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Short communication

Combinatorial screening employing nylon loops and micro-X-ray powder diffraction

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Abstract

A combinatorial screening method is described that employs nylon loops and micro-X-ray powder diffraction. The nylon loops are used to position the sample in a three-circle X-ray diffractometer in such a way as to allow for Gandolfi-like scans. These scans maximize mechanical tumbling of the sample, which in turn results in higher quality data. The diffractometer utilizes a pinhole source and two-dimensional area detection for rapid data collection. The method can be readily automated by employing single-crystal screening procedures. The combinatorial analysis of the three known polymorphs of D-mannitol is discussed and a simple procedure to produce all three polymorphs from the same starting solutions is presented.

Keywords: Combinatorial screening; Combinatorial chemistry; Micro-X-ray powder diffraction; High throughput screening; Polymorph screening; Mannitol polymorphism

1. Introduction

Combinatorial chemistry methods employ rapid screening procedures to identify new materials and processes. The search for new polymorphs is a major driving force for the development of new screening techniques that provide high throughput with good accuracy [1]. X-ray powder diffraction (XRPD) is one of the most useful of these screening methods because of its high sensitivity, non-destructive nature and reliability [2]. XRPD provides valuable structural information, which in turn affords indisputable evidence of discovery. Unfortunately, conventional XRPD methods require relatively large amounts of sample (milligrams), which limits the combinatorial procedure. Micro-X-ray powder diffraction (μXRPD) on the other hand, requires only micro-grams of material and therefore provides an alternative to conventional XRPD for combinatorial screening [3].

The μXRPD experiment employs a small diameter (<0.5 mm) collimated X-ray beam, transmission geometry and an area detection system in order to acquire data. The area detection system provides for rapid data collection and compensates for non-homogenous scattering from non-ideal sample orientations. The sample can be positioned in the X-ray beam in a variety of manners. One of the most useful techniques is to place the sample in a supported thin nylon loop that is fastened to a metal pin [4]. This method allows for mechanical tumbling of the sample with respect to the X-ray beam. The tumbling procedure allows more crystallite orientations to be positioned in the X-ray beam and thus produces a more homogeneous scattering pattern, which in turn generates more accurate powder patterns.

With better powder patterns, it is possible to structurally characterize the powder by determination of its molecular structure. If possible, every powder pattern produced should be of high enough quality to attempt structural characterization, due to the fact that the polymorph may be difficult to make and the investigator may never have the opportunity to study it again.

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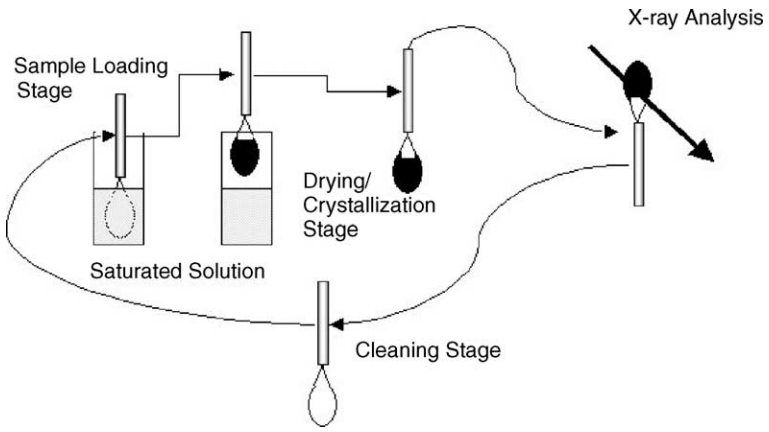

Scheme 1. Automated polycrystalline generation and X-ray analysis in a nylon loop.

Nylon loops also provide an opportunity for quick reproducible sample mounting. The loop shape makes it easy to enclose the sample and quickly relocate it to the X-ray instrument. The loops coupled with magnetic bases also allow for rapid placement of the sample in the X-ray beam and rapid removal and storage. In fact, the entire process can be automated with robotic devices originally developed for single crystal screening.

The loops also provide a support for in situ polymorph formation. The loop can be dipped in a liquid solution containing the compound of interest (see Scheme 1). When removed, the solution that clings to the loop will dry and form a polycrystalline mass in the loop. The polycrystalline material can then be relocated to the instrument and analysed. This process can be easily automated to produce a limited user intervention system for screening polymorphs.

D-Mannitol was chosen to test the procedure because it is know to have three polymorphs (α, β, and δ), that have been well characterized and studied [5–7]. Unfortunately, a simple procedure for producing all three polymorphs from the same starting solutions has not been clearly proposed. In this case, combinatorial methods followed by rapid screening can test for a useful method for the generation of all known polymorphs of D-mannitol.

Several methods for polymorph production have been reported and a complete review is available [8]. The best method to test the combinatorial screening procedure presented in this manuscript is the evaporation from mixed solvent systems. This method affords a fully automated pathway to produce and screen polymorphs with little or no user intervention.

