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A high-throughput multi-microfluidic crystal generator (MMicroCryGen) platform for facile screening of polymorphism and crystals morphology for pharmaceutical compounds

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Abstract

In this work a novel multi-microfluidic crystallization platform called MMicroCryGen is presented, offering a facile methodology for generating individual crystals for fast and easy screening for polymorphism and crystal habit of solid compounds. The MMicroCryGen device is capable of performing 8×10 cooling crystallization experiments in parallel using 8 disposable microcapillary film strips, each requiring less than 25 μL of solution. Compared to traditional microfluidic systems the MMicroCryGen platform does not require complex fluid

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handling, it can be directly integrated with a 96 wells microplate and it can also work in "dipstick" mode. The produced crystals can be safely and directly observed inside the capillaries with optical and spectroscopic techniques. The platform was validated by performing a number of independent experimental runs for: (1) polymorph and hydrate screening for ortho-aminobenzoic acid, succinic acid and piroxicam; (2) co-crystal form screening for the p-Toluenesulfonamide/triphenylphosphine oxide system; (3) studying the effect of o-toluic acid on ortho-aminobenzoic cooling crystallization (effect of structurally related additives). In all three cases, all known solid forms were identified with a single experiment using ~200 μL of solvent and just few micrograms of solid material. The MMicroCryGen is simple to use, inexpensive and it provides increased flexibility compared to traditional crystallization techniques, being an effective new microfluidic solution for solid form screening for pharmaceutical, fine chemicals, food and agrochemical industries.

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Introduction

Polymorphism is known as the ability of a compound to exist in more than one crystalline structure and is very common in the crystallization of active pharmaceutical ingredients (APIs) (1). Polymorph screening is therefore essential during the development of new drugs since the formation of the wrong polymorph can affect solubility, rate of dissolution and toxicity of the API (1-3). The discovery of possible polymorphic forms for newly synthesized compounds is usually done by running independent crystallization experiments in small, bench scale (1-100 mL) crystallizers or via high-throughput devices based on arrays of wells, tubes or vials. This task requires a large number of steps and it is often difficult and expensive to automate because of the complex fluid handling involved (4, 5). Robotic liquid handling systems provided the mean to automate and increase throughput for screening of novel compounds in a drug discovery environment (6); however, the high cost of such systems means the technology is only accessible to few.

In recent years microfluidic devices have been used to study several crystallization steps, including nucleation and growth (7-11), polymorphism (12-14) and co-crystal formation (15). Microfluidic technologies offer the possibility of running several experiments in parallel using small quantities of API, which is particularly important during the early stages of clinical trials when only small quantity of API is available. Nevertheless, current microfluidic devices still require interface to complex fluid handling (16). Furthermore, the extraction of crystals from the microfluidic channels for off-line analysis can also be problematic.

Droplet-based devices are the most commonly used microfluidic tools for measuring nucleation rate, metastable zone width as well as solubility, and for the screening of polymorphs (7, 16-21). The main advantage of using droplets for crystal formation is that a small volume of solution can be isolated in an easy way such as using a manual or electronic pipette (20), or by segmenting the solution flow with an immiscible liquid or gas in a

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microchannel (17-19). The supersaturation necessary to form crystals can then be generated by decrease the temperature of the droplets (7), adding an anti-solvent or a reactant (19) or via solvent evaporation (20). Each droplet can be considered as a single crystallizer allowing a systematic determination of the primary nucleation kinetic as well as an easier determination of the polymorphism of each crystal nucleated. Furthermore, the wells containing the droplets for evaporation-based devices can be chemically functionalized via self-assembled monolayers of specific organic compounds. In this way polymorphism can be controlled via templated heterogeneous nucleation (20, 21). Despite these advantages, the droplet-based devices that use micro-channels to mix immiscible liquids require the use of external pumps, precise flow control for the mixing of the two immiscible phases and complex microchannel geometry. Additionally, in the case of evaporation-based microfluidics, a controlled atmosphere around the droplets is necessary for a slow and controlled removal of the solvent.

Both cooling and anti-solvent crystallization processes have been conducted in microfluidic channels with precise control of both temperature and solute concentration (12). Because of the reduced amount of crystallizing material needed, microfluidic devices are particularly convenient for the study of expensive compounds such as proteins (22, 23), or for safe handling of toxic and/or carcinogenic compounds (16). Compared to larger scale crystallizers, microfluidic crystallizers provide a micro-environment with laminar only mixing conditions that enable the formation of high quality crystals, with defined habit and limited structural defects or amorphicity. Such crystals can easily be analysed via optical microscopy, vibrational spectroscopy and, in some cases X-ray diffraction (16). Microfluidic devices are also particularly suited for polymorph screening. In fact, the reduced volume and the absence of turbulent mixing promotes primary over secondary nucleation and favours the discovery of a larger number of polymorphs and co-crystals (13-16). Furthermore, in small

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volumes the nucleation and persistence of highly unstable polymorphs is more likely compared to large scale crystallizers (12-14, 16, 24). It is worth noticing that the rate of nucleation in small volumes is always slower than for large crystallizers, and the formation of crystals that are large enough to be analysed takes a longer time.

Despite the evident advantages of microfluidics technologies, current devices remain expensive to produce at small scale, require bulky fluid handling equipment and present solvent-compatibility issues.

In this work a novel multi-microfluidic platform for the generation of individual separated crystals (MMicroCryGen) that allows rapid and facile screening for polymorphism and crystal habit from a small sample volume is presented for the first time. In contrast to existent droplet-based microfluidic devices for polymorph screening (17-20) the MMicroCryGen does not require external pumps as sample is loaded by capillary action (similar to a conventional 'dipstick' test) and crystals are generated by cooling, with smoother control of the supersaturation during nucleation.

The MMicroCryGen device shown in Figure 1a was specifically designed to perform multiple cooling crystallization experiments in parallel, with the option of real-time, *in-situ* observation of the crystals using optical or spectroscopic sensors. This platform can be used for the screening of hydrates, salts and co-crystals, which are often used as expedients to improve the rate of dissolution and solubility of an API, as well as to study the morphology of the crystals in different solvents.

