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Revisit of solubility of oxytetracycline polymorphs. An old story in the light of new results

Dóra Tempfli[a], Enikő Borbás[b], Hajnalka Pataki[b], Dóra Csicsák[a], Gergely Völgyi[a], Bálint Sinkó[c], Krisztina Takács-Novák[a,*]

[a] Department of Pharmaceutical Chemistry, Semmelweis University, Hőgyes Endre u. 9., Budapest H-1092 Hungary
[b] Department of Organic Chemistry and Technology, Budapest University of Technology and Economics, Műegyetem rakpart 3., Budapest, H-1111, Hungary
[c] Pion Inc. Billerica, Massachusetts 01821, United States

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ABSTRACT

In the literature the therapeutic nonequivalence of oxytetracycline hydrochloride (OTCH) capsules and tablets was attributed to the different aqueous solubility of polymorphs without their comprehensive study. Our aim was to reveal the effects of polymorphism on equilibrium solubility, dissolution kinetics and the supersaturation of two OTCH polymorphs (stable Form A and metastable Form B).The equilibrium solubility was measured in biorelevant pH range 4–7.4 by the standardized saturation shake-flask method. We also studied the solubility in SGF at pH 1.2 and the effect of the pH change from 1.2 to 5.0 on solubility. The dissolution was studied using real-time concentration monitoring with an ATR probe attached to a UV spectrophotometer (μDISS Profiler). A wide spectrum of solid phase analysis methods (SEM, IR, XRPD, Raman) was applied for characterization of polymorphs and to identify which form is present at the equilibrium solubility. Identical equilibrium solubility values were obtained at the same pHs in region 4.0–7.4 using the two polymorphs as starting materials. The XRPD analysis of the isolated solid phases proved that both polymorphic forms were converted to dihydrate form. *In situ* monitoring of the dissolution at pH 5.0 showed immediate dissolution, no difference in supersaturation, and short equilibration time for both forms indicating the immediate conversion. In SGF (pH 1.2) Form B dissolved better than Form A and showed significantly different dissolution kinetic and stability. A long-lasting, false chain-citation stating that Form B dissolves 28x better in water than Form A, was cut by the present study (i) revealing that the cited data was measured in IPA not in water, and (ii) proving that only the intrinsic solubility of OTC dihydrate can be measured in water due to conversion of polymorphs under the experimental conditions of solubility measurement. However this conversion is inhibited below pH 1.5, so the differences in solubility and dissolution kinetic found at pH 1.2 may contribute to the interpretation of the different serum-levels reported at solid formulations.

API	active pharmaceutical ingredient
ATR	attenuated total reflection
BR	Britton–Robinson buffer
CCD	charged-coupled device detector
IPA	isopropyl alcohol
IR	infrared spectroscopy
OTC	oxytetracycline
OTCH	oxytetracycline hydrochloride
PK/PD	pharmacokinetic/pharmacodynamics
SD	standard deviation
SEM	scanning electron microscopy
SGF	simulated gastric fluid
SSF	saturation shake-flask method
XRPD	x-ray powder diffraction

1. Introduction

Polymorphism and the effect of different polymorphic forms of drugs on PK/PD properties have gained substantial interest in the past 50 years in pharmaceutical sciences and the industry. The theoretical background and the practical aspects are comprehensively discussed in books [e.q. Brittain, 1999; Bernstein, 2002; Hilfiker et al., 2006] and reviews [e.q. Raw et al., 2004; Singhal and Curatolo, 2004; Saifee et al., 2009; Pangarkar et al., 2013]. In these papers those examples are

* Corresponding author.
E-mail address: novak.krisztina@pharma.semmelweis-univ.hu (K. Takács-Novák).

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surveyed in detail where polymorphism of API in formulated and marketed drugs caused serious safety problems, such as stability issue, failed dissolution, different plasma levels, and sometimes toxicity. Among these well-known examples (from chloramphenicol palmitate, through carbamazepine, enalapril, etc. up to ritonavir) one of the cited compounds is oxytetracycline.