2. Experimental

D-Mannitol (98%) was purchased from Aldrich and was used without further purification. A saturated aqueous solution (135 mg/ml) was prepared by dissolving excess mannitol in 100 ml of distilled water followed by double filtration of the solution through sintered glass (coarse followed by fine). A saturated ethanol solution (0.16 mg/ml) was also prepared by dissolving excess mannitol in 100 ml of absolute ethanol followed by double filtration of the solution through sintered glass (coarse followed by fine).

A spot plate was cleaned and used for the combinatorial search. Into the first well of the plate was placed 10 drops of the saturated ethanol/mannitol solution (100%). Into the second well was placed ten drops of a solution that was 50 parts ethanol/mannitol solution and one part water/mannitol solution (98%). Into the third well was placed 10 drops of a solution that was 20 parts ethanol/mannitol solution and one part water/mannitol solution (95%). Into the fourth well was placed 10 drops of a solution that was nine parts ethanol/mannitol solution and one part water/mannitol solution (90%). This process was repeated for wells five to eight with ratios of 8/2, 7/3, 6/4 and 5/5 of ethanol/mannitol to water/mannitol, respectively. The spot well was then set aside in a dry evaporation container and the solutions were allowed to evaporate to dryness. The procedure is summarized in Table 1.

A spatula was employed to remove a random sample from each of the eight wells. The eight samples were used as is and were not ground or crushed. Each sample was then mounted on an individual nylon loops (see Fig. 1). The eight mounted samples were then moved to the instrument and each sample was measured in turn.

Table 1
Spot plate evaporation method

Well no.	$EtOH/H_2O$	Ethanol (%)
1	100/0	100
2	50/1	98
3	20/1	95
4	9/1	90
5	8/2	80
6	7/3	70
7	6/4	60
8	5/5	50

A saturated solution of D-mannitol in ethanol and water is mixed in the ratios stated below and 10 drops of each solution was added to individual spot plate wells.

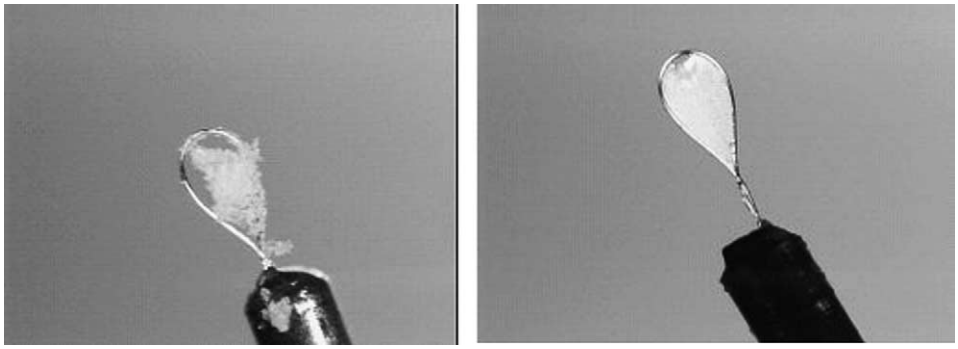

Fig. 1. Lose powder and polycrystalline sample grown in nylon loop.

A second method to produce polymorphs without using a spot plate is to dip a clean loop into each solution produced in method one and to allow the solution to dry in the loop. This produces a polycrystalline mass inside the loop (as seen in Fig. 1). Unfortunately the low surface tension of ethanol will restrict the formation of "useable" samples when the mixture exceeds 80% (ethanol/water).

3. Data collection

A three-circle BRUKER X-ray diffractometer was employed for polymorph screening. The goniometer was controlled using the GADDS® software suite. The sample was optically centred with the aid of a video camera such that no translations were observed as the powder was rotated through all positions. The detector was set at 12.0 cm from the sample (multi-wire proportional counter, 1024 × 1024 pixel). The X-ray radiation employed was generated from a Cu sealed X-ray tube (K_α = 1.54178 Å with a potential of 40 kV and a current of 40 mA) and filtered with a graphite monochromator in the parallel mode (175 mm collimator with 0.5 mm pinholes). The beam stop was positioned as close to the sample as possible to reduce the background X-ray scattering.

The data was collected with a Gandolfi-like method [9] (Guggenheim, S. ACA Annual Meeting, Chicago, IL, abstract, 2004). For the data collection the detector was positioned at -30° 2θ and a full 180° omega scan was undertaken while the phi-axis was rotated independently. The two axes were not coupled and care was taken so that the rates of rotation of both axes were not similar. The exposure time was fixed to 300 s per scan. Conical area integration yielded a one-dimensional powder pattern for each data run.

For a few selected samples, a still data collection and a Debye data collection method were also taken. For the still and Debye data collection the sample was positioned so that the loop mount was perpendicular to the beam. The Debye data collection involved a constant rotation of the sample around phi-axis while the still data collection involved no rotation.

