Application of a Fiber Optic Refractometric Sensor to Measure the Concentration of Paracetamol in Crystallization Experiments

Liliana Soares, Patrícia Cruz, Susana Novais, António Ferreira, Orlando Frazão, and Susana Silva

A refractometric sensor was applied to measure in real-time the concentration of Active Pharmaceutical Ingredients (APIs) in crystallization experiments. Paracetamol was used as a model system due to the extensive literature available for this API. The refractometric sensor was fabricated by a simple and inexpensive method that consisted in splicing a short section of a multimode fiber to a single mode fiber. The compact geometry of this sensor, with an external diameter of just 125 µm, allowed it to measure the concentration of paracetamol, both in a stirred tank crystallizer operating in batch and in an oscillatory flow crystallizer operating continuously. The proposed technique shows the potential to monitor the concentration of APIs in crystallizers of different sizes and geometries as an alternative to more expensive and complex analysis equipment.

Introduction to Process Analytical Technology in Crystallization

Crystallization, with its ability to control the yield, polymorphic form, purity, particle size and shape of the final product, is one of the most important operations involved in the manufacturing of Active Pharmaceutical Ingredients (APIs). Nevertheless, it is still a relatively poorly understood and poorly controlled process and so can benefit greatly from the use of Process Analytical Technology (PAT) [1].

The term PAT covers a variety of tools that can be used to design, analyze, and control the manufacturing processes with the aim of ensuring the quality of the final product. In the early stages of development, the use of PAT contributes to the mechanistic understanding of the process. In the transition to pilot scale, PAT can be used for process monitoring and verification, to ensure that the process runs as expected, to monitor batch-to-batch variability, and also to facilitate the process transfer between different vessels or sites. Finally, in the commercial manufacturing stage, PAT can be used for process control and troubleshooting, to enable continuous improvement and detect robustness issues [2].

The implementation of PAT requires sensors that measure the variables of interest. Depending on the location of the sensor, it can be classified as off-line (when the measurement is performed on a sample extracted from the process stream), on-line (when the measurement is performed in real-time on a diverted sample stream, which may be returned to the process after measurement), in-line (when the measurement is performed in real-time on the process stream, but the process stream might be disturbed) and non-invasive (when the measurement is performed in real-time on the process stream and the process stream is not disturbed). Real-time measurements are more appealing than off-line measurements because they avoid sample preparation and time delays. They are also preferred when the sample is difficult to access, when the off-line sample may not be representative, when the process of sampling changes the material, or even when frequent sampling is required [3]. Nevertheless, sometimes real-time measurements are not feasible, particularly when the vessel is very small, and the probes cannot be immersed in the process stream. An example is monitoring the concentration of an API in an Oscillatory Flow Crystallizer (OFC). The OFC consists of a tubular crystallizer that contains evenly spaced orifice baffles which are transversely assembled to a periodically oscillating flow. These crystallizers can be operated batchwise or continuously in horizontal tubes/channels or vertical columns. The development and application of OFCs has received significant attention in the past few decades due to the rise of continuous manufacturing in the pharmaceutical industry [4].

However, the use of PAT in these crystallizers is still limited by the diameters of the probes which typically exceed the diameter of the tubes/channels. In the literature it is possible to find studies in which the concentration of paracetamol was measured in real-time by a dielectric constant sensor [5], a densitometer [6], attenuated total reflectance Fourier transform

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infrared (ATR-FTIR) spectroscopy [7], ATR ultraviolet/visible (ATR-UV/Vis) spectroscopy [8], ATR mid-infrared (ATR-Mir) spectroscopy [9], and near-infrared (NIR) spectroscopy [10]. However, all these works were performed in Stirred Tank Crystallizers (STC), whereas the use of PAT in OFC-like crystallizers remained forgotten.

In view of the need to monitor the concentration of APIs in real-time and in crystallizers such as OFC, in this work, a refractometric sensor was applied to measure the concentration of paracetamol in crystallization experiments. The refractometric sensor works as follows: the light propagates inside the fiber by total internal reflection; when the light reaches the fiber/liquid interface, it is partly transmitted into the liquid and partly reflected into the fiber. The sensor measures the spectrum and the optical power of the reflected wave light [11].

The concentration of paracetamol was monitored in real-time in two different crystallizers: in a STC operating in batch and in an OFC operating continuously. Monitoring in the STC was carried out to compare the results obtained in this crystallizer with the results obtained during the monitoring in the OFC and thus, to validate the developed technique. Paracetamol was used as a model system due to the extensive literature available for this API.

