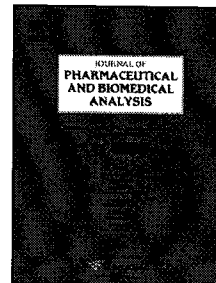


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**Tracking of crystalline-amorphous transition of carvedilol in rotary spun
microfibers and their formulation to orodispersible tablets for in vitro
dissolution enhancement**

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Highlights

- Carvedilol loaded microfibers were prepared via high speed rotary spinning
- Microfiber based orodispersible tablets were manufactured by direct compression
- Carvedilol embedded in microfibers was in amorphous state
- Dissolution improvement of drug was observable in case of microfiber based tablets
- Carvedilol loaded microfibers have shown high stress tolerance capacity

ABSTRACT

Physicochemical characterization of microfibers including powder X-ray diffraction, differential scanning calorimetry, attenuated total reflectance Fourier transform infrared spectroscopy, and positron annihilation spectroscopy were used to track the crystalline-amorphous transition of carvedilol during formulation and stability testing. The applied methods unanimously indicated the amorphous transition of carvedilol in the course of rotary spinning, furthermore a supramolecular ordering of chains of polymer matrix was revealed out by positron annihilation spectroscopy. The accelerated stability study (40 ± 2 °C/ $75 \pm 5\%$ RH, for 4 weeks) indicated a large stress tolerance capacity of fibers, since only a partial crystallization of the active compound was observable at the last sampling point. To demonstrate possible utilization of microfibers, orodispersible tablets containing 10 mg of carvedilol were successfully prepared by direct compression applying common tableting excipients. All of the investigated tablet parameters (hardness, friability, in vitro disintegration time) complied with the pharmacopoeial requirements. The performed dissolution (pH 1.0 and 6.8) study indicated that the drug dissolution from the microfiber based formula was rapid, complete and independent from the pH of the applied media, while the dissolution from the control tablets, containing crystalline carvedilol was incomplete and was strongly influenced by the pH of the applied media.

Keywords: FTIR; X-ray diffractometry (XRD); positron annihilation spectroscopy (PALS), differential scanning calorimetry (DSC), rotary-spun microfiber, dissolution enhancement

1. Introduction

The increasing presence of molecules of undesirable physicochemical characteristics among new drug candidates forces pharmaceutical industry to consider new technological approaches during drug development [1, 2]. Poor water solubility and the consequent insufficient extent of oral bioavailability are of the most urgent issues to be handled in pharmaceutical research.

This troublesome phenomenon has been recognized as the consequence of molecular obesity, which term refers to the flourishing proportion of large and lipophilic molecules among active pharmaceutical ingredients [3, 4]. Dissolution enhancement is generally accepted as a useful way for the formulation of pharmaceuticals involved in biopharmaceutical drug classification system (BCS) class II, where oral bioavailability is confined by the limited aqueous solubility [5].

During the past decade several types of techniques and dosage forms, such as hot melt extrusion, nanostructured lipid carriers have been developed and investigated thoroughly in order to improve drug dissolution and oral bioavailability [6-8]. Recently, an increasing interest has been paid to polymeric micro- and nanofibers as potential drug delivery systems owing to their high specific surface area, high porosity, and the ability to incorporate pharmaceuticals in amorphous state [9]. Electrospinning is the most studied technique used for fiber production, where the formation of fibers is induced by the high voltage applied on the polymeric solutions [10]. Although this method provides a great control over fiber characteristics, the limited productivity is one of its major disadvantages [11]. In high-speed rotary spinning, where polymeric solution is put into a rotating reservoir, the fiber formation

is induced by the formed centrifugal force. The centrifugal force presses the polymeric solution through the orifices on the wall of the spinneret, the intensive solvent evaporation facilitates the fiber formation [12]. Both aqueous and organic solutions can be applied, and high-speed rotary spinning as well as electrospinning is capable for the preparation of micro and nanofibers. Polymer concentration of the solution, the solvent employed and rheological-textural properties are the key parameters affecting the fiber formation [12, 13].

Since the stability determines the functionality related characteristics, such as dissolution profile, the physical and chemical stability of different dosage forms is a pivotal issue. Crystallinity of the active compound and the supramolecular structure of the polymeric matrix, thus the size of free volume holes are the crucial parameters in case of drug loaded fibrous systems. It has been pointed out in previous papers that the polarity of the employed solvent mixture and the solubility of the active ingredient, the polymer crystallinity, as well as the chosen process parameters have an impact on the crystallization of the drug in the course of electrospinning [14-16]. On the other hand, aging of the polymeric matrix can also result in the alteration of the drug release kinetics; therefore it is essential to obtain structure-related characteristics of the polymer during stability test. In preceding studies, Zelkó et al. have demonstrated that by means of positron annihilation spectroscopy, the aging of polymeric excipients can be tracked by the following up of the size associated changes of the free volume holes [17-19]. The changes in crystallinity and supramolecular structure can engender the alteration of the intermolecular (drug-polymeric and polymer-polymer) interactions, which can be tracked by Fourier transform infrared spectroscopy (FTIR) [20].

Carvedilol is a nonselective beta and alpha-1 adrenoceptor antagonist drug, which is used in hypertension, coronary artery disease and reduces morbidity and mortality in patients with chronic heart failure [21-24]. The oral bioavailability of carvedilol (below 25 %) is strictly limited due to its poor aqueous solubility ($27.11 \pm 1.14 \mu\text{g/ml}$ in pH 6.8 buffer) and the

intensive first-pass metabolism [25, 26]. Several formulation approaches can be found in the literature focusing on the dissolution improvement of carvedilol. Planinška et al. have developed a porous silica based solid dispersion, where the improved dissolution was attributed to the amorphous state of the pharmaceutical [27]. Formulation of a surfactant (lauroyl polyoxyglycerides) based solid dispersion of the active compound also resulted in enhanced solubility and dissolution rate [28]. It was found that augmentation of bioavailability can be achieved by the incorporation of carvedilol into self-nano-emulsifying drug delivery systems or solid lipid nanoparticles [29]. Solid lipid nanoparticles are nanosized lipid spheres consisting of a solid lipid matrix have also emerged as a promising solution for the bioavailability issue [30, 31].

