Rapid nano-gram scale screening method of micro-arrays to evaluate drug-polymer blends using high-throughput printing technology

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ABSTRACT

A miniaturized, high-throughput assay was optimized to screen polymer-drug solid dispersions using a 2-D Ink-jet printer. By simply printing nanoliter amounts of polymer and drug solutions onto an inert surface, drug:polymer micro-dots of tunable composition were produced in an easily-addressable micro-array format. The amount of material printed for each dried spot ranged from 25 ng to 650 ng. These arrays were used to assess the stability of drug:polymer dispersions with respect to recrystallization, using polarized light microscopy. One array with a panel of 6 drugs formulated at different ratios with Poly (vinylpyrrolidone-vinyl acetate) copolymer (PVPVA) was developed to estimate a possible bulk (gram-scale) approximation threshold from the final printed nano amount of formulation. Another array was printed at a fixed final amount of material to establish a literature comparison of one drug formulated with different commercial polymers for validation. This new approach may offer significant efficiency in pharmaceutical formulation screening, with each experiment in the nano-micro-array format requiring from 3 up to 6 orders of magnitude lower amounts of sample than conventional screening methods.

INTRODUCTION

Due in part to the use of high-throughput and combinatorial screening approaches in drug discovery development pipelines, over 40% of APIs (Active Pharmaceutical Ingredients) result in poorly water soluble highly permeable candidates, i.e. assigned to CLASS II according to the Biopharmaceutics Classification System (BCS)[1]. High lipophilicity linked with markedly low water solubility ($\leq 1\mu g/ml$) limits formulation approaches, clinical applications and marketability because of low dissolution and bioavailability of new APIs. [1,2] A variety of formulation methodologies have been investigated to overcome the problem of poor aqueous solubility, [1] amongst them, amorphous solid dispersions have been considered one of the most promising strategies.[3,4] Taking the broad concept of a solid dispersion, the drug is molecularly dispersed in an inert carrier, commonly a hydrophilic polymer to give an amorphous presentation of the drug to maximize aqueous apparent solubility and

dissolution rate.[5] In particular, amorphous drugs show a higher solubility and better dissolution profile compared to their crystal forms.[6] Despite the advantages of solid dispersions, the number of available marketed products remain low due to a number of issues including scale-up and physicochemical instability of formulations that lead to phase segregation and possible further recrystallization of the API, particularly during the storage period. In a successful solid dispersion the polymer not only stabilizes the amorphous drug state against recrystallization but also improves the rate of dissolution of the API.[7] Developing such dispersions can also be difficult[1,8] both due to the timescales involved for assessment of stability and the preparation of solid dispersions using realistic large scale methods. These approaches such as melt extrusion and spray-freeze drying, can require a substantial amount of carrier-API combinations that can be time consuming, costly and end up with batches with different physicochemical properties.[9,10] In addition, the stability of the amorphous state of drugs in a solid dispersion can be affected by the ease by which the APIs themselves undergo recrystallization. In relation to this, Taylor's group reported a further API categorization.[11,12] Drug-like organic molecules can be classified according to their crystallization tendencies. Classification can be determined either by solvent evaporation[12] following deposition e.g. by spin-coating or by monitoring the crystallization behaviour of the amorphous forms from the undercooled melt by DSC.[11,13] These two methods allow the classification of molecules into three groups: the easily crystallizing non-Glass formers GFAI and the Glass-formers (GFAII and GFAIII). GFAII shows substantial crystallization only upon 7 days of storage while GFAIII shows either little crystallization or no crystallization at all. While some optimization may be related just to formulation, the polymer itself is also a key parameter because of the need for physicochemical miscibility between drug and polymer[8] which needs to be determined and optimized. Assessment methods commonly present in the pharmaceutical-related literature are usually based on trial and error evaluations employing a single model drug against a small range of polymers.[8] Key analytical techniques,[1] such as TGA, DSC and XRPD are routinely adopted to evaluate sample stability, drug recrystallization and thus, the amorphous state of the drug. These assessments may require samples to be prepared on a

milligram to gram scale. The analyses coupled with selection and validation of methods to produce the dispersion are quite inefficient if not properly combined[14] and there are no reliable methods for predicting the miscibility of a large library of drug:polymer solid dispersions. In view of the potential of polymer dispersions as a suitable formulation, and the increasing prevalence of large poorly soluble drug molecules, it would be useful to have a better screening process.[8,9,10] In this regard, Taylor's group[8] reported an elegant small scale screening method, based on spin coating of 200 µl of polymer-drug solutions onto coverslips (at concentration of 0.2-0.3 M[12], thus, approximately 10-20 mg of a drug with 250-500 g/mol molecular weight for each sample). The ability of 7 chemically diverse polymers to inhibit the crystallization of 8 drugs prone to quick crystallization was tested. They concluded that miniaturized screening can be adopted as a powerful technique to evaluate the role of drug-polymer chemistry in the stabilization of amorphous solid dispersions. Nevertheless, there are some limitations of this method, such as the storage space for all the used coverslips, difficulty in automating the sample preparation and the control of the exact amount of materials deposited on the coverslips. A similar approach was adopted recently by Scoutaris et al (CrystEngComm, 2016,18, 5079-5082) for high throughput screening of pharmaceutical cocrystals of Indomethacin and Saccharin. Different parameters were investigated, such as solvent drug–coformer stoichiometric ratios, solvent grade and solid content amounts while the cocrystals obtained were subsequently characterised by DSC, XRPD and FT-IR.

An inspiring and pioneering work from Bradley's group[15] linked for the first time a high throughput 2D printing technique with the crystallization screening of commercial drugs. In their work, carbamazepine, sulfamethoxazole and 2-[(2-nitrophenyl) amino]-3-thiophenecarbonitrile were printed onto a microarray of hundreds of commercial polymers in order to generate or trigger different polymorphic forms. Raman spectroscopy was successfully used to characterize different polymorphic forms, although the crystal habits of the API solids were demonstrated to be a poor indicator of polymorphic form. Nonetheless, it was reported that 27 µg of each polymer-support and 1 mg (around ca. 15 µg/dry spot) of the selected drugs were enough to achieve rapid and effective solid form screening. Moreover, inkjet technology has recently aroused interest as an

alternative drug formulation method[16] and for preparing medicines by printing the active pharmaceutical ingredients (APIs). Inkjet printing encompasses a range of versatile, inexpensive fully automated methods to place small liquid drops with high precision onto a surface.[17] A plethora of different materials have been successfully printed by inkjet including cells,[18] colloids,[19] curable-antifouling monomers,[20] genes[21,22] or proteins,[23] polymers[24] and screening of polymer features such as polymer functionalities or compositions for determining materials suitability in microarray manufacturing,[25] nanomaterials and pharmaceutical formulations.[26,27] Inkjet printing technology has been exploited particularly in the broad areas of new biomaterials[28] and drug discovery, namely combinatorial chemistry and high-throughput screening,[17,29] but not yet, in the screening of amorphous solid dispersions. As depicted, the main limitations in the screening of drug: polymer formulations reside not only in the scaling-down the amount of materials but also minimization of storage space. Therefore, we propose here a new screening process capable of combining all the advantages of the previous screening methods, namely, the miniaturization,[8] the addressability and high-throughput.[15] Six GFAI drugs were adopted in the present work, due to their ability to easily crystallize during preparation and storage of formulations. The GFA1 class of drugs can be considered the most challenging to formulate as solid dispersions, hence are ideal model molecules to discriminate in the ability of polymers to inhibit crystallization. We have chosen PVPVA since it is available commercially, is low cost, widely used in literature for this aim, marketed in solid dispersions (KALETRA©) and present as a common polymer in the literature of interest. Briefly, three different nano/micro arrays were printed onto gold sputtered glass slides. The first array was developed in order to evaluate drug recrystallization tendency at different printed amounts by depositing DMSO solutions of pure drugs and PVPVA as a polymer reference in volumes ranging from 2.5 to 65nL. The second array was produced by printing blends at different weight/weight ratios of the six drugs and PVPVA. Once the threshold printed amount required to characterize bulk behaviour was assessed, the third array was produced to establish a literature comparison and

complete the validation. We hope that this will also lead to the development of useful methodology to analyze solid dispersions for their bulk behaviour.

