FINAL REPORT
Preparation, characterization and pharmatech application of drug loaded nanofibers
NKFIH - PD108975
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According to the determined work plan we conducted our researches in the field of drug loaded nanofibers in the three-year period. The main aim of this project was to map the technological/scientific capability and limitations of the promising electrospinning method for producing new drug delivery systems with improved drug bioavailability and/or patient compliance. It was hypothesized in the project proposal that electrospinning has better amorphization efficiency than melt extrusion, spray drying or film casting.

Detailed aims described in the project proposal were as follows:

"- Preparation and characterization of electrospun orally dissolving webs of drugs with poor water solubility;

- Preparation and characterization of drug loaded nanofibers with high drug content and comparison to spray dried systems;

- Preparation and characterization of fast dissolving cyclodextrin-based fibers;

- Preparation and characterization of drug loaded nanofibers with high drug content and comparison to spray dried systems;

- Development of adequate polymer matrices for the preparation of melt electrospun drug loaded fibers;

- Investigation of the possibilities of the preparation of electrospun fiber-based solid dosage forms;

- Investigation and comparison of different fiber manufacturing techniques and scale-up possibilities."

Scientific output

In the three-year period with the support of this project **27 scientific articles** were published in journals indexed by Web of Science. Most of these articles were published in journals with impact factor over 2.5 (such as Int. J. Pharm. if: 3.994, J. Pharm. Sci. if: 2.61, J. Pharm. Biopharm. Anal. If: 3.169, etc.). Overall more than 15 undergraduate and 5 PhD students were involved and contributed in the research project.

Detailed description of the results

Dissolution enhancement by amorphous solid dispersions

In the first period according to the determined aims of the proposed project we mainly focused on dissolution enhancement of drugs with poor water solubility.

Fast-release nano- and microfibers of lipophilic spironolactone were prepared in a continuous manner by electrostatic spinning, in which the application of polyvinylpyrrolidone K90 as matrix polymer enabled formation of solid solutions. However, instead of the anticipated immediate drug release, temporary precipitation was observed. The polyvinylpyrrolidone web gelled immediately after wetting, hindering drug diffusion and aiding the crystallisation of the solvated amorphous spironolactone. These local supersaturations could be successfully avoided by using hydroxypropyl-β-cyclodextrin. The dependence of fiber diameter and dissolution rate on the complexing agent–polymer ratio was also studied. A small addition of hydroxypropyl-β-cyclodextrin proved enough for a dramatic enhancement of the extent of drug release even in the case of high drug loaded formulations. Transmission Raman spectroscopy (TRS), differential scanning calorimetry and X-ray powder diffraction showed that the drug was totally amorphised during processing in all formulations. Polymer-free hydroxypropyl-β-cyclodextrin fibers containing spironolactone were also electrospun from an ethanolic solution, which is a new way of dissolution improvement in the case of poorly water-soluble drugs. This novel approach ensured nearly total drug release in a minute, making the system a suitable age-appropriate orally dissolving formulation. (Vigh et al.: Polymer-free and polyvinylpirrolidone-based electrospun solid dosage forms for drug dissolution enhancement, **EUR J PARM SCI 49, 595-602; 2014**)

Based on the promising sensitivity of the transmission Raman spectrometry method on the physical state of spironolactone experienced in the case of electrospun PVP-CD-Spir nanofibrous materials the residual crystallinity, drug degradation were investigated using TRS in Eudragit E matrix in melt extruded solid dispersions. Good prediction was achievable on the residual crystallinity, drug degradation, glass transition temperature and even on drug release based on Raman spectra. (Vigh et al.: Predicting final product properties of melt extruded solid dispersions from process parameters using Raman spectrometry, **J PHARMACEUT BIOMED ANAL 98, 166-177; 2014**)

Manufacturing of Eudragit E – spironolactone solid dispersions using supercritical CO_2-aided melt extrusion was also developed and it resulted in better grindability and increased surface area. (Vigh et. al.: Effect of supercritical CO_2 plasticization on the degradation and residual crystallinity of melt-extruded spironolactone, **POLYM ADVAN TECHNOL 25: (10) 1135-1144, 2014**)

