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A Rapid Method for Screening Crystallization Conditions and Phases of an Active Pharmaceutical Ingredient

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Abstract:

Here we present a useful methodology for rapid screening of crystallization conditions and phases in the pharmaceutical industry using a multiwell setup with video-microscopy developed in our laboratory. This methodology which detects, from small quantities of an active pharmaceutical ingredient, crystal habit modification, nucleation and phase transition could be used for rapid and reliable screening of crystallization media, particularly for an early stage of pharmaceutical development.

1. Introduction

Early in the development of a process to produce an active pharmaceutical ingredient (API) crystallization conditions and the phase to be produced must be defined. This prerequisite means determining the system thermodynamics and kinetics, i.e. the phase diagram (number of polymorphs and/or phases and their relative thermodynamic stability) and the phase transition kinetic. For a new API, polymorph screening is necessary. In reality, it would be more accurate to call this phase screening since the emergence of a new phase can seriously compromise the process developed, as was the case for Norvir,[1,2] or other examples.[3] Thus, it is necessary to develop an experimental strategy in order to study the respective influence of temperature, supersaturation, medium (chemical conditions) and hydrodynamics on the API crystallization. Currently, high-throughput screening (HTS), initially developed for biocrystallization,[4] is commonly used to accelerate the identification of the API phases.[5] Here we present a methodology for screening crystallization conditions and phases using a multiwell setup with video-microscopy developed in our laboratory. Using this method, we tested 45 media of crystallization in a temperature range of 20−60 °C. Nineteen solubility curves were estimated, four different crystal habits were obtained, and eleven different phases were nucleated with less than 5 g of API in 4 weeks.

Table 1. **List of solvents for the screening**

compound
water
ethanol, 2-propanol, methanol, butanol, pentanol, acetone, methyl isobutyl ketone (MIBK)
hexane, heptane, cyclohexane, methylcyclohexane, toluene, xylene
ethyl acetate, butyl acetate
diethyl ether, isopropyl ether, petroleum ether, methyl *tert*-butyl ether (MTBE), dimethoxyethane (Glyme), triethylene glycol dimethyl ether (Triglyme), 1,4-dioxan
1,2-dichloroethane, dichloromethane, chloroform, chlorobenzene
acetonitrile, dimethyl sulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF), tetrahydrofuran (THF), *N*,*N*-dimethylacetamide (DMA)

Table 2. **Solubilities for the first screening (pure solvents)**

	solubility		
cmpd	at 20 °C (g/L)	at 40 °C (g/L)	at 60 °C (g/L)
water	$10 < S < 15$	$20 < S < 25$	$55 < S < 60$
ethanol	$S < 5$	$S < 5$	$5 < S < 10$
methanol	$5 < S < 11$	$5 < S < 11$	$11 < S < 15$
butanol	$S < 5$	$S < 5$	$5 < S < 11$
petroleum ether	$140 < S < 160$	$320 < S < 360$	$500 < S < 520$
DMSO		$250 < S < 265$	$265 < S < 335$
DMF	$5 < S < 10$	$5 < S < 10$	$16 < S < 21$
DMA	$10 < S < 15$	$15 < S < 30$	$15 < S < 30$

Table 3. **List of solvent mixtures for the second screening**

compound mixed with water in a ratio 50/50 %w
ethanol, methanol, acetone
hexane, toluene
ethyl acetate
isopropyl ether
dichloromethane, chloroform
acetonitrile, DMF, THF, DMA

2. Experimental Section

2.1. Materials. The API (398 g/mol), a hydrochloride, is supplied as a crystalline powder by Oril Industrie and used as received. Crystals were observed under a scanning electron microscope (SEM) JEOL 6320F. Moreover, all the solid phases were characterized by X-ray diffraction INEL CPS 120. The references pattern micrograph and X-ray powder diffraction (XRPD) are shown in Figure 1 and correspond to phase A. The chemical properties of the API cannot be released for reasons of confidentiality.

