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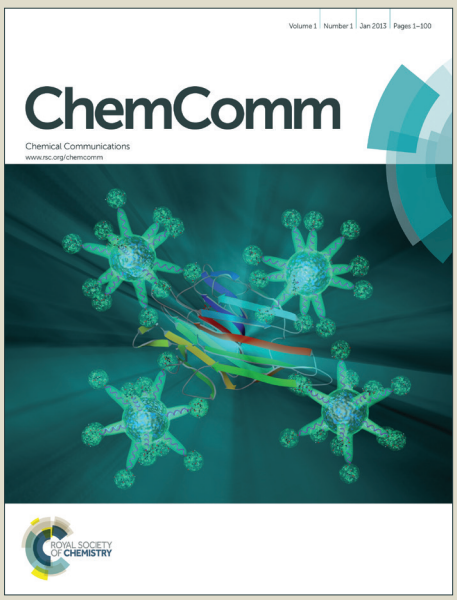

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ARTICLE TYPE

Co-crystal formation between poly (ethylene glycol) and small molecular drug griseofulvin

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Most of the pharmaceutical co-crystals are formed between drug molecules and small molecular compounds. Here, we demonstrated that small molecular drug griseofulvin and poly (ethylene glycol) can also form co-crystals.

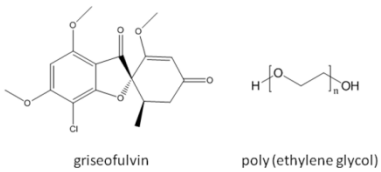

Fig.1 Structural formulas of griseofulvin and poly (ethylene glycol).

Pharmaceutical co-crystals are usually referred to crystalline molecular complexes formed between drug molecules and other pharmaceutically accepted compounds.[1] In recent years, pharmaceutical co-crystals have received increasing attention as a powerful means to improve the performance of drugs, especially the dissolution rate of poorly-soluble drugs.[2] However, so far most of the pharmaceutical co-crystals are composed of drugs and small molecular co-formers[3] such as succinic acid, nicotinamide, and saccharin. In contrast, co-crystals between drugs and polymers has only been reported in a few cases, including hydroxybenzenes-PEG co-crystals[4,5] and mavacoxib-PEG co-crystals[6], and it would be interesting to see if the drug-polymer co-crystal can be a general structure category and applicable to more drugs.

Here, we report co-crystal formation between small molecular drug griseofulvin (Gris) and polyethylene glycol (PEG) (Fig. 1). Gris is an antifungal drug, which has three known crystal forms (Form I, II and III) with melting points at 220, 214 and 205 °C, respectively[7]. PEG is a widely used polymer excipient in oral and injection drug products. In fact, Gris has been marked since 1970s in the form of a solid dispersion product as "ultramicrosized griseofulvin" dispersed in PEG matrix[8], but surprisingly few reports exist on the detailed crystallization behavior of this system.

In our study, a small amount of drug/polymer physical mixture (50/50 w/w, PEG MW 6000, see Supporting Information for details) between two glass cover slips was melted at 220 °C, quenched to predesigned temperatures and then annealed on a hot-stage equipped with a polarized optical microscope. When annealed at temperatures below 100 °C, spherulite crystals formed in the Gris/PEG co-melt. On heating, these crystals

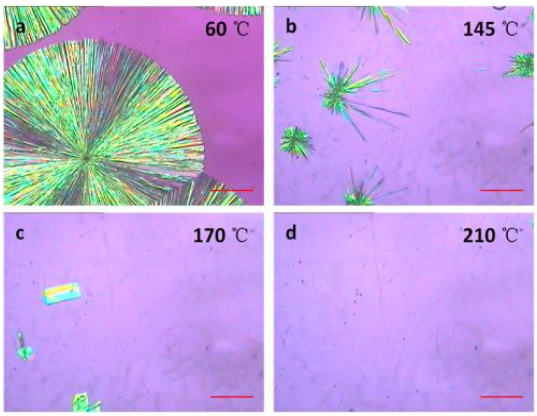

Fig.2 Crystallization behavior of Gris/PEG co-melt (50/50, w/w) under a hot stage microscope. **a** Spherulite crystals formed (60 °C); **b** Spherulite crystals melted (145 °C); **c** Recrystallization into flake-like crystals (170 °C); **d** Flake-like crystal completely melted (210 °C). The bars in these pictures represent 200 μm.

