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A potentiometric titration method for the crystallization of drug-like organic molecules

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Abstract

It is generally accepted, that crystalline solids representing a low energy polymorph should be selected for development of oral dosage forms. As a consequence, efficient and robust procedures are needed at an early stage during drug discovery to prepare crystals from drug-like organic molecules. In contrast to the use of supersaturated solutions, we present a potentiometric crystallization procedure where saturated solutions are prepared in a controlled manner by pH-titration. Crystallization is carried out under defined conditions using the sample concentration and experimental pK_a values as input parameters. Crystals of high quality were obtained for 11 drugs selected to demonstrate the efficiency and applicability of the new method. Technical improvements are suggested to overcome practical limitations and to enhance the possibility of obtaining crystals from molecules in their uncharged form.

Keywords: Crystal; Crystallization; Solubility; Potentiometric titration

1. Introduction

Crystallization is an important purification and separation technique in a variety of commercial processes, as for example biotechnology, mineral processing, waste treatment, energy storage, production of new materials and electronic chemicals (Garside and Tavare, 1986). In the pharmaceutical industry, crystals from potential drug candidates are used as a starting point for the development of oral dosage forms and their characterization with respect to polymorph formation, thermodynamic solubility and dissolution rate in different buffer systems (Singhal and Curatolo, 2004).

Crystallization can occur in solution, from vapor or from melt. Most processes in the chemical industries use crystallization from solution. The starting point for crystallization is the creation of a saturated solution. However, formation of a saturated solution is a time-consuming process since it may take several days until the equilibrium between the compound's soluble and insoluble forms has been reached. As a consequence, supersaturated and not saturated solutions are used frequently as the starting point for crystallization. If not well controlled, however, crystallization from supersaturated solutions may fail and lead to the formation of amorphous material or crystal defects (Shekunov and York, 2000). In general, with decreasing level of supersaturation, the crystal growth becomes slower and the crystal quality improves (Smakula, 1962). In view of these limitations, a protocol was designed which allows for the controlled preparation of saturated solutions by potentiometric titration. Such crystallization procedures using pH variation are often used for the crystallization of proteins (McPherson, 1985; Baird, 1999; Berisio et al., 1999; Stewart and Baldock, 1999; Wiencek, 1999; Gray et al., 2001), but rarely for drug molecules (Wang and Berglund, 2000).

The present crystallization method enables the efficient generation of saturated solutions using pH-titration. Fine pH variations are applied based on experimental pK_a values to obtain the saturated solution. The crystallization process can thus be smoothly initialized and controlled, a key prerequisite for fur-

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ther optimization and application in a commercial environment. The advantages of the new method are, first, avoidance of buffer systems in the crystallization of compounds, second, improved control of crystal growth due to the use of the saturated solution and, third, reduction of the risk to obtain non-crystalline (amorphous) materials.

2. Materials and methods

2.1. Materials

Diclofenac, famotidine, flurbiprofen, furosemide, hydrochlorothiazide, ketoprofen, propranolol and quinine were commercial compounds used for crystallization.

Cyclopenthiazide and codeine are compounds with known polymorphic forms. Additionally, an internal compound with known polymorphs was included in the study. Their solubilities were determined via a potentiometric method. Crystalline materials of all compounds were successfully obtained using the invented new crystallization method.

The pSol instrument (pION INC., Woburn, USA), usually foreseen for potentiometric solubility measurements, was used here to study the crystallization processes and to obtain an additional pH-solubility profile.

2.2. Methods

2.2.1. Potentiometric pK_a assay

A potentiometric titration method was used for the pK_a determination via the GlpKa equipment (Sirius Analytical Instruments Ltd., East Sussex, UK) (Avdeef and Comer, 1993). Usually, a blank titration is performed at the beginning of the measurement to calibrate the electrode. Afterwards, precisely known volumes of a standardized strong acid or base are added to a vigorously-stirred solution of a protogenic substance, while the pH is continuously measured with a pH-electrode. The results of an experiment deliver two potentiometric titration curves, one with and one without sample as shown in Fig. 1a.