Materials and Methods

Caffeine (CAF) (CAS 58-08-2), Flutamide (FLU) (CAS 13311-84-7) and Flufenamic acid (FLA) (CAS 530-78-9) were purchased from SIGMA-ALDRICH (UK). Mefenamic acid (MEF) (CAS 61-68-7) and Finasteride (FIN) (CAS 98319-26-7) were obtained from Alfa Aesar (US, Ward Hill, MA). Carbamazepine (CBZ) (CAS 298-46-4) was purchased from MP Biomaterials, LLC (France). All drugs were used as received. Acetone HPLC grade, used in the bulk experiments, and Dimethylformamide HPLC grade were obtained from Fisher (UK). Polyvinylpyrrolidone-vinyl acetate copolymer or Copovidone64 as Kollidon®64 (PVPVA) was used as received from BASF. Polyvinylpyrrolidone (PVP), (Hydroxypropyl)methyl cellulose (HPMC), Poly-(acrylic) acid (PAA) and Dimethyl sulfoxide (DMSO) were purchased from SIGMA-ALDRICH and the latter used as a common solvent to dissolve all the printable materials and their blends. Gold sputtered slides were prepared employing a Leica EM SCD005 coating unit. The sputtering chamber was filled with Argon, subsequently, a layer of 10-20 nm of gold was deposited onto the glass slide under vacuum at 25 Volt resulting in an average contact angle of 43.5 ± 0.5. This procedure provides a surface with a higher contact angle compared to bare glass limiting DMSO droplet splashing and is cheaper than commercial gold-coated glass slides. Polymer and drug solutions in DMSO were separately deposited on gold sputter-coated microscope slides, with a piezo electric inkjet printer (Sciflexarray S5, Scienion) using a 90 μm orifice nozzle. The droplet size was controlled by the values of the voltage and electrical pulse. In a routine experiment DMSO solution droplets with nominal volumes ranging from 250-280 pl, were dispensed at a 3 kHz jetting frequency by adjusting the voltage and pulse between 98-105 Volt and 45-55 μs respectively. The nozzle was washed with DMF in between each printing cycle, as part of the automated printing-washing loop. Printed DMSO solutions were allowed to evaporate overnight in the

printer cage at around 25 °C and 55 % of RH and subsequently stored in a desiccator avoiding moisture contamination. DMSO normally evaporates in a time frame of 100-120 minutes for the biggest printed droplets (around 65-70 pl). DMSO was chosen both due to its high evaporation point that avoids clogging of the printer nozzle and also because it acts as a common solvent for all the drugs and polymers used in this study. The resulting dried spots, comprising variable amounts of polymer and drug from 25 up to 700 ng, were investigated using an Advanced Polarizing Microscope (HS1 microscope), Prior LuxPOLTM with 12V and 30W halogen lamp with variable brightness control to analyze the crystallinity of the drugs in these amounts. All the printed solid spots and the formulated powders were analyzed by POM[30] which has been adopted as a routine benchmark analytical technique in the pharmaceutical field, to detect drug-recrystallization quickly.

Microarray development

A schematic representation of drug/polymer solution preparation and printing is shown in Figure 1. The initial drug and PVPVA stock solutions were prepared by simply dissolving the drugs and PVPVA separately in DMSO, in order to reach a final concentration of 10 mg/ml. High solubility of all the tested materials was found at this concentration. All the solutions were subsequently pipetted into a 384-well plate, used as the cartridge of the printer system. Three different microarrays were developed in order to decipher the best conditions to either predict drug:polymer bulk behaviour or align printed outcomes with pre-existing literature results. A first reference micro-array (1-PURE) was created to investigate how the pure formulation components behaved when printed onto the gold-coated slides. Secondly a drug:polymer micro-array was prepared from drug:PVPVA solutions as outlined above (2-DRUG:PVPVA array) For each of the six selected drugs (MEF, CBZ, FIN, FLU, CAF and FLA). A range of spot sizes (total formulation mass 25-28, 125-140, 625-700 ng) were used to allow direct comparison with the micro-array 1-

PURE described earlier. Each drug was formulated at drug:polymer ratios of 5, 10, 15, 25, 50 % w/w drug:polymer. The "2-DRUG:PVPVA" array was printed by dispensing different volumes, namely, 10-50-250 droplets, in order to determine the optimum amount to predict the bulk behaviour. The microarray procedure adopted was as follows: as a first step, the desired amount of PVPVA solution was printed onto the gold-coated slides. Subsequently, the different drug solutions were printed onto the previously deposited polymer-DMSO droplets, to give 5/95, 10/90, 15/85, 25/75 and 50/50 drug/polymer w/w% ratios. To reach this protocol two different methodologies were tried. In the first protocol, 10, 50 and 250 droplets of DMSO solutions were printed. The slides were left overnight inside the printer cage, at around 55% relative humidity (RH) and 25 °C in order to allow total DMSO evaporation. After DMSO evaporation, drug crystallization was monitored by using POM after 1 day and 7 days. The arrays were stored in a desiccator employing silica as drying agent, in order to prevent humidity affecting drug recrystallization. To dispense 6 pure drugs, PVPVA and the various blends, in triplicate in one single slide, the entire process required around 45 minutes and less than 1.5 μg per drug. Secondly, the effect of premixing solutions upon drug crystallization was evaluated. Drug-polymer solutions were pipetted into a 384-well plate to give solutions at 5/95, 10/90, 15/85, 25/75 and 50/50 drug/polymer w/w% ratios. This latter approach resulted in a longer process (at least 2 hours), using a more sustained volume of drug-polymer solutions, without showing any difference from the first array building approach. This was expected as the polymer solution does not evaporate and no polymer film is observed in the time required for printing. Additionally, the amount of DMSO added with the drug is enough to completely re-wet the spot area. The first and faster approach was therefore adopted for the present work.

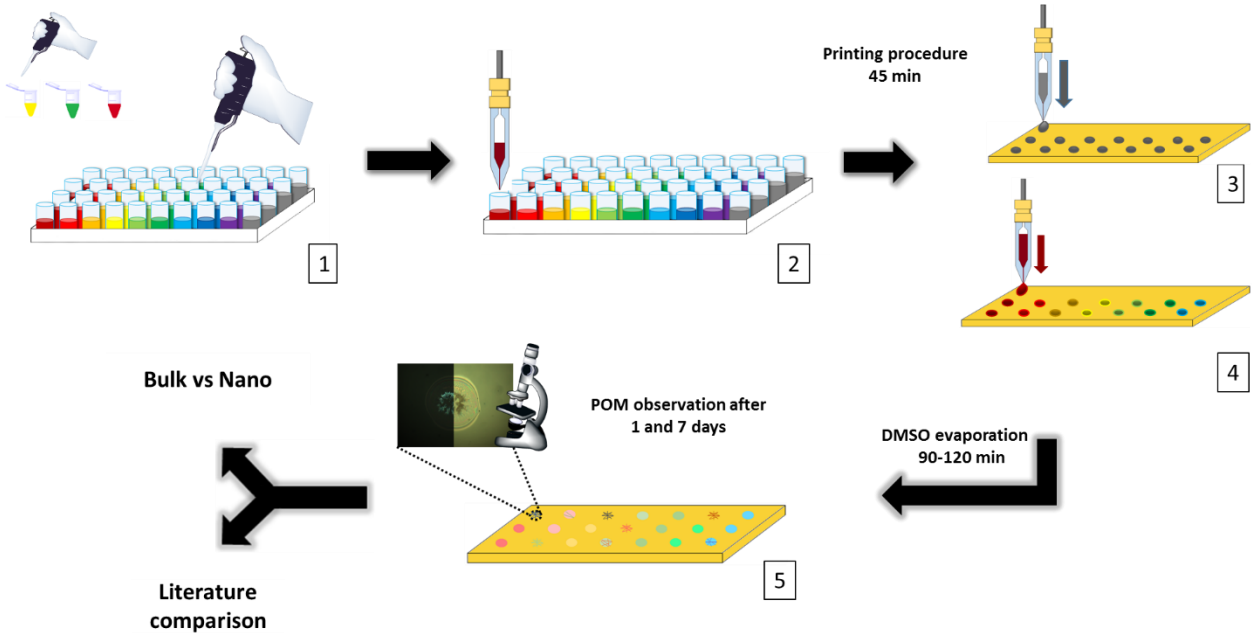

Figure1. Schematic representation of the sequential steps needed to develop a solid dispersion micro-array. (1) Drug and polymer DMSO solutions are separately prepared and pipetted into a well-plate. (2-3) PVPVA solution is firstly withdrawn by the printer nozzle and dispensed at the required amount at the desired slide surface location. (2-4) Subsequently the drug solutions are printed using the desired amount onto the polymer solution droplets. After evaporation of DMSO, (5) each solid spot was analyzed by POM.

Evaluation of drug recrystallization of printed spots

Crystallization was simply evaluated by POM, mainly employing a 4X or 10X objective. A simplified semi-quantitative categorization of the spots, produced in the present work, was adopted from the well-established method described by Eerdenbrugh and Taylor[31] for spin-coated drug-polymer films. As shown in Figure 2, different classes of relative degree of drug crystallization can be observed, despite the complexity and the wide variety of forms and shapes, (a) completely amorphous (‘‘AAAA’’), (b) some minor crystallisation observed (“AAAC”), (c) slightly crystalline (‘‘AACC’’), (d) mainly crystalline (‘‘ACCC’’), or (e) completely crystalline (‘‘CCCC’’). Due to the spot conformation, and the miniaturized size of each sample it was possible to observe the whole spot/blend with one snapshot. Spots were imaged with and without crossed polarized filters 1 and 7 days after printing. In this time-frame few variations in crystallization were found by POM throughout the set of arrays (an example is given in Figure 1SI). Exploiting this latter classification, and aligning to the pre-existing literature[8,31] a further semi-quantitative numerical value was calculated and defined as the ‘amorphicity index percentage’ (AI%) of the drug-polymer spots. AI % is simply defined as the relative number of A’s counted in the categorizations of the various drug/polymer ratios and different time points, expressed as a percentage.[8,31] AI% can be applied for each drug:polymer spot or as an average amongst all the formulations of the same drug and polymer. A high value of AI% indicates a good relative ability of a particular polymer to keep a particular drug in an amorphous state, as AI% decreases the ability of the polymer to maintain the drug in an amorphous form drops as well. Due to the stability of the printed formulations in the present work the amorphicity index has only been assessed after 7 days (AI%-7D). This latter was analyzed for each spot.

