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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.

2.5. Measurement of the droplet size

The size of emulsion droplets was investigated by two methods, a light microscopy and a static laser light scattering. For light microscopy, the emulsions were dropped on a glass slide and covered afterward with a coverslip. Fifty emulsion droplets were captured by a calibrated Motic Image Plus 2.0 program and the Martin diameter of droplets was measured, then median size was calculated. By static light scattering method (Laser scattering particle size distribution analyzer LA-950, Horiba, Japan), the emulsions were dispersed or diluted in deionized water with gentle stirring. The median particle size was measured under continuous stirring and obtained from the measurements of at least three batches of emulsions.

2.6. Zeta potential measurement

The zeta potential of ITZ-loaded nanoemulsions was measured by zeta potential analyzer (ZetaPlus, Brookhaven, USA). (Nano)Emulsions were dispersed in deionized water at the ratio of 1:50 (v/v) and the electric field applied was 1 V. The average and standard deviation of the measurement of three batches of emulsions were reported.

2.7. Small angle X-ray scattering (SAXS) measurement

The experiments were performed with the small angle X-ray scattering (SAXS) beamline BW4, installed at the synchrotron source HASYLAB/DESY in Hamburg, Germany (Roth et al., 2006). The scattering patterns were acquired using a position sensitive area detector (MarCCD165, Marresearch GmbH, Norderstedt, Germany) with the pixel size of 79.1 μm × 79.1 μm at the wavelength of 0.13808 nm. The liquid of (nano)emulsions both from CCT and chloroform of about 20 μL was injected into a glass capillary (Hilgenberg GmbH, Malsfeld, Germany) with a diameter of 2.5 mm and a wall thickness of 0.01 mm. The capillaries were sealed with a flame and measured at ambient temperature (22 °C) without vacuum condition. The raw data were treated with the Fit2D-Program, version 12.077 (Andy Hammersley, European Synchrotron Radiation Facility, Grenoble, France) but analyzed beforehand by the Origin-Program, version Pro 8.0 (OriginLab Corporation, Northampton, USA) in order to determine the peak-maximum.

2.8. Stability test

All emulsions were kept in the glass vials. The (nano)emulsions were separated into two groups, the first group was kept at ambient temperature (22 °C) while the other group was kept at 4 °C in a refrigerator. The stability of (nano)emulsions in both groups was examined after 7 days by calculation of percent creaming using the following equation:

$$\%\,\text{creaming} = \frac{V_t - V_s}{V_t} \times 100 \quad (1)$$

where V_t is the total volume (mL) of the sample, and V_s is the volume (mL) of the lower phase layer. According to this equation, it is worth noticing that a greater value of the % creaming is an indication of a more stable emulsion.

The size of the emulsion droplets after stability test was also measured by a light microscopic method as described above.

2.9. Statistical analysis

Analysis of variance (ANOVA) and Levene's test for homogeneity of variance were carried out using SPSS version 10.0 for Windows (SPSS Inc., USA). *Post hoc* testing ($p < 0.05$) of the multiple comparisons was performed by either the Scheffé or Games-Howell test depending on whether Levene's test was insignificant or significant, respectively.

3. Results and discussion

3.1. Solubility study

ITZ has low solubility in both oil and water. The solubility of ITZ is 65 μg/mL and 140 μg/mL in olive oil and caprylic/capric triglyceride (CCT), respectively while that in deionized water is only 2.8 μg/mL. Good solubility of ITZ has been observed in organic solvents, i.e., 30,720 μg/mL, 28,400 μg/mL and 18,600 μg/mL for chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2) and carbontetrachloride (CCl_4), respectively. Among the organic solvents tested in this study, ITZ in chloroform demonstrates the highest solubility; therefore, chloroform was selected as an internal phase for the formation of nanoemulsions in this study. CCT was also used for comparison purpose.

3.2. Formation of (nano)emulsions

In this study (nano)emulsions were formed by a simple, conventional homogenization using Ultra-Turrax homogenizer, at a speed of 24,000 rpm. In general, the size of emulsion droplet formed by homogenization is controlled by the interplay between droplet break up and droplet coalescence (Dickinson, 2003; McClements, 2004). Droplet break up is controlled by the type and amount of shear applied to the droplets as well as the droplets resistance to deformation which is determined by the surfactant. The rate of droplet coalescence is determined by the ability of the surfactant to adsorb on the surface of newly formed droplets; this is governed by the surfactant concentration and the surface activity (McClements, 2004).