Drug administration is often confined in certain populations, e.g. in elderly, due to the physiological and morbidity related changes, resulting low adherence [32]. Reduced dexterity and difficulties in swallowing by cause of mucosal degeneration or decreased saliva production are considered as common problems in geriatrics [33]. Numerous advantages, such as rapid disintegration, easy administration, improvement in oral bioavailability make the formulation of orodispersible tablets a reasonable choice [34].

The present study aims at the formulation of drug loaded rotary spun microfiber based orodispersible tablets containing carvedilol, which belongs to the biopharmaceutical classification system class II, in order to enhance its dissolution. Moreover, the authors' intention was to track the physical stability changes of the prepared fibers under stress conditions with complex characterization of drug loaded microfibers applying the combination of four different physico-chemical characterization methods (powder X-ray diffraction, differential scanning calorimetry, attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy, and positron annihilation spectroscopy (PALS)).

Complex physico-chemical characterization was applied in order to explore the supramolecular changes of drug-loaded rotary spun microfibers in the course of storage.

2. Materials and methods

2.1 Materials

Hydroxypropyl cellulose (Klucel[®] ELF, Mw ~ 40000, moles of substitution of 3.8, Ashland, USA), citric acid monohydrate (Molar Chemicals, Hungary) was used in the fiber formation process. Carvedilol (EGIS Pharmaceuticals Plc, Hungary, Ph. Eur.) was selected as a poorly water soluble drug. In the preparation of the carvedilol stock solution diluted ethanol (3:1 volume ratio) was made by the mixing of 75 ml of ethanol (96 % v/v%, Reanal, Hungary) and 25 ml of distilled water. Potassium dihydrogen phosphate (Molar Chemicals, Hungary), sodium hydroxide (Molar Chemicals, Hungary) and distilled water were applied in the preparation of the dissolution media. The excipients of the orally disintegrating tablets were as follows: microcrystalline cellulose (Vivapur[®] 102 MCC) as filler and disintegrant, spray-dried lactose monohydrate (Flowlac[®] 100, Meggle GmbH, Wasserburg, Germany) as filler, magnesium stearate (Ph.Eur. Hungaropharma, Budapest, Hungary) as lubricant, equimolar mixture of milled citric acid anhydride and sodium bicarbonate as effervescent agent and croscarmellose sodium (Vivasol[®], JRS Pharma) as superdisintegrant.

2.2 Preparation of drug stock solution

5.000 g of carvedilol was measured into a 50.00 ml volumetric flask, and then it was suspended with about the half of the necessary amount of diluted ethanol (3:1 volume ratio). When a lactescent dispersion was formed, 2.590 g (equimolar to carvedilol) of citric acid monohydrate was added. The dispersion was carefully shaken until a clear solution was obtained, then the solution was diluted to 50.00 ml with the solvent mixture.

2.3 Preparation of hydroxypropyl cellulose gels

The optimum polymer concentration was given to 50 % w/w which was determined in our previous study by texture analyzer [35]. Gels containing carvedilol was prepared by the addition of the same amount of Klucel ELF hydroxypropyl cellulose and the carvedilol stock solution. After careful homogenization the gels were covered with paraffin tape, and stored at room temperature (25 °C) for one hour. These samples were used in the preparation of the fibers by high-speed rotary spinning.

2.4 High-speed rotary spinning technique

For producing fibers high-speed rotary spinning technique was performed, where the samples were put in a perforated rotating reservoir, which is made of aluminum. The rotating reservoir was powered by a motor WSE 602M (AEG, Germany), and the fiber formation was carried out at 10500 RPM. The rotational speed was controlled with a toroidal transformer and determined with laser revolution counter (DT-10L, Voltcraft, Germany). The internal diameter of the two side wall orifices were 0.5 mm.

2.5 Morphology study

For morphological characterization of the prepared drug loaded fibers, optical microscope and scanning electron microscope were employed. In case of optical microscope (LCD Micro type; Bresser; Germany) magnifications were: 40x, 100 x. The produced digital images were analyzed using the computer program Image Pro Plus 4.5 (Media Cybernetics, Bethesda, U.S.). A standard micrometer scale was used for the calibration. Minimum of 20-25 fibers could be photographed at a time. Fifty measurements were taken and average fiber diameter was obtained from values of diameter of 50 different, individual fibers.

For detailed morphological analysis SEM (Amray 1830-D4, equipped with a tungsten electron gun and EDAX-PV 9800 energy-dispersive spectrometer) was employed. The

applied parameters were as follows: acceleration voltage of 15 kV, beam current of 0.1-0.5 nA . The samples were gold coated with JEOL JEE-4B vacuum evaporator.

2.6 Milling process

Citric acid anhydrate, sodium bicarbonate and the carvedilol loaded microfibers were milled with a Gorenje SMK 150 B grinder for 6 min with 24000 rpm (determined with DT-10L laser revolution counter, Voltcraft, Germany). Afterwards, the milled materials were sieved through a mesh sieve (nominal wire diameter 320 μm).

2.7 Determination of the particle size distribution

A laser scattering particle size distribution analyzer LA-950V2 (Horiba Co., Kyoto, Japan) was used with a dry feeder unit to measure the particle diameter of the grinded excipients, and the milled fibers in the range of 0.011-3000 μm . A measurement was performed with the following parameters: air: 0.1 MPa; feeder intensity (0-200): 80; relative refractive index: 1.60. The distribution values were the averages of 3 determinations of each sample. The results represented as volume distribution of 3x5000 particles. To describe the width of the distribution span values were calculated according to Eq (1):

$$Span = \frac{(D_{90\%} - D_{10\%})}{D_{50\%}} \quad (1)$$

where $D_{10\%}$, $D_{50\%}$ and $D_{90\%}$ are the particle diameters at 10, 50 and 90% of the cumulative particles undersize plot. The results are the averages of five parallel measurements.

