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Characterization of Itraconazole Semisolid Dosage Forms Prepared by Hot Melt Technique

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The objective of this study was to formulate itraconazole semisolid dosage forms and characterize their physicochemical properties. Itraconazole and excipients such as polysorbate 80, fatty acids, fatty alcohols, oils and organic acids were melted at 160°C. The fused solution was then cooled immediately at -10°C to make wax-like semisolid preparations. Their physicochemical attributes were first characterized using differential scanning calorimetry, Fourier transform infrared spectroscopy and nuclear magnetic resonance spectrometry. The solubility of itraconazole in semisolid preparations and their dispersability in the simulated gastric fluid were also determined. Our semisolid preparations did not show any distinct endothermic peak of a crystalline form of itraconazole around 160-163°C. This suggested that it was changed into amorphous one, when it was formulated into semisolid preparations. In addition, the distinctive functional peaks and chemical shifts of itraconazole were well retained after processing into semisolid preparations. It could be inferred from the data that itraconazole was stable during incorporation into semisolid preparations by the hot melt technique. In particular, itraconazole semisolid preparations composed of polysorbate 80, fatty acids and organic acids showed good solubility and dissolution when dispersed in an aqueous medium. It was anticipated that the semisolid dosage forms would be industrially applicable to improving the bioavailability of poorly water-soluble drugs.

Key words: Itraconazole, Semisolid dosage forms, Instrumental characterization, Emulsion size, Solubility

INTRODUCTION

Itraconazole is a potent antifungal agent used to treat various fungal diseases including histoplasmosis, blastomycosis and onychomycosis (Koks *et al.*, 2002). On the tenets of the Biopharmaceutics Classification Systems, itraconazole belongs to class II drugs characterized with low water solubility and high permeability (Amidon *et al.*, 1995). Because of poor dissolution in the gastrointestinal tract, its oral administration is faced with large inter-individual variations in bioavailability. Therefore, enhancing the dissolution rate of itraconazole is an important task for its formulation development (Serajuddin, 1999). In many cases, the low drug solubility arises from its crystalline structure. Changing a crystalline form into an amorphous state often enhances drug solubility and dissolution, which are prerequisites for reliable and good therapeutic responses (Rambali *et al.*, 2003; Heo *et al.*, 2005; Six *et al.*, 2005).

To improve dissolution rate, itraconazole has been formulated with various carriers including Eudragit E100, HPMC, phosphoric acid and hydroxypropyl-b-cyclodextrin by hot-melt extrusion (Woo and Yi, 2000; Peeters et al., 2002; Rambali *et al.*, 2003; Six *et al.*, 2005). Even though itraconazole became amorphous, these solid-type preparations were often too brittle or rigid to be processed into final dosage forms. It was also reported elsewhere that common excipients such as oils, fatty acids, surfactants and organic acids could be used as semisolid carriers to enhance dissolution of various hydrophobic drugs (Kim *et al.*, 2002; Cao *et al.*, 2003; Heo *et al.*, 2005; Wang *et al.*, 2005). It is emphasized that the usefulness of a semisolid preparation depends on the ability to form a fine, submicron-sized emulsion *via* self-emulsifying process when exposed

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to gastrointestinal fluids (Charman *et al.*, 1992; Nazzal *et al.*, 2002; Heo *et al.*, 2005).

This study aimed at developing itraconazole semisolid dosage forms containing a mixture of surfactant and fatty acid to increase its solubility. A hot melt technique was employed to make semisolid preparations with use of various excipients. Their characteristics were then evaluated by DSC, FT-IR and ^{1}H-NMR experiments. Physical behaviors of semisolid preparations were also investigated in terms of solubility, dispersability, and propensity for self-emulsification in the simulated gastric fluid.

