Electrospun biphasic drug release polyvinylpyrrolidone/ethyl cellulose core/sheath nanofibers

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ABSTRACT

The capability of core/sheath nanofibers prepared using coaxial electrospinning to provide adjustable biphasic drug release was investigated. Using ketoprofen (KET) as the model drug, polyvinylpyrrolidone as the sheath polymer, and ethyl cellulose as the core matrix, the coaxial process could be conducted smoothly and continuously without spinneret clogging. Scanning electron microscopy and transmission electron microscopy revealed linear nanofibers with homogeneous and clear core/sheath structures. Differential scanning calorimetry and X-ray diffraction verified that the core/sheath nanofibers were nanocomposites, with the drug present in the polymer matrix in an amorphous state. Attenuated total reflectance–Fourier transform infrared spectra demonstrated that the sheath polymer and core matrix were compatible with KET owing to hydrogen bonding. In vitro dissolution tests showed that the core/sheath nanofibers could provide typical biphasic drug release profiles consisting of an immediate and sustained release. The amount of drug released in the first phase was tailored by adjusting the sheath flow rate, and the remaining drug released in the second phase was controlled by a typical diffusion mechanism. The present study shows a simple and useful approach for the systematic design and fabrication of novel biomaterials with structural characteristics for providing complicated and programmed drug release profiles using coaxial electrospinning.

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1. Introduction

In clinical applications, the desired drug release profiles should obey biological rhythm for effective and safe drug delivery and convenient administration. For some pharmaceutical ingredients such as non-steroidal anti-inflammatory drugs (NSAID), as well as antihypertensive, antihistaminic and anti-allergic agents [1,2], an initial release of a fraction of the dose in the shortest time after administration is favored for relieving the symptoms of the disease. Meanwhile, sustained release of the remaining dose over a defined period can optimize the therapy and avoid repeated administration, for the patients’ convenience [3,4]. During biphasic release, a drug is released at two rates or in two periods. A typical biphasic release system can provide immediate drug release followed by a constant release.

In recent decades, different traditional pharmaceutical techniques such as tabletting, casting and spraying have been investigated for preparing biphasic drug delivery systems (DDS). Some of these systems include mixed films, multi-layered films, multiple tablets, multi-layered tablets, film-coated tablets and eluting stents [5–8]. Meanwhile, advanced technologies are continuously exploited in the literature to produce novel materials or DDS for furnishing biphasic release, taking advantage of more accurate time-programmed administration of active ingredients and fulfilling the specific therapeutic needs of some diseases. These techniques include three-dimensional printing, nanotechniques [9–12] and single fluid electrospinning [13].

Electrospinning has attracted considerable attention as a nanotechnology for nanofiber production because of its simplicity and cost effectiveness. It can produce nanofibers with unique properties and versatile applications [14–21]. One of the most significant breakthroughs in this area is coaxial electrospinning, in which a concentric spinneret can accommodate two liquids [22,23]. Coaxial electrospinning is widely used in controlling the secondary structures of nanofibers, encapsulating drugs or biological agents into polymer nanofibers, preparing nanofibers from materials that lack filament-forming properties, enclosing functional liquids within the fiber matrix, manipulating the size of self-assembled nanoparticles, preparing ultrafine fibers from concentrated polymer solutions, and improving the quality of nanofibers [24–29]. Recently, advanced functional materials fabricated using electrospinning have attracted considerable attention because of their ability to allow the controlled release of multiple active ingredients [30,31]. Electrospinning could be used to control the microstructure and spatial deposition of components. Thus, with the appropriate
polymer matrix, it is hypothesized that this technique could also be used to develop nanoproducts with biphasic drug profiles.

Polyyvinylpyrrolidone (PVP), a hydrophilic polymer excipient with a wide variety of applications in medicine, food, pharmacy and cosmetics, was selected as the filament-forming matrix of the sheath part for immediate drug release [32–34]. Ethyl cellulose (EC) is an inert, non-toxic and stable hydrophobic polymer suitable for sustained release matrices [13,35].