The story began in the late 60s when Brice and Hammer (1968) first reported the therapeutic nonequivalence of oxytetracycline hydrochloride (OTCH) capsules marketed in the UK. 16 lots of OTCH capsules from 13 suppliers produced lower antibiotic level in the blood serum than the originator product (Terramycin, Pfizer). Seven of the lots produced blood levels below the generally accepted minimum therapeutic level. Similar results were found by Blair et al. (1971) testing 10 OTCH capsules from different manufacturers supplying the US market. Only 3 were equivalent to the reference product, serum concentrations of the remaining seven products were significantly lower. Subsequently, Groves (1973) investigated other orally used solid forms and reported large differences in the *in vitro* dissolution performance of OTCH tablets sourced from different suppliers. Although the existence of various polymorphic forms of OTCH has been known [Burger et al., 1986], polymorphism was not mentioned as a possible reason for discrepancies in dissolution and serum level in the above studies. First Liebenberg et al. (1999) studied the effect of polymorphism on dissolution properties of chemically equivalent OTCH powders. Six OTCH bulk samples from randomly selected suppliers in South Africa were characterized by solid phase analysis, and two samples were found as Form A, and four as Form B. The two polymorphic forms had different dissolution properties in water and 0.1 M HCl. This difference became more substantial when the dissolution from tablets prepared with microcrystalline cellulose in 5:1 (OTCH: Avicel PH 200) ratio was compared. Tablets prepared from Form A dissolved significantly slower in acidic medium than the others, for example, at 30 min, these tablets exhibited ~ 55% dissolution, while the Form B containing tablets achieved ~ 95% dissolution. These important findings - first showing to the scientific community the possible consequence of polymorphism of OTCH on bioavailability - however were attributed to the low and different intrinsic solubility of OTCH polymorphs cited from ref. [Burger et al., 1986]. The statement in the reference article *'Form A is 28 times less soluble than Form B'* was however not referring to solubility measured in water, but in IPA. With this statement, a long-lasting, false citation chain has been started. Subsequent reviews [e.q. Singhal et al., 2004; Censi and Di Martino, 2015] mentioned the above aqueous solubility difference as a fact. The characterization of several polymorphs of OTCH was published [Toro et al., 2007], but the effect of OTCH polymorphism on solubility/dissolution and its role in therapeutic nonequivalence has not been revealed. Recently Censi and Di Martino (2015) have commented on the topic the following way: 'further studies characterizing the physical and chemical properties of oxytetracycline polymorphs would be useful, as no recent works are available in the literature'.

In this study, our aims were to analyse the solubility of two polymorphic forms of OTCH, namely, Form A and B. We attempted to measure the equilibrium solubility of the samples in aqueous buffers in the biorelevant pH range of 4–7.4 by the standardized saturation shake-flask (SSF) method to reveal the solubility difference between them. We also studied the solubility in simulated gastric fluid (SGF) at pH 1.2 and the effect of the pH change from 1.2 to 5.0 on dissolution. Another purpose of ours was the real-time monitoring of the dissolution profile using an ATR probe *in situ*, attached to a UV spectrophotometer (μDISS Profiler™). A wide spectrum of solid phase analysis methods (SEM, IR, XRPD, Raman) was applied for characterizing the polymorphs and to identify which form is present at the solubility equilibrium.

2. Materials and methods

2.1. Materials

OTCH Form A was purchased from Sigma-Aldrich Co. LLC. (St. Louis, MO, USA) and used without further purification. Form B was prepared by us from Form A according to the literature method (see below). Both forms were identified by IR, Raman and XRPD methods. Both samples were crystalline material, Form A used as was supplied, Form B after sieving to assure the homogeneity of particle size. Distilled water of Ph. Eur. grade was used. All other reagents were of analytical grade.

A Britton–Robinson (BR) buffer stock solution (a mixture of acetic acid, phosphoric acid, and boric acid, each at 0.04 M) was prepared, and the required amount of 0.2 M NaOH or 1 M NaOH was added to reach the pH specified for the solubility experiments.

2.2. Preparation of Form B

Form B was prepared in our laboratory according to Burger et al., 1986. The recrystallization was made in a reactor with *noninvasive* Raman monitoring (Horiba Jobin Yvon, France). Real-time Raman spectra were fixed in 292–1540 cm^{-1} spectral range with a 300 mW laser (785 nm) source and air-cooled CCD detector. 5 g of Form A was mixed with 20 mL of a solvent mixture consisting of 0.2 M HCl and MeOH (1:3). The system was heated until getting a clear solution, then it was cooled using a linear (1 °C/min) rate cooling program. The temperature was controlled using a Stardom FCN-type PLC manufactured by Yokogawa (Japan) and a monofluid thermostat developed in our Department. A Eurostar power control-visc type stirrer, made by IKA (Germany), was also controlled by the PLC. The precipitated crystals were filtered and washed with 2 × 5 mL cold MeOH and dried. The identification of the polymorphic form was confirmed by IR, XRPD, and SEM as well.

2.3. Determination of thermodynamic solubility by SSF method

The equilibrium solubility of the samples in pH region 4.0–7.4 was determined by the standard protocol of SSF method [Baka et al., 2008; Avdeef et al., 2016]. The sample was added in excess to the aqueous buffer solutions to produce a suspension. The amount of solid added was accurately measured: 40–50 mg/5–10 mL. At a controlled temperature of 25.0 ± 0.1 °C the suspension was vigorously stirred for a period of 6 h, followed by a further 18 h of sedimentation period (stirrer turned off). For separation of the phases sedimentation was used to avoid the filtration. Sedimentation has advantages from both theoretical and practical point of view, because it does not perturb the dynamic equilibrium of solid dissolution/solute precipitation and there is no adsorption to the filter material what is common upon filtration [Völgyi et al., 2018]. During the 24 h incubation the sample was protected from light. At pH 5.0, the solubility measurement was also performed at 37.0 ± 0.1 °C.

