Solvent-Free Drug Crystal Engineering for Drug Nano-& Micro suspensions

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ABSTRACT

Poor water-solubility is becoming the leading hurdle for novel drug molecules to reach the market. Enhancing the surface-to-volume ratio by reducing the drug particles size has emerged as a powerful method to enhance the drug dissolution rate of poorly water-soluble drugs. Here we present several approaches to produce micro- and nano-suspensions of febantel and itraconazole, as poorly water-soluble model drugs, in the presence of the self-emulsifying excipient Gelucire 44/14 as additional solubility enhancing agent. Two top-down approaches involving either ball milling or ultrasound treatment, to reduce the size of existing drug crystals, were used as reference processes. Both techniques allowed to significantly reduce the size of the drug crystals and enhance the dissolution of febantel with the ultrasound treated formulation performing the best. In case of itaconazole, no influence of both processing techniques was observed, which is likely to be attributed to its extremely low water-solubility. To address this challenge, we developed a novel bottom up approach to produce nanosuspensions. This approach involved first dissolving the drug in molten Gelucire 44/14 followed by atomization into cold water. During the atomization, cavitation was induced by ultrasonication. This process yielded milky suspensions in the submicrometer range. Furthermore a fraction of the drug was found to be in amorphous state. Nanosuspensions produced by this technique showed improved dissolution behavior, both in case of febantel and itraconazole.

Key terms: drug formulation, nanoparticles, oral dosage form, drug dissolution, self-emulsifying, ultrasound

1. INTRODUCTION

One of the major causes of new drug molecules failing to reach the market is poor bioavailability. Indeed, many of the newly discovered drug candidates suffer from low to extremely low water solubility and hence are prone to limited bioavailability. [1-3] To cope with these issues, drug formulation scientists are developing formulation strategies that enhance the dissolution, and thus bioavailability of these poorly water-soluble drugs. [4-10] These strategies include, formation of inclusion complexes with cyclodextrins, using amphiphillic excipients such as block copolymer micelles, formulation in lipids, salt formation, transformation of crystalline to amorphous, particle size reduction, etc...

Amongst these techniques, particle size reduction is highly promising. [9-10] Micronization of drug crystals to the lower micron range improves drug dissolution by various mechanisms. [11] According to the Noyes-Whitney equation, the dissolution rate increases by increasing the specific surface area. Decreasing the particles size also increases the saturation solubility according to the Ostwald-Freundlich equation. Furthermore, the Prandtl equation predicts that a decrease in particle size will results in a thinned hydrodynamic layer around the particles and an increase of the surface-specific dissolution rate. For the simplified case of spherical particles, size reduction with a factor 10 (e.g from 100 µm to 10 µm), a 100-fold increase in surface area is obtained. However, for extremely hydrophobic drug molecules, micronization is not sufficient to achieve high enough bioavailability [12,13]. Therefore, reducing particle size to the nanorange (increasing the surface area of the drug crystals with multiple orders of magnitude) has been investigated through several approaches. Basically, these can be divided into two main groups: top-down and bottom-up techniques. In a top-down approach, nanoparticles are produced by exposing drug particles to mechanical energy, either through impact, shear or ultrasound induced cavitation. Drawbacks of these approaches are the limited control over the final particle size and morphology and the risk for potential contamination originating from the grinding media or ultrasonication set-up. Bottom-up approaches involve controlled recrystallization of dissolved (typically in organic solvents) drug molecules into nanoparticles via e.g. spray-drying or solvent-displacement. These approaches allow, by optimizing solvent and stabilizer (e.g. surfactants) a better control over particle size

and morphology. However, a major drawback is the use of organic solvents that holds the risk of residual traces of organic solvents that can hardly be removed.

When producing crystalline drug nanosuspensions, several considerations should be taken into account. First of all, colloidal stability of the nanosuspension is an important issue as reaggregation of hydrophobic nanocrystals readily takes place when these are not stabilized by means of surface active molecules. Reaggregation decreases the total drug surface area, thereby decreasing drug dissolution kinetics and should thus be avoided. Secondly, Ostwald ripening which involves the dissolution of smaller particles in favour of the growth of larger ones should be avoided. Third, drug concentration plays an important role as for practical reasons excessive dilution of the nanosuspensions should be limited. Related to the latter is the further processing of the nanosuspensions into a solid form, while still allowing a nanosuspension to reform in aqueous physiological medium (e.g. in the gastrointestinal tract).

This study aims to evaluate different solvent-free approaches to produce nanosuspensions of poorly water-soluble drugs in order to enhance their dissolution behaviour. As model drugs, itraconazole (**Figures 1A)**; and febantel (**Figures 1B**) were chosen due to their extremely low water solubility. In order to stabilize the drug nanosuspensions, we will coformulate the drug nanocrystals with non-ionic surfactants. First we present two top-down approaches based on either simple grinding or ultrasonic treatment of crystalline drug suspended in liquid surfactant. In the bottom-up approach, drug dissolved in a liquid surfactant is atomized in water under ultrasonic cavitation to control the size of the formed drug precipitates. Cavitation is the formation of gas bubbles in a liquid phase in regions where the pressure of the liquid falls below its vapour pressure. Due to acoustic waves, the gas bubbles oscillate in size and upon collapse of the bubbles, a shock wave is produced. These shock waves have sufficient power to erode crystalline material into smaller particles as well as avoiding nanoparticle aggregation. [14]

2. MATERIALS AND METHODS

2.1 Materials

Febantel was obtained from Fagron (Belgium) and itraconazole was obtained from Johnson & Johnson (Belgium). To allow proper comparison between the different formulations we have determined the solubility of both drug molecules experimentally under the same conditions as those that will be used for dissolution experiments, i.e. 0.1 M HCl and 37 °C. These values were found to be respectively 0.51 ± 0.05 mg/mL for febantel and 0.0012 ± 0.007 mg/mL for itraconazole. **Figure 1** shows the molecular structure of both drugs. Febantel is a broad-spectrum probenzimidazole that is widely used against gastrointestinal nematodes and lung worms in live stock [15-19]. It is a prodrug that is metabolized *in vivo* to fenbendazole and further to oxfendazole, 4-hydroxyfembendazole, 4-hydroxyfendazole, and an inactive metabolite febendazolesulfone [20-25]. Itraconazole is a triazole antifungal drug, used in the treatment of fungal infections.

