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How to stop disproportionation of a hydrochloride salt of a very weakly basic compound in a non-clinical suspension formulation

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ABSTRACT

Our objectives were to stabilize a non-clinical suspension for use in toxicological studies and to develop methods to investigate the stability of the formulation in terms of salt disproportionation. The compound under research was a hydrochloride salt of a practically insoluble discovery compound ODM-203. The first of the three formulation approaches was a suspension prepared and stored at room temperature. The second formulation was stabilized by pH adjustment. In the third approach cooling was used to prevent salt disproportionation. 5 mg/mL aqueous suspension consisting of 20 mg/mL PVP/VA and 5 mg/mL Tween 80 was prepared for each of the approaches. The polymer was used as precipitation inhibitor to provide prolonged supersaturation while Tween 80 was used to enhance dissolution and homogeneity of the suspension.

The consequences of salt disproportionation were studied by a small-scale *in vitro* dissolution method and by an *in vivo* pharmacokinetic study in rats. Our results show that disproportionation was successfully suppressed by applying cooling of the suspension in an ice bath at 2–8 °C. This procedure enabled us to proceed to the toxicological studies in rats. The *in vivo* study results obtained for the practically insoluble compound showed adequate exposures with acceptable variation at each dose level.

1. Introduction

Many of the new drug candidates are poorly water-soluble, which may lead to low oral bioavailability and consequently insufficient exposures *in vivo*. This problem is frequently encountered during the non-clinical development phase (Benet et al., 2006). Salt formation, when possible, is one of the methods used to increase solubility, to elevate dissolution rate, and thus to enhance bioavailability (Serajuddin, 2007). The stability of the salt form is critical for the quality of the pharmaceutical product and for the *in vivo* performance of poorly water-soluble weakly acidic or basic drugs. If a salt undergoes disproportionation to the free form solid phase, its bioavailability may be reduced (Zannou et al., 2007; Merritt et al., 2013). In terms of salt stability, namely disproportionation, the difference between the pKa values of the basic drug compound and the acid forming the salt is crucial. Hence, development of the stable salt form of a very weakly basic drug compound with low pKa is ambitious (Gould, 1986). Stephenson et al. (2011) explored more than 200 marketed drugs that had weakly basic compounds as their API. According to their research, all APIs as a salt form within the formulation, had pKa values higher than 4.6. They concluded

Abbreviations: API, active pharmaceutical ingredient; AUC, area under the curve; C_{max}, maximum concentration; K_2EDTA, dipotassium ethylenediaminetetraacetic acid; FaSSIF, fasted state simulating intestinal fluid; FeSSIF, fed state simulating intestinal fluid; FGFR, fibroblast growth factor receptor; HCl, hydrochloride; μDISS, micro dissolution apparatus; NIR, near infrared spectroscopy; PK, pharmacokinetic; PTFE, polytetrafluoroethylene; PVP/VA, polyvinylpyrrolidone/vinyl acetate; ssNMR, solid state nuclear magnetic resonance spectroscopy; SIF, simulated intestinal fluid; UV, ultraviolet; VEGFR, vascular endothelial growth factor receptor; XRPD, x-ray diffraction.

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that formulation development using the salt form of weak bases with pKa values of less than five, would be challenging.

Careful selection of excipients in the formulation of basic salts is important since they can increase the microenvironmental pH above the pH_{max} (Fig. 1). Microenvironmental pH above the pH_{max} is thermodynamically favorable condition for disproportionation of the salt form of a weak base (Badawy, 2001; Guerrieri and Taylor, 2009; Stephenson et al., 2011; John et al., 2013; Merritt et al., 2013; Hsieh and Taylor, 2015; Koranne et al., 2020). In addition to the microenvironmental pH, there are other crucial factors responsible for disproportionation. The studies discussing the roles of relative humidity, temperature change, particle size and surface area, salt solubility and volatilization of counter-ion have been thoroughly reviewed by Thakral and Kelly (2017).

Enhanced solubility and dissolution rate as well as transient supersaturation induced by the salt form of a compound may provide adequate bioavailability of a poorly soluble compound. Polymers like PVP/VA (polyvinylpyrrolidone/vinyl acetate) have been used to prevent precipitation and to retard crystallization in the gastrointestinal (GI) tract after dissolution. It is thus possible to extend supersaturation and thereby achieve higher bioavailability (Brouwers et al., 2009; Liu et al., 2016). This idea is known as “spring” (initial dissolution) and “parachute” (prolonged supersaturation) (Guzmán et al., 2007; Ilevbare et al., 2013; Ueda et al., 2013).