2.2. Experimental Setup. The apparatus presented here is homemade (available commercially - ANACRISMAT) and is

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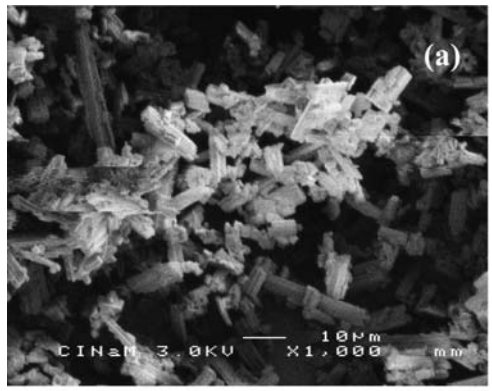

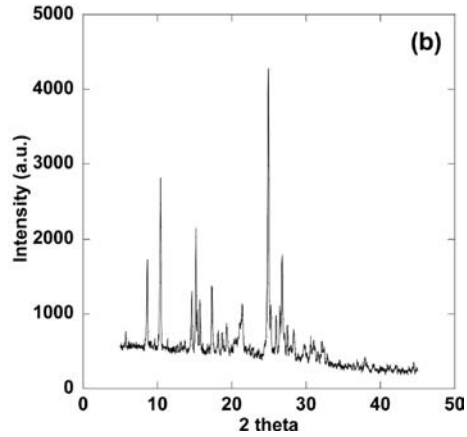

Figure 1. **(a) IPA phase A observed by SEM and (b) XRD.**

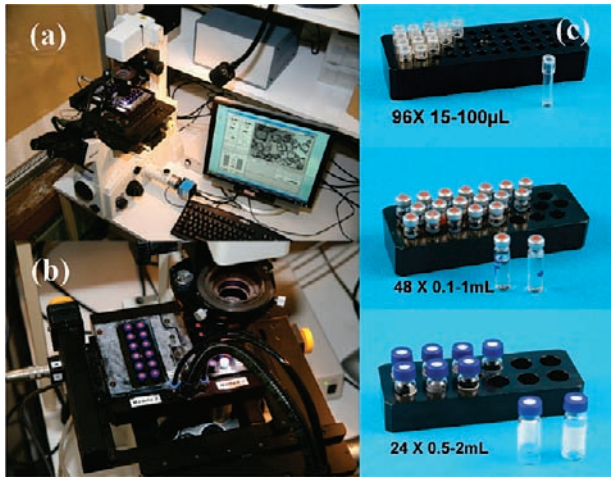

Figure 2. **(a) Multiwell setup, (b) $X-Y$ translation stage, (c) blocks of 6, 8, and 12 mm vials.**

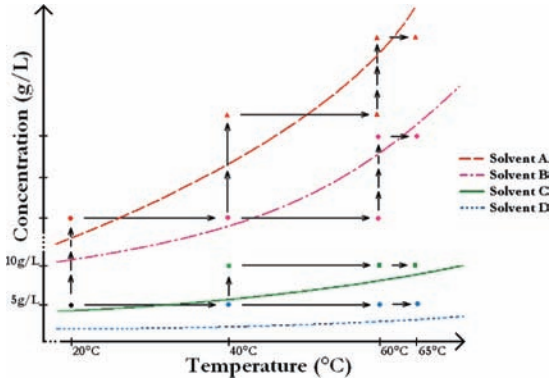

Figure 3. **Schematic representation of solubility estimation, horizontal arrows for case 1 and vertical arrows for case 2. Solubility curves are bracketed at a given temperature by upper point and upper point −5 g/L.**

shown in Figure 2. Three types of vials can be used (Figure 2c); for this screening, we used standard HPLC glass vials with 1 mL of solvent. The vials are inserted into two blocks (for 1 mL vials, each block contains 12 vials) thermostatted independently by Peltier elements and observed by a microscope (Nikon Eclipse TE2000-U). The whole assembly is mounted on an $X-Y$ translation table. Sequential image acquisitions are performed automatically and periodically (from minutes to hours). At the end of the experiment crystals are analyzed by XRPD.

