PROJEKT SZAKMAI ZÁRÓJELENTÉS (OTKA 121143)

In the three years of the project, we have completed the work described in the workplan in three different topics: crystallization in the presence of formulation additives, development of camera image analysis for crystallization monitoring and development of other continuous crystallization procedures.

The work schedule for the first year of the project was started to investigate the effect of formulation additives in different drug crystallizations and study the processes with inline spectroscopic methods. Crystallizations in the presence of additives were performed using famotidine, carvedilol, piracetam and acetylsalicylic acid drugs. The applied formulation additives were polyvinylpyrrolidone, cellulose derivates (HPC, HPMC), detergents (SLS), lactose, polyethylene glycol, microcrystalline cellulose. Among the listed drugs, morphological changes could be identified by crystallizing famotidine and carvedilol only using polyvinyl-pyrrolidone at different molecular weights. The crystallized products had excellent or good flowability creating direct tableting possibility[1]. In one case, polymorphism could be changed using PVP where additive produced the thermodynamically stable Form A polymorph of famotidine.

We developed continuous crystallization procedures (MSMPR, mixed suspension mixed product removal reactor; IJ-MSMPR impinging jet coupled with an MSMPR reactor; PF, ultrasonicated plug flow reactor) for direct processing of a multicomponent synthetic mixture and provided pure, homogeneous crystalline products for further formulation steps. Firstly, the solid-liquid separation and the purification of the acetylsalicylic acid from the multicomponent mixture were accomplished in a single-stage mixed suspension mixed product removal (MSMPR) continuous crystallizer equipped with an overflow and an inner buffer element to ensure the representative withdrawal of the product suspension. The effect of process parameters such as the operating temperature and the length of residence time (RT) on product quality and quantity were studied. Investigating these parameters, we found that higher operating temperatures (25 °C) and longer residence time (47 min) assisted appropriate purity (>99.5%) and narrow crystal size distribution. By reducing the operating temperature (2.5 °C), the yield improved slightly (approximately 77%), and polydisperse products were characterized[2]. The second continuous crystallization was a novel non-submerged triple impinging jet mixer coupled with a small MSMPR reactor to provide a longer aging period for the crystals. We also examined the effect of the same process parameters (temperature, residence time and antisolvent to ASA solution ratio) on product quality and process productivity. It was found that due to the intensive initial mixing achieved with triple impinging jet, significantly smaller crystal size (<180 μm) with narrower unimodal crystal size distribution and higher maximum yield (83.1%) could be obtained compared to the conventional MSMPR technique. Furthermore, the developed continuous crystallization was accomplished in smaller equipment with the same productivity compared to previous MSMPR results[3]. The third continuous crystallization procedure was the ultrasonicated continuous plug flow crystallizer. A 2^3 full factorial experimental design was used to explore the process parameters' effect on crystalline acetylsalicylic acid quality and quantity. It was found that ultrasonication resulted in robustness against process parameter modification and producing

[1] G. Marosi, E. Hirsch, K. Bocz, A. Toldy, B. Szolnoki, B. Bodzay, I. Csontos, A. Farkas, A. Balogh, B. Démuth, Z. K. Nagy, H. Pataki. *Period. Polytech. Chem. Eng.* **2018**, 62 *(4)*, 457-466.

[2] K. Tacsi, H. Pataki, A. Domokos, B. Nagy, I. Csontos, I. Markovits, F. Farkas, Z. K. Nagy, G. Marosi. *Cryst. Growth Des.* **2020**, *20* (7), 4433–4442.

[3] K. Tacsi, Á. Joó, É. Pusztai, A. Domokos, Z. K. Nagy, G. Marosi, H. Pataki. *Chem. Eng. Process. Intensif.* **2021**, *165*, 108446.

purified (<1% salicylic acid), small (<50 µm), consistent product quality while enabling to reach 89% yield even with 30 sec residence time[4]. Acetylsalicylic acid of various crystalline size and size distributions was obtained with adequate purity in each case by developing various continuous crystallizations to process a multicomponent reaction mixture. Taking advantage of the three different technologies, we finally created an integrated continuous crystallization technique in which the rapid dissolution property was coupled with the excellent flowability of the product. For this purpose, the PF crystallizer was paired with the MSMPR crystallizer, where the individual crystals coming from the first tubular reactor formed aggregates in the second MSMPR stage by continuously adding a polymer solution. (These results are under publication.)