Accordingly, the present study investigates the preparation of core/sheath nanofibers for providing biphasic drug release using coaxial electrospinning. Ketoprofen (KET), a NSAID active ingredient with poor water solubility [36,37], was used as the model drug loaded into the sheath and core parts of the nanofibers.

2. Experimental

2.1. Materials

PVP K60 (M_w = 360,000) was purchased from Shanghai Yuhong Pharmaceutical Aids and Technology Co. Ltd. (Shanghai, China); KET was purchased from Wuhan Fortuna Chemical Co. Ltd. (Hubei, China). EC (5 mPa s to 9 mPa s) was obtained from Aladdin Chemistry Co. Ltd. (Shanghai, China). Methylene blue, N,N-dimethylacetamide (DMAc) and anhydrous ethanol were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All other chemicals used were analytical grade, and water was doubly distilled before use.

2.2. Electrospinning

The core solutions were prepared by dissolving 24 g EC, 3 g KET and 2 mg methylene blue in 100 ml ethanol. The sheath solution was prepared by placing 9 g PVP and 1 g KET in 100 ml of a solvent mixture of DMAc and ethanol in a volume ratio of 1:9. Two syringe pumps (KDS100 and KDS200, Cole-Parmer, IL, USA) and a high-voltage power supply (2GF 60 kV/2 mA, Shanghai Sute Corp., Shanghai, China) were used for coaxial electrospinning. All electrospinning processes were carried out under ambient conditions (22 ± 3 °C with relative humidity 58 ± 5%). A homemade concentric spinneret [38] was used to conduct both single fluid (adjusting the core or sheath fluid flow rate to 0 ml h⁻¹) and coaxial electrospinning processes. The electrospinning process was recorded using a digital video recorder (PowerShot A490, Canon, Tokyo, Japan). For optimization, the applied voltage was fixed at 15 kV, and the fibers were collected on an aluminum foil at a distance of 20 cm. All other parameters are listed in Table 1.

2.3. Characterization

2.3.1. Morphology

The morphology of the fiber mats was assessed by field emission scanning electron microscopy (FESEM) using an S-4800 microscope (Hitachi, Tokyo, Japan). Prior to the examination, the samples were platinum sputter-coated under a nitrogen atmosphere to render them electrically conductive. Images were recorded at an excitation voltage of 10 kV. The average fiber diameter was determined by measuring their diameters in FESEM images at more than 100 places, using the NIH Image J software (National Institutes of Health, MD, USA). Before platinum coating, the cross sections of the fiber mats were prepared by placing them in liquid nitrogen before they were manually broken.

Transmission electron microscopy (TEM) images of the samples were recorded on a JEM 2100F field emission transmission electron microscope (JEOL, Tokyo, Japan). TEM samples of the core/sheath nanofibers were collected by fixing a lacy carbon-coated copper grid on the collector.

2.3.2. Physical status and compatibility

Differential scanning calorimetry (DSC) was carried out using an MDSC 2910 differential scanning calorimeter (TA Instruments Co., USA). Sealed samples were heated at 1 °C min⁻¹ from 20 °C to 250 °C. The nitrogen gas flow rate was 40 ml min⁻¹.

The X-ray diffraction (XRD) analysis was conducted using a D/ Max–BR diffractometer (Rigaku, Japan) with Cu Kα radiation in a 2θ range of 5–60° at 40 mV and 300 mA.

Attenuated total reflectance–Fourier transform infrared (ATR-FTIR) spectroscopy was carried out on a Nicolet-Nexus 670 FTIR spectrometer (Nicolet Instrument Corporation, Madison, USA) at a range of 500 cm⁻¹ to 4000 cm⁻¹ and a resolution of 2 cm⁻¹.