Table 4. **Solubilities for the second screening (solvent mixtures)**

	solubility		
cmpd	at 20 °C (g/L)	at 40 °C (g/L)	at 60 °C (g/L)
water + ethanol	$15 < S < 25$	$25 < S < 50$	$80 < S < 120$
water + methanol	$15 < S < 25$	$25 < S < 40$	$40 < S < 70$
water + acetone	$160 < S < 190$	$160 < S < 190$	$335 < S < 385$
water + hexane	$5 < S < 10$	$5 < S < 10$	$5 < S < 10$
water + toluene	$5 < S < 10$	$5 < S < 10$	$5 < S < 10$
water + isopropyl ether	$5 < S < 10$	$5 < S < 10$	$5 < S < 10$
water + dichloromethane	$5 < S < 10$	$5 < S < 10$	$5 < S < 10$
water + acetonitrile	$380 < S < 400$	$795 < S < 805$	$1175 < S < 1220$
water + DMF	$30 < S < 40$	$210 < S < 245$	$495 < S < 525$
water + THF	$230 < S < 255$	$430 < S < 470$	$845 < S < 875$
water + DMA	$S < 5$	$65 < S < 85$	$300 < S < 340$

2.3. Procedure. Measurements of solubility are performed using glass vials with 5 mg of solute +1 mL of solvent. Slurry solutions in different solvents are placed inside the two blocks of the multiwell setup.

In the first stage, temperature is fixed at 20 °C. The evolution of the suspension is followed over time until, case 1, the crystals dissolve completely or, case 2, the crystals no longer dissolve. For the samples in case 1, 5 mg of API is added repeatedly until the crystals no longer dissolve (case 2). Now all the samples contain slurry, and the solubility at 20 °C ($S_{20\ °C}$ in mg/mL) of the API is bracketed according to the following formula:

$$C_{20\ °C} - 5 < S_{20\ °C} < C_{20\ °C} \qquad (1)$$

with $C_{T\ °C}$ the ratio of the total mass added to (mg) in a given vial at a given temperature T, $m_{T\ °C}$, by the volume of solvent, 1 mL.

In the second stage, temperature is rapidly increased to 40 °C. During this process the samples are observed, and images are acquired regularly to monitor possible dissolution. If during this T-variation all the crystals in a sample disappear (case 3) the temperature is noted (T_n) and:

$$S_{T_{n20\ °C}} > C_{20\ °C} \qquad (2)$$

At 40 °C, the procedure is repeated: stepwise addition of 5 mg of API to samples in case 1. Note that before API addition the temperature is decreased to 20 °C in order to avoid evaporation; after addition the temperature is rapidly increased to 40 °C. When all the samples contain slurry, the solubility of

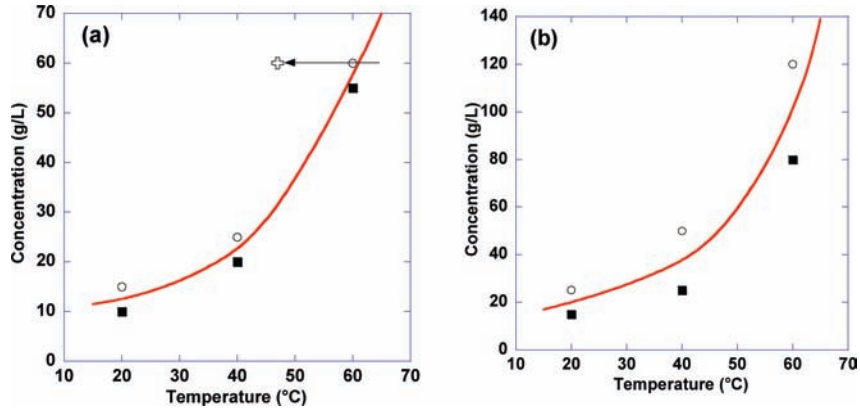

Figure 4. **API-solubility estimated in (a) water and (b) water/ethanol (50/50 %w). The arrow in (a) represents the last step of the procedure, and the cross represents the nucleation point.**

the API at 40 °C ($S_{40\ °C}$) is bracketed according to the following formula:

$$C_{40\ °C} - 5 < S_{40\ °C} < C_{40\ °C} \qquad (3)$$

In the third stage, temperature is rapidly increased to 60 °C. If during this *T*-variation all the crystals in a sample disappear (case 3) the temperature is noted as explained in the second stage.

