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Fabrication of pectin-based nanoemulsions loaded with itraconazole for pharmaceutical application

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ABSTRACT

The aim of this study was to prepare nanoemulsions containing itraconazole (ITZ), a poorly water-soluble drug, using pectin as a polymeric emulsifier. Nanoemulsions were prepared by simple homogenization to avoid high-pressure conditions. The influences of type of internal phase, type and concentration of pectin on the droplet size, morphology, and zeta potential of the pectin-based emulsions were also examined. Nanoemulsions were achieved when chloroform was used as an internal phase while using caprylic/capric triglyceride can produce only micron-sized emulsions. Pectin with high degree of esterification offered good emulsion properties because of its high amount of hydrophobic molecules. The droplet size of emulsions decreased with the increased pectin concentration. The addition of ITZ to the emulsion formulation was essential to obtain the nano-sized emulsions, resulting from the molecular association between ITZ and pectin. It appears that 3% (w/w) pectin provided the most stable emulsion with the highest percent creaming. The obtained nanoemulsions may be subsequently developed as a self-emulsifying drug delivery system.

1. Introduction

Nanoemulsions have received a growing attention as colloidal drug carriers for pharmaceutical applications. The use of nanoemulsions for oral administration to increase the bioavailability of poorly water-soluble drugs due to an enhancement of the intestinal absorption of the drug is well documented (Bates & Carrigan, 1975; Constantinides, 1995). It has been also found that the absorption in the gastrointestinal tract is improved by a small droplet size (Toguchi, Ogawa, & Shimamoto, 1990). Nanoemulsions are often referred to emulsions with droplet sizes in the nanometric scale, generally 100–200 nm (Solans, Izquierdo, Nolla, Azemar, & Garcia-Celma, 2005). Ultrafine emulsions, submicron-sized emulsions and miniemulsions are also used to describe emulsions with fine disperse droplets (Gramdorf et al., 2008; Solans et al., 2005). Nanoemulsions can be prepared by two major techniques, i.e., high-energy and low-energy emulsifications. High-energy emulsification methods include high-shear stirring, high-pressure homogenization and ultrasound generators. It has been reported that the apparatus supplying the available energy in the shortest time and having the most homogenous flow produces the smallest sizes (Walstra, 1996). Although high-pressure homogenizer meets the requirements, the damaging of long chain molecules could occur when very high pressure was applied. The emulsification methods making use of the chemical energy stored in the components, also named low-energy emulsification methods, are receiving increased attention. In these methods, nanoemulsions are obtained as a result of phase transitions produced during the emulsification process (Sadurní, Solans, Azemar, & García-Celma, 2005). In practice, a combination of high-energy and low-energy emulsification methods has proved to be an efficient way to obtain nanoemulsions with small and very uniform droplets (Nakajima, 1997).

The primary stabilizing mechanism may occur in the bulk aqueous phase or at the surface of the droplets, depending on the chemical nature of the particular ingredients involved. There are two broad classes of emulsifying agents, i.e., small-molecule surfactants and macromolecular emulsifiers. For a polymer or biopolymer to be effective as an emulsifying agent, it must be surface-active, i.e., it must have the capacity to lower the surface tension at the oil–water interface, both rapidly and substantially when it

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presents at the concentrations typically used during emulsification (Dickinson, 2003).

Pectin, a natural polymer, is extracted from plant cell walls, especially in apple pomace, citrus fruits and sugar beet root. It has commonly been used as a gelling agent, a thickening agent and a colloidal stabilizer in food industry (Van Buren, 1991). Its applications in the pharmaceutical industry were increased in the last decade (e.g., Sriamornsak, Sungthongjeen, & Puttipipatkhachorn, 2007; Sriamornsak, Thirawong, & Puttipipatkhachorn, 2005; Sriamornsak, Thirawong, Weerapol, Nunthanid, & Sungthongjeen, 2007; Sungthongjeen, Sriamornsak, Pitaksuteepong, Somsiri, & Puttipipatkhachorn, 2004). High-methoxyl pectin (≥50% degree of esterification or DE) forms gels under acidic conditions in aqueous media of high sugar content, whereas low-methoxyl pectin (<50% DE) forms gels in the presence of calcium ions (Guo, Skinner, Harcum, & Barnum, 1998). The presence of surface-active molecules in pectin provides the emulsification properties. In addition, pectin is capable of reducing the interfacial tension between an oil phase and a water phase and can be effective in the preparation of emulsions (Sriamornsak, Thirawong, & Puttipipatkhachorn, 2004; Sriamornsak et al., 2005). Dea and Madden (1986) reported that sugar beet pectin was more surface-active than commercial high-methoxyl or low-methoxyl pectins due to the substantially hydrophobic character of the acetyl groups (2–9%), and hence to be readily capable of producing and stabilizing fine vegetable oil-in-water emulsions. Even with a low acetyl content (<0.8%), pectins from citrus fruits and apples can also exhibit good surface activity and emulsion stabilizing characteristics if the average molecular weight is reduced to <80 kDa (Mazoyer, Leroux, & Bruneau, 1999). Depolymerized pectin of molecular weight 70 kDa and 70% DE has been used to make highly stable fine oil-in-water emulsions, based on time-dependent changes in emulsion droplet-size distribution and serum separation, at a relatively low pectin/oil ratio (1:5) (Akhtar, Dickinson, Mazoyer, & Langendorff, 2002). Emulsion stability was found to be reduced on increasing the pH from 4.7 to 7, or by substantially increasing (or decreasing) the pectin molecular weight.