Pectin having amphiphilic character was used as a natural surface-active agent in this study. As a hydrocolloid, pectin contains hydrophobic groups that are numerous enough and sufficiently accessible on a short timescale to enable the adsorbing molecules to adhere to and spread out at the interface, thereby protecting the newly formed droplets (Dickinson, 2003). Hydrophobic characters attributable to methyl ester groups, acetyl groups, and amide groups should be considered in the case of pectin. Their limited emulsifying capacity can be attributed to poor solubility and/or insufficient amphiphilic character to produce substantial and rapid lowering of the interfacial tension during droplet break up. Moreover, pectin is a polysaccharide well known to effectively stabilize emulsions, e.g., acid drink milk products (May, 1999). Kravtchenko, Parker, and Trespoey (1995) reported that pectin can stabilize casein micelles above a concentration called 'the critical pectin level' by interacting with the protein thus preventing their aggregation as a result of steric repulsion forces which prevent the micelles from approaching each other. Dalgleish and Hollocon (1997) also observed that high DE pectin can stabilize emulsions containing caseinate against precipitation upon acidification, and this was attributed to the formation of a pectin layer on the casein-coated oil droplet surface as a result of interactions with absorbed casein molecules through electrostatic forces. Pectin may stabilize emulsion system against phase separation by increasing the viscosity of the aqueous phase and, therefore, retard droplet or particle movement. This stabilizes the system as long as its value remains high enough to counteract the forces responsible for phase separation (Parker, Gunning, Ng, & Robins, 1995). In this case, pectin adsorption leading to steric stabilization of oil droplets can take place.

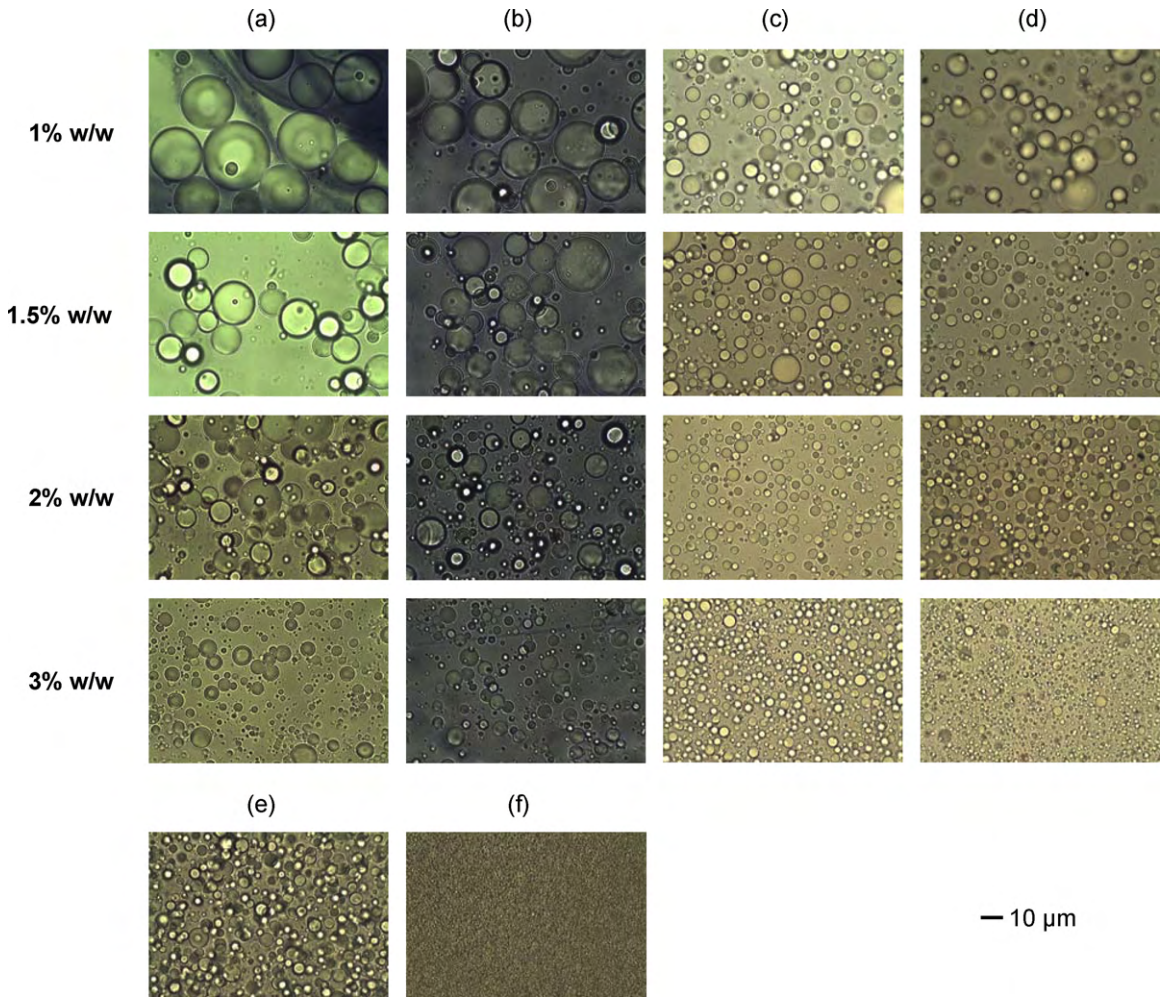


Fig. 1. Microscopic images of emulsions containing various types and concentrations of pectin; CCT-based emulsions without ITZ, prepared from (a) LMP, (b) ALMP, (c) HMP, and (d) emulsions with ITZ, prepared from HMP, chloroform-based emulsions containing 3% (w/w) HMP; (e) emulsions without ITZ, and (f) with 0.5% (w/w) of ITZ. Different concentrations of pectin were studied, i.e., 1, 1.5, 2 and 3% (w/w).