Bulk drug:PVPVA dispersion preparation and bulk validation

To probe whether the crystallization/stable amorphous behaviour of the "2-DRUG:PVPVA" array can be further considered as predictive of bulk conditions, two drug/PVPVA ratios for each drug were selected for a scaled-up formulation. Solutions of pure drugs, pure PVPVA and mixtures at drug/PVPVA ratios of 10 and 50% w/w were prepared by dissolving a final total amount of 2 g of materials in 10-15 ml of acetone. Acetone was selected because, as for DMSO, it is an aprotic polar solvent but with a lower boiling point. Consequently, acetone evaporation time scale in bulk experiments was similar to DMSO evaporation from printed spots. Upon full powder dissolution, different volume aliquots were dispensed in glass vials. Solvent was removed by centrifugal-rotary-evaporation (at 25 °C) and the vials stored in desiccators under silica. Each sample was prepared in triplicate and the extent of recrystallization of the different scaled-up drugs was analyzed by POM.

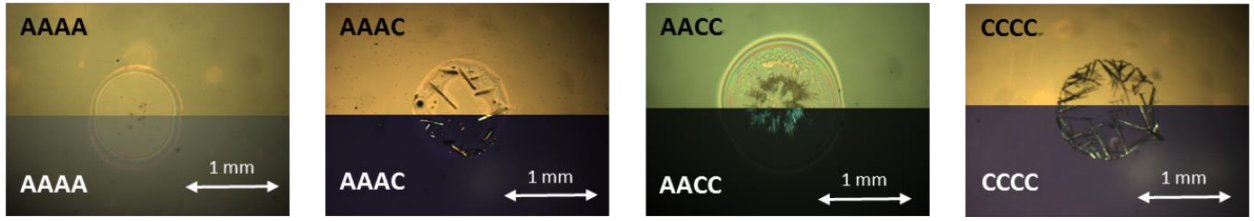

Figure2. Examples of different API recrystallization profiles are presented. All the pictures are reported both without (top part) and with (bottom part) a polarized filter. (i) AAAA stands for entirely amorphous, (ii) AAAC, mainly amorphous, (iii) AACC, mainly crystalline and (iv) CCCC, entirely crystalline.

Literature Comparison

In order to align the present printing methodology to the pre-existing literature a third microarray was produced ("3-FLA:LITERATURE" array) to match some formulations and weight ratios reported in work by Taylor's group.[8,12] In the 3-FLA:LITERATURE array : only spots formed by ca. 650 ng were printed for the literature comparison. Hence 3-FLA:LITERATURE array was shaped. Solutions of FLA and different polymers, namely, HPMC, PAA, PVP and PMMA as well as PVPVA, were printed both to further test the reported high throughput nano-miniaturized method and to compare with the already well established literature.[8] Pure polymer and FLA solutions in DMSO were prepared and pipetted into a 384-well plate. Polymer solutions were printed onto a gold coated glass slide and the FLA solution was printed upon the polymer-solution-droplets to give final drug/polymer ratios of 10/90, 25/75, 40/60, 50/50, 60/40, 75/25 and 90/10 (w/w). After evaporation, all the spots were observed by POM. The calculated AI% was compared to the values previously published by Van Eerdenbrugh and Taylor.[8]

Results and discussion

Pure Drug and PVPVA microarray printing

The first microarray contained only pure drug solutions printed at different volumes/number of droplets in order to evaluate the miniaturization effect upon drug recrystallization. In particular, different amounts of 6 different pure drugs were initially deposited onto the gold sputtered slides alongside PVPVA (Figure 2SI). Starting from solutions at 10 mg/ml spots with an average of 25-28 ng for 10 droplets (2.5-2.8 nl), 125-140 ng for 50 droplets (12.5-14 nl) and 625-700 ng for 250 droplets (62,5-70,0 nl) were obtained in all the printing experiment controls. As depicted in Figure 2SI, the whole set of drugs showed recrystallization with different and unique patterns at each of the printed volumes, as expected from

their previously established GFAI classification.[8] On the other hand, as expected, PVPVA did not show any birefringence at any printed amount, as it is widely reported in the literature to exist in the amorphous state, as is common for many polymers.[8,32,33]

Effect of spot size, drug:PVPVA ratio and time on drug recrystallization within PVPVA matrix

An example of the different ng amount for drug:PVPVA in each solid spot is reported in Figure 3 a sub-array of the 2-DRUG:PVPVA array. From inspection of Figure 3 it is clear that the presence of crystals in the spots appears to be a function of both increasing drug loading and increasing spot size, with crystals much more prevalent in the spots to the lower right portion of the figure than at the upper left. Despite the use of a similar final amount of drug and polymer mixture, there are differences in spot sizes. This was observed throughout all arrays and seems to be affected by the amount of crystallization, with greater crystallinity shrinking the spot size and amorphicity resulting in greater spread. This may be due to a variation in properties such as variation of contact angle and the nature of different crystals.

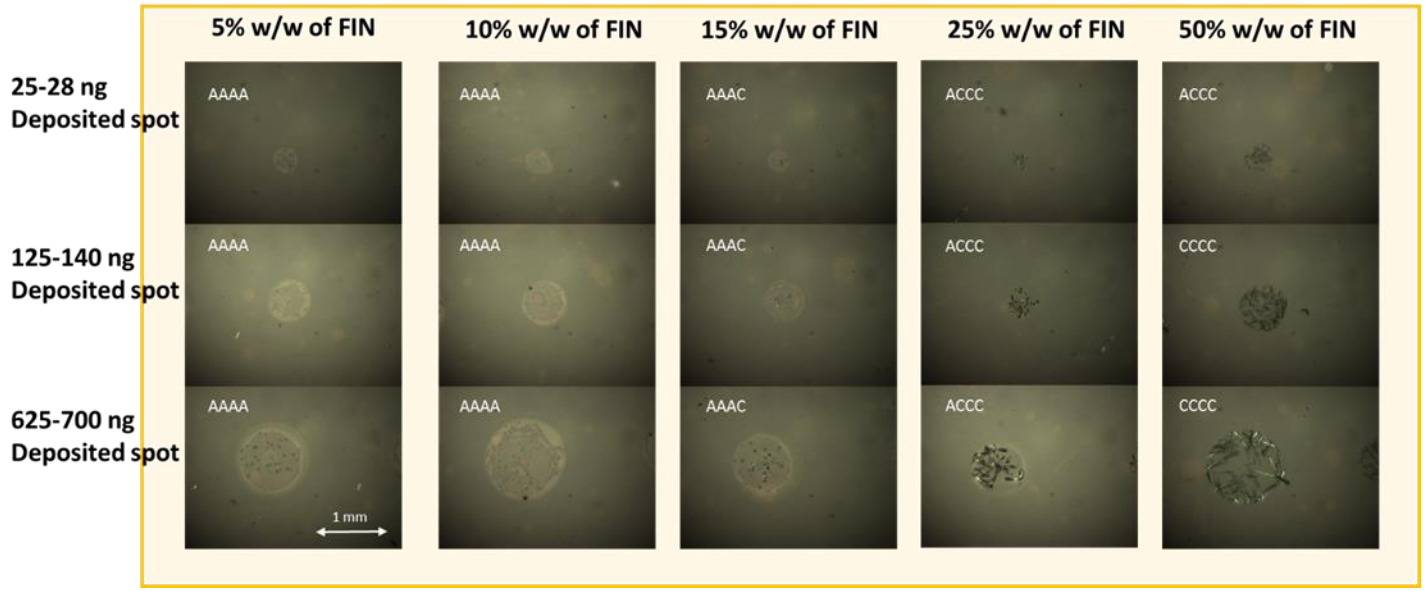

Figure 3. Example of an array section, all the POM pictures are reported with the cross polarized filters in place. Pictures of all Finasteride-PVPVA spots at all the different ratios are depicted after 7 days from the solvent evaporation. As shown in each row there is the same amount of printed materials (25-28ng top row, 125-140ng center and 625-700ng bottom row) and in each column there are spots containing the same drug/polymer ratio.

