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Research Article

Co-crystal integrity and pharmaceutical role of Cremophor ELP

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ABSTRACT

Co-crystals are sensitive to dissociation in aqueous microenvironment losing their effects on bioavailability increase before oral administration. The integrity of the fumaric acid co-crystal of SAR1 active pharmaceutical ingredient (API) was studied after a wet granulation process with four formulations containing the same qualitative and quantitative composition. Standard pharmaceutical excipients, particularly water and Cremophor ELP were used in different addition order to evaluate the robustness of the manufacturing process. Slight dissociation of fumaric acid co-crystal was measured by XRPD in all cases, lowest dissociation was observed when Cremophor ELP was added to the granulation liquid. Dissolution profiles of the formulations were analysed by flow through dissolution method. The *in vitro* dissolution profile of the experimental formulation showing the best co-crystal integrity was approximately 10% lower compared to the formulation with the highest integrity.

Keywords: wet granulation; co-crystal integrity; flow through dissolution

1. INTRODUCTION

1.1. General introduction

The advantages of pharmaceutical co-crystals are better solubility and dissolution kinetic profiles than that of free base or acid forms (Good *et al.*, 2009, McMahon *et al.*, 2005, Horst *et al.*, 2009, Mohamed *et al.*, 2009). Using co-crystals within formulations gives the opportunity to increase oral bioavailability of APIs, especially when free acid or base forms show very low aqueous solubility such as BCS Class II and IV actives (Lipinski, 2000, Amidon *et al.*, 2006). The target of pharmaceutical development is to administer pharmaceutical co-crystals in formulations, in which the integrity of the co-crystal is ensured as much as possible. Most preferred granulation process from industrial manufacturing point of view is the wet granulation.

The target of this study was to evaluate how the physical integrity of the co-crystal during a high shear wet granulation process is affected. In addition, the influence of Cremophor ELP on physical stability and dissolution was studied. Cremophor ELP is commonly used as solubiliser and is known to ensure the integrity of the co-crystals (Yalkowsky, 1999, Dai *et al.*, 2008, Eastman, 2005, Venczel *et al.*, 2012). Cremophor ELP has been demonstrated to be a well tolerated

pharmaceutical excipient via oral route (Tatou *et al.*, 1996, Giorgi *et al.*, 2003).

SAR1 fumaric acid co-crystal was used as model active pharmaceutical ingredient in the present study. Increased bioavailability of fumaric acid co-crystal versus the free base could be confirmed in a pharmacokinetic study (Venczel *et al.*, 2012).

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Active pharmaceutical ingredient

SAR1, a co-crystal with fumaric acid, was used in the study as a model material (Fig.1).

As a weak base with pKa₁ of 2.9 and pKa₂ of 3.5 salt formation is only feasible with strong acids however, under strong acidic conditions, hydrolysis occurs. Salt formation process has not further been taken into consideration to avoid chemical degradation of the parent compound.

The batch of the fumaric acid co-crystal was manufactured in 0.7 kg scale. Particle size distribution of SAR1 showed 15.7 μm at D(90) measured by laser diffraction.

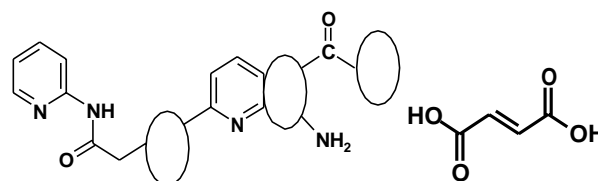


Figure 1: SAR1 fumaric acid co-crystal as a model active pharmaceutical ingredient (API)

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2.1.2. Dissolution buffer

Acetate buffer solution, pH=4.5 with 0.5 % sodium dodecyl sulphate was prepared according to USP recommendations. Sodium dodecyl sulphate was ordered from Fluka. Discriminative properties of the dissolution method were evaluated in a separate study (Venczel *et al.*, 2012).

2.1.3. Pharmaceutical excipients

Cremophor ELP was ordered from BASF. Cremophor ELP, a purified grade of Cremophor EL was specially developed for sensitive active ingredients, as the higher purity was found to improve their stability (Amidon *et al.*, 2006).

Pharmaceutical excipients such as mannitol, microcrystalline cellulose, HPMC, croscarmellose sodium and stearyl fumarate sodium, all compliant to pharmacopeial requirements, were ordered from the internal warehouse of Sanofi. Excipients were selected based on results of chemical and physical compatibility pre-tests containing 1 % of active as the most sensitive concentration after 30 days storage at 50°C and 50°C, 75% R.H.