3.3. *Effect of internal phase*

As the solubility of ITZ in CCT is about 140 μg/mL, only 0.003% (w/w) of ITZ could be loaded in the formulation. The emulsion formulations those used chloroform as an internal phase could encapsulate higher amount of ITZ than those using CCT. Owing to very high solubility (about 30,720 μg/mL) of ITZ in chloroform, 0.5% (w/w) of ITZ was added in the formulation. Fig. 1 shows the microscopic images of emulsions containing various types and concentrations of pectin (without and with ITZ), using CCT or chloroform as an internal phase. All emulsions showed spherical polydispersed oil droplets. The morphology and size of ITZ-loaded CCT emulsions (Fig. 1d) were about the same as the emulsions without ITZ (Fig. 1c).

Different results were obtained when using different internal phases, i.e., the nano-sized emulsions were achieved when chloroform was used as an internal phase. The nanoemulsions obtained were spherical in shape and showed a polydisperse in the droplet size. ITZ-loaded formulation revealed very small droplets (Fig. 1f); the size was lesser than 900 nm, comparing to the size of the formulations without ITZ which were more than 10 μm. This indicated that the addition of ITZ to the emulsion formulation was very important to obtain the nano-sized emulsions. The reason for this behavior could be the influence of ITZ in the molecular association with pectin. It was reported that ITZ presents an amine or triazole group that may interact with the carboxyl group of pectin (Yi et al., 2007). As the non-polar region of ITZ lay into oil droplet, it would enhance the van der Waals forces between the hydrophobic parts of pectin chain (particularly HMP). Therefore, ITZ could work as an additional surfactant, and a close-packed film composed of pectin and ITZ would take place. Fig. 2 demonstrates the possible mechanisms of the formation of

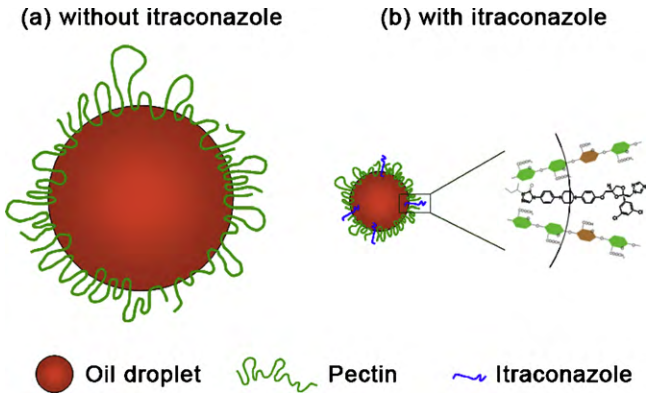
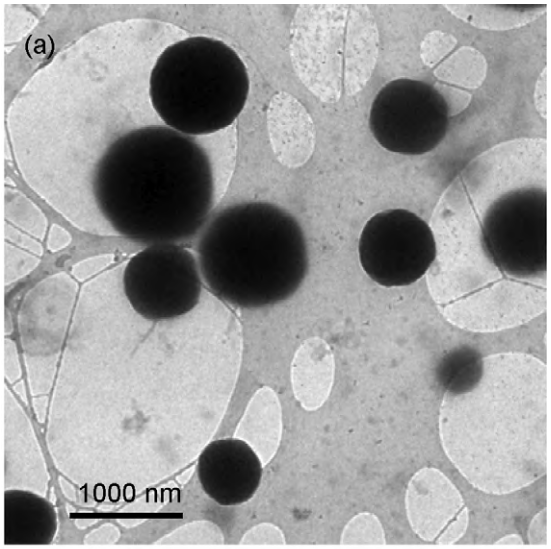
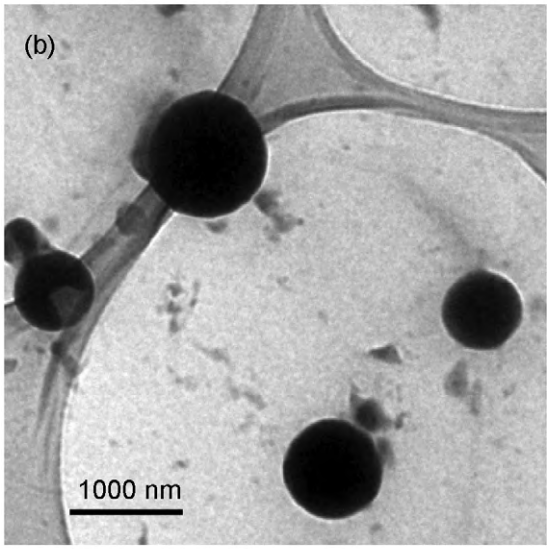


Fig. 2. Diagram showing possible mechanisms of the formation of nano-sized emulsions, using pectin as a polymeric emulsifier, loaded with itraconazole.