MATERIALS AND METHODS

Materials

Itraconazole was kindly donated from Choongwae Pharma. Corp. (Seoul, Korea). Polysorbate 80, sodium lauryl sulfate, citric acid and malic acid were purchased from Sigma Chemical Co. Ltd. (St. Louis, U.S.A.). Medium chain triglyceride (MCT; Captex®) was purchased from the Nissan Oil Mills, Ltd (Tokyo, Japan). Transcutol was obtained from BASF (Ludwigshafen, Germany). Oleic acid and lauric acid were purchased from Showa Chemical Co. Ltd. (Tokyo, Japan). Polyethylene glycol with a molecular weight of 400 (PEG400) was obtained from Hayashi Pure Chemical Industry Ltd. (Osaka, Japan). Other inorganic and organic compounds were of reagent grade and used as received without further purification.

Preparation of semisolid forms by a hot melt technique

Semisolid preparations consisting of binary components were made by mixing itraconazole and excipients at 1:3 ratios, melting at 160°C and immediately placing inside a deep freezer (-10°C) for 10 min. In addition to binary semisolid preparations, four different types of semisolid preparations were also produced according to the following formulations: M1, itraconazole/oleic acid/citric acid/polysorbate 80 (1/1/2/10, by weight); M2, itraconazole/lauric acid/citric acid/polysorbate 80 (1/1/2/10); M3, itraconazole /oleic acid/polysorbate 80 (1:3:10); and M4, itraconazole/ oleic acid/malic acid/polysorbate 80 (1:1:2:10). The final semisolid preparations were aged at room temperature for 24 h. The wax-like semisolid preparations were milled three times with Three Roll Milling Unit (Exakt Co., Norderstedt, German)

DSC study

The thermal behaviors of itraconazole, various excipients and semisolid preparations were investigated using DSC (DSC2910, TA Instruments, Dupont, U.S.A. in the Central Laboratory, Kangwon National University) with nitrogen as purge gas. Before analysis, the instrument was calibrated for temperature and heat flow using indium. An accurately weighed sample was placed inside a standard open aluminum pan and heated from 20 to 200°C at a heating rate of 10 °C/min.

FT-IR study

FT-IR spectra were measured using an IR spectrophotometer (Excaliber UMA-500, Bio-Rad, Cambridge, U.S.A. in the Central Laboratory, Kangwon National University). About 10 mg of a sample was mixed with 200 mg of KBr and compressed into a pellet using a hydraulic press. FT-IR spectra over the scanning range of 400 to 3600 cm^{-1} were obtained with the resolution of 2 cm^{-1} (Excaliber series; Bio-Rad, Cambridge, U.K).

^{1}H-NMR study

Chemical shifts of itraconazole and its semisolid preparations dissolved in $CDCl_3$ were measured with a Varian Gemini 200 (Central Laboratory, Kangwon National University) at 200 MHz in a Fourier transform mode.

Physical properties of semisolid preparations

Solubility

The solubility of itraconazole in semisolid preparations was investigated by a dispersion method using the gastric fluid. One hundred milligram of a semisolid preparation was added into a test tube containing 10 mL of the gastric fluid (pH 1.2 ± 0.1). The mixture was vortexed to disperse homogeneously and then centrifuged at 15,000 g for 20 min. The content of intraconazole in the supernatants, defined as the degree of solubility in this study, was measured by the HPLC method given below.

Dispersability and compatibility

One hundred milligram of a semisolid preparation was placed inside a UV cell to which was added 0.8 mL of the simulated gastric fluid. When the mixture was shaken, the semisolid preparation was spontaneously transformed into a fine emulsion. The dispersability of a semisolid preparation into emulsion droplets and its propensity for precipitation were observed by visual inspection immediately and after 1 week.

Emulsion size

The size of emulsion droplets was determined immediately and after 1 week. The instrument used for this experiment was a Par-III laser particle analyzer (Otsuka, Japan).

Assay of itraconazole

A reverse phase HPLC system was used for itraconazole analysis. HPLC consisted of the pump (Jasco PU-980), the UV-VIS spectrophotometric detector (Jasco UV-975), the autosampler (Jasco AS-950-10), the reverse

phase column (Hansil® ODS 150 × 4.6 mm, 5 mm) and the integrator (Borwin® 1.20 software). The mobile phase was composed of acetonitrile and 0.1% diethylamine solution (65:35, by v/v) and its flow rate was maintained at 1 mL/min. Twenty microliter of a sample was injected by the autosampler, and the elution of itraconazole was detected at 263 nm. Econazole was used as an internal standard. A standard calibration curve was constructed