Prior to the development of a clinical formulation, a safe but high exposure non-clinical formulation needs to be developed for pharmacological and toxicological studies (Li and Zhao, 2007). Regarding the quality of a formulation, the shelf life is one of the important considerations during non-clinical studies. Besides the dosage strength and chemical stability, physical stability of the formulation may be crucial. In non-clinical studies, suspension is the prevailing formulation approach (Neervannan, 2006). In such water-based formulations, however, the risk of disproportionation is high and may lead to unacceptably slow dissolution and variable drug absorption levels.

Traditionally disproportionation is studied in solid pharmaceutical powders and formulations. The experimental techniques to verify salt disproportionation include conventional x-ray diffraction (XRPD) and vibrational spectroscopy (Dharani et al., 2019; Figueroa et al., 2019), advanced solid state nuclear magnetic resonance spectroscopy (ssNMR) (Hirsh et al., 2018), synchrotron x-ray diffractometry (Koranne et al., 2017), non-linear imaging (Strachan et al., 2011) and even machine-vision-based methods (Štukelj et al., 2020). In addition, chemometric methods based on near infrared (NIR) and raman spectroscopy data have been shown to produce precise models to accurately determine the fractions of base and salt forms in the drug product (Dharani et al., 2018). It is more challenging, but possible, to use these methods also to detect salt disproportionation in suspension formulation. However, while these methods can be sensitive to the disproportionation process, they may not detect the detrimental consequences of even minor disproportionation at the surface of drug compound particles on dissolution and consequently, on bioavailability.

The effect of disproportionation on *in vitro* dissolution has been previously studied in detail (Ewing et al., 2015; Wray et al., 2015; Rahman et al., 2018). Chiang et al. (2009) concluded that formulation

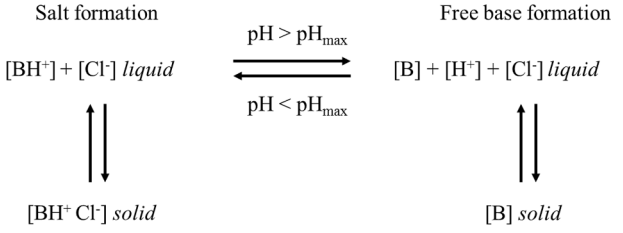

Fig. 1. The conversion between the solid salt and free base versus pH. Figure adapted from Pudipeddi et al. (2002).

containing no water can be used as an approach to hinder the salt form conversion in non-clinical formulations. On the contrary, they found in their toxicological formulation study that it is important to consider the quantity of water present *in vivo*. Insufficient amount of water slows down dissolution and leads to insufficient exposures.

A clinical example was presented by Unger who reported a study in which prasugrel HCl salt was found to convert from salt to free base in high extent. In this case, however, conversion of the salt did not have a clinically important effect on the performance of the drug (Unger, 2009).

The aim of this work was to find a way to stabilize the non-clinical suspension formulation of an HCl salt of a practically insoluble and very weakly basic compound. During non-clinical development, it had been proven that absorption of the compound is dramatically solubility limited. Adequate exposures were only achieved with solution formulation containing strong solvents that are unacceptable in toxicological studies. The concentration of the suspension intended for use in toxicological studies was high (5 mg/mL *i.e.* 50 mg/kg) with respect to the intrinsic solubility of the free base (0.2 μg/mL). In addition, there is a substantial solubility enhancement of the salt over the free base at pH above the pH_{max} promoting disproportionation. In the literature, some formulation stabilization methods like pH adjustment have been presented (Shah et al., 2014). However, for the salt we study, the pH stabilization is not an option because the pKa (3.1) and consequently the pH_{max} (1.3) of the studied salt are too low. According to the pH_{max} theory, a physiologically unacceptably low pH (less than1.3) is needed to stabilize the suspension formulation by the pH adjustment. In the following sections, we address (1) the possibility to stop disproportionation of the HCl salt of a weakly basic compound in a non-clinical suspension formulation by cooling and (2) the consequences of disproportionation on *in vitro* dissolution and *in vivo* exposure. To our knowledge, combination of these topics has not been reported in the literature previously.