2.8 Positron lifetime measurements

For the microstructural characterization of fibers positron annihilation lifetime spectroscopy (PALS) was applied as a unique method since it is exceptionally sensitive to the free volume. The measurement is based on the interaction of the free volume holes and the so-called ortho-positronium atom [36]. Positron as the antiparticle of the electron lives only a few hundred

picoseconds in materials. However, several positrons form ortho-positronium (o-Ps), a hydrogen-like exotic atom, before their annihilation. These positrons live a “very long” time, usually a few nanoseconds. In polymers the formed o-Ps atoms tend to be trapped in free volume holes and their annihilation is not governed by their intrinsic lifetime but by the electron density in the holes. Their lifetime is associated with the size of the free volume around them [37]:

$$\tau = \frac{1}{2} \left[1 - \frac{R}{R + \Delta R} + \frac{1}{2\pi} \sin \left(\frac{2\pi R}{R + \Delta R} \right) \right]^{-1} \quad (2)$$

where τ is the positronium lifetime, R the radius of the free volume hole, and ΔR a constant.

As a very crude guess, we can say that a longer lifetime indicates a larger hole.

For positron lifetime measurements, a positron source made of carrier-free $^{22}\text{NaCl}$ was used.

Its activity was around 10^6 Bq. Lifetime spectra were measured with a fast-fast coincidence system based on BaF_2 /XP2020Q detectors and Ortec® electronics. Every spectrum was recorded in 4096 channels of an analyzer card for 3×900 s and each contained about 1.8×10^6 coincidence events. Three parallel spectra were measured at each composition to increase reliability. After summarizing the parallels, spectra were evaluated by the RESOLUTION computer code [38]; the indicated errors are the deviations of the lifetime parameters obtained. Three lifetime components were found in all the samples. The powder blend (physical mixture) consisting of 86.3 % w/w Klucel ELF hydroxypropyl cellulose, 4.7 % citric acid monohydrate and 9 % w/w carvedilol was measured as control of drug loaded fibers.

2.9 UV-VIS spectroscopic analysis

Carvedilol content of milled fibers was determined applying Jasco 530 UV-Vis spectrophotometer (Tokyo, Japan) at 241 nm using 0.1 M hydrochloric acid as solvent. The

drug content of the samples was measured on the basis of the calibration curve recorded earlier. Five parallel measurements were performed.

2.10 ATR-FTIR spectroscopic examinations

ATR-FTIR spectra were collected on Jasco FT/IR-4200 spectrophotometer between 4000 and 2000 cm^{-1} with an ATR PRO470-H single reflection accessory (Jasco) equipped with flat pressure tip. The spectral measurements were performed in absorbance mode. 200 Scans at a resolution of 2 cm^{-1} were co-added by the FT-IR software (Spectra Manager-II, Jasco).

2.11 Powder X-ray diffraction

For crystallinity study powder X-ray diffraction patterns of samples (fibers, physical mixture of same composition, and carvedilol) were obtained by using X'Pert Pro diffractometer (PANAnalytical, Almelo, The Netherlands) system with Cu K α i radiation ($\lambda = 1.5406 \text{ \AA}$) over the interval 2.0000-40.0014°. The measurement conditions were as follows: target of Cu; filter of Ni (thickness was 0.02 mm); voltage of 40 kV; current of 40 mA; angular step of 0.0334°; counting time of 40.005 s.

2.12 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (Seiko Exstar 6000/6200, Seiko Instruments, Japan) was performed in the temperature range from 6°C to 200°C at a scanning rate of 5°C/min under air atmosphere. Approximately five milligrams was weighed from each sample into an open aluminum pan.

2.13 Preparation of orodispersible tablets

Orodispersible tablets of 10 mg carvedilol with a weight of 600 mg of different compositions were prepared by direct compression technique according to the formula given in Table 1,

where microfiber based tablets were assigned as TF, and control tablets consisting physical mixture of hydroxypropyl cellulose and carvedilol were assigned as TPM. All of the excipients were sieved through a mesh sieve (nominal wire diameter 320 μm).

The substances were homogenized in a Turbula (T2F model; Willey A Bachofen AG, Maschinenfabrik, Basel, Switzerland) using a 1200 ml cylindrical container at 23 rpm for 30 min. Tablets were prepared by a single-punch tableting machine (Diaf TM20, Copenhagen, Denmark), with a shallow concave round punch of 13.5 mm. In order to produce tablets of a specified hardness (40-45 N) the applied pressures were adjusted for each formula.

2.14 Tablet parameters

5 tablets of each composition were measured by employing tablet hardness tester (8M, Dr. Schleuniger Pharmatron, Switzerland).

The friability of the tablets was determined by weighing ca. 6.6 g of dedusted tablets and moved for 4 mins with a revolution speed of 25 rpm in an Erweka friability tester (TAP, Offenbach/Main, Germany). The percentage friability was calculated by the reweighting of the dedusted tablets.

In vitro disintegration times were determined by using Erweka Disintegration Tester (ZT 4, Germany). The applied media was 900 ml of demineralised water; the measurement was carried out at 37 ± 2 °C by visual observation. Six tablets from each composition were evaluated for their disintegration times. The observed minimum and maximum values are reported in results and discussion.

2.15 Dissolution test

Dissolution tests of the orodispersible tablets were carried out in a Hanson SR8-Plus (Hanson Research, Chatsworth, USA) type dissolution tester. The temperature of the dissolution fluid

was 37 ± 1 °C and the rotation speed was 50 rpm, using rotating paddles. The tests were made with two different dissolution mediums: 500 ml of a solution of hydrochloric acid (pH 1.0, 0.1, Ph. Eur. 8.) and 500 ml of a phosphate buffer (pH 6.8, 0.05 M, Ph. Eur. 8.). 3.00 ml of samples were taken at predetermined time points using a Biohit Proline 5.00 ml pipette. The samples were filtered through a 10 μ m UHMW polyethylene cannula dissolution filter. The drug content of the samples was measured with an UV-VIS spectrophotometer (Jasco 530 UV-Vis spectrophotometer, Tokyo, Japan) at 241 nm at the characteristic wavelength of the carvedilol on the basis of the calibration curve recorded earlier.

2.16 Comparison of the dissolution curves

Mathematical comparison of the drug release profiles of different compositions in each media was carried out by calculating the difference (f_1) and similarity (f_2) factors according to Eqs. (3) and (4) proposed by Moore and Flanner [39] and implemented by Food and Drug Administration Center for Drug Evaluation and Research (FDA CDER).