The potentiometric titration curve depicts the measured pH against titrant volume added. The shape can give information on the amount of substance present and its characteristic acid–base ionization properties. To reveal overlapping pK_as, it is necessary to transform the titration curves into Bjerrum plots. Such a plot can be obtained by subtracting a titration curve containing no sample, "blank" titration, (left curve in Fig. 1a), from a titration curve with sample, (right curve in Fig. 1a), at fixed pH-values. The difference between the total and the free concentrations is equal to the concentration of the bound hydrogen ions. The latter concentration divided by that of the sample gives

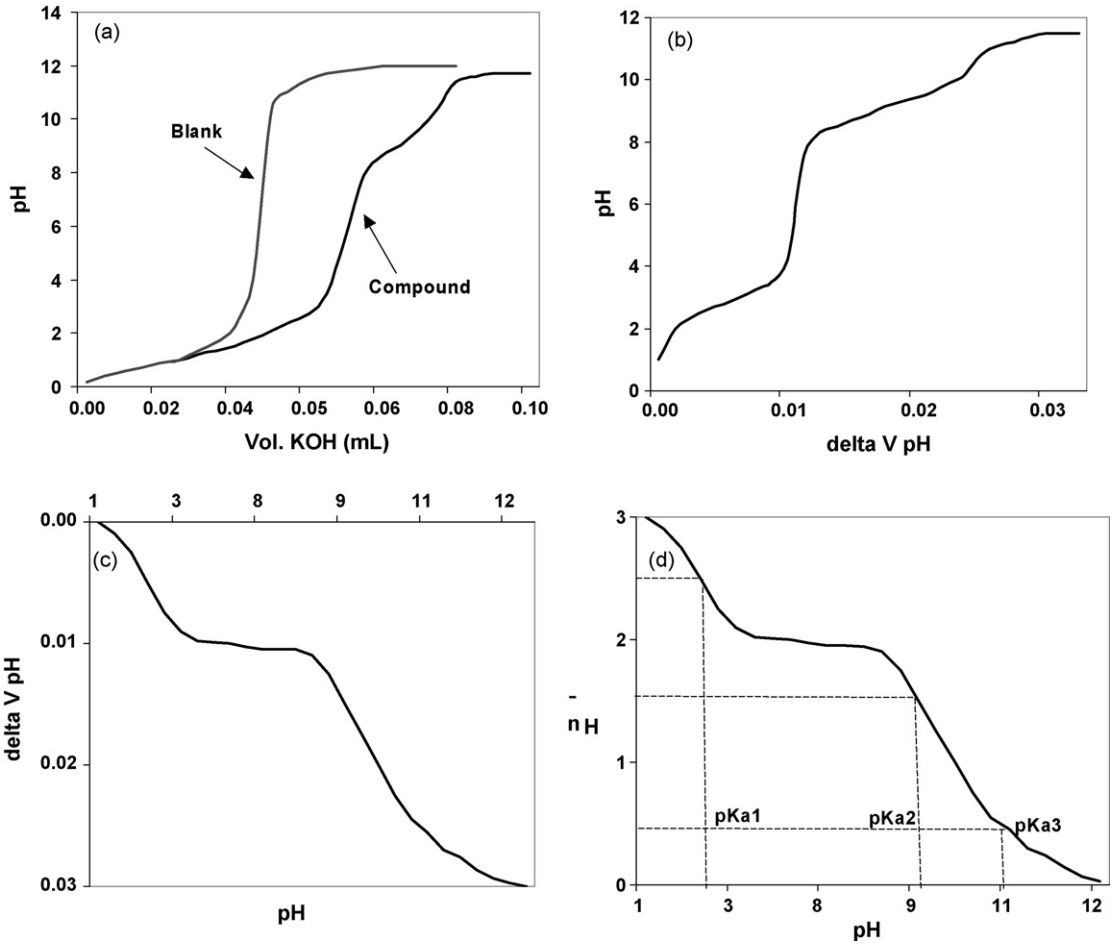

Fig. 1. Four step construction of the Bjerrum difference plot for a molecule with three pK_a values, whose constants are observed in the simple titration curve.

the average number of bound hydrogen atoms per molecule of substances, $\bar{n}_H$. The Bjerrum curve is a plot of $\bar{n}_H$ versus p_cH. It reveals all the pK_as as p_cH values at half-integral $\bar{n}_H$.

