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Brief/Technical Note

Study of Polymorphs of Progesterone by Novel Melt Sonocrystallization Technique: A Technical Note

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Abstract. A large number of pharmaceuticals exhibit polymorphism; 23% steroids, 60% sulfonamides, and 70% of barbiturates have shown this property. In this study, we have investigated and compared a new technique termed as melt sonocrystallization (MSC) with melt and sonocrystallization (SC) for induction of polymorphism in progesterone (PRG). Polymorphs were characterized by DSC, XRD, FT-IR, and FT Raman spectroscopy. Melt sonocrystallized progesterone (MSC-PRG) contained both the polymorphs, more soluble form II along with less soluble form I, whereas melt progesterone (M-PRG) and sonocrystallized progesterone (SC-PRG) contained only form I. Improvement in dissolution characteristics of both the polymorphs were compared and form II was found to be more readily soluble than form I in deionized water. Reduction in mean particle size of PRG during SC was also determined using laser diffractometer. During stability testing (40°C/75% RH) for 1 month, metastable form II of MSC-PRG was found to be transformed into its more stable state. MSC technique was thus found as a useful tool for induction of polymorphism.

KEY WORDS: melt sonocrystallization; polymorphism; progesterone; sonocrystallization.

INTRODUCTION

Polymorphism is a characteristic feature which can affect various characteristics of a molecule-like solubility, dissolution rate, stability, hygroscopicity, and bioavailability (1). As different polymorphs may have different bioavailability, much emphasis has been given on their study by both scientific and regulatory communities. A special attention was paid on drug polymorphism during the International Conference on Harmonization (ICH) 1999, under ICH Q6A, where more focus was given on the control of crystal form and use of appropriate analytical procedure to detect and characterize different forms of a drug substance (2). Classical epitome of drug polymorphism is chloramphenicol palmitate, wherein metastable form B has eightfold higher bioactivity than form A. It has also been reported that form B may cause fatal side effects (3).

The study of drug polymorphism has become vital, as it can directly affect the drug's *in vivo* performance. Considering this impact, new methods have been evaluated to predict the nature of drug polymorphism, both during drug and drug product development stage. Progesterone (PRG) is prescribed in various gynecological conditions. It has now been proven that PRG exhibits polymorphism. Two polymorphs, form I (α-form) and form II (β-form), of PRG have already been reported. Form I has melting point at 128°C, whereas form II has melting endotherm at 122°C (4–8). Thermodynamically, form I (α-form) is more stable than form II (β-form). Considering PRG's poor oral bioavailability, the study and selection of appropriate polymorph of PRG are one of the promising ways to improve its *in vivo* performance (9,10). Therefore, PRG was selected as a model drug for this study.

Particle engineering techniques based on ultrasound have recently been coined in the field of pharmaceutical technology to improve drug solubility. Application of ultrasound during crystallization leads into uniform mixing, which results in homogenous growth of crystals with low variation in particle size and morphological characteristics. Due to its effect on initial nucleation process, it reduces the width of metastable zone and initiates the process of nucleation at lower level of supersaturation (11–13). In our previous study, we obtained porous ibuprofen and celecoxib particles by use of melt sonocrystallization (MSC) technique (14–16). However, application of MSC as a tool to predict the polymorphism needs to be explored in depth. Therefore, this study was undertaken to check the utility of MSC and sonocrystallization (SC) as tool to investigate drug polymorphism.

MATERIALS AND METHODS

Materials

Natural micronized PRG (form I) was supplied by Uni-Sankyo Ltd. Loteparshuram, India. All other reagents used were commercially procured and were of the purest grade.

