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FORMULATION OF FUROSEMIDE-LOADED ELECTROSPUN NANOFIBROUS BUCCAL DRUG DELIVERY SYSTEMS

Ph.D. thesis

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LIST OF ABBREVIATIONS

API	Active Pharmaceutical Ingredient
ASD	Amorphous Solid Dispersion
ATR-FTIR	Attenuated Total Reflectance - Fourier Transform Infrared Spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy
ANOVA	One-Way Analysis of Variance
BCS	Biopharmaceutical Classification System
CD	Cyclodextrin
DDS	Drug Delivery System
FUR	Furosemide
GI	Gastro-Intestinal
GIT	Gastro-Intestinal Tract
HPC	Hydroxypropyl Cellulose
logP	Logarithm of Partition Coefficient
MDR1	Multidrug Resistance P-Glycoprotein
NaOH	Sodium Hydroxide
NF-FUR-NaOH	Furosemide-, and Sodium Hydroxide-Containing Nanofibrous Sample
NF-FUR-TEA	Furosemide-, and Triethanolamine-Containing Nanofibrous Sample
NF-PLACEBO-NaOH	Sodium Hydroxide-Containing Nanofibrous Sample Without Furosemide
NF-PLACEBO-TEA	Triethanolamine-Containing Nanofibrous Sample Without Furosemide
***o*-Ps**	o*rtho*-Positronium
PALS	Positron Annihilation Lifetime Spectroscopy
PgP	P- Glycoprotein
PM-FUR-HPC/PVP	Physical Mixture of Furosemide and Fiber-Forming Polymers

PM-FUR-HPC/PVP-TEA	Physical Mixture of Furosemide, Triethanolamine and Fiber-Forming Polymers
PM-HPC/PVP	Physical Mixture of Fiber-Forming Polymers
PM-HPC/PVP-TEA	Physical Mixture of Triethanolamine and Fiber-Forming Polymers
PVP	Poly(vinylpyrrolidone)
SEM	Scanning Electron Microscopy
TEA	Triethanolamine
XRD	X-Ray Diffraction

1. INTRODUCTION

1.1. Challenges in the Pharmaceutical Developments

Pharmaceutical developments have high costs, take a long time and full of challenges and a higher or lower level of risks. These challenges are not just in case of original drug developments but also in the case of added value medicines. Nowadays, most of the potential drug candidates have poor water solubility and poor permeability owing to the high throughput screening and combinatorial chemistry (1, 2). Sometimes these molecules require newer and more complex technologies for a proper formulation (3, 4). Some of these technologies can take place in the pharmaceutical industry due to the up-scaling possibility (4). On the other hand, the possibility of the upscaling is not enough, to develop a unique formulation it is also needed more depth academic research for a better understanding of the new technologies, a widespread knowledge of the excipients, the knowledge about the human body and the knowledge and consideration of the regulations (4-8).

1.2. Biopharmaceutical Classification System (BCS)

The Biopharmaceutical Classification System (BCS) was made by Amidon et al. in 1995. BCS is about the classification of the drugs from the point of their solubility and permeability. They created four groups based on these parameters (9).

- Class I: high solubility – high permeability
- Class II: low solubility – high permeability
- Class III: high solubility – low permeability
- Class IV: low solubility – low permeability

The importance of the BCS is to predict the *in vivo* behaviour of the active pharmaceutical ingredient (API) based on their solubility and permeability. It can help to the pharmaceutical developments during the formulation developments, the developments of *in vitro* absorption predictor tests after a peroral application of the drug and also to the *in vitro-in vivo* correlation (10, 11).

The US Food and Drug Administration defined the official testing parameters during the examination of the water solubility and permeability of the drugs (12).

1.2.1. Solubility

In the BCS the solubility is always the water solubility. The test is conducted under the following conditions: the highest dose of the drug (immediate release dosage form) in its highest clinical strength, 37 °C, pH 1-6.8 (which is the physiological pH range), max 250 mL solvent (which represents the drug-taking with a glass of water). Good water solubility means that the drug solubility is well in these conditions, and poor water solubility if it is not soluble (12).

1.2.2. Permeability

For the prediction of absorption from the gastrointestinal tracts (GIT), mostly from the absorption from the small intestine, the permeability is of great impact.

According to the U.S. Food and Drug Administration, we are talking about good permeability if the absorption is higher than 85% compared to the intravenous administration (12).

1.3. Pharmaceutical Approaches to Overcome the Disadvantageous Properties of Drugs Belonging to BCS Class II and IV.

1.3.1. Strategies for Dissolution Enhancement

Figure 1 is summarizing the different classes, including some drug examples of the BCS classes and some possible solutions for the BCS classes to reach a better bioavailability of the APIs. There are several strategies for the improvement of the dissolution of drugs belonging to BCS II and IV class. The evolving of the drug effect requires the drug in its dissolved form. On the other hand, sometimes the permeability enhancement challenging also the pharmaceutical experts in the case of BCS III and IV class (9, 13).

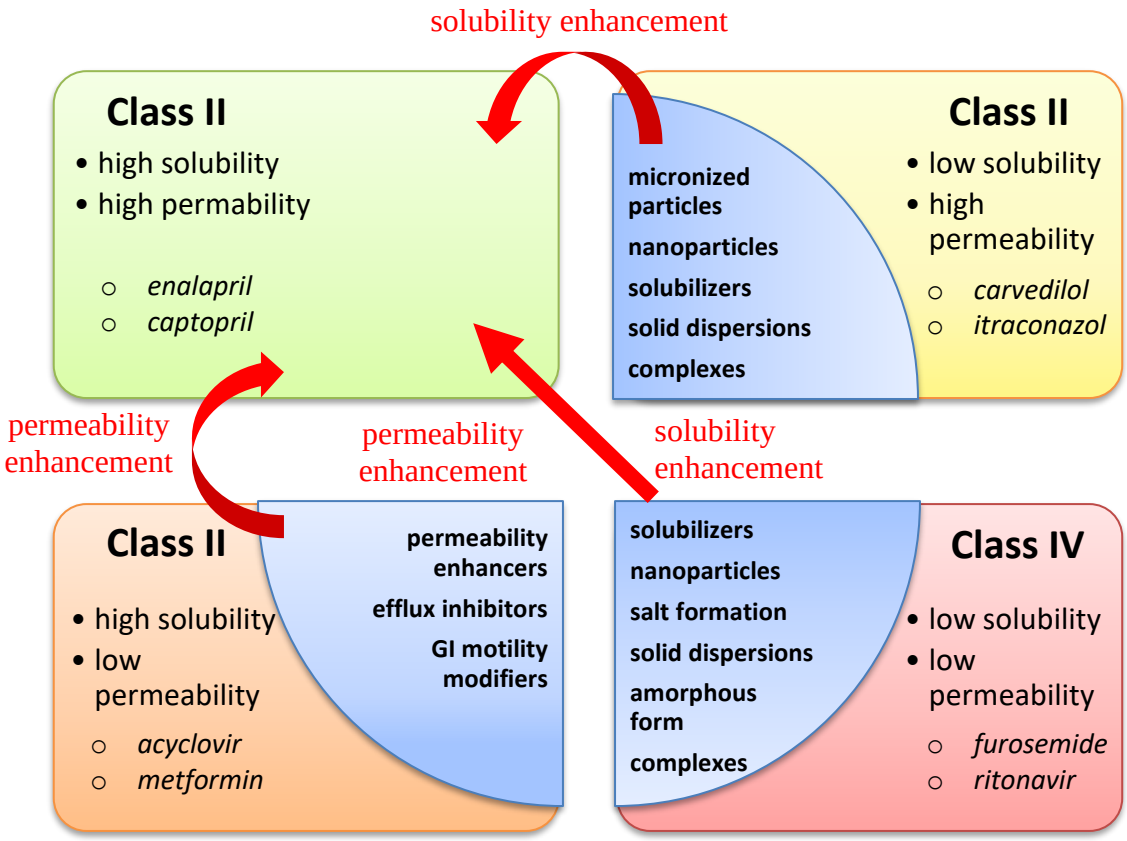

Figure 1 *– Illustration of the Biopharmaceutical Classification System, including some examples of the classes, and the possibilities to improve the bioavailability of the formulations (based on (13)).*

The dissolution enhancement of the poorly water-soluble drug helps also the drug release and absorption. Various physical (e.g.: particle size reduction), physicochemical (e.g.: solubilizing) and chemical methods (e.g.: chemical structure modifying, salt-forming) are available for the dissolution and solubility enhancement, from which the most frequently used listed below, and some of them also directly affect the permeability (14):

- *Modifying the chemical structure of the drug*

  Structure modifying is the competence of chemists; it can be the addition of polar chemical groups or prodrug formation, which means that the active form of the drug is formed in situ in the human body. We should keep in mind to preserve the original effect of the drug, during any type of chemical modification (14).

- *Salt formation*

  In general, during salt formation, the pharmacological effect can remain, and it also should be. On the other hand, it can be a difference in the intensity of the drug effect, so it is inferred different biopharmaceutical and pharmacokinetic properties. Various salt forms are used in the field of pharmaceutical drug development, but the choice of the proper salt form is based on experiments (15-19). It could also have an impact from the point of chemical stability, to avoid the deterioration of the physical properties and the processability. Besides the water solubility enhancement, there could be other reasons for the application of the salt form of the drug, like crystallization difficulties, low melting point, high hygroscopicity, or low chemical stability (15-17).

- *Use of different crystal form of the drug*

  Different crystal forms have different physical-chemical properties, including water solubility, so polymorphism can define the biopharmaceutical properties of the drug (20). Amorphous state is a special crystal form, which is characterized by the lack of molecularly ordered structure, which results higher water solubility. The amorphous form is a thermodynamically unstable state of the drug, which has higher entropy, enthalpy and free energy (10, 21).

- *Solvents and solubilizer excipients*

  Cosolvents are used for solubility enhancement (by changing the dielectric constant of the solution), by adding the cosolvent the polarity of the aqueous system is decreasing; the solubility of the lipophilic drug is enhancing; the solubility of the polar drug is decreasing. Generally applied cosolvents are the followings: propylene glycol, polyethylene glycol, ethanol, sorbate. On the other hand, by diluting the solvent mixture, precipitation can occur, and some of the solvents can be a tissue damage effect (22-25).

  In general, the solubilizer excipients are amphipathic molecules, and they have a polar and apolar part and can be forming micelles in an aqueous media, as well. After reaching the critical micelle concentration, the surfactant excipients enhance the water solubility in a high amount, and after that point, the solubility is increasing linearly with the micelle forming. The lower critical micelle concentration value means the more stable formed micelle. Surfactants help not

just the solubilization but also the absorption. In the human body system, cholic acids and salt are also working as surfactants (26, 27).

- *Complex forming*

  Solubility enhancement can be achieved by complex forming-excipients including cyclodextrins (CDs). CD should be highlighted, because of their great role in the innovative formulations. CDs are special complex-forming excipients, which can interact with the guest molecule and in this way enhance its water solubility. The interaction can happen in the inner or on the outer part of the CD. CDs are widely used in combination with other innovative technologies and applied in the various route of application (28-31).

- *Particle size reduction and nanotechnology*

  The solubility enhancement effect takes place in case of the particle size is in nanoscale, when the solid-humid surface area is enhancing, thus increasing the solubility. Particle size reduction technologies and nanotechnology include various methods, such as micronization, nanoprecipitation, milling, high pressure homogenization (32-35).