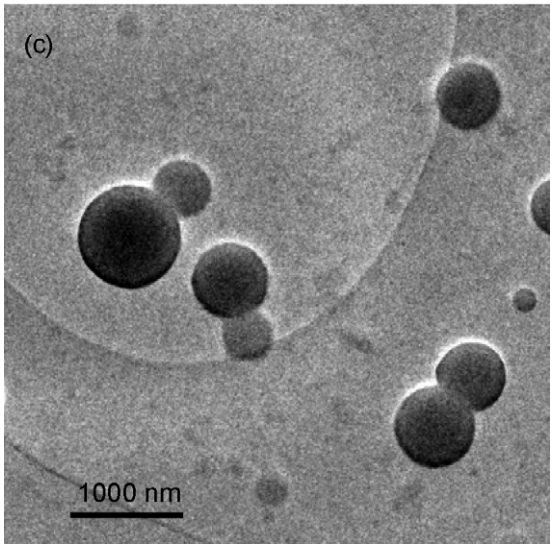
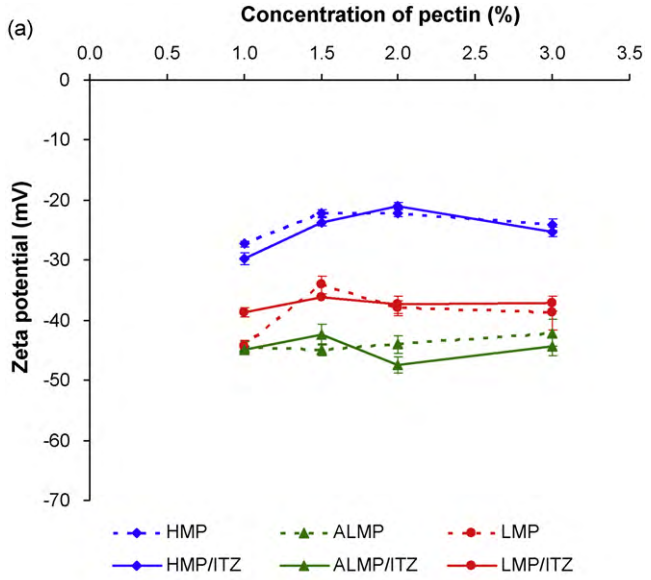


Fig. 3. Transmission electron microographs of ITZ-loaded nanoemulsions, using chloroform as an internal phase, containing various pectin solutions; (a) 3% (w/w) LMP, (b) 3% (w/w) ALMP and (c) 3% (w/w) HMP. The drug loading is 0.5% (w/w).



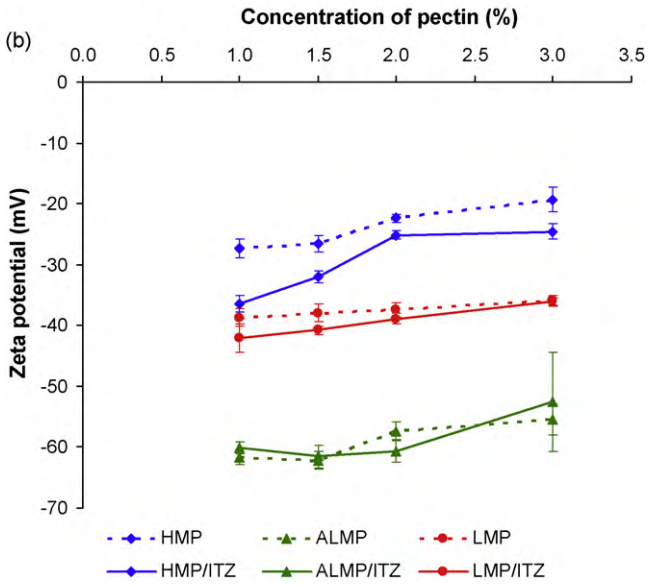


Fig. 4. Zeta potential (mV) of emulsions containing various types and concentrations of pectin, using using (a) CCT or (b) chloroform as an internal phase, without and with ITZ ($n = 3$). The zeta potential of pectin solutions was -27.06 ± 1.13 mV for HMP, -33.66 ± 1.26 mV for LMP and -58.85 ± 1.08 mV for ALMP.

nanoemulsions, using pectin as a polymeric emulsifier, loaded with ITZ.

Nano-sized emulsions were selected and investigated by transmission electron microscope (TEM). Fig. 3 shows transmission electron micrographs of nanoemulsions prepared from 3% (w/w) of various types of pectin, loaded with 0.5% (w/w) ITZ, after air-drying on TEM grid and coating with carbon film. The TEM images revealed that the oil droplets were spherical and their size was less than 900 nm. Moreover, the dark appearance of the ITZ-loaded nanoemulsion droplets may result from the metal elements with higher atomic number (i.e., Cl) of ITZ which increased electron density in the droplets resulting in higher contrast. This phenomenon is not observed in the emulsion droplets without ITZ (data not shown).