MEF, CBZ, FIN and FLA acid showed different recrystallization behaviour upon printing as a function of deposited volume and drug/polymer ratios adopted (Figure 4SI). CAF appeared to recrystallize at all the drug/polymer ratios and independently from the volume of solution deposited. In contrast, FLU showed full amorphicity at all the ratios and also at the highest ng amounts explored (Table 1SI). Full data are available in Fig 4SI. Two examples of mixed recrystallization tendency are reported in Figure 4. For clarity and reasons of space, a vertical section at particular

weight/weight % ratio of MEF:PVPVA and FIN:PVPVA formulations are reported in Figure 4. MEF did not show any evidence of recrystallization at any drug/polymer ratio up to 50 printed droplets, namely, 125-140 ng of final drug/polymer spot on the surface. Crystals were first observed only at 50% w/w and after printing an amount of formulation in the range 625-700 ng (Figure 4SI). On the other hand, FIN showed recrystallization, in a time frame of 1 day after printing, at 25-28 ng at a ratio of 15% w/w (Figure 4). For the two latter MEF and FIN formulations, taken as examples, higher final volumes between 125 and 140 nl (final spot containing around 1.5 µg of drug and polymer) were dispensed in order to check whether spots of around 650 ng are the minimum amount needed for bulk behaviour. As shown in Figure 4 the same trend evaluated for 62.5-70 nl was observed with 125-140 nl (example in Figure 4).

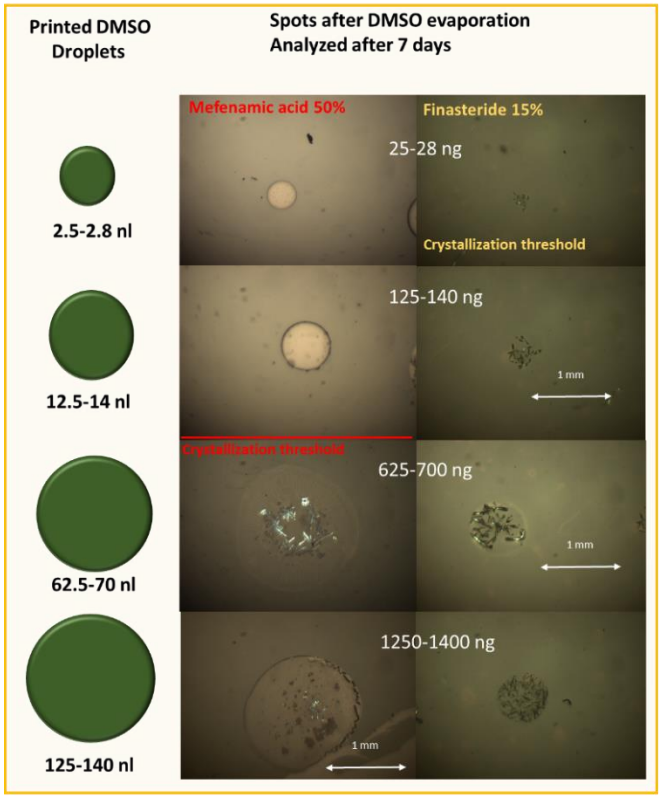

Figure 4. Section of MEF:PVPVA and FIN:PVPVA subarrays showing examples of different recrystallization behaviour. Left column: Mefenamic Acid showed re-crystallization from PVPVA mixture at 50% w/w of concentration and final deposited volume threshold of 62.5-70 nl. Right column: Finasteride phase segregated from PVPVA at a concentration of 15 % w/w showing the crystallization threshold at 2.5-2.8 nl. Final spot diameter may result from several variables such as amount of polymer, rate and extent of drug crystallization and polymer-surface interaction.

This trend for larger spots with higher drug loading to crystallize earlier than the smaller spots with lower drug loading likely arises simply from the probability of nucleation being, to a first approximation, a function of the total number of drug molecules present in a spot. Physical separation of drug molecules by polymer will also reduce the nucleation probability in the samples with lower drug loading. Spots were depicted with and without polarized filters, 1 and 7 days after printing. However, in this time-frame few variations were found by POM throughout the set of arrays (MEF is reported as main example in Figure 1SI). Since the aim of the present work is mainly to develop a high throughput screening method for the prediction of the stability of solid dispersions regardless of the nature of the polymer drug pairs, we have not attempted to provide possible in depth explanations for particular experimental observations. In addition, by simply plotting the AI%-7D for each pair of drug:PVPVA it is easy to immediately establish differences of recrystallization amongst the selection of drugs within the PVPVA matrix (Figure 3SI). Taking into account the above drug/polymer experimental evidence, spots formed by 625-700 ng, were considered as the smallest required for evaluation of different drug recrystallization behavior within the polymer matrix. As an interesting first observation of the arrays, it is evident that each drug/polymer combination showed a threshold of amorphous stability at different w/w % ratios with little variation over time (MEF is reported as an example Figure 1SI). Moreover, for some stable spots amorphicity was also observed up to 25-50% drug loading (MEF, CBZ, FLU and FLA) (Figure 3SI). This latter evidence supports previous reported experimental observations where a printer was adopted to formulate PVP-Felodipine solid dispersions, which has shown an intimate mixing and homogeneity up to almost 70% of drug loading.[34]

Bulk prediction of drug:PVPVA formulations

In order to assess ink-jet printing as a predictive technique to identify useful drug-polymer formulations suitable as solid dispersions, two drug:polymer ratios 10/90 and 50/50, were selected to be further analyzed for all the drug-like molecules, at a gram-scale. POM was adopted to evaluate the presence or absence of crystals inside the different solid dispersions without approximating any semi-quantitative ranking as reported for the printed samples. The bulk scale-up was carried out simply to assess the screening outcomes and compare recrystallization at both nano- and gram-scale. Dissolution of drug and polymer in acetone and removal by rotary evaporator to constant weight took around 60 to 90 mins. The temperature and the evaporation time frame was similar to that for evaporation of DMSO from the slide for 62.5-70 nl, that was of 100 to 120 minutes. The resulting powders were stored in a desiccator for 7 days and then analyzed and compared to the selected spots. All the formulated powders were rapidly analyzed by POM. [30] At ratios of 10/90, POM observation of the stored powders revealed that only the CAF formulation showed birefringence. No evidence of birefringence was found for the all the other 10/90 ratios, this matched with the printed samples, as seen in Figure 5A. At a ratio of 50/50 in the bulk samples, the POM observation showed no evident birefringence for FLU, fairly weak for CBZ and FLA whilst remarkable birefringence was obvious for all the other samples (Figure 5B). This mirrored the printed sample observations well, where only the FLU spot was completely amorphous while all the others showed small molecule recrystallization to various extents that was weak or particularly limited for CBZ and FLA, well visible for MEF and extensive in the cases of FIN and CAF (Figure 5).

In the present work it has been found that a printed threshold of some 650 ng of physical blend is needed to align with bulk-gram scale re-crystallization behaviour.

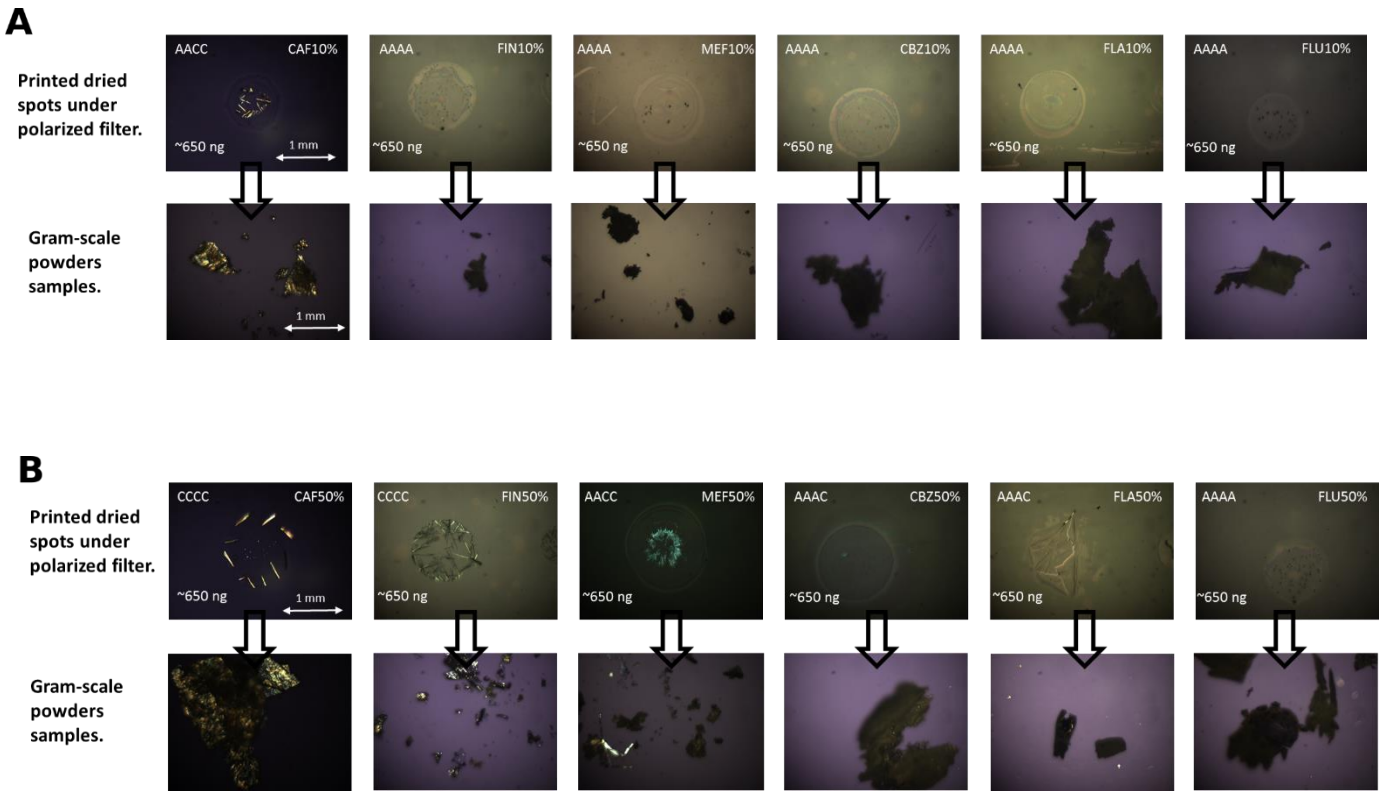
A
Printed dried spots under polarized filter.
Gram-scale powders samples.
AACC CAF10%
AAAA FIN10%
AAAA MEF10%
AAAA CBZ10%
AAAA FLA10%
AAAA FLU10%
~650 ng
1 mm
B
Printed dried spots under polarized filter.
Gram-scale powders samples.
CCCC CAF50%
CCCC FIN50%
AACC MEF50%
AAAC CBZ50%
AAAC FLA50%
AAAA FLU50%
~650 ng
1 mm