Continuing the MSMPR crystallization results, an end-to-end continuous pharmaceutical manufacturing process was developed to produce conventional direct compressed tablets. The MSMPR crystallizer was directly connected to a continuous filtration carousel device. Thus the crystallization, filtration and drying of acetylsalicylic acid were carried out in an integrated 2-step process. The filtered crystals were ready for further processing in a following continuous blending and tableting experiment due to the good flowability of the material. At the end of the production chain, 500 mg ASA-loaded tablets were compressed with 100 mg dose strength. Thus, starting from raw materials, the final drug product was obtained by continuous manufacturing steps with appropriate quality[5]. In another publication, modeling this batch type continuous filtration process was presented to improve the understanding of this process and aid the integration of continuous crystallization and filtration steps. The developed model utilizes the generalized form of Darcy's law with the calculation of the cake porosity and specific cake resistance based on the full crystal size distribution (CSD) of the slurry, without the need for experimental filtration data. The model is also integrated with a population balance model of crystallization. The effect of the slurry properties (solid concentration, CSD) and the crystallization and filtration process parameters (crystallization temperature, flow rates, filter pressure difference, carousel rotation time) on the specific cake resistance, filtration time and residual moisture content are determined. The simulation results showed good agreement with real integrated continuous crystallization and filtration experiments of acetylsalicylic acid[6]. As a continuation of this work, a dynamic model of the integrated „end to end" continuous manufacturing of acetylsalicylic acid was formed based on real continuous unit operations (synthesis, crystallization, filtration, drying, capsule filling). In addition, the model of the *in vitro* dissolution test of ASA capsules was also integrated into the flowsheet model to analyze the integrated manufacturing in light of the dissolution specifications of an immediate-release formulation. The systematic optimization studies performed on the integrated process and separately on the continuous unit operations resulted in the threefold increase in the overall productivity and the parallel decrease in the required reactant excess. The study revealed that crystallization temperature emerged as one of the most critical parameters, which variation could even result in the failure of the dissolution specification. These results clearly show the significance of integrated flowsheet modeling approaches to develop optimal manufacturing technologies[7].

[4] K. Tacsi, G. Stoffán, É. Pusztai, B. Nagy, A. Domokos, B. Szilágyi, Z. K. Nagy, G. Marosi, H. Pataki. *Powder Technol.* **2021** (minor revision)

[5] A. Domokos, B. Nagy, M. Gyürkés, A. Farkas, K. Tacsi, H. Pataki, Y. C. Liu, P. Firth, B. Szilágyi, G. Marosi, Z. K. Nagy, Z. K. Nagy. *Int. J. Pharm.* **2020,** *581*, 119297.

[6] B. Nagy, B. Szilágyi, A. Domokos, K. Tacsi, H. Pataki, G. Marosi, Z. K. Nagy, Z. K. Nagy. *Chem. Eng. J.* **2021**, *413*, 127566.

[7] B. Nagy, B. Szilágyi, A. Domokos, B. Vészi, K. Tacsi, Z. Rapi, H. Pataki, G. Marosi, Z. K. Nagy, Z. K. Nagy. *Chem. Eng. J.* **2021**, *419*, 129947.

The research plan included the development of a camera-based imaging system for nucleation detection and real-time characterization of crystal size distribution in drug crystallization. This new image analysis system consisted of a high-speed process camera, a fiber optic illuminator and an in-house developed image analysis software. It was developed and tested firstly in wet granulation process. The size and size distribution of the obtained particles were successfully monitored. The validation of the developed system showed that the particle size analysis tool could determine the size of the granules with an error of fewer than five µm[8]. Subsequently, this image analysis system was further improved for an image-based mass flow measurement system and tested in an ultra-low dose powder feeding using a single-screw microfeeder. The mass, mass flow and sizes of the granule particles were successfully monitored and controlled in real-time by the developed videometric system[9]. Applying this imaging system in crystallization was difficult since the reactor glass wall has a distorting effect on the sharpness of the different parts of the image during the off-reactor detection. Thus, this imaging system was tested in the case of the overflow MSMPR reactor. In this work, a steep glass plate was placed under the overflow outlet, onto which the slurry was dripping on its surface. The glass plate spread and guided the droplets towards the product collection filter. A high-speed process camera was mounted above the glass plate to capture images of the crystals. Several light sources were tested in various positions to find the appropriate experimental setup for the optimal image quality. Samples were taken during continuous operation for off-line particle size analysis to compare to the crystal size distributions calculated from the images. The results were in good agreement, and the trends of the process could be followed well using the images. As a subsequent step, image analysis was operated throughout the entire continuous crystallization experiment collecting a huge quantity of information from the process. The crystal size distribution of the product was calculated every 30 seconds, which provided a thorough and detailed insight into the crystallization process[10].