Gelucire 44/14 (i.e. PEG-32 glyceryllaurate) was obtained from Gattefossé (France). Gelucire 44/14 is an amphiphilic semisolid with a melting point of 44 °C and an HLB of 14. Gelucire 44/14 is GRAS (generally recognized as safe) and is obtained by polyglycolysis of hydrogenated palm kernel oil with polyethylene glycol 1500. Due to its unique composition, comprising mono- and diesters of PEG, mono-, di- and triglycerides, it spontaneously forms a microemulsion upon contact with water [26]. These properties allow Gelucire 44/14 to be used in the solubilization enhancement of poorly soluble drugs and increase of their solubility rate [27, 28].

2.2 Preparation of the nanosuspensions

The composition of the nanosuspensions containing itraconazole and febantel is displayed in **Table 1**.

2.2.1. Wet milling

Drug, Gelucire 44/14 and water were added to a 500 mL zirconium dioxide grinding bowl loaded with 25 zirconium dioxide grinding beads of 20 mm in diameter. The bowl was closed with a zirconium dioxide lid, placed in a ball mill type Pulverisette 6 (Fritsch, Idar-Oberstein,

Germany) and allowed to rotate at 350 rpm for respectively 15, 30, 45 and 60 min. For further dissolution experiments, we used samples that were milled for 60 min.

2.2.2 Indirect sonication

First, Gelucire 44/14 was dispersed in water followed by the addition of the crude crystalline drug powder. The experimental set-up is schematically shown in **Figure 2.** The ultrasonic mini flow cell was obtained from Hielscher Ultrasonics (Teldow, Germany) and attached to a titanium sonotrode type BS2d22 (HielscherUltrasonics) and an ultrasound generator type UIP1000 (Hielscher Ultrasonics), operating at 20 kHz (± 1kHz) and an intensity of 20 W/cm². The ultrasonic mini flow cell consists of a glass tube connected to a closed tubing circuit in which the aqueous drug/Gelucire 44/14 suspension is injected through a syringe connected to a valve. **Table 1** shows the composition of the starting suspension. From this 30 mL was injected into the system. The tubing is inserted in a peristaltic pump to allow circulation of the suspension at a flow rate of 30 mL/min. The residence time of the suspension in the flow cell can be controlled via the peristaltic pump. Additionally, the ultrasonic mini flow cell contains a water jacket (room temperature), surrounding the glass tube (4 mm in diameter), connected with a pressurized water reservoir. This water jacket allows propagation of the generated ultrasonic waves, transmitting them to the suspension within the glass tube. Furthermore, the water jacket allows controlling the temperature of the glass tube limiting the heating caused by the ultrasonication. Samples were prepared that were processed for respectively 30, 60, 90 and 120 min. Those subjected to 120 min of processing were used for dissolution experiments.

2.2.3 Ultrasonic melt precipitation

Figure 3 shows a schematic representation of the experimental set-up to produce drug nanosuspensions via ultrasonic melt precipitation. Itraconazole and febantel were first dissolved in Gelucire 44/14 at respectively 150°C and 140°C, which is above their respective melting point. Subsequently, the liquid phase was atomized at an air pressure of 5 bar through a 2 fluid nozzle into a dual wall stainless steel flow cell containing 150 mL water. The flow rate was 4 mL/min for tfebantel suspensions and 6 mL/min for itraconazole suspensions. The water was

thermostatized at 10°C to allow prompt cooling of the atomized liquid. The temperature was monitored by an in line temperature probe. During the atomization process, cavitation was induced to the system by means of an ultrasonic probe (HielscherUltrasonics, Teldow, Germany) composed of a titanium sonotrode type BS2d22 (HielscherUltrasonics), a booster type B2-1.4 (HielscherUltrasonics) and an ultrasound generator type UIP1000 (HielscherUltrasonics) operating at 20 kHz ± 1 kHz, and an intensity of 100W/cm^2. After 5 minutes of sonication, milky suspensions of both drugs were obtained and used for particle size measurements and dissolutions experiments.

2.3 Particle size distribution

The particle size distribution of the drug crystals in the nanosuspensions was determined by laser diffraction using a Malvern Mastersizer S (Malvern Instruments, Spring Lane South, UK) equipped with a small volume dispersion unit and a 300 RF lens. Water was used as dispersion medium for all measurements.

2.4 Optical microscopy

Optical microscopy images were recorded under brightfield and polarized light using a Leica 2500P microscope equipped with 10x and 63x oil-immersion objectives, a DFC360FX CCD camera and a Linkham THMS600 heating stage. To allow proper visualization, drug crystals and nanosuspensions were squeezed between two glass cover slides.

2.6 *In vitro* drug dissolution

Drug release from the produced nanosuspensions was evaluated in a VK7010 dissolution bath (VanKel Industries, NJ, USA) equipped with a VK8000 automatic sampling station (VanKel Industries, NJ, USA). All dissolution tests were run in triplicate. Note that since this study focused on the development of formulations that enhanced the dissolution rate of extremely poorly water-soluble drugs, sink conditions were not met. As dissolution medium, 900 ml 0.1 M HCl in demineralized water was used at a temperature of 37°C. At 5, 10, 15, 20, 30, 45 and 60 minutes, 5 ml samples were withdrawn from the dissolution vessels without medium

replacement, filtered through a 200 nm filter to remove particulate matter and analyzed by spectrophotometry for their drug content. The febantel concentration was measured at 280 nm and itraconazole concentration was measured at 260 nm using a double beam spectrophotometer (Shimadzu UV-1650 PC, Shimadzu CO., Kyoto, Japan). It was verified that Gelucire 44/14 did not interfere with neither febantel nor itraconazole at the respective wavelengths used to determine the drug concentration. The analytical methods were validated according to the ICH Harmonized Tripartite Guideline – Validation of Analytical Procedures (1994).

2.7 *X-ray diffraction (XRD)*

X-ray diffractograms were recorded on a PANalytical X'Pert PRO X-ray diffractometer (Siemens). XRD patterns were obtained with Cu KR radiation (45 kV x 40 mA; λ = 1.5406 A) at a scanning speed of 25° (2θ)/min and step size of 0.03° (2θ). Measurements were done in the reflection mode in the 2θ range of 5-40°.