The concentration of the saturated solution was measured by UV spectroscopy using JASCO V 550 UV/vis spectrophotometer. Three aliquots were carefully withdrawn from the liquid (using a fine glass pipette), and were diluted with the solvent as necessary. Three replicate solubility measurements were carried out under each of the tested conditions. Since OTCH has a pH-dependent UV spectrum, the specific absorbance ($A^{1\%1cm}$, the absorbance of 1 g/100 mL solution over a 1 cm optical pathlength at a given wavelength) of OTCH in each BR buffer used for solubility measurement was determined separately at a selected wavelength using 12 points of two parallel dilution series, from the linear regression equation (Lambert–Beer law), where the regression coefficient (r) was higher than 0.9998 in each case. The specific absorbance data are shown in Table 1.

Table 1
Spectroscopic data of OTCH at different pH values.

pH	λ_{max}	$A^{1\%}_{1cm}$
1.2	354	280
4.0	354	331
5.0	355	331
6.5	359	331
7.4	362	349

2.4. Real-time in situ monitoring of dissolution, supersaturation, precipitation and equilibration time using ATR UV-probe

The drug solution concentration *versus* time (0–24 h) profile was investigated at 25 ± 0.1 °C in BR buffer pH 5.0 with ATR UV-probe (Hellma Analytics, Germany) attached to the Rainbow Dynamic Dissolution Monitor of the μDISS Profiler™. Originally the μDISS Profiler™ instrument (Pion Inc. Billerica MA, US) was designed to measure real-time concentration with UV fibre optic dip probes inserted into 8 temperature-controlled 20 mL vessels, stirred with magnetic stirrer and applying different (2–5–10–20 mm) tips, depending on the concentration of the solution measured. It is suitable for the analysis of poorly soluble compounds [Borbás et al., 2018; Takács-Novák et al., 2019], but cannot be used for OTC due to the high concentration of its saturated solution even at the pH of the isoelectric point. Therefore, the ATR UV-probe was immersed into a temperature-controlled 20 mL vessel, where 50 mg of the samples (Form A or Form B) were added to 10 mL of BR buffer solution pH 5.0 assuring the excess of the solid. The UV spectra were registered using the following protocol: 1 spectrum/min for 2 h, 1 spectrum/10 min for 4 h, and 1 spectrum/30 min for 18 h. The stirrer was turned off at 6 h. For the evaluation of the concentration, previously determined calibration data and second derivative spectra were used. Three parallel measurements were performed with both polymorphs. Light protection was assured.

2.5. Solubility measurement in SGF

Though OTCH dissolves very well at low pH values, the solubility was investigated in simulated gastric fluid (pH 1.2) as well. 1.0 g sample (Form A or Form B) was added to 2.0 mL SGF producing a very turbid, colloidal, foamy, dark brown suspension. After shaking for 20 min, the solubility sample was allowed to stand at a controlled temperature of 25.0 or 37.0 °C for 1 h. The concentration of the saturated solution was measured after 500x dilution, with a UV fibre optic dip probe (2 mm tip) coupled with μDISS Profiler™. For evaluation previously determined calibration data and second derivative spectra were used.

The real-time monitoring was performed with an ATR-UV probe. 2.5 g of Form A or Form B was added to 3 mL of SGF, and the spectra were registered using the same protocol as described above. The stirrer was turned off at 1 h. For the evaluation of the concentration, previously determined calibration data and second derivative spectra were used. One measurement was performed with both polymorphs. Light protection was assured.

2.6. Study of pH-change from 1.2 to 5.0 on solute concentration

A high concentration (20 mg/10 mL) clear solution was prepared from Form A in SGF. After 20 min, the pH was changed to 5.0 by adding 1.2 mL of concentrated phosphate solution (used for the same purpose in ref. [Borbás et al., 2018] and called media B), 500 μL of 1 M NaOH, and 600 μL of 0.2 M NaOH. The opalescence of the solution was visually assessed, and the precipitation was also followed by the registration of the UV spectrum in the moment of pH change, after 1 h and after 24 h.

2.7. Methods for analysis of solid phase

2.7.1. XRPD

Following the solubility measurement, small amount of the solid phase was isolated and dried to a glass plate. X-ray powder diffraction patterns of these samples were recorded using a PANalytical (Amelo, The Netherlands) X'pert ProMDP X-ray diffractometer using Cu-Kα radiation (1.524 Å) and a Ni filter. The applied voltage was 40 kV, while the current was 30 mA. The samples were analysed between 4° and 42° 2Θ.

2.7.2. SEM

Morphology of the samples (crystalline Form A and Form B) was studied by SEM using JEOL JSM-5500 LV electron microscope (JEOL Ltd., Tokyo, Japan) with an excitation voltage of 5–20 kV.