2.2. Methods

2.2.1. Chemical manufacturing of the co-crystal

The reactor was charged with acetone (12 L), SAR1 base Form III (592 g) and fumaric acid (600 g). The slurry was stirred at room temperature for 24 hours, the crystals were filtered off, washed with water (1 L) and ethanol (1 L), and dried in a vacuum at 80°C for five hours.

The obtained yield was 723 g (94.0%) as pale yellow powder. The purity of the product was 98.9% determined by HPLC.

2.2.2. Analytical methods

2.2.2.1. Dissolution method

Experimental dissolution work was carried out in an opened, Sotax type flow through dissolution equipment (Venczel *et al.*, under publication). The temperature of the media was 37.0 ± 0.5 °C. Dissolution samples were collected by a fraction collector followed by a spectrophotometric analysis. Samples were collected up to 120 minutes. Flow through dissolution was performed on 100 mg mass tablets with 10% API load.

2.2.2.2. Spectrophotometric method

The analysis of dissolution samples was performed by an Agilent 8453 type spectrophotometer. Samples were measured at 342 ± 2 nm undiluted (90 and 120 minutes dissolution) or after 200-fold (until 20 minutes dissolution), 40-fold (30 minutes dissolution) or 60-fold (until 60 minutes dissolution) dilution with dissolution medium acetate buffer.

2.2.2.3. XRPD method

The X-ray diffractograms were recorded on a PANalytical X'PertPro diffractometer, using Cu-K α (without monochromator) radiation. The granulated samples were loaded to a 25mm standard holder and measured in the range of 3- 35°2 θ with 0.007°/min scan speed. Starting materials and the centrifuged suspension samples were measured on silicon zero background holder with 0.05°/min scan speed.

2.2.3. Pharmaceutical formulations

The composition and function of each formulation are summarized in Table 1.

2.2.3.1. High shear granulation

Manufacturing of the different formulations were performed in Mi-pro miniaturized high shear granulator (Pro-C-ept). The speed of the impeller was 500 rpm while the chopper rpm was 3000.

Four experimental compositions were manufactured in 30g miniaturized scale with 10% API load. The integrity of the co-crystal was studied from granules.

2.2.3.1.1. F1 formulation

API and the excipients of the internal phase were sieved through 0.63 mm sieve size. Cremophor ELP was added to the internal phase as last excipient and granulation was performed with water. Drying of the wet internal phase was performed at 50°C until 45 minutes. Calibration of the granules was made on 1 mm sieve size and finally stearyl fumarate sodium of the external phase was added to the granules.

2.2.3.1.2. F2 formulation

The active and the excipients of the internal phase were sieved on 0.63 mm sieve size. Cremophor ELP was added to the granulation liquid. Drying of the wet internal phase was performed at 50°C until 45 minutes. Calibration of the granules was made on 1 mm sieve size and finally the excipient of the external phase was added to the granules.

2.2.3.1.3. F3 formulation

Water was added directly to the active followed by the excipients of the internal phase. Cremophor ELP was added to the internal phase as the last excipient.

Drying of the wet internal phase was performed at 50°C until 45 minutes. Calibration of the granules was made on 1 mm sieve size and finally the excipient of the external phase was added to the granules.

2.2.3.1.4. F4 formulation

Cremophor ELP was added directly to the active followed by the excipients of the internal phase. Granulation was performed with water. Drying of the wet internal phase was performed at 50°C until 45 minutes. Calibration of the granules was made on 1 mm sieve size and finally the excipient of the external phase was added to the granules.

Table 1: Formulation compositions and function of ingredients

Formulations	Function of ingredients	F1	F2	F3	F4
Internal phase					
SAR1 fumaric acid co-crystal	active pharmaceutical ingredient	10 %*	10 %*	10 %*	10 %*
mannitol	diluent	49 %	49 %	49 %	49 %
microcrystalline cellulose	diluent	25 %	25 %	25 %	25 %
Hypromellose	binder	5 %	5 %	5 %	5 %
croscarmellose sodium	disintegrant	4 %	4 %	4 %	4 %
Cremophor ELP	surfactant solubiliser	5 %	5 %	5 %	5 %
granulation liquid	-	water	water + Cremophor ELP	water	water
position of water	-	Added to the internal phase	added to the internal phase	added to the active directly	added to the internal phase
position of Cremophor ELP	-	Last excipient of the internal phase	part of the granulation liquid	last excipient of the internal phase	added to the active directly
External phase					
stearyl fumarate sodium	glidant	2 %	2 %	2 %	2 %
Total	-	100 %	100 %	100 %	100 %
Mass of tablets	-	100 mg	100 mg	100 mg	100 mg

* expressed as free base, fumaric acid parts are corrected from quantity of the diluents

Loss on drying values were measured at 105°C until 20 minutes three times during the manufacturing process: after mixing of the internal phase without Cremophor ELP, after the wet granulation process and after drying. Comparable loss on drying results were reached for the internal phase and after the drying process.

