarte, C.M.M., 2006. Preparation of controlled release microspheres using supercritical fluid technology for delivery of anti-inflammatory drugs. Int. J. Pharm. 308, 168–174.
Gao, P., 2008. Amorphous pharmaceutical solids: characterization, stabilization, and development of marketable formulations of poorly soluble drugs with improved oral absorption. Mol. Pharm. 5, 903–904.
Gong, K., Darr, J.A., Rehman, I.U., 2006. Supercritical fluid assisted impregnation of indomethacin into chitosan thermosets for controlled release applications. Int. J. Pharm. 315, 93–98.
Gurusamy, S., Kumar, V., Mishra, D.N., 2006. Preparation, characterization and *in vitro* dissolution studies of solid systems of valdecoxib with chitosan. Chem. Pharm. Bull. 54, 1102–1106.
He, W., Guo, X., Zhang, M., 2008. Transdermal permeation enhancement of N-trimethyl chitosan for testosterone. Int. J. Pharm. 356, 82–87.
Hertzberg, R.P., Caranfa, M.J., Hecht, S.M., 1989. On the mechanism of topoisomerase I inhibition by camptothecin: evidence for binding to an enzyme–DNA complex. Biochemistry 28, 4629–4638.
Huang, H.-N., Li, T.-L., Chan, Y.-L., Chen, C.-L., Wu, C.-J., 2009. Transdermal immunization with low-pressure-gene-gun mediated chitosan-based DNA vaccines against Japanese encephalitis virus. Biomaterials 30, 6017–6025.
Joung, S.N., Yoo, C.W., Shin, H.Y., Kim, S.Y., Yoo, K.P., Lee, C.S., Huh, W.S., 2001. Measurements and correlation of high-pressure VLE of binary CO_2–alcohol systems (methanol, ethanol, 2-methoxyethanol and 2-ethoxyethanol). Fluid Phase Equilib. 185, 219–230.
Jung, J., Leboeuf, F., Clavier, J.Y., Perrut, M., 2002. Equipment design for supercritical fluid micronization in compliance with GMP for pharmaceutical applications. In: Proceedings of the Fourth International Symosium in High-Pressure Process Technology and Chemical Engineering, Italy.
Kang, H.-W., Tabata, Y., Ikada, Y., 1999. Effect of porous structure on the degradation of freeze-dried gelatin hydrogels. J. Bioact. Compat. Polym. 14, 331–343.
Kazarian, S.G., Chan, A., 2003. Molecular states of drugs in formulations processed with supercritical fluids: in situ ATR-IR spectroscopy and spectroscopic imaging study. PMSE Prepr. 89, 628–629.
Kazarian, S.G., Vincent, M., West, B., Eckert, C., 1998. Partitioning of solutes and cosolvents between supercritical CO_2 and polymer phase. J. Supercrit. Fluids 13, 107–112.
Kluge, J., Fusaro, F., Muhrer, G., Thakur, R., Mazzotti, M., 2009. Rational design of drug–polymer co-formulations by CO_2 anti-solvent precipitation. J. Supercrit. Fluids 48, 176–182.

Ladet, S., David, L., Domard, A., 2008. Multi-membrane hydrogels. Nature (London, U.K.) 452, 76–79.
LeClair Ellis, J., Tomasko, D.L., Dehghani, F., 2008. Novel dense CO_2 technique for beta-galactosidase immobilization in polystyrene microchannels. Biomacromolecules 9, 1027–1034.
Lee, J.-Y., Tan, B., Cooper, A.I., 2007. CO_2-in-water emulsion-templated poly(vinyl alcohol) hydrogels using poly(vinyl acetate)-based surfactants. Macromolecules 40, 1955–1961.
Lee, P.-W., Peng, S.-F., Su, C.-J., Mi, F.-L., Chen, H.-L., Wei, M.-C., Lin, H.-J., Sung, H.-W., 2008. The use of biodegradable polymeric nanoparticles in combination with a low-pressure gene gun for transdermal DNA delivery. Biomaterials 29, 742–751.
Liu, C.-X., Hou, W.-G., Li, Y., Li, L.-F., 2008. Synthesis and characterization of camptothecin intercalated into Mg/Al layered double hydroxide. Chin. J. Chem. 26, 1806–1810.
Lopez-Periago, A., Argemi, A., Andanson, J.M., Fernandez, V., Garcia-Gonzalez, C.A., Kazarian, S.G., Saurina, J., Domingo, C., 2009. Impregnation of a biocompatible polymer aided by supercritical CO_2: evaluation of drug stability and drug-matrix interactions. J. Supercrit. Fluids 48, 56–63.
Lovrecich, M., 1995. Supported drugs with increased dissolution rate, and a process for their preparation, US 5449 521.
Ma, P.X., 2008. Biomimetic materials for tissue engineering. Adv. Drug Deliv. Rev. 60, 184–198.