  Nanomedicine is the applied nanotechnology in the field of medicine, it can be also therapeutic and diagnostic. The dimension of the applied nanoparticles is in the nanoscale range (generally with the size from 10 to 1000 nm), and they can be used not only to increase the solubility of poorly water-soluble drugs but also with the aim of modified drug release, easier membrane transport or even active targeting. The nanoparticles can be divided into two major groups, the first is the polymeric drug delivery systems (DDS), like the polymeric micro-, and nanostructures, microgels, dendrimers, polymer conjugates, and the other group is the nanoparticles formed by a spontaneous association of molecules, like liposomes (4, 36-42).

- *Amorphous solid dispersions (ASDs)*

  The ASD is a dispersion of drug(s) in an inert matrix at solid-state, it can be prepared by several methods such as melting or electrospinning. The ASDs contain also the drug and the carrier in amorphous form, but in 2 phases. On the other hand, there is a subcategory of the ASDs, called “solid solutions” where the drug is molecularly dispersed in the amorphous matrix. The extent and rate

of dissolution from an ASD is higher than from the product which contains the API in its crystalline form. Improved wettability can be achieved with hydrophilic polymer, and the amorphous state of the API can be stabilized in the matrix, which leads to longer stability of the product is contrary to the purely amorphous drugs (10, 26, 43-46).

1.3.2. Strategies for Permeability Enhancement

Lipinski et al. described the "*rule of 5*", which predict the poor permeation or absorption of the drug if two or more states are valid from the followings: there are more than 5 H-bond donors in the molecule, the molecular weight is over 500, the logP value is over 5, and there are more than 10 H-acceptors in the molecule. So the physicochemical properties of the drug fundamentally determine the permeability, however, there are some technological options to increase the permeability (2).

The chemical permeability enhancers are a large group of the permeation enhancer excipients, which work in different ways. Some of them are fatty acids acting by the disruption of the intercellular lipid layer. The different surfactants can extract the membrane components, fluidize the membrane, and creating channels in them. Alcohols can also influence on the membrane by changing the conformation of the proteins. CDs can be also applied in case of permeation enhancement. There are some other ways to achieve a better permeation like physical methods, which mostly applied in the case of transdermal application, but in the case of oral application efflux inhibition or gastrointestinal (GI) motility modulators can be used (47-50).

1.4. Furosemide

1.4.1. Therapeutic Application of Furosemide

Furosemide (FUR) is used as a loop diuretic, mostly in the treatment of hypertension and renal, cardiac or hepatic oedematous status. It is commonly applied in oral and intravenous therapies, and on the other hand, during the traditional administration routes, several systemic side effects occurred including polyuria, mouth-drying, dizziness, GI problems (51, 52). Besides these disadvantages there are other limiting factors in the use of conventional tablets of FUR, like the application in emergency

situations is not suitable, hence its low oral bioavailability and the slow onset of action (even 60 minutes) (53).

1.4.2. Physical-Chemical Characteristic of FUR

FUR is a weakly acidic molecule (Figure 2), which contains hydrogen acceptor functional groups, with 3.5 and 9.9 pKa values FUR has different polymorphic forms, from which there are three crystalline forms, two solvates and an amorphous form (51, 54-59). In the pharmaceutical industry, the use of polymorphic forms of the active ingredients has an important role, because of their different physical-chemical properties, which influence the stability, bioavailability and the onset of the side effects (60, 61).

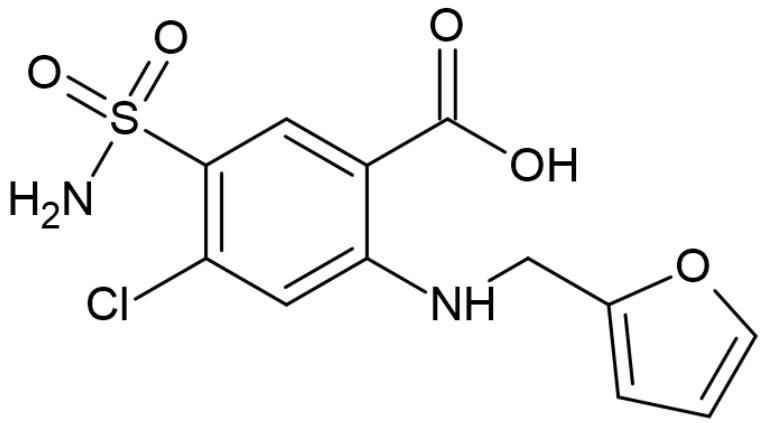

Figure 2*- Chemical structure of furosemide*

1.4.3. Biopharmaceutical Characteristics of FUR

FUR belongs to the BCS IV class, owing to its poor water solubility properties (5-20 μg/mL at pH=7) and the low membrane permeability. Due to the aforementioned properties and the low and variable absorption (stomach and upper part of the duodenum) from the gastrointestinal tract (GIT), and also because of the presence of the intestinal efflux proteins (62), FUR has poor bioavailability that makes its therapeutic use difficult (51, 54-59). The cause of the variable absorption in the GIT is the different amount of expression of the MDR1 (*multidrug resistance* P-glycoprotein; PgP) efflux proteins, and the ionization rate differences in the different parts of the duodenum. As a consequence, the absorption in the ileum is 2.5 times slower than in the jejunum (62).

1.4.4. FUR-Containing DDSs

Among with the FUR-containing medicines mostly tablets (40 mg, 80 mg, 500 mg) injections and oral solutions are registered by the authorities in Hungary (63) and also in worldwide (64, 65). However, there are several approaches to develop new special formulations of FUR with the aim of bioavailability enhancement. Some of these formulations are fast dissolving tablets (53), supramolecular complexes (56, 59), products by melt extrusion (66), micro/nanoparticles for drug delivery (67), mucoadhesive microspheres (55), halloysites (68) and there are also nanofibrous formulations (69).

The mucoadhesive buccal DDSs could be promising options for the formulation of drugs with low bioavailability such as FUR. The buccal application can be usable in clinical emergency situations, without invasive administration or it can be also a good opportunity in the paediatrics fields. For paediatric use, there are not available FUR-containing solid formulations in proper doses in the markets (70). The transmucosal applications like buccal formulation provide an opportunity for enhancing the bioavailability of FUR, by circumventing the first-pass metabolism, so the decreasing of the applied dosage can be possible. On the other hand, it is also needed some further problem-solving regarding permeability enhancement (47, 71, 72).

Electrospun nanofibrous DDSs are offering some advantages of nanotechnology and the ASD which made them promising alternative formulations in the case of FUR or other drugs from the BCS IV class. Their advantages, like high porosity, high surface to volume ratio, the preservation of the drug in amorphous form, thus enhancing its solubility (69, 73-75) and the addition of non-ionic surfactants either can improve the solubility and the permeability of FUR.

A FUR-loaded buccal nanofibrous DDS combines the beneficial properties mentioned above, which may offer a new, even more favorable opportunity to use a low-dose paediatric formulation (69, 76-78).

1.5. Buccal DDSs

1.5.1. Advantages and Disadvantages of the Intraoral Administration of Drugs

The use of buccal administration provides a unique opportunity by circumventing the first-pass metabolism, and the drug can easily take to the systemic circulation as a consequence of the rich vascularization of the buccal mucosa. However, the buccal epithelium also has a barrier function against the microorganism and harmful agents, which makes the penetration difficult. Consequently, the application of permeation enhancers is necessary for the buccal formulations (47, 71, 72).

There are also some advantages of the buccal DDSs over the sublingual administration such as the larger surface area, shorter turnover of the epithelium, higher blood flow, less mobile surface which is suitable for the application of buccal patches for longer time (72, 79, 80).

1.5.2. Anatomical and Physiological Aspects of the Intraoral Route of Administration

The successful formulation of a buccal DDS requires the knowledge of the anatomical properties of the oral cavity, the buccal mucosa and the environmental properties. There are several factors which have to be considered when designing a buccal formulation, such as the presence of the saliva, its volume, the pH, the enzymes in it, the surface, the permeability and the turnover of the mucosa. Saliva is an aqueous fluid which contains only 1-5% organic and inorganic components. The dominant macromolecules in the saliva are the glycoproteins. At the physiological pH range in the oral cavity (pH 5.5 – 7) the mucus forms a strongly cohesive network and hence to the negatively charged sialic acid and sulphate groups it can be bound to the epithelia, creating a covering gel surface (47, 72, 81).

The cells of the oral mucosa build a so-called „leaky epithelia”, and creating non-keratinized epithelia. The buccal epithelia are of a lack of tight junctions, and just looser intercellular connections are there, such as gap junction. The latter indicates that the permeability of the buccal mucosa 4-4000 times higher than of the skin. Thanks to the highly vascularized mucosa, the surface of the buccal mucosa offers a good opportunity for drug transport, by circumventing the first-pass metabolism (47, 72, 81).

1.5.3. Type of the Drug-Loaded Buccal Films

The drug-loaded buccal films can be divided into matrix and reservoir type formulations. In the matrix type formulations, the drug is dispersed in a polymeric matrix and the drug-release occurs by diffusion, while the reservoir types contain a membrane by which modified drug-release can be achieved. Based on the number and the function of the layers of the formulations, other groups can be differentiated:

- *Single layered sheets:* Where the bioadhesive matrix contains the drug and the drug-release is multidirectional.
- *Simple coated sheets:* It is made up of a water-impermeable coated layer and a mucoadhesive matrix, which contains the drug.
- *Coated sheets with unidirectional drug-release*: In this case the bioadhesive drug loaded matrix attached to the buccal mucosa, and every other surface are coated with an impermeable layer.

The choice and the application of these different types of buccal formulations based on the expected effects, side effects but also the taste of the API (48, 72, 81-83).

1.5.4. Requirements for Buccal Sheets

Several requirements must be considered, when developing a buccal formulation, including stability, safety, not irritability, provide the wanted drug release, and permeability, it should be tasteless and odourless and it can be able to adhere to the mucosa (47).

1.5.4.1. Permeability

Permeability is a key issue as the mucosa also performs a barrier function, protecting the body against various microorganisms, so it is necessary to increase the permeability with penetration enhancers to achieve the appropriate absorption of the drug. The most widely used permeation enhancers are surfactants, bile salts chelators, CDs, fatty acids, thiolated polymers. The different permeation enhancers exert their effects in different ways. These ways could be the followings (47, 84, 85):

- *Changing mucus rheology:* by reducing the viscosity of the mucus and saliva
- *Increasing the fluidity of lipid bilayer membrane:* by disturbing the intracellular lipid packing, thus the intracellular transport of the drug can be increased

- *Acting on the desmosomes*
- *By overcoming the enzymatic barrier*: by inhibiting the various enzymes in the mucosa, such as peptidases and proteases.
- *Increasing the solubility of the drug:* which leads to increased thermodynamic activity resulting in better absorption (47).

1.5.4.2. Taste-masking

When releasing a drug in the oral cavity, it is important to avoid an unpleasant taste, as this could lead to a decrease in patient compliance mostly in the case of paediatric use. Care should be also taken not to reduce bioavailability with taste-masking. There are some taste-masking methods, such as the application of sweeteners and flavouring agents or reducing the sensitivity of taste buds. Another widely used method is the inhibition of the drug-dissolution in the oral cavity, with some kind of physical covering method, or with complex-forming (77, 86-89).