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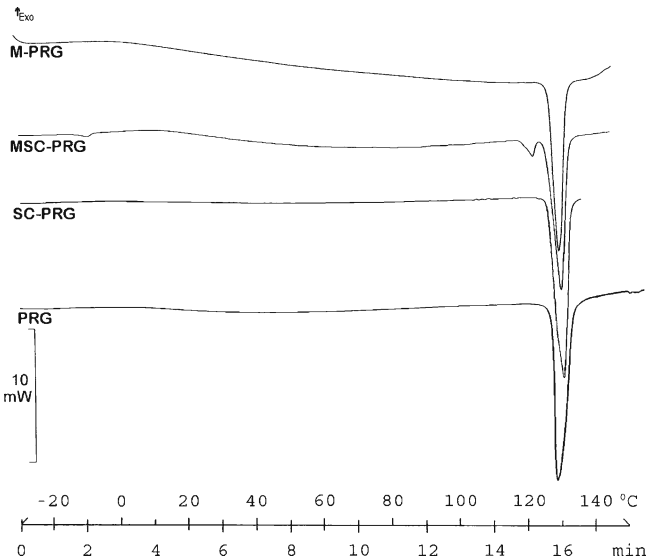

Fig. 1. DSC thermogram of PRG, SC-PRG, MSC-PRG, and M-PRG of progesterone

Methods

Melt Sonocrystallized Progesterone

PRG was allowed to melt at 150°C, following which it was poured into 50 ml of deionized water maintained at 5°C using cryostatic bath Phoenix C25P (Haake, Karlsruhe, Germany). It was sonicated for 2 min using probe ultrasonicator IKASONIC U 200S (IKA Labortechnik, Staufen, Germany) operated at an amplitude of 80% and cycle of 0.8 per second. The solidified product was separated by filtration and dried overnight at 40°C.

Melt Progesterone

Melt progesterone (M-PRG) was prepared by the same procedure; the only difference is that sonication was not performed.

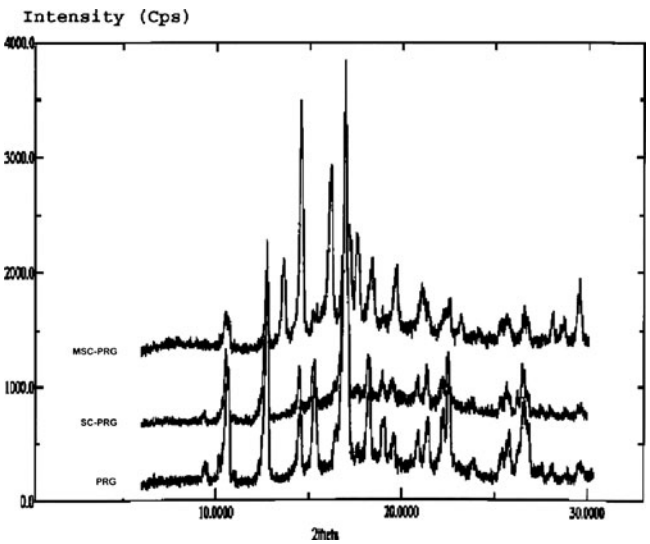

Fig. 2. X-ray diffractogram of three different samples of progesterone

Table I. Comparison of X-Ray Diffraction Patterns for SC-PRG and MSC-PRG with Their Corresponding Values Reported for Form I and Form II

d-Spacing (Å)	I/I_0 (%)	d-Spacing (Å)	I/I_0 (%)	d-Spacing (Å)	I/I_0 (%)
Reported (7)		JCPDS-37-1690		SC-PRG	
9.3706	4	9.29043	2	9.4204	6
8.3231	31	8.26839	37	8.4020	41
6.9479	62	8.00005	2	6.9806	79
6.0826	22	6.90544	64	6.1161	25
5.7711	22	6.28131	1	5.7974	26
5.2387	100	6.05082	22	5.4102	9
5.0178	5	5.73906	26	5.2603	100
4.8728	17	5.36622	6	5.2172	71
4.6620	9	5.22187	100	5.0377	5
4.5391	8	5.17348	74	4.8781	24
Reported (7)		JCPDS-37-1691		MSC-PRG	
8.2457	5	11.2484	1	8.4259	14
6.3747	26	8.39354	9	8.2842	12
5.9405	100	6.44507	59	6.9479	27
5.4102	86	6.02626	100	6.4911	35
5.1010	23	5.60910	8	6.0785	100
4.9787	41	5.46479	96	5.8088	7
4.7612	24	5.13188	37	5.6396	5
4.4556	21	5.01104	61	5.4868	65
4.1467	16	4.80126	38	5.2325	52

JCPDS Joint Committee on Powder Diffraction Standards

Sonocrystallized Progesterone

Sonocrystallized progesterone (SC-PRG) was prepared by the same procedure; the only difference is that PRG was dissolved in the minimum volume of methanol instead of melting.