2.7.3. IR

Infrared spectra (4000–400 cm^{-1}) of solid samples were recorded using a Bruker Tensor 37 type Fourier transform infrared (FTIR) spectrometer (Bruker Corporation, Billerica, MA, USA) equipped with DTGS (deuterated triglycine sulphate) detector with a resolution of 4 cm^{-1}. Before testing, the samples were mixed with potassium bromide (KBr) powder and cold-pressed into a suitable disk for FTIR measurement.

2.8. Statistical analysis

The concentrations were expressed as means ± SD, and were compared using 'two-sample' Student's *t*-test. The differences were considered statistically significant when $p < 0.05$.

3. Results and discussion

Oxytetracycline hydrochloride, a broad-spectrum antibiotic of the tetracycline family was introduced into therapy by Pfizer in 1950. For decades, it was commonly used all over the world in the treatment of infections caused by Gram positive or Gram negative bacteria. As of today, some strains of bacteria had developed resistance against it, its usage has decreased. However, due to its low price, it is still prevalent in developing countries and in veterinary medicine. OTC is a triprotic ampholyte compound, having two acidic (pK_{a1}: 3.23 and pK_{a2}: 7.22) ionisation sites and a basic (pK_{a3}: 8.82) one [Tam and Takács-Novák, 2001]. Its isoelectric point is at pH 5.2, where the compound is present in zwitterionic form. The distribution profile of different protonation forms of OTC is shown in Fig. 1. As visible, the ionisation of the compound happens mostly in the physiologically relevant pH range (1–8), its solubility is strongly pH-dependent.

Several polymorphic forms of OTCH are known and characterized [Toro et al., 2007], but out of them two, namely, Form A and Form B, are used in pharmaceutical formulations. They are in monotropic relationship and exhibit different morphology (Fig. 2). Form A is the thermodynamically stable polymorph, having brownish-yellow colour, 204 °C melting point, and 1.496 g/cm^3 density, while Form B is a metastable (kinetic) polymorph, a hygroscopic, brilliant yellow colour powder with a melting temperature of 180–195 °C, and 1.440 g/cm^3 density. SEM images show different crystal habits of the two forms (Fig. 2). IR spectra (Fig. 3) were used for identification compared with those published by Burger et al. (1986). According to *Burger* the hygroscopicity of the two polymorphs differs greatly, this way they can be identified based on the water peak in the IR spectra. Form B is more hygroscopic, showing a characteristic broad peak between 3400–3600 1/cm.

3.1. Equilibrium solubility measured in pH range 4–7.4

The equilibrium solubility of OTCH Form A and B as starting

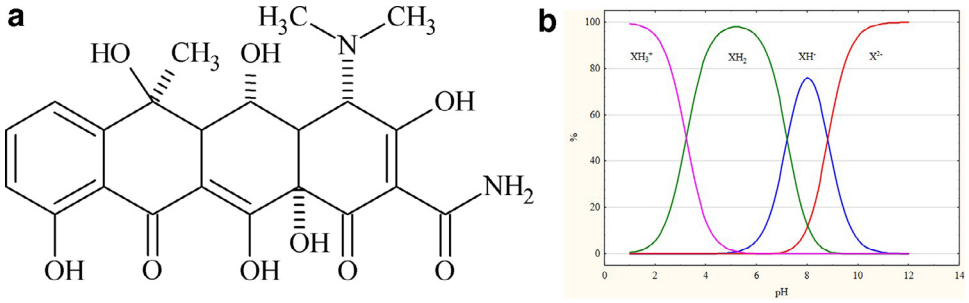

Fig. 1. Structure (a) and distribution profile of OTC (b).

materials was measured by the standard protocol of SSF method at 4 different points in pH range 4.0–7.4, at 25 °C. In the experiments, we followed all the recommendations suggested in the recently published consensus-based 'white paper' about the good practice of solubility measurements in order to improve the quality of solubility data [Avdeef et al., 2016]. The S_{pH} ± SD results are expressed in μg/mL unit as the average value of 3 parallel measurements, and are summarized in Table 2. The standard deviation was within the range of 1.5–6.7%, average SD: 3.0%. This SD value fully corresponds to that found in the validation study of SSF method using the standardized protocol (6 h stirring followed by 18 h sedimentation) [Baka et al., 2008]. No significant difference was found between the S_{pH} values of the samples in the pH region 4.0–7.4. This can be explained with the transition of polymorphs to a same polymorph under the experimental conditions, as supported by the solid phase analysis (see below).