1.5.4.3. Mucoadhesivity

Mucoadhesiveness is essential for buccal formulations, as it is crucial to held drug-loaded formulation on the surface of the mucosa for a specified time in order to the active substance can be absorbed. Adhesion to the mucosa is mediated through various interactions (electrostatic, hydrogen-bonding, covalent bonding). Several theories have been described in the literature in connection with the forces involved in mucoadhesiveness (wetting theory, electronic theory, adsorption theory, diffusion theory, fracture theory, etc.). The consideration of these factors can help to understand the mechanism and to select the appropriate excipients during the formulation. Factors influencing the mucoadhesiveness of the buccal formulation include the mucus turn-over rate, the applied mucoadhesive polymer and its concentration, molecular weight, the flexibility of the polymeric chains, stereochemistry of the polymer, swelling factor and the pH of the saliva (72, 81, 90, 91).

1.5.4.4. Polymers

The most frequently used polymers for buccal formulations are natural (sodium alginate, hyaluronic acid etc.), semisynthetic (chitosan, hydroxypropyl cellulose, hydroxypropylmethyl cellulose) and synthetic polymers (poly(vinyl alcohol,

poly(ethylene oxide), poly(acrylic acid)-based polymers, poly(vinylpyrrolidone)). The polymer choice contributes to the mucoadhesivity, and thus allows the API-containing formulation to be present on the buccal mucosa for a sufficient time, so the transmucosal absorption of the drug can take place (47, 81, 90, 91). Most of the polymers mentioned above are suitable for electrospinning as well (73, 75, 92, 93), which could be a good opportunity to combine the advantages of electrospinning and the buccal application.

1.6. Electrospinning

The first study of micro and nanofibrous structures as DDSs was in 2002 by Kenawy et al., who incorporated tetracycline-hydrochloride into polymeric nanofibers for dentistry application (94). Since then several publications have appeared year after year, and the micro and nanofibrous DDSs became intensively researched medical and pharmaceutical areas (95-97).

A widespread application of nanofibrous structures in the pharmaceutical and medical fields has become possible due to their unique micro-, and macrostructural characteristics, which allow either the drug delivery in an amorphous state or the tissue replacement hence to their similar structure with the extracellular matrix. The nanofibrous formulations are good options for solubility enhancement of the drugs belongs to BCS II and BCS IV class, even local and systemic effects can be achieved by the proper applications of them. On the other hand, the advantages of the fibrous structures are not just in the pharmaceutical development but also other medical fields with the use of implants, tissue-engineering, wound healing and wound dressings, transmucosal drug-delivery etc (73, 75, 93, 96, 98-101).

1.6.1. Process of Nanofibrous API-Formulation by Electrospinning Technique

Electrospinning is one of the most frequently used fiber forming technology (46, 93, 99, 102). The mechanism of the electrospinning is the following. During the electrospinning, the API, different excipients and generally polymer containing precursors (aqueous or organic) are filled into a syringe which is connected to a needle with specified needle diameter. The system provides a high voltage supply which is connected to the needle and a grounded collector, which is placed in front of the needle, and it is suitable for collecting the forming fibers. The precursor fluid passes through

the needle (and a tube, if it is connected between the syringe and the needle) with the help of a pump and forms a droplet at the tip of the needle. In the case of optimal process parameters, environmental properties and precursor properties, the droplet started to deform. When the electrostatic energy overcomes the surface tension of the liquid the so-called Taylor-cone is formed from the droplet, it starts to elongate, and it makes a continuous fiber. The forming fibers near the needle moving in a straight-line motion while in a longer distance towards the grounded collector the moving changes to whipping motion, and the fibers landed on the collector. During this time, the solvents evaporating from the polymeric jet and the solid product contains the drug in an amorphous state, because there is no time for the crystallization due to the very rapid evaporation and drying (46, 73, 99). The schematic figure of the electrospinning process is placed below, Figure 3.

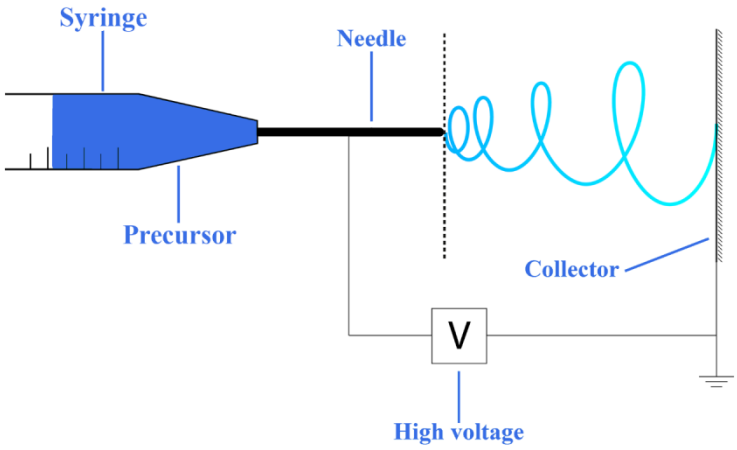

Figure 3 *– Schematic illustration of the electrospinning process*

1.6.2. Characteristics of the Electrospun Fibers

The advantages of the prepared nanofibrous formulations include the preservation of the APIs in their amorphous form, the resulting high surface area, the porous structure that allow the rapid dissolution, the increasing of the apparent water solubility thus enhancing the bioavailability (46, 74). Some new studies describe also the permeability-increasing effect of the drugs from the properly chosen polymeric based nanofibrous DDSs (85, 103, 104). In addition to these beneficial properties, a modified or sustained drug release can be achieved with the help of properly chosen polymers (considering the

hydrophobicity and glass transition temperature (T_g) of the polymer), surface functionalization or with co-axial fiber formation (74, 75, 99, 105).

The fiber formation is one of the technologies by which the API can be preserved in the amorphous state. The process is based on solvent evaporation to form an ASD or amorphous solid solution. The properly chosen polymers allow the drug stabilization in its amorphous form by the reducing of the molecular mobility. The strength and the number of interactions between the polymers and the drugs define the stability of the product, by influencing the molecular mobility. But these interactions are depending on the ratio and the amount of the applied polymer(s) and drug(s). On the other hand, the polymers can be also contributing to the stabilization of the system by their antiplasticizing effect. This means that in the ASDs the T_g of the drug is increasing, while the T_g of the polymer is decreasing, thus stabilize the drug in its amorphous state. So, the choice of a polymer with high T_g can be convenient during the formulation (10, 44-46).

It is important to emphasize, that the ASDs are more stable than the purely amorphous drugs. Therefore, the production of purely amorphous drugs is mostly feasible only on a laboratory scale and shows short-term stability (45), while because of the aforementioned properties the ASDs can stabilize the amorphous drug in a long-term (46).

The effective characterisation of nanofibrous structures requires a multidisciplinary approach using complementary analytical methods. Several micro- and macrostructural characterization methods can be used from this purpose. Morphological studies are essential in the initial stages of nanofibrous product development, such as various microscopic methods (scanning electron microscopy, atomic force microscopy, transmission electron microscopy) to investigate the quality of the fibrous structure (106, 107). Intermolecular information is gained by directly employing techniques such as thermal methods (differential scanning calorimetry, thermogravimetric analysis (108-110)) and X-ray diffraction measurements (106, 110-112), which analyse the sample on the particulate level. Solid-state nuclear magnetic resonance (108, 109), Fourier-transform infrared spectroscopy (110, 111), Raman-mapping (113, 114), positron annihilation lifetime spectroscopy (106) which are primarily intramolecular methods probing the sample at the molecular level.

1.6.3. Influencing Factors and the Possible Errors of the Electrospinning

The electrospinnability and the prepared electrospun fibrous formulation are depending on several properties, including process, material and environmental properties. To achieve an optimal fiber formation it is necessary to find the appropriate settings of the process parameter. The electrospinnability of the precursor means that its composition is suitable for fiber forming in the case of a given device, is determined by the physical-chemical properties of the raw materials and defines the quality of the product. The relative humidity and the temperature should not be neglected during the experimental design in the point of the fiber formation, as the electrospinning is based on solvent evaporation, so these two environmental properties can have a significant effect on the process. Temperature can also influence by modifying the viscosity of the precursor. Some of the most important influencing factors are summarized in Table 1 based on some recent publications (75, 115-117).

Table 1 *– The influencing factors of the electrospinning (based on (75, 115-117))*

Process parameters of the electrospinning	**Properties of the applied raw materials**		**Environmental properties**
flow rate	precursor properties		relative humidity
applied high voltage		rheological properties	temperature
the diameter of the needle		surface tension	
type of the collector		conductivity	
collector-needle distance	polymer(s)		
		chemical structure	
		concentration	
		molecular weight	
	solid content		
	solvent(s)		

Various errors may occur during fiber formation if the choice of parameters is not appropriate. From the appearance of these errors, the existing problem can be deduced, and the abnormal fiber formation can be diminished by eliminating the existing problem, such as low polymer concentration, low or high applied voltage. “Bead-on-string” structure is one of the most characteristic defects in fiber formation, but the ribbon-like structure, fiber-merging, film-forming, or the appearance of spray-dried droplets could also occur. These malformations can be eliminated by changing the composition or the process parameters of the fiber formation in the appropriate direction, thus a homogeneous randomly oriented fiber structure can be achieved (75, 113, 115-120).

2. OBJECTIVES

The aim of the thesis was the formulation and optimization of a FUR-containing buccal nanofibrous DDS. For buccal formulations the proper elastic and mucoadhesive behaviour of the structure is essential, therefore, the polymer choice fell on a hydroxypropyl cellulose (HPC, M_w~80000)-containing composite. To improve the electrospinnability poly(vinylpyrrolidone) (PVP, M_w ~1000000–1500000) was also added to the precursor in different amounts. Further preliminary physico-chemical studies of the precursors were performed to determine the appropriate composition for the electrospinning (121).

The objectives of this part of the thesis were the followings:

- to formulate FUR-containing nanofibrous sheets for buccal administration
- to investigate the correlation between the compositions with different HPC/PVP ratio and the rheological properties of the prepared precursors
- to morphologically and solid-state characterize the different samples
- to predict the electrospinnability based on rheological properties
- to choose the composition with the best fiber-forming ability from the examined formulations based on the preformulation studies.

To develop the chosen composition with different solubility enhancers were applied in a comparative study (122).

The objectives were the followings:

- to prepare sodium hydroxide (NaOH) or triethanolamine (TEA) containing nanofibers
- to morphologically and solid-state characterize the nanofibrous samples containing different solubility enhancers
- to find a correlation between the microstructures and the functionality-related characteristics of the samples
- to examine the effect on the electrospinning and the stability features of FUR-loaded nanofibrous DDSs containing different solubility enhancing excipients.

3. RESULTS

3.1. Preformulation Study

3.1.1. Rheological Properties of the Examined Precursors

Dynamic moduli measurements were carried out to investigate the correlation between the compositions with different HPC/PVP ratio and the rheological properties of the precursors. Figure 4 displays the loss (G")/storage (G') moduli as a function of the HPC/PVP polymer ratio of the examined aqueous precursors for electrospinning.