The main consumable of the MMicroCryGen is a set of multiplex, microfluidic strips obtained via cutting a longer MicroCapillary Film (MCF). The MCF is a flat film containing an array of ten parallel microcapillaries fabricated from fluorinated ethylene propylene co-polymer (Teflon-FEP®) using a novel continuous melt-extrusion process (25, 26). Photos of

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the MCF strips are shown in Figure 1b and c. Due to the geometry and optical properties of FEP-Teflon®, the microcapillaries are uniquely transparent in a very broad range of wavelengths including UV, VIS and NIR. Additionally, the MCF material is available in different inner internal diameters as reported elsewhere (27). Although FEP-Teflon® is naturally hydrophobic, internal coating of the microcapillaries with a hydrophilic hydrogel (28) enables the aspiration of fluid sample from a conventional microtiter plate by capillary action. Small volumes of API solutions (around 25 μL) can be prepared and aspirated into the microcapillaries without the use of pumps or syringes, similarly to conventional 'dip stick' tests. The small volume, low cost, transparency and hydrophilicity of the MCF material makes it an optimal consumable capillary element for the MMicroCryGen platform. The device uses a Peltier heat exchanger for carrying out controlled cooling crystallizations, enabling the study of both crystal morphology and polymorphism. In this work, the MMicroCryGen platform was validated by carrying out polymorph screening of succinic acid (SA) and ortho-aminobenzoic acid (OABA) (29-32), by determining the effect of o-toluic acid (OTA) on the polymorphism of OABA and by studying the formation of hydrates of piroxicam and co-crystals of p-Toluenesulfonamide (p-TSA) and Triphenylphosphine oxide (TPPO) (33). The data presented show that the MMicroCryGen is a valuable microfluidic solution for facile polymorph screening of APIs since it requires a smaller amount of drugs and solvents and considerably less and cheaper additional instrumentation compared to standard techniques. The device can be particularly useful during the early stages of drug development when only a small amount of material is available and for the study of toxic, genotoxic or carcinogenic APIs.

Experimental

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Materials

For the screening experiments succinic acid (SA, from Sigma Aldrich, ≥99.0 % purity), ortho-aminobenzoic acid (OABA, from Sigma Aldrich, purity >98%), piroxicam (from Hangzhou Hyper Chemicals Limited, 99 % purity, anhydrous), o-toluic acid, (OTA, from Sigma Aldrich, 99% purity) and p-Toluenesulfonamide (p-TSA, 99 %) and triphenylphosphine oxide (TPPO, 99 %) was sourced from Sigma-Aldrich (Dorset, UK) and used as received. Deionized water (Millipore ultrapure water system) isopropyl alcohol (IPA 99.97 % grade, Fisher Scientific, Loughborough, UK), acetone (Fisher Scientific, >99.98 % grade) and acetonitrile (Fisher Scientific, grade 99.9 %) were used as solvents.

Multi-Microfluidic Crystal Generator (MMicroCryGen)

Figure 2 shows the miniaturised MMicroCryGen device, developed and built in house at Loughborough University (UK). The main element is a re-usable frame (60.0 mm×78.8 mm, as shown in the drawings provided in the Supporting Information) composed of an aluminium plate, a central transparent Perspex cover and two side aluminium edges used for sealing the MCF strips and keeping them in a flat position (Figure 2a and 2d). The frame has 8 slots for the insertions of 70-80 mm long MCF strips. The slots are spaced by 8.36 mm centre-to-centre, enabling the direct interface with a 96 wells microplate (Figure 2d). Each MCF strip has an internal volume of approximately 25 μL and each 2.5 μL microcapillary can be considered as a single crystallizer with no mixing. Although the MMicroCryGen was operated manually, it offers the opportunity for easy automation with a robot arm, and does not require any fluid handling equipment/pumps. The MCF strips are kept in a flat position on the plate and sealed at both ends to avoid solvent evaporation by compressing the central Perspex cover and the two lateral aluminium edges with three sets of four screws. The lateral edges are provided with 8 pointy protrusions that compress both edges of the MCF strips. Although in this work the whole frame was kept in position using three sets of screws (Figure

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2b), the design could be further simplified with a one-step, 'clip' mechanism. The capillaries could be observed in real time through the Perspex cover using optical or spectroscopic sensors.

The whole aluminium frame containing the MCF strips lay on a polyoxymethylene plate that contains a Peltier unit for temperature control (Meerstetter Engineering TEC-1090, Rubigen, Switzerland, shown in Figure 2b). The Peltier element (ThermaTEC^{TM} Series HT4, Laird Technologies, London United Kingdom) allows a range of temperatures in the microcapillaries between -20 and 120 °C and a maximum cooling rate of 5 °C/min. The Peltier unit is connected to a fan (BIG Shuriken 2, model SCBSK-2100 purchased from Scythe, Tokyo, Japan) and a controller (Meerstetter Engineering TEC-1091 running with TEC Service v3.0) for temperature regulation. Multiple units could be arranged in parallel and controlled independently for a higher number of simultaneous experiments.

Fabrication of hydrophilic microfluidic, MCF strips

The MCF material was manufactured by Lamina Dielectrics Ltd (Billingshurst, West Sussex, UK) from Teflon® FEP (Dupont, USA) and consists of an array of 10 parallel microcapillaries with a mean hydraulic diameter of 206 ± 12.6 μm (34). The raw MCF strips are hydrophobic (28), therefore they would require a pressure driven flow for sample loading; however in this work the MCF strips were made hydrophilic by coating the inside of each capillary with an hydrogel, poly(vinyl alcohol) (PVOH) (28). In this way the MCF strips could be filled simply by capillary action ('dip stick' mode). During the coating procedure 100 mL of an aqueous solution of PVOH (MW 13 000–23 000, >98 % hydrolysed from Sigma-Aldrich, Dorset, UK) with concentration of 5 mg/mL was recirculated through a 6 m

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long MCF at a flowrate of 50 mL/h for 12-15 hrs using a FPLC P-500 Pharmacia Biotech (Piscataway, USA) pump and Upchurch flangeless tube fittings (Kinesis, Saint Neots, United Kingdom). After that, the PVOH coating was crosslinked with glutaraldehyde by manually filling the MCF strip with a freshly prepared solution of PVOH (5 mg/mL) containing 5 mM of glutaraldehyde (Sigma-Aldrich, UK) and 5 mM HCl (Sigma-Aldrich, UK). The solution was kept inside the strips for 2 hours at 37 °C, after which the MCF strips were manually washed and dried with air using a 50 mL syringe (34).