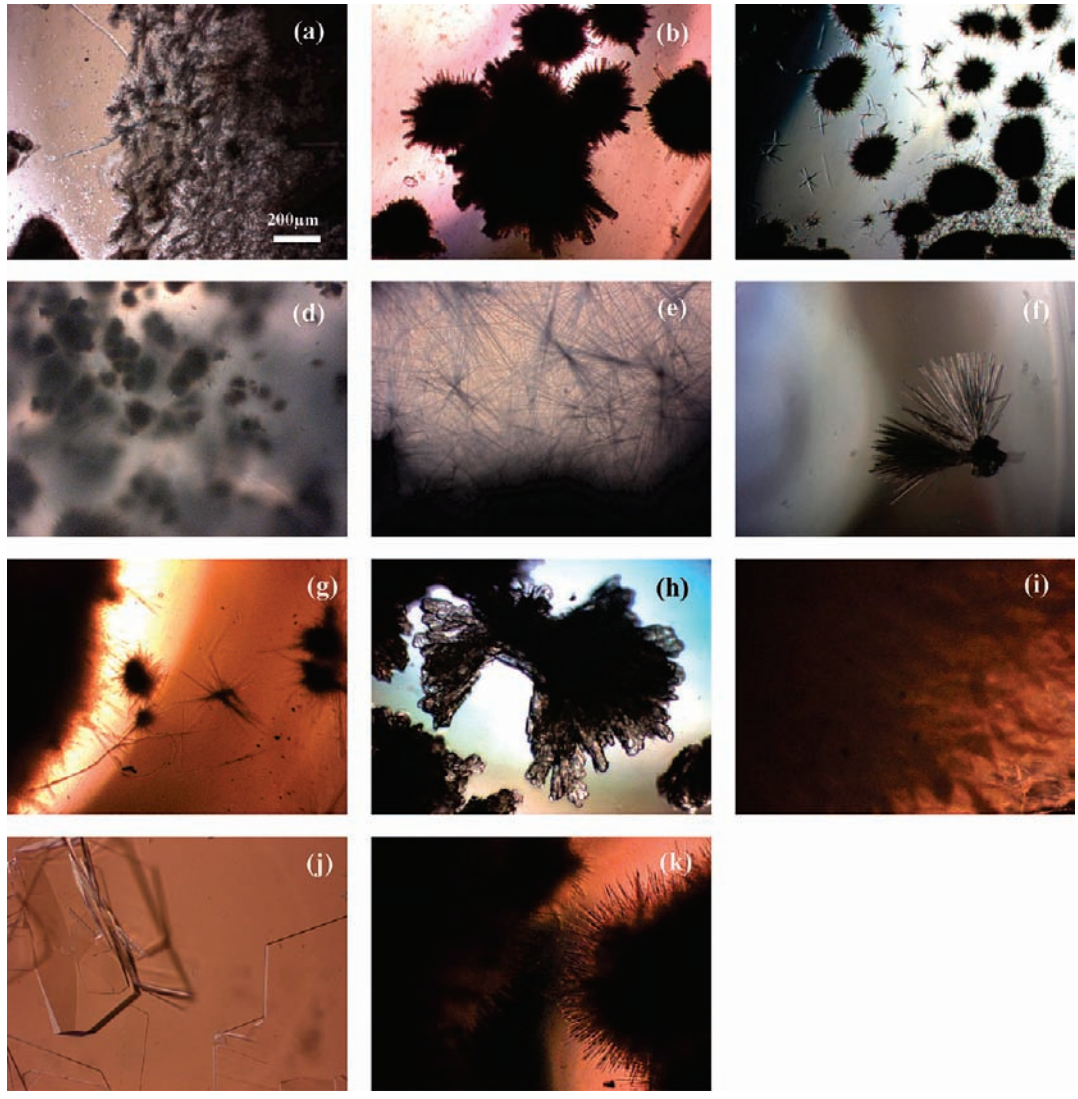

Figure 5. **Summary of crystal forms of the API, forms A to K: (a) to (k), respectively. All the micrographs are at the same magnification.**