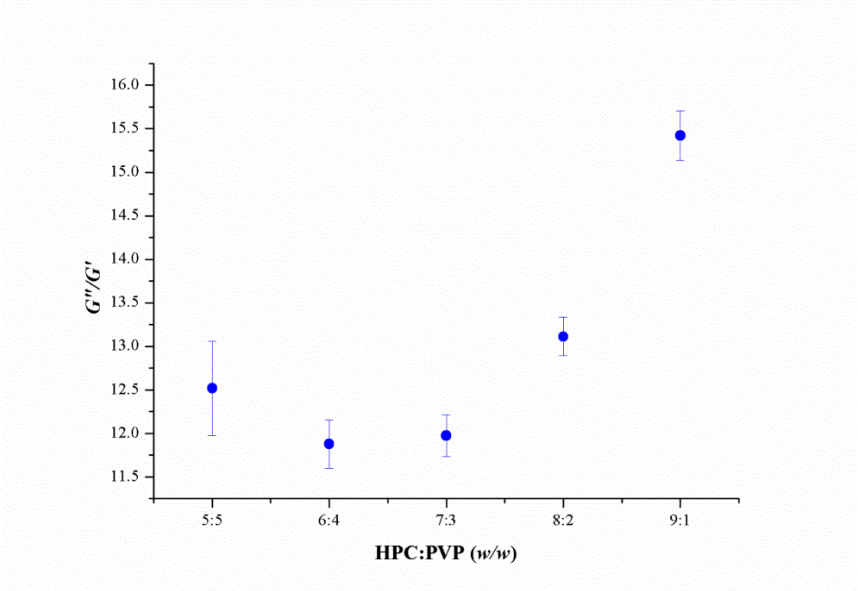

Figure 4 *- The ratio of loss (G") and storage (G') moduli of 1 w/w% FUR-loaded polymeric aqueous precursors as a function of HPC/PVP mass ratio with 15 w/w% total polymer concentration (HPC: M_w~80000, PVP: M_w ~1000000–1500000). Three parallel oscillatory shear measurements were carried out at a frequency 1.995 Hz) (121).*

3.1.2. Solid-State Characterization

3.1.2.1. Morphological Analysis

Morphological analysis was performed in order to observe the *fibrous structure* of the prepared formulations. In the SEM images (Figure 5), nanofibers and spray-dried droplets were distinguished, of which amount is related to the composition of the samples. In Figure 5 A and B we can observe fibrous structures, but the sample corresponding image A (HPC/PVP 5/5) contains more merging fibers, like the sample

of image B (HPC/PVP 6/4), which is more homogenous. In samples C and D (HPC/PVP 7/3, 8/2 respectively) nanofibrous structures and droplets can be found, as well, while in sample E (HPC/PVP 9/1) almost fully spray-dried droplets were prepared.

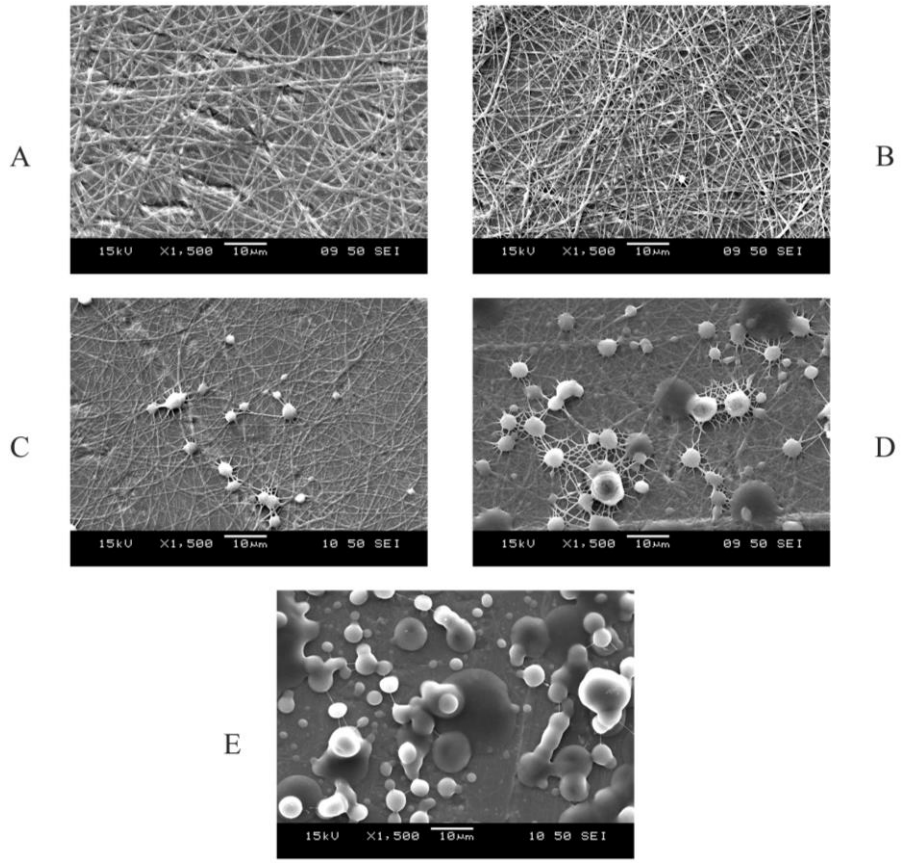

Figure 5 *– SEM images of the fibers consisting of different HPC/PVP mass ratio: (A) HPC/PVP 5/5 mass ratio; (B) HPC/PVP 6/4 mass ratio; (C) HPC/PVP 7/3 mass ratio; (D) HPC/PVP 8/2 mass ratio; (E) HPC/PVP 9/1 mass ratio (121).*

3.1.2.2. Statistical Analysis of the Fiber Diameters

Based on the results of the SEM measurements, only the fibrous samples were used for the statistical analysis of the fiber diameters. As the structure of the sample of HPC/PVP 9:1 molar ratio was almost totally electrosprayed, the statistical analysis was not performed in this case. The distribution of the other four samples (HPC/PVP 5/5; 6/4; 7/3; 8/2 molar ratios) were tested by using Kolmogorov–Smirnov test and the normality was confirmed in all the examined cases (the corresponding p values were 0.964; 0.992; 0.641; 0.410). Significant differences were found ($p < 0.001$) between the distributions of the samples by one-way analysis of variance (ANOVA). Further

pairwise comparisons were performed by using the Bonferroni and Scheffe post hoc tests. The sample of HPC/PVP 5:5 molar ratio was shown a significant difference from the other three samples ($p < 0.001$). The other samples (HPC/PVP 6:4; 7:3; 8:2 molar ratios) were all normally distributed compared to each other. The results of the statistical analysis are illustrated in Figure 6.

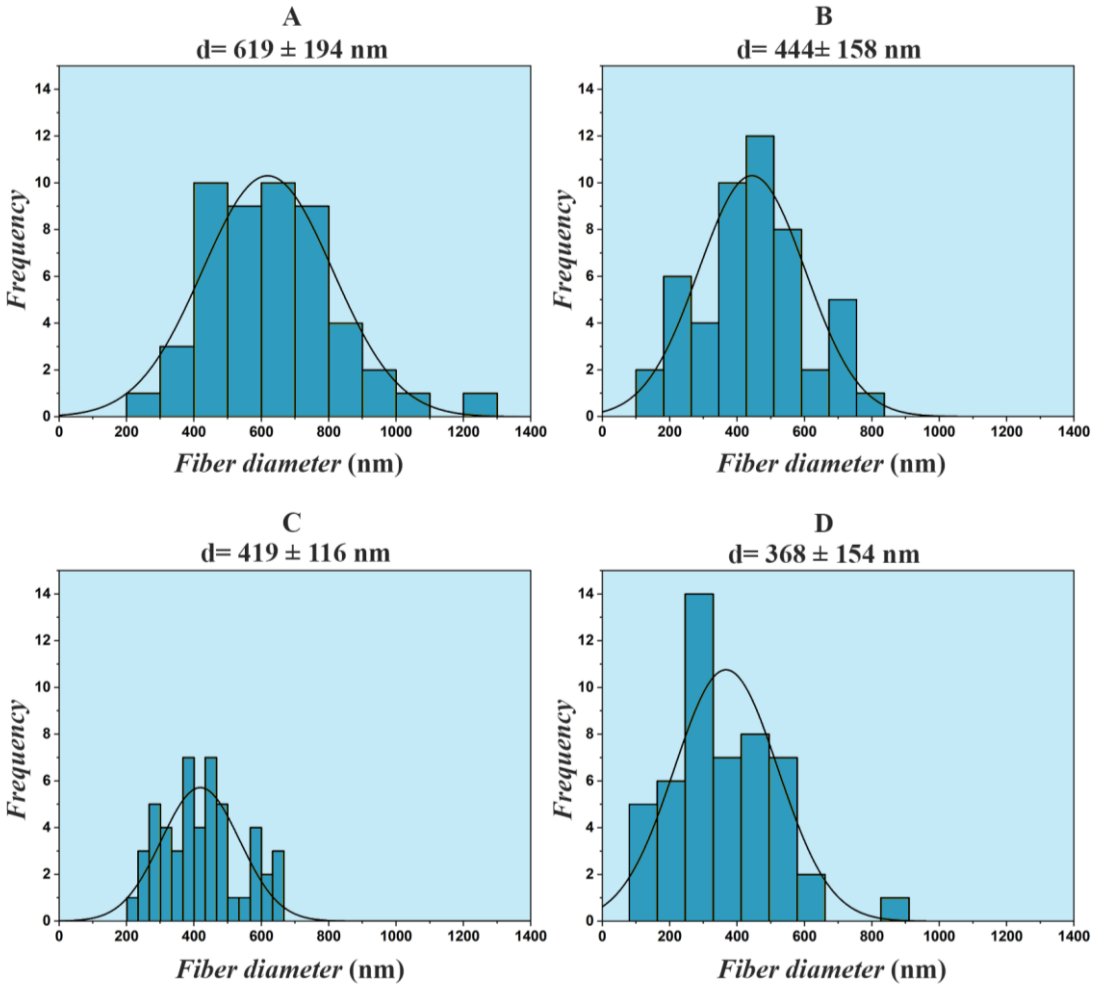

Figure 6 *- Distribution of fiber diameters (fifty individual fiber diameters) of the fibers consisting of different HPC/PVP mass ratio. (**A**) HPC/PVP 5/5 mass ratio; average diameter: 619 ± 194 nm;(**B**) HPC/PVP 6/4 mass ratio; average diameter: 444 ± 158 nm; (**C**) HPC/PVP 7/3 mass ratio; average diameter: 419 ± 116 nm; (**D**) HPC/PVP 8/2 mass ratio; average diameter: 368 ± 154 nm (121).*

3.1.2.3. Attenuated Total Reflectance - Fourier Transform Infrared Spectroscopy (ATR-FTIR) Analysis

To reveal the appearance or the lack of the characteristic peaks belonging to the changes of the secondary bindings in the drug-loaded formulations, FTIR measurements

were applied. Figure 7 shows the FTIR spectra of the raw materials (HPC, PVP, TEA, FUR), the powder physical mixture of the materials, and the prepared formulation of HPC/PVP 6/4 mass ratio. In the range of 3400–3200 cm^{-1} FUR has three characteristic peaks, which belong to the sulphonamide NH stretching at 3397 cm^{-1} and 3281 cm^{-1}, and to the secondary amine NH stretching at 3349 cm^{-1}, which are in good agreement with previous studies (59, 123, 124).These characteristic peaks appear in the powder physical mixture, although these are missing in the prepared fibrous formulation.