Itraconazole (ITZ), a triazole antifungal agent, was used as a model poorly water-soluble drug in this study. It has a broad spectrum of activity against a variety of pathogens, including *Aspergillus* species, *Candida albicans*, *Cryptococcus neoformans*, *Coccidioides immitis*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, and *Sporothrix schenckii* (Saag & Dismukes, 1988), which are the major cause of opportunistic infection in human immunodeficiency virus (HIV) infected patients. The mechanism of action of ITZ is similar to all other azole antifungals. It inhibits cytochrome P450 of the fungi and thus interferes the synthesis of ergosterol, a vital component of the fungal cell membrane, leading to cell death (Gestel & Beule, 2001). ITZ is a white to slightly yellowish powder. It has a molecular formula $C_{35}H_{38}Cl_2N_8O_4$ and molecular weight of 705.64. It is a weak basic drug ($pK_a = 3.7$) which is virtually ionized at only low pH, possessing extremely low water solubility (about 1 ng/mL at neutral pH and about 6 μg/mL at pH 1). The log partition coefficient of ITZ in a system of n-octanol and an aqueous buffer solution of pH 8.1 is 5.66 (Peeters, Neeskens, Tollenaere, Van Remoortere, & Brewster, 2002), indicating a very high lipophilicity. According to the biopharmaceutics classification system (BCS), ITZ is one of a class II compound suggesting that its oral bioavailability is determined by dissolution rate in gastrointestinal tract (Amidon, Lennernäs, Shah, & Crison, 1995). Therefore, it is a good model to study the characteristics of nanoemulsions containing poorly water-soluble drug.

The objective of this study was to prepare nanoemulsions containing poorly water-soluble drug, ITZ, by using pectin as a polymeric emulsifier. Nanoemulsions were prepared by a simple homogenization to avoid high-pressure conditions. The influences of type of internal (oil) phase, type and concentration of pectin on the physicochemical characteristics of the pectin-based nanoemulsions were also examined. The obtained liquid formulations could be subsequently developed as a self-emulsifying drug delivery unit dose formulation.

2. Materials and methods

2.1. Materials

Pectins were a gift from Herbstreith & Fox KG (Germany), namely low-methoxyl pectin (referred as LMP) with DE of 38, amidated low-methoxyl pectin (referred as ALMP) with DE of 29 and degree of amidation of 20, and high-methoxyl pectin (referred as HMP) with DE of 70. The molecular weight of LMP, ALMP and HMP was 70 kDa, 150 kDa and 200 kDa, respectively. Caprylic/capric triglyceride (Miglyol® 812) was a gift from Sasol GmbH (Germany) and referred as CCT. ITZ was from Nosch Labs Private (India). Chloroform was supplied by Carl Roth GmbH (Germany). Deionized water was used as an aqueous phase in all preparations. All other chemicals used in this study were of pharmaceutical grade and used as received without further purification.

2.2. Solubility studies

Solubility of ITZ was determined in water and various solvents by adding ITZ in 1 mL of a pure solvent in Pyrex culture tubes. The drug suspension was equilibrated at 25 °C in a thermostatically controlled bath for 48 h. After equilibration, the tubes were centrifuged at 3500 rpm for 15 min and the clear supernatants were analyzed for ITZ with a high performance liquid chromatography, HPLC (Agilent, USA) using Alltima™ C18 column (5 μm, 25 cm × 4.6 mm) (Alltech, Italy). The mobile phase consisted of acetonitrile:water (37:63, v/v) adjusted to pH 2.45 with phosphoric acid and was filtered through a membrane filter (0.22 μm), and degassed in a sonicator bath before use. The flow rate was 1.0 mL/min, and the UV detection wavelength was 263 nm.

2.3. Preparation of (nano)emulsions containing ITZ

Oil-in-water or chloroform-in-water emulsions were prepared by using simple homogenization. ITZ was dissolved in either CCT or chloroform at the different concentrations depending on its solubility in CCT or chloroform. Twenty percent of CCT or chloroform were mixed with pectin solution using homogenizer (Ultra-Turrax® T50 Basic, IKA, Germany) at a speed of 24,000 rpm for 20 min in an ice-bath to avoid over heating. Three types of pectin with different degrees of esterification were used in this study, i.e., ALMP, LMP and HMP with DE of 29, 38 and 70, respectively. The effect of concentration of pectin (0.5–3%, w/w) was also investigated in this study.

2.4. Morphology

The morphology of all emulsions was investigated by a light biological microscope (Motic BA 300, Motic China Group, P.R. China). The emulsions were dropped on a glass slide and covered afterward with a coverslip, then the photos of the emulsion droplets were taken and investigated by the Motic Image Plus 2.0 program. Additionally, chloroform-in-water nanoemulsions were examined under transmission electron microscope (Tecnai G2-20 TEM, FEI Company, USA). Nanoemulsions were dropped and dried on the TEM copper grid, and then coated with carbon film under the ambient condition (22 °C). The samples were determined at 200 kV.