For better characterization of solid dispersion Raman-based chemical imaging technique was developed further using SERS (surface enhanced Raman spectrometry) technique and detailed chemometric analysis to get more accurate quantitative results from Raman mapping (Firkala et al.: Quantification of low drug concentration in model formulations with multivariate analysis using surface enhanced Raman chemical imaging, **J PHARMACEUT BIOMED ANAL 107: 318-324, 2015**; Nagy B et. al.: Quantification and handling of nonlinearity in Raman micro-spectrometry of pharmaceuticals, **J PHARMACEUT BIOMED ANAL 128: 236-246, 2016**).

Development of fiber production from melts

As a potential method for scaling-up of formation fibrous drug delivery systems melt electrospinning (MES) and melt blowing (MB) were investigated to prepare fast dissolving

fibrous solid dispersions and compared with solvent-based electrospinning (SES) using carvedilol as a model drug and Eudragit E or PVPVA64 as matrix formers. The HPLC studies demonstrated that all of the materials produced by the different techniques passed the regulatory purity requirements. The fibers exhibited ultrafast drug release. The melt-blown sample dissolved within 2 min owing to its large specific surface area. The obtained results confirmed the applicability of MES and MB as a novel formulation technique for polymer-based drug delivery systems. (Balogh et. al.: Plasticized Drug-Loaded Melt Electrospun Polymer Mats: Characterization, Thermal Degradation, and Release Kinetics, **J PARM SCI 103 (4), 1278-1287; 2014**) (Balogh et al.: Melt-Blown and Electrospun Drug-Loaded Polymer Fiber Mats for Dissolution Enhancement: A Comparative Study, **J PHARM SCI 104: (5) 1767-1776, 2015**).

Comparison of amorphization efficiencies

We compared the amorphization efficiency of electrospinning, electroblowing and spray drying which is a widespread technology in the pharmaceutical industry for preparation of amorphous solid dispersions. Using electrospinning the burst release of the model drug from the matrix (PLA, PLGA) was avoidable owing to the its better amorphization efficiency compared to spray drying (Sóti et al.: Preparation and comparison of spray dried and electrospun bioresorbable drug delivery systems, **EUR POLYM J 68: 671-679, 2015**) Quantitative characterization of this composition was also developed using micro-Raman spectrometry coupled with multivariate linear regression methods. (Farkas et al.: Comparison of multivariate linear regression methods in micro-Raman spectrometric quantitative characterization, **J RAMAN SPECTROSC 46: (6) 566-576, 2015**). At higher drug loadings in the case of itraconazole (drug) and Eudragit E (matrix) we found similar amorphization phenomenon. Electrospinning and electroblowing provided more stable amorphous solid dispersions compared to spray drying (Sóti et al.: Comparison of spray drying, electroblowing and electrospinning for preparation of Eudragit E and itraconazole solid dispersions, **INT J PHARM 494: (1) 23-30, 2015**). We also applied electrospinning and electroblowing to prepare a powder for reconstitution injection and we compared these techniques to the generally applied freeze drying. According to the scanning electron microscopic images, X-ray diffraction, differential scanning calorimetry no traces of crystallinity of the drug were detectable in the fibers as opposed to the freeze-dried product. Reconstitution tests of the fibers showed fast dissolution obtaining clear solutions equivalent to the marketed and later withdrawn (because of instability) liquid-based bolus injection (Balogh et al.: Electroblowing and electrospinning of fibrous diclofenac sodium-cyclodextrin complex-based reconstitution injection, **J DRUG DELIV SCI TEC 26: 28-34, 2015**).

Biodrug formulation using electrospinning

Electrospinning is a very gentile drying technique and it is worth to study the applicability of electrospinning for drying of biodrugs such as bacterica, peptides, proteins.