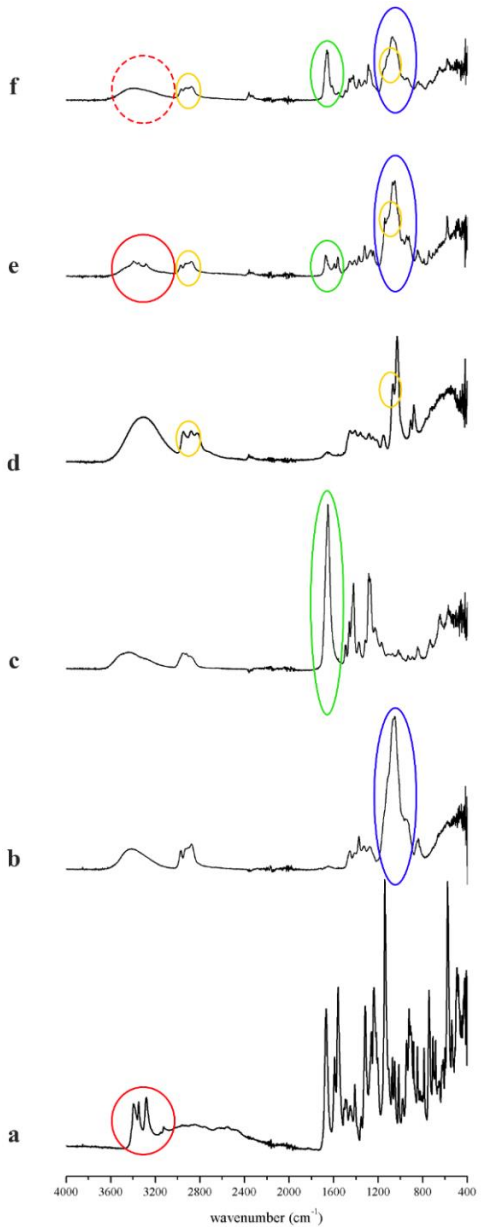

Figure 7 *- FTIR spectra of the raw materials, the FUR-containing physical mixture and the corresponding fibrous sample. (a) FUR; (b) HPC; (c) PVP; (d) TEA; (e) FUR-, and TEA-containing physical mixture (HPC/PVP 6/4 mass ratio); (f) FUR-, and TEA-containing fibrous sample (HPC/PVP 6/4 mass ratio) (121).*

3.2. Comparison Study of Different Solubility Enhancing Excipient-Containing Formulations

3.2.1. Results of the Morphological Analysis

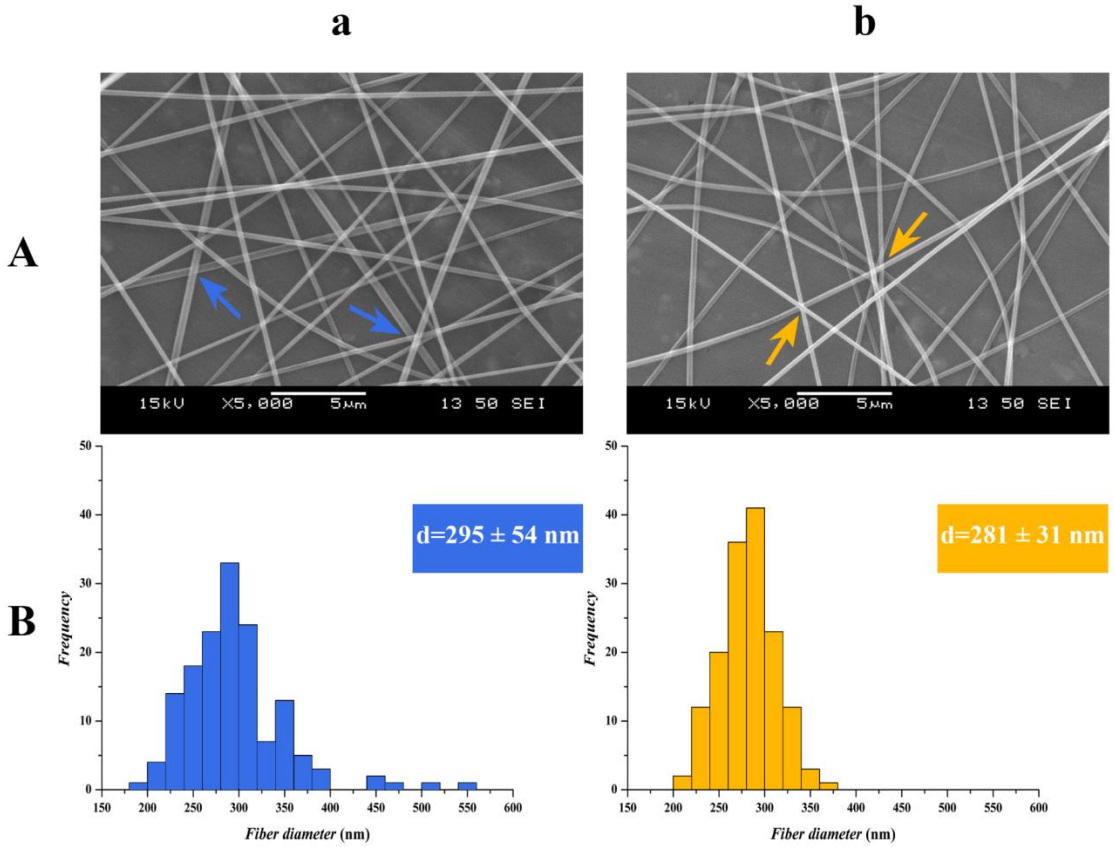

Figure 8 *- (A) Scanning electron microscopic (SEM) images and (B) fiber diameter distributions with the mean fiber diameters of the two drug-loaded nanofibrous formulations; (a) NF-FUR-TEA; (b) NF-FUR-NaOH (122).*

Based on the preliminary studies (121), the chosen composition (HPC/PVP 6/4 molar ratio) was developed with different solubility enhancers (1.5 w/w% TEA or approximately 2 w/w% NaOH (2 M concentration) solution) in the precursors. To define the optimal polymer concentration (17 w/w%) in the case of the NaOH-containing formulation, and to compare the structure of the two different solubility enhancer-containing formulations, SEM measurements were applied for visualizing the possible morphology changes. Based on Scanning electron microscopic (SEM) images (Figure 8A), the nanofibers formed mostly homogenous structure with some beads from both two different compositions of viscous precursors. The average fiber diameter of FUR-, and TEA-containing nanofibers (NF-FUR-TEA) (d= 295 ± 54 nm) and FUR-,

and NaOH-containing nanofibers (NF-FUR-NaOH) were similar (d= 281 ± 31 nm), and statistical analysis confirmed the normality of the fiber diameter distribution (Figure 8B) in case of NF-FUR-NaOH (p=0.909), while NF-FUR-TEA showed approximately normal distribution (p=0.048), confirmed by Kolmogorov – Smirnov test. One-way analysis of variance (ANOVA) was used to determine the possible differences between the two formulations, and it verified the significant differences between the formulations (p=0.041).

The differences between TEA-containing composition and NaOH-containing composition become more dominant during storage (40 ± 2°C, 75 ± 5% relative humidity). Both formulations are started to go through some changes by time. However, it was much more significant change in case of NF-FUR-TEA. The unstored sample of NF-FUR-TEA showed flat ribbon-like structures (Figure 8Aa) at the touching point of the fibers, which were become more extended during storage (Figure 9). While in case of NF-FUR-NaOH the unstored sample showed uniform cylindrical shaped fibers (Figure 8Ab), during the storage time these fibers also started to slightly widening and merging, but these changes were much slighter compared to the more dominant changes of NF-FUR-TEA (Figure 9).

After the first week of storage, the individual fibers of NF-FUR-TEA had already started to be merging and widening, mostly at the contact points of the fibers, while after the fourth week of storage these changes were so significant, that the fibrous structure was disappeared and massive film-forming was observed.

While the fibrous structure of the NF-FUR-NaOH was almost the same as in the initial state after the first week of storage and the fibrous structure was also preserved until the end of the storage time with some widening and merging.

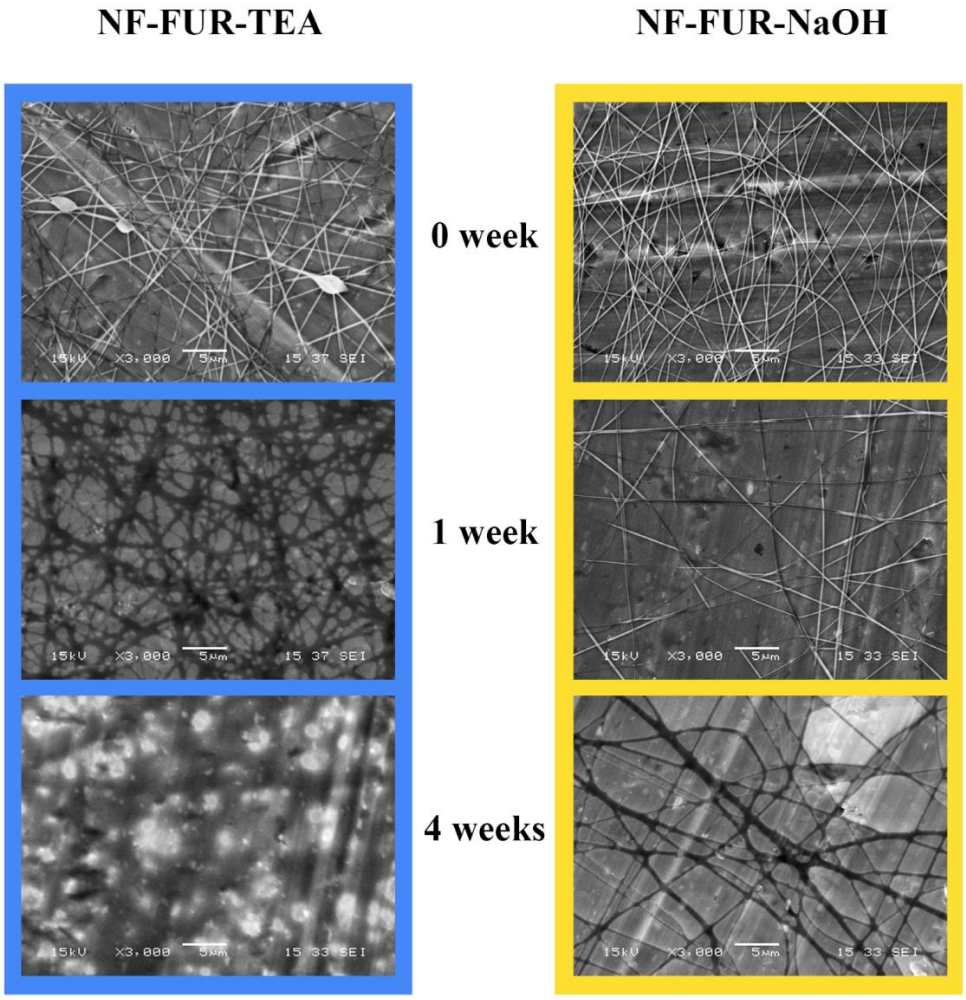

Figure 9 *– Scanning electron microscopic (SEM) images of the fibrous samples without storage and after 1 and 4 weeks of storage in case of NF-FUR-TEA and NF-FUR-NaOH formulations (122).*

3.2.2. Results of the Solid-State Characterisation

3.2.2.1. Results of the X-ray Diffraction (XRD) Patterns of the Fibers

The XRD phase analysis of the examined samples is displayed in Figure 10. The patterns of the pure crystalline FUR showed several sharp peaks. These peaks appear in case of physical mixtures, while in the FUR-containing nanofibrous formulations, even in TEA-, and NaOH-containing samples, these characteristic peaks are missing.