Solid form screening experiments in the MMicroCryGen platform

For each experiment different solutions were prepared and loaded in the MMicroCryGen platform using the same procedure. The central Perspex cover was positioned on the aluminium frame and secured with four screws, then 8 hydrophilic or hydrophobic MCF strips of 70-80 mm length were inserted in the slots. For hydrophilic strips, the frame was positioned vertically above a 96 wells microplate loaded with clear API solutions and then lowered in order to fully immerse the tip of each strip in the wells. For hydrophobic strips, the microcapillaries were aspirated using a multi-syringe aspiration device reported elsewhere (34). On both cases, after complete filling of the capillaries with solution, the frame was placed in horizontal position and the lateral aluminium edges were secured with 8 screws (4 at each side), automatically sealing both ends of the microcapillaries. The frame containing the MCF strips was then placed on the Peltier heat exchanger and the desired temperature or cooling profile set from the temperature control software. On-line monitoring of the experiments was performed with a USB Microscope (5.0 Megapixels supplied by MAOZUA, China). The MMicroCryGen device allows 80 independent parallel crystallization experiments in one go, this can be easily further expanded by increasing the number of strips.

Once crystals were formed within the microcapillaries, the whole frame was removed from the Peltier unit and the MCF strips were individually analysed for crystal morphology and

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polymorphism. A Raman microscope DXR 780 nm (Thermo Scientific equipped with OMNIC 8 software) equipped with a Linkam hot-stage was used to identify the structure of the crystals produced.

For the polymorph screening experiments succinic acid (SA) and ortho-aminobenzoic acid (OABA) were used as solid compounds. Only deionized water was the solvent for succinic acid, while mixtures of deionized water and isopropyl alcohol were used for OABA. A solution of SA in water with concentration of 0.145 g/g solvent was prepared at 50 ºC and loaded in one row of a standard 96-wells microplate before being interfaced with the MMicroCryGen. The MCF strips were then cooled down to 5.0 °C at a rate of -0.5 °C/min. For OABA polymorph screening solutions at different concentrations of OABA were prepared and loaded into the capillaries at 60 °C, before being cooled down to 3.0 °C at a rate of -1.0 °C/min.

Piroxicam in mixtures of deionized water and acetone was used in the hydrate screening experiments. Four solutions at different concentrations of piroxicam as well as solvent composition were prepared at 50 °C and loaded in duplicated MCF strips. The temperature was reduced to 3.0 °C at a rate of -1.0 °C/min.

In the co-crystal screening experiments, p-Toluenesulfonamide and triphenylphosphine oxide were used with acetonitrile as solvent. Two solutions containing both components in specific stoichiometric ratios were prepared at 50.0 °C, and each loaded into four MCF strips. After that, the temperature was reduced to 3.0 °C at a rate of -1.0 °C/min.

Finally, a set of experiments was conducted in the presence of o-toluic acid (OTA) in order to determine the effect of a structurally related additive on the crystallization of OABA. Four different aqueous solutions with concentration of OABA of 0.010 g/g water and different

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ratios OTA/OABA w/w were prepared at 50 ºC and loaded into the MCF strips. After that the temperature was reduced to 5.0 °C at a rate of -1.0 °C/min.

In all experimental sets, the MMicroCryGen was further incubated overnight at 5.0 °C enabling full growth of crystals upon completion of the cooling stage.

Results and discussion

Polymorph screening of SA and OABA

SA is known to have two polymorphic forms, α and β, which are enantiotropically related. The triclinic α-form is usually obtained by solid transformation from the β-form at temperatures above 137 °C while the monoclinic β-form is the polymorph that generally precipitates from water in various morphologies (29, 30). Microphotographs for SA crystals nucleated in the MMicroCryGen platform are shown in Figure 3b-3e, whereas Figure 3a shows plate-like crystals of β-form only nucleated from an aqueous solution of the same concentration in a 350 mL vessel with overhead stirring (29). Both high aspect ratio needles and plate-like crystals were observed in the MCF strips. Raman spectroscopy analysis (Figure 4) confirmed the needle SA crystals were of α-form. The spectra of these crystals were also compared with those obtained from hot-stage experiments performed in the available Raman microscope. SA β-form crystals were heated up above the transition temperature of 137 ºC and then quickly cooled down to ambient temperature to allow the formation of the α-form on the hot-stage. Crystallisation in the MCF strips resulted in formation of SA crystals in their α-form, which is usually not observed in cooling crystallization in aqueous solutions. This is presumably due to both the reduced volume and the absence of convection and turbulent mixing in the microfluidic strips of the MMicroCryGen device. Such conditions promote

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primary over secondary nucleation, allowing the formation of a larger number of possible crystal structures, which is ideal for polymorph screening.

The Raman spectra for both polymorphs of SA obtained in the MCF strips are shown in Figure 4, and compared to the spectrum of an empty microcapillary. FEP-Teflon® has distinct, clear peaks that can be easily identified and subtracted from the total final spectra; strong intensity FEP-Teflon® peaks can be found at values of Raman shift of 290, 385, 730, 1225, 1300, 1380 cm^{-1}. Depending on the objective and the size of the analysed crystals, it is possible to focus the Raman laser on a single crystal, avoiding the signal from the MCF strip. Both hydrophilic and hydrophobic capillaries were tested and no differences were observed in respect to SA polymorph formation. In fact, both structures were always obtained, suggesting that the chemistry of the capillary's inner surface did not have a templating effect on the nucleation of this compound.