The two factors are:

$$f_1 = \frac{\sum_{t=1}^{n'} \|R_t - T_t\|}{\sum_{t=1}^{n'}} \times 100 \quad (3)$$

$$f_2 = 50 \times \log \sqrt{1 + \frac{\sum_{t=1}^{n'} (R_t - T_t)^2}{n'}} \times 100 \quad (4)$$

where n is the number of time points, R is the dissolution value of the reference sample at time t (compressed physical mixture), and T is the dissolution value of the test sample at time t (microfiber based formula). For curves to be considered similar, f_1 values should be close to 0, and f_2 values should be close to 100. Generally, f_1 values up to 15 (0–15) and f_2 values greater than 50 (50–100) ensure sameness or equivalence of the two curves and, thus, of the performance of the test and reference samples.

2.17 Accelerated stability studies

Sealed snapcap vials of freshly prepared carvedilol loaded microfibers were placed in stability chamber (Sanyo type 022, Leicestershire, UK) maintained at 40 ± 2 °C/ 75 ± 5 % RH. Samples subjected to stability test were analyzed over 4 weeks period by means of differential scanning calorimetry, powder X-ray diffraction, and Fourier transform infrared (ATR-FTIR) spectroscopy and positron annihilation spectroscopy (PALS).

3. Results and discussion

3.1 Characterization of the carvedilol-loaded microfibers

Formation of carvedilol loaded microfibers was successfully carried out employing 50 % w/w hydroxypropyl cellulose gels via high speed rotary spinning, with an average diameter of 12.1 ± 3.5 μm . In our previous study, hydroxypropyl cellulose fibers containing model drug of BCS II with similar average diameter were produced, which refers to that the fiber diameter is not affected by the nature of the applied drug, rather by the concentration of the polymeric gel [35]. The appearance of microfibers in the optical microscopic images indicates that transparent fibers with no observable beads were formed (Fig. 1A-B). The fibrous structure is also confirmed by the obtained SEM images representing fibers of smooth surface, with no perceptible crystals on it, suggesting the distribution of carvedilol in the polymeric matrix (Fig. 1C-D). The latter confirms that the chosen solvent mixture and the acidic compound were appropriate for both the drug and the polymer allowing their complete miscibility. The carvedilol content of fibers was 9.01 ± 0.26 % w/w based on UV spectrophotometric assay, which can be considered as a relative high drug loading among rotary spun fibers intended for pharmaceutical applications [35, 40, 41].

The performed thermal analysis of microfibers, physical mixture of the same composition and the drug substance implies that crystalline-amorphous transition of carvedilol occurred during

the fiber formation, since the definite endothermic peak representing the melting point of the active compound disappeared in each fibrous sample. Fig. 2A clearly illustrates the softening temperature of hydroxypropyl cellulose (140-150 °C), which is consistent with previous papers [42, 43]. A slight melting point depression of carvedilol can be observed in case of the physical mixture, since the polymeric excipient acts as an impurity exerting disturbing effect on the melting point.

The obtained powder X-ray diffraction patterns also confirmed the changes of the crystalline state of carvedilol (Fig. 3A). An ordered crystalline lattice scatters X-ray beams in certain directions, resulting in the development of characteristic high intensity peaks in the X-ray diffraction pattern. Numerous high intensity sharp peaks of the pure drug substance and the physical mixture indicate the crystalline state of carvedilol, while diffuse peaks of hydroxypropyl cellulose implies the amorphous nature of the polymer. In the matter of the carvedilol loaded microfibers, the lack of these peaks reveals the amorphous state of the drug.

The results of the ATR-FTIR measurements were also in good agreement with the thermal analysis and the X-ray diffraction patterns. Characteristic peaks of carvedilol at 3200-3300 cm^{-1} (-NH stretching vibration) and 2900-3100 cm^{-1} (-CH stretching vibrations) can be observed in the physical mixture (Fig. 4A). With respect to the microfibers, merging and broadening of the peaks were detected. The latter can be traced back to the amorphous transition of carvedilol. It is known, that the lack of an ordered crystalline structure, such as an amorphous dispersion allows numerous types of conformations, which can result in the widening and merging of the expected peaks.

Changes in the supramolecular structure are also indicated by the results of PALS analysis, where significant difference was found between the microfibers and the physical mixture of the same composition. Based on the reduction of o-Ps lifetimes (Fig. 5), thus the size of free

volume holes, it can suggest that during the fiber formation a more ordered structure was developed. One of the possible reasons could be the altered orientation of the polymeric chains of hydroxypropyl cellulose due to the formation of intra- and intermolecular secondary binding forces. It can be proposed, that citric acid interacts with the hydroxyl groups of the polymer via the formation of hydrogen-bonds [44].

3.2 Formulation of orodispersible tablets from drug-loaded microfibers

Carvedilol loaded rotary-spun microfibers were intended to utilize in the formulation of orally disintegrating tablets, therefore the cotton-like microfibers were milled. In order to obtain information about particle size characteristics, the milled microfibers and the milled excipients employed in the formulation process were subjected to particle size measurements.

The results are summarized in Table 2, indicating that the size of the milled substances is comparable to the commercial tableting excipients. SEM images of milled microfibers demonstrate that the fibrous structure was retained after the milling process (Fig. 1E-F).

Tablet parameters of TF and TPM orodispersible tablets are shown in Table 3, from which it can be seen that all of the investigated parameters comply with the pharmacopoeial requirements.

As can be seen from Fig. 6, complete and rapid dissolution of carvedilol was obtained in each dissolution media in the matter of microfiber based formula (TF), while the dissolution from TPM mixture was incomplete. The calculated difference and similarity factors also confirm that there is a significant difference between the dissolution profiles of the investigated formulas in each dissolution media (Table 4). In contrast to TPM, the drug dissolution from the microfibers was independent from the pH of the applied medium. The enhanced dissolution of the poorly water soluble carvedilol can be attributed to the amorphous state of

the drug, and the altered supramolecular structure of the polymeric chains of hydroxypropyl cellulose providing easier access to water molecules.

3.3 Stability study of drug loaded microfibers

Carvedilol loaded microfibers were subjected to accelerated stability test (40 ± 2 °C/ $75 \pm 5\%$ RH). Crystallization of active substance is not indicated by the thermograms, since there is no observable characteristic endotherm peak (Fig. 2B). Furthermore there cannot be found significant change, i.e. reduction in the softening temperature of the polymer (ca. 140-150 °C), which could imply notable water absorption.