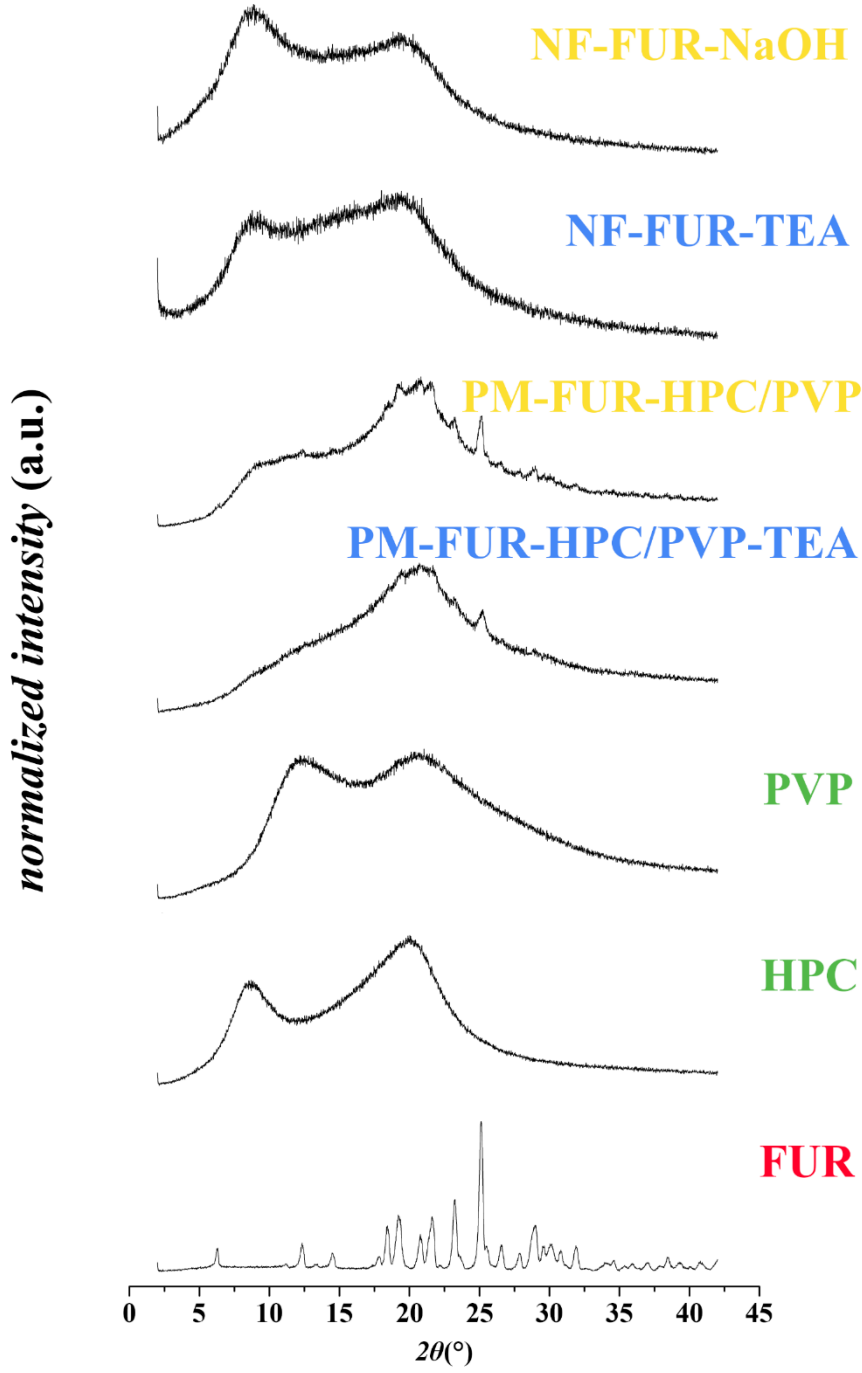

Figure 10 *- X-ray diffractograms (XRD) of various samples: powder substances; physical mixtures and drug-loaded nanofibrous samples (122).*

3.2.2.2. Results of the FTIR Spectroscopic Analysis

To compare the microstructure of the TEA-, or NaOH-containing nanofibrous formulations and to obtain additional information about any possible interactions between the excipients in the formulations, FTIR measurements were performed. The FTIR spectra of the raw FUR, the different physical mixtures with and without drug, and the corresponding nanofibrous samples are displayed in Figure 11 and Figure 12. We can observe several characteristic peaks of FUR not even in the pure crystalline drug, but also in the FUR-containing physical mixtures, although these peaks are disappeared in case of the FUR-containing fibrous samples. The aforementioned characteristic peaks are the followings: NH stretching vibration of the sulphonamide group (3395 cm^{-1} and 3281 cm^{-1} in pure FUR, 3397 cm^{-1} and 3285 cm^{-1} in physical mixtures), NH stretching vibration of a secondary amine group (3348 cm^{-1} in pure FUR, 3349 cm^{-1} in physical mixtures) (59, 123, 124).

On the other hand, the analysis of the fingerprint region reveals some interesting differences between the spectra. In our study, the C=O characteristic peak appeared at 1659 cm^{-1} in the case of FUR-containing NF-FUR-TEA nanofibrous sample and the corresponding neat fibers (NF-PLACEBO-TEA), and 1657 cm^{-1} in FUR-containing NF-FUR-NaOH nanofibrous sample and also in the corresponding neat fibers (NF-PLACEBO-NaOH). Although in the physical mixture of FUR and the fiber-forming polymers (PM-FUR-HPC/PVP) the C=O brand appears at 1667 cm^{-1}. A new peak appeared at 1613 cm^{-1} in the case of NF-FUR-TEA and at 1614 cm^{-1} in the case of NF-FUR-NaOH nanofibrous samples, respectively. However, it can also be observed at 1610 cm^{-1} in the physical mixture of composition-TEA (PM-FUR-HPC/PVP-TEA).

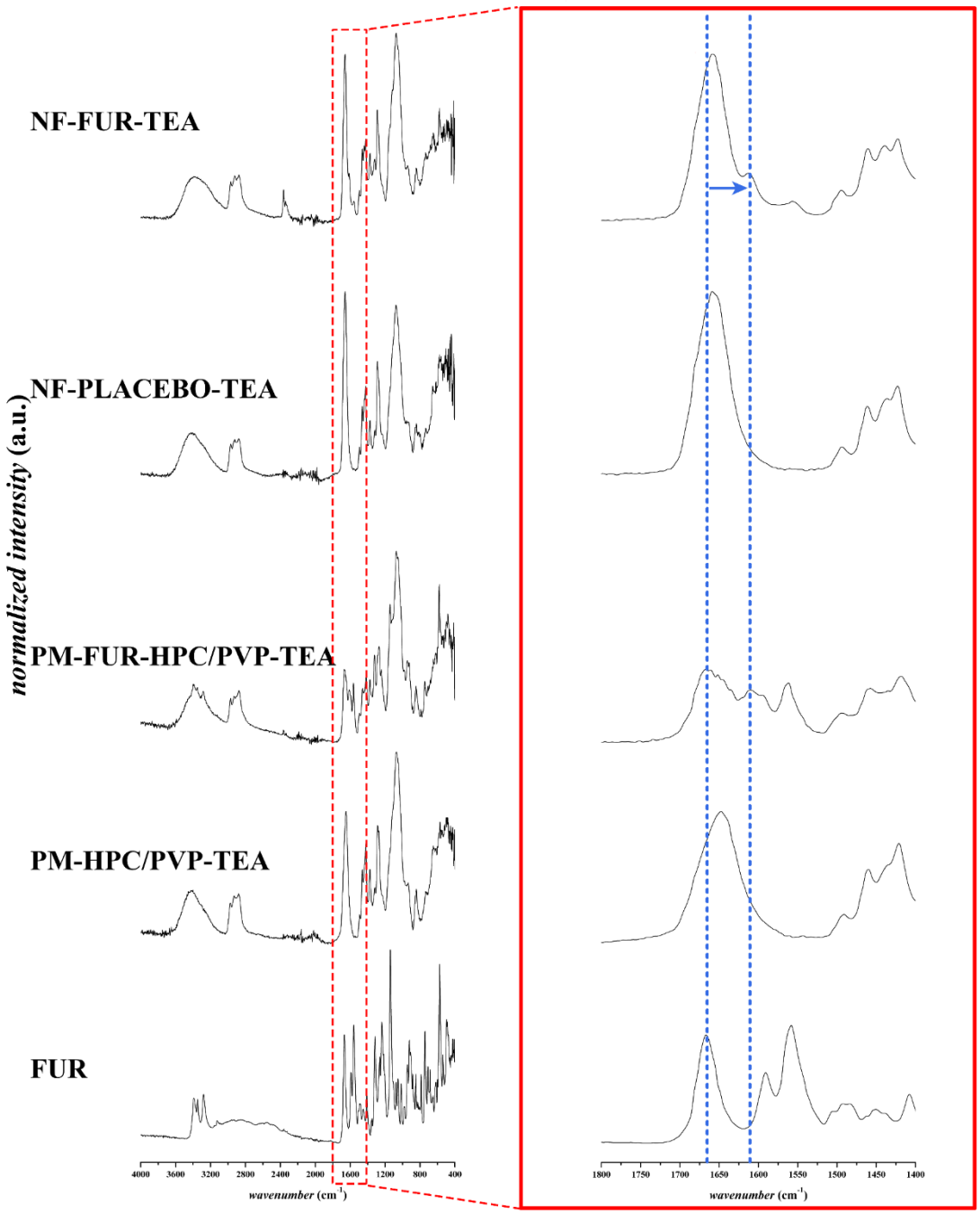

Figure 11 *- Fourier transform infrared spectroscopy (FTIR) spectra of the pure crystalline FUR and the TEA-containing compositions from 400 to 4000 cm^{-1}, and the corresponding spectra in 1400-1800 cm^{-1} range. The shifting peak in the fingerprint region was shown with the blue dashed line and the arrow (122).*