2.2.3.1.5. Reference

A suspension formulation was prepared as reference to the solid experiments.

For the 10 mg/ml concentrated suspension formulation API was manually suspended in a mortar in 5% Cremophor ELP in methyl cellulose water solution.

2.2.3.2. Tableting process

Tableting was performed on Korsch excentrical tableting machine with 3-15 kN pressure force. Flat, rimmed tablets were pressed with 30-35 N hardness. The diameter of the tablets were 6 mm. The temperature of the plant was 21°C and the relative humidity was 23 %.

3. RESULTS AND DISCUSSION

In a previous work, it was shown that Cremophor ELP can have protective effects against rapid dissociation of

fumaric acid co-crystal of SAR1 as active pharmaceutical ingredient (Venczel et al., 2012).

Cremophor ELP was included in the formulations at three different positions. It was the last excipient of the internal phase in two cases (F1 and F3), one time it was added directly to the API (F4) and one time it was the part of the granulation liquid (F2). Different addition orders of water within the formulations were investigated as well. In order to evaluate the effect on the integrity of the co-crystals, water, as standard granulation liquid, was added to the internal phase in three cases (F1, F2, F4) and in one case it was added directly to the active (F3).

The crystallinity of API in the granules was examined by XRPD (Figure 2).

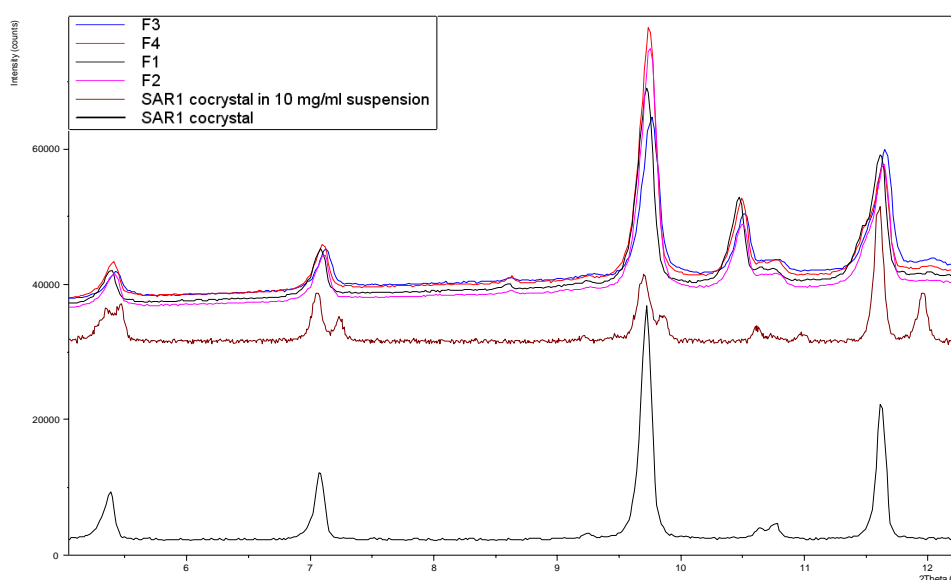
The appearance of a peak at $\sim 12.0^\circ 2\theta$ not related to any starting phase was observed with different intensities in the granules. This new peak corresponds to a

Table 2: Intensity % of peak 12.0° 2 Θ compared to peak 11.6° 2 Θ (100%)

Samples	Intensity %
F1	4.0
F2	2.9
F3	6.7
F4	4.3
SAR1 co-crystal	0.0

Table 3: XRPD and dissolution results of the four test formulations

Formulations	F1	F2	F3	F4
Integrity of SAR1 fumaric acid co-crystal by XRPD method	F1 and F4 same level of integrity	highest level of integrity	lowest level of integrity	F1 and F4 same level of integrity
Dissociation of SAR1 fumaric acid co-crystal by XRPD method	signs of the dissociated co-crystal	signs of the dissociated co-crystal	highest level of dissociation	signs of the dissociated co-crystal
Dissolution profiles	reference profile	≈ 10 % decrease	comparable with F1	≈ 10 % decrease

**Figure 2: XRPD patterns of the test formulations F1 – F4 compared to SAR1 co-crystal and SAR1 co-crystal reference suspension formulation**

disproportionated free base observed in the reference formulation (10 mg/ml SAR1 suspension). Based on the results of our studies the fumaric acid to API ratio was shown to decrease in parallel with the intensity increase of peak 12.0° 2 Θ in the XRPD pattern of centrifuged suspension samples. It suggests that in the granulated samples a minor part of the API disproportionates to base and fumaric acid.