The results exhibit the typical solubility-pH profile of an ampholyte molecule. The solubility is the lowest at (and near to) the *i.e.* point pH

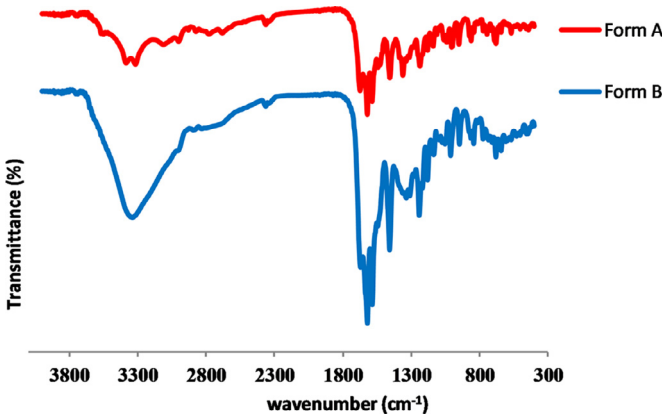

Fig. 3. IR spectra of OTCH polymorphs.

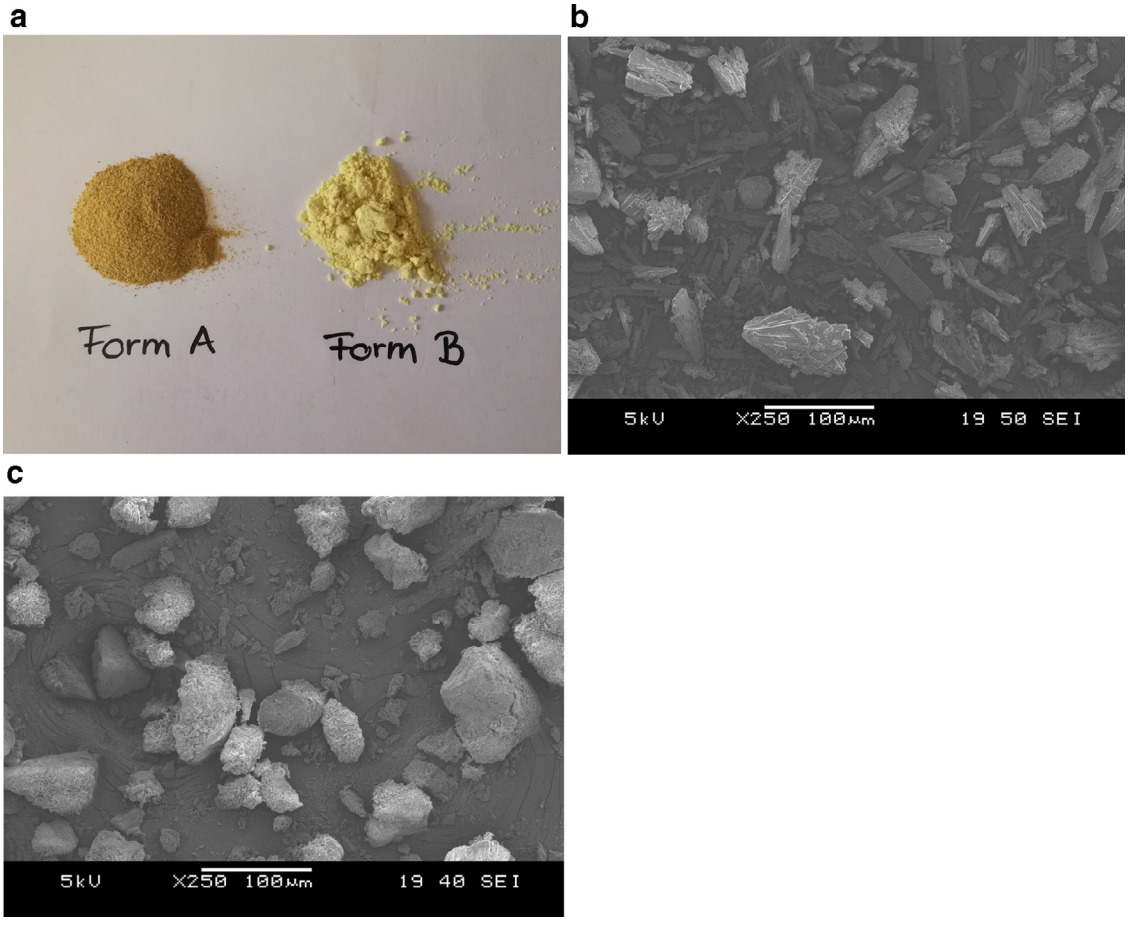

Fig. 2. Morphology of OTCH polymorphs (visual appearance of the solid (a); SEM images: (b) Form A, (c) Form B).

Table 2
Equilibrium solubility of OTC dihydrate in Britton–Robinson buffer at 25 °C measured by SSF method using Form A and Form B as starting materials.

pH	S_{pH} ± SD (μg/mL) Form A	Form B
4.0	345 ± 7	325 ± 22
5.0	328 ± 5	298 ± 5
6.5	668 ± 36	693 ± 10
7.4	1721 ± 40	1816 ± 60

(5.2 ± 0.5 in this case) and this value corresponds to the intrinsic solubility (S_o) of the compound. Towards the lower and higher pH values, - due to ionization - the solubility increases as determined by the Henderson–Hasselbalch (HH) equation [Völgyi et al., 2010]. The average solubility value obtained at pH 5, at 25 °C, is 313 ± 21 μg/mL ($\log S_o$ [M]: −3.200) (n = 6), which can be considered as the intrinsic solubility of OTCH in aqueous medium.