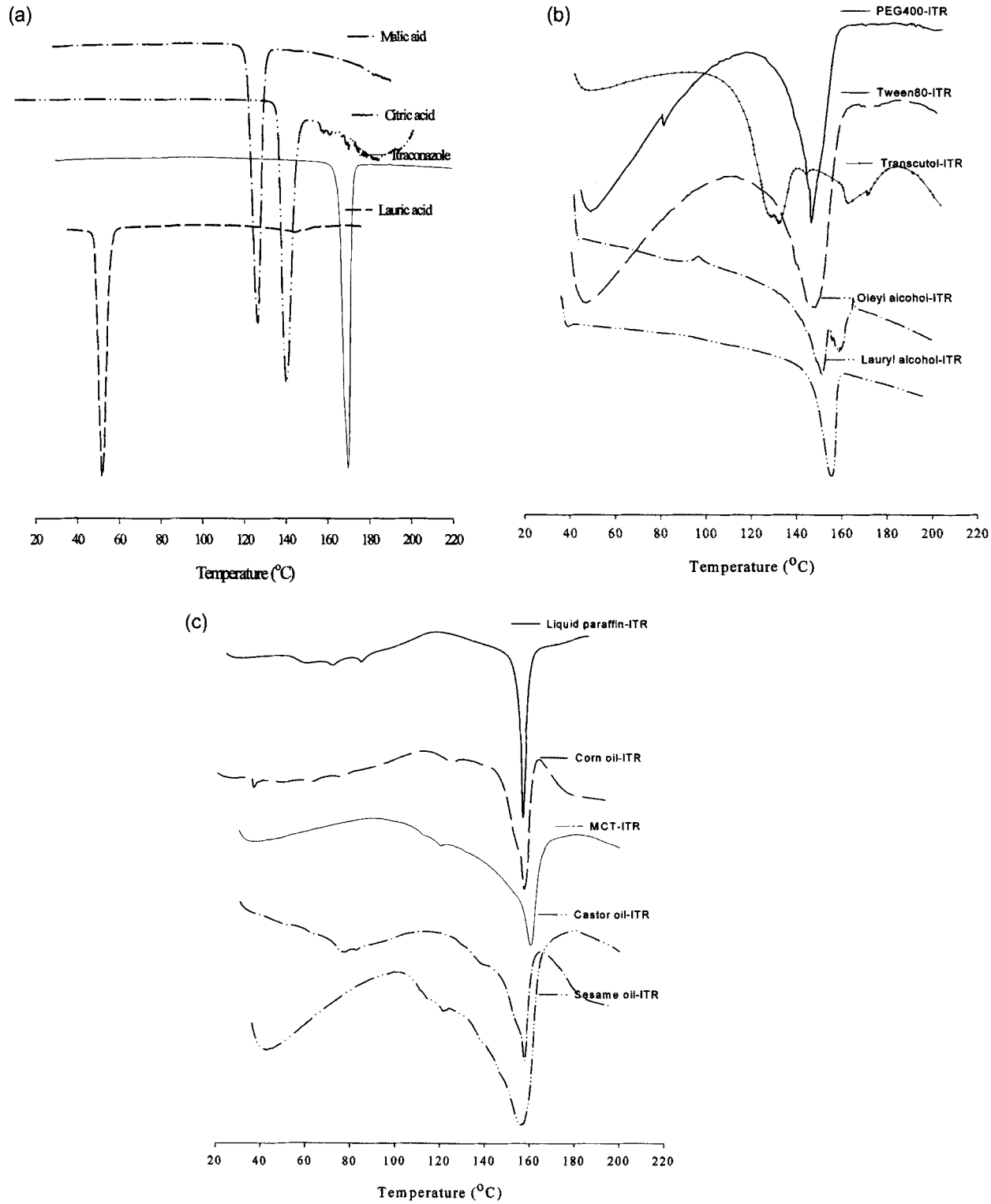

Fig. 1. DSC thermograms of itraconazole, excipients, and its semisolid preparations: (a) shows thermograms of individual samples before processing into semisolid preparations. (b), and (c) illustrate thermograms of binary semisolid preparations composed of itraconazole and various excipients.

using known itraconazole concentrations ranging from 0.05 to 25 mg/mL.