2. Materials

2.1. Study compound

The compound of interest was ODM-203 (Fig. 2) which is a small molecule with balanced inhibitory effects on both FGFR 1–4 and VEGFR 1–3 subtypes (Holmström et al., 2019). Inhibitors of fibroblast growth factor receptors (FGFR) are being developed for the treatment of solid tumours with FGFR genetic alterations. The compound has entered phase II studies in human (Bono et al., 2018). The HCl salt of ODM-203 was used for the toxicological study formulation. Polymorph 3 three was selected for the studies. The form was, due to practical reasons, the only form suitable for synthesis up-scaling. The physicochemical properties of ODM-203 are presented in Table 1.

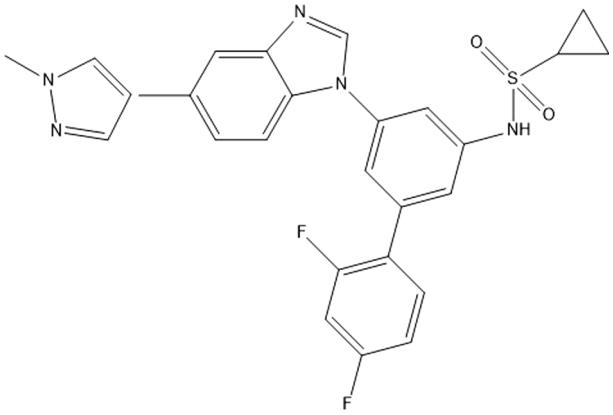

Fig. 2. Chemical structure of ODM-203.

Table 1
Physicochemical properties of ODM-203.

Solubility (base form) – pH profile	Solubility at 25 °C (μg/mL)
pH 2.0 HCl	2.0
pH 3.0 phosphate buffer	0.5
pH 4.1 acetate buffer	0.2
pH 4.5 acetate buffer	0.2
pH 6.5 phosphate buffer	0.2
pH 8.0 phosphate buffer	0.2
pH 10.0 borate buffer	15
0.1 M NaOH	848
Water	0.2
SGF pH 1.2	1.4
FaSSIF pH 6.5	1.4
FeSSIF pH 5.0	6.2
pH 1.0 (HCl salt) at 37 °C	15
pH 1.0 (HCl salt) at <1 °C	3.4
pKa	3.1 (basic) 8.1 (acid)
pH_{max}	1.3
LogP	3.6

2.2. Toxicological suspension formulation

Three different suspension formulations were prepared for the comparison studies. They have the same chemical composition, but different conditions of preparation and use (Table 2). In the formulations, the polymer (PVP/VA) was used as precipitation inhibitor to provide the "parachute" effect (Guzmán et al., 2007; Ilevbare et al., 2013; Ueda et al., 2013;) while Tween 80 was used as surface active ingredient to enhance dissolution and homogeneity of the suspension (Balakrishnan et al., 2004).

3. Description of the methods

3.1. Determination of pH_{max} and suspension pH

The solubilities of the free form and the HCl salt of ODM-203 (C_s and C_{sS}, respectively) were calculated using the following equations:

$$C_s = K_s\left(1+\frac{[H^+]}{K_a}\right) \quad (1)$$

$$C_{sS} = \sqrt{K_{sS}\left(1+\frac{K_a}{[H^+]}\right)} \quad (2)$$

where K_s is the intrinsic solubility of ODM-203 free form (0.4 μM), K_a the acid dissociation constant (basic) of ODM-203 (3.1) and K_{sS} the solubility product of the ODM-203 HCl salt (625 μM). These equations allow the theoretical pH_{max} to be evaluated from Eq. (3) (Kramer and Flynn, 1972; Bogardus and Blackwood, 1979; Serajuddin, 2007; Stephenson et al., 2011)

$$pH_{max} = pKa + \log\left(\frac{K_s}{\sqrt{K_{sS}}}\right) \quad (3)$$

Table 2
Description of the formulations 1–3 prepared for the comparison study.

Formulation no	Description of the formulation
Formulation 1	ODM-203 5 mg/mL aqueous suspension consisting of 20 mg/mL PVP/VA and 5 mg/mL Tween 80
Formulation 2	ODM-203 5 mg/mL aqueous suspension consisting of 20 mg/mL PVP/VA and 5 mg/mL Tween 80. pH of the vehicle was adjusted to 1.2 with HCl.
Formulation 3	ODM-203 5 mg/mL aqueous suspension consisting of 20 mg/mL PVP/VA and 5 mg/mL Tween 80. The suspension was continuously stirred in an ice bath during preparation (temperature of the suspension being 2–8 °C).