Solubility of OTCH shows very slight temperature-dependence between 25 and 37 °C, experiments performed at the physiological temperature resulted in $\log S_o$ [M]: −3.02 (n = 4). Therefore further experiments were conducted at 25 °C.

3.2. Solid phase analysis in pH range 4–7.4

First the X-ray powder diffraction patterns of the starting materials, Form A and Form B were registered (see in Fig. 4). Next, the solid phases - isolated and dried from the solubility suspension after incubation at different pHs - were studied. Diffractograms shown in Fig. 4 demonstrate that the samples are not identical with the starting polymorphic forms in either case, since the characteristic peaks of the starting polymorphs cannot be found in the diffractogram of the isolated sample. The results indicate that both polymorphs recrystallized to a common form. It was identified as oxytetracycline dihydrate with the help of the Cambridge Structural Database (reference code: OXTETD). The above results of solid phase analysis confirm and can explain the equilibrium solubility data measured experimentally (Table 2). The conversion of different polymorphic forms to an identical product is a common, possible behaviour of drugs: a similar phenomenon was found recently in the case of venlafaxine hydrochloride [Takács-Novák et al., 2019].

3.3. Real-time monitoring of dissolution, supersaturation and equilibration time at pH 5.0

As the determination of the equilibrium solubility of the polymorphs in water is prohibited by the conversion to dihydrate, next, we studied the dissolution kinetics of the two forms in BR buffer at pH 5.0. The *in situ* real-time monitoring of dissolution was conducted

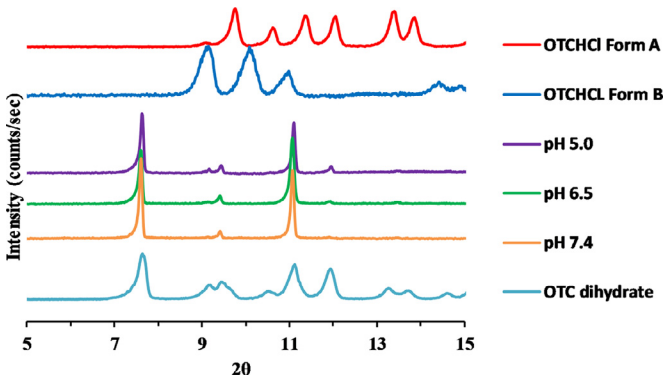

Fig. 4. XRPD patterns of OTCH Form A and B and the solids isolated from solubility suspensions at different pH values.

immersing an ATR-UV probe to the solubility suspension and attaching to the μDISS Profiler™. The application of the ATR-UV probe was necessary, because the concentration of the OTCH solutions was too high for the direct measurement with the smallest (2 mm) available tip of the μDISS instrument. ATR-UV is a specially designed and crafted probe containing a sapphire crystal and fibre optic. It makes measuring highly concentrated solutions possible, because the UV light goes through only a few μm pathlength of the sample (Simon et al., 2015).

In a pilot experiment, the 0–24 h monitoring of dissolution showed immediate dissolution and a short (~10–12 min) time needed to reach the equilibrium. Therefore, further parallel experiments were conducted up to 60 min. The average concentration values and the SD bars are presented in Fig. 5. *a* panel. Enlarging the 0–10 min period (in Fig. 5. *b* panel) shows the identical dissolution performance of the two forms. At pH 5.0 no significant difference is in the extent of supersaturation (S_{max}: 549 μg/ml Form A *vs* 536 μg/ml Form B) which has been reached around 1 min. Precipitation starts at this point and reaching the solubility equilibrium around 10 min, the concentration of the saturated solution (that is, the solubility) has not changed. The identical dissolution kinetic supports the immediate conversion of the two forms to the common dihydrate product at this pH. Intrinsic solubility measured by the ATR-UV probe, at 25 °C, is 308 ± 26 μg/mL ($\log S_o$ [M]: −3.207) (n = 6), which is in perfect agreement with S_o obtained by the SSF method.

3.4. Solubility analysis in SGF

As the above results show, neither the intrinsic solubility nor the dissolution kinetic at pH 5.0 can explain the therapeutic nonequivalence of OTCH polymorphs. So, we extended the study to the solubility analysis in SGF, despite the fact that in acidic pH, OTCH dissolves very well, and the high-concentration saturated solution may behave as a non-ideal liquid. Further on, the decomposition of OTCH in acidic medium in such concentration may also cause difficulties, even though - according to literature data - the diluted aqueous solutions of OTCH at pH 1.0–2.5 are stable for at least 30 days at 25 °C [The Merck Index, 2001].