Polymorph Characterization

Differential Scanning Calorimetry. Differential scanning calorimetry (DSC) thermograms of M-PRG, melt sonocrystallized progesterone (MSC-PRG), SC-PRG, and PRG were obtained using Mettler-Toledo DSC 821e (Mettler-Toledo, Greifensee, Switzerland) instrument equipped with an intracooler RP-110 (LabPlant, Huddersfield, England). Indium standard was used to calibrate the temperature and enthalpy scale. Powder samples were hermitically sealed in perforated aluminum pans. They were cooled to −30°C and subsequently heated with a constant rate of 10°C/min up to a temperature of 150°C. Inert atmosphere was maintained by purging nitrogen at the flow rate of 100 ml/min. Results were analyzed with the software STAR^e (Mettler-Toledo, Greifensee, Switzerland).

X-ray Powder Diffraction. The X-ray powder diffraction (XRD) patterns of PRG, MSC-PRG, and SC-PRG were recorded on a Miniflex goniometer (Rigaku Corporation, Tokyo, Japan). Samples were analyzed between 3° and 30° (2θ). Experimental conditions were as follows: X-ray Cu; 30 kV and 15 mA; scattering and receiving slit, 4.2 deg. and 0.3 mm, respectively. The scan range and scan speed were 4×10^3 CPS and 2°/min, respectively. Kb filter was used during continuous-scan mode.

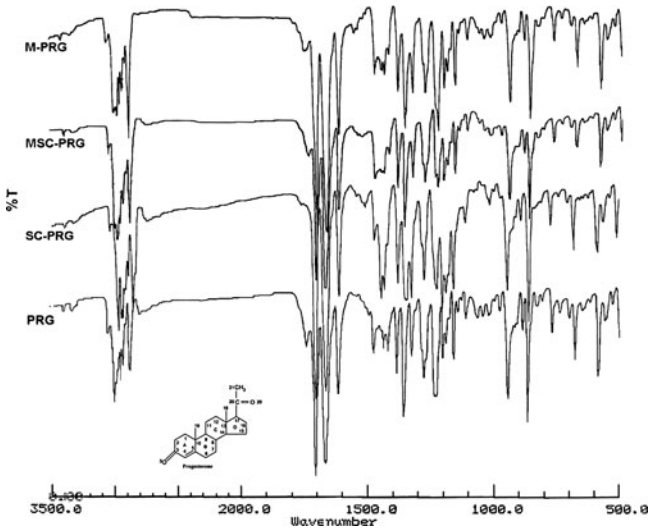

Fig. 3. FT infrared spectrum of PRG, SC-PRG, MSC-PRG, and M-PRG and molecular structure of progesterone

Fourier Transformed Infrared Spectroscopy. The pellets were prepared on KBr press (Spectra Lab, Thane, India). The Fourier transformed infrared spectroscopy (FT-IR) spectra were recorded over the wavenumber range of 3,500–500 cm^{-1} using Jasco V5300 FT-IR (JASCO Inc., Tokyo, Japan).

Fourier Transformed Raman Spectroscopy. FT Raman spectra of samples were recorded on portable series R-3000HR FT Raman Spectrometer (Raman System Inc., Austin, USA) equipped with a gallium arsenide (GaAs) laser diode (785 nm) as the excitation source, thermoelectrically cooled, low-resolution diffraction grating, and high-sensitivity diode array detector. The powder samples were examined using the fiber-optics probe accessory. Sample was placed into a small self-sealing transparent polybag. For each spectrum, 10 scans were performed at a resolution of 6 cm^{-1} over the dynamic range of 2,400 (stokes)–200 cm^{-1} (antistokes).

Particle Size Analysis. Particle size was measured by laser diffractometer, Mastersizer 2000 Ver.2.00 (Malvern Instruments, Malvern, UK). Analysis was done in triplicate, and mean results are presented. PRG-saturated deionized water was used as dispersant media, and obscuration was not less than 10% for each measurement. The measurements were based on Mie theory and refractive index of PRG was 1.52 and that of dispersant was 1.33. Analysis was performed under the size range of 0.02~2,000 μm. Size distribution was expressed as volume median diameter (VMD) and span. Data analysis was done by Malvern Software (Ver 5.2).