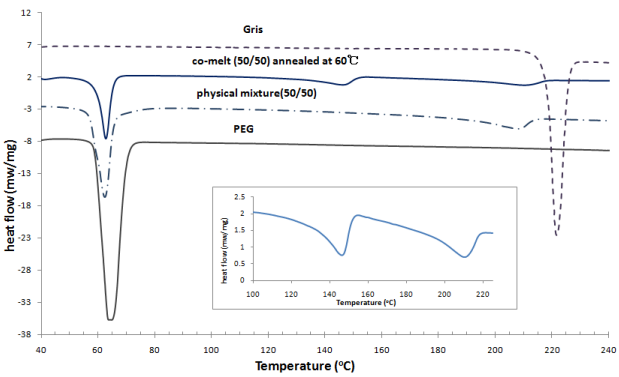

Fig.3 DSC thermograms of PEG 6000, griseofulvin and Gris/PEG 6000 systems (w/w) measured at a heating rate of 10 °C/min. The heating curve of the annealed sample showed an additional thermal event at about 145 °C (more clearly shown in the inset).

melted at about 145 °C, followed by recrystallization into flake-like crystals, which melted above 200 °C (Fig. 2). Compared with the known melting behavior of Gris crystals in the PEG matrix[9], the flake-like crystals are identified as Form I of Gris, while the spherulite crystals seemed to be a new crystal form. In contrast, when the sample was annealed at a temperature above 100 °C, the flake Gris crystals formed directly (data not shown).

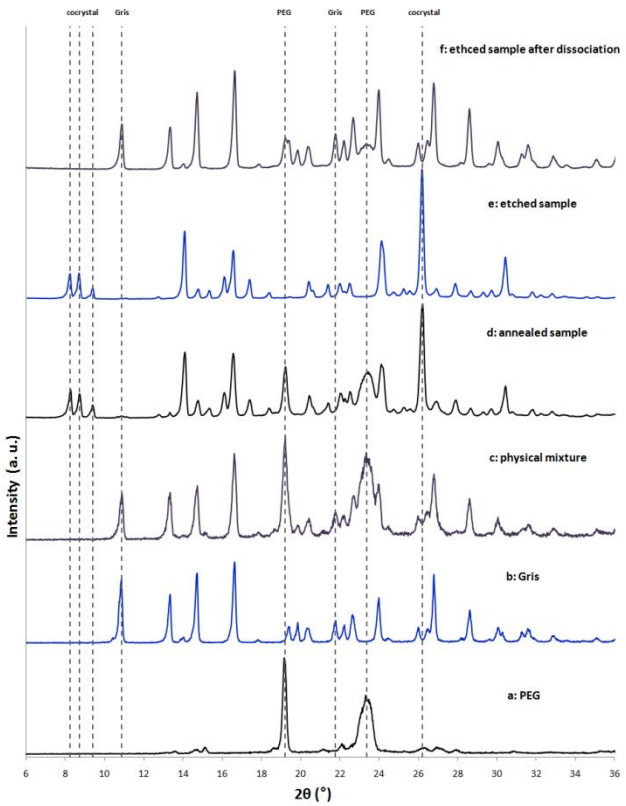

Fig.4 Powder X-ray diffraction patterns of **a** PEG 6000; **b** griseofulvin raw materials; **c** physical mixture (50/50, w/w); **d** co-melt (50/50, w/w) annealed at 60 °C; **e** annealed sample with excess PEG being etched by de-ionized water; **f** etched sample after dissociation.