Table 5. **Summary of crystal forms of the API, identified/discriminated by XRPD**

phases	medium
form A	ethanol, 2-propanol, methanol, butanol hexane, heptane, cyclohexane, methylcyclohexane, toluene, xylene butyl acetate diethyl ether, isopropyl ether, MTBE, Glyme,1,4-dioxan chlorobenzene water/ethanol, water/methanol
form B	water
form C	pentanol, Triglyme
form D	acetonitrile
form E	1,2-dichloroethane, dichloromethane
form F	chloroform
form G	DMF water/DMF
form H	THF
form I	water/acetonitrile, water/THF water/DMA
form J	water/acetone−water/acetonitrile, water/THF
form K	water/DMA water/acetone

At 60 °C, the procedure is repeated: stepwise addition of 5 mg of API to samples in case 1; before API addition the temperature is decreased to 20 °C and rapidly increased to 60 °C. When all the samples contain slurry, the solubility of the API at 60 °C ($S_{60\ °C}$) is bracketed according to the following formula:

$$C_{60\ °C} - 5 < S_{60\ °C} < C_{60\ °C} \quad (4)$$

NB: (1) The whole procedure is summarized in Figure 3. (2) When the solubility is high, as indicated by fast dissolution at low concentration, the quantity of each addition is gradually increased, to a maximum of 50 mg.

In the fourth and last stage, temperature is increased to 65 °C for 1−2 h in order to dissolve crystals remaining in the samples. Then the temperature is slowly decreased to 10 °C, with a ramp of 1 °C/h, in order to induce nucleation and estimate metastable zone width (Figure 4a). Solids obtained are filtered (Millipore FG 0.2 μm) and characterized by XRDP.

3. Results and Discussion

A first set of 32 solvents tested is presented in Table 1 with data in Table 2 for solvents which dissolved crystals. However, for the following solvents solubilities were very low, lower than 5 mg/mL, for the whole temperature range tested (20−60 °C): 2-propanol, acetone, MIBK, hexane, heptane, cyclohexane, methylcyclohexane, toluene, xylene, ethyl acetate, butyl acetate, diethyl ether, isopropyl ether, MTBE, Glyme, Triglyme, 1,4-dioxan, 1,2-dichloroethane, dichloromethane, chloroform, chlorobenzene, acetonitrile and THF.

