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Generation and precipitation of paclitaxel nanoparticles in basil seed mucilage via combination of supercritical gas antisolvent and phase inversion techniques

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ABSTRACT

In recent years, plant derived polymers have evoked tremendous interest in the field of drug delivery. In this work, a promising anticancer drug, paclitaxel, was precipitated in the basil seeds mucilage (BSM) using supercritical carbon dioxide (SC-CO_2). The employed SC-CO_2 process in this research is a combination of gas antisolvent and phase inversion techniques and consists of two steps: (1) casting solution preparation, a uniform mixture of BSM, water, paclitaxel and dimethyl sulfoxide (DMSO), (2) simultaneous generation and precipitation of nanoparticles in BSM structure using SC-CO_2 as antisolvent. The effect of DMSO/water ratio (4 and 6 (v/v)), pressure (10–16 MPa) and CO_2 addition rate (1–3 mL/min) on mean particle size (MPS), particle size distribution (PSD) and drug loading efficiency (DLE) were studied. Particle analyses were performed by scanning electron microscopy (SEM) and Zetasizer. High performance liquid chromatography was utilized for studying DLE. Nanoparticles of paclitaxel (MPS of 117–200 nm depending on process variables) with narrow PSD were successfully precipitated in BSM structure with DLE of 56.8–78.2%. The FTIR spectra confirmed that paclitaxel actually precipitated in basil seeds mucilage. Experimental results indicated that higher DMSO/water ratio, pressure and CO_2 addition decreased MPS and DLE.

1. Introduction

Paclitaxel (PX) is a promising anticancer drug with excellent therapeutic efficacy against a wide spectrum of cancers, especially ovarian, breast, lung, colon, head, and neck cancers [1–3]. It works through the inhibition of DNA synthesis by stabilizing microtubule assembly [2]. However, its clinical application has been limited due to its low solubility in water. Its current formulation requires the use of an adjuvant, Cremophor® EL (polyethoxylated castor oil), which has been associated with several undesirable side effects including severe hypersensitivity reactions, myelosuppression and neurotoxicity [2–4]. Recently, several alternative PX formulations without the use of adjuvant have been adopted, including using colloidal nanoparticle carriers such as PX albumin-bound nanoparticle suspension [5], and encapsulating of PX in biodegradable polymers such poly lactic acid (PLA) [1] or poly lactic-co-glycolic acid (PLGA) [6,7].

In recent years, both synthetic and natural polymers have been successfully investigated and employed for the design of novel drug delivery systems. However, the use of natural origin polymers for pharmaceutical applications is more attractive because they are non-toxic, biocompatible, potentially biodegradable, chemical modifications capability [8,9], freely available and inexpensive in comparison to synthetic polymers [9–11].

Among the natural origin polymers, in recent years, plant derived polymers have evoked tremendous interest in the field of drug delivery application [12,13]. The plant based polymers have been studied for their application in different pharmaceutical dosage forms like matrix controlled system, buccal films, film coating agents, microspheres, nanoparticles, viscous liquid formulations like ophthalmic solutions, suspensions and implants [10,14,15].

Mucilages are plant derived natural polymers which mainly consist of polysaccharides, proteins and uranides [16]. Mucilage is a gelatinous substance with a wide range of applications in the pharmaceutical industries such as adjuvant, thickeners, suspending agents, binders, granulating agent, and also used as matrices for sustained and controlled drugs delivery [16–19]. Plant

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mucilages and gums, concentrated form of mucilage, are well known since ancient times for their medicinal use. Acacia, tragacanth, gum karaya and ocimum bascilium are popular examples of plant mucilages with a wide range of pharmaceutical applications [20–22]. *Ocimum basilicum* L. also known as basil is an annual herb plant which grows in several regions around the world. Basil seed is black in colour and oval in shape with mean dimensions of 3.11 ± 0.29 mm (length), 1.82 ± 0.26 mm (width) and 1.34 ± 0.19 mm (height) [23]. When the basil seeds are soaked in water, the outer pericarp swell into a gelatinous mass, called mucilage, which could be extracted from the seeds and concentrated or dried for further applications. Prajapati et al. [10] reviewed the pharmaceutical applications of various natural gums, mucilages and their modified forms for the development of various drug delivery systems. Archana et al. [17] characterized mucilage polysaccharide from waste of *Abelmoscus esculentus* for biomedical applications. Srinivas et al. [24] studied ocimum basilicum as disintegrates in the formulation of dispersible tablets. In this work, the extracted mucilage from basil seeds was used as a cost effective polymeric bed for precipitation of PX for controlled drug delivery.