Materials and Methods

Portable Interrogation System

A short section of a multimode fiber (MMF GIF 625 µm, supplied by Thorlabs, Newton, NU, USA) spliced to a single mode fiber (SMF 28e, supplied by Thorlabs, Newton, NJ, USA) was used as a refractometric sensor to measure the concentration of paracetamol in crystallization experiments. These fibers have a germanium doped silica core with a pure silica cladding and a mechanically strippable acrylate coating. The SMF has a core diameter of 8.2 µm and the MMF has a core diameter of 62.5 µm. Both fibers have a cladding diameter of 125 µm and a coating diameter of 242 µm. The refractometric sensor was fabricated by splicing a section of MMF with a length of 10 cm to a SMF by means of a splicing fusion machine (TYPE-72C), thus forming a MMF fiber tip. The MMF length was chosen to ensure that its entire core was excited. The refractometric sensor was connected to an optical interrogator (BraggMETER FS22), and the response was recorded by a data acquisition software (BraggMONITOR SI) at 1550 nm. The portable interrogation system, and the refractometric sensor operating in reflection, is presented in Fig. 1.

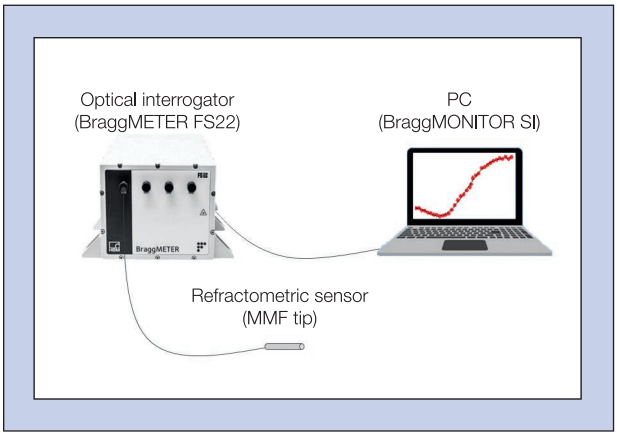

Fig. 1. Portable interrogation system and the detail of the refractometric sensor working in reflection.

Calibration Curve

Ten standard solutions of paracetamol (CAS number 103-90-02, min. 99% purity, supplied by Sigma-Aldrich) in a mixture of 40% (v/v) ethanol/water were prepared at room temperature (~20 °C). The concentration of the solutions ranged from 53 to 261 g/kg (g of paracetamol per kg of solvent), corresponding to an index range of 1.3634 RIU to 1.3947 RIU. The refractive index of each solution was measured by an Abbe refractometer (ATAGO, DR-A1).

The sensing head was vertically immersed in each standard solution and its optical power response was monitored. Through this optical power response, the calibration curve relating the concentration and optical power was plotted. Posteriorly, this calibration allowed the concentration of paracetamol in the crystallizers to be monitored during the crystallization process.

In situ Monitoring

The concentration of paracetamol was monitored in real-time, under the same experimental conditions, in a STC operating in batch and in an OFC operating continuously. The purpose was to compare the results obtained in the two monitoring situations to prove the potential of the developed technology to monitor API concentrations in crystallizers, regardless of their geometry. The experimental setups for the STC and the OFC are presented in Fig. 2.

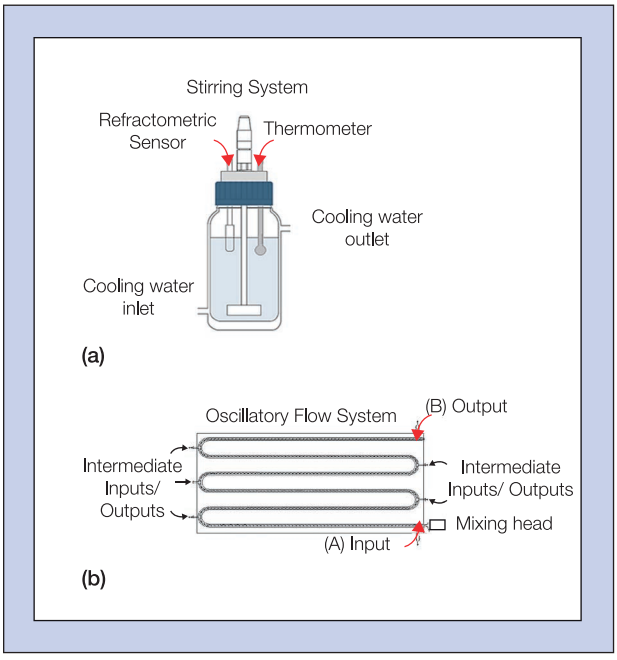

Fig. 2. Experimental setups. (a) STC; (b) OFC.

The STC consists of a 1 L jacketed glass flask with a built-in stirring system. The temperature inside the flask is measured by a thermometer and controlled by a water-cooling bath circulating in the jacket. The concentration of paracetamol in the mother liquor is measured by the refractometric sensor. The experimental setup is presented in Fig. 2a.