3. RESULTS AND DISCUSSION

3.1 Preparation of nanosuspensions

As mentioned above, itraconazole and febantel are used in this study as model poorly water-soluble drugs. Both drugs are highly crystalline with a mean diameter D(v, 0.5) of respectively 26 µm (itraconazole) and 232 µm (febantel) as measured by laser diffraction. A first step in our formulation strategy was suspending the crystalline drug in Gelucire 44/14 and water. The rationale behind this is to use Gelucire 44/14 as a biocompatible surface-active agent that would aid in the formation of nanosuspensions by covering newly formed particle surface, preventing immediate re-aggregation. A first series of preliminary screening experiments served to determine the Gelucire 44/14 to drug ratio that allows processing via all three formulation processes. The respective formulation parameters are shown in **Table 1**.

3.1.1 Wet milling

Wet milling is frequently used to produce drug nanosuspensions, and was used in this work as reference process. The crude aqueous drug/Gelucire 44/14 suspensions were added to a bowl containing zirconium dioxide as grinding medium, and were subsequently placed during 1 h in a ball mill at 350 rpm. **Figure 4A**, shows the evolution of the particle size distribution and the mean particle diameter, measured by laser diffraction, during the wet milling process. These data demonstrate that for both febantel and itraconazole the initial particle population decreases as a function of process time, while a new sub-micron population emerges. Although efficient to produce drug nanosuspensions, the wet milling process bears several disadvantages. First of all, relatively long milling times are required. Secondly, there is a potential risk of contaminating the drug formulation with particulate matter resulting from erosion of the milling components. A third limitation is the possibility of only batch wise production.

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3.1.2 Indirect sonication

To cope with the above mentioned issues related to wet milling, we aimed to design a process that allows to generate energy input without exposing the drug molecules to potential sources of contamination. Therefore, we constructed in-house an ‘indirect sonication’ set-up. An advantageous aspect of this process the shielding of the suspensions from the sonication tip

by a glass wall. This prevents contamination by eroded metal nanoparticles from the sonication time after prolonged processing times. Additionally, this process can potentially be operated in continuous fashion by putting multiple sonication cells in series.

Figure 5 displays the evolution of the particle size, measured by laser diffraction. Similar to the wet milling process, the particle size decreased as function of the process time. Again, febantel showed a more profound down-sizing of the particle diameter than itraconazole. We also observed that the efficiency of down-sizing can be optimized by extending the residence time of the liquid in the flow cell by controlling the pump rate. Lower pump rates lead to longer residence times in the flow cell and allowed to shorten the total process time. The indirect sonication process clearly has the advantage that the drug is not in contact with the physical energy source, thus avoiding potential contamination. Furthermore, this process is expected to be more suitable for up-scaling, either by enlarging the flow-through cell or by putting several cells in series. Additionally, this process should allow working in a continuous way with constant in-and-out flow of fresh drug suspension, while the earlier described wet milling process is a batch process. Nevertheless, the indirect sonication process still suffers from relatively long, i.e. 2h, process times that are required to significantly reduce the drug crystal size. To summarize, **Table 2** gives an overview of the volume distributions (D values) of the drugs particles during processing via wet milling and indirect sonication.

3.1.3 Ultrasonic melt precipitation

To cope with the issue of prolonged process times associated with both wet milling and indirect sonication, we developed a novel bottom up approach that we termed 'ultrasonic melt precipitation'. In this process, drug nanosuspensions are generated by dissolving the drug in a molten phase of Gelucire 44/14 at respectively 140 °C (itraconazole) and 150°C (febantel). The process of drug dissolution in a molten Gelucire 44/14 phase was deeper analyzed by hot stage microscopy and presented in **Figure 6**. At 60 °C a liquid Gelucire 44/14 phase exists in which the crystalline drug is suspended. As soon as the melting point of the respective drugs is reached, they dissolve in the liquid Gelucire 44/14 phase. Upon cooling to room temperature, the drug

(or a part of the drug) recrystallizes from the solution into very fine particles in case of febantel and larger precipitates in case of itraconazole, as shown in **Figure 6**.

During the ultrasonication process, the molten drug/Gelucire 44/14 mixture was atomized through a two-fluid nozzle into a flow cell containing water thermostatized at 10°C. Furthermore, during atomization of the molten drug/Gelucire 44/14 phase, cavitation is induced by immersion of an ultrasonic probe. After 5 min of sonication, milky suspensions of both drugs were obtained. **Figure 3** gives a schematic representation of the experimental set-up.

As shown in **Figure 7** on the size distribution graphs, the ultrasonic melt precipitation approach allowed to dramatically reduce the drug particle size. Importantly, whereas wet milling and indirect sonication yielded a bi-modal size distribution, the ultrasonic melt precipitation approach resulted in a mono-modal drug nanosuspension. In case of itraconazole, the D(v,0.5) reduced from 20.6 µm (i.e. the crude crystalline material before melting in Gelucire 44/14) to 0.51 µm, while in case of febantel the D(v,0.5) was reduced from 153.7 µm to 0.38 µm (**Table 3**). This is due to a series of events that occur during the process. First, the drug is dissolved in a molten Gelucire 44/14 phase through heating. Secondly, the atomisation of liquid Gelucire 44/14 droplets into cold water under intense cavitation induces flash precipitation of the drug into small crystals. Cavitation plays a crucial role in this process, preventing agglomeration of the newly formed crystals [29]. Indeed, when we conducted control experiments in absence of ultrasonication, the atomized liquid immediately gelified upon contact with the cold water, followed by nucleation of drug crystals and further on the formation of large crystalline precipitates. By contrast, performing the process under ultrasonication readily allowed the Gelucire 44/14 to be distributed over the whole aqueous phase and to control the nucleation of drug crystals, avoiding aggregation and retain the size of the formed drug precipitates within the nano-range. The interesting aspects of this approach are (1) the short processing times that are required to produce a drug suspension with a mean particle size below 1 µm and (2) the potential to be operated in continuous fashion.

3.2 In vitro drug dissolution of drug nanosuspensions

Finally, the itraconazole and febantel nanosuspensions were evaluated for their *in vitro* dissolution behaviour. The cumulative release curves of the respective formulations and their controls are shown in **Figure 8**. **Table 4** summarizes the maximum concentration of drug that was dissolved after 60 min in 0.1M at 37°C. Crude drug as well as a physical mixture of drug and Gelucire 44/14 give very low drug concentrations, for both febantel and itraconazole. By contrast, the cumulative drug release curves depicted in **Figure 8A**, show a pronounced enhancement in total drug release for febantel processed via all three formulation strategies. Febantel formulated via ultrasonic melt precipitation achieves the highest total drug dissolved followed by indirect sonication and wet milling in a third place. Importantly, maximum drug concentration is reached within 10 min after addition of the nanosuspensions to the dissolution medium. In case of itraconazole only ultrasonic melt precipitation (**Figure 8B**) was able to increase drug dissolution.