The appearance of the disproportionated phase in the granules are represented by the intensity % of peak 12.0° 2 Θ compared to peak 11.6° 2 Θ (Table 2). The most intense change was observed in the F3 sample, where the API was mixed with water in a mortar before granulation, which is similar to the preparation of

the suspension. The highest level of co-crystal integrity was measured for F2 and F4 formulations where the SAR1 was granulated with the mixture of water and Cremophor ELP (F2) and when Cremophor ELP was added directly to SAR1 (F4).

When the dissolution kinetics were measured, about 10% dissolution decrease were observed with F2 compared to the F1 formulation (Table 3 and Fig. 3). The difference in dissolution among the F2, F4 and F1, F3 formulations is significant at P=0.95 confidence level. A slight decrease in dissolution could have a negative impact on bioavailability that is why it is proposed to increase the content of the disintegrant within the formulation when Cremophor ELP is used.

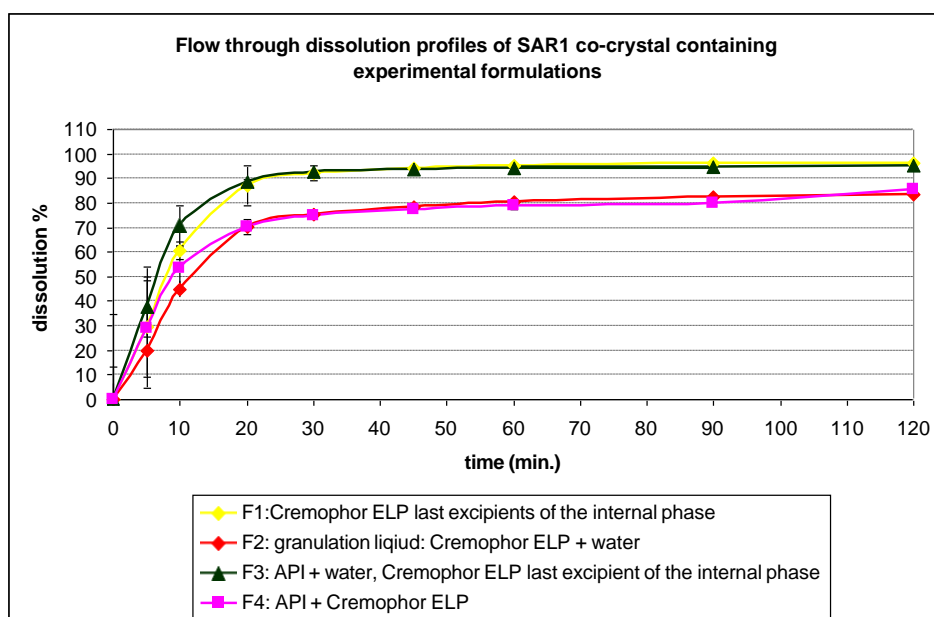


Figure 3: Flow through dissolution profiles of SAR1 co-crystal formulations F1 – F4

4. CONCLUSION

Keeping the integrity of co-crystals as pharmaceutical ingredients after the manufacturing process is essential to ensure advantages like faster dissolution kinetic and higher bioavailability (Remenar *et al.*, 2007).

As the physical interaction between the active and its co-crystal former, these pharmaceutical co-crystals are sensitive to rapid or slow dissociation in aqueous microenvironment. Four experimental formulations were manufactured to study the influence of water and Cremophor ELP order of addition in the formulation process.

Based on XRPD results higher integrity of the active as co-crystal was measured when granulation process was performed with the mixture of Cremophor ELP and water. Fast dissolution kinetic were obtained with all formulations containing the co-crystal form. This suggests that Cremophor ELP is a suitable pharmaceutical excipient to increase the physical stability of co-crystals and to ensure a positive effect on bioavailability. Dissolution profiles of Cremophor ELP containing formulations needs to be monitored regularly as Cremophor ELP has both an effect on co-crystal integrity and on dissolution kinetics. However from biological effect point of view, ensuring co-crystal integrity is more important than a slightly lower dissolution profile.

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