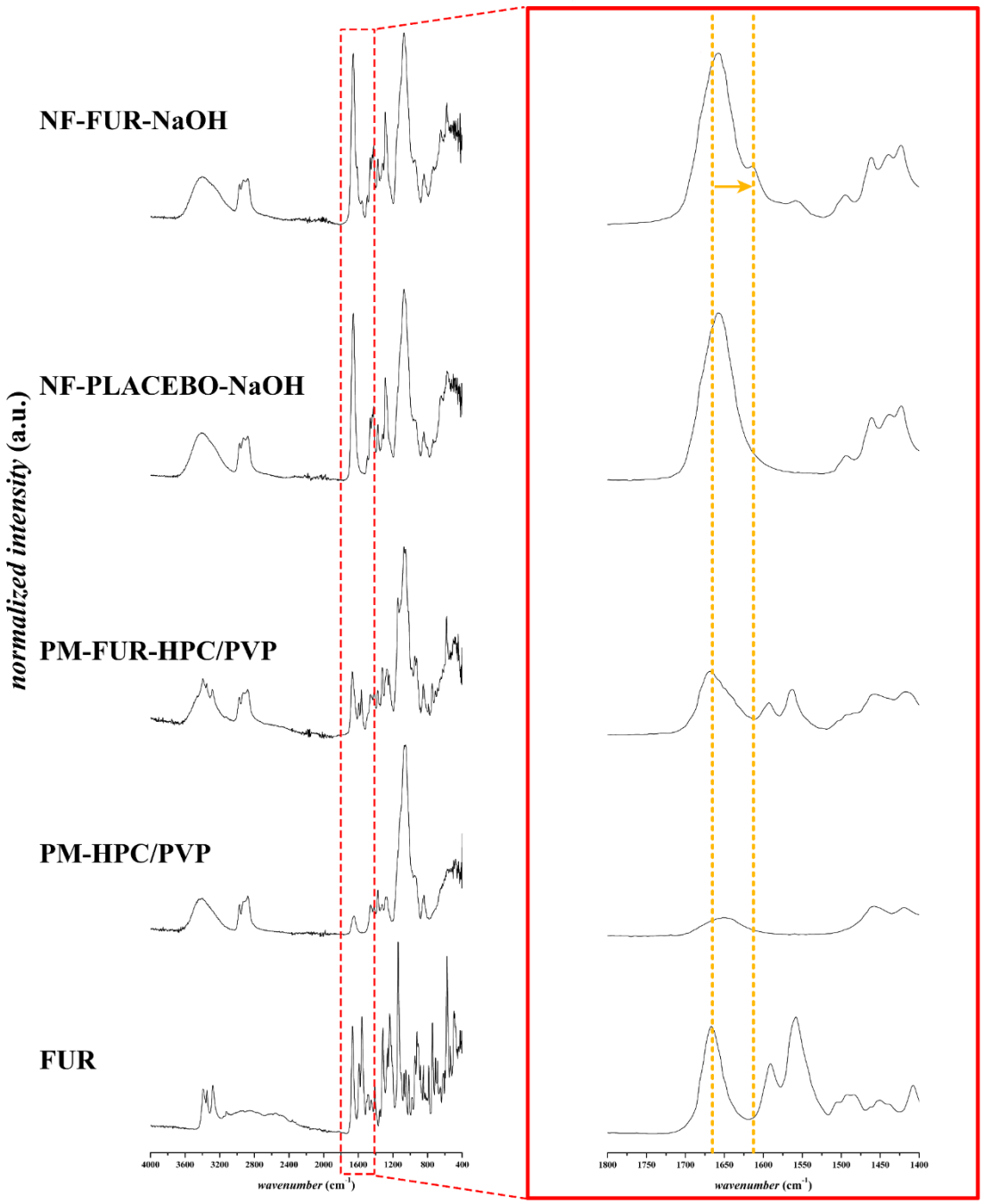

Figure 12 *- Fourier transform infrared spectroscopy (FTIR) spectra of the pure crystalline FUR and the NaOH-containing compositions from 400 to 4000 cm^{-1} and the corresponding spectra in 1400-1800 cm^{-1} range. The shifting peak in the fingerprint region was shown with the yellow dashed line and the arrow (122).*

3.2.2.3. Results of the Positron Annihilation Lifetime Spectroscopy (PALS)

PALS measurements were used to follow the microstructural changes in different formulations and physical mixtures. Figure 13 summarized the data of the average discrete *o*-Ps lifetime values. The *o*-Ps lifetime values were similar to the physical mixtures as they were within the limit of standard deviation and the corresponding nanofibrous samples also show similar values compared to each other. On the other hand, the values of the physical mixtures and the corresponding fibrous formulations were over standard deviation limits. Furthermore, between the TEA and NaOH-containing samples, a slight difference can be observed, since the TEA-containing samples have higher *o*-Ps lifetime values.

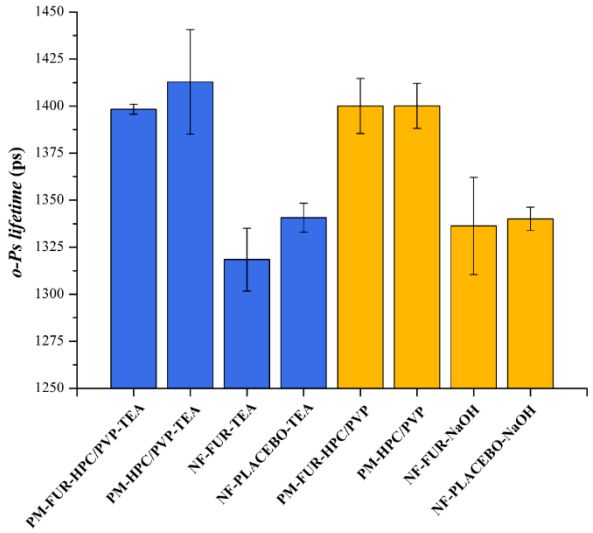

Figure 13 *- Average ortho-positronium (o-Ps) lifetime values (+/-standard deviation) of the physical mixtures and nanofibers of the two different compositions (122).*

Lifetime distribution data are displayed in Figure 14 and Figure 15, where we can define three lifetime components, a short, a medium (positron) and a longer (*o*-Ps) lifetime component. Moreover, the characteristic values of the *o*-Ps lifetime distributions of the examined samples are summarized in Table 2. In order to obtain further information regarding any possible interactions between the applied excipients in different formulations, or slight structural changes, we analysed the intensity of second and the third lifetime component. In the case of the nanofibrous formulation of composition-TEA, the difference between the second and third lifetime components was considerably higher than in the case of composition-NaOH.

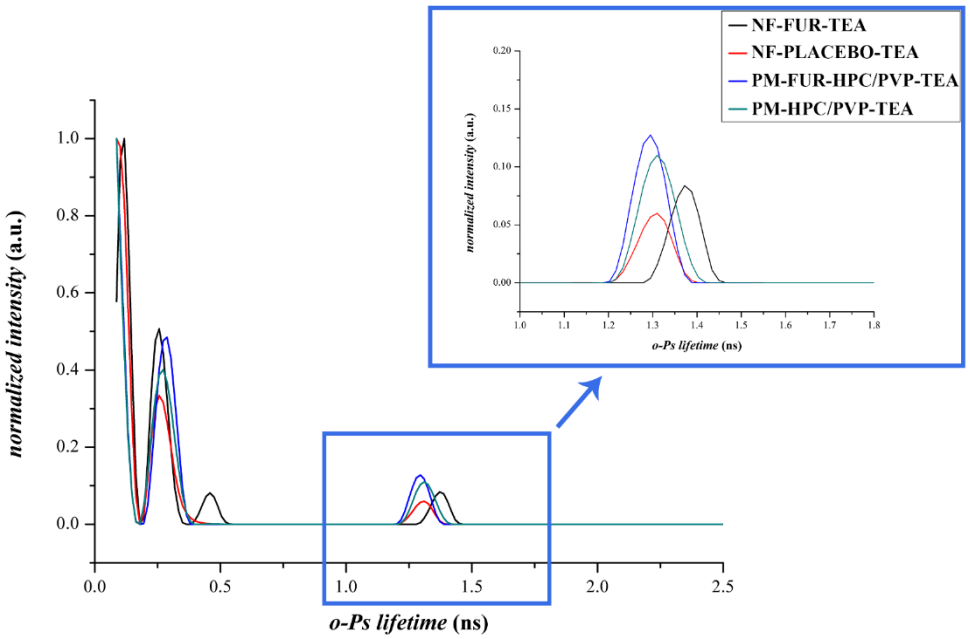

Figure 14 *- Distribution of ortho-positronium (o-Ps) lifetime of the physical mixture and nanofibers of the FUR-loaded, TEA-containing composition and the corresponding placebo (122).*

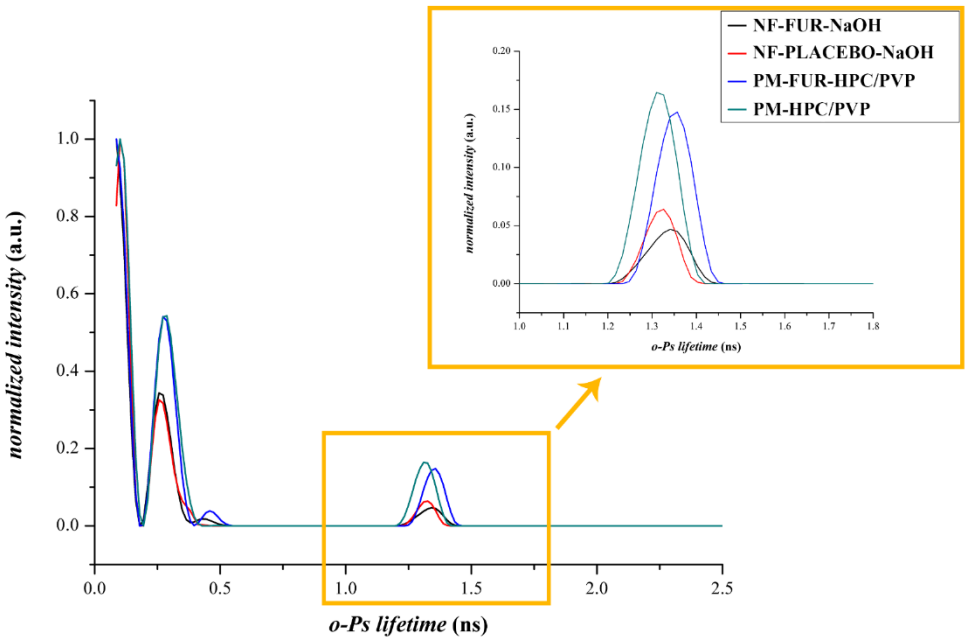

Figure 15 *- Distribution of ortho-positronium (o-Ps) lifetime of the physical mixture and nanofibers of the FUR-loaded, NaOH-containing compositions and the corresponding placebo (122).*

Table 2 *- Summarized the data of the ortho-positronium(o-Ps) lifetime distribution, as the o-Ps lifetime peak maximum, the max intensity (height) of o-Ps lifetime, full width at half maximum (FWHM) and the area under the curve (A) of various samples (122).*

Sample name	*o*-Ps lifetime (ns)	height (a.u.)	FWHM (ns)	A (a.u.)
PM-FUR-HPC/PVP-TEA	1.29325	0.13188	0.08653	0.01215
PM-HPC/PVP-TEA	1.3108	0.11264	0.09452	0.01133
NF-FUR-TEA	1.37286	0.08635	0.07866	0.00723
NF-PLACEBO-TEA	1.30485	0.06128	0.0869	0.00567
PM-FUR-HPC/PVP	1.35086	0.15345	0.09621	0.01571
PM-HPC/PVP	1.31382	0.17043	0.09771	0.01773
NF-FUR-NaOH	1.33622	0.04768	0.10874	0.00552
NF-PLACEBO-NaOH	1.31933	0.06599	0.08334	0.00585

3.2.2.4. Results of the Dissolution Study

The results of dissolution were summarized in Figure 16. The dissolution was rapid and complete within 3 minutes in each electrospun formulations independently of the applied solubility enhancer. In the first minutes the standard deviations of the parallel measurements are relatively high, which may due to the manual sampling and the inhomogeneity of the solutions.