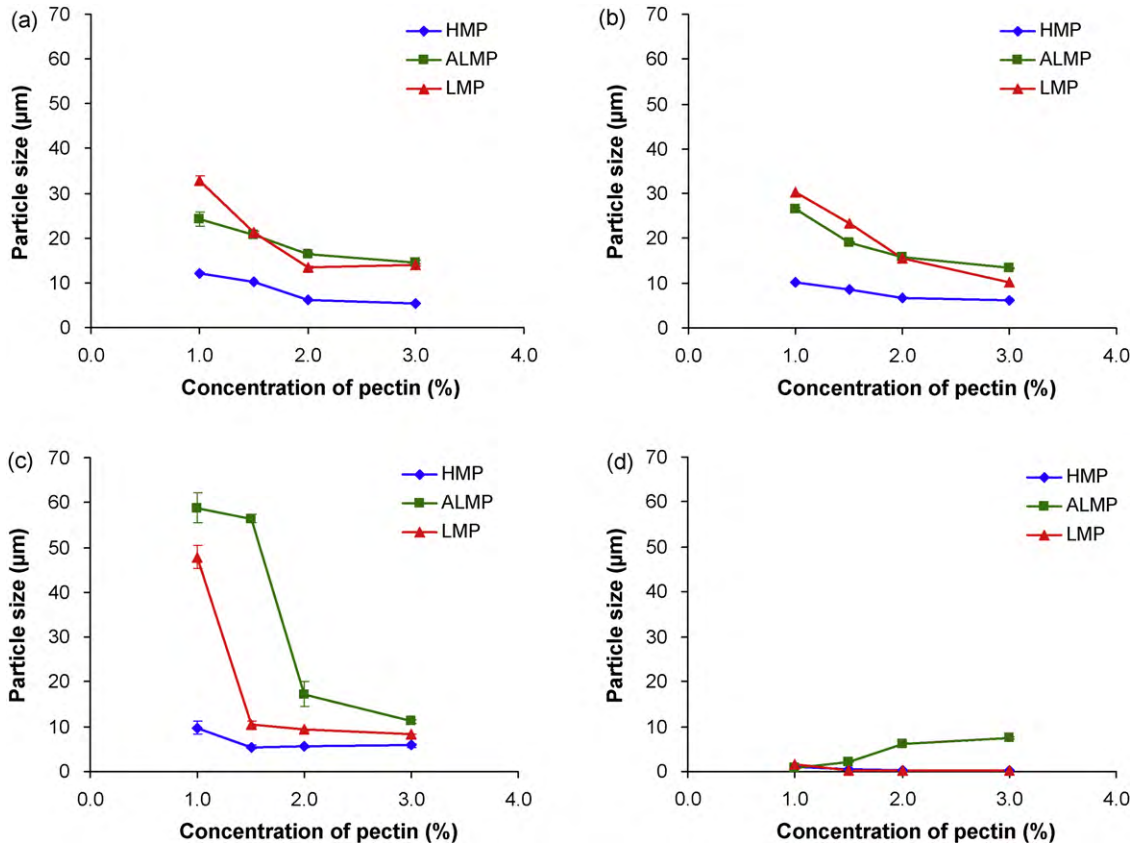


Fig. 5. Effect of type and concentration of pectin and the addition of ITZ on particle size (as investigated by light scattering method) of emulsions prepared by the simple homogenization; emulsions, using CCT as an internal phase, (a) without ITZ, (b) with ITZ, and emulsions, using chloroform as an internal phase, (c) without ITZ, and (d) with ITZ.

3.4. *Effect of pectin type*

Pectin typically showed negative charge due to carboxyl groups embedded on its molecules. Fig. 4 shows the zeta potential values (or surface charge) of the emulsions containing various types and concentrations of pectin, using CCT or chloroform as an internal phase. Zeta potential of emulsions was changed upon pectin type, i.e., the zeta potential of emulsions containing HMP was about −20 mV to −27 mV, while that of HMP was -27.06 ± 1.13 mV. LMP showed more negative charge due to the higher amount of free carboxyl group which was not substituted by methyl group therefore its zeta potential was -33.66 ± 1.26 mV. ALMP demonstrated the lowest zeta potential of -58.85 ± 1.08 mV. The zeta potential of emulsions containing LMP or ALMP was insignificantly changed when different concentrations were used; the zeta potential was similar to which was investigated in pectin alone.

CCT-based formulations using HMP provided emulsions with smaller droplet size, about 2–10 μm (Fig. 1c) while those using LMP and ALMP showed similar droplet size, ranged from 8 μm to 25 μm. For instance, the formulations containing 1% (w/w) LMP or ALMP provided emulsion droplets with the median diameter of about 20 μm while those containing HMP presented smaller oil droplets with the median diameter of about 10 μm (Fig. 1). The formulations prepared from chloroform, with no ITZ, showed the same results as those prepared from CCT (data not shown). Size of emulsions containing various types and concentrations of pectin, using CCT as an oil phase, is shown in Fig. 5. Emulsion prepared from CCT demonstrated similar droplet sizes regardless of the addition of ITZ (Fig. 5a and b). This is probably due to a very small amount of ITZ was loaded in the oil phase. HMP revealed remarkable size reduction effect, which the smallest droplets were obtained when using 3% (w/w) pectin. However, nanoemulsions could not be prepared from CCT by the simple homogenization method. The smallest droplet size obtained was about 5 μm, as investigated by the static light scattering method.