In Vitro Dissolution Studies. In vitro dissolution study was performed using USP 26 Type II dissolution test apparatus (Electrolab TDT-06P, Mumbai, India). PRG, SC-PRG, M-PRG, and MSC-PRG equivalent to 10 mg drug were suspended in 10 ml of the dissolution media and placed in the dissolution vessel containing deionized water as dissolution media, and final volume was made to 900 ml, maintained at 37±0.5°C, and stirred at 100 rpm. Samples were collected periodically and replaced with a fresh dissolution medium. Aliquots were filtered through 0.45-μm filter paper; concentration of PRG was determined spectrophotometrically at 248 nm. Dissolution study was conducted in triplicate (n=3) and reported as mean±standard deviation. Analysis of data was done using "PCP Disso v2.08" software (Poona College of Pharmacy, Pune, India).

Stability Study. Stability study of MSC-PRG was performed by storing it in a Petri dish with perforated foil at 40± 0.5°C and 75±5% RH for 30 days as per ICH guidelines. It was evaluated using DSC.

RESULTS AND DISCUSSION

Melting of PRG followed by rapid cooling and sonication during MSC technique produces agglomerates whereas SC technique produces fine powder of PRG. Slight changes in sonication parameters (amplitude and cycle) did not exhibit major difference in results; therefore, all data have not been shown.

Differential Scanning Calorimetry

DSC thermogram of the samples is shown in Fig. 1. The observed average value for melting endotherm of SC-PRG and M-PRG were 130°C and 129°C, respectively, which is consistent with the reported value for form I. Also, the heat of fusion of SC-PRG and M-PRG was 88.50 and 85.66 J/g, respectively, slightly higher than the reported value of 83.2 J/g for form I (7). The appearance of two endothermic peaks at 124.69°C and 131.74°C in the case of MSC-PRG gives clear indication of the presence of two polymorphs in the sample.

The secondary endothermal peak at 124.69°C of MSC-PRG corresponds to form II (reported value 122°C), having variable enthalpy, which was 3.13 J/g lower than the reported value of 68.73 J/g (7). This may be due to the incomplete transformation in the crystal habit of PRG molecule during ultrasonication. Ultrasonic energy produces a significant increment in kinetic energy of the molecule, which brings about rapid collision among the particles, thus causing phase transformation. Enthalpy was used as parameter for quantitative calculation of polymorph, and it was calculated that MSC-PRG contains only 6.38% of form II (7). This could be due to incomplete transformation of one polymorph to another.

Table II. Comparison of –C=O– Stretching Mode at C-3 and C-20 Positions for PRG, SC-PRG, M-PRG, and MSC-PRG Obtained by FT-IR Spectrophotometer

S. no.	Carbon position	PRG (cm^{-1})	SC-PRG (cm^{-1})	M-PRG (cm^{-1})	MSC-PRG (cm^{-1})
1	C_3	1,662	1,658	1,662	1,662
2	C_{20}	1,700	1,700	1,701	1,708