The obtained powder X-ray diffraction patterns are presented in Fig. 2A. The high intensity peaks are lacking from the X-ray diffraction patterns of the samples of first three weeks. However, in case of the sample stored for four weeks, similar peaks can be observed than in the physical mixture (Fig. 3B). These results suggest that crystallization of the active compound and/or the citric acid has occurred as a consequence of the applied stress conditions. Although, it can be noted that only partial crystallization took place, since the presence of the crystalline compounds was below the sensitivity of the DSC analysis. The latter was sensitive enough to detect the melting of the crystalline compound of the physical mixture.

The results of the FTIR spectra are consistent with the previous findings. The characteristic peak of carvedilol at $3200\text{-}3300\text{ cm}^{-1}$ (-NH stretching vibration) appeared only after four week-storage. The presence of the appearing slight peak indicates that the possibility of the several molecular conformations is confined by the formation of a long-range order in a crystalline lattice (Fig. 4B). The peak intensity also suggests that only a partial crystallization occurred.

Ageing of the polymeric drug matrix is also represented by results of the PALS measurements. A water-induced progressive change in supramolecular structure was found along with storage, since o-Ps lifetimes, thus the size of the free volume holes increased as a function of storage time (Fig. 5). The phenomenon can be traced back to the partial crystallization of drug, owing to the disturbing effect of the crystalline particles on the supramolecular ordering. On the other hand it is well known in the literature that the ageing of polymeric excipients leads to supramolecular changes. Therefore, the increase of the free volumes can be a consequence of the altered ordering of the polymeric chains of hydroxypropyl cellulose during stress conditions. The latter is in agreement with the results of the previous papers, where it has been shown that water absorption by polymeric excipients can result in longest mean o-Ps lifetime values [18, 19].

4. Conclusion

The results indicate that crystalline to amorphous transition of carvedilol occurred in the course of high speed rotary spinning process. After milling the fibrous structure remained without damage which enabled the formulation of orodispersible tablets reserving the fiber-derived advantageous properties. The performed dissolution study in two distinct, biorelevant dissolution media indicated that in the matter of microfiber based tablets an enhanced and pH-independent release was developed, while the reference tablets made of the compression of the physical mixture of the same composition showed incomplete and strongly pH-dependent release profiles.

The drug loaded microfibers were subjected to accelerated stability study, demonstrating that the fibers can be characterized with a large stress tolerance capacity, since only a partial crystallization of carvedilol was observable at sample stored for four weeks.

Our results point out the importance of rotary-spun fiber based drug delivery systems in a novel formulation approach of pharmaceuticals of poor water solubility.

Figure captions

Figure 1: Optical (A, B) and scanning electron (C and D) microscopic morphology of carvedilol loaded rotary spun microfibers, and scanning electron microscopic morphology of the milled carvedilol loaded microfibers (E and F)

Figure 2: A: Differential scanning calorimetry curves of carvedilol, hydroxypropyl cellulose, physical mixture and microfibers B: Stability study: DSC curves of the stored microfibers (0-4 weeks)

Figure 3: A: Powder X-ray patterns of carvedilol (a), citric acid monohydrate (b), hydroxypropyl cellulose (c), physical mixture (d) and microfibers (e) B: Stability study: powder X-ray patterns of the investigated samples: carvedilol (a), freshly prepared microfiber (b), microfibers stored for 1 (c), 2 (d), 3 (e) and 4 (f) weeks

Figure 4: A: FTIR spectra of carvedilol, hydroxypropyl cellulose, citric acid, physical mixture and microfibers B: Stability study: FTIR spectra of the stored microfibers (0-4 weeks) in the spectral range between 2000 and 4000 cm^{-1}

Figure 5: Ortho-positronium lifetime (o-Ps) values of the investigated samples: physical mixture, freshly prepared microfibers and the stored microfibers (1-4 weeks)

Figure 6: In vitro dissolution analysis of orodispersible tablets carried out at dissolution media of two distinct pH values: (A): pH 1, (B): pH 6.8.

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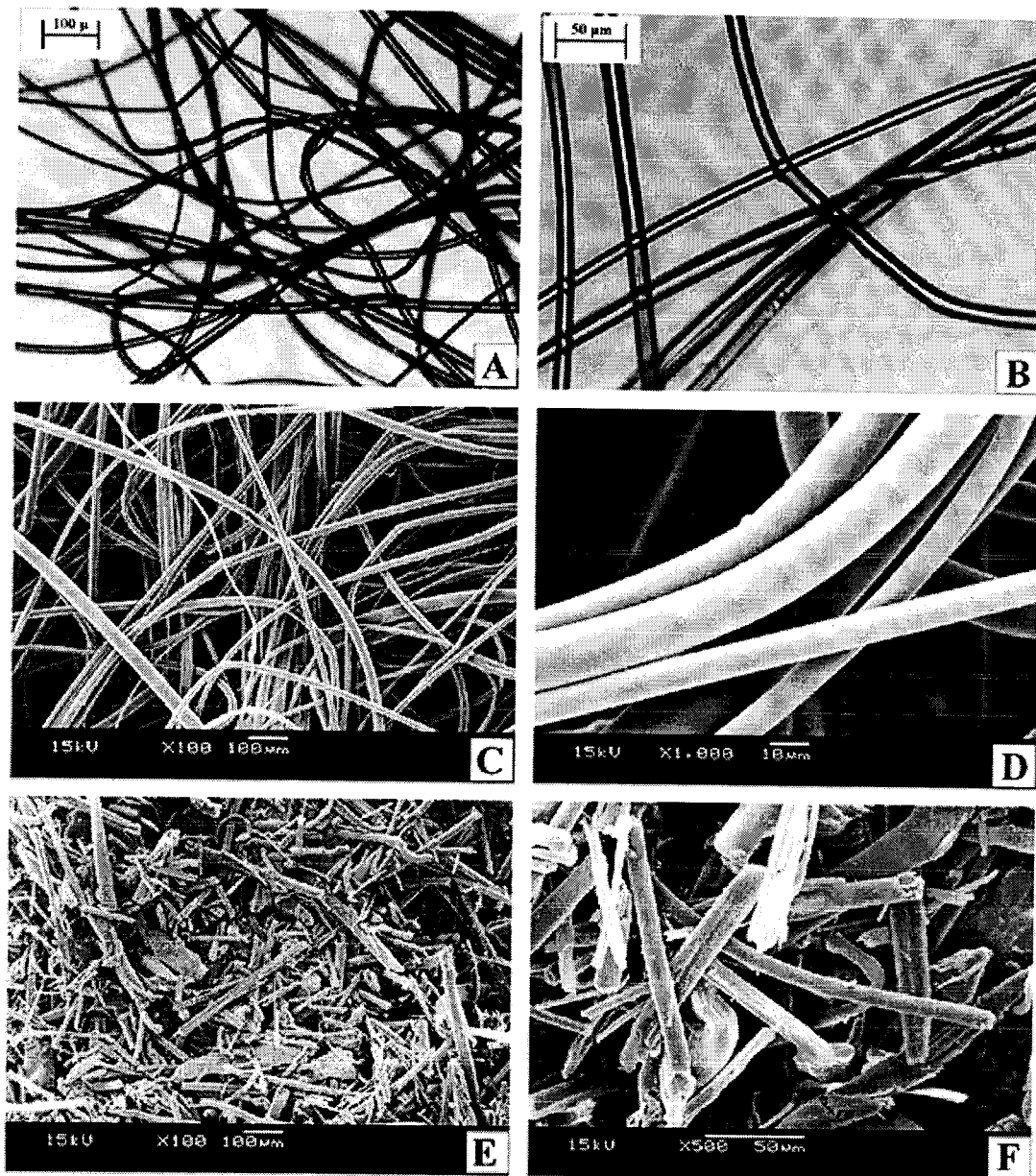