Thermal analysis by differential scanning calorimetry (DSC) confirmed the hot-stage microscope (HSM) observations (Fig. 3). In the DSC heating scan, Gris/PEG (50/50, w/w) physical mixture showed two endothermal events around 64 °C and 205 °C, corresponding to the melting point of PEG and the depressed melting point of Gris crystals in PEG matrix, respectively, consistent with those observed in literature[9]. However, Gris/PEG co-melt (50/50, w/w) annealed at 60 °C showed an additional endothermal event around 145 °C during the heating scan, which corresponds to the melting behavior of spherulite crystals observed by HSM. Further searching the literature on griseofulvin crystallization, we found that similar phenomena were mentioned by Ford, in which two thermal events of crystallization were also observed in Gris/PEG physical mixtures during heating, but without further investigation on the nature of these phenomena[10].

Powder X-ray diffraction (PXRD) were used to distinguish between different crystal forms (Fig. 4, all measurements were performed at room temperature). The PXRD pattern of Gris raw material (Fig. 4b) was consistent with that of its Form I, and its physical mixture with PEG (50/50, w/w) (Fig. 4c) showed a simple combination of all the peaks from Gris and PEG. However co-melt of the same composition after annealed at 60 °C showed a different diffraction pattern (Fig. 4d), with absence of the main Bragg peaks characteristic of the pure Gris crystal form (Form I, e.g., at 2θ 10.8°, 21.7°), and presence of several new Bragg reflections (e.g., at 2θ 8.2°, 8.7°, 9.4° and 26.2°). The

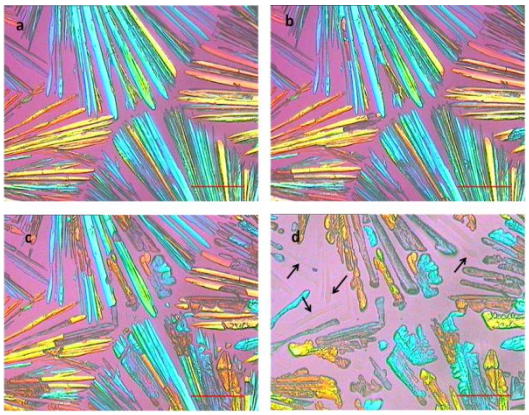

Fig. 5 Dissociation of the etched samples on a heating process. **a** Image of crystals prior to dissociation (r. t.). **b c** Images of crystals during dissociation (110 °C and 120 °C, respectively). **d** Image of crystals after dissociation (140 °C). Amorphous phase had been released from the crystal lattice as marked by black arrows. The bars in these pictures all represent 50 μm.

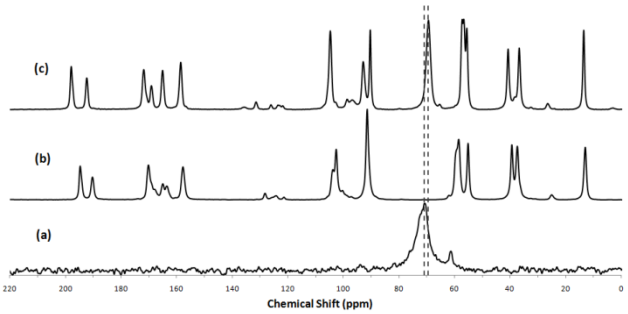

Fig.6 CP/MAS ^{13}C NMR spectra of **(a)** pure PEG 6000; **(b)** Gris Form I; **(c)** Gris/PEG co-crystal. The resonance peak of methylene carbon of PEG appeared at 70.8 ppm for neat crystalline PEG and 69.5 ppm for Gris/PEG co-crystal, as indicated by the dashed lines.

diffractogram is also different form the two recently discovered polymorphs of Gris[7] (e.g., Form II at 2θ 7.2° and 8.1°, and Form III 7.5°). The broad peaks at 2θ 19.1° and 23.3° characteristic of crystalline PEG still exist, suggesting excess PEG in this system.