2.2.2. Potentiometric solubility assay

The potentiometric solubility assay requires an ionizable compound as reactant and a strong acid or a strong base as titrant. A blank titration is performed at the beginning of the measurement, similar to the procedure described for the determination of ionization constants. Afterwards, a certain amount of compound is placed in a reaction beaker and dissolved in a given volume of solvent. A titration is then performed in the direction of complete dissolution. During the measurement, the pH-value is continually determined via a pH-electrode. Similar to the potentiometric pK_a assay, two potentiometric titration curves are obtained, and the corresponding Bjerrum plot is derived. Thus, the value of apparent pK_a, (pK_a^{App}), can be determined at the half-integral $\bar{n}_H$ positions of Bjerrum plot. In case of weak acid, the apparent ionization constant, K_a^{App}, is defined as Eq. (1).

$$K_a^{APP} = \frac{[A^-][H^+]}{([HA] + [HA]_s)} = K_a \frac{[HA]}{([HA] + [HA]_s)} \quad (1)$$

[HA] is the concentration of the molecule HA in the solution. $[HA]_{(s)}$ is the moles of the molecule HA, which precipitated per liter of aqueous solution.

At the half-integral $\bar{n}_H$ positions of Bjerrum plot, half of the total amount of the substance is protonated, thus, the concentration of the free acid, HA, equals that of the conjugate base, A^- (Eq. (2)).

$$[HA] + [HA]_s = [A^-] = \frac{C}{2} \quad (2)$$

$[A^-]$ is the concentration of the conjugate base, A^- in the solution. C is the total amount of substance in the solution and solid phase.

Combining Eqs. (1) and (2) together, the value of intrinsic solubility can then be deduced from the Eq. (3) using the experimentally determined pK_a value and the sample concentration, C, as input parameters.

$$\log S_0 = \log[HA] = \log\frac{C}{2} - pK_a^{App} + pK_a \quad (3)$$

2.2.3. Description of the crystallization assay

Crystallization is considered as a kinetic process and illustrated with the help of the pH-solubility profile using a weak base as an example (Fig. 2).

In region A, the compound is in equilibrium and solubility stays constant. Eq. (4) describes the equilibrium in region A.

$$BH^+ \rightleftarrows B \rightleftarrows B_{(s)} \quad (4)$$

In region B, solubility rises with the increasing amount of BH^+, when pH changes from high to low. This means, when a basic compound is titrated from its insoluble to its soluble form, an increasing amount of uncharged precipitate $B_{(s)}$ will go into solution with increasing hydrogen concentration $[H^+]$. This will continue, until point 2 is reached. At point 2, a "perfect" buffer system (Avdeef, 2001) exists. The simultaneous presence of solid free base and its solid conjugate acid force the pH and solubility to be constant, as long as the two interconverting solids are present. This special pH point has been designated as the Gibbs' pK_a (pK_A^{GIBBS}) (Avdeef, 2001). The equilibrium equation associated with this phenomenon is Eq. (5).

