# FINAL REPORT

# Development of new formulation technologies for the preparation nanofibrous drug delivery systems

## NKFIH – FK 132133

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According to the determined work plan we conducted our research in the field of the formulation development of drug loaded nanofibrous dosage forms in the four-year period. The main aim of this project was to map the technological/scientific capability and limitations of the promising high-speed electrospinning method which can supply high quality nanofibrous material for development of stable solid biodrug formulations, improved dissolution and flux of poorly water-soluble drugs with better patient compliance. To achieve these goals continuous formulation technologies were developed and tested as the most promising approach in pharmaceutical technologies.

**The main achievements of the research** project are the followings:

- Aqueous high-speed electrospinning of monoclonal antibody resulting in a stable, reconstitutable powder for injection using HPβCD as stabilizing matrix; Similar HPβCD-based powder for injection was developed for doxycycline drug delivery.
- For the treatment of periodontal diseases orally dissolving web of Lactobacillus paracasei was developed using PVA-PEO matrix system.
- The better amorphization efficiency of electrospinning was proved in the case of spironolactone PVPVA64 compared to spray drying due to the faster evaporation.
- Continuous downstream processing of itraconazole-based electrospun amorhous solid dispersion was developed with PAT control to achieve powder vials for reconstitution injection or tablets.
- Different spectroscopy and artificial intelligence based analytical methods were developed to support the PAT controlled downstream processing concept.
- Absorption driven drug formulation concept was introduced for the formulation development of electrospun telmisartan fibers using simultaneous dissolution-permeation experiments.

**Scientific output in numbers**

In the four-year period with the support of this project **26 scientific articles** were published in journals indexed by Web of Science (cumulative impact factor: >100). Most of these articles were published in journals with impact factor over 4 (such as Int. J. Pharm. if: 5.8, Eur. J. Pharm. Sci. if: 4.6, AAPS J. If: 4.5, etc.). Overall, more than 10 undergraduate and 5 PhD students were involved and contributed to the research project.

## Detailed description of the results

In the first year of the project according to the determined aims of the proposed project we developed of an electrospun solid formulation of a monoclonal antibody. Solid formulations of monoclonal antibodies present several advantages, such as improved stability and increased shelf-life as well as simpler storage and transportation. We presented a gentle drying technology for monoclonal antibodies, applying the water soluble 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) as matrix, to prepare a solid reconstitution dosage form. High-speed electrospinning of an aqueous infliximab-containing HP-β-CD solution was carried out at 25°C resulting in fibers with an average diameter of 2.5 μm. The mAb-loaded electrospun fibers were successfully prepared to preserve the stability of infliximab in solid form. The results of size exclusion chromatography and gel electrophoresis indicated no significant increase in aggregate formation during the electrospinning process compared to the initial matrix solution. The binding activity of infliximab was preserved during electrospinning compared to the reference liquid formulation. Due to the enhanced surface area, excellent reconstitution capability, i.e. clear solution within 2 min without any vigorous mixing, could be achieved in a small-scale reconstitution test. The results demonstrated that high-speed electrospinning is a very promising technique to manufacture the solid formulation of monoclonal antibodies for applications such as fast reconstitutable powders. (Domján et al.: ***Monoclonal antibody formulation manufactured by high-speed electrospinning***, Int. J. Pharm., 2020).

Similarly high-speed electrospinning was applied using 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) as a matrix for solid formulation of doxycycline (DOX). A new intravenous (i.v.) bolus dosage form was prepared by electrospinning. The new solid formulation could be produced with high (~80 g/h) productivity rate using high-speed electrospinning (HSES) from a water-based precursor solution. Freeze-dried DOX-HP-β-CD was also prepared from the same precursor solution as HSES for comparison. Raman mapping showed that the amorphous DOX was uniformly distributed in the fibrous powder making precise dosing of the API possible. The new formulation's viability as an i.v. bolus product was examined with reconstitution test. The samples contained 100 mg of pure DOX (similarly to the products currently on the market). To ensure i.v. bolus applicability, the dissolution was carried out in 1.5 mL water. The final DOX concentration was 66.7 mg/mL, which is 7 times higher than the currently marketed formulation. The drug release was followed by UV–VIS spectrophotometry. The results confirmed that the reconstitution solution could be applied as an i.v. bolus dosage form. Moreover, the work confirmed that the continuous high-speed electrospinning process can be a viable high productivity alternative to freeze-drying. (Kiss et al.: ***A solid doxycycline HP-beta-CD formulation for reconstitution (i.v. bolus) prepared by scaled-up electrospinning***, Int. J. Pharm., 2020).

In the first year of the project we also investigated the continuous upstream and downstream possibilities for the preparation of amorphous solid dispersion containing final solid dosage

forms. In this period we mainly investigated the continuous blending of powders from technological and quality control point of view. In our experiments a twin-screw continuous high-shear blender was used. We described several residence time models (Gyürkés et al.: ***Process Design of Continuous Powder Blending Using Residence Time Distribution and Feeding Models***, Pharmaceutics, 2020). We developed a new mass flow control mechanism for micro-feeding which is applicable for continuous blending (Madarász et al.: ***Videometric mass flow control: A new method for real-time measurement and feedback control of powder micro-feeding based on image analysis***, Int. J. Pharm., 2020). Continuous blending using a twin-screw blender was also applied in an end-to-end continuous manufacturing process. The blending uniformity was monitored by in-line NIR spectrometry. (Domokos et al.: ***End-to-end continuous manufacturing of conventional compressed tablets: From flow synthesis to tableting through integrated crystallization and filtration***, Int. J. Pharm., 2020). Furthermore, in the first year of the project we reviewed and published the available technologies for scaled-up electrospinning and the related applications in oral drug delivery (Vass et. al.: ***Scale-up of electrospinning technology: Applications in the pharmaceutical industry***, Wiley Interdis. Reviews – NanoMed. NanoBioTech., 2020).

In the second year of the project according to the determined aims of the proposed project we developed an orally dissolving nanofibrous probiotic formulation. The main achievements of this study are the followings:

- High-speed electrospinning was used for the scaled-up production of an orally dissolving dosage form of probiotics.
- Lactobacillus paracasei was successfully encapsulated in PVA-PEO nanofibers containing stabilizing excipients.
- Excipients can improve survival rate during electrospinning and long-term storage.
- Excellent storage stability of encapsulated bacteria was achieved using skim milk and mannitol.

Our results were published in Food and Bioproducts Processing scientific journal.
(Hirsch Edit et al.: **Probiotic bacteria stabilized in orally dissolving nanofibers prepared by high-speed electrospinning**, FOOD AND BIOPRODUCTS PROCESSING, 2021).
In another study within a collaboration with Prof. Van den Mooter (KU Leuven) high speed electrospinning was applied for the preparation of amorphous solid dispersions and the technology was compared to spray drying. Electrospinning was more effective in amorphization and dissolution enhancement than the spray drying due to the faster solvent evaporation. (Szabó et al.: **Comparison of Amorphous Solid Dispersions of Spironolactone Prepared by Spray Drying and Electrospinning: The Influence of the Preparation Method on the Dissolution Properties**, MOLECULAR PHARMACEUTICS, 2021).
We also investigated the downstream processing of the nanofibrous amorphous solid dispersions. Our aim was to develop a continuous downstream technology. Continuous feeding, blending and tableting of electrospun fibers was accomplished. In-line monitoring of

the amorphous solid dispersion content was performed. The prepared tablets passed the USP <905> content uniformity test, thus continuous manufacturing of milled electrospun materials to tablets proved feasible. (Szabó et al.: **Continuous downstream processing of milled electrospun fibers to tablets monitored by near-infrared and Raman spectroscopy**, EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES 2021).

In the field of pharmaceutical analysis spectroscopy and computer vision based analytical methods were developed for fast, non-destructive determination of the critical quality attributes of tablets and granules. Raman and NIR spectroscopy were also applied as PAT tools for drug dissolution prediction from sustained release tablets using artificial neural networks as a surrogate model of dissolution testing. (Galata et al.: **Real-time release testing of dissolution based on surrogate models developed by machine learning algorithms using NIR spectra, compression force and particle size distribution as input data**, INTERNATIONAL JOURNAL OF PHARMACEUTICS 2021). Machine vision was applied for indirect monitoring of ultralow dose API content. (Ficzere et al.: **Indirect monitoring of ultralow dose API content in continuous wet granulation and tableting by machine vision**, INTERNATIONAL JOURNAL OF PHARMACEUTICS 2021).

The integrated continuous technologies were reviewed in this research period focusing on amorphous solid dispersions and electrospun systems among other formulation technologies. (Domokos et al.: **Integrated Continuous Pharmaceutical Technologies—A Review**, ORGANIC PROCESS RESEARCH & DEVELOPMENT 2021)

In the third year of the project according to the determined aims of the proposed project we developed and tested a continuous powder filling system for the formulation of electrospun powders. The main achievements of this study are the followings:

- Vial filling proved to be possible with high-speed electrospun material.
- Different methods were compared for milling of electrospun materials.
- Correlations were observed between the material properties and feeding responses.
- A vibratory feeder is suitable for automatic vial filling with low mass variance.
- Reconstitution dosage forms can be prepared from electrospun materials.

Our results were published in International Journal of Pharmaceutics scientific journal. (Szabó et al.: **Powder filling of electrospun material in vials: A proof-of-concept study**, INTERNATIONAL JOURNAL OF PHARMACEUTICS, 2022)

We also investigated the new possibilities of real-time quality control in the case of continuous downstream processing of drug loaded materials. We published a review in this field (Nagy B. et al.: **Application of Artificial Neural Networks in the Process Analytical Technology of Pharmaceutical Manufacturing—a Review**, AAPS JOURNAL, 2022) and three research articles about different analytical possibilities (Horkovics-Kovats et al.: **Raman-based real-time dissolution prediction using a deterministic permeation model**, INTERNATIONAL JOURNAL OF PHARMACEUTICS, 2022), (Madarász et al.: **In-line particle size measurement based on**

**image analysis in a fully continuous granule manufacturing line for rapid process understanding and development**, INTERNATIONAL JOURNAL OF PHARMACEUTICS, 2022), (Mészáros et al.: **UV/VIS imaging-based PAT tool for drug particle size inspection in intact tablets supported by pattern recognition neural networks**, INTERNATIONAL JOURNAL OF PHARMACEUTICS, 2022).

As a continuation of the second year, we investigated of the continuous formulation and downstream processing possibilities of probiotic bacteria using twin-screw wet granulation line as a potential technology providing mild and continuous drying conditions. (Vass et al.: **Processing of thermosensitive biological API from suspension using an integrated continuous granulation - Drying - Milling line into powder ready for tableting**, DRYING TECHNOLOGY, 2023)

In the field of drug passive diffusion, we tested drug permeations through artificial membranes for the investigation of the driving force of the permeation. The results were published in collaboration with Semmelweis University and PION Inc. (Kádár et al.: **Flux-Based Formulation Development-A Proof of Concept Study**, AAPS JOURNAL, 2022).

In the last year of the project we investigated a continuous granulation process which is suitable for effective granulation without the use of water. PEG was applied in a twin-screw granulator as a melting agent to achieve powder agglomeration. A fully continuous powder-to-tablet line was developed and the possibilities of scaling up was also tested up to 8 kg/h. (Záhonyi et al.: **Integrated continuous melt granulation-based powder-to-tablet line: Process investigation and scale-up on the same equipment**, EUROPEAN JOURNAL OF PHARMACEUTICS AND BIOPHARMACEUTICS 2023).

For the controlled realization of the technological steps of formulation efficient analytical methods are needed as there is no control without measurement. For this purpose, powder blends and tablets were investigated machine vision or spectroscopy methods to monitor the critical quality attributes of them such as blend uniformity, particle size distribution, drug content and dissolution. (Ficzere et al.: **Image-based simultaneous particle size distribution and concentration measurement of powder blend components with deep learning and machine vision**, EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES 2023), (Galata et al.: **Comparing the Performance of Raman and Near-Infrared Imaging in the Prediction of the In Vitro Dissolution Profile of Extended-Release Tablets Based on Artificial Neural Networks**, PHARMACEUTICALS 2023), (Péterfi et al.: **Artificial Intelligence-based Prediction of In Vitro Dissolution Profile of Immediate Release Tablets with Near-infrared and Raman Spectroscopy**, PERIODICA POLYTECHNICA-CHEMICAL ENGINEERING 2023).

Last but not least in collaboration with some experts of electrospinning from different countries we elaborated an in-depth scientific review article on electrospinning technology and we published it in Advanced Material Technnologies scientific journal. (Keirouz et al.: **The History of Electrospinning: Past, Present, and Future Developments**, ADVANCED MATERIALS TECHNOLOGIES, 2023).