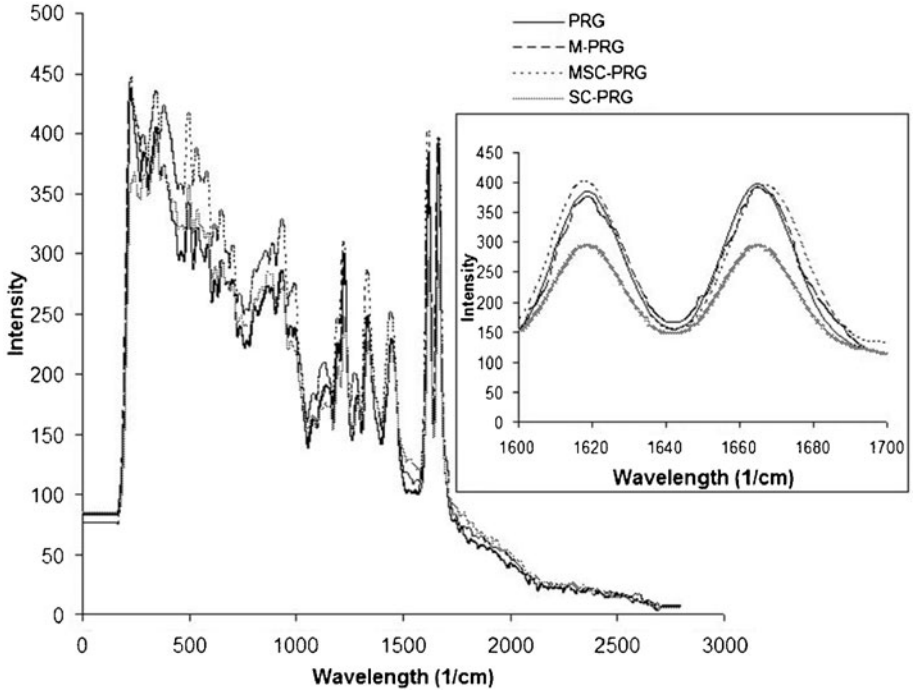

Fig. 4. Normal and expanded Raman spectrum of PRG, M-PRG, MSC-PRG, and SC-PRG

X-Ray Powder Diffraction

Diffractograms of PRG, SC-PRG, and MSC-PRG are shown in Fig. 2. Major d-spacing and I/I_0 ratio values in the Table I, for SC-PRG *vs* form I and MSC-PRG *vs* form II, were compared with those of the Joint Committee on Powder Diffraction Standards (7). The comparative values of d-spacing and $I/I_o(\%)$ for SC-PRG with form I could easily be observed (values underlined) in Table I. The absence of prominent d-spacing for form II in the SC-PRG data reveals the formation of form I as a lone polymorph of PRG during SC.

The same comparison of d-spacing and $I/I_o(\%)$ has been made for the MSC-PRG with form II (values underlined) in Table I. Surprisingly, the occurrence of d-spacing for form II along with form I has been seen for sample of MSC. XRD patterns in Fig. 2 also approve the presence of polymorphic form II in MSC-PRG samples. The verdict of hypothesis has been tried and tested that the melting of PRG and cooling rapidly below their melting point disturb the rearrangement of atoms. Atoms try to rearrange themselves in the most natural phase. Molten state of PRG was expected to show a random pattern of molecular arrangement, whereas rapid cooling of melt with the power of ultrasonication may accelerate the rearrangement pattern, which results in another unstable polymorphic form. However, the crystal habit is a dynamic and reversible phenomenon, which depends on ambient condition of storage. It was felt that melting along with sonication holds the necessary value for polymorphic transformation and enthalpy changes, as discussed previously under thermal analysis.

Fourier Transformed Infrared Spectroscopy

FT-IR spectra (Fig. 3) of PRG, SC-PRG, MSC-PRG, and M-PRG showed the presence of vibrations in the region of 1,600–1,900 cm^{-1} due to –C=O– stretching at C-3 and C-20 positions in PRG (17). The presence of the two most important stretching peaks, 1,662 and 1,700 cm^{-1}, at C-3 and C-20, respectively, in FT-IR of PRG has been shown (Table II). Table II shows a red shift at 1,658 cm^{-1} peak from its original one (1,662 cm^{-1}) and no change at 1,700 cm^{-1} peak in SC-PRG. The occurrence of two carbonyl stretching peaks (Fig. 3) gives the strong evidence of the presence of form I polymorph in PRG, SC-PRG, and M-PRG (18–20). However, the cause for the red shift in SC-PRG at C-3 was uncertain.

Appearance of two carbonyl peaks at 1,662 and 1,708 cm^{-1} in the FT-IR spectra of MSC-PRG confirmed the presence of both of ketonic groups at C-3 and C-20. Unexpected blue sift of C-20 at 1,708 cm^{-1} in MSC-PRG exhibits a change in the bonding state at molecular level. It is assumed that the blue shift of carbonyl peak by 8 cm^{-1} in MSC-PRG was associated with change of polymorphic form, i.e., form II along with form I (Table II). FT-IR spectrum revels that sonication or melting can produce single

Table III. Comparison of Stretching Mode in PRG, SC-PRG, M-PRG, and MSC-PRG; Obtained by Raman Spectrometer