The kinetic solubility in SGF was determined with a simplified approach (described in Section 2.5). The temperature effect (37 *vs* 25 °C) on solubility at this pH was also negligible (less than 30%) thus we discuss the results obtained at 25 °C in order to be comparable with the literature data. The two polymorphs have different values: $S_{pH1.2}$: 205 mg/mL for Form A *vs* 385 mg/mL for Form B. The higher kinetic solubility of the metastable form can be explained by its 2.6 times higher specific surface relative to the other form [US Patent, 1959, US2867661]. Though these solubility values can be considered as approximate data, as the number of analysed samples was limited due to the excessive amount of OTCH required at this pH, they reflect the (~1.9 times) better solubility of Form B. This perfectly corresponds with the ratio of metastable/stable polymorph solubility found to be typically less than 2 by Pudipeddi and Serajuddin (2005) surveying literature data.

The real-time analysis of dissolution with the ATR-UV probe supported the above results. The drug concentration *versus* time profile in the first 0–1 h period is shown in Fig. 6. The metastable Form B dissolves fast and reaches a substantial supersaturation in minutes (S_{max}: 718 mg/mL at 7 min) then precipitation causes a decrease in the solution concentration. At about 30 min, a sharp decrease in the concentration accompanied by a distortion of the UV spectrum can be observed indicating either the decomposition or the transition of the solute. The behaviour of the stable Form A is markedly different, showing immediate dissolution (S_{max}: 436 mg/mL at 6.5 min) without supersaturation. The UV spectrum of this sample is unchanged during the solubility experiment.

Solid phases were analysed by XRPD with samples taken out from the solubility suspension at 1 h and 24 h, and shown in Fig 7. While in

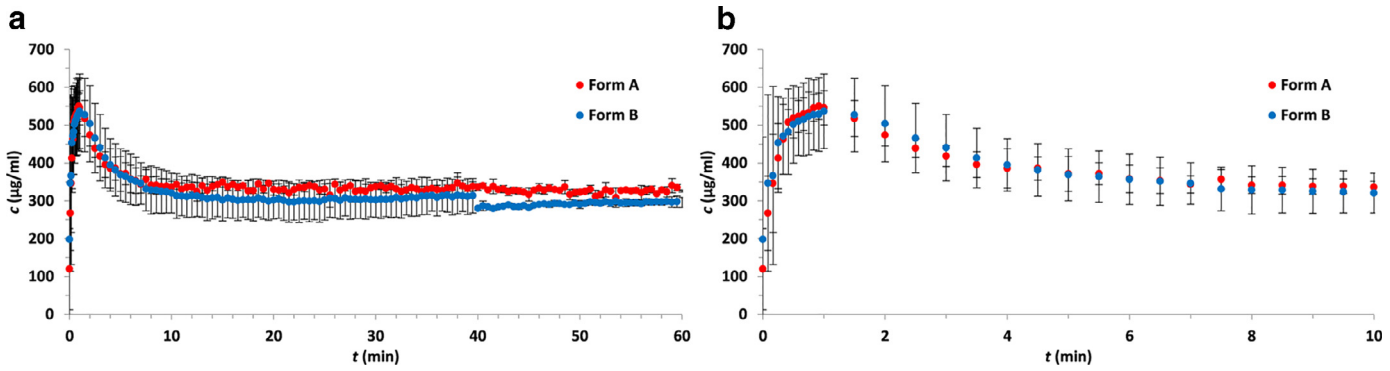

Fig. 5. Real-time monitoring of concentration *vs* time profile of OTCH Form A and Form B in BR buffer pH 5.0 with ATR-UV probe (*a* panel: 0–60 min, *b* panel: 0–10 min).

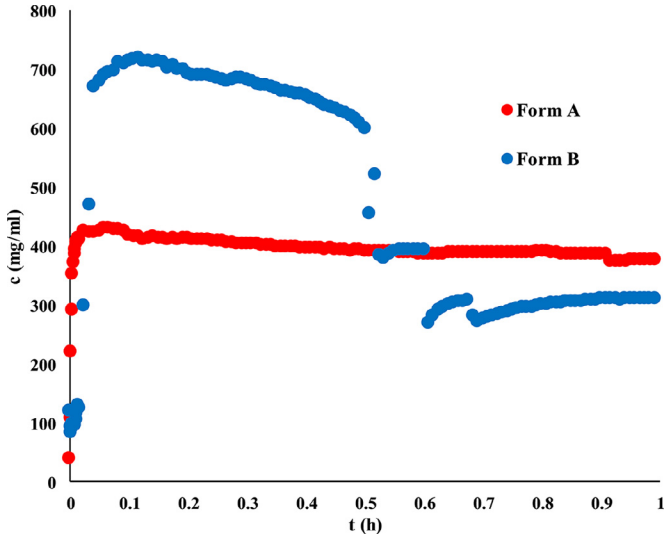

Fig. 6. Real-time monitoring of concentration *vs* time profile of OTCH Form A and Form B in SGF pH 1.2 with ATR-UV probe.