For febantel, on the first sight, merely reducing the particle size (**Figure 7**) is effective to enhance drug dissolution, however, this is not the case for itraconazole. Probably this is due to the extremely poor water solubility of itraconazole, which is even several orders of magnitude lower than febantel that has a water. To further investigate why the ultrasonic melt precipitation is the only process that effectively enhances the dissolution of itraconazole we assessed the crystallographic state of the formulated drug by X-ray diffraction (XRD). **Figure 9** shows the corresponding X-ray diffractograms for all febantel and itraconazole formulations and the respective controls. These diffractograms show, relative to a physical mixture of drug/Gelucire 44/14 in the same ratio as used for the respective formulations, a significant reduction in crystallinity only for the formulations (of both drugs) that were processed via ultrasonic melt precipitation. The other formulations strategies had no detectable influence on the crystalline state of the drug. Due to the fact that febantel and itraconazole dissolve in Gelucire 44/14 upon heating it was found not possible to quantify via differential scanning calorimetry (DSC) the amount of amorphous drug that was produced during the ultrasonic melt crystallization process. Although, when looking at literature [30] it is clear that amorphous itraconazole has a much higher solubility, with values that are in the same order as those values

that were obtained in our present study. Hence, it is likely that ultrasonic melt precipitation produces a certain fraction of amorphous drug, that contributes to the observed enhanced drug dissolution

4. CONCLUSIONS

In this paper, we have evaluated several solvent-free strategies to produce micro- and nanosuspensions of poorly water-soluble drugs. To enhance the water-solubility of these drugs, Gelucire 44/14, a self-emulsifying biocompatible excipient, was used. Wet milling and indirect sonication yielded drug suspensions with a bimodal size distribution, comprising submicron particles and particles in the lower micron range. Ultrasonic melt precipitation was able to yield a monomodal drug suspension with submicron drug particles. All three strategies to reduce the size of the drug crystals in the presence of Gelucire 44/14 strongly enhanced the dissolution rate and total amount of dissolved drug for febantel . In case of itraconazole, only ultrasonic melt precipitation was able to enhance the dissolution rate and total amount of dissolved drug. This was attributed to the lower water solubility of itraconazole, relative to febantel, and the fact that ultrasonic melt precipitation produces a fraction of amorphous drug that is stabilized by Gelucire 44/14.

TABLES

Table1. Composition of the crude febantel and itraconazole suspensions

formulation components	**(wt %)**	**(g)**
Gelucire 44/14	10,1	20
water	75,7	150
febantel	14,2	28,14
Gelucire 44/14	11,5	20
water	85,9	150
itraconazole	2,6	4,54

Table 2. Overview of the D values, measured by laser diffraction, of itraconazole and febantel drug suspension after different process times during respectively wet milling and indirect sonication.

process time [min]		**D(v,0.1)** [µm]		**D(v,0.5)** [µm]		**D(v,0.9)** [µm]	
		itraconazole					
milling	*sonication*	*milling*	*sonication*	*milling*	*sonication*	*milling*	*sonication*
15	30	0.35	0.4	2.29	4.51	7.08	20.21
30	60	0.3	0.33	1.4	2.08	4.54	6.57
45	90	0.3	0.3	1.26	1.3	4.13	4.29
60	120	0.29	0.28	1.07	0.96	3.66	3.36
		febantel					
milling	*sonication*	*milling*	*sonication*	*milling*	*sonication*	*milling*	*sonication*
15	30	0.29	0.89	1.53	4.1	4.11	8.95
30	60	0.27	0.29	0.7	1.16	2.75	4.78
45	90	0.28	0.27	0.91	0.92	3.36	3.72
60	120	0.26	0.34	0.58	0.65	2.2	3.06

Table 3. Overview of the D values, measured by laser diffraction, of itraconazole and febantel before and after ultrasonic melt precipitation.

	D(v,0.1) [µm]	**D(v,0.5)** [µm]	**D(v,0.9)** [µm]
		itraconazole	
crude	9.1	26	71
processed	0.26	0.51	1.25
		Febantel	
crude	36	232	480
processed	0.26	0.38	0.56

Table 4. Maximal amount of drug dissolved within 60 min in 0.1 M HCl at 37°C obtained via the respective formulation strategies.

Formulation	Febantel [mg/ml]	Itraconazole [mg/ml]
crude drug	0.55 ± 0.05	0.0015 ± 0.001
physical drug/Gelucire 44/14 mixture	0.8 ± 0.05	0.0028 ± 0.002
wet milling	8.8 ± 0.5	0.0063 ± 0.001
indirect sonication	17.45 ± 1.5	0.003 ± 0.002
ultrasonic melt precipitation	21.3 ± 0.5	0.45 ± 0.05

FIGURES

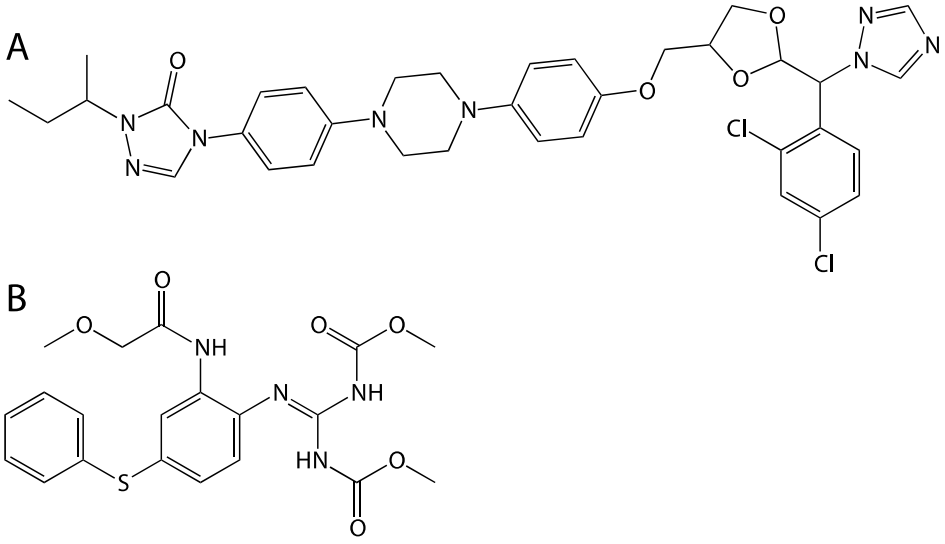

Figure 1. Molecular structure of (A) itraconazole and (B) febantel.