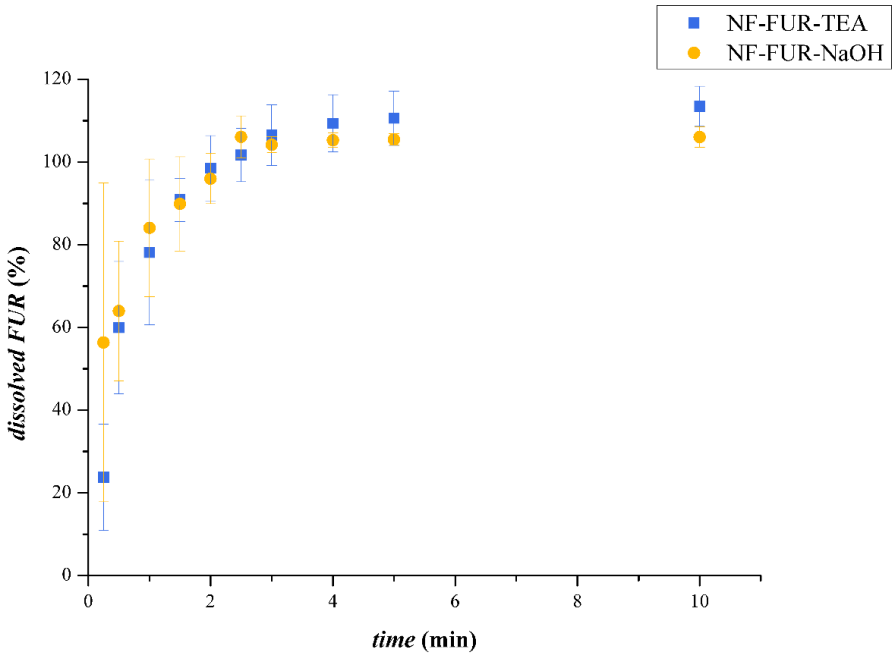

Figure 16 *- Comparison of in vitro dissolution of FUR-containing nanofibrous samples. The dissolution parameters were: 20 ml pH 6.8 KH_2PO_4 (0.025 mol/dm^3) buffer, 37 ± 1°C, 50 rpm stirring rate, n=3 ± SD (122).*

4. DISCUSSION

4.1. Preformulation Studies of the Different HPC/PVP Ratio Aqueous Precursors and the Fibers Made from Them

SEM images show that in the case of the samples with a lower HPC/PVP ratio (Figure 5/A, B), the fibrous structures are more dominant, like in the samples with a higher HPC/PVP polymer ratio, where spray-dried droplets and fibers can be found, as well (Figure 5/C, D, E). As the HPC content of the samples is increasing, the droplets become more and more dominant. Along with the increasing of the HPC ratio in the composites, more droplets formed in the fibrous mats. Based on the SEM images, and the statistical analysis (Figure 6), the sample B (6/4 HPC/PVP) can be considered the most homogeneous fibrous system. This suggests that the HPC/PVP 6/4 ratio is the most suitable concentration for electrospinning from the examined under the given preparation conditions.

The results of the rheological measurements (Figure 4) and the SEM (Figure 5) images of the corresponding electrospun fibrous samples suggest the existence of an optimal viscoelastic range of the polymeric precursors which occurs a better electrospinnability.

These results and the correlation are similar to previous studies (125). The best fiber-forming ability belongs to the most elastic sample (6/4 HPC/PVP) and results in a more homogenous fibrous structure with randomly-oriented individual fibers. In this case, the arrangement of the macromolecules could be more advantageous in the course of the fiber formation. Otherwise, the inappropriate viscoelasticity of the precursors could be disadvantageous for the homogenous fiber formation if during the electrospinning the resulting electrical field is not able to form a properly stable viscoelastic elongation of the polymer jet.

Based on the FTIR measurements (Figure 7) the crystallinity of the FUR cannot be confirmed in the nanofibrous samples, while in the physical mixture of the components the crystalline form of the drug can be distinguished.

4.2. Morphological Analysis of NaOH-, and TEA-Containing Fibers

Based on SEM images (Figure 8), the nanofibers formed mostly homogenous structure with some beads from both compositions. A slight difference can be visually

observed between the two compositions, NF-FUR-TEA and NF-FUR-NaOH and the statistical analysis also confirmed the differences. The differences between compositions become more dominant during storage as both formulations are started to go through some changes (Figure 9). As the unstored sample of NF-FUR-TEA (Figure 8/Aa) showed flat “ribbon-like” structures at the touching point of the fibers, which were become more extended during storage (Figure 9) the reason of that phenomena could be a fiber-to-film transition, which can be explained by the plasticizer effect of the solubilizer excipient TEA (126).

4.3. Solid-State Characterisation and Dissolution Properties of NaOH-, and TEA-Containing Fibrous Formulations

The pattern of the X-ray diffraction phase analysis (Figure 10) showed that the applied FUR is crystallized as Form I in triclinic structure (123, 127). The characteristic peaks of the FUR have appeared in case of the drug-containing physical mixtures which refer to the presence of a crystalline form of the active ingredient. On the other hand, in both drug-containing formulations, the lack of any sharp characteristic peaks in the X-ray patterns indicates the amorphous state of FUR. Not only the signs of the pure crystalline drug disappeared, but neither the signs of the crystalline sodium or the TEA salt appeared in the nanofibrous formulations, which was in good agreement with the results of the previous studies (58, 128).

Based on the results of FTIR analysis (Figure 11 and Figure 12) cannot be observed any sign of the acidic form of the FUR in the fibrous samples, while the spectra of the corresponding physical mixtures of the drug and the polymers, confirm the contrary. These results are in good agreement with the XRD results.

The results of the fingerprint region analysis of the FTIR spectra suggest an amorphous salt formation of FUR in case of both nanofibrous samples (1613 cm^{-1} and 1614 cm^{-1}) and in the TEA-containing physical mixture (1610 cm^{-1}). The latter may be due to the addition and the mixing of the liquid TEA to the powder excipients, which caused a partial salt formation of the drug. In our study the C=O characteristic peak which appeared at 1659 cm^{-1} and 1657 cm^{-1} in case of drug-containing nanofibrous samples and their corresponding neat fibers (NF-FUR-TEA, NF-PLACEBO-TEA and

NF-FUR-NaOH, NF-PLACEBO-NaOH respectively) refers to a C=O group of the applied PVP polymer for fiber-forming

A similar phenomenon was observed by Nielsen et al., who described the amorphous salt formation of FUR by spray drying its aqueous solution of NaOH in contrary to the application of methanol as a solvent. In their study, a peak at 1608 cm^{-1} refers to the formation of an amorphous sodium salt of FUR by using NaOH as a solvent, while in case of methanolic solution, a peak at 1670 cm^{-1} refers to the amorphous free acid form of the FUR (58). Furthermore, Abraham et al. applied TEA for salt formation of FUR and they also describe a new peak at 1612 cm^{-1} in the FTIR spectra, which belongs to COO^- group (128).

The results of the PALS measurements confirmed the difference between the physical mixtures and the corresponding nanofibrous samples, while significant differences cannot be distinguished based on the discrete *o*-Ps lifetime values (Figure 13) between the two physical mixtures or between the two nanofibrous samples. In case of nanofibrous formulations the lower values of *o*-Ps lifetime, compared to the values of physical mixtures, refer to a denser supramolecular structure with smaller average free volume holes owing to the formation of polymer-drug weak bonds (129).

Lifetime distribution data (Figure 14, Figure 15 and Table 2) help to gain more depth information about the interactions between the excipients. These evaluations indicate that TEA- and NaOH-containing nanofibrous formulations differed from each other in their supramolecular interactions. We can observe that there is a much higher difference in case of TEA-containing nanofibers than in case of NaOH-containing nanofibers based on the difference of the second and third lifetime component. This phenomenon may concern the presence of sulphonamide group of FUR. The sulphonamide group could have a role of positronium inhibitor during the measurements. This effect is more pronounced in the TEA-containing formulations, which may refer to a micellar structure of FUR and the solubilizer excipient TEA (130).

The o-Ps lifetime distribution data included in Table 2 indicates that there are differences between the supramolecular structure of the FUR-containing fibrous formulations and the neat fibers. Difference was also found in case of the two FUR-containing fibers. NF-FUR-NaOH formed a more dense supramolecular structure compared to NF-FUR-TEA, which formed a less dense microstructure. The less-dense

supramolecular ordering involves the increasing of the free volume holes between the polymeric chains, which could represent by the drug-solubilizer effect of TEA. The latter can affect the molecular mobility and thus plasticizing the formulation.

The dissolution was rapid from both two different electrospun formulations and it was complete in 3 minutes in each case (Figure 16).

5. CONCLUSION

In the case of a poorly water-soluble and poorly permeable drug, which belongs to the BCS IV class, a buccal formulation provides an opportunity to improve the oral bioavailability by circumventing the first-pass metabolism. From this point of view, the amorphous state of the API also contributes to the enhancement of bioavailability by the increased solubility. To improve the oral bioavailability of soluble FUR, HPC-PVP composite electrospun nanofibrous systems were successfully prepared. The optimal composition was chosen based on the preformulation, rheological and morphological measurements of the precursors and the corresponding fibers, respectively. The obtained formulation could enable transmucosal drug delivery could be a promising formulation for paediatric use.

With the addition of NaOH or TEA as solubility enhancers, amorphous FUR salt-loaded electrospun nanofibers were formed with similar morphology. Based on the *o*-Ps lifetime distributions, differences were observed in the microstructure of the fibrous samples, which indicate various distributions of free volume holes. The different supramolecular arrangements of the formulated drug-loaded fibers may lead to various long-term stability. The NaOH-containing formulation could be a promising alternative for the development of buccal nanofiber-based dosage form containing outer cover sheet to ensure the one-way drug transport toward the oral mucosa

6. SUMMARY

The thesis aimed to prepare FUR-loaded nanofibrous buccal formulations, which could be promising options to improve the bioavailability of the poorly soluble and permeable crystalline FUR, chosen from BCS IV class.

For the electrospinning, HPC was chosen as a water-soluble mucoadhesive polymer, and PVP was also added to the FUR-containing solution to achieve a better electrospinnability. Different formulations were prepared with the change of the HPC/PVP ratio (5/5, 6/4, 7/3, 8/2, 9/1), the total polymer concentration was kept constant at 15% (w/w). Rheological and SEM measurements were performed to define the optimal composition of the aqueous polymeric precursor for fiber-forming. A correlation was found between the rheological properties of the precursors and their electrospinnability. A transition from the spray-dried droplets to the fibrous structures was observed by decreasing the HPC ratio of the samples, thus indicating a better fiber-forming ability. The FTIR spectra revealed no evidence of crystalline FUR in either sample. Based on the results, the appropriate ratio of HPC/PVP at 15% (w/w) total polymer concentration was determined to 6/4 and selected to improve the DDS.

Further comparative examinations were carried out to choose an additional solubility enhancer excipient. In one case TEA was used as a solubilizer, while in the other NaOH was applied, as a pH-modifier. The SEM morphological characterization of the fibrous structures revealed a similar mean fiber diameter. However, further statistical analysis confirmed the differences between them. The morphological differences between NF-FUR-TEA and NF-FUR-NaOH become more dominant during storage, which can be explained by the plasticizing effect of the TEA. For microstructural characterization of the prepared formulations, FTIR, XRD and PALS measurements were performed. The XRD measurements did not confirm the presence of crystalline FUR in the samples. The results of the FTIR analysis indicate the salt formation of the FUR in both cases. On the other hand, the PALS measurements revealed differences in the average *o*-Ps lifetime values and the distributions of the FUR-containing fibrous formulations.

The two amorphous FUR salt-loaded electrospun nanofibrous formulations showed similar macrostructural, but different microstructural characteristics depending on the type of the applied solubility enhancers, thus leading to altered long-term storage stability.

7. REFERENCES

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8. LIST OF PUBLICATIONS

8.1. Publications Relevant to the Dissertation

I. Kovács A, Démuth B, Meskó A, Zelkó R. (2017) Preformulation Studies of Furosemide-Loaded Electrospun Nanofibrous Systems for Buccal Administration. Polymers, 9: 643.
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II. Kovács A, Palcsó B, Zelkó R. (2018) Elektrosztatikus szálképzéssel előállított szálas hatóanyag-hordozó rendszerek vizsgálatának lehetségei. Acta Pharm Hung, 88: 27-44.

III. Kovacs A, Kazsoki A, Demuth B, Sziranyi B, Madarasz J, Suvegh K, Zelko R. (2020) Influence of Aqueous Solubility-Enhancing Excipients on the Microstructural Characteristics of Furosemide-Loaded Electrospun Nanofibers. Pharmaceutics, 12: 385.
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