Figure 5. 2-DRUG:PVPVA array POM pictures with polarized filters of printed dried spots compared to gram-scale powder samples.

A. Amongst all the 10/90 samples only CAF10% showed birefringence, confirmed by the corresponding bulk sample. All the other formulations printed or bulk showed no birefringence.

B. Both printed and bulk CAF50%, FIN50% and MEF50% formulations showed remarkable birefringence. Weak recrystallization of the printed CBZ50% and FLA50% was confirmed by POM of the corresponding bulk samples, while no birefringence was observed for FLU50% either as printed spot-ng sample or in gram quantity.

Literature comparison of FLA:polymer formulations

A comparison to the pre-existing literature was made. Taking into account the bulk prediction results, dry spots with an average of 650 ng of final blend were adopted. In particular, FLA DMSO solution was printed at different ratios against PAA, HPMC, PVP and PVPVA solutions producing an array named 3-FLA:LITERATURE. The resulting dry spots were compared to previously published outcomes obtained by spin coating.[8] AI% of each FLA formulation is reported in Table 2SI as a mean value for each ratio against the values reported in the literature. An

average AI% for the different FLA/polymer pairings is reported and presented in Figure 6 to simplify the drug polymer compatibility rank and to easily evaluate differences with respect to the published data. The same AI% trend, PVP>PVPVA>HPMC>PAA, was found both in the experimental results collected in the present work and the previously published data[6]. In addition, in table 2SI both the AI% for each FLA formulation at each weight ratio mediated after 1 and 7 days and the AI% average are reported. The main advantage in the present work resides in the amount of material needed for analysis and prediction. In fact, for each formulation of FLA/polymer at all the seven explored ratios, a maximum of 2.4 µg was deposited against an approximate value of 5-15 mg following the methodology in the Taylor group papers.[8,12] There are only minor differences between the two sets of data (Table 2SI), most likely due to the intrinsic differences between the two techniques used, resulting in a greater time to solidify the samples in the presently reported method which may lead to the slightly increased amount of crystallization seen.

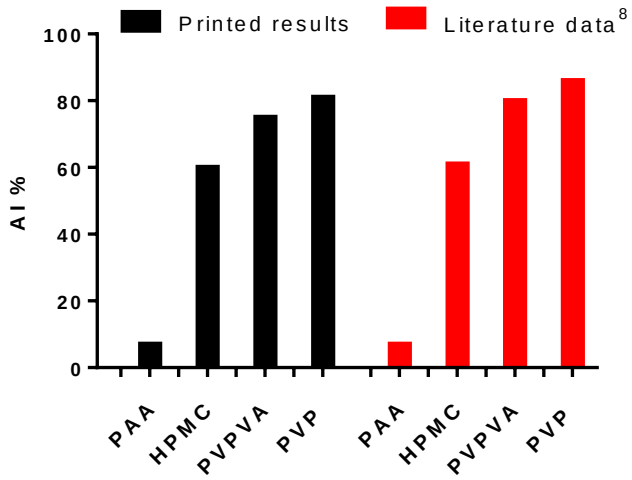

Figure 6. Average AI% value for FLA-PAA, FLA-HPMC, FLA PVPVA and FLA-PVP printed in the present paper (black) and extracted from spin coated previously published data (red) are shown in the figure. The same stability-affinity trend was found comparing the two sets of data, in particular, PVP>PVPVA>HPMC>PAA.

While relatively small numbers of drugs and polymers were used for the present work, 400-500 spots can be readily accommodated on a single microscope slide, and these can be printed at a rate of approximately 1000spots per hour. POM analysis and making a photographic record of a single slide could be accomplished in about 45minutes. Reproducibility is within 5-10 AI%. This new technique might be exploited, not only to save precious or unknown/newly synthesized organic compounds, but also to reduce the assay space to just few slides and facilitate much more rapid sample preparation and analysis. Taken together, the tailored flexibility in terms of materials and solvents adopted,[35] high degree of automation and speed of execution[36] combined with low error[37] and wastage,[17] inkjet printing thoroughly meets all the characteristics and demands for a high-throughput-miniaturized screening approach not only in the drug-solid dispersion field but in all those areas where recrystallization needs to be evaluated.

Conclusions

In the present work, ink-jet technology was exploited for the first time to build nano-arrays to determine drug nucleation within a polymeric matrix (PVPVA). Drug nucleation was investigated with respect to the amount of drug mixture required, the drug/polymer ratio and assessed with

respect to the existing methodology and literature. , It was noted that, after solvent evaporation, drug nucleation can be triggered by either the final amount of drug printed (bulk amount) or to the ratio of drug with respect to the polymer, (due to incompatibility of the drug inside the polymer matrix). It was observed that, for the reported set of drug:PVPVA, spots with a final quantity of 650 ng of materials provided a high correlation of screening compared to the behaviour of gram scale drug-polymer blend. This screening ability was further assessed against literature data produced by spin coating with solvent fast evaporation. In the 3-FLA:LITERATURE array PVPV, PVPVA, HPMC and PAA were mixed at different w/w% ratios with FLA, printed by producing final dry spots of ca. 650 ng of final blend and compared with AI% values extracted from the literature. The same trend of amorphicity stability was found, in particular, PVP>PVPVA>HPMC>PAA. In the printed array 2-DRUG:PVPVA an average of 390 ng (10 and 50% of drug) were used, hence, <0.05% sample than a bulk experiment, where around 1.1 g were used, showing that the screening process was not just high throughput, but also very sparing on use of precious material. The entire array was printed in triplicate in less than 45 min in a fully automated way. Using the final format (an average spot of 65-70 nl) DMSO evaporation was about 100 to 120 min, and it is possible to start POM evaluation immediately after this, making the nanoprocedure very rapid and efficient.

Furthermore, the extended amorphous lifetime obtained by printing amounts ranging between 25 and 140 ng, might lead to future applications. An interesting idea might be to use manufacturing technologies such as 3D printer to separate drug/polymer into small restricted and defined areas and coating of small devices. It might facilitate a better understanding of the drug/polymer interactions leading to more predictable and more stable polymer dispersions. Finally, it might be considered as an easy and powerful method to assess nucleation probability in both drug:polymer blends and for pure drugs, which is possible with a final printed material amount well below 1 μg.

Supporting Information

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

Data access statement

All raw data created during this research are openly available from the corresponding author (martin.garnett@nottingham.ac.uk) and at the University of Nottingham Research Data Management Repository (https://rdmc.nottingham.ac.uk/) and all analyzed data supporting this study are provided as supplementary information accompanying this paper.

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Supplementary information

Table 1. Number of A and C values for each drug/polymer blend at all the w/w% ratios after 7 days.

Drug	Drug/PVPVA w/w%					AI%-7D
	5%	10%	15%	25%	50%	
Flutamide	AAAA	AAAA	AAAA	AAAA	AAAA	100
Carbamazepine	AAAA	AAAA	AAAA	AAAA	AAAC	95
Flufenamic	AAAA	AAAA	AAAA	AAAA	AAAC	95
Mefenamic	AAAA	AAAA	AAAA	AAAA	AACC	90
Finasteride	AAAA	AAAA	AAAC	AACC	CCCC	60
Caffeine	AAAC	AACC	AACC	CCCC	CCCC	35

As depicted from the table, it is possible to evaluate the extent of amorphicity for each drug/polymer spot and easily calculate the AI% -7D average to determine differences of stable amorphous miscibility. The table was produced considering only spots formed for a volume between 60-70 nl.