A 200 g/kg solution of paracetamol in 40% (v/v) ethanol/water was stirred at 150 rpm and kept at 45 °C. The temperature of the solution gradually decreased when the temperature of the cooling bath was reduced to 25 °C. When the temperature of the solution reached 25 °C, seeds of paracetamol were introduced. The addition of seeds prevented the formation of crystals on the surface of the refractometric sensor, which could interfere with the signal measured by the sensor.

The experimental setup of the OFC is presented in Fig. 2b. The OFC was constructed with three plates: one Teflon plate at the base, one Teflon plate at the middle, and one polycarbonate plate at the top. In the middle plate, two channels were carved: one channel designed with smooth periodic constrictions at the top where the crystallization takes place and a straight channel at the base where the water of a cooling bath circulates. The channel where the crystallization takes place comprises a volume of ~70 mL. Besides its optimized geometry, the OFC also has optimum dimensions that assure the reduction of waste and reagents requirements during the crystallization process [12].

Initially, the OFC was filled with ethanol and thermostated at 25 °C. The frequency and the amplitude of oscillation were set at 4 Hz and 4 mm (peak-to-peak in the piston), respectively. Then, a 200 g/kg solution of paracetamol in 40% (v/v) ethanol/water at 45 °C was fed into the OFC by a peristaltic pump (BT300-2J, supplied by Longer Pump) at 40 mL/min. The solution became supersaturated due to the decrease of temperature, which provided the driving force required for crystallization. The crystals of paracetamol were then collected at the end of the OFC. The concentration of paracetamol was measured by two refractometric sensors, placed at the input and the output of the OFC system as it is identified in Fig. 2b.

Results and Discussion

Calibration Curve

The concentration of the standard solutions of paracetamol increases from 53 to 261 g/kg, which leads to the increase of optical density of solutions. This variation corresponds to a refractive index variation in a range from 1.3634 RIU to 1.3947 RIU.

Fig. 3 presents the optical power response of the refractometric sensor and its correlation to the refractive index and concentration of the measured standard solutions. This response was obtained with a sensitivity to paracetamol refractive index of $-1.53\times10^{-2} \pm 0.05\times10^{-2}$ (RIU/dB) and a resolution of 5.06×10^{-4} RIU, which corresponds to a concentration sensitivity of -101.7 ± 3.58 ((g/kg)/dB) and a resolution of 3.36 Kg/g, respectively.

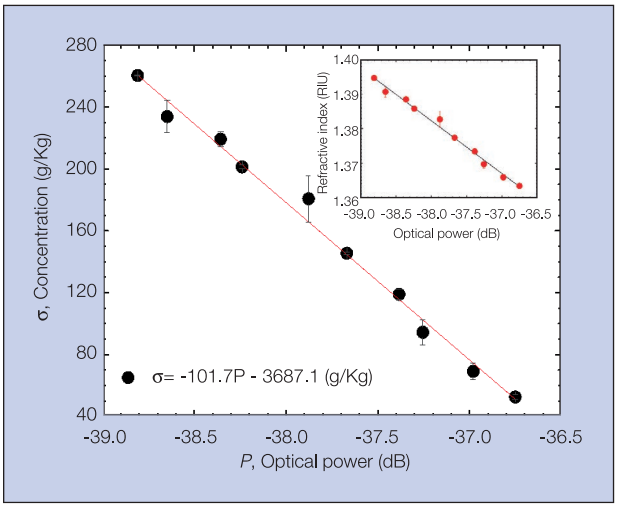

Fig. 3. Paracetamol concentration as a function of the optical power. (Inset: Refractive index of each paracetamol standard solutions as a function of the optical power).

As expected, the optical power decreased as the refractive index and the concentration increased, which is justified by the decrease of the amplitude of the reflected light at the fiber/liquid interface. Since the optical power measured by the refractometric sensor varies linearly with the concentration within the range of concentrations under study, a concentration versus optical power calibration curve could be used to monitor the concentration of paracetamol in the crystallizers.

Stirred Tank Crystallizer

The refractometric sensor monitored the evolution of the optical power during crystallization in the STC. Then, the concentration of paracetamol in the mother liquor was calculated from the calibration curve, as shown in Fig. 4.