Madihally, S.V., Matthew, H.W.T., 1999. Porous chitosan scaffolds for tissue engineering. Biomaterials 20, 1133–1142.
Maestrelli, F., Zerrouk, N., Chemtob, C., Mura, P., 2004. Influence of chitosan and its glutamate and hydrochloride salts on naproxen dissolution rate and permeation across Caco-2 cells. Int. J. Pharm. 271, 257–267.
McKelvey, S.A., Koros, W.J., 1996. Phase separation, vitrification, and the manifestation of macrovoids in polymeric asymmetric membranes. J. Membr. Sci. 112, 29–39.
Mura, P., Zerrouk, N., Mennini, N., Maestrelli, F., Chemtob, C., 2003. Development and characterization of naproxen-chitosan solid systems with improved drug dissolution properties. Eur. J. Pharm. Sci. 19, 67–75.
Nambu, N., Sawayanagi, Y., Nagai, T., 1983. Dissolution properties and bioavailability of poorly soluble drugs from ground mixtures with chitin or chitosan. Expo.—Congr. Int. Technol. Pharm., 3rd. 1, 259–267.
Natu, M.V., Gil, M.H., de Sousa, H.C., 2008. Supercritical solvent impregnation of poly(e-caprolactone)/poly(oxyethylene-b-oxypropylene-b-oxyethylene) and poly(e-caprolactone)/poly(ethylene-vinyl acetate) blends for controlled release applications. J. Supercrit. Fluids 47, 93–102.
Osman, Z., Arof, A.K., 2003. FTIR studies of chitosan acetate based polymer electrolytes. Electrochim. Acta 48, 993–999.
Overhoff, K.A., Johnston, K.P., Williams III, R.O., 2006. Improvement of dissolution rate of poorly water soluble drugs using a new particle engineering process: spray freezing into liquid. ACS Symp. Ser. 924, 305–319.
Puttipipatkhachorn, S., Nunthanid, J., Yamamoto, K., Peck, G.E., 2001. Drug physical state and drug–polymer interaction on drug release from chitosan matrix films. J. Control. Release 75, 143–153.
Quirk, R.A., France, R.M., Shakesheff, K.M., Howdle, S.M., 2005. Supercritical fluid technologies and tissue engineering scaffolds. Curr. Opin. Solid State Mater. Sci. 8, 313–321.
Saetern, A.M., Skar, M., Braaten, A., Brandl, M., 2005. Camptothecin-catalyzed phospholipid hydrolysis in liposomes. Int. J. Pharm. 288, 73–80.
Sawayanagi, Y., Nambu, N., Nagai, T., 1983. Pharmaceutical interactions in dosage forms and processing, XXXIX. Enhancement of dissolution properties of prednisolone from ground mixtures with chitin or chitosan. Chem. Pharm. Bull. 31, 2507–2509.
Shih, H.-H., Lee, K.-R., Lai, H.-M., Tsai, C.-C., Chang, Y.-C., 2003. Production of porous biodegradable polymers using supercritical fluid, US 2003064156.
Thacharodi, D., Panduranga Rao, K., 1996a. Collagen–chitosan composite membranes controlled transdermal delivery of nifedipine and propranolol hydrochloride. Int. J. Pharm. 134, 239–241.
Thacharodi, D., Panduranga Rao, K., 1996b. Rate-controlling biopolymer membranes as transdermal delivery systems for nifedipine: development and in vitro evaluations. Biomaterials 17, 1307–1311.
Thacharodi, D., Rao, P., 1995. Development and in vitro evaluation of chitosan-based transdermal drug delivery systems for the controlled delivery of propanolol hydrochloride. Biomaterials 16, 145–148.
Thassu, D., Deleers, M., Pathak, Y., 2007. Nanoparticulate Drug Delivery Systems. CRC Press, p. 64.
Thomas, J., 2001. Australian Prescription Products Guide, p. 1.
USP-NF, The United States Pharmacopedia-National Formulary (USP-NF).
VandeVord, P.J., Matthew, H.W.T., DeSilva, S.P., Mayton, L., Wu, B., Wooley, P.H., 2002. Evaluation of the biocompatibility of a chitosan scaffold in mice. J. Biomed. Mater. Res. 59, 585–590.
Wang, A., Ao, Q., Wei, Y., Gong, K., Liu, X., Zhao, N., Gong, Y., Zhang, X., 2007. Physical properties and biocompatibility of a porous chitosan-based fiber-reinforced conduit for nerve regeneration. Biotechnol. Lett. 29, 1697–1702.
Yurkovetskiy, A.V., Hiller, A., Syed, S., Yin, M., Lu, X.M., Fischman, A.J., Papisov, M.I., 2004. Synthesis of a macromolecular camptothecin conjugate with dual phase drug release. Mol. Pharm. 1, 375–382.