The empirical pH_{max} determination was carried out by monitoring the slowly decreasing pH of the supersaturated salt slurry. In order to follow disproportionation in the suspensions, pH of the samples was recorded (pH meter, Mettler Toledo, SevenCompact, Switzerland).

3.2. In vitro dissolution

The dissolution-precipitation behaviour of the formulations 1–3 was studied as follows. In the test 3 mL of suspension were added to 12 mL of the dissolution medium with a nominal concentration of the sample solution thus being 1.0 mg/mL of ODM-203. A micro dissolution instrument (μDISS) with *in situ* fiber optics (pION Inc., USA) was used to frequently measure the ODM-203 concentration at a wavelength of 328 nm against a pre-prepared standard curve. A second derivative of the ultraviolet (UV) spectrum was used in the quantification of the results to avoid interference of the undissolved particles (Van Eerdenbrugh et al., 2011). As the rats were treated in the fed state *in vivo*, FeSSIF was selected as the *in vitro* dissolution medium. The FeSSIF Version 1 medium was prepared by dissolving commercially available SIF powder acquired from Biorelevant.com (Biorelevant.com Ltd, UK) in an acetate buffer solution pH 5.0 (Reagena, Finland). The UV detection of the dissolution samples was done at one-minute intervals. Dissolution analysis of formulation one was performed immediately after the suspension preparation and after 60 and 120 min. Formulations 2 and 3 were subjected to dissolution analysis immediately after the suspension preparation and after 15, 30, 45 and 60 min.

3.3. XRPD analysis for suspension stability

The traditional qualitative phase analysis was conducted by comparison of the XRPD patterns with the known patterns of the solid-state forms of ODM-203 HCl salt and its free base form. We used a conventional XRPD incorporated in the Philips X'Pert PRO multi-purpose θ-θ diffractometer (Panalytical, the Netherlands) equipped with the RTMS (Real Time Multiple Strip) detector and filtered Kα radiation from a Cu tube at 40 mA and 45 kV.

The concentration of ODM-203 suspension formulations in the PK study was 5 mg/mL (Table 2). However, the XRPD signal for *in situ* measurements in suspension was insufficient. For this reason, the solid fraction was extracted by vacuum filtering the suspension sample with 0.2 μm filters (Millipore, type GTBP, USA). The extracted solid fraction was placed on the sample holder and analysed. In order to enhance the signal-to-noise ratio, 5 subsequent x-ray patterns were superposed. The stability measurements for formulations 1 and 3 were performed as soon as the suspension was prepared (within 10 min) and then after 60, 120, 240, 360 and 1440 min.

3.4. Dosing in pharmacokinetic studies in rats

The *in vivo* PK protocol is presented in brief in Table 3.

Pharmacokinetic studies were carried in two laboratories, in

Table 3
Protocol for the formulation PK study in rats.

Animals	Male Wistar Rats (8–10 weeks old), $n = 3$
Dose	50 mg/kg
Route of administration	Oral under complete fed condition
Formulation	Formulations 1 and 3
Dosing Time	Formulation 1 administered less than 15 min and post 30, 60, 90, 120 & 180 min of formulation preparation Formulation 3 administered less than 60 min after formulation preparation
Blood collection time points	Pre-dose, 120, 240, 360 & 1440 min or 60, 120, 360, 480 & 1440 min.
Dose volume:	10 mL/kg

Aurigene Discovery Technologies Ltd, Bangalore, India and in Covance Inc., Harrogate, UK. The studies were approved by Institutional Animal Ethic Committee (IAEC) of Aurigene Discovery Technologies Ltd, Bangalore (Aurigene/IAEC/PCD/01E-11/03-2013) and conducted in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India. At Covance Inc. the studies were carried in accordance with the requirements of the Animals (Scientific Procedures) Act 1986. Local ethical review was maintained.

Liquid chromatography-tandem mass spectrometry (LC/MS/MS) was used to determine concentrations of ODM-203 in plasma. Blood samples were taken at predefined time points into blood collection tubes containing K_2EDTA (dipotassium ethylenediaminetetraacetic acid) as anticoagulant. Plasma was obtained following centrifugation of the whole blood, transferred to uniquely labelled polypropylene tubes and deep-frozen at −80 °C nominal. At the time of analysis, the study samples were collected from the deep freezer and allowed to thaw to room temperature. Following protein precipitation with acetonitrile the samples were analyzed with an AB Sciex API 4000 triple quadrupole mass spectrometer (Sciex Ltd., USA). The lower limit of quantitation was 10 ng/mL.