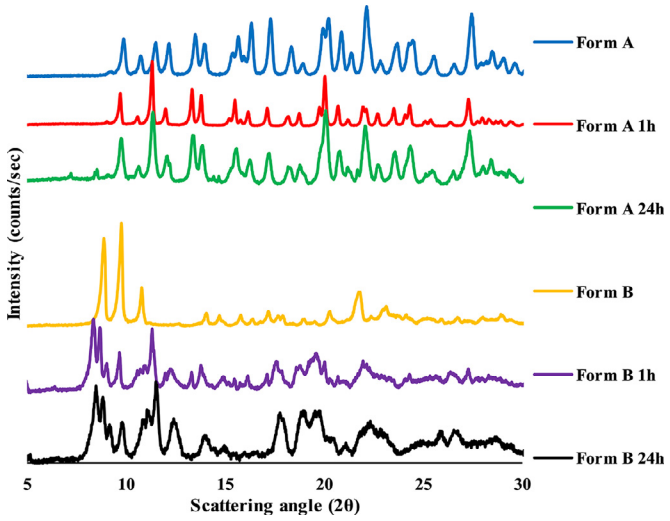

Fig. 7. XRPD patterns of OTCH Form A and B and the solids isolated from solubility suspensions at pH 1.2.

the case of Form A, the solid phase has not changed, and its pattern is identical with the starting material, in case of Form B, solid phase already at 1 h shows a different pattern, indicating a decomposition/transition process of the metastable polymorph. Consequently, from real-time monitoring experiments, the equilibrium solubility could not be obtained, but the dissolution kinetic in SGF at least in the first 1 h (which is relevant with the gastric residence time) revealed significantly different performances of the two polymorphs.

Above results are in accordance with findings reported by Burger et al. (1986). They suggested keeping the pH below 1.5 in order to avoid the conversion of these polymorphs to dihydrate, where the process is hindered or very slow.

3.5. Effect of pH-change from 1.2 to 5.0

For simulating the gastric-small intestine transfer, the effect of pH change was investigated. Considering that the oral dose of OTCH is 500 mg and the gastric fluid in the stomach is estimated to be around 250 mL according to recent studies, a 2 mg/mL OTCH solution was prepared of Form A in SGF (pH 1.2). (This concentration is well below the solubility of both polymorphs in SGF). The acidic media was converted to duodenum pH 5.0, where precipitation can be expected based on the intrinsic solubility of the API. Surprisingly, the supersaturated solution with the concentration of 1.63 mg/mL (around 5 times the concentration of the intrinsic solubility) was stable for over 1 h, while no precipitation was observed with the spectrophotometer. Since the molecule is neutral (in zwitterionic form) at this pH, having the least polarity/highest lipophilicity, the absorption from this compartment of the GI tract can be expected. Supersaturation/precipitation is influenced by the individual intestinal pH and/or the food consumed, which can lead to different absorption and blood-level.

4. Conclusion

In this study, we conducted the comprehensive solubility analysis of two polymorphic forms of OTCH including equilibrium solubility measurements by the SSF method in a biorelevant pH range, and monitoring the dissolution behaviour of the two polymorphic forms in real-time at different pH values. Our work demonstrates the importance of solid phase analysis in the solubility investigation of drug polymorphs providing reliable information about which form is present in solubility equilibrium.

We proved that the determination of the equilibrium solubility of OTCH polymorphs in pH range 4–7.4 is hindered by the conversion of the starting materials to a common dihydrate form during solubility equilibration. The obtained results indicate the pH-dependent solubility of OTC dihydrate.

With this, we put an end to a long-lasting, false citation chain about the 28 times aqueous intrinsic solubility difference of OTCH polymorphs. We revealed that the cited data from ref. Burger et al. (1986) was originally measured in isopropanol, not in water. In IPA the polymorphs keep their original form during the solubility experiment and the metastable form dissolves much better in this organic solvent. But

this phenomenon does not exist in aqueous medium.

The real time monitoring of dissolution at pH 5.0 and 1.2 with an ATR-UV probe attached to the μDISS Profiler provided further information about the dissolution performance of polymorphs being in complete agreement with the SSF results.

The difference in the kinetic solubility and dissolution performance of the polymorphs found in SGF and supersaturation occurring on pH-change 1.2 → 5.0 may contribute to the interpretation of the therapeutic nonequivalence of solid OTCH products.

CRediT authorship contribution statement

Dóra Tempfli: Investigation, Formal analysis, Data curation, Visualization. **Enikő Borbás:** Methodology, Investigation, Visualization. **Hajnalka Pataki:** Investigation. **Dóra Csicsák:** Investigation, Visualization. **Gergely Völgyi:** Methodology, Visualization, Supervision, Project administration. **Bálint Sinkó:** Conceptualization, Methodology, Supervision, Project administration. **Krisztina Takács-Novák:** Conceptualization, Methodology, Writing - original draft, Supervision, Project administration.

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