Formulation and characterization of floating cilostazol-loaded electrospun nanofibers

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ABSTRACT
(Cilostazol is considered a potential anti-platelets drug exhibiting also antithrombotic effects. Unfortunately, cilostazol shows low oral bioavailability due to its low aqueous solubility. Nanofibers are a promising new class of drug delivery materials that can improve the solubility of poorly soluble medications and prolong their release, thereby creating a gastro-retentive delivery system. This can be achieved by appropriate picking of polymers in the fabrication of the nanofibers. Consequently, the goal of this work was to create eudragit/ethyl cellulose-based nanofibers for enhancing and managing the release of the medication cilostazol. Scanning electron microscopy (SEM) was used to characterize the nanofibers' shape. Also floating behavior, drug content, drug solubility and drug release profile were determined for evaluation of the nanofiber. The Scanning electron microscopy (SEM) showed that the cilostazol-loaded nanofibers were smooth and uniform. Solubility study showed that nanofibers enhanced the cilostazol aqueous solubility by more than ten folds. The floating study showed that the nanofibers developed extended floating time. Drug content was satisfactory and reached 87.6%. Eudragit/ethyl cellulose nanofiber enhanced the solubility of cilostazol reaching 43.5% while only 11.6% for the unprocessed cilostazol after 24 hours exhibiting also sustained drug release profile.

Keywords: [Electrospinning, Scanning electron microscopy, eudragit, floating time]

1. Introduction
(Cilostazol is a quinolinic derivative drug which has dual mechanism as it can both inhibit cellular phosphodiesterase III and suppress the degradation of cyclic adenosine monophosphate (CAMP) thus, increasing CAMP concentration in platelets and blood vessels leading to the inhibition of platelet aggregation and vasodilation (Patel and Rajput 2009). Accordingly, cilostazol is powerful in controlling ischemic symptoms associated with peripheral arterial occlusive diseases (Jinno et al., 2005). Cilostazol exhibits low oral bioavailability due to its low water solubility. Cilostazol is

Variant techniques have been employed to enhance cilostazol solubility and bioavailability including spray drying, supercritical fluid techniques (Kim et al., 2005) complexation with cyclodextrins sulfonate and mesylate salts of cilostazol(Bae et al., 2015). However these techniques decreased the particle size only to micro range and did not exhibit sustained release pattern. Electrospinning is a potential technique which is implemented to produce nano scaled fibers for drug delivery[Saadatmand et al., 2019]. The nanometer-scaled fibers exhibit high surface-to mass ratio [Sasmal et al., 2019]and high porosity [Liao et al., 2015] which gives rise to floating behavior in addition to enhancing drug solubility. Floating delivery systems intended to postpone the drug's release so that it can only be taken once daily and to avoid a marked rise in the drug's blood concentration, which would lessen the drug's adverse effects. Current formulations of cilostazol are provided twice daily, which has the drawback of low patient
compliance. When standard cilostazol immediate-release preparations are used, there is a sudden increase in blood concentration and accompanying side effects, such as headache and tachycardia. Also, the total bioavailability of the typical release control formulation may be lowered due to cilostazol's poor solubility and decreasing absorption rate in the lower portion of the small intestine (Am J Cardiol 2001). Therefore, Nano fibers are promising dosage forms used for not only enhancing solubility of poorly soluble drugs but also sustaining drug release through extended gastric residence. Eudragit and ethyl cellulose are examples of polymers which can be implemented to prepare the polymeric solutions used for producing electro spun nanofibers. Eudragit (ES100) is methacrylate copolymer characterized by pH dependency and biocompatibility. When ES100 comes in contact with the biological fluids, it swells. Ethyl cellulose (EC) is inert water-insoluble cellulose ether characterized by high tensile strength and high elasticity (Amani et al., 2023). Considering these advantages, this work aimed to produce eudragit/ethyl cellulose-based nanofibers for extending the release of cilostazol drug.