Polymorph screening in different solvent mixtures was performed with OABA. The solvent compositions and OABA concentrations for each MCF strip are summarised in Table 1. In contrast to OABA crystallisation experiments previously conducted at larger scale, where only form I and II could be precipitated in water/IPA mixtures (31), all three known polymorphs of OABA were obtained in the capillaries, similarly to what was observed with SA. It can also be noted that, due to the slower kinetics of nucleation in small volumes, some capillaries did not show any visible crystals in the time scale used for experiments (around 18-24 hrs).

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278 **Table 1: Experimental conditions and results for polymorph screening of OABA.**

MCF strip number	Solvent composition (% of IPA in water, w/w)	OABA concentration (g/g solvent)	Crystals nucleated in each MCF strip
1	0	0.010	0
2	2%	0.010	1 (form I)
3	5%	0.010	0
4	10%	0.015	0
5	15%	0.020	2 (form II)
6	20%	0.030	2 (form II) 5 (form III)
7	25%	0.055	4 (form II) 2 (form III)
8	30%	0.080	1 (form II) 3 (form III)

279

280 The MMicroCryGen device allowed to identify all the three known forms of OABA from a
281 small amount of material, demonstrating the advantage of using this novel methodology for
282 polymorph screening. The area in the Raman spectrum that unequivocally identifies the three
283 OABA polymorphs is between 1500 and 1700 cm^{-1} (Figure 5). Form I presents a peak at
284 1665 cm^{-1} and a doublet at 1600 cm^{-1}, form II has a strong peak at 1565 cm^{-1} and form III
285 presents the same peak but shifted to 1560 cm^{-1}. Finally, form III is characterized by a strong
286 peak at 1610 cm^{-1}, which is not present in form I and it is weaker in form II.

287

288 The polymorphs screening experiments performed in the MMicroCryGen platform show the
289 advantage of using small volumes in the absence of mixing for the discovery of new crystal
290 structure.

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Hydrate detection for Piroxicam

Piroxicam was crystallized in different mixtures of acetone and water in order to identify the possible polymorphs and confirm the presence of hydrate forms. Table 2 shows the experimental conditions and the different polymorphs identified by Raman spectroscopy, all known polymorphs and the hydrate form (35-37) were detected in the microcapillaries. Microscopic images of the two polymorphs are shown in Figure 6b, with the thin needles characteristic of form II clearly distinguishable from the prismatic form I crystals. The monohydrate form of piroxicam was obtained in all solutions containing water. These crystals have a characteristic bright yellow colour as shown in Figure 6a.

Table 2: Experimental conditions and results for polymorph screening of piroxicam.

MCF strip number	Solvent composition (% w/w)	Concentration (g/g solvent)	Crystals nucleated in each MCF strip
1-2	100% acetone	0.040	Anhydrous form I and form II
3-4	5% water in acetone	0.035	Anhydrous form II (needles) and monohydrate (prism)
5-6	20% water in acetone	0.013	Piroxicam monohydrate
7-8	30% water in acetone	0.009	Piroxicam monohydrate

Raman spectroscopy was also used to distinguish among the polymorphs and the monohydrate form of piroxicam within the MCF strips. Figure 6c shows the region between 1500 and 1700 cm^{-1}, where the three forms can be identified without interference from the FEP-Teflon®. The monohydrate form is characterized by a single strong peak at 1590 cm^{-1},

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whereas form I has a high intensity peak at 1520 cm^{-1} and finally, form II is the only polymorph with a peak at 1540 cm^{-1}.

In the case of piroxicam, the MMicroCryGen enabled simultaneous identification of the two known polymorphs and the hydrate form in a single experiment with four different solutions of piroxicam in acetone and water mixtures.

Screening for co-crystals

A further set of experiments was carried out aiming to demonstrate the application of the MMicroCryGen device to identify co-crystal structures, using p-Toluenesulfonamide (p-TSA) and Triphenylphosphine oxide (TPPO) as model compounds.

Table 3: Experimental conditions and structures obtained during the screening of TSA/TPPO co-crystals.

Solvent composition (% w/w)	Concentration p-TSA (g/g solvent)	Concentration TPPO (g/g solvent)	Crystals nucleated in each MCF strip
100% acetonitrile	0.02987	0.01492	3:2 co-crystal form
100 % acetonitrile	0.01154	0.01489	1:1 co-crystal form

The two co-crystals known for those chemicals, with molecular ratio p-TSA/TPPO 3:2 and 1:1 (33), were quickly and successfully identified in a single experiment in the MMicroCryGen device, as confirmed by Raman spectroscopy according to data available from literature (33). Experimental conditions are described in Table 3, whereas the Raman spectra of the two co-crystal forms obtained are shown in Figure 7. The Raman spectra for

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pure p-TSA and TPPO were compared to data available in literature (33, 38), confirming the formation of two co-crystal forms. The characteristic regions of the Raman spectrum for the unequivocal identification of the two co-crystal forms are shown in Figure 7. Characteristic peaks for the 1:1 co-crystal are located at 317, 460, 507, 934 and 1151 cm^{-1}, whereas the 3:2 co-crystal is characterized by specific peaks at 310, 915 and 1149 cm^{-1}(Figure 7).

These experimental demonstrate the use of 'dip stick' microcapillaries for discovery of new co-crystal structures. Furthermore, the use of the MMicroCryGen platform allowed the identification of such co-crystal forms running a single experiment, with the use of limited amount of chemicals as well as solvents.

Screening of the effect of additives on polymorphism

Additives can have an effect on the polymorphic outcome of crystallization processes. These additives can act by inhibiting nucleation or growth of a certain polymorph of the main crystallizing compound, therefore stabilizing a different form. They can also stereo-selectively bind to specific surfaces of the targeted crystal nuclei, inhibiting their growth and therefore modifying the morphology of the crystals (32). The MMicroCryGen device was used to determine the effect of a structurally related additive (o-toluic acid, OTA) on the polymorphic outcome of OABA. Experiments at larger scale conducted with this compound and a similar molecule (benzoic acid) showed that a structurally related additive promoted nucleation of OABA form III, which can normally be obtained only by sublimation or polymorphic transformation at temperatures higher than the transition one (around 60 °C) (32). Four aqueous solutions at different ratios OTA/OABA were loaded into the MCF strips before starting the cooling profile. Table 4 shows the polymorphs detected in the capillaries.