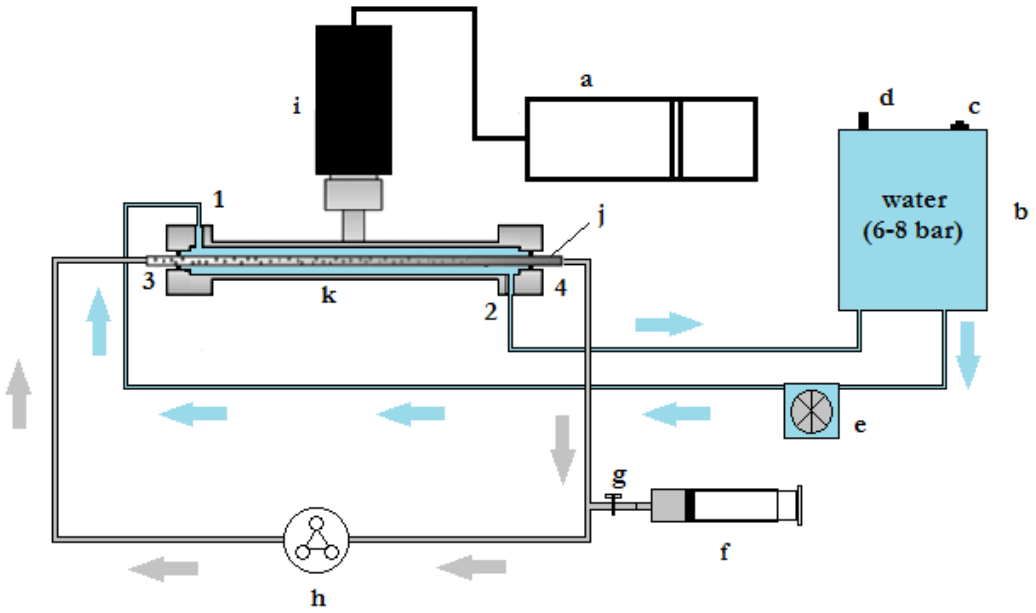

Figure 2. Schematic representation of the experimental set-up for indirect sonication of drug suspensions. (a) ultrasonic processor, (b) pressurized water tank, (c) security pressure valve, (d) compressed air input, (e) water pump, (f) syringe, (g) close cycle valve, (h) peristaltic pump, (i) sonotrode, (j) glass tube, (k) ultrasonic mini flow cell, (1:2) pressurized water input/output, (3;4) suspension input/output.

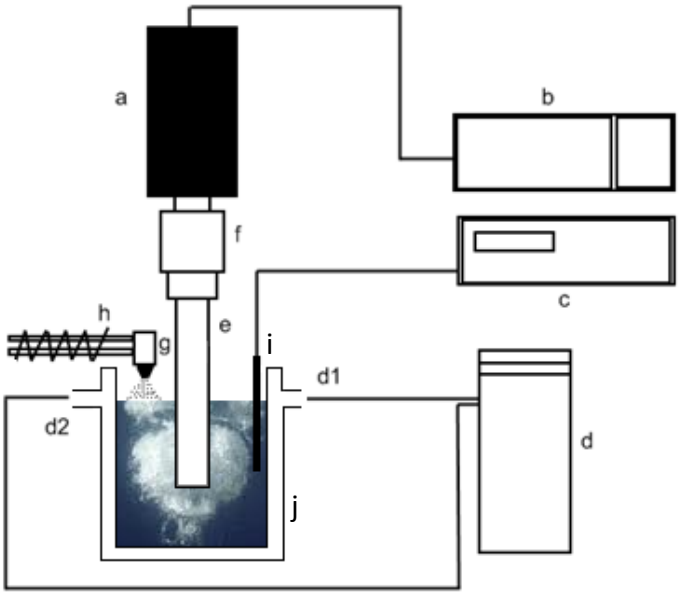

Figure 3. Schematic representation of the ultrasonic melt precipitation set-up. (a) sonotrode, (b) ultrasonic processor, (c) digital thermometer, (d) cooling system, (e) titanium tip, (f) booster, (g) two fluid nozzle, (h) heating cord, (i) temperature probe, (j) dual wall flow cell, (d1, d2) inputs for the cooling circuit.