The results for the data collections are illustrated in Figs. 2 and 3. Fig. 2 displays the combinatorial screening

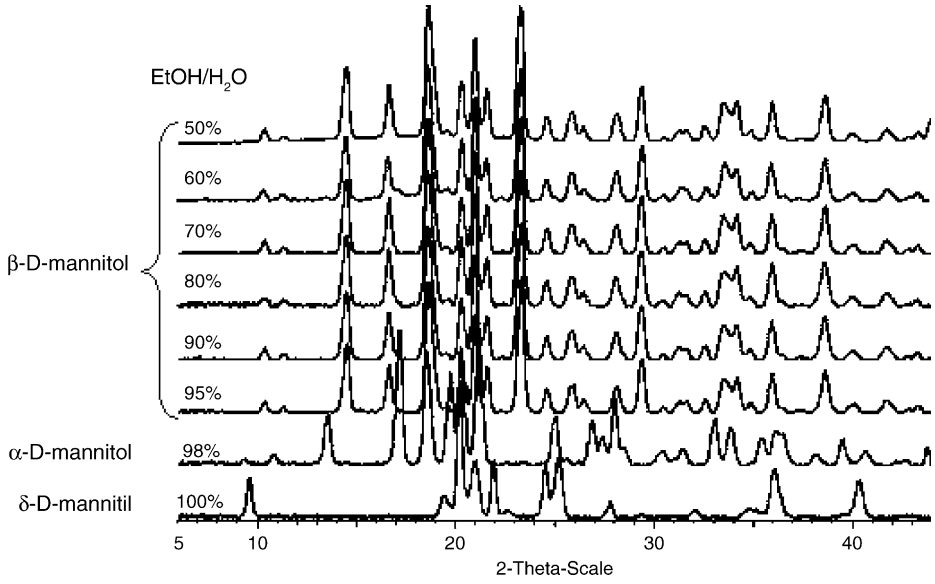

Fig. 2. Combinatorial XRPD screening for the three polymorphs of D-mannitol. Results from the spot plate evaporation method.

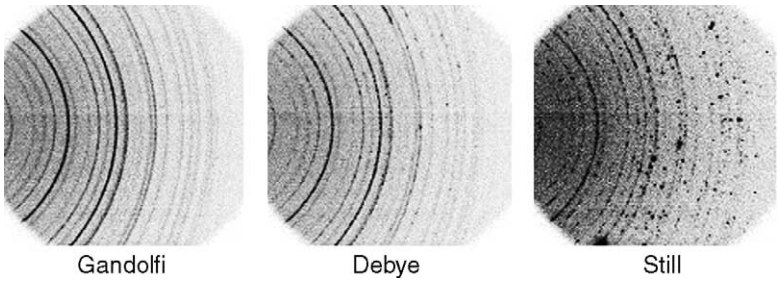

Fig. 3. Three frames collected by the Gandolfi, Debye and still data collection method from a powdered sample of β-D-mannitol.

results for D-mannitol. Fig. 3 shows the raw frame data for the Gandolfi, Debye and the still data collection.

4. Discussion

The procedures discussed in this paper will produce the best powder patterns possible from the samples described. As seen in Fig. 3, the Gandolfi method is far superior (far less grainy) to the still or Debye method for producing a homogenous scattering pattern. This is because the method will tumble the sample and expose the crystallites to more possible crystallite orientations than the other techniques. Other than grinding the sample, the tumbling procedure clearly provides the best method to produce mechanical randomness in the sample orientations. The method also involves little on no user intervention, since grinding the sample is not required, and it can be readily automated.

The combinatorial screening results are also of interest. The three known polymorphs of D-mannitol can be produce on the same spot plate by simply varying the ethanol/water ratio. If the sample is kept free of any trace water contaminate (100% absolute ethanol) and allowed to rapidly dry then only the δ polymorph is formed, without detectable amounts of the α or β polymorph forms. If a solution that is 50 parts saturated ethanol/mannitol is mixed with one part saturated water/mannitol solution (to produce a 98.04% by volume ethanol/water solution) is allowed to rapidly evaporate then only the α polymorph form is seen (no trace of β or δ is detected). Finally, if a 20 parts saturated ethanol/mannitol is mixed with one part saturated water/mannitol solution (to produce a 95.24% by volume ethanol/water solution) is allowed to rapidly evaporate then only the β form of the polymorph is detected. Further spot plate experiments indicate that the 99% by volume mixture of ethanol/mannitol and water/mannitol will form a mixture of δ and α polymorphs and solutions between 97 and 96% will form a mixture of the α and β polymorphs.

In conclusion the combination of a nylon loop, transmission geometry and Gandolfi data collection produces the best X-ray powder patterns for the limited amounts of material available. Employing this method, data can be collected in tens to hundreds of seconds, which allows for rapid materials screening. The method avails itself to automation and can be employed with no or limited user intervention. In general, no grinding or sieving of the sample is necessary and results can be of very high quality, without the errors associated with other X-ray diffraction techniques (especially preferred orientation) [10]. In practice the method could be limited only to the number of samples one could mount at one setting and thus hundreds of samples could be examined on a daily basis.

Acknowledgements

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