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Table 4: Polymorphic outcome of cooling crystallization of OABA in the presence of the structurally related molecule OTA.

MCF strips number	Ratio OTA/OABA w/w	OABA polymorphs identified in MCF
1-2	0	Form I and II
3-4	5%	Form I, Form II, Form III
5-6	10%	Form III
7-8	15%	Form III

Similarly to previously reported observations at larger scale experiments with benzoic acid (32), the addition of a structurally related additive promotes the formation of OABA form III in water. In fact, only form I and II were nucleated in water in the absence of additive, whereas preferential nucleation of only form III could be observed at increasing concentrations of OTA. This indicates that, similarly to benzoic acid, OTA has an effect on the polymorphic outcome of OABA crystals generated via cooling. Crystallization experiments in the MMicroCryGen platform allowed easier determination of the effect of a structurally related additive on the crystallization of OABA using a considerably lower amount of solvent and materials compared to experiments conducted at larger scale (32).

Conclusions

The presented microfluidic MMicroCryGen crystallization device represents a valuable novel solution for polymorph and co-crystal screening of small molecules, requiring a smaller amount of compound and solvents and considerably fewer and cheaper fluidics and electronic instrumentation compared to standard microfluidic techniques, as well as larger scale equipment. The device and methodology proposed has the ability of enabling up to 80 parallel crystallization experiments without any pipetting or complex fluid handling, which

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could be particularly useful during the early stages of drug development when only a small amount of material is available, and for the study of particularly toxic, genotoxic or carcinogenic APIs. In this paper the MMicroCryGen platform was validated for solid form screening of SA, OABA, Piroxicam and the TSA/TPPO system. All known polymorphs, hydrates and co-crystals of the systems studied were identified with a reduced number of experiments and using very small amounts of material. The MMicroCryGen platform could also be used for more complex experiments including the study of the effect of additives on crystal forms resulting from cooling crystallization.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgments

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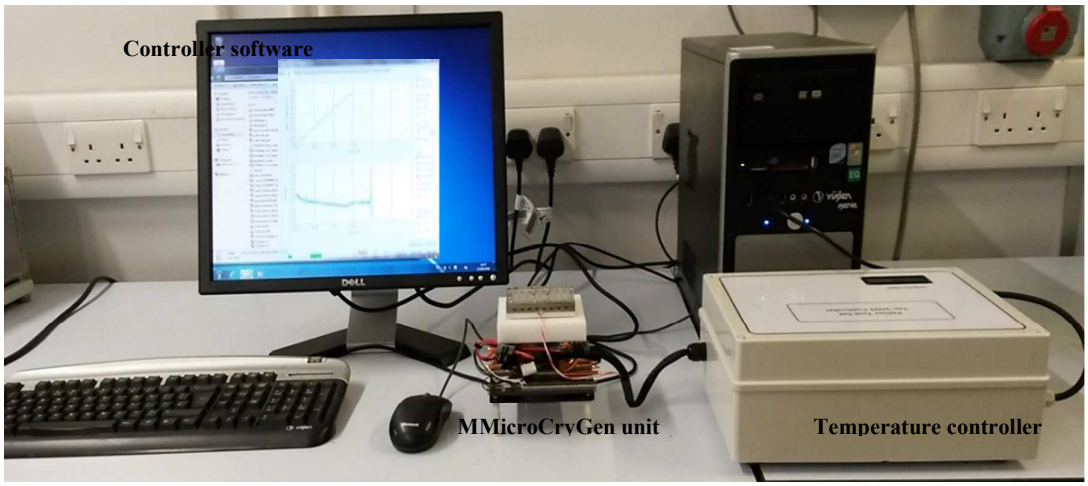

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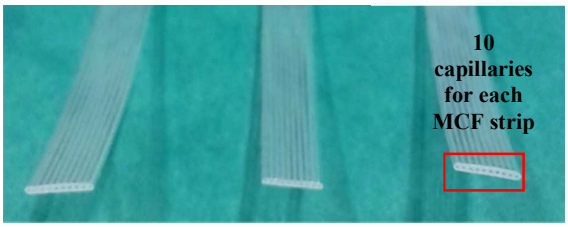

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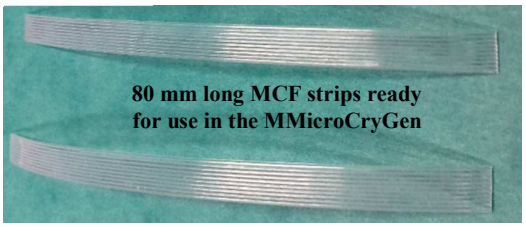

(c)

Figure 1: (a) Photographs of the MMicroCryGen unit with temperature controller for remote temperature setting; (b-c) Photographs of the MCF strips and detail of the 10 internal capillaries