The work schedule has contained the application of PAT tools during continuous crystallization (i.e. ATR-UV/Vis or Raman spectroscopy, imaging system). For this purpose, a new PAT system was developed to control the polymorphic purity during crystallization processes. The efficiency of the developed polymorphic concentration control (PPC) utilizing combined signals of inline Raman and ATR-UV/Vis sensors was tested in a batch cooling crystallization of carvedilol. Polymorphic concentrations were obtained by calculating the current solid-phase concentration from UV/Vis data then this value was proportionated to the Raman spectral concentrations of different polymorphs. The calculated polymorphic concentrations governed the control of cooling and reheating cycles of the crystallization using a programmable logic controller. The developed control approach was successfully adapted to produce pure polymorphic forms such as the kinetically preferred Form II or the thermodynamically stable Form I crystal form via seeding of Form I[11]. In the future, we also plan to test this PAT in continuous crystallization. As a further technological development in the case of MSMPR crystallization, turbidimetric level control was realized for the first time.

[8] L. Madarász, Z. K. Nagy, I. Hoffer, B. Szabó, I. Csontos, H. Pataki, B. Démuth, B. Szabó, K. Csorba, G. Marosi. *Int. J. Pharm.* **2018,** *547,* 360-367.

[9] L. Madarász, Á. Köte, M. Gyürkés, A. Farkas, B. Hambalkó, H. Pataki, G. Fülöp, G. Marosi, L. Lengyel, T. Casian, K. Csorba, Z. K. Nagy. *Int. J. Pharm.* **2020**, *580,* 119223.

[10] A. Domokos, L. Madarász, G. Stoffán, K. Tacsi, D. Galata, K. Csorba, P. Vass, Z. K. Nagy, H. Pataki. *Org. Process Res. Dev.* **2021**, DOI: 10.1021/acs.oprd.1c00372. (accepted for publication).

[11] K. Tacsi, M. Gyürkés, I. Csontos, A. Farkas, E. Borbás, Z. K. Nagy, G. Marosi, H. Pataki. *Cryst. Growth Des.* **2020**, *20* (1), 73-86.

During the preparation of the hydrate form of spironolactone the feed and removal rates were controlled by pumps using the signal of a turbidimeter in noncontact mode to maintain a constant fluid level and long-term steady-state operation. The temperature and antisolvent ratio were found to be the significant process parameters. Crystals were produced with monomodal distribution and d_{90} ranging from 7 to 62 µm[12].

Improving the previously developed intensity-based Raman spectroscopic process control in crystallization, a new PLS (partial least square regression) calibration-based feedback control was developed and tested in ethanol fermentation via maintaining glucose concentration at a proper level. The control of glucose concentration during fed-batch fermentation resulted in increased ethanol production. The results show that the use of Raman spectroscopy for the determination of dissolved components of yeast fermentation is a promising way to enhance process understanding and achieve a consistently high production yield[13].

In addition to the planned topics of the project, I was involved in performing crystallization tasks to produce different polymorphs of a selected drug. The aim of this study was to reveal the effects of polymorphism on equilibrium solubility, dissolution kinetics and the supersaturation of two oxytetracycline polymorphs (stable Form A and metastable Form B). The dissolution was studied using real-time concentration monitoring with an ATR probe attached to a UV spectrophotometer. A broad spectrum of solid-phase analysis methods (SEM, IR, XRPD, Raman) was applied for the characterization of polymorphs and to identify which form is present at the equilibrium solubility[14].

There are further publications that are not closely related to the research plan of the project[15,16,17].

[12] M. H. Bosits, Z. Szalay, H. Pataki, G. Marosi, Á. Demeter. *Org. Process Res. Dev.* **2021**, *25* (4), 760–768.

[13] E. Hirsch, H. Pataki, J. Domján, A. Farkas, P. Vass, C. Fehér, Z. Barta, Z. K. Nagy, G. Marosi, I. Csontos. *Biotechnol. Prog.* **2019**, *35* (5), e2848.

[14] D. Tempfli, E. Borbás, H. Pataki, D. Csicsák, G. Völgyi, B. Sinkó, K. Takács-Novák. *Eur. J. Pharm. Sci.* **2020**, *149*, 105328.

[15] E. Borbás, S. Kádár, K. Tsinman, O. Tsinman, D. Csicsák, K. Takacs-Novak, G. Völgyi, B. Sinko, H. Pataki. *Mol. Pharm.* **2019,** *16* (10), 5120-4130.

[16] M. Gyürkés, L. Madarász, A. Domokos, D. Mészáros, Á. K. Beke, B. Nagy, G. Marosi, H. Pataki, Z. K. Nagy, A. Farkas. *Pharmaceutics,* **2020**, *12*, 1119.

[17] D. Csicsák, E. Borbás, S. Kádár, P. Tőzsér, P. Bagi, H. Pataki, B. Sinkó, K. Takács-Novák, G. Völgyi. *New J. Chem.* **2021**, *45*, 11618-11625.