Initially, the sensor measures a concentration close to 200 g/kg when paracetamol is fully dissolved. When the seeds are introduced, there is a slight increase of concentration due

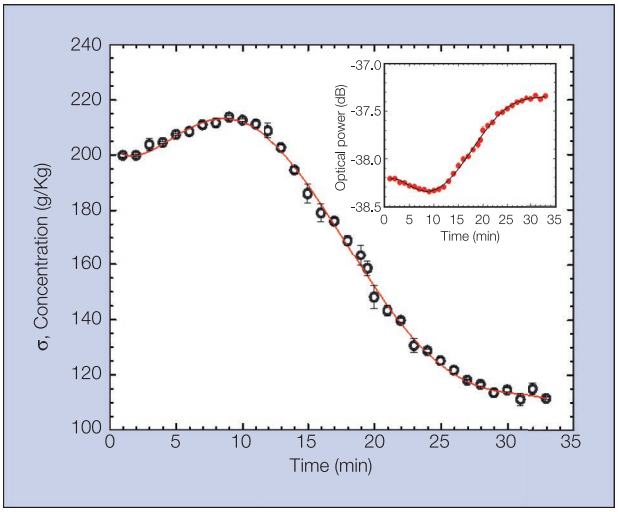

Fig. 4. Paracetamol concentration during crystallization in the STC. (Inset: Evolution of the optical power during crystallization).

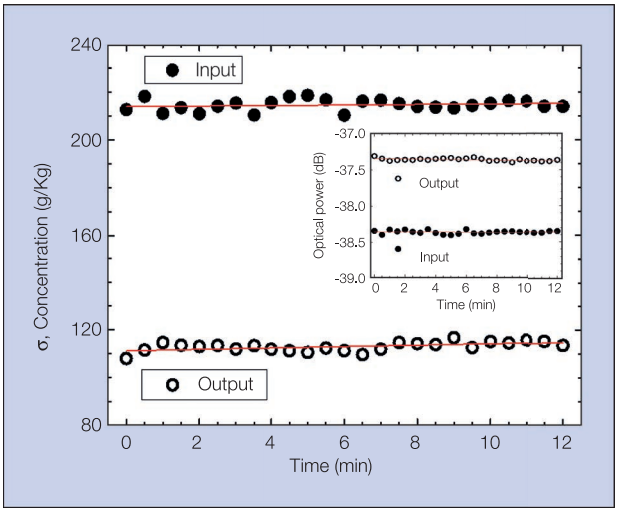

Fig. 5. Paracetamol concentration during crystallization in the OFC. (Inset: Evolution of the optical power during crystallization).

to the increase of optical density. Then, the concentration of paracetamol decreases due to crystal growth and secondary nucleation, which reduces the optical density and the refractive index of the solution. When the concentration approaches the solubility of paracetamol at 25 °C, the system reaches the thermodynamic equilibrium, and the concentration of paracetamol stabilizes at 100-120 g/kg, respectively.

Oscillatory Flow Crystallizer

The optical power was monitored in the OFC by two refractometric sensors, one at the input and another one at the output. The concentration of paracetamol in the mother liquor was then calculated from the calibration curve (Fig. 4). As expected, the signal of optical power measured by the sensor at the input is lower than that measured by the sensor at the output. The signal is virtually constant at both locations, which suggests that crystallization occurs at steady state.

At the input, the concentration of paracetamol fluctuates between 200 and 220 g/kg, when paracetamol is fully dissolved. Then, the solution cools inside the OFC and becomes supersaturated. The supersaturation is then gradually consumed as paracetamol crystallizes. At the output, the concentration fluctuates between 100-120 g/kg, which suggests that the solution reaches solubility before leaving the OFC.

The results obtained in the OFC are in agreement with those obtained in the STC as the concentration at the input of the OFC is comparable to the concentration at the beginning in the STC (200-220 g/kg), and the concentration at the output of the OFC is comparable to the concentration at the end in the STC (100-120 g/kg). These results prove the viability of the portable interrogation system to monitor the concentration of paracetamol in STC-like systems operating in batch or in OFC-like systems operating continuously.

Most previous studies on the crystallization of paracetamol in OFCs have not reported the use of any PAT to monitor the concentration of the mother liquor [13]. The study of Jiang and Ni [14] is the exception, but even in this case, the concentration of paracetamol had to be measured offline in an HPLC system coupled to an UV detector. Therefore, this is the first time that the concentration of paracetamol was measured in real-time in an OFC.

Conclusions

In this work, a portable interrogation system was used to monitor the concentration of paracetamol in an OFC system in real-time. To measure the concentration, a refractometric sensor was used, based on a small section of an MMF capable of measuring the optical power as a function of the concentration. It was found that the optical power varies linearly with the concentration of paracetamol in the concentration range of 53- 261 g/kg. Therefore, the concentration of paracetamol can be obtained directly as a function of the optical power without diluting the solution. The refractometric sensor was also used in an STC to calibrate the paracetamol crystallization process. This solution allows it to be applied in OFC systems due to the reduced dimensions, because it does not disturb the process inside of the crystallizer. The results obtained in this work show the potential of this sensor in monitoring in real-time the concentration of APIs in crystallizers of different sizes and geometries, such as the OFC. This technique can respond to the needs pointed out in this sense, being an alternative to more expensive and complex analysis equipment.

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