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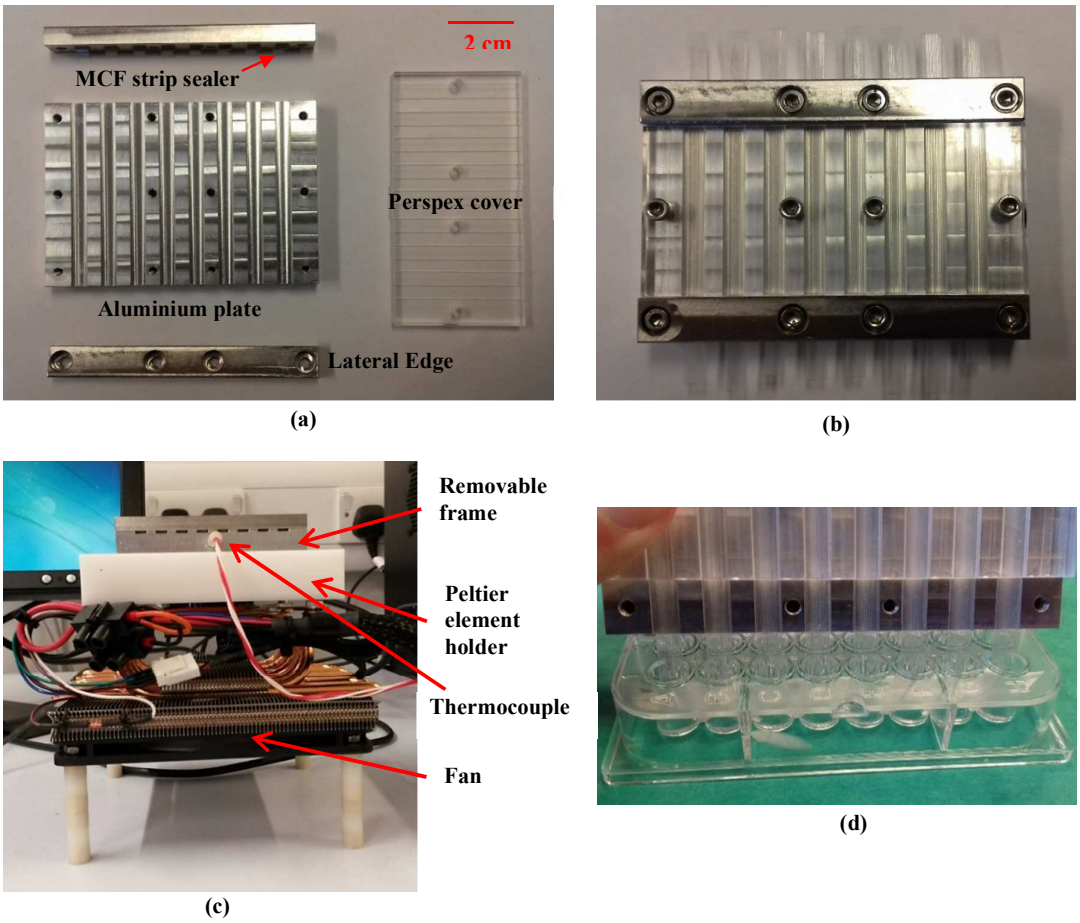

Figure 2: (a) Photograph showing the four elements of the removable frame of the MMicroCryGen platform; (b) Assembled frame; (c) Removable frame positioned on the Peltier element holder and fan; (d) Solutions loading procedure for hydrophilic capillaries.

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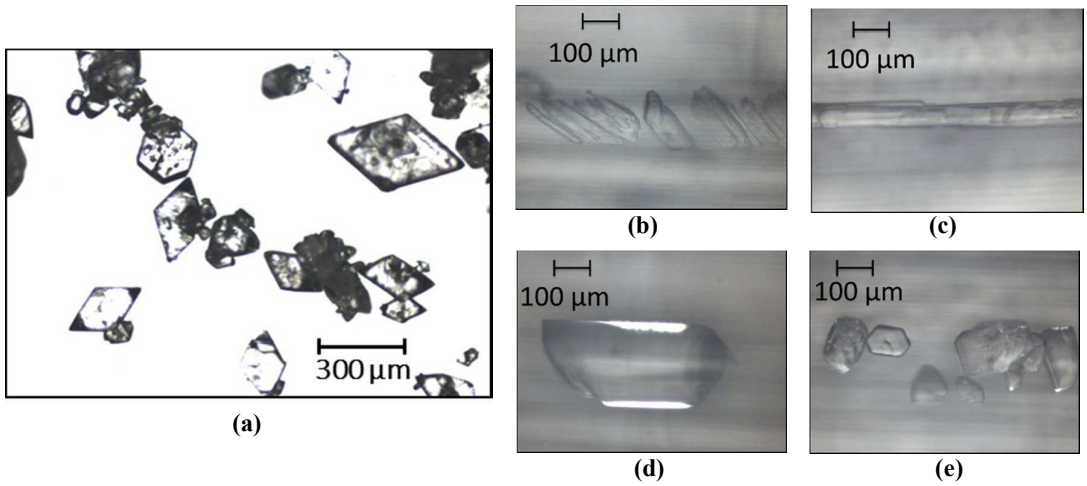

Figure 3: (a) Microscopic image of β SA crystals obtained from water in a 350 mL stirred vessel; (b), (c) crystals of α SA inside an MCF strip; (d), (e) crystals of β SA inside an MCF strip.

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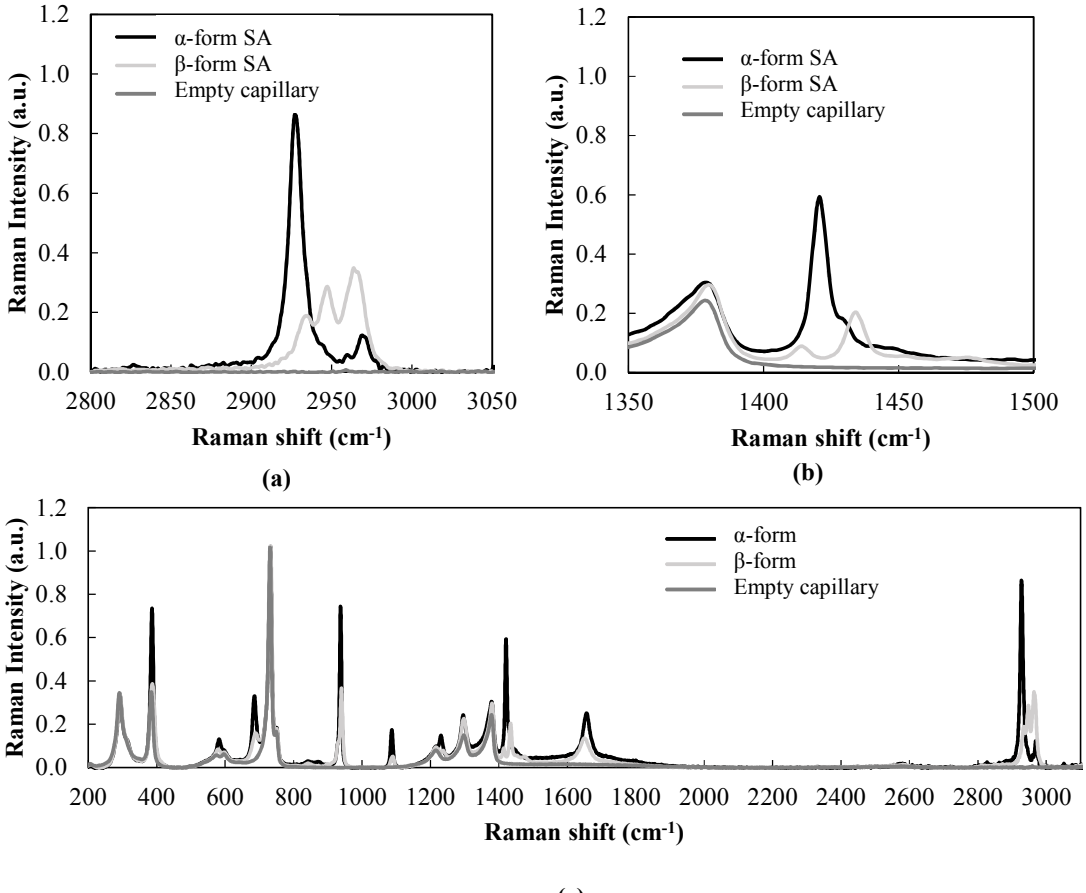