Fig. 5c and d shows the size of emulsions containing various types and concentrations of pectin using chloroform as an internal phase. Using only pectin, without ITZ, cannot produce nanoemulsions (discuss above). The droplet sizes were decreased significantly (Fig. 5d) when ITZ was added to the formulations. It

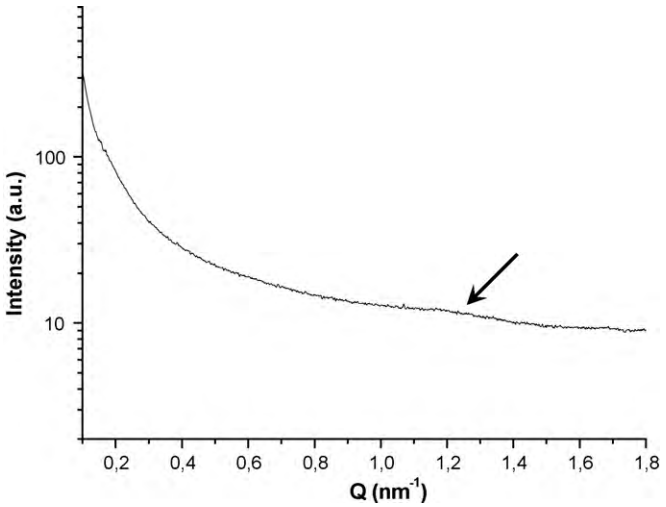


Fig. 6. A typical SAXS-scattering curve of emulsions: emulsions without drug prepared from 3% (w/w) HMP, using CCT as an internal phase.

is thought that the incorporation of ITZ into internal phase led to an additional input of surfactant at the o/w interface, resulting in breaking the droplets into smaller ones (Formiga et al., 2007). The formulations using both HMP and LMP showed the same effect. It should be noted that, in some cases, there is a difference between the droplet size measured by a light microscopic method and that by a light scattering method. The size investigated by a light scattering method was about 6 times larger than that measured by a light microscope, especially in the formulations without ITZ that used ALMP and LMP (data not shown). This may be due to the instability of the emulsions during sample preparation process which the emulsions had to be diluted with water before measurement by a light scattering method. The oil droplet may coalesce or fuse together, hence, the larger droplets were formed. However, this effect was less found in emulsions prepared using HMP and ITZ-loaded formulations. The results suggested that, using HMP or loading of the drug into the formulation resulted in more stable emulsions via steric effect (Lutz, Aserin, Wicker, & Garti, 2009).

With ITZ loading, the diameter of spherical droplets from chloroform-based nanoemulsions using HMP was about 200–900 nm (Fig. 3c). The higher amount of hydrophobic groups in the HMP may result in a greater emulsifying properties capable of making smaller droplets. Using LMP in the formulation caused slightly larger droplets (Fig. 3a). Although LMP has lower amount of hydrophobic groups compared to HMP, its lower molecular mass with better solubility in the aqueous continuous phase might help to increase the emulsifying properties and thus cause small droplets. On the other hand, the oil droplets obtained from the formulation using ALMP (Fig. 3b) were larger than those using HMP and LMP. Moreover, the fragments of pectin wall and drug crystals were observed, resulting from the rupture of oil droplets during the air-drying process for TEM observation.

The results from the size measurement and TEM images indicated that pectin with high DE (HMP) may provide superior property for preparation of nanoemulsions as it offers suitable properties, e.g., high amount of hydrophobic molecules which can