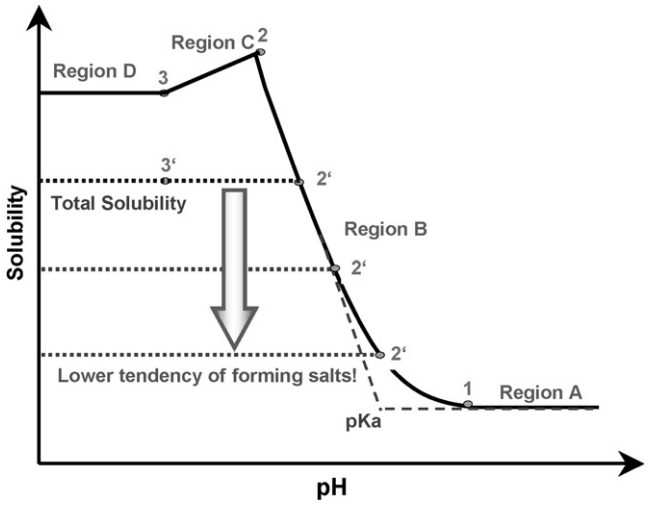

Fig. 2. Solubility-pH profile of a weak base. B is the soluble form of the weak base. $B_{(s)}$ is the solid form of the weak base. BH^+ is the charged form of the weak base. Reduction in concentration of a compound leads to lower tendency of forming salts.

$$BH^+_{(s)} \rightleftarrows B_{(s)} + H^+ \quad (5)$$

$$K_a^{GIBBS} = \frac{\{H^+\}\{B_{(s)}\}}{\{BH^+_{(s)}\}} \quad (6)$$

The solubility at point 2 is $S = S_0 + S_i$. The constants S_0 and S_i are intrinsic and salt solubility (Avdeef, 2001).

From point 2 on, (in region C), $BH^+_{(s)}$ will be only won in credit of B in the solution and solubility decreases with $B + H^+ \rightarrow BH^+_{(s)}$, until region D is reached. In region D, no more B will be changed into $BH^+_{(s)}$ and the minimum of [B] is achieved. The equilibrium existing there is described by Eq. (7):

$$BH^+_{(s)} \rightleftarrows BH^+ \rightleftarrows B + H^+ \quad (7)$$

However, during the potentiometric titration, one is not very frequently able to observe the phenomenon of the "perfect" buffer system, because in order to get a good titration, it is always recommended to use a small amount of compound. And this leads to the situation, that the whole amount of compound is dissolved before the maximal concentration of salt in solution ($[BH^+]_{max}$ at point 2) is reached. The point where the whole amount of compound dissolved in the solution is signified as 2′ in Fig. 2; at this point, the compound reaches its total solubility. The total solubility does not change with pH and is signified in Fig. 2 by the blue dashed line.

According to the new crystallization method, crystals can be easily obtained, when the direction of titration described above is

reversed. In the case of a weakly basic compound B, one starts with an unsaturated solution of the compound at a low initial pH-value as illustrated in Fig. 2 by means of point 3′, which can be varied by the amount of the compound used. Subsequently, the pH-value is gradually increased by adding a strong basic titrant to the solution. This leads to an increasing deprotonation of BH^+ to B, but initially, there is no precipitation of solid phase. By reaching point 2′, the titration is stopped. At this target point 2′, the concentration of the uncharged form has reached its maximal value $[B]_{max}$, which is equal to the intrinsic solubility S_0. Therefore, a saturated solution of the compound of interest has been reached that may serve to carry out a crystallization under substantially saturated conditions. Hence, at the point 2′, the probability for the formation of the neutral form is at its maximum.

The point of saturation can be precisely identified via the pH-solubility profile and can be easily reached using pH-titration. Therefore, generation of saturated solution is no more a time-consuming process since the advent of the invented new crystallization method. Furthermore, the newly developed method uses saturated solution instead of highly supersaturated solution as the starting point for the crystallization. Hence, the shortcoming of the currently known method can be avoided. The control of the crystal growth can be improved and the possibility to obtain non-crystalline form can be reduced using the new crystallization method. However, due to practical limitations, it may be difficult to reach the target point 2′ very precisely. If too much base is added, the pH-value goes beyond the targeted pH-value corresponding to point 2′ and a supersaturated solution is formed. Therefore, the titration is usually stopped at a point very close to the solubility-pH profile that corresponds to a slightly unsaturated solution. By keeping the solution at defined conditions allowing controlled slow solvent evaporation, the concentration of the solution will slowly increase so that the saturated state is reached. In order to obtain good crystallization results and reduce the risk of forming amorphous solid materials, an improved system might be of advantage for monitoring the concentration of the uncharged form and regulating the pH-value so that the concentration of the uncharged form is kept within a predefined tolerance range above the intrinsic solubility. Alternatively, the improved system may monitor the total concentration of the compound and regulate the pH-value so that the total concentration is kept within a predefined tolerance range above the predetermined total solubility profile.