2.3.3. In vitro dissolution tests

In vitro dissolution tests were carried out according to the Chinese Pharmacopoeia (2005 edn.) Method II, which is a paddle method using a RZ-8A dissolution apparatus (Tianjin University Radio Factory, Tianjin, China). Drug-loaded nanofibers (200 mg) were placed in 600 ml physiological saline (PS, 0.9 wt.%) at 37 ± 1 °C. The instrument was set to 50 rpm, providing sink conditions with C_i/C_s < 0.2C. At predetermined time points, 5.0 ml aliquots of the samples were withdrawn from the dissolution medium and replaced with fresh medium to maintain a constant volume. After filtration through a 0.22 μm membrane (Millipore, MA, USA) and appropriate dilution with PS, the samples were analyzed at 260 nm using a UV–vis spectrophotometer (UV-2102PC, Unico Instrument Co. Ltd., Shanghai, China). The concentration of released KET was back-calculated from the data obtained against a predetermined calibration curve.

The actual content of KET in the fibers was quantified by dissolving each sample in ethanol for extraction and later proceeding to the above-mentioned procedure. The cumulative percentage of drug released from the electrospun fibers was calculated using the following equation:

\[ P(%) = \frac{P_k \times V_o + \sum_{n=1}^{N} P_i \times V}{Q_o} \times 100 \]

where \( V_o \) is the volume of the dissolution medium (ml), \( V \) is the volume of the withdrawn sample (ml), \( Q_o \) is the total amount of KET in

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<th>Table 1</th>
<th>Experimental parameters for the fabrication of different nanofibers.</th>
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<td>No.</td>
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\(^a\) Sheath fluid consists of 9% (w/v) PVP K60 and 1% (w/v) KET in a mixture of ethanol and DMAc (1:9, v/v).

\(^b\) Core fluid consists of 24% (w/v) EC and 3% (w/v) KET in ethanol.

\(^c\) In this column, “Linear” morphology refers to nanofibers with few beads or spindles.
of the fluids to 0 ml h$^{-1}$. The experiments were carried out six times, and the cumulative percentage reported as mean values was plotted as a function of time ($T$, h).

### 3. Results and discussion

#### 3.1. Coaxial electrospinning

A schematic diagram of the modified coaxial electrospinning process is shown in Fig. 1a. A homemade concentric spinneret was employed (Fig. 1b). The critical voltage applied to a fluid to initiate Taylor cone formation and straight thinning jet ($V_c$) has a close relationship with the diameter of the sheath part of the concentric spinneret [39]:

$$V_c \sim \sqrt{\frac{d^2}{\kappa R}}$$

where $V_c$ is the critical voltage for a jet emanating from the meniscus tip, $d$ is the electrode separation, $\kappa$ is the permittivity, $\gamma$ is the surface tension and $R$ is the principal curvature of the liquid meniscus. A small diameter spinneret orifice gives a high $R$ value. Thus, only a small $V_c$ is needed to initiate electrospinning. The homemade spinneret used in this work has outside and inner diameters of 1.2 and 0.3 mm, respectively (Fig. 1b), facilitating the initiation of coaxial electrospinning.

A digital image of the apparatus arrangement for the coaxial electrospinning process is shown in Fig. 2a. Two syringe pumps were used to drive the sheath and core fluids independently. An alligator clip was used to connect the inner stainless steel capillary of the spinneret to the high-voltage power supply (Fig. 2b).

The coaxial electrospinning process could be changed to the single fluid electrospinning process by adjusting the flow rate of one of the fluids to 0 ml h$^{-1}$. Both the sheath and core solutions had good electrospinnability under the electrical field and the selected conditions for the preparation of the corresponding drug-loaded composite nanofibers F1 and F2. The electrospinning process of the core solutions was smoothed by occasionally removing the semi-solid skin formed around the nozzle of the spinneret to avoid clogging. Clogging is a critical but common problem during electrospinning, especially when a highly volatile solvent is used to prepare a polymer solution [40,41]. However, the sheath fluid could be run smoothly and continuously without user intervention. The sheath solvent, consisting of DMAc and ethanol, could effectively prevent spinneret clogging during the electrospinning because of the presence of DMAc with a high boiling point of 165.9 °C.