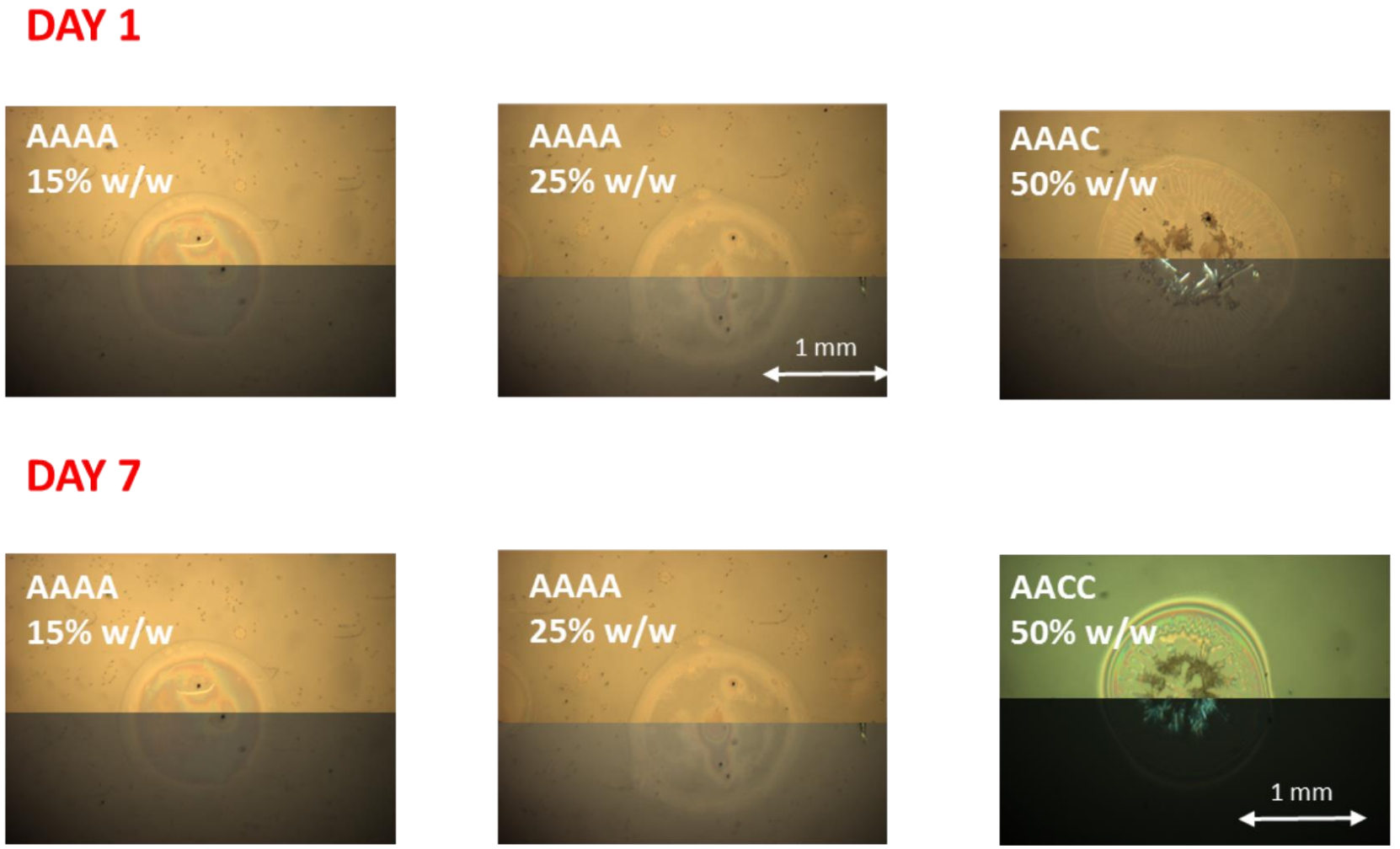


Figure 1SI. PVPVA/MEF blends at 15, 25 and 50% w/w ratios after 1day (top row) and 7 days (bottom row) were selected to show both an example of "A" degree due to polymer/drug ratios and the variation of AI% with time. Printed volume ranged from 62.5 to 70 nl (625-700 ng of final materials present on the slide).

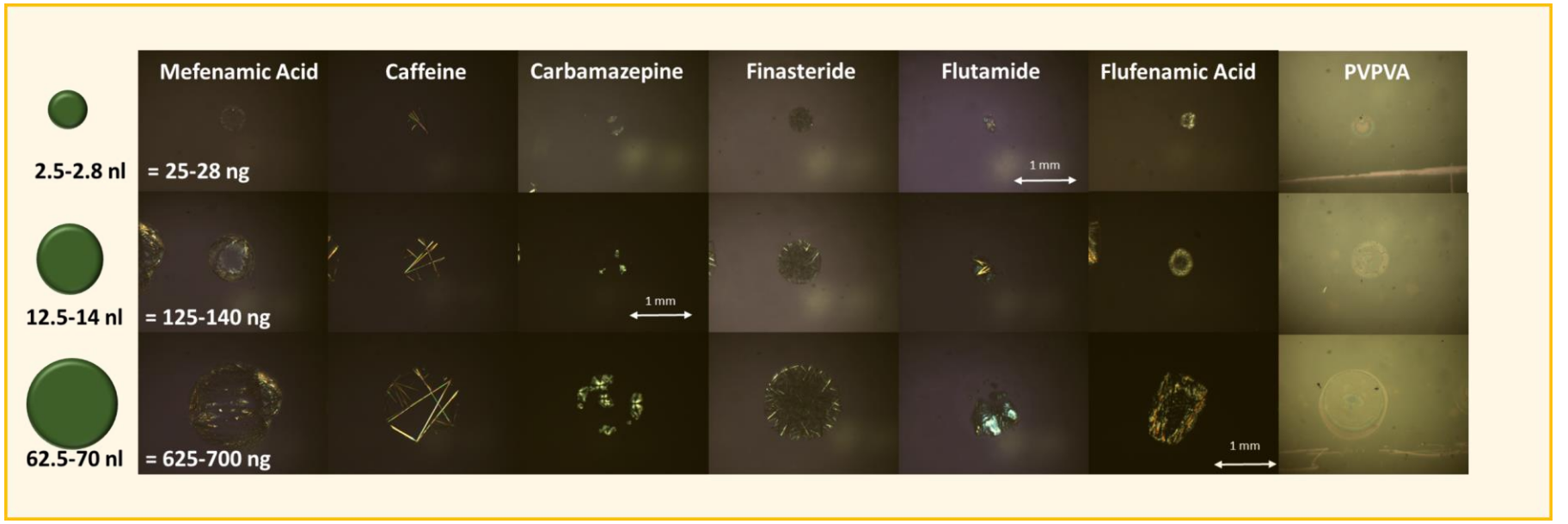


Figure 2SI. Pure drugs and PVPVA microarray depicted by POM with the polarizing filters on. From the top line: spots produced by depositing a volume solution between 2.5-2.8 nl (25-28 ng). Middle line: spots printed using 12.5-14 nl (125-140 ng). Bottom line: spots printed with a volume ranging 62.5-70 nl (625-700 ng). All the drugs but not the amorphous polymer show a characteristic birefringent pattern. In fact, PVPVA is barely visible with the polarizing filters on.