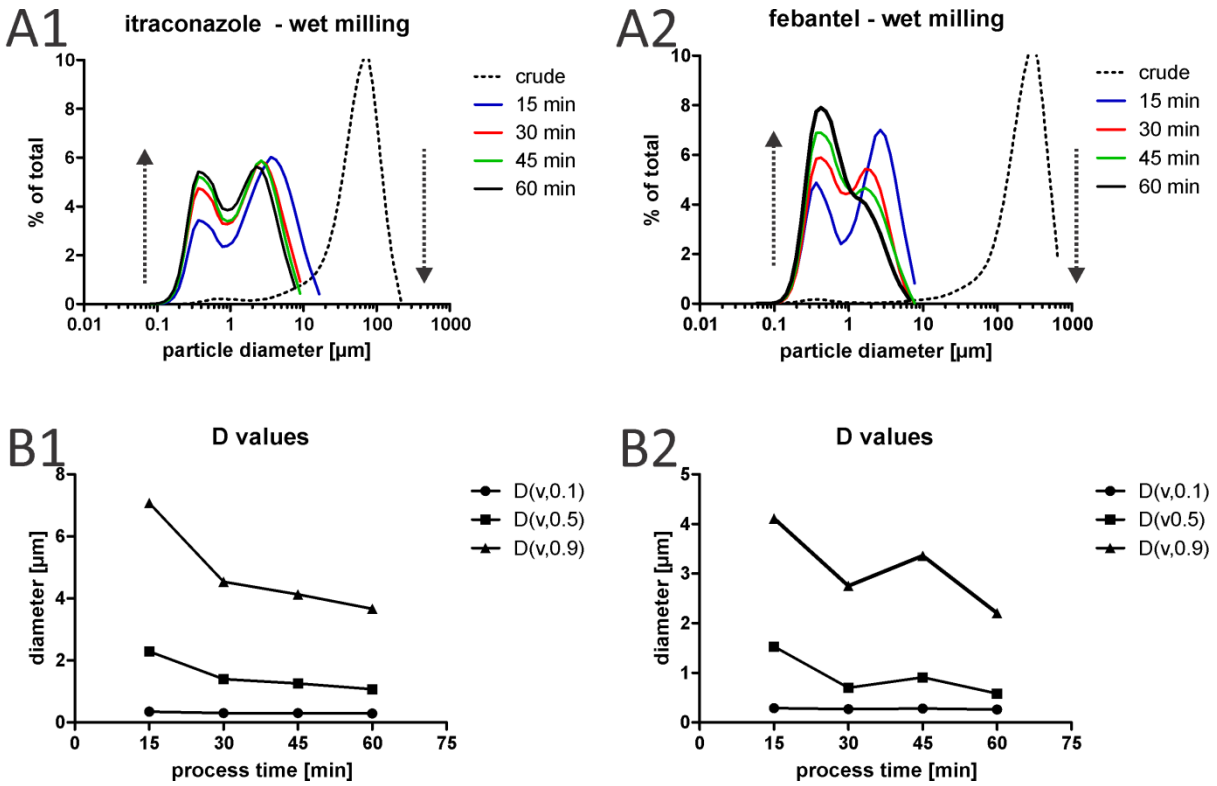

Figure 4.(A) Size distribution, measured by laser diffraction, at different time points during wet milling of respectively itraconazole and febantel suspensions in Gelucire 44/14 . The dotted arrows indicate the 'micro' drug crystal population to diminish as function of process time in favour of the 'nano' population. (B) Corresponding evolution of the D values as a function of process time. (C) Optical microscopy images of the corresponding drug crystal suspensions before and after wet milling.

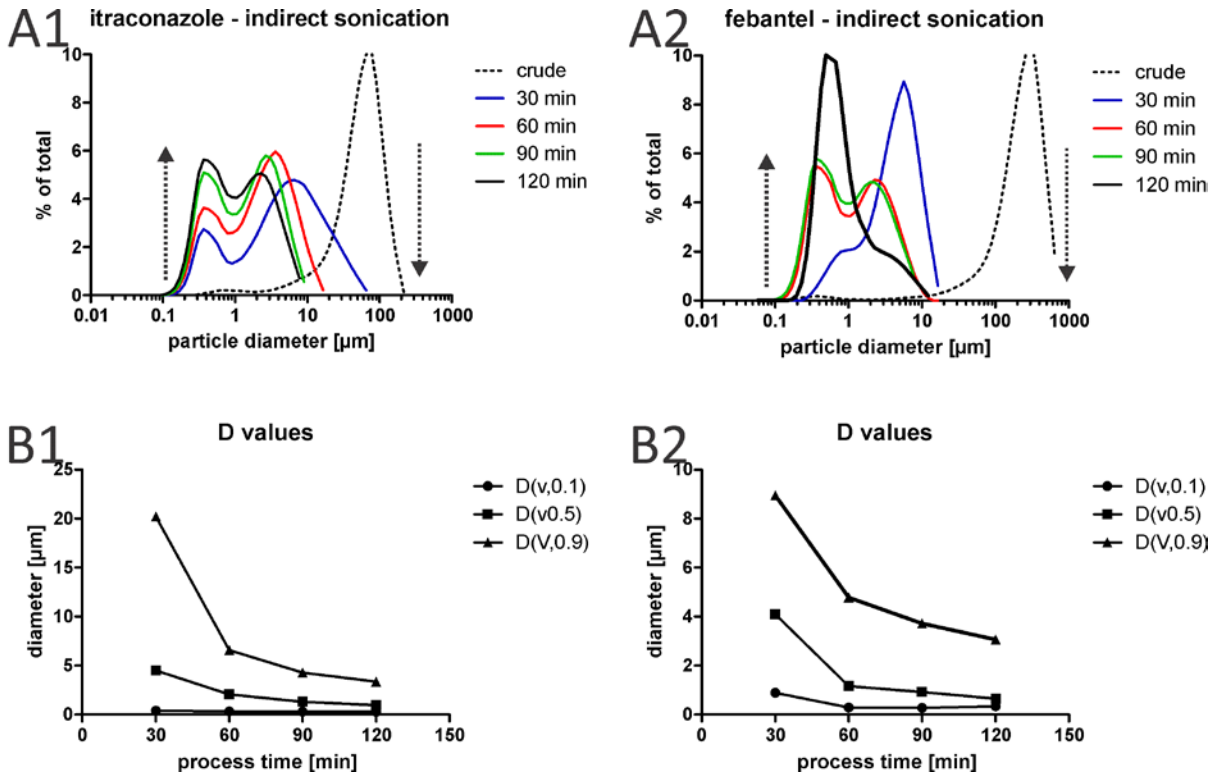

Figure 5. (A) Size distribution, measured by laser diffraction, at different time points during indirect sonication of respectively itraconazole and febantel suspensions in Gelucire 44/14 . The dotted arrows indicate the 'micro' drug crystal population to diminish as function of process time in favour of the 'nano' population. (B) Corresponding evolution of the D values as a function of process time. (C) Optical microscopy images of the corresponding drug crystal suspensions before and after indirect sonication.