3. Results and discussion

3.1. Crystallization of known drugs

In drug development and in particular for the design of appropriate formulation strategies, test compounds of high chemical purity and defined crystalline state are needed. Crystals of organic molecules are thereby often obtained using saturated or supersaturated solutions. The actual degree of saturation needed to promote slow crystal growth leading to crystals of high purity and quality varies from compound to compound and is often determined empirically in time-consuming series of experiments. Sub-optimal levels of saturation lead thereby either to a very slow rate of crystallization or to an accelerated crystallization process resulting in the formation of amorphous material or crystals of poor quality due to crystal defects. It was therefore the aim of the present work to overcome these limitations and disadvantages of currently known methods for crystallization of weakly acidic and/or weakly basic compounds. Test compounds are thereby dissolved in water at an initial pH chosen such that the compound is present predominantly in its charged form. Gradual titration of the pH-value in a direction that leads to a decrease of the number of ionized functional groups, and thus to an increase in neutral forms, results in a substantially saturated solution, which is maintained in this state while allowing for the formation of crystals. The present gradual pH-titration method can be automatized, reduces the time needed to identify optimal conditions for crystallization and reduces the amount of test compound since no serial dilutions of stock solutions have to be prepared.

Table 1
Ionization constants and intrinsic solubility of famotidine, diclofenac, flurbiprofen, furosemide, hydrochlorothiazide, ketoprofen, propranolol and quinine

Compounds	ApK_a	BpK_a	S_0 (μg/mL)
Famotidine	6.74	11.19	1100
Diclofenac	3.99		0.98
Flurbiprofen	4.03		14.7
Furosemide	3.52		6.9
Hydrochlorothiazide	8.87		979.1
Ketoprofen	3.98		118
Propranolol		9.53	51.6
Quinine		8.53	427.4

ApK_a is the acidic pK_a, BpK_a is the basic pK_a. S_0 is the intrinsic solubility.

The known drugs famotidine, diclofenac, flurbiprofen, furosemide, hydrochlorothiazide, ketoprofen, propranolol and quinine were used to verify the readiness and applicability of the new crystallization method. The total amount of test compound needed for each experiment was in the range of 0.5–2 mg. The incubation volume (initial conditions) was 1.8 mL. Ionization constants and intrinsic solubility data were available and were required for the identification of the initial point of the crystallization (Table 1). Crystals with high quality were obtained for all these eight ionizable compounds. The microscopic pictures of ketoprofen, quinine and famotidine are taken here as examples to demonstrate that the new crystallization method cannot only be applied for acidic, basic, but also for ampholytic compounds. (Fig. 3)

3.2. Crystallization of internal development compound

In order to validate the newly developed crystallization method under conditions as typically encountered in the pharmaceutical industry, a proprietary development compound was analyzed. Compound 1 is a compound which readily adopts amorphous as well as several polymorphic crystal forms. The well characterized polymorphic forms include modification A (which is known to be the most stable form obtained via transition