Figure 1 .

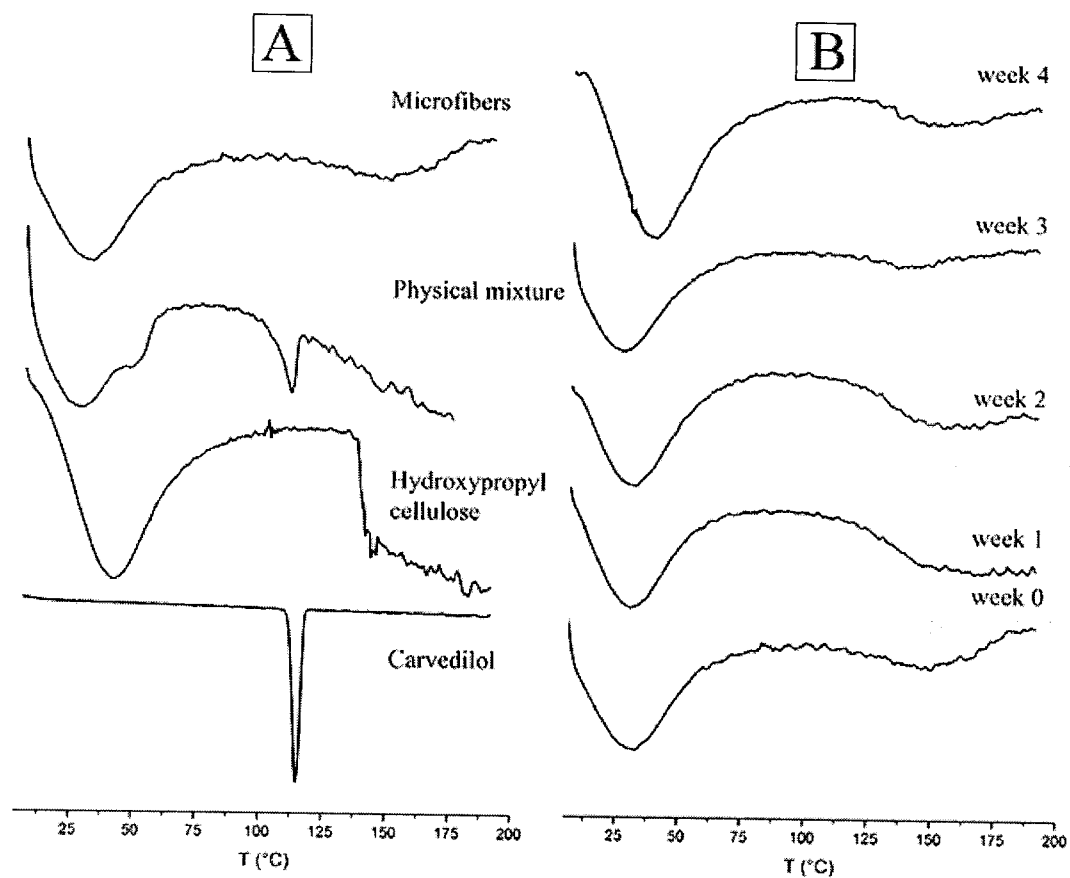


Figure 2 .