Polymorph	Reported (cm^{-1}) (1,16)	Sample	Observed (cm^{-1})
–C=C– stretching at position 4 (C-4) of progesterone			
Form I	1,617	PRG	1,617
		SC-PRG	1,617
Form II	1,617	M-PRG	1,617
		MSC-PRG	1,617
–C=O– stretching at position 3 (C-3) of progesterone			
Form I	1,662	PRG	1,664
		SC-PRG	1,664
Form II	1,667	M-PRG	1,664
		MSC-PRG	1,667

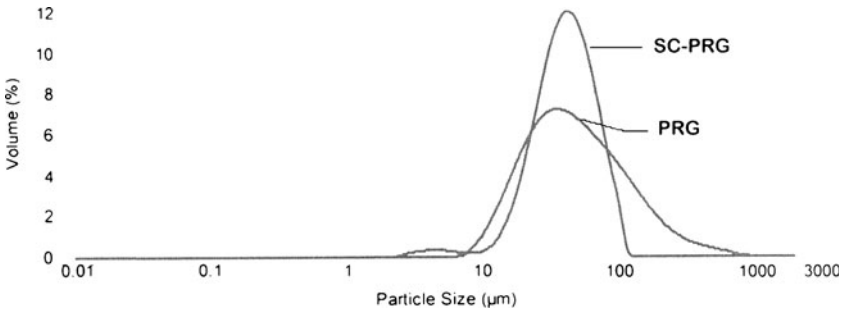

Fig. 5. Particle size analysis of PRG and SC-PRG

polymorph, whereas combination of both in MSC results into both the polymorphs of PRG. FT-IR study also revealed that both of the polymorphs differed with respect to bond length at their molecular state.

FT Raman Spectroscopy

Conjugation theory could explain the difference of Raman spectra between form I and form II (21). Delocalization of π electrons between –C=O– at position 3 and –C=C– at position 4 in the structure of PRG causes absorption at lower wavelength as compared to unconjugated system. Resonance of π electron along with bond dissipates the double-bond character for both of the positions in PRG. This delocalization of π electron causes vibration in the molecule and therefore results into absorption at lower wavenumber than those of the unconjugated system. Peak shift of polymorphs was used to set a calibration to correlate the peak position and to identify the concentration of polymorph in the processed sample (1). Polymorph induction was monitored using Raman spectroscopy, and obtained spectrum was analyzed (Fig. 4).

Stretching of –C=C– bond, due to delocalization of π electrons, was found at 1,617 cm^{-1} for both of polymorphic forms of PRG. The obtained peaks for PRG, SC-PRG, M-PRG, and MSC-PRG were exactly similar to the reported value of –C=C– stretching (Table III) (1). In Table III, a comparison has been made for –C=O– stretching at C-3 position. A slight deviation of peak 1,664 cm^{-1} was observed in PRG, SC-PRG, and M-PRG with respect to the reported value 1,662 cm^{-1} for form I (Fig. 4). However, strong resemblance of Raman values has been found between MSC-PRG and form II. The observed peak (Fig. 4) for –C=O– stretching of MSC-PRG was at 1,667 cm^{-1}, which was exactly similar to reported value for form II (1).

Raman shift indicates the difference of molecular packing/arrangement between the polymorphs at their molecular level. Successful Raman spectroscopy in Fig. 4 was utilized for the prediction of polymorphs of PRG in our samples.

Particle Size Analysis

With application of sonication, VMD was reduced from 71.067 μm (PRG) to 44.708 μm (SC-PRG) (Fig. 5). The key property of ultrasonication is cavitation, which causes microstreaming and forced particle acceleration under the influence of ultrasound shock wave. In SC, ultrasonication produces particles of narrow size range. The size range value of $PRG_{d(0.1)–d(0.9)}$=17.29–149.65 μm, whereas the power of ultrasonication was able to reduce size of particles under the size range of $SC\text{-}PRG_{d(0.1)–d(0.9)}$=20.58–74.85 μm. Alone, the span of SC-PRG ($Span_{SC–PRG}$=1.315) was much lower than that of PRG ($Span_{PRG}$=2.939). Particles collide with each other with such great force, which is responsible for breaking of particles. The magnitude of energy and time of exposure play the major role in the breakdown of particles and in obtaining particles of desired micromeritics (13). However, melting and rapid cooling offers the opportunity for the formation of irregular agglomerates of PRG during melting and MSC technique. These irregular agglomerates were big enough for the breakdown under the force of ultrasonication (data have not been shown).