Statistical analysis

All data were presented as mean ± standard deviation. The statistical significance of differences was performed using student t-test. A probability level at 5% ($p < 0.05$) was considered to be statistically significant.

RESULTS AND DISCUSSION

DSC thermal analysis

The physiochemical interaction between itraconazole and various excipients was evaluated by DSC. Fig. 1 shows DSC thermograms of itraconazole, excipients, and binary semisolid preparations. Itraconazole powders showed a sharp endothermic peak at 163°C, representing its crystalline nature. The endothermic peaks of citric acid and malic acid were noticed at 135-150°C. The binary semisolid preparations composed of itraconazole and polysorbate 80, PEG400, fatty alcohols, or saturated aliphatic oils failed in changing a crystalline form of itraconazole into an amorphous form: an endothermic peak of itraconazole was still observed with the binary systems.

Interestingly, semisolid preparations composed of itraconazole, fatty acids, organic acids and polysorbate 80 drastically changed thermal behaviors of itraconazole (Fig. 2). An endothermic peak of the crystalline itraconazole form was not observed with semisolid formulations with M1, M2, M3 and M4. This is indicative of a physical change of itraconazole from crystalline to amorphous forms. It is likely that fatty acids including oleic acid and lauric acid play a vital role in the structural change. Organic acids such as citric acid and malic acid seem to have synergic effects. It was anticipated that the formation of an amorphous structure in the semisolid preparations would contribute to enhancing the solubility and dissolution of highly lipophilic drugs such as itraconazole.

FT-IR analysis

FT-IR analysis was made to itraconazole before and after processing to semisolid preparations, in order to detect interactions between itraconazole and excipients (Fig. 3). Major, specific FT-IR spectra of itraconazole powders were noticed at 400-1800 cm^{-1}. They might have arisen from the stretching and vibration of functional groups such as -C = C- of aromatic groups. A peak observed at 1600-1800 cm^{-1} is attributed to -C = O stretching and vibration, whereas peaks for alkane and amine groups were noticed at 2800-3200 cm^{-1}. Peaks of lauric acid, oleic acid, and polysorbate 80 at 2800-3000 cm^{-1} were still observed with M1, M2, M3 and M4 semisolid preparations. Also, major peaks observed with

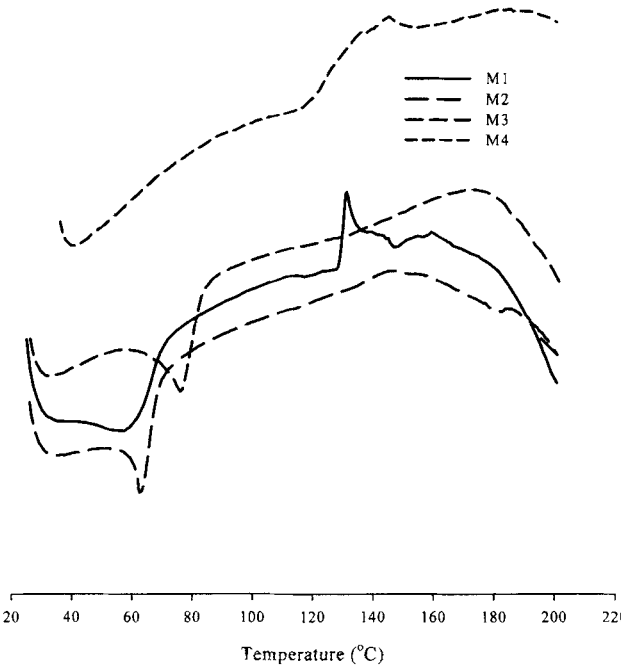

Fig. 2. DSC thermograms of the semisolid preparations with M1, M2, M3, and M4 formulations