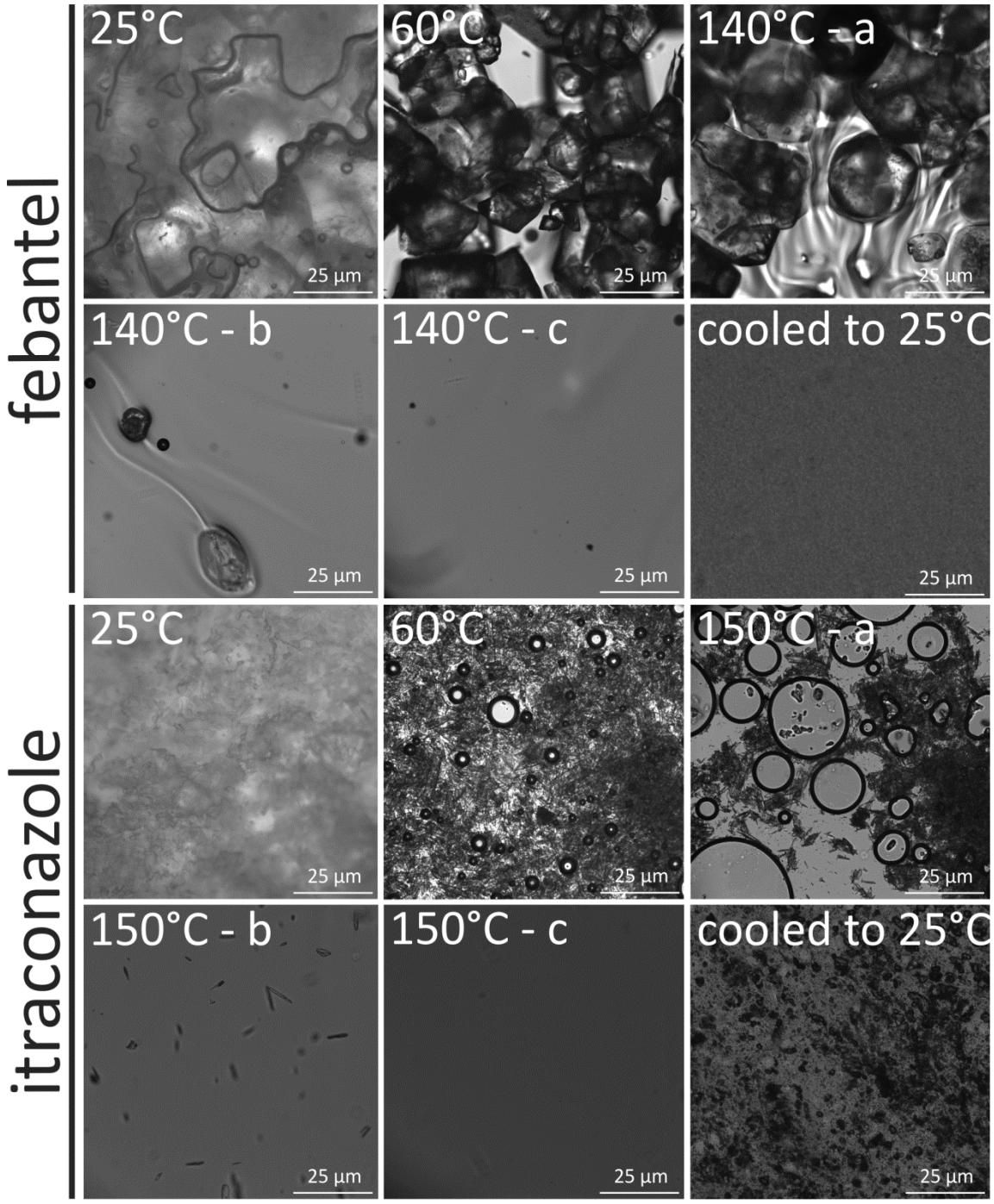

Figure 6. Optical microscopy images recorded using a hot stage microscopy set-up, demonstrating the dissolution of febantel, respectively itraconazole in a liquid Gelucire 44/14 phase as soon as the melting point of the drug is reached. The panels a, b and c show images at 10 s time intervals. Upon cooling to room temperature, (partial) re-crystallization occurs.

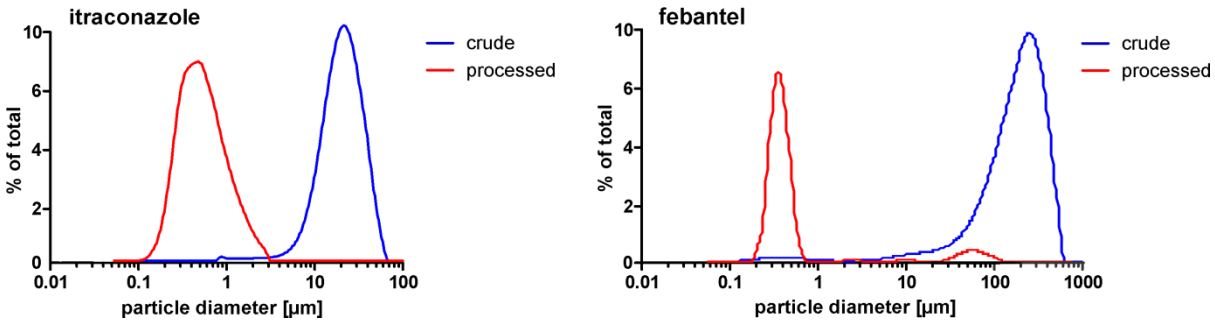

Figure 7. Size distribution, measured by laser diffraction, of the drug (itraconazole, respectively febantel) crystals in crude state (red curves) and after ultrasonic melt precipitation.

