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FABRICATING AND PROPERTIES OF RIFAMPICIN-LOADED ETHYL CELLULOSE COMPOSITES VIA ELECTROSPRAY

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ABSTRACT. Rifampin (Rif) is usually applied as first-line anti-tubercular drugs but has limited bioavailability. Herein, Rif-loaded ethyl cellulose (EC) composites was designed and fabricated by electrospray to improve therapy effectiveness and duration. A novel stable disk-like drug delivery system was prepared, and characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC), thermogravimetric (TG) analysis as well as *in vitro* release tests in phosphate buffer solution (PBS) at pH= 4.0 and pH= 7.4. The SEM results suggested EC/Rif composites had uniform circular surface and particle size distribution with an average size ranged from 7.11 μm to 7.37 μm. Rif can be physically and molecularly dispersed and incorporated into the EC matrix, as confirmed by the XRD, FTIR, DSC, and TG results. At pH 7.4, the rate of Rif release in EC/Rif composites improved with the increasing Rif content. For EC/Rif sample with equal mass ratio, the highest cumulative release of Rif

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reached 64.6% at the end of 24 h, while at pH 4.0 the Rif release was slower to 24.8% at 24h. These results suggested that EC/Rif composites fabricated by electrospray could be a promising strategy for controlling Rif delivery.

Keywords: *ethyl cellulose; rifampicin; electrospray; amorphous, drug delivery.*

INTRODUCTION

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* seriously threatens public health around the world. 10.6 million new TB infections and 1.6 million deaths were reported in 2021 [1]. TB is a major global health concern and the incidence is still on the rise [2]. Many anti-TB agents have been developed and applied for the treatment of TB infections [3]. Rifampicin (Rif) with ansa ring structure represents one of the most powerful first-line anti-tubercular drugs because of its unique activity to inhibit bacterial RNA polymerase [4]. However, the Rif efficacy is limited mainly due to its insoluble in water, relatively short plasma half-life (2h) after oral administration and hepatotoxicity [5-6]. An effective strategy to improve therapy effectiveness and duration of Rif is to form micro/nano drug delivery systems by various methods. Rif-loaded nanoparticles with branched poly(lactic-co-glycolic acid) (PLGA) were prepared by nanoprecipitation method [7]. The research showed that Rif was molecularly dispersed and sustained Rif release could be ensured. Many steps such as centrifugation, redispersion cycles and freeze-dry involved in preparing samples may be the applicable limitation of the method. In another study, Rif-loaded PLGA particles were fabricated by solvent evaporation method [8]. The drawback during the sample preparation may be the excessive use of solvents. Tse J. Y. et al prepared Rif-loaded in branched cyclic dextrin dry powder inhalers using the spray-drying technique [9]. The relatively high inlet temperature at 130 °C may be detrimental to the activity of Rif during the preparation course.

Electrospraying, as a cost-effective and one-step technique, uses electrostatic force to prepare non-agglomerated micro/nanoparticles under room temperature [10]. At the moment, electrospraying has been a promising platform for pharmaceutical development and biomedical application [11-12]. Ethyl cellulose (EC) is permitted in good manufacturing without limited acceptable daily intake and widely used in pharmaceutical industry for controlled release of drugs [13]. Regrettably, there are few published reports on preparing EC/Rif drug delivery systems by electrospray method. Therefore, in this study, we loaded Rif into EC matrix by electrostatic spray. The detail properties of the EC/Rifs were explored through the characterization of morphology, structure

and thermal properties, as well as release behavior under acidic (pH 4.0) and physiological (pH 7.4) conditions. This study would add insights for the further development and application of EC/Rif delivery systems.

RESULTS AND DISCUSSION