Thus, we investigated the suitability of electrospinning for drying of living bacteria and for development an electrospinning-based method to produce vaginal drug delivery systems. Lactobacillus acidophilus bacteria were encapsulated into nanofibers of three different polymers (polyvinyl alcohol and polyvinylpyrrolidone with two different molar masses). Shelf life of the bacteria could be enhanced by the exclusion of water and by preparing a solid dosage form, which is an advantageous and patient-friendly way of administration. The formulations were stored at –20, 7 and 25°C, respectively. Viability testing showed that the nanofibers can provide long term stability if they are kept at (or below) 7°C. Furthermore, all kinds of nanowebs prepared in this work dissolved instantly when they got in contact with water, thus the developed biohybrid nanowebs can provide new potential ways for curing bacterial vaginosis. (Nagy et al.: Nanofibrous solid dosage form of living bacteria prepared by electrospinning, **EXP POL LETT 8 (5), 352-361; 2014**)

As a continuation of these results in the field of bacteria containing formulation development we tested film coating technology using Lactobacillus acidophilus containing PVA-based coating dispersion to manufacture administrable probiotic tablets in one step and to enhance the survival rate of bacteria and their viability during storage. After one year, 20% of the embedded bacteria were active when stored at –20°C, and viability dropped only one order of magnitude when stored at 7°C (Wagner et al.: Film Coating as a New Approach to Prepare Tablets Containing Long-Term Stable Lactobacillus acidophilus, **PERIOD POLYTECH CHEM ENG 59: (1) 96-103, 2015**).

Based on the positive results obtained using the bacterium-type biodrug we further investigated this gentile drying technique for solidification and formulation of proteins.

Suitability of electrospinning for biodrug formulation was investigated in order to develop an electrospinning-based method for producing oral dosage form of β-galactosidase. β-galactosidase-loaded polymeric (polyvinyl alcohol, polyvinylpyrrolidones with two different molar masses, and polyethylene glycol) nanofibers were prepared by electrospinning. Based on the activity of the encapsulated β-galactosidase, the most suitable polymer was polyvinylpyrrolidone with higher molecular weight, because 97% of the original activity remained in this case. Kinetic behaviour of β-galactosidase did not show any alteration after encapsulation, and the pH and temperature profiles were not changed either. Time course of viability testing showed that the nanofibrous formulation can provide long-term stability for β-galactosidase; the activity of the enzyme decreased only 4% after a year. Furthermore, scaling-up and tableting had no influence on activity and long-term stability; thus, the developed drying technology and tablets, containing enzyme-loaded nanofibers, can provide a new promising way of oral biodrug delivery (Wagner et al.: Stable formulation of protein-type drug in electrospun polymeric fiber followed by tableting and scaling-up experiments, **POLYM ADVAN TECHNOL 26: (12) 1461-1467, 2015**). Real-time monitoring of enzyme activity of lactase was developed as well using a Raman probe. Based on that feedback control of lactose hydrolysis was achievable (Hirsch et al.: Raman-Based Feedback Control of the Enzymatic Hydrolysis of Lactose, **ORG PROCESS RES DEV 20: (10) 1721-1727, 2016**). This kind of Raman-based feedback control was also developed in the

case of an oximation reaction. The real-time feedback control approach is very important in the establishment of continuous pharmaceutical manufacturing which is in the focus of the most big pharma companies (Csontos et al.: Feedback control of oximation reaction by inline Raman spectroscopy, **ORG PROCESS RES DEV 19:(1) pp. 189-195, 2015**).