Most of previous techniques proposed for processing of polymeric materials present some limitations such as, time-consuming, presence of organic solvents that are difficult to be eliminated and that can remain entrapped inside the polymeric network. To overcome these shortcomings, supercritical fluid techniques have been explored for processing of polymeric materials due to their high liquid-like dissolving power and gas-like transport properties [25]. For pharmaceutical applications, the supercritical carbon dioxide (SC-CO_2) is usually preferred, because it is non-reactive, non-toxic, non-flammable, environmentally benign, inexpensive, abundance and has favourable critical properties [1,26,27]. The SC-CO_2 has been used as an alternative non-solvent in phase inversion processes to generate polymeric and biopolymeric porous structure [28]. SC-CO_2 rapidly forms supercritical mixtures with many organic compounds at temperatures and pressures (for example, at 100 bar and 40 °C) readily obtainable from the processing point of view and (especially temperatures) compatible with thermal stability of polymers. However, SC-CO_2 shows a very limited compatibility with polar components, like water, at the ordinary temperatures and pressures used in SC-CO_2 processing; for example, at 40 °C and 100 bar, water solubility is around 0.5% [29]. Therefore, the common supercritical polymer processing method, drug encapsulating and porous structure generating, is not directly applicable to water soluble polymers. To overcome this limitation, CO_2-assisted phase inversion [30] and water substitution and SC-CO_2 gel drying processes [31,32] have been recently employed. In both of aforementioned techniques, to increase water solubility in SC-CO_2, a polar co-solvent was used. In this work, a modified SC-CO_2 method based on Temtem et al. [30] technique was used to simultaneously generate and precipitate nano sized particles of PX in the BSM as a low cost, biocompatible plant derived polymer. The final PX products of this study were characterized from a microscopic point of view using SEM imaging. Then, the particle size and drug loading efficiency of these PX products were compared with similar works. The effect of the process variables such as pressure, co-solvent to water ratio and CO_2 addition rate, on the particle size, size distribution and drug loading were investigated.

2. Materials and method

2.1. Materials

Paclitaxel was purchased from Sobhan Oncology Co. (Iran). Basil seeds used in this study were purchased from Isfahan local market in Iran. Dichloromethane (DCM) (HPLC/Spectro Grade), acetonitrile (ACN) (HPLC/Spectro Grade), dimethyl sulfoxide (DMSO) (≥99%) and ethyl alcohol (≥96%) were obtained from Merck. Industrial grade carbon dioxide (≥99.9%) was purchased from Zamzam Co. (Isfahan, Iran). All materials were used as received.

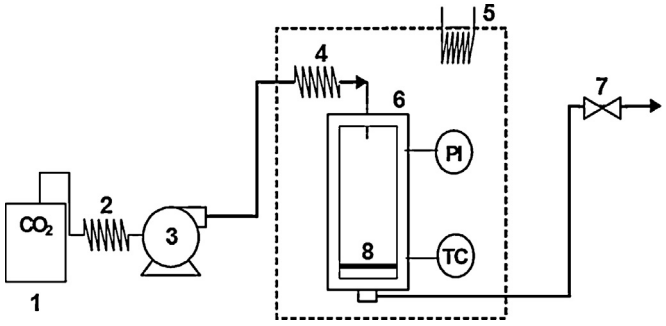

Fig. 1. Schematic diagram of employed SC-CO_2 process: (1) cylinder, (2) cooler, (3) high pressure pump, (4) heat exchanger, (5) oven, (6) vessel, (7) back pressure regulator, and (8) porous disc.

2.2. Procedure of BSM preparation

The extraction of mucilage (Mu) from basil seeds was performed using a modified method based on Razavi et al. study [33]. The basil seeds were soaked and swelled in distilled water at 65 °C and a water/seed ratio of 60:1 at pH 8 which is adjusted using a 0.2 M NaOH aqueous solution. The mixture was stirred with a rod paddle mixer until the seeds were completely swelled (20 min agitation, 100 rpm). In order to remove ash content, the swelled seeds were washed with ethyl alcohol/water (volumetric ratio of 90/10) solution. Seeds solution was passed through an extractor (a rotating mixer with rough blades) to scrape the Mu layer off the seeds surface. The separated Mu was passed through a sieve and then centrifuged at 5000 rpm (Himac CR22GII, Hitachi Koki Co. Ltd., Takeda, Hitachinaka city, Japan) for 5 min at ambient condition to remove all likely seed residuals. After filtration, ethyl alcohol was added to the extracted Mu in the 4:1 ratio and left overnight at 4 °C. Finally, the crude extract was concentrated at 55 °C with rotary vacuum evaporator (IKA, RV 10, Deutschland, Germany) to remove extra water/ethyl alcohol content and dried in laboratory oven to obtain the final BSM material.

2.3. SC-CO_2 experimental set-up

The experimental set-up of this work is similar to our previous publications for GAS process [26,27]. Fig. 1 shows a scheme of this set-up. Carbon dioxide was drawn from the cylinder (1) and liquefied in a cooler (2). Liquid carbon dioxide was pumped with a high pressure pump (3) (Jasco–Milroyal B, France). High pressure carbon dioxide was heated in a spiral heat exchanger (4) located in an oven (5) before charging into the 15 mL stainless steel high pressure vessel (6). The pressure was controlled by a back pressure regulator (7) (Tescom, USA). A metallic porous disc (8) was placed at bottom of the vessel to collect polymeric structure containing the precipitated particles.

2.4. Experimental procedure

As mentioned, Temtem et al. [30] developed a new continuous steady state SC-CO_2 phase inversion method to load gentamicin into the chitosan membranes. In their work, a non solvent stream, mixture of SC-CO_2 and ethanol as co-solvent, was added continuously to the casting solution (aqueous solution of polymer and drug) to form drug loaded polymeric structure. This work is a modified semi batch version of the Temtem et al. procedure. In this work,