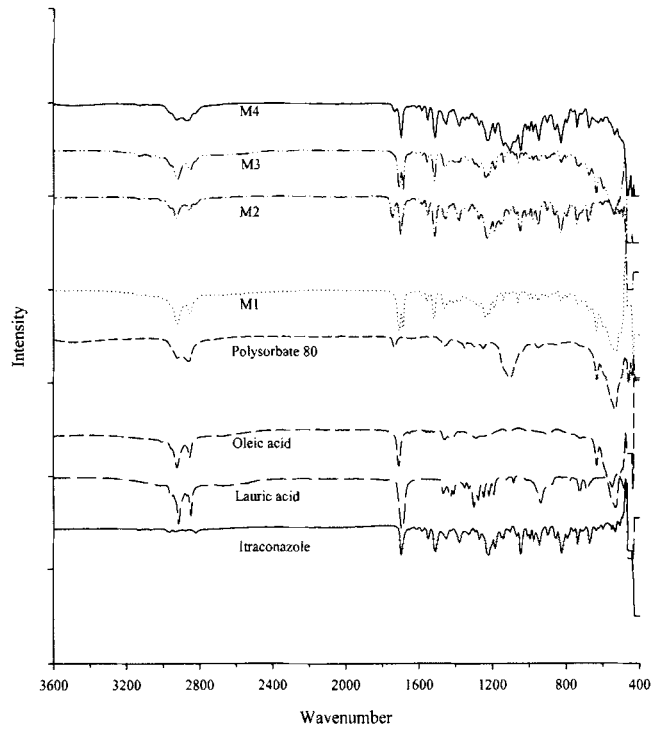

Fig. 3. FT-IR spectra of itraconazole, excipients, and its semisolid preparations

itraconazole before and after loading to the semisolid preparations at 400-1800 cm^{-1} were almost superimposible. This suggested the absence of any significant interactions between itraconazole and the excipients used to make the semisolid preparations.

^{1}H-NMR analysis

Fig. 4 shows ^{1}H-NMR spectra of itraconazole and semisolid preparations. Even though slight peak shifts and overlapping occurred due to molecular interactions between itraconazole and excipients, the NMR spectra of the semisolid preparations demonstrated the intrinsic peaks of itraconazole. Therefore, it could be concluded that any pronounced chemical reactions did not occur when a crystalline form of itraconazole was converted into an amorphous form by the semisolid preparations.

Physical properties

Shown in Table I are physical properties of some semisolid preparations with regard to solubility, emulsion size and dispersability in the gastric fluid. The solubility of a binary mixture consisting of itraconazole and polysorbate 80 was 0.57 mg/mL in the gastric fluid. Previously, it was reported elsewhere that the aqueous solubility of itraconazole at pH 1.2 was 4 mg/mL (Peeters *et al.*, 2002). Considering such negligible water solubility of itraconazole, it is clear that the binary system brings about a substantial increase in its solubility. It was also found that, under our experimental condition, the nonionic surfactant had much better solubilizing power than did an ionic surfactant such as sodium lauryl sulfate. The binary system also showed a relatively good stability without any precipitation during storage. After 1 week's storage, however, the average size of emulsion droplets slightly increased.