The coaxial process could be undertaken continuously under a voltage of 12 kV and a flow rate of 0.5 ml h$^{-1}$ for the sheath and core fluids. A typical fluid jet trajectory was created. The jet trajectory consisted of a straight thinning jet emitted from a compound Taylor cone, as indicated by the methylene blue marker in Fig. 2c, followed by a bending and whipping instability region with loops of increasing size (Fig. 2d). The applied voltage was further increased to 15 kV, which produced a similar jet trajectory with a shorter straight jet (Fig. 2e). Given that nanofibers become thinner with increasing applied voltage, all experiments were conducted under an applied voltage of 15 kV.

#### 3.2. Morphology and structure of nanofibers

Fig. 3 shows that all four types of nanofibers had smooth surfaces and uniform structures without any beads-on-a-string morphology. No drug particles appeared on the surface of the fibers, indicating good compatibility between the polymers and KET.

The nanofibers F1 and F2 prepared through single fluid electrospinning had average diameters of 710 ± 130 nm (Table 1; Fig. 3a and b) and 910 ± 240 nm (Table 1; Fig. 3c and d), respectively. The core/sheath nanofibers F3 and F4 had average diameters of 780 ± 90 nm (Table 1; Fig. 3e and f) and 940 ± 80 nm (Table 1; Fig. 3g and h), respectively. These results verified that PVP and EC had good electrospinnability in the selected solvent systems and concentrations, and that the coaxial electrospinning process consisting of two electrospinnable fluids could be carried out smoothly.

As expected, the FESEM images of the cross sections of the core/sheath nanofibers F3 (Fig. 4a and b) and F4 (Fig. 4c and d) were round and smooth. This finding suggests that these nanofibers are nanocomposites with a homogeneous structure. Similar to single fluid electrospinning, coaxial electrospinning is a one-step “top-down” fabrication process, in which electrical energy is exploited to dry and solidify micro-fluid jets. The processes produce nanosized fibers very rapidly, often at ~ 10$^{-2}$ s [45]. As a result, the physical state of the components in the liquid solutions was propagated into the solid nanofibers without any discerned nanoparticles generated from phase separation.

The nanofibers F3 and F4 had clear core/sheath structures (Fig. 5). Similarly to the FESEM results, no nanoparticles were discerned in the sheath and core parts. This finding suggests that these nanofibers have a homogeneous structure. However, the nanofibers of F4 (Fig. 5b) had larger diameters and thicker sheath parts than those of F3 (Fig. 5a). This difference could be attributed to the larger sheath flow rate in F4 than in F3. The fast drying electrospinning process not only propagated the physical state of the components in the liquid solutions into the solid nanofibers, but also duplicated the concentric structure of the spinneret on a
macroscale to nanoproducts on a nanoscale. As a result, the components in the sheath and core fluids occurred in the sheath and core parts of the nanofibers, respectively, with weak diffusion.

3.3. Physical status and compatibility of components

DSC and XRD tests were conducted to determine the physical status of KET in the four nanofibers. DSC thermograms are shown in Fig. 6a. The DSC curve of pure KET exhibited a single endothermic response corresponding to its melting point of 96.24 °C (ΔHf = -114.72 J g⁻¹). As amorphous polymers, PVP and EC did not show any fusion peaks or phase transitions. The DSC thermograms of the composite nanofibers F1, F2 and F3 did not show any characteristic peaks of KET. This finding suggests that the drug was no longer present as a crystalline material, but was converted into an amorphous state in all the nanofibers, regardless of the generation process.