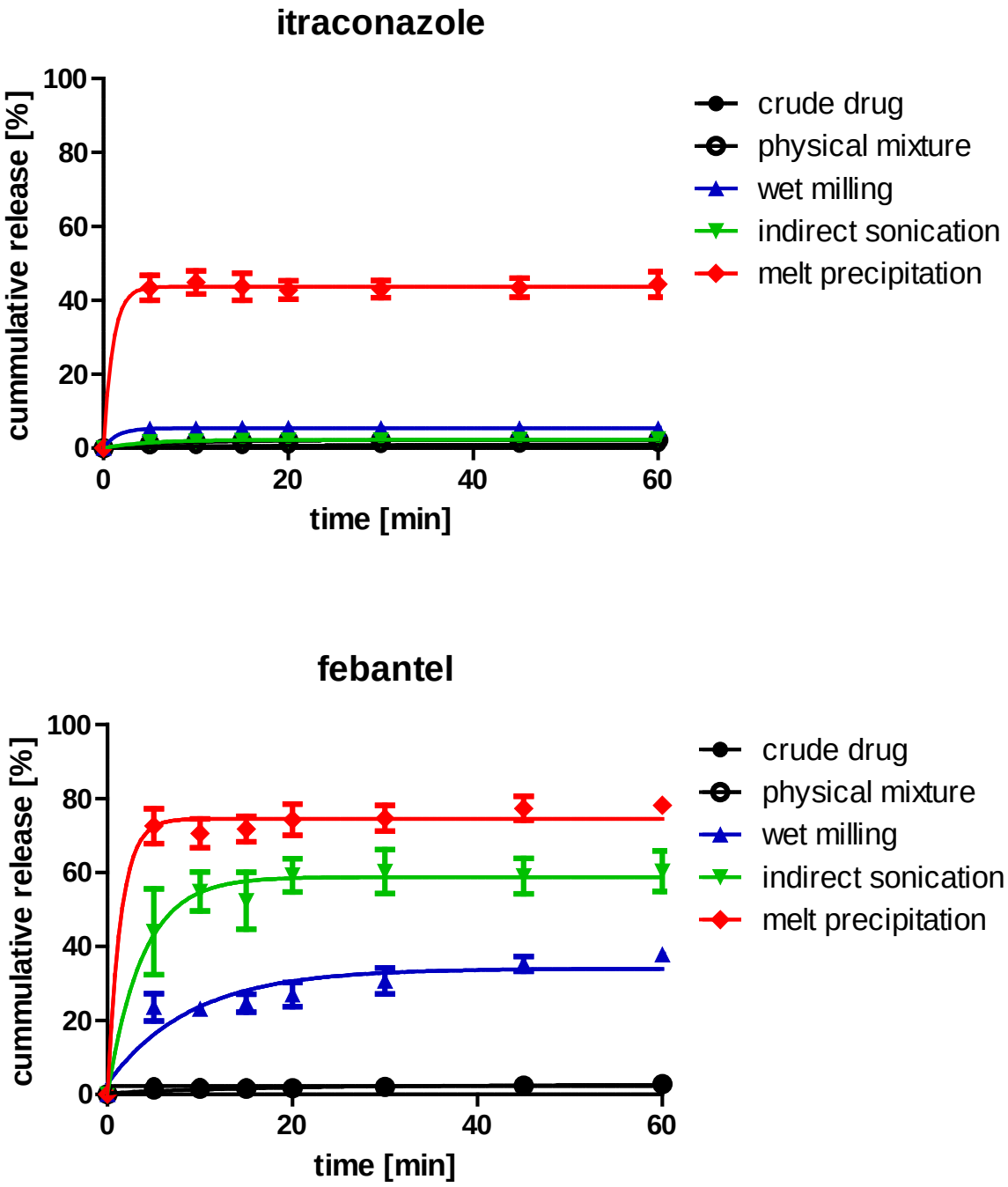

Figure 8. Cumulative *in vitro* in 0.1 M HCl at 37 °C release of (A) febantel and (B) itraconazole formulated via either wet milling, indirect sonication or melt precipitation. As controls, crude unprocessed drug and a physical mixture of drug and Gelucire 44/14 were taken.

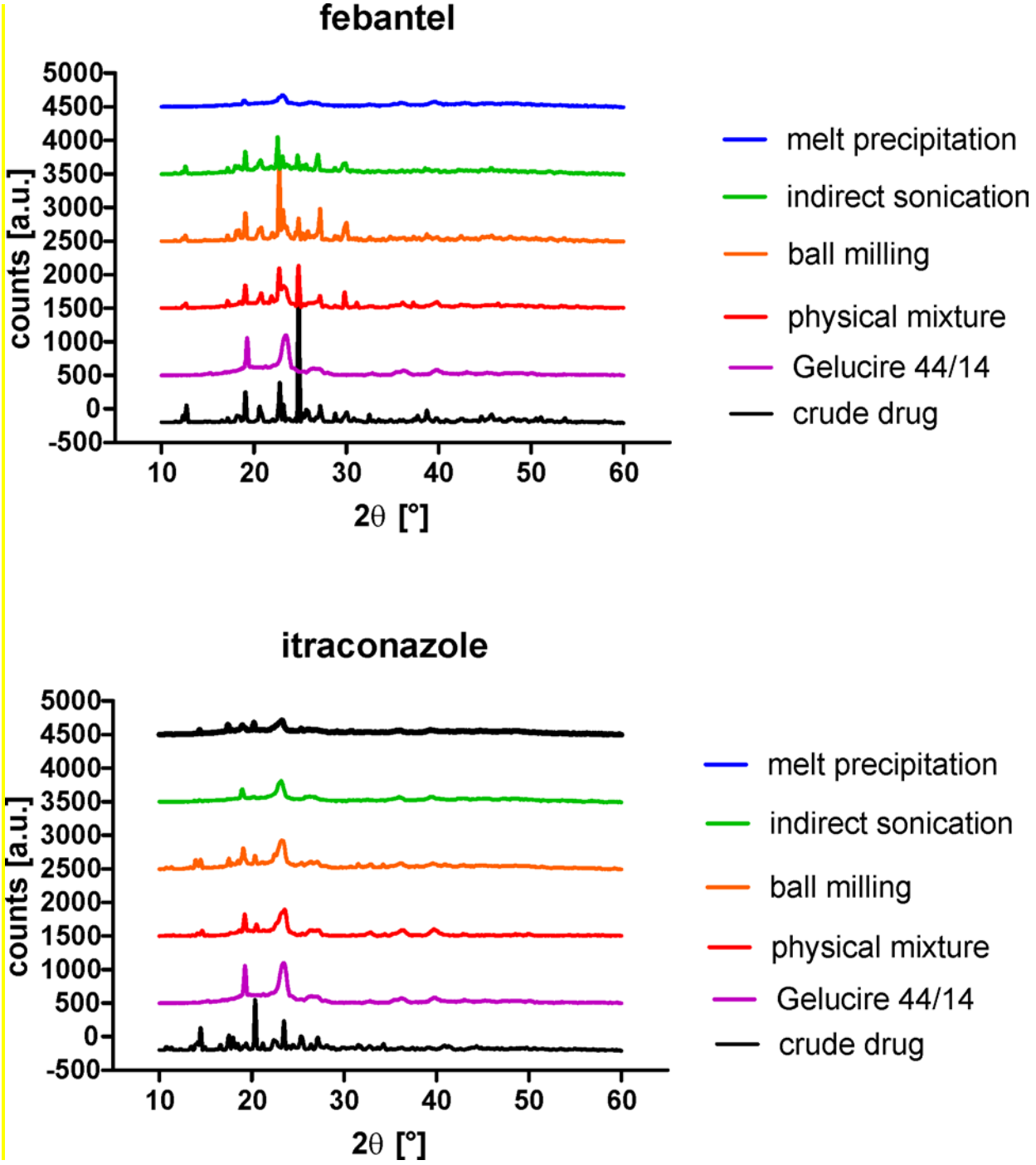

Figure 9. X-Ray diffractograms of the respective (A) febantel and (B) itraconazole formulations.

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