2. Material and methods

2.1. Materials and reagents
Cilostazol was obtained as a gift sample from Delta Grand Pharma, Cairo, Egypt. Ethyl cellulose and Eudragit (ES100) were donated by Sigma for Pharmaceutical Industries, Quesna, Egypt. Ethanol (analytical grade) and dimethyl formamide (DMF) were purchased from El Nasr Chemical Company, Cairo, Egypt.

2.2. Preparation of the medicated polymeric solutions
The polymeric solution was prepared by dissolving eudragit and ethyl cellulose polymers with percentage 20% w/v and 2.5% w/v respectively in a mixture of solvents dimethyl formamide and ethanol with ratio (4:1). The solution was magnetically stirred at room temperature and cilostazol was added to the polymeric solution with concentration of 50 mg/ml. The mixture was stirred gently at room temperature to ensure a complete dissolution of the drug and eventually obtain homogeneous polymer/drug solutions for electrospinning (NANON-01A, MECC CO., made in JAPAN). For electrospinning, 5 mL of the polymer/drug solution was placed in a 5 mL syringe fitted with a stainless-steel blunt needle of 22G, and the injection rate was adjusted to 0.6 ml/hr. The needle tip of the syringe was connected to a high voltage power supply with the applied voltage of 25 kV. Randomly oriented nanofibers were simply collected by a flat collector wrapped with aluminum foil which was kept at a distance of 15 cm from the needle tip. The criteria for selecting the concentrations of eudragit and ethyl cellulose to prepare the polymeric solution, flow rate and the applied voltage was producing nanofibers matt free of beads. Several concentrations of eudragit and ethyl cellulose to prepare the polymeric solution were tried (15%, 2.5%), (17.5%, 2.5%), (20%, 2.5%) and (22.5%, 2.5%) and the optimum was (20%, 2.5%). Also several voltages and flow rates of the electrospinning device were tried till getting the optimum one.

Assay of Cilostazol
Stock solution containing 1mg/ml of the cilostazol was prepared in ethanol. Serial dilutions were prepared in (0.1 N HCL) to prepare a series of concentrations in the range of 5 to 20 µg/ml. The absorbance values were recorded at 257 nm employing UV-visible spectrophotometer (JENWAY, Staffordshire, UK) (Antunes et al., 2021). The recorded absorbance values were plotted as a function of cilostazol concentration to construct the standard calibration graph showing linearity in the tested range.

2.3. Characterization of fibers
2.3.1. Scanning electron microscopy (SEM)
Scanning electron microscope (SEM) was utilized for morphological characterization of cilostazol loaded nano fiber at an accelerating voltage of 15 KV. A square of (1*1 cm2) Cilostazol loaded nanofibrous sheet was cut, put in SEM sample mount and covered with platinum for 10 minutes then photographed by SEM (Meng et al. 2011).

2.3.2. Saturation Solubility study
Saturation solubility of cilostazol and cilostazol-loaded nanofiber was calculated by putting an excess amount of cilostazol and cilostazol-loaded nanofiber to certain volume of distilled water in airtight screw-capped test tubes and maintaining stirring at room temperature for 24 h. Filtered clear samples were measured spectrophotometrically utilizing UV spectrophotometer (UV-1700 Pharma Spec, Shimadzu, Japan) at 257 nm (Yu et al. 2009b).

2.3.3. Floating time
Floating time can determined by recording the time of buoyancy of the dosage form in the dissolution medium. A square of 3*3 cm² nanofiber film was cut off and placed into the 0.1 N HCL (pH 1.2) at 37°C (Jaimini et al. 2007).
2.3.4 Actual drug content
Cilostazol-loaded nanofiber was added and dissolved in ethanol. Cilostazol concentration was measured spectrophotometrically utilizing a UV-Vis spectrometer at a wavelength 257 nm and calculated with the aid of the calibration curve. (Meng et al. 2011).