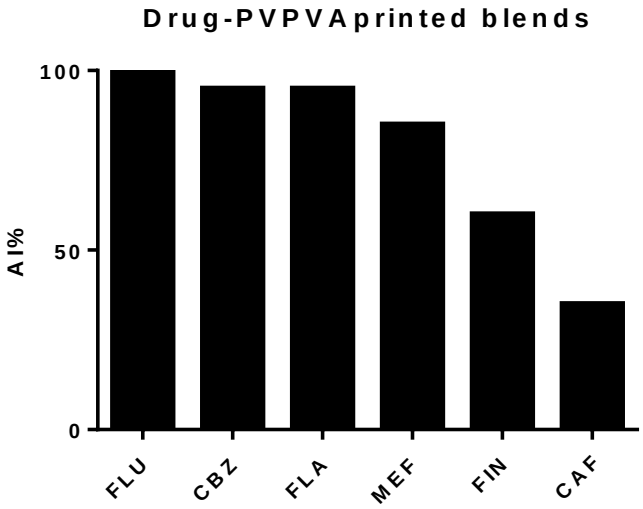
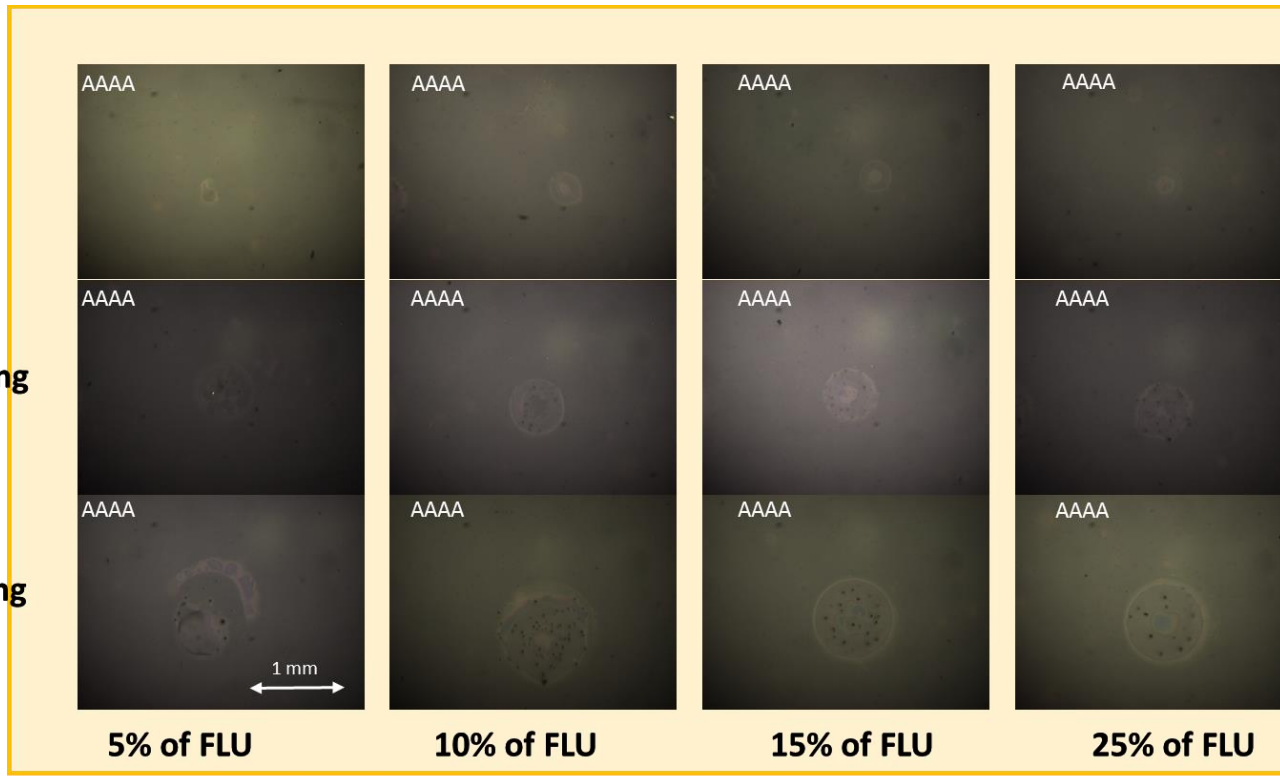
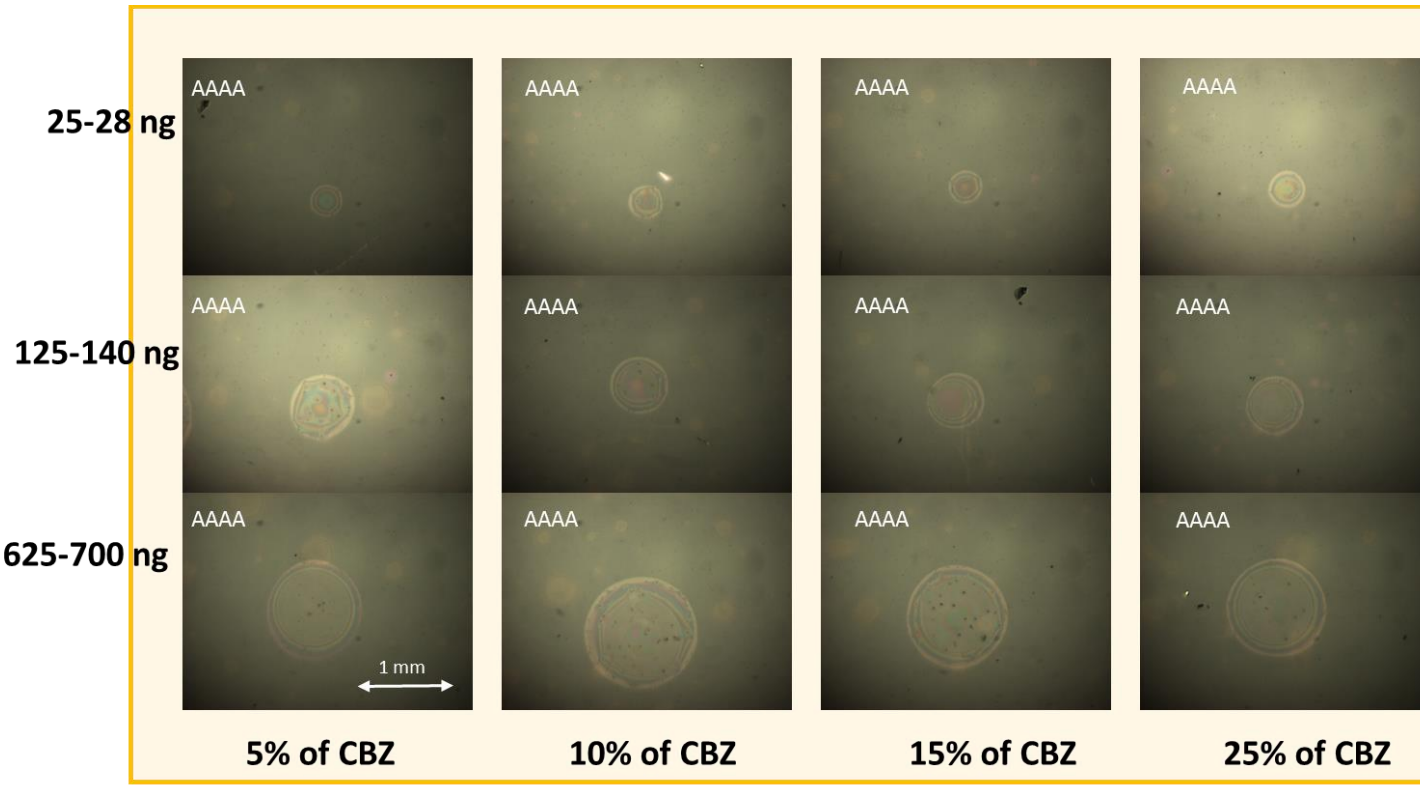


Figure 3SI. All the drugs showed an AI%-7D above 50% apart from CAF that re-crystallized easily at all the ratios with a final AI% of 35%.

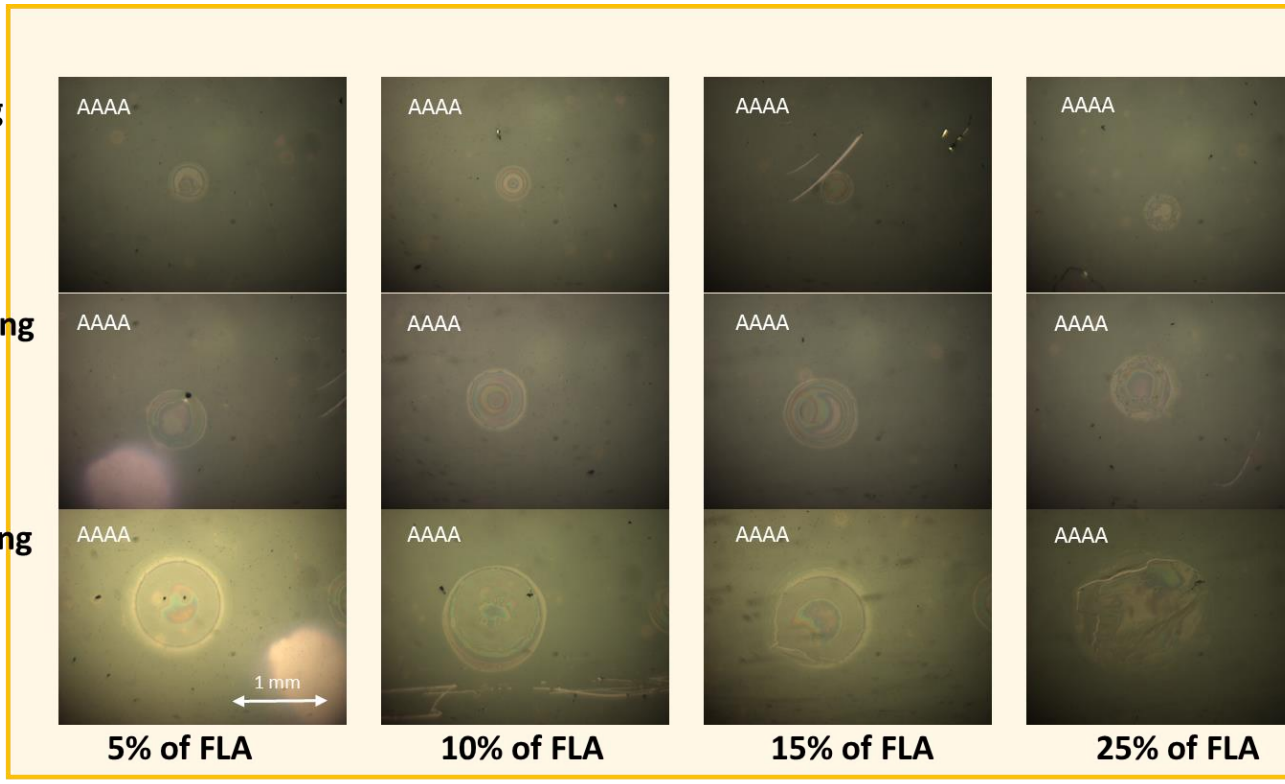
FLU-PVPVA


25-28 ng
125-140 ng
625-700 ng
AAAA
1 mm
5% of FLU
10% of FLU
15% of FLU
25% of FLU