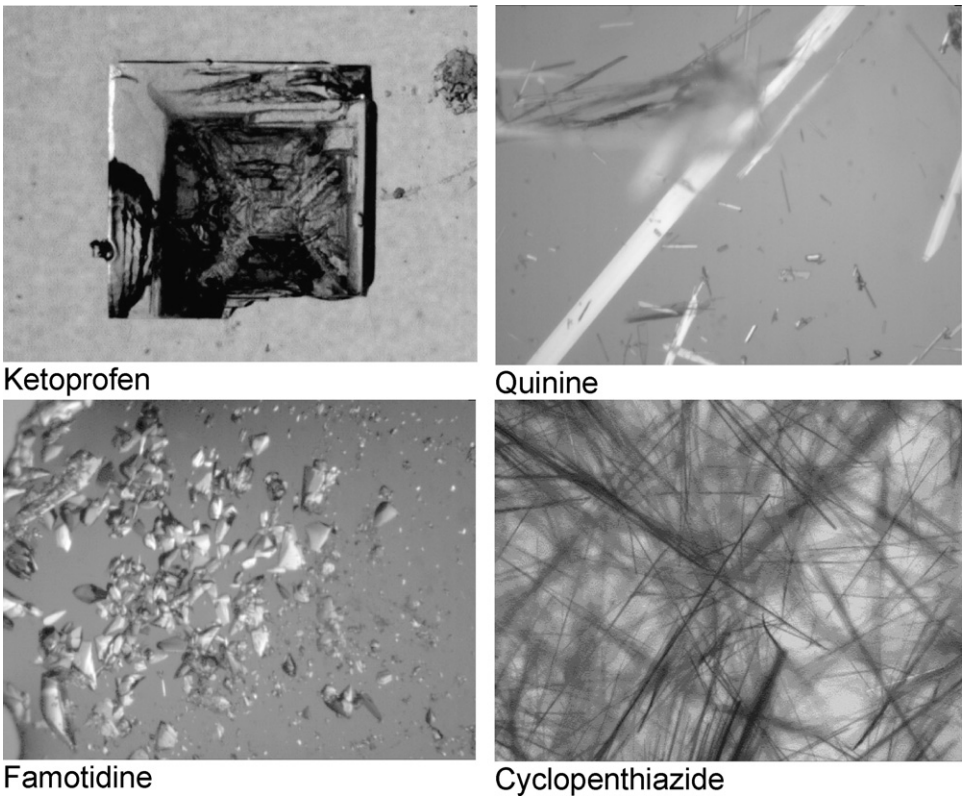

Fig. 3. Crystals of ketoprofen, quinine, famotidine and cyclopenthiazide obtained using the new crystallization method.

from modification B), modification B (an anhydrate) and modification C (a hydrate).

The crystals obtained via the potentiometric crystallization method in aqueous solution had the form of yellow needles. No formation of amorphous material was observed. Through the comparison with the reference data, the obtained crystals were characterized by powder diffraction as modification C (Fig. 4). The formation of the hydrate modification C is in full agreement with our expectations and corresponds to a crystal form obtained by crystallization in an aqueous solvent.