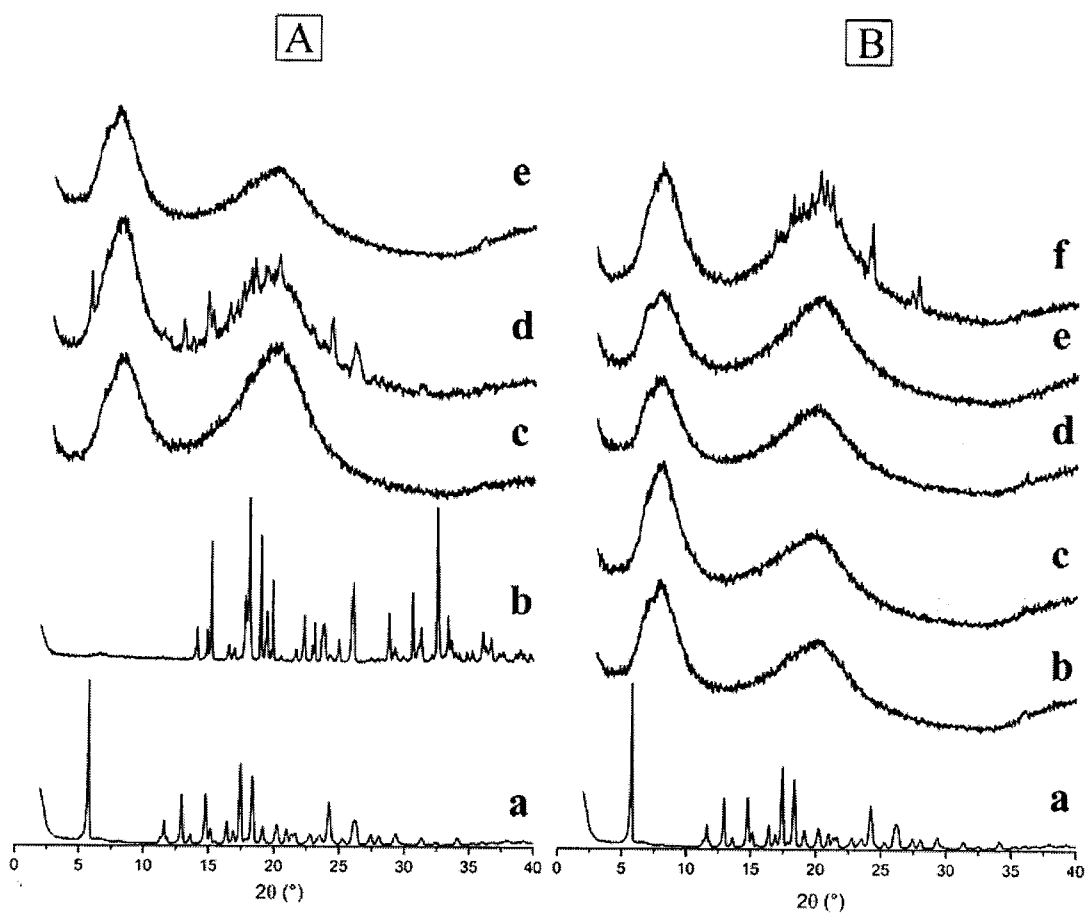


Figure 3 .

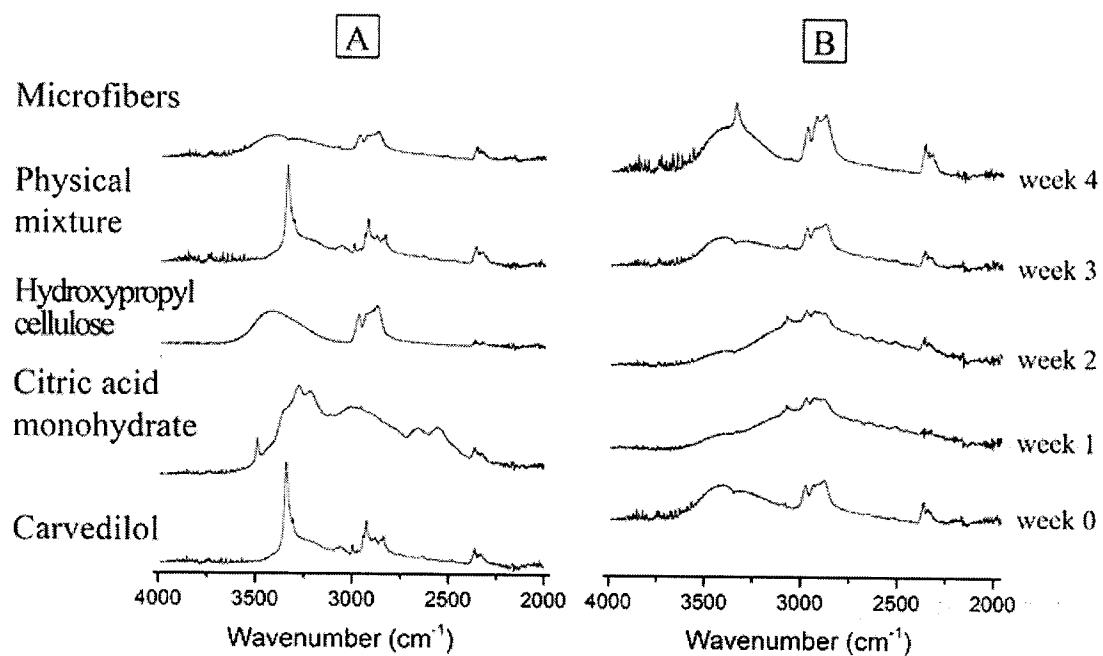


Figure 4 .

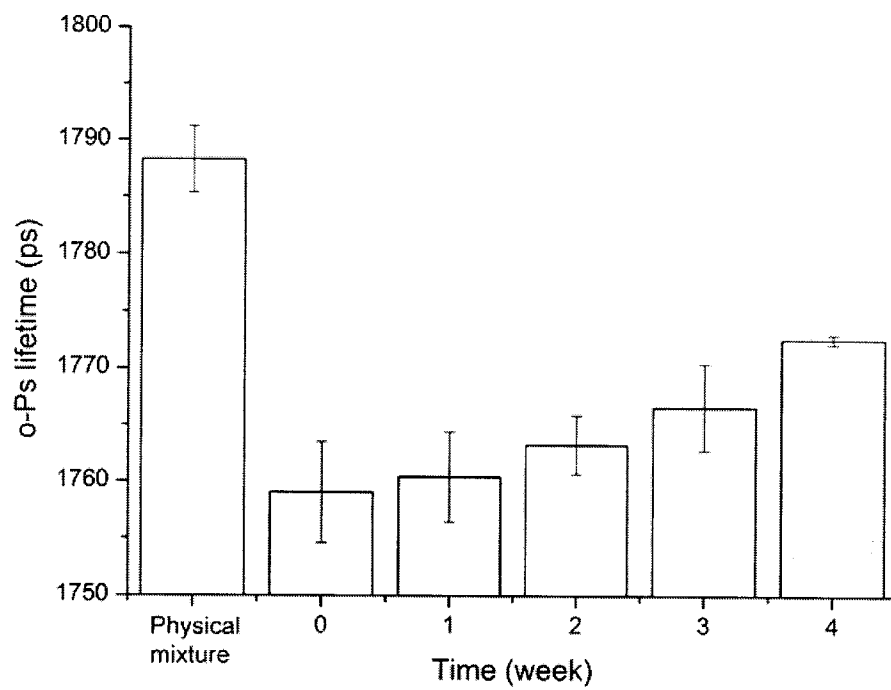


Figure 5 .