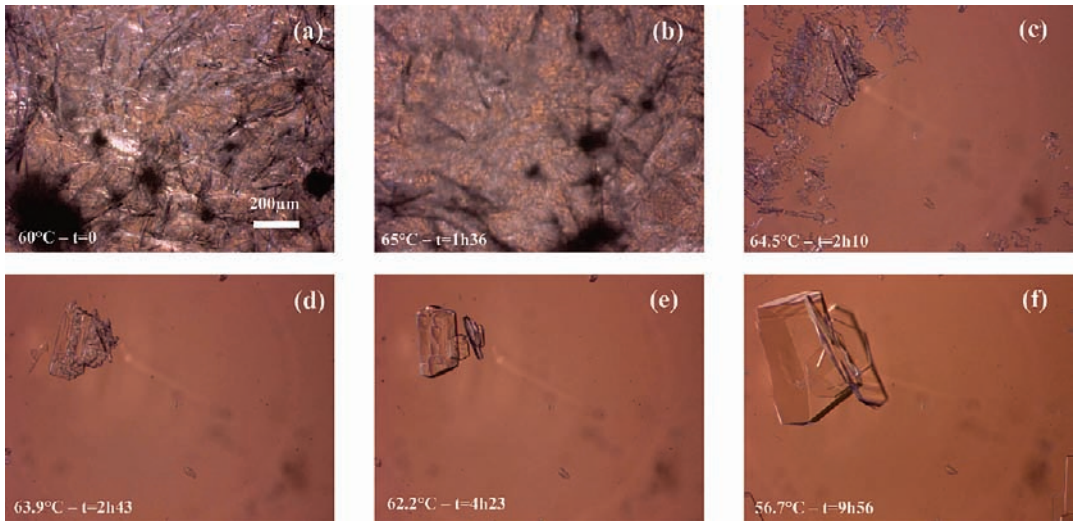

Figure 6. **In situ observation under optical microscopy of the solution-mediated phase transition of the API in water/acetone; temperature and timing are noted on the images. (a) Only one phase (form A) is observable, (b) the second phase (platelet habit-form J) has appeared, (c) and (d) dissolution of the metastable phase (form A) and growth of the stable phase (form J), (e) and (f) growth of the stable phase.**

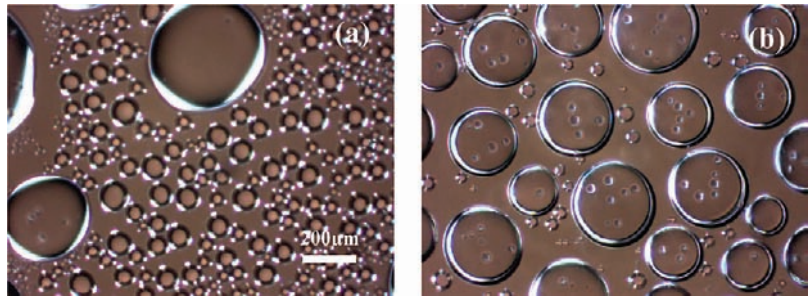

Figure 7. **In situ observation under optical microscopy of the demixion of the API in (a) water/chloroform and (b) in water/dichloromethane.**

Therefore, a second set of experiments was realized by testing mixtures of two solvents, one solvent in which API-solubility was high and varied with temperature (a good solvent for crystallization) and the other in which API-solubility was very low. Since water was a good solvent for this API (Figure 4a) as well as a good antisolvent choice for chemical processes in general, 13 mixtures with water at 50/50 %w ratio were tested (Table 3): data are shown in Table 4, and an example of the API-solubility is presented in Figure 4b. In two cases, for water/ethyl acetate and water/chloroform, the solubility is less than 5 mg/mL, for the whole temperature range tested (20−60 °C).

NB: With hexane, toluene, ethyl acetate, isopropyl ether, dichloromethane and chloroform miscibility is very weak (from 0.013% for hexane to 8.7% for ethyl acetate) leading to emulsion in which the API dissolves in the aqueous phase.

Table 5 summarized the different crystallization media for the 11 different phases obtained from the solvent screen and characterized by XRPD. However, for the moment, we cannot differentiate polymorphs and solvates. Four different crystal habits were observed (Figure 5): needle-like (forms A, C, D, E, G, I and K), rod-like (forms B and F), aggregated tabular (form H) and platelet (form J).

Here video-microscopy allows us to monitor the evolution of the slurry over time and with temperature. In the case of the water/acetone mixture a phase transition was observed during the last stage of the screening. The total amount of API (form A) added was 385 mg (Table 4), the slurry temperature was increased to 65 °C and was immediately decreased by 1 °C/h. The sequence in Figure 6 shows the solution-mediated phase transition obtained by changing the temperature. At the end of the experiment, the stable phase (form J) was in suspension.

Figure 7 presents two examples of demixion of the API in water/chloroform and water/dichloromethane media. When a solvent mixture is used as crystallization medium, the probability of having a miscibility gap in the phase diagram increases,[6] i.e. a liquid−liquid phase separation or demixion appears. The impact of such a phenomenon on the crystal nucleation can be disastrous.[7−9]

4. Conclusion

By permitting rapid screening of crystallization medium and temperature for API crystallization, our method allows us to select from the solvents tested those that generate the optimum crystal habit for downstream processes, storage and handling. Moreover, image acquisitions performed automatically during the experiment allow us first, to monitor the suspension during dissolution and second, to monitor the solution during cooling. We can therefore detect any crystal habit modification, nucleation and phase transition.

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