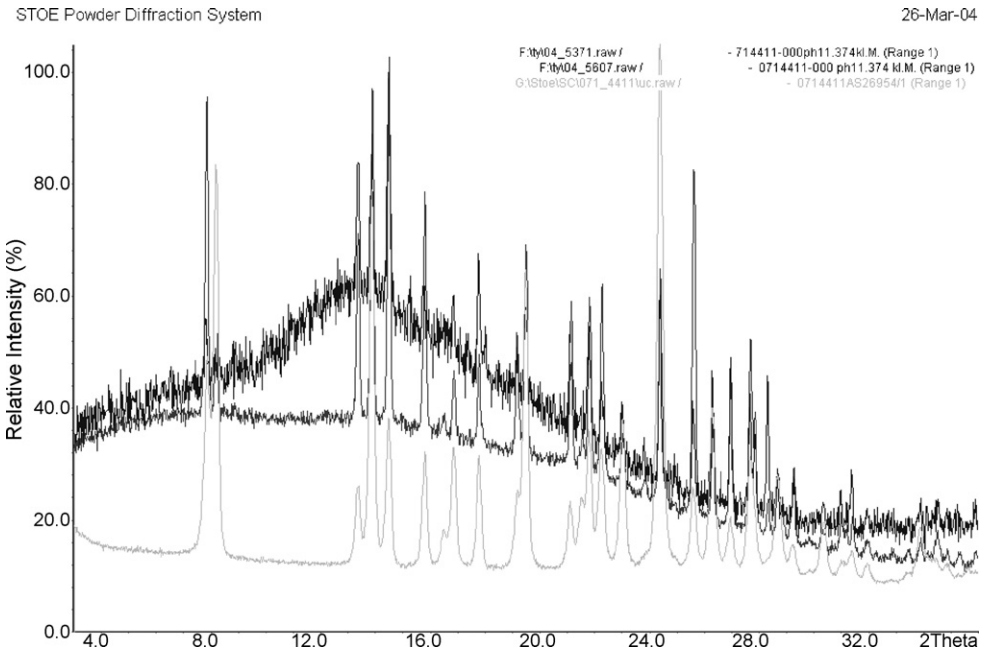

Fig. 4. Powder diffraction diagram of crystal forms (upper and middle curve) of compound 1 obtained via crystallization method. The obtained crystals show the same diffraction pattern as crystals in modification C (lower curve) in reference diagram.

Table 2
Physicochemical properties of diverse cyclopenthiazide polymorphic forms

Polymorph	Melting point (°C)	Solubility in water (μg/mL)
I	239.33	34.7
II	223.03	61.8
III	187.87 and 233.48	17.15

3.3. Crystallization of external polymorphs

In another set of experiments, the new crystallization method was applied to reference compounds, which are characterized by big differences in aqueous solubility of their polymorphic forms. Pudipeddi and Serajuddin (2005) has shown that in a data set of 72 compounds with different polymorphic forms, usually small differences in their solubilities were determined. The described differences in the solubilities were often in the range of the experimental error of the high-throughput solubility measurements in the early drug discovery phase. However, literature searches allowed us to identify compounds showing large differences in the measured solubilities of their polymorphic subtypes. Thus, several interesting drugs could be found with large solubility differences and were subsequently used to challenge and further validate the new crystallization method. One of those examples is premafloxacin (Schinzer et al., 1997). There is a 30-fold solubility difference described between polymorphic form I and III of premafloxacin. The other examples are codeine (El-Gindy and Ebian, 1978) and cyclopenthiazide (Gerber et al., 1991) with a 13-fold difference for codeine between hydrate and other crystal forms. A four-fold difference was described between the polymorphic form II and III of cyclopenthiazide. Both cyclopenthiazide and codeine were available for further characterization and application of the new crystallization method.

The physicochemical properties of the three cyclopenthiazide polymorphic forms, according to Gerber et al. (1991), are summarized in Table 2. After the crystallization, white needles were obtained (Fig. 2) with a melting point of 233 °C. No powder diffraction diagram was described for cyclopenthiazide by Gerber et al. (1991). Therefore, a direct comparison between obtained crystals and those described in the literature was not possible. However, based on the agreement in melting points and crystal morphologies, it can be assumed that the obtained crystals belonged to the polymorphic form III. This result is in agreement with our expectations since polymorphic form III is characterized by the lowest solubility.

The solubility of three polymorphic forms of codeine was described previously (Ebian and El-Gindy, 1978; El-Gindy and Ebian, 1978) and is summarized in Table 3. We obtained white