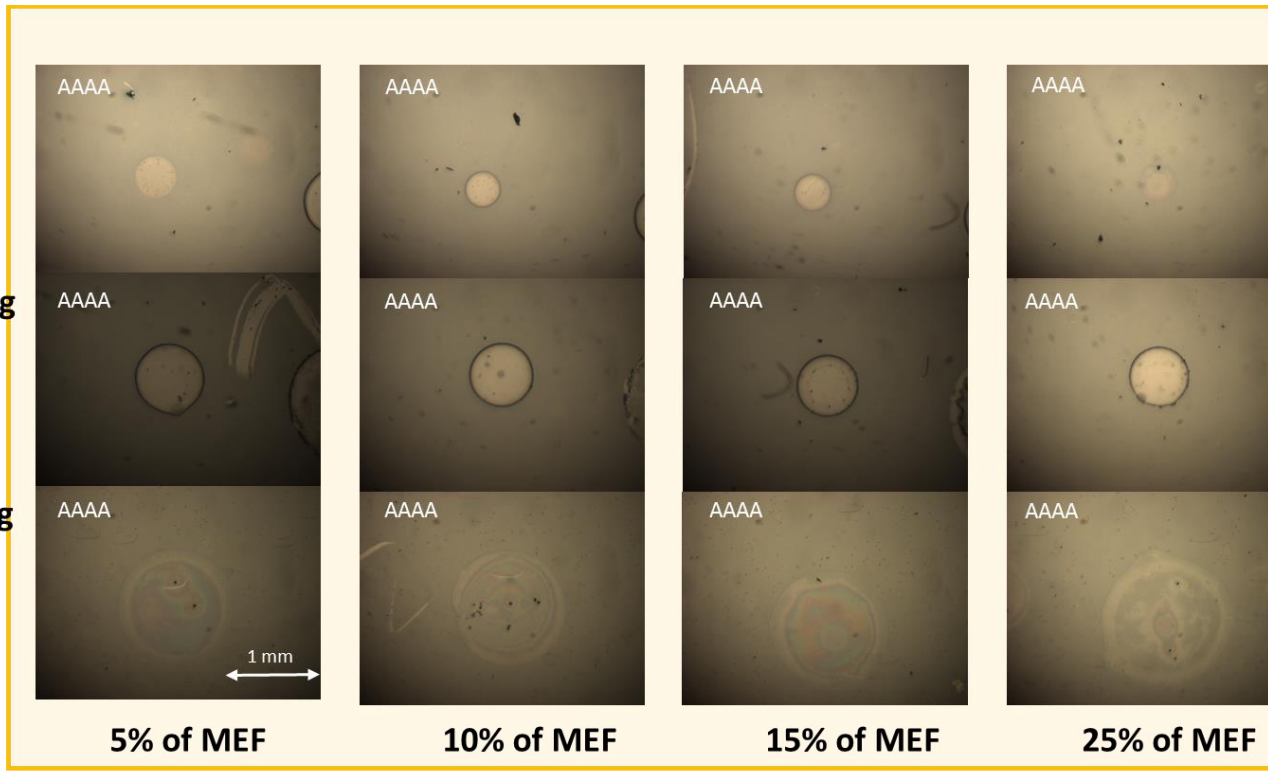
CBZ-PVPVA


25-28 ng
125-140 ng
625-700 ng
AAAA
1 mm
5% of CBZ
10% of CBZ
15% of CBZ
25% of CBZ

FLA-PVPVA



MEF-PVPVA


25-28 ng
125-140 ng
625-700 ng
AAAA
1 mm
5% of MEF
10% of MEF
15% of MEF
25% of MEF

CAF-PVPVA

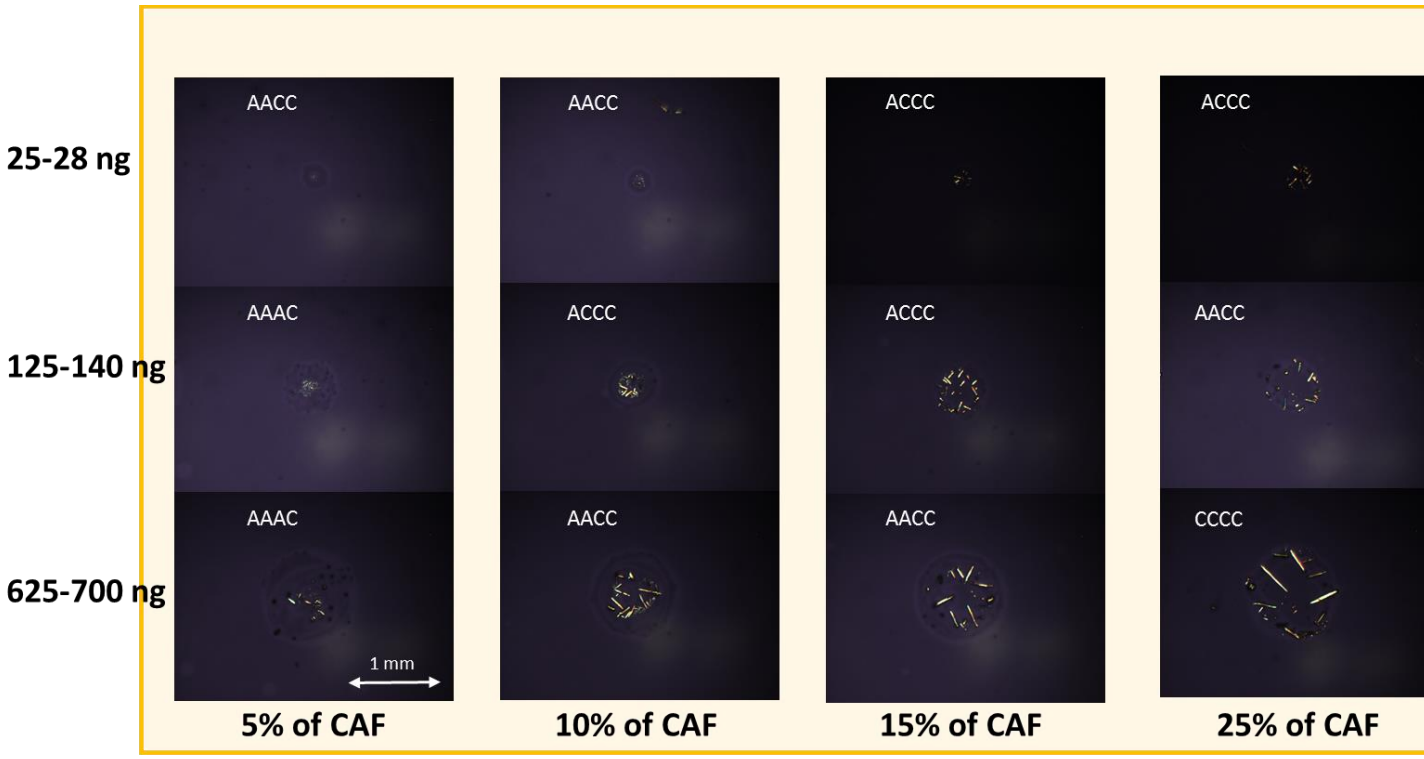


Figure 4SI. Drug-PVPVA spot array POM pictures, varying both printed amount (number of droplets) and drug polymer ratio.

Table 2SI. AI% of FLA against PAA, HPMC, PVPVA and PVP, at each w/w% ratio and as final average. The AI% for each drug\polymer formulation was calculated as an average between day 1 and 7.

	10/90 AI% 1-7days	25/75 AI% 1-7days	40/60 AI% 1-7days	50/50 AI% 1-7days	60/40 AI% 1-7days	75/25 AI% 1-7days	90/10 AI% 1-7days	Average AI%
FLA/PAA	25	25	0	0	0	0	0	7
	25	25	0	0	0	0	0	7
FLA/HPMC	100	100	100	50	25	0	0	54
	100	100	100	100	25	0	0	61
	100	100	100	75	50	0	0	61

FLA/PVPVA	100	100	100	100	100	63	0	80
FLA/PVP	100	100	100	100	75	25	0	71
	100	100	100	100	100	100	0	86

In the table both experimental (black) and literature (red) AI% values are reported. It is possible to exploit AI% to follow the presence of amorphicity stability trends amongst both polymers and between data generated by printing and literature results.