4. Results

4.1. pH_{max} and pH of the suspensions

Bogardus and Blackwood (1979) stated that, for a basic drug, the saturation solubilities of free base and its salt form will be equal at pH_{max}. By solving the relevant equations for pH_{max}, they derived the relationship expressed in Eq. (3). Based on that equation, the pH_{max} of the ODM-203 HCl salt is approximately 1.3.

The pH_{max} was also experimentally determined by monitoring the decrease in pH of the supersaturated ODM-203 suspension. The suspension was mixed for 3 days. After the mixing the pH of the suspension was 1.39 and after 4 days 1.34.

The suspension pH was measured in parallel with the investigative dissolution analyses. The pH of the formulation 1 decreased from 2.78 to 2.34 in 180 min whereas for the formulation 3 pH remained stable at a value of about 3. The pH remained also stable for formulation 2 at a pH of 1.2.

4.2. In vitro dissolution and XRPD results

The dissolution profile of formulation 1 changed dramatically depending upon the sample storage time at room temperature (Fig. 3a). The highest concentration was achieved for the freshly prepared suspension, approximately 0.3 mg/mL at 20 min. The sample stored at room temperature for 60 min reached only 0.06 mg/mL at 10 min and the profiles for samples stored longer than 60 min were flat, *i.e.,* no detectable concentrations were obtained.

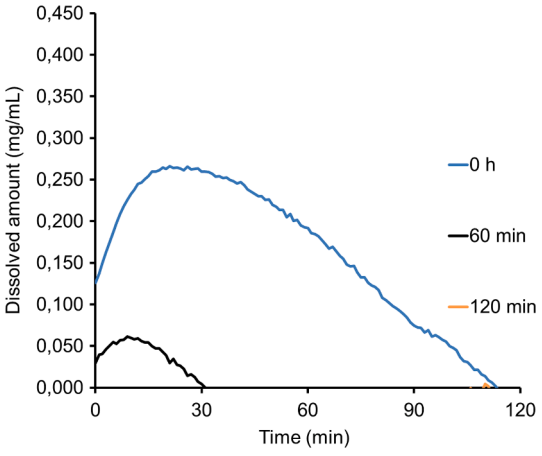

Fig. 3a. Formulation 1: Dissolution in FeSSIF. Profiles of the freshly prepared sample and the samples stored at room temperature for 60 and 120 min.

The x-ray diffraction results of formulation 1 (Fig. 3b) show that no disproportionation of the salt in the suspension analyzed was detected at the 10 min time point but the typical peaks of the free base (2Θ = 25–26°) appeared already after 60 min. Their relative intensities grew with time so that after 360 min only the free base can be detected. The characteristic peaks of the ODM-203 HCl salt (2Θ = 7.2°; 9.2°; and 11.7°) are difficult to detect in the samples measured after 240 min of the sample shelf life.

The width of the diffraction peaks of the free base is considerably larger than that of undissolved HCl salt and the reference free base sample. This demonstrates the lower degree of crystallinity of the free base. The visual appearance of suspension also changed. At the start, the suspension was almost transparent and after 120 min it had milky opacity.

Formulation 2, the suspension with a pH adjusted to less than the estimated pH_{max} value, produced enhanced dissolution profiles for all samples with different storage times (Fig. 4). The maximum dissolved concentration was 0.4 mg/mL independently of storage time.

A greatly enhanced dissolution rate was also obtained for the cold stabilized formulation 3 (Fig. 5a). Compared to the dissolution profiles of the room temperature samples (formulation 1), the maximum dissolved concentration was enhanced for the samples prepared and stored at 2–8 °C up to 60 min. The maximum dissolved concentration was 0.4 mg/mL similarly as for formulation 2. Also, the time ODM-203 stayed in solution was prolonged.

The x-ray diffraction study of the cold stabilized formulation 3 (Fig. 5b) showed that the characteristic peaks of the ODM-203 HCl salt (2Θ = 7.2°; 9.2°; and 11.7°) can still be detected after 1440 min storage time and the small peaks typical to the free base (2Θ = 25–26°) can only be detected after 240 min. Their relative intensity grew very slowly with time so that after 1440 min there was a mixture of at least two solid phases: the salt polymorph 3 and the free base. Moreover, in the pattern measured at 1440 min we observe the presence of another crystalline form, but this form does not match any known references of the ODM-203 free base.