2.3.5 In vitro drug release
The in vitro release study of cilostazol was accomplished by using USP type II paddle dissolution apparatus (Copley, NG 42JY, Nottingham, UK). 900 ml of 0.1N HCl (pH=1.2) at 37 ± 0.5 °C were implemented as dissolution medium in order to mimic the gastric environmental conditions. 50 mg of pure cilostazol and certain weight of nanofibers holding 50 mg of cilostazol were placed in dissolution vessels with revolved paddles at a rate of 75 rpm. Aliquots were taken, filtered and replenished at specific time spans (1,2,3,4,5,6,12,24 hrs). The cilostazol content in the withdrawn samples was analyzed via UV spectrophotometer at 257 nm. The dissolution profiles were assembled by plotting the cumulative amount of the dissolved cilostazol versus time.

3. Results

3.1. Scanning electron microscopy (SEM)
The electron micrographs showed uniform fibers with smooth discrete surfaces. The diameter of fibers was in the range of (100-260 nm) (fig 1). The behavior of nanofibers in an aqueous environment and dissolution media is controlled by their shape and thickness, which are significant features. Nano-diameter and uniform fibers has enhanced the solubility of cilostazol significantly.

3.2. Solubility study
Nanofibers enhanced the aqueous cilostazol solubility by more than ten times. The aqueous solubility of cilostazol alone was recorded to be 0.45 µg/ml while that of cilostazol loaded nanofiber was 5.3 µg/ml. This significant increase in solubility may be owed to change in the crystalline form of cilostazol during the phase transition from polymeric liquid solution to electro-spun solid nanofibers through the electrospinning process. Also, nanofibers exhibit higher surface area which leads to increasing cilostazol solubility (Yu et al. 2009b).

3.3. Floating time
The floating time of cilostazol loaded nanofibers extended for more than 72 hours as the nanofibers remained buoyant on the dissolution medium surface for more than 3 days. The extended floating time of cilostazol loaded nanofibers is accredited to the nano size which resulted in high surface area to volume ration and decreased bulk density (Yu et al. 2009a).

3.4. Actual drug content
The drug content of the cilostazol nanofibers was found to be 87.6% (2.69). This may be attributed to the loss in the collected nanofibers on the collector plate during the electrospinning process (Malik et al., 2016).

3.5. In vitro release study
The release profiles of unprocessed cilostazol powder and cilostazol loaded nanofibers were displayed in figure 2. Cilostazol loaded nanofibers displayed significant enhancement in % cilostazol release compared to the unprocessed cilostazol (p<0.05) reaching 43.5%(0.86) by the end of the experiment( after 24 hours) while only 11.6%(0.97) for the unprocessed cilostazol. The significant increase in the dissolution of cilostazol from the electro-spun nanofibers is attributed to large surface area and high porosity of nanofibers that subsequently increased the exposed cilostazol molecules to the dissolution media and facilitated the entry of the dissolution media through the fiber matrix respectively resulting in increasing cilostazol wettability. In addition to that, the phase change from liquid polymeric solution to the solid nanofibers during electrospinning process resulted in decrease in the cilostazol crystalline nature and subsequently decreasing the energy required to overcome the crystal lattice energy for dissolution process (Reda et al., 2017).The extended release profile of cilostazol loaded nanofibers could be attributed to eudragit and ethyl cellulose polymeric blend which has gastro-resistance properties as previously reported (Gowda et al., 2011). Cilostazol release pattern from electro-spun nanofibers was mostly governed by a diffusion mechanism.
Conclusion
Eudragit and ethyl cellulose were capable of formulating electro-spun nanofibers. Incorporation of cilostazol in the ethyl cellulose / eudragit based nanofibers enhanced and extended the release of cilostazol relative to cilostazol suspension due to large surface area of nanofibers and gastro-resistance properties.

References


Disclosure
The author reports no conflicts of interest in this work.

Tables and graphs

Fig. 1 photomicrograph of cilostazol loaded nanofibers

![Fig. 1 photomicrograph of cilostazol loaded nanofibers](image)

Fig. 2 *In-vitro* cilostazol release profiles from of pure cilostazol powder and cilostazol loaded nanofibers

![Fig. 2 *In-vitro* cilostazol release profiles](image)