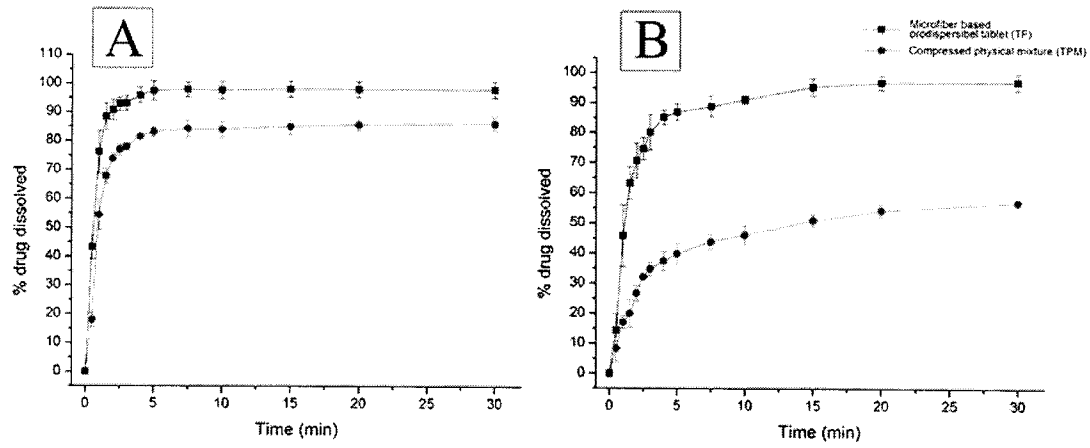
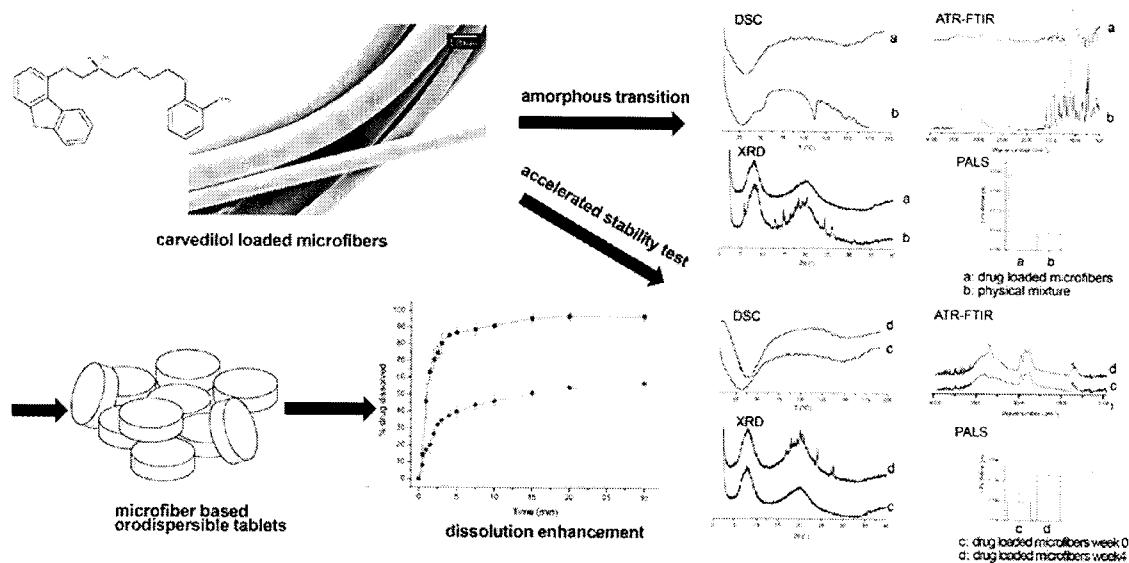


Figure 6 .



Graphical abstract .

| Component (% w/w) | Formulation series | |
|-------------------------------------|--------------------|-------|
| | TF | TPM |
| Milled carvedilol loaded microfiber | 11.10 | - |
| Vivapur [®] 102 | 30 | 30 |
| Magnesium stearate | 1 | 1 |
| Vivasol [®] | 3 | 3 |
| Effervescent agent* | 5 | 5 |
| FlowLac [®] | 49.90 | 49.90 |
| Klucel ELF | - | 9.58 |
| Carvedilol | - | 1 |
| Citric acid monohydrate | - | 0.52 |

Table 1 Composition of the investigated tablets: the microfiber based formula, TF and control tablets made of its corresponding physical mixture, TPM

*Equimolar mixture of milled citric acid anhydrate and sodium bicarbonate

| Milled substance | Mean size (μm) | Size distribution span |
|-----------------------|-----------------------------|------------------------|
| Microfibers | 135 \pm 1 | 2.85 \pm 0.03 |
| Citric acid anhydrous | 168 \pm 37 | 2.34 \pm 0.31 |
| Sodium bicarbonate | 141 \pm 19 | 1.54 \pm 0.24 |

Table 2 Particle size characteristics of the milled carvedilol loaded microfiber and the milled excipients

Table 3 Tablet parameters of the investigated orodispersible tablets

| Reference | TPM | Test TF | | | | |
|-----------|-----|---------|--------|-------|--------|-------|
| | | pH 1 | pH 1 | | pH 6.8 | |
| | | | f1 | f2 | f1 | f2 |
| | | 21.78 | 39.05 | 7.89 | 58.66 | |
| | | pH 6.8 | 149.63 | 13.12 | 111.62 | 19.04 |

| Tablet parameters | TF | | TPM | |
|-------------------------|---------------------|-------|---------------------|-------|
| Hardness (N) | 42.2 \pm 2.4 | | 40.3 \pm 1.7 | |
| Friability (% w/w) | 0.54 | | 0.48 | |
| Disintegration time (s) | 27.11 | 29.08 | 24.47 | 28.21 |
| Mass (g) | 0.5997 \pm 0.0124 | | 0.6029 \pm 0.0102 | |

Table 4 Difference (f1) and similarity (f2) factors calculated according to Eqs (3) and (4)