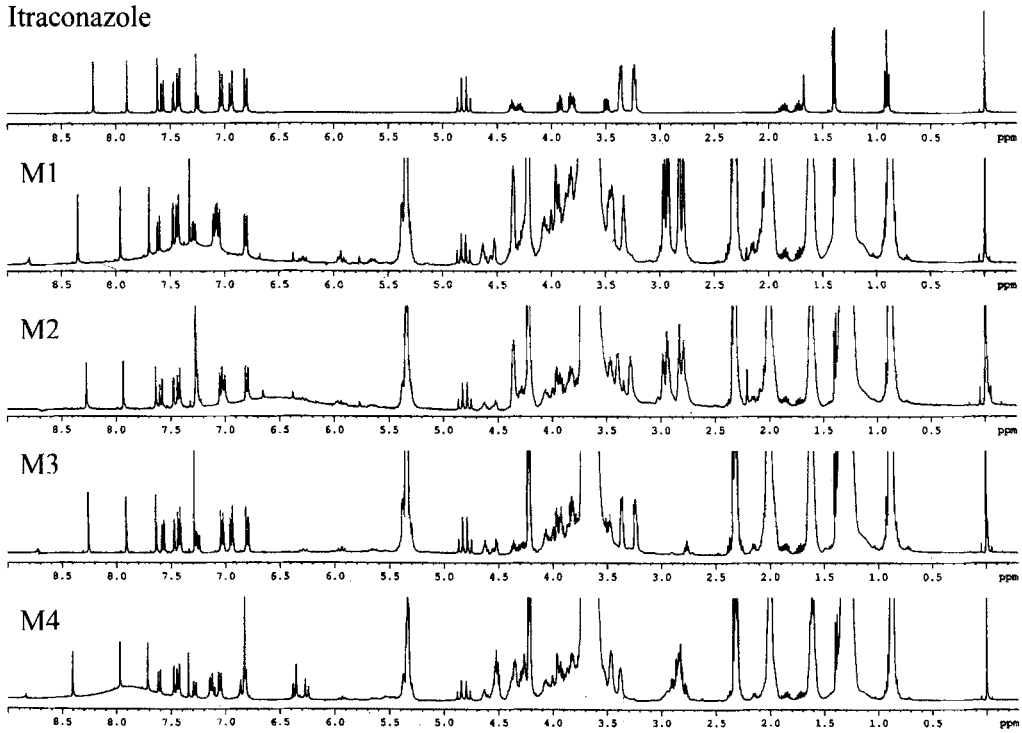

Fig. 4. ^{1}H-NMR spectra of itraconazole and its semisolid preparations dissolved in $CDCl_3$

Table I. Formulation effects of semisolid preparations upon itraconazole solubility at the gastric fluid (pH 1.2) and the size of their emulsion droplets observed at days 0 and 7

Compositions	Solubility (mg/mL)	Emulsion size (nm)	
		day 0	day 7
Itraconazole/polysorbate 80	0.57±0.62	240±41	310±65[b]
Itraconazole/polysorbate 80/oleic acid	0.92±0.81[a]	230±32	250±32
M1 (itraconzaole/oleic acid/citric acid/polysorbate 80)	2.10±0.29[a]	200±12	220±25
M2 (itraconazole/lauric acid/citric acid/polysorbate 80)	1.24±0.31[a]	190±31	200±27
M3 (itraconazole/oleic acid/polysorbate 80)	1.52±0.12[a]	250±23	280±41
M4 (itraconazole/oleic acid/malic acid/polysorbate 80)	0.95±0.13[a]	200±19	250±35[b]

[a]Significantly different from the itraconazole/polysorbate 80 ($p<0.05$).
[b]Significantly different from the day 0 ($p<0.05$).

Surprisingly, the solubility and dispersability of semisolid preparations containing polysorbate 80, fatty acids and organic acids were significantly improved as compared with ones without organic acids. As discussed before, a change into an amorphous form of itraconazole in hot melted dispersions might have played a role in enhancing its solubility (Woo and Yi, 2000; Rambali *et al.*, 2003; Six *et al.*, 2005). Furthermore, when dispersed in the gastric fluid, the semisolid preparations were readily transformed into submicron-sized emulsions with an aid of fatty acid and surfactant (Charman *et al.*, 1992; Nazzal *et al.*, 2002; Heo *et al.*, 2005;). At this time, neither aggregation nor precipitation were noticed. The sizes of emulsion droplets ranged from 200 to 300 nm, depending on the formulation of semisolid preparations. In particular, the M1 semisolid preparation showed the greatest improvement in itraconazole solubility. Furthermore, there was no significant change in the size of emulsion droplets after 1-week storage.

CONCLUSIONS

The semisolid preparations composed of a highly lipophilic itraconazole and various excipients were produced, and their physicochemical properties were characterized. Fatty acids, organic acids and polysorbate 80 were found to be effective excipients useful for their preparation. The semisolid dosage forms made it possible to change a crystalline form of itraconazole into an amorphous one. The data of FT-IR and NMR indicated that the chemical stability of itraconazole was well retained after processing into the semisolid preparations. When dispersed in the gastric fluid, the semisolid preparations were readily transformed into submicron-sized emulsions. It was anticipated that this simple, easily scalable semisolid preparations would be industrially applicable to enhancing the bioavailability of itraconazole.

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