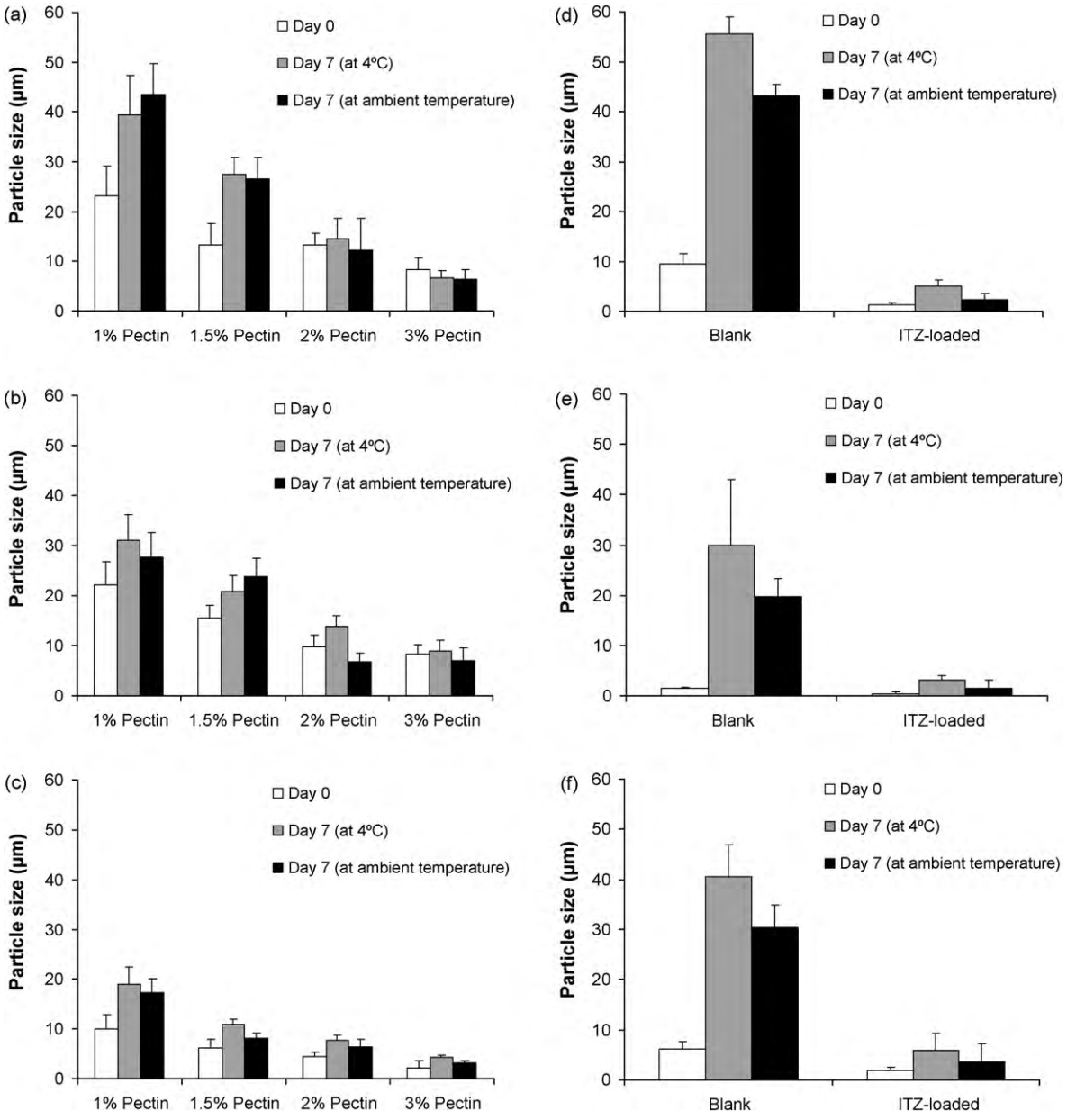


Fig. 7. Effect of storage conditions on the particle size of ITZ-loaded emulsions; CCT-based emulsions containing various concentrations of (a) LMP, (b) ALMP, (c) HMP, and chloroform-based emulsions containing various concentrations of (d) LMP, (e) ALMP, and (f) HMP.

cause better emulsifying effect, high molecular weight which provided more emulsion stability. ALMP may not be suitable for the nanoemulsion formulations due to its low emulsifying properties. Addition of ITZ demonstrated very high effect on decreasing of emulsion droplet size. It is possible that ITZ acts as a fine solid particle, in the same manner as bentonite and veegum (Akhtar et al., 2002; Funami et al., 2007), that could locate on the surface of oil droplets and exhibited emulsifying properties.

3.5. Effect of pectin concentration

Increasing of pectin concentration in the formulation tended to decrease zeta potential of emulsions. It is thought that the increase of a negatively charged polysaccharide like pectin in the emulsion formulation results in an increase in negative repulsive forces (electrostatic and steric) between oil droplets. Therefore, the presence of pectin can reduce the interfacial tension and form a cohesive interfacial film around the emulsion droplets thereby retarding emulsion instability (Funami et al., 2007). Higher concentrations of pectin produced more stable emulsions in which pectin was tightly bound onto the oil droplets, then the amount of free carboxyl group was decreased. The results were the same between the emulsions those used CCT and chloroform, suggesting that the type of the internal phase was not significantly influenced the zeta potential.

As seen from Fig. 1, the increased concentration of pectin in CCT-based emulsions decreased the diameter of oil droplets to about 8 μm for LMP and ALMP, and 2 μm for HMP. These results suggested that the DE have a greater effect to the size of emulsion droplets than the type of substituted molecule on pectin chain. The results from a static light scattering (Fig. 5) also showed a decrease of the droplet size with the increased concentration of pectin. For the formulations that used chloroform as an internal phase, the pectin concentration of 1.5–3% (w/w) yielded nano-sized emulsions with the droplet size of about 200–400 nm. The size of emulsion droplets also decreased with the increased concentration of the pectin solution (Fig. 5).

The relationship between emulsion droplet size and pectin concentration can be explained in terms of polymeric surfactant surface coverage. At low concentrations of pectin, there was insufficient pectin, newly formed droplets coalesced and the emulsion droplet size was influenced by the pectin concentration. Initially increasing pectin concentration resulted in a large decrease in particle size (Fig. 5) because more pectin is able to stabilize the newly formed droplets. Once there is an excess of pectin, at about 2% (w/w) pectin, the rate of the decrease in emulsion droplet size with increasing pectin concentration was diminished. This is because the concentration of pectin in the bulk is sufficient to allow rapid diffusion and adsorption of the pectin to newly formed droplets. The further increase in pectin concentration only led to a small increase in pectin adsorption and hence a small decrease in droplet coalescence (Dickinson, 2003).

3.6. SAXS experiments

Fig. 6 shows a typical SAXS-scattering curve of the nanoemulsions. The other formulations of the emulsions both from CCT and chloroform also show only a broad peak at the scattering vector Q of about 1.2 nm^{-1}, hence the emulsions were formed as a simple emulsion without any crystalline structure. These results were also in agreement with that from the microscopic measurements. If the emulsion has an ordered system, e.g., lamellar liquid crystalline phase, cubic phase or hexagonal phase, there would be typical reflections at the characteristic spacing ratios, i.e., $\sqrt{2}:\sqrt{4}:\sqrt{6}:\ldots$ or $\sqrt{2}:\sqrt{3}:\sqrt{4}:\sqrt{6}:\ldots$ or $1:\sqrt{3}:\sqrt{4}:\sqrt{7}:\ldots$, respectively, as reported in our previous studies (Gramdorf et al., 2008; Willumeit et al., 2005). On the contrary, in some reports (Gramdorf et al., 2008;

Table 1
Percent creaming of CCT-based and chloroform-based emulsions without and with itraconazole (ITZ), prepared from various types of pectin.