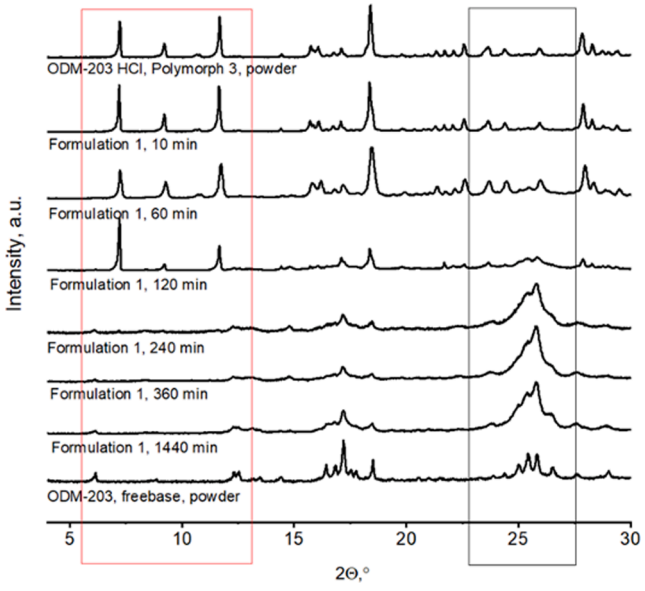

Fig. 3b. Formulation 1: X-ray patterns of the suspension stored for 10, 60, 120, 240, 360 and 1440 min at room temperature and the X-ray patterns of the free base and salt polymorph 3.

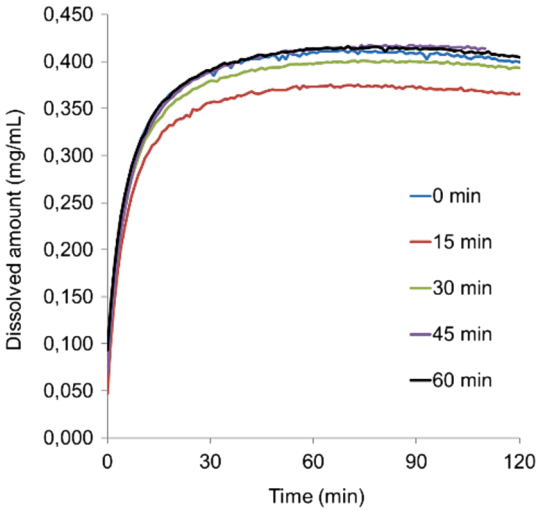

Fig. 4. Formulation 2, pH adjusted to 1.2: Dissolution in FeSSIF. Profiles of the freshly prepared sample and the samples stored at room temperature for 15, 30, 45 and 60 min.

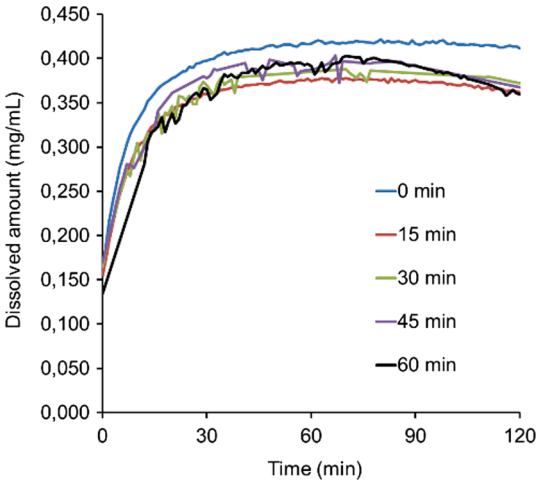

Fig. 5a. Formulation 3, cold stabilized: Dissolution in FeSSIF. Profiles of freshly prepared sample and the samples stored in an ice bath at 2–8 °C for 15, 30, 45 and 60 min.

4.3. Results of pharmacokinetic studies in rats

The results of the PK study obtained in the case of administration of formulation 1 are shown in Table 4. These PK results exhibit dramatic differences in the exposures after different storage times at room temperature. The maximum plasma concentration (C_{max}) after dosing of the freshly prepared formulation 1 was approximately 9700 ng/mL while it was only 360 ng/mL after administration of the 120 min dosage. The corresponding decrease in the area under the curve (AUC) was from a peak of 145,000 h*ng/ml to less than 5000 h*ng/mL Only 3% of exposure after 180 min storage was achieved compared to the exposure obtained when the suspension was administered within 15 min after its preparation. Table 4 also shows the high exposure obtained after administrating the cold stabilized formulation 3 within 60 min after preparation. The values of AUC and C_{max} were like the those obtained in the case of administration of the freshly prepared suspension, which was stored at room temperature for less than 15 min.