The diffraction pattern of this new crystal form can be seen more clearly after the excess PEG was etched away by rinsing the sample with de-ionized water (Fig. 4e). No PEG diffraction peaks could be observed after etching, indicating the complete removal of excess PEG, at least at the XRD detection level. However, ^{1}H-NMR characterization of the etched samples (Fig. S1 in the Supporting Information) revealed that PEG still exists in these crystals even after etching, as indicated by the characteristic peak corresponding to CH_2 groups on PEG chains on ^{1}H-NMR spectrum, with the ethylene oxide/Gris molar ratio around 2. This suggests that, the new crystal form might not be a new polymorph of Gris, but a multi-component crystal with PEG, i.e., a Gris-PEG co-crystal.

Further evidence of the Gris-PEG co-crystal formation was found in thermal characterization of the etched sample. Observed under hot stage microscope, the crystals dissociated into two phases at about 110 °C during heating, with spontaneously release of an amorphous phase and the recrystallization of Gris as a separate phase (Fig. 5). The same phenomenon was also observed in DSC scans (Fig. S2). PXRD pattern of the dissociated sample

Table 1 Comparison of the measured ^{1}H T_1 and ^{1}H $T_{1\rho}$ values for Gris (Form I), bulk PEG 6000, and Gris-PEG co-crystal[a]

			Gris-PEG co-crystal	
	Gris (Form I)[b]	PEG	Gris[b]	PEG
^{1}H T_1 (s)	0.837 (0.012)	4.91 (0.03)	0.899 (0.028)	0.958 (0.019)
^{1}H $T_{1\rho}$ (ms)	307 (2.4)	0.365 (0.007)	9.47 (0.07)	8.42 (0.13)

[a] The numbers in parentheses indicate the standard error associated with the fit.
[b] Resonance peak of Gris in the region of 202-195 ppm was selected for this comparison, and data of other peaks can be found in Table S1 of the Supporting Information.

confirmed that, the re-formed product were PEG and Gris Form I (Fig. 4f). It should be noted that the dissociation occurs only after excess PEG was etched away, while in the PEG matrix, melting of the co-crystal was observed at a higher temperature (Fig.2 and 3)

The CP/MAS ^{13}C NMR spectra are used to investigate the state of PEG chains in crystal lattice, as shown in Fig. 6. The resonance peak of methylene carbon of PEG appeared at 70.8 ppm for pure semi-crystalline PEG and 69.5 ppm for the new crystal form. The upfield shift of PEG resonance peak suggests the chemical environment of PEG chains in this new crystal form is different from that of pure PEG. In addition, the PEG peak of the co-crystal sample is much narrower than that of semi-crystalline PEG, suggesting the PEG chains are in a more uniform conformation in the co-crystal[11]. The significant change of chemical shift characteristic of carbon atoms of Gris (e.g., around 57 ppm and 89 ppm) also suggests a different molecular packing mode in the new crystal form, consistent with that observed in PXRD patterns.

Solid state NMR spin-lattice relaxation times T_1 (^{1}H) and $T_{1\rho}$ (^{1}H) (in the laboratory and rotating frames, respectively) are commonly used to characterize the homogeneity of a mixture[12]. Table 1 shows such relaxation time data of the Gris-PEG co-crystal sample and the two pure components. It can be seen that in the co-crystal sample, the relaxation times of Gris and PEG were different from those of the pure components, and became similar to each other. Using the more sensitive $T_{1\rho}$ (^{1}H) data for calculation, a common relaxation time around 8-10 ms means that the Gris and PEG molecules are within the distance shorter than the spin diffusion length scale $L = (6DT)^{0.5} = 2.4$ nm, where D is the spin diffusion coefficient (often assumed to be 10^{-12} cm^2/s) and T is the relaxation time[11,12]. Considering that the molar ratio between Gris and ethylene oxide (EO) units is about 1:2 (hence the PEG weight percent around 20%), and the diameter of a single PEG chain (molecular weight 6000 Da) in its random coil conformation is about 3.6 nm[13], it is highly unlikely that the PEG chains exist as a separate phase. Instead the Gris and the PEG chains should be homogeneously mixed in the molecular level. More data on the relaxation times are included in the Supporting Information as Table S1.