Internal phase	Drug	Pectin concentration (%)	HMP			ALMP			LMP		
			Day 0	Day 7 (at 4 °C)	Day 7 (at ambient temperature)	Day 0	Day 7 (at 4 °C)	Day 7 (at ambient temperature)	Day 0	Day 7 (at 4 °C)	Day 7 (at ambient temperature)
CCT	Without ITZ	1	100	87.8	31.71	100	37.5	36.59	100	35.56	34.09
		1.5	100	97.56	92.68	100	61.54	52.38	100	75	41.86
		2	100	100	97.67	100	100	95	100	100	92.11
		3	100	100	100	100	100	100	100	100	100
	With ITZ	1	100	81.4	32.43	100	36.36	36.59	100	60	34.09
		1.5	100	97.67	88.37	100	50	50	100	80	41.86
		2	100	100	97.67	100	100	75	100	100	91.43
		3	100	100	100	100	100	100	100	100	100
Chloroform	Without ITZ	1	95	5	5	N/A	N/A	N/A	N/A	N/A	N/A
		1.5	97.5	5	5	N/A	N/A	N/A	N/A	N/A	N/A
		2	97.5	5	6.67	N/A	N/A	N/A	N/A	N/A	N/A
		3	100	5	6.67	100	13.16	15	100	12.5	100
	With ITZ	1	100	12.5	11.43	N/A	N/A	N/A	N/A	N/A	N/A
		1.5	100	87.5	100	N/A	N/A	N/A	N/A	N/A	N/A
		2	100	95.24	100	N/A	N/A	N/A	N/A	N/A	N/A
		3	100	100	100	100	15	20	100	86.84	100

Note: N/A, not applicable.

Rodríguez-Abreu et al., 2007; Wörle et al., 2007) the emulsions or dispersions have shown the well-ordered system. This may be due to the type of the triglyceride used or other components that have been added.

3.7. Stability test

The stability of emulsions was investigated. Freshly prepared emulsions were milky white in color and showed 100% cream in all preparations. For the CCT-based emulsions, after 7-day storage, the size of oil droplets changed to larger when kept at the ambient temperature (22 °C) and at 4 °C (in a refrigerator). Different types of pectin did not influence the stability of emulsions in both conditions, according to the size of oil droplets (Fig. 7), and percent creaming (Table 1). However, it was influenced by the concentration of pectin. Using at least 2% (w/w) of pectin could produce the stable oil-in-water emulsions for 7 days when kept at 4 °C. It appears that pectin at a high concentration is efficient for stabilization of the CCT-based emulsions, i.e., the formulations using 3% (w/w) pectin showed the highest percent creaming (100% cream) when kept at 4 °C or at ambient temperature (22 °C). Emulsions using low concentration of pectin, e.g., 1 and 1.5% (w/w), were separated into two phases in both conditions within 7 days.

The results indicated that when ITZ was added into the formulations, not only the droplet size was reduced but also the stability (against the creaming) was enhanced to some extent, especially for chloroform-based nanoemulsions using HMP. Although freshly prepared emulsions were found to be stable in all formulations, after the stability test the emulsions with ITZ provided more stable emulsions with low creaming rate (i.e., high percent creaming), as shown in Table 1 and Fig. 7. The storage conditions also affected the emulsion stability; emulsions kept at 4 °C were more stable than at ambient temperature, especially the ITZ-loaded nanoemulsions consisted of 3% (w/w) HMP. This may be due to the same reason as the size reduction effect which occurred when ITZ was added into the emulsions, ITZ may play a role as a co-emulsifier that could improve emulsion stability. ITZ-loaded nanoemulsions also demonstrated smaller droplet size and higher percent creaming than the nanoemulsions without ITZ, in all types of pectin. HMP-based nanoemulsions showed the most stable characteristics and may be the most suitable formulation for further study in the future. The addition of a lipophilic or amphiphilic agent in a submicron-sized emulsion can lead to an important physicochemical change on its behavior (Yi et al., 2007). Sometimes, this procedure can induce an increase on the stability of the emulsion, as also observed in this study.

4. Conclusions

In this study, nanoemulsions were formed by a simple homogenization. Pectin, which is a natural surface-active agent, was used as a polymeric emulsifier. Nano-sized emulsions were achieved when chloroform was used as an internal phase while using CCT can produce only micron-sized emulsions. Pectin with high DE (HMP) provided good emulsion properties according to 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 important to obtain the nano-sized emulsions, resulting from the molecular association between ITZ and pectin. Using HMP or loading of the drug into the formulation resulted in more stable emulsions via steric effect. It appears that 3% (w/w) pectin provided the most stable emulsion with the highest percent creaming. The obtained nanoemulsions could be formulated as self-emulsifying drug delivery system (SEDDS) as a unit dosage form. We are continuing our experiments with these systems, in particular to fabricate dried nanocapsules from nanoemulsion templates.

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