Numerous distinct reflections were found in the XRD pattern of pure KET (Fig. 6b), demonstrating that the pure drug is a crystalline material. The diffraction patterns of PVP and EC showed a diffuse background pattern with two diffraction halos, indicating that the polymer is amorphous. No characteristic reflections of KET...
were found in the patterns of nanofibers F1, F2 and F3. This observation indicates that KET was no longer present as a crystalline material, but was converted into an amorphous state.

The results of DSC and XRD both confirmed that KET was highly distributed in the nanofiber matrix in its amorphous state and that the original structure of the pure materials was lost. These results agree with the FESEM and TEM results, verifying that the electrospun drug-loaded nanofibers are nanocomposites or drug nanosolid dispersions that could be used to resolve dissolution problems of poorly water-soluble drugs [42–44].

Compatibility among the components, which was investigated through FTIR analysis (Fig. 7), is essential for producing high-quality and stable nanofibers. Second-order interactions such as hydrogen bonding, electrostatic interactions and hydrophobic interactions improve compatibility. KET, PVP and EC molecules possess free hydroxyl (acting as potential proton donors for hydrogen bonding) and carbonyl (potential proton receptors) groups (Fig. 7b). Therefore, hydrogen-bonding interactions may occur within the KET-loaded PVP nanofibers (F1), EC nanofibers (F2) and their core–sheath nanofibers (F3) (Fig. 7c).

The ATR-FTIR spectra of the components and their nanofibers are shown in Fig. 7a. Two well-defined sharp peaks at 1697 and 1655 cm\(^{-1}\) were observed for pure crystalline KET. The former was assigned to the stretching vibration of the carbonyl group in the KET dimer (Fig. 7c), whereas the latter was assigned to the stretching of the ketone group. The peak at 1697 cm\(^{-1}\) was observed because KET molecules in crystalline form are bound together in dimers. However, the peak at 1696 cm\(^{-1}\) disappeared in the spectra of F1, F2 and F3, indicating the breakage of the KET dimers and the formation of hydrogen bonds between the PVP/EC carbonyl group and the KET hydroxyl group (Fig. 7c). By interacting with the polymer, KET molecules are less likely to form the dimers that are essential for the formation of a crystal lattice.

3.4. In vitro drug release profiles

KET has a UV absorbance peak at 260 nm. Thus, the amount of KET released from the fibers was determined by UV spectroscopy, using a predetermined calibration curve
\[
C = 15.27A - 0.0034 \quad (R = 0.9996),
\]
where \(C\) is the concentration of KET (\(\mu\)g ml\(^{-1}\)), and \(A\) is the solution absorbance at 260 nm (linear range 2–20 \(\mu\)g ml\(^{-1}\)).

The actual content of KET in all the fibers was equivalent to the theoretical calculation value, suggesting no drug loss during the electrospinning process. The in vitro drug release profiles of the four types of nanofibers are shown in Fig. 8a and b. The nanofibers of F2 disappeared instantly after they were placed in the dissolution media. In vitro dissolution tests verified that KET was dissolved completely in the bulk media in the first minutes. Yu et al. [33] reported that PVP nanofiber mats loaded with the poorly water-soluble drug ibuprofen are good oral fast-integrating membranes that can release the drug completely in several seconds. The reasons can be concluded as follows. First, PVP has hygroscopic and hydrophilic properties, and polymer–solvent interactions are stronger than polymer–polymer attraction forces. Thus, the polymer chain can absorb solvent molecules rapidly, increasing the volume of the polymer matrix and allowing the polymer chains to loosen out from their coiled shape. Second, the three-dimensional continuous web structure of the membrane can offer a huge surface area for PVP to absorb water molecules, greater porosity for the water molecules to diffuse into the inner part of the membrane, and void space for the polymer to be swollen and disentangled and for the dissolved KET molecules to disperse into the bulk dissolution medium. Third, the drug and the matrix polymer formed composites at the molecular level. Thus, KET molecules can dissolve simultaneously with PVP molecules. That is, the ability of these nanofibers to improve significantly
the dissolution rate of poorly water-soluble drugs is attributable to the synergistic effects of nanosized fibers, the web structure of the membranes and the drug composites with the filament-forming matrix.
As expected, the nanofibers of F3 and F4 could provide typical biphasic drug release profiles with immediate release percentages of 30.7% and 41.2%, respectively, after they were placed in the dissolution media for half an hour. They later released 50.6% and 48.5% of the remaining KET sustainably for 24 h (Fig. 8a and b). The surfaces of the nanofibers remained smooth without discerned nanoparticles, suggesting that KET in the sheath part was freed into the dissolution medium synchronously with the matrix PVP through a polymer-controlled erosion mechanism. The KET release profiles from the KET-loaded EC nanofibers (F1) and the core part of the core/sheath nanofibers (F3 and F4) were analyzed using the Peppas equation [46]:

\[ Q = k t^n \]

where \( Q \) is the drug release percentage, \( t \) is the release time, \( k \) is a constant reflecting the structural and geometric characteristics of the fibers, and \( n \) is the release exponent that indicates the drug release mechanism.

The regressed result for F2 is \( Q = 20.8t^{0.43} \) (\( R^2 = 0.9947 \)), indicating that the drug release from the KET/EC nanofibers was controlled via a typical Fickian diffusion mechanism by a value of the release exponent 0.18 (<0.45). For the core parts of nanofibers F3 and F4, the regressed equations are \( Q = 13.7t^{0.41} \) (\( R^2 = 0.9963 \)) and \( Q = 12.6t^{0.40} \) (\( R^2 = 0.9951 \)), respectively. These results demonstrated that the second phasic release of F3 and F4 were still to be controlled by a typical Fickian diffusion mechanism. Thus, the biphasic release profiles could be achieved through a core/sheath structure, wherein the sheath hydrophilic polymer provides fast initial release and the core matrix furnishes the later sustained release. However, the drug release mechanism was still determined by the properties of the drug-loaded matrices.

The present study demonstrated that the amount of drug released could be controlled by adjusting the sheath flow rate. Other parameters such as drug concentrations in the sheath or core fluids can also be exploited to tailor the amount of drug released at different phases. The matrix in the core part can be selected to manipulate drug release, e.g., by using a pH-sensitive polymer matrix to produce a delayed release of the second phase or a colon-targeted drug delivery. Further in vivo experiments on animals will be conducted to investigate the effects of these biphasic drug release core/sheath nanofibers.

4. Conclusion

Coaxial electrosprinning was carried out to prepare core/sheath nanofibers that could provide biphasic drug release profiles, using PVP as the sheath polymer and EC as the core matrix. With the selection of suitable sheath and core fluids, the coaxial electrosprinning process could be conducted smoothly and continuously without spinneret clogging, and the generation of clear core/sheath structures could be achieved. XRD and DSC verified that all the components were present in the core/sheath nanofibers in their amorphous states. ATR-FTIR spectra demonstrated that the sheath polymer and core matrix were compatible with KET, owing to hydrogen bonding. In vitro dissolution tests showed that the core/sheath nanofibers could provide typical biphasic release profiles consisting of an immediate and sustained release. The amount of drug released in the first phase could be tailored by adjusting the sheath flow rate. The remaining drug released in the second phase was controlled by a typical diffusion mechanism. The present study showed a simple and useful approach for the systematic design and fabrication of novel biomaterials with structural characteristics for providing complicated and programmed drug release profiles using coaxial electrosprinning.

Acknowledgments

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Appendix A. Figures with essential colour discrimination

Certain figures in this article, particularly Figs. 1–3, and 6–8, are difficult to interpret in black and white. The full colour images can be found in the on-line version, at http://dx.doi.org/10.1016/j.actbio.2012.10.021.

References