Since the Gris and the PEG molecules are very different, one likely structure of their co-crystal could be similar to the extensively-studied urea-PEG[14] and cyclodextrin-PEG[15] inclusion complexes, where the packing of the small molecules forms continuous channels and the PEG chain reside within them. Gris is known to form channel-structured solvates with various solvents, including 1,2-dichloroethane, acetonitrile, nitromethane, and nitroethane[16]. Indexing the diffraction pattern of the Gris-PEG co-crystal (*Jade 6.0 software*, Materials Data, Inc.), and the best fit was obtained with the following parameters: monoclinic crystal, space group C2, a = 22.169 Å, b = 8.716 Å, c = 11.730 Å, $\beta = 112.73^o$, which are close to those of the Gris-nitroethane (1:1) solvate[16] (monoclinic, space group C2, a=21.931 Å, b=8.654 Å, c=11.792 Å, $\beta = 117.53^o$). The indexing and the fitting results were shown in Fig. S3 and Table S2, and all the diffraction peaks fit the calculated distances very well with all errors ($|d(cal)-d(exp)|/d(cal)$) smaller than 0.3%. Furthermore, a large spherulite of the Gris-PEG co-crystal was obtained and the peripheral part of this spherulite was used for the X-ray fiber diffraction. The 2D diffraction pattern is shown in Fig. 7. The diffraction spots can all be well indexed with the same set of unit cell parameters (all errors smaller than 1.0%). PEG is a linear and highly flexible polymer, and it can be included in narrow channels such as those of α-cyclodextrin with minimum internal diameter of 4.4 Å[17], similar to those of the Gris-nitroethane (1:1) solvate. Hence it is possible that the Gris-PEG co-crystal may adopt a crystal structure very similar to that of the Gris-nitroethane (1:1) solvate, but a single crystal sample is still needed to conclusively determine its structure.

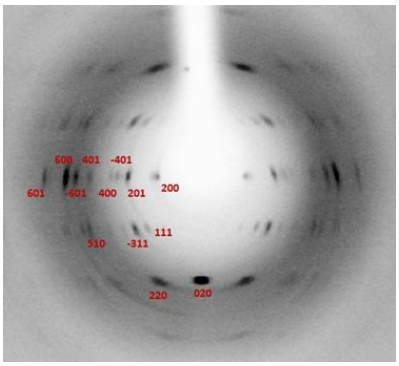

Fig.7 Fiber diffraction pattern obtained at the peripheral part of a spherulite of the Gris-PEG co-crystal. The average direction of the fiber cluster is vertical. The X-ray beam is perpendicular to the plane of the spherulite. The index is given using the same unit cell parameters as those used in the powder XRD indexing.

Besides the aforementioned experimental conditions, similar Gris-PEG co-crystals were observed in Gris/PEG co-melt under a wide range of conditions, including the variation of annealing temperatures (Fig. S4 and S5), compositions (Fig. S6) and the molecular weight of PEGs used (Fig. S7). To investigate the stability of Gris-PEG co-crystal at room temperature, long-time storage (up to 10 months) was carried out, and a slow transformation of co-crystal to separate phases of PEG and Gris was identified by PXRD (Fig. S8), indicating the co-crystal is a metastable form at room temperature.

Conclusions

In conclusion, this study demonstrated the co-crystal formation of the small molecular drug griseofulvin and PEG, adding to the short list of drug-PEG co-crystals. Since PEG is widely used in pharmaceutical products, its drug co-crystals should pose no safety restriction to their clinical use. As a pharmaceutical co-crystal co-former, PEG is very different from the conventionally-

used small molecules, and hence the dissolution and stability behavior of such drug-polymer co-crystals should be studied. On the other hand, other pharmaceutically-acceptable polymers should also be studied.

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Notes and references

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† Electronic Supplementary Information (ESI) available. See DOI: 10.1039/b000000x/

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