Figure 4: Raman spectra of the two polymorphs of SA inside an MCF strip and an empty capillary of MCF strip. Region (a) 2800- 3050 cm^{-1}; (b) 1350-1500 cm^{-1} and (c) full spectrum.

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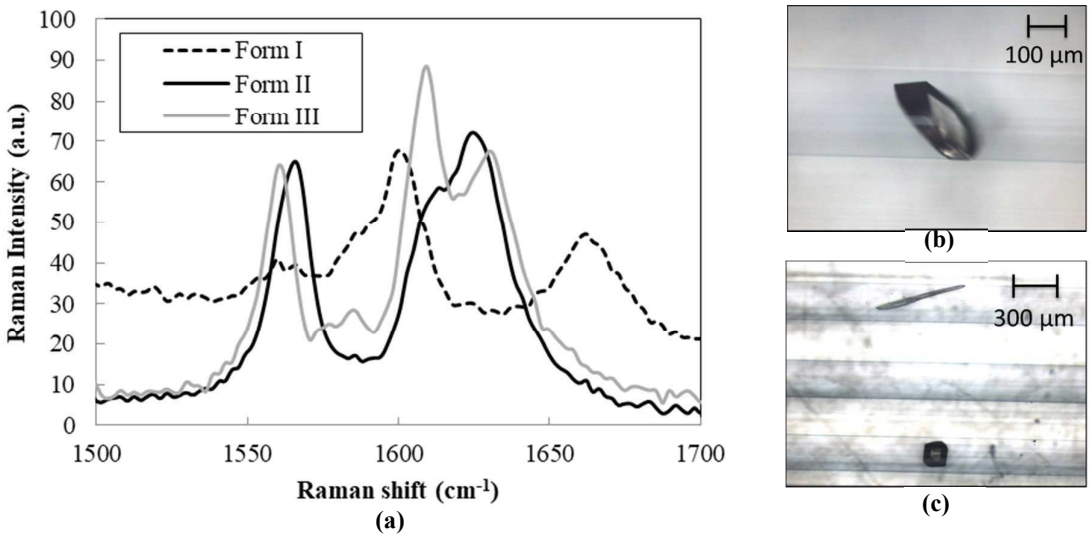

Figure 5: (a) Raman spectra of the different polymorphs of OABA identified in the capillaries (b) Crystal of form III, (c) Crystals of form III on the top capillary and form II on the bottom one.

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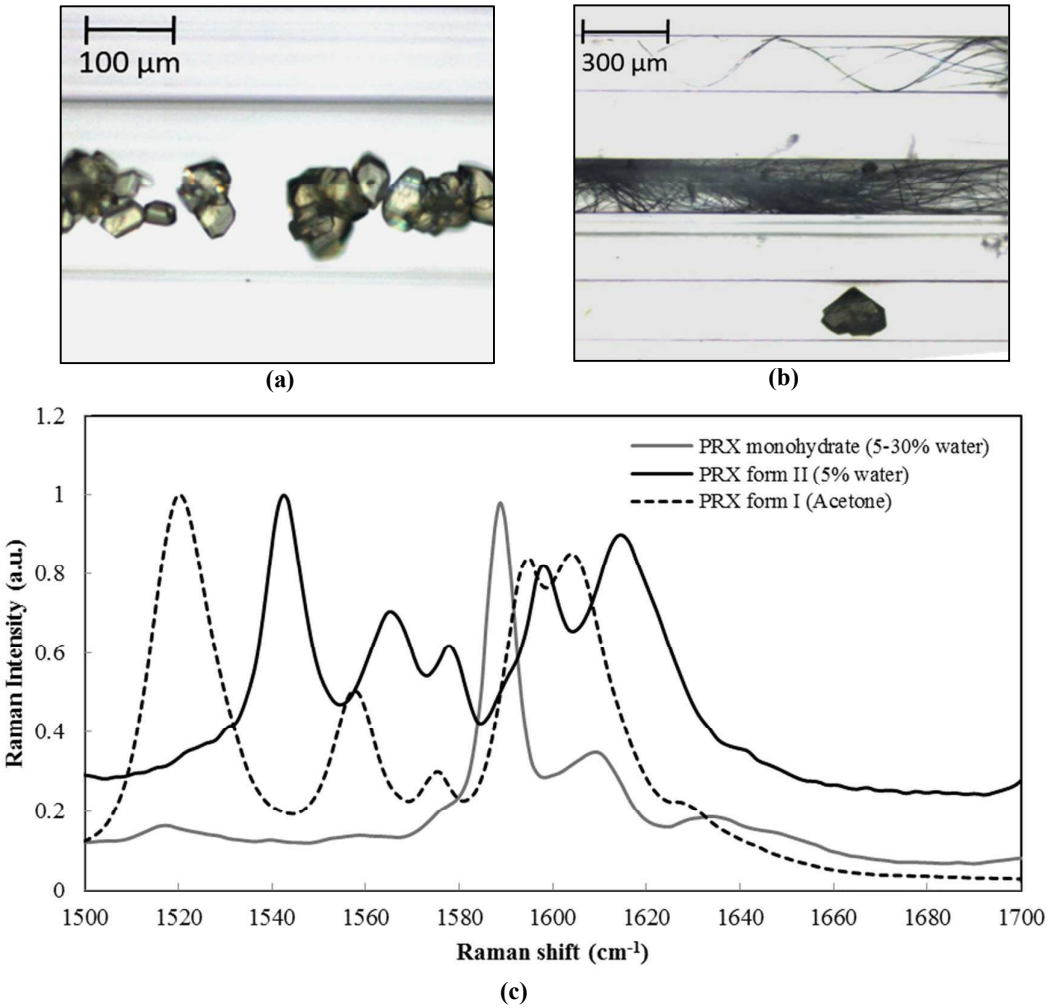