Table 3
Solubility of diverse polymorphic forms of codeine

Polymorph	Solubility in water (g/mL)
I	8.103
II	11.123
III	80.431

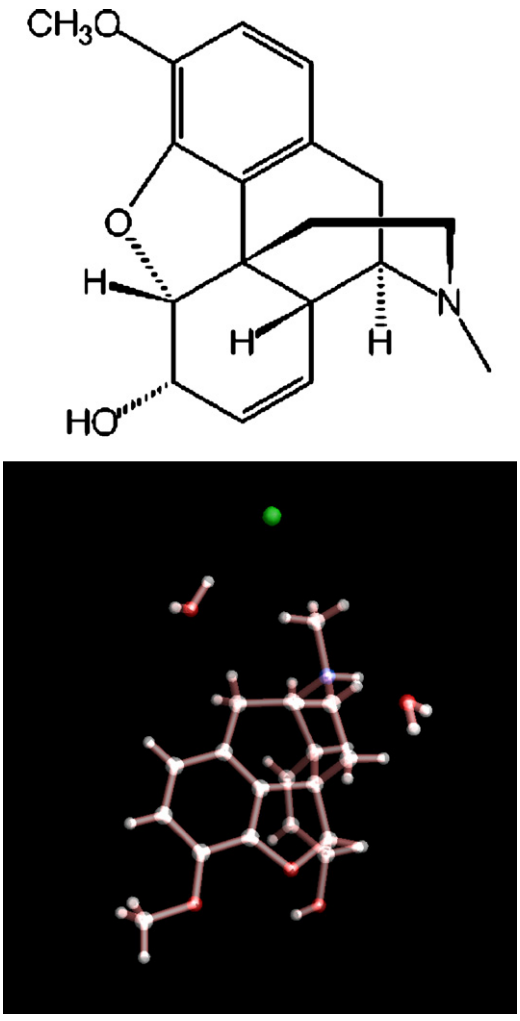

Fig. 5. Structure identification by single crystal X-ray analysis of codeine using crystals obtained by the new method: 2D chemical structure of codeine (upper panel) and 3D structure of codeine hydrochloride dihydrate (lower panel).

needles of codeine by applying the new crystallization method. The 3D structure of crystalline codeine was solved by single crystal X-ray analysis (Fig. 5b). Fig. 5 shows, that instead of one of the expected crystal forms of the free base, a chloride salt form of codeine with two water molecules in the crystal packing was obtained. This unexpected result demonstrates the practical limitations of the newly developed crystallization method. According to the description of the new method, the titration is stopped at a point of the pH-solubility profile, which corresponds to a slightly unsaturated solution (point 2′ in region B of Fig. 2). This point 2′ is considered to be the starting point for the crystallization. The pH-value at which point 2′ is reached depends thereby on the concentration of the compound in solution. At high concentrations, point 2′ is reached at a pH where the relative amount of ionized compound is still high. At low concentrations, point 2′ is reached at a pH where the relative amount of uncharged form of the test compound is higher. As a consequence, the likelihood of interactions between test compound and counter ions (such as chloride ions present in the incubation) changes and thus influences the crystallization process and the obtained crystal form. At high concentrations, the

crystallization process favors crystallization of the charged salt form since this ionized species has generally a stronger crystal lattice than the uncharged solid form. This can be explained by strong ionic interactions between cations and anions and the resulting stabilizing effect on the crystal lattice. At low concentrations, the uncharged species starts to compete with the salt form and eventually dominates the crystallization process. The conclusions from these experiments with codeine are twofold. First, the used concentration of test compound may have an influence on salt formation. Second, in order to enhance the possibility of obtaining crystals of the free base, a reduced concentration of the test compound is recommended to be utilized for the crystallization.

In conclusion, a new crystallization method has been developed for weak acidic and basic compounds. According to this method, one can rapidly proceed to a situation in which the solution is in a substantially saturated state, by gradually changing the pH-value of the solution in a direction that leads to a decrease of said compound's solubility. In particular, one can avoid the drawbacks associated with crystallization from a supersaturated state, because crystallization is then carried out under the most desirable conditions, by maintaining the solution in a substantially saturated state. In all the 11 analyzed cases, crystals could be obtained easily in solution by pH-titration using the sample concentration and experimental pK_a as input parameters. In combination with fully automated assay systems, the presented method offers the possibility to prepare efficiently crystals from drug-like organic molecules and therefore facilitates the development of oral dosage forms in drug discovery and development.

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