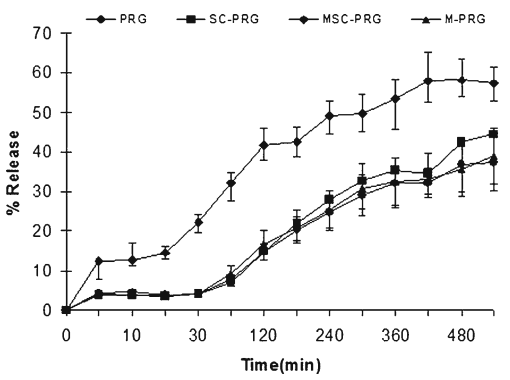

Fig. 6. *In vitro* dissolution study of PRG, SC-PRG, MSC-PRG, and M-PRG. *Bars* represents mean±SD (n=3)

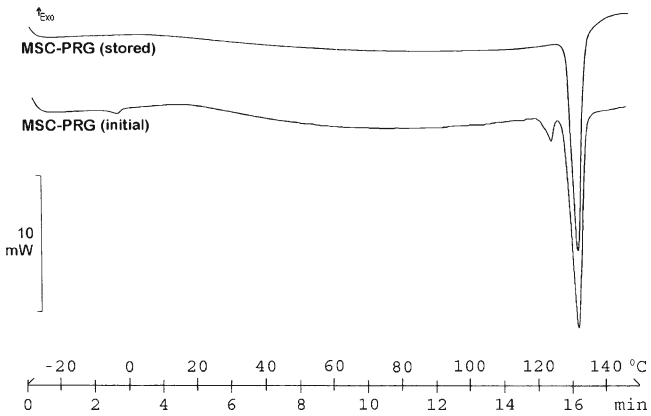

Fig. 7. DSC thermogram of MSC-PRG before and after storage at 40°C/75%RH for 1 month

Dissolution Studies

A 22% dissolution rate was observed in MSC-PRG whereas it was nearly 4% for PRG, SC-PRG, and M-PRG in 30 min (Fig. 6). Further, MSC-PRG, SC-PRG, and M-PRG were dissolved up to 54%, 45%, and 39%, respectively, as compared to 40% dissolution of PRG in 9 h. The presence of more soluble form II in MSC-PRG may be responsible for the observed enhancement in the dissolution as compared to form I (19). Further, MSC-PRG with form II might have provided faster dissolution if we could have obtained loose particles with lower particle size rather than agglomerates. The application of ultrasound might have resulted into porous surface structure and therefore lead to slight improvement in the dissolution of SC-PRG as compared to PRG and M-PRG, although all three possessed the same polymorph (form I).

Stability Studies

Disappearance of melting endotherm at 124.69°C of MSC-PRG strongly supports the metastable character in form II of PRG (Fig. 7). The presence of endothermic peak at 131.8°C (normalized energy 59.53 J/g) in 1-month storage sample of MSC-PRG revealed that form I was stable at ambient temperature. It was assumed that thermodynamic transformation of form II of MSC-PRG into form I occurred during storage. Literature also supports our finding for the lesser stable form II of PRG (1,19,20).

CONCLUSION

This study has coined the new application of MSC for the induction of drug polymorphism. MSC was successfully developed and utilized for the induction of metastable form II in PRG. SC was also carried out and was found to be useful for the comminution of poorly water-soluble drug. DSC, XRD, FT-IR, and Raman spectrum have confirmed the absence of form II in SC-PRG and M-PRG samples, which limits its role. Interestingly, the dissolution study revealed that dissolution rate of metastable form II was higher than form I. Thus, this study provides opportunity for generation of more soluble and subsequently more bioavailable polymorph of drug substances. Therefore, the MSC technique can be used as a tool to screen the possibility of existence of more soluble and stable drug polymorph. Although form II converted into form I during stability study, use of additional excipients can be used to stabilize form II. Thus, this study promotes the particle engineering technique like MSC for induction of drug polymorphism.

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