Figure 6: (a) Piroxicam hydrate crystals nucleated from a solution of 30% water in acetone w/w, (b) crystals of Piroxicam anhydrous form II (needles) and monohydrate (prism) nucleated in a solution of 5% water in acetone w/w; (3) Raman spectra of form I, form II and piroxicam monohydrate.

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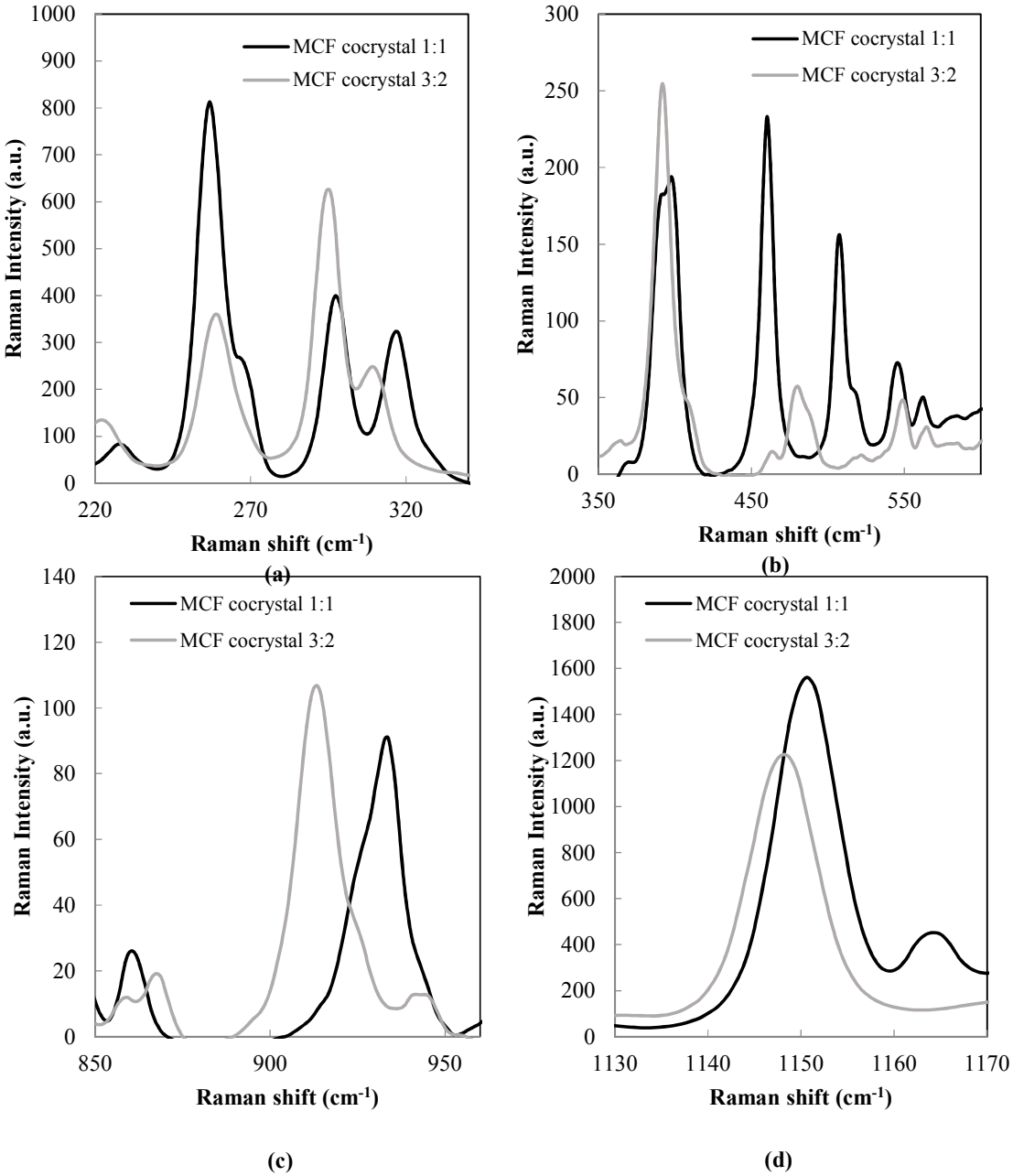

Figure 7: Raman spectra of the different forms of p-TSA/TPPO co-crystals identified in the capillaries. Regions between (a) 220-330 cm^{-1}; (b) 350-560 cm^{-1}; (c) 850-960 cm^{-1} and (d) 1100-1250 cm^{-1}.

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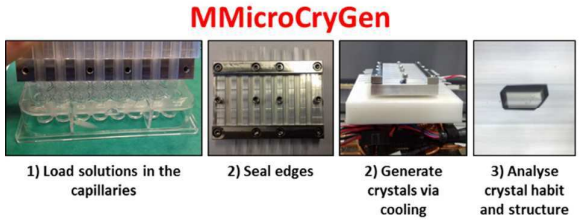
The MMicroCryGen allows single crystal generation and screening using 200 µL of solvent and without complex fluid handling ('dipstick' mode).



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