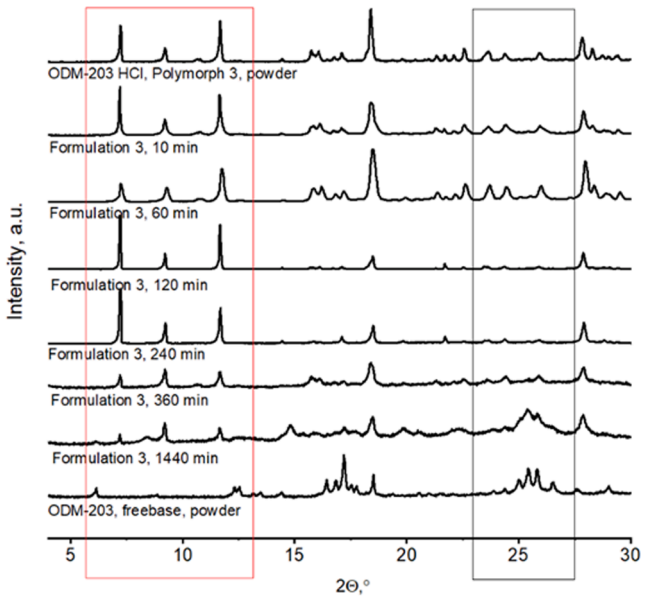

Fig. 5b. Formulation 3, cold stabilized: X-ray patterns of the suspension stored for 10, 60, 120, 240, 360 and 1440 min in an ice bath 2–8 °C. X-ray patterns of the free base and salt polymorph 3.

5. Discussion

Our aim was to study the stability of a suspension formulation in terms of salt disproportionation. The suspension has a solid hydrochloride salt suspended in a medium that is highly supersaturated with respect to the free base. Three different approaches were used. The first formulation was a suspension prepared and stored at room temperature. It was prone to fast disproportionation limiting its utility *in vivo*. The dissolution results showed a decrease in the dissolved concentration of ODM-203, which was already apparent after storage for 1 h of the sample at room temperature, indicating progressive disproportionation in the formulation (Fig. 3a). After 2 h of storage, the dissolved concentration in the dissolution test was negligible. In the XRPD analysis disproportionation was detected after 60 min storage. Further, the XRPD results showed that ODM-203 HCl salt had been completely converted to the free base after approximately 240 min (Fig. 3b).

In the second approach, the supersaturation with respect to the free base was controlled by decreasing salt solubility by common ion effect and by lowering the pH of the suspension to a value less than pH_{max} (1.3). This approach stabilized the formulation. Reproducible dissolution profiles were obtained for the suspension (Fig. 4). That was in accordance with the pH_{max} theory (Fig. 1). In addition, the *in vitro* dissolution data suggests that the potential solubility limitation due to the common ion effect is not crucial in this case. Such extreme pH condition, however, is outside the recommended range (pH 2–9) for *in vivo* studies. The formulation administration in this case may lead to diarrhea, vomiting, tissue ulceration or necrosis and pain on administration *in vivo* (Li and Zhao, 2007; Turner et al., 2011).

In the experiments with the third formulation, disproportionation was successfully suppressed by applying cooling of the suspension in an ice bath at 2–8 °C. While a decrease of pH of the suspension stored at room temperature indicated the presence of disproportionation, the same was not observed in the cold suspension. In this case the dissolution profiles (Fig. 5a) were similar to the profiles of the pH adjusted suspension 2. The suppression of disproportionation of the suspension 3 by cooling was confirmed by the XRPD results (Fig. 5b).

Our results show the higher sensitivity of the dissolution method to disproportionation compared to the XRPD method. This is likely because the x-ray beams diffract from both particle surfaces as well from internal bulk material. So, the large changes on the surfaces are a small change in

Table 4
PK parameters derived from plasma samples taken from the rats (n = 3). Formulation 1, stored at room temperature, and formulation 3, stored in an ice bath at 2–8 °C. The time points (minutes) refer to the storage time of the formulations.

	<15 min	30 min	60 min	90 min	120 min	180 min	60 min
Formulation	1	1	1	1	1	1	3
C_{max} (ng/mL)	9680	6020	1520	540	360	310	12,000
$AUC_{0\text{-}24}$h*ng/mL	144,750	81,210	17,560	7400	4740	3840	142,000

C_{max} = maximum plasma concentration.
$AUC_{0\text{-}24}$ = area under the curve between 0 and 24 h.

the whole XRPD pattern.

The extent of salt disproportionation is highly dependent on the value of pH_{max}. Cooling has a tendency to increase the pH_{max} (Eq. (3)) by increasing pKa and decreasing the salt solubility more than the base solubility (Hsieh et al., 2015; Hsieh and Taylor, 2015). In our case, the solubility of the salt decreased from 15 μg/mL (37 °C) to 3 μg/mL (less than 1°C). It is also well-recognized that crystallization depends on supersaturation and solubility. Thus, the nucleation rate increases with increasing solubility of the precipitating solid. However, the rate of nucleation and crystallization starts to increase when the temperature is lowered below a critical temperature. Furthermore, when the drop in temperature continues, the nucleation rate reaches its maximum and starts to decrease due to kinetic reasons, such as slow molecular motion and decreased molecular collision rate (Schmelzer et al., 2015).

Suspension was prepared by sequentially adding suitable quantities of vehicle (aqueous 2 % PVP/VA, 0.5 % Tween 80) to ODM-203 HCl salt with continuous trituration in mortar. Cold stabilized formulation 3 was prepared by adding cold (≤5 °C) vehicle. The preparation methods are expected to lead to at least partial amorphization and disproportionation of API particle surfaces and possibly to formation of amorphous solid dispersion on these surfaces. Potential mechanisms of crystallization inhibition with polymers in amorphous solid dispersions include: antiplasticising effect, reduced molecular mobility, drug-polymer interactions like hydrogen bonding and hydrophobic interaction, and steric hindrance. Corresponding crystallization inhibition mechanisms by polymers in solution include: cosolvent effect that reduces supersaturation, covering the growing crystal faces, and thin film formation around the amorphous particles (Surwase et al.; 2015; Novakovic et al. 2020). Lower temperatures reduce molecular mobility and strengthen drug-polymer interactions (Plaizier-Vercammen and De Nève, 1981; Warren et al., 2010).

The *in vivo* exposures obtained in the rat PK study were in line with the phenomena seen in the *in vitro* dissolution tests. The cooled suspension formulation 3 met the expectations of high *in vivo* exposure, which were based on *in vitro* dissolution results. These results show the importance of the *in vitro* dissolution method in the detection of the consequences of disproportionation.

6. Conclusions

A novel method to limit disproportionation of a HCl salt in a non-clinical suspension formulation has been proposed and successfully verified. We showed that the stability of the suspension formulation of ODM-203 HCl salt affect critically to the degree of exposure *in vivo*. We discovered that disproportionation of the HCl salt in a suspension formulation is a rapid process that leads to negligible dissolution and consequently inadequate exposures *in vivo*. We proved that disproportionation can be significantly impeded by cooling the suspension.

In the literature, there are examples of carefully designed small-scale *in vitro* dissolution studies that enable detection of supersaturation propensity and the effectiveness of precipitation inhibitors (Palmelund et al., 2016; Plum et al., 2017; Gesenberg et al., 2019). Indeed, in our work, with the aid of the *in situ* fiber optic dissolution method, it was possible to elucidate the consequences of disproportionation on the dissolution-precipitation rate of ODM-203 and to predict *in vivo* exposure.

The cooled formulation was stable for up to 60 min. The toxicological study in rats was successfully conducted and the toxicokinetic results showed that adequate exposures with acceptable variation were achieved at each dose level of the administered cold suspension. Our results provide powerful and convenient tools to study and mitigate the disproportionation risk of salt forms in non-clinical suspensions.

CRediT authorship contribution statement

Krista Ojala: Conceptualization, Methodology, Validation, Investigation, Writing - original draft. **Jukka Salmia:** Conceptualization, Methodology, Validation, Investigation, Writing - original draft. **Anna Shevchenko:** Conceptualization, Methodology, Validation, Investigation, Writing - original draft. **Johanna Ylikotila:** Conceptualization, Methodology, Validation, Investigation, Writing - review & editing. **Timo Korjamo:** Validation, Investigation, Writing - review & editing. **Bert van Veen:** Writing - review & editing. **Piritta Koistinen:** Conceptualization, Methodology, Validation, Investigation, Writing - review & editing. **Chira Malmström:** Investigation, Writing - review & editing. **Sirpa Laakso:** Investigation, Writing - review & editing. **Indu Bansal:** Investigation, Writing - review & editing. **D.S. Samiulla:** Investigation, Writing - review & editing. **Anne Juppo:** Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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