Similarly to the lactase enzyme lipase enzyme was successfully encapsulated into PLA- and PVA-based nanofibers using electrospinning and it could be used as biocatalyst. (Sóti et al.: Electrospun polylactic acid and polyvinyl alcohol fibers as efficient and stable nanomaterials for immobilization of lipases, **BIOPROC BIOSYST ENG 39: (3) 449-459, 2016**; Weiser et al.: Bioimprinted lipases in PVA nanofibers as efficient immobilized biocatalysts, **TETRAHEDRON 72: (46) 7335-7342, 2016**)

Development of scaled-up electrospinning methods

We also focused on the scaling-up of electrospinning. **Alternating current electrospinning** (AC-ES) is a promising technique for scaled-up production of nanofibers, thus, we investigated the manufacturability of electrospun drug delivery systems prepared by AC-ES and we compared them with samples prepared by DC-ES. (Balogh et al.: Alternating current electrospinning for preparation of fibrous drug delivery systems, **INT J PHARM 495: (1) 75-80, 2015**)

(Balogh et al.: AC and DC electrospinning of hydroxypropylmethylcellulose with polyethylene oxides as secondary polymer for improved drug dissolution, **INT J PHARM 505: (1-2) 159-166, 2016**)

Corona electrospinning developed at BME was tested for scaling-up. The productivity of corona ES technique can be two orders of magnitude higher than that of the SNES method.

(K. Molnar, Z.K. Nagy: Corona-electrospinning: Needleless method for high-throughput continuous nanofiber production, **EUR POLYM J 74: (1) 279-286, 2016**)

High speed electrospinning (HSES), as a high through-put electrospinning method compatible with pharmaceutical industry, was developed and used to demonstrate the viability of the preparation of drug-loaded polymer nanofibers with radically higher productivity than the known single-needle electrospinning (SNES) setup. Poorly water-soluble itraconazole was formulated with PVPVA64 matrix polymer using four different solvent-based methods such as HSES, SNES, spray drying and film casting. Despite the significantly increased productivity of HSES, the obtained morphology was very similar to the SNES nanofibrous material. ITRA transformed into an amorphous form, according to the DSC and XRPD results, in most cases except the FC samples. The limited dissolution of crystalline ITRA could be highly improved: fast dissolution occurred (>90% within 10 min) in the cases of both (the scaled-up and the single-needle) types of electrospun fibers, while the improvement in the dissolution rate of the spray-dried microspheres was significantly lower. Production of amorphous solid dispersions with the HSES system might open new routes for the development of industrially relevant nanopharmaceuticals (Nagy et al.: High speed electrospinning for scaled-up production of amorphous solid dispersion of itraconazole, **INT J PHARM 480: (1-2) 137-142, 2015**). Longer term (1 year) stability of these samples was investigated as well. The electrospun drug-loaded samples were stable after 1 year storage. (Démuth et al.: Detailed stability investigation of amorphous solid dispersions prepared by single-needle and high speed electrospinning, **INT J PHARM 498: (1-2) 234-244, 2016**)

Downstream processing of nanofibers to generate tablet formulations

After the scaled-up production of amorphous solid dispersions by the means of electrospinning another challenge is the downstream of the produced fibrous material to tablet formulation. Thus we reviewed the achieved results in this field (Démuth et al.: Downstream processing of polymer-based amorphous solid dispersions to generate tablet formulations, **INT J PHARM 486: (1-2) 268-286, 2015**)

After the literature review we developed tablet formulations from electrospun amorphous solid dispersions. DC technology could be used for tablet formation and scaled-up tableting was also performed. (Démuth et al.: Lubricant-Induced Crystallization of Itraconazole From Tablets Made of Electrospun Amorphous Solid Dispersion, **J PHARM SCI 105: (9) 2982-2988, 2016**)

Investigation of membrane transport from supersaturated solutions

Last but not least we investigated the membrane transport of drugs from supersaturated solutions obtained using electrospun ASDs. The difference in degree of supersaturation (defined as the ratio of dissolved amount of the drug to its thermodynamic solubility in the actual solution) between the donor and acceptor side, was found to be the driving force of membrane transport. (Borbás et al.: Investigation and mathematical description of the real driving force of passive transport of drug molecules from supersaturated solutions, **MOL PHARM 13: (11) 3816-3826, 2016**)
(Borbás et al.: In vitro dissolution–permeation evaluation of an electrospun cyclodextrin-based formulation of aripiprazole using µFlux™, **INT J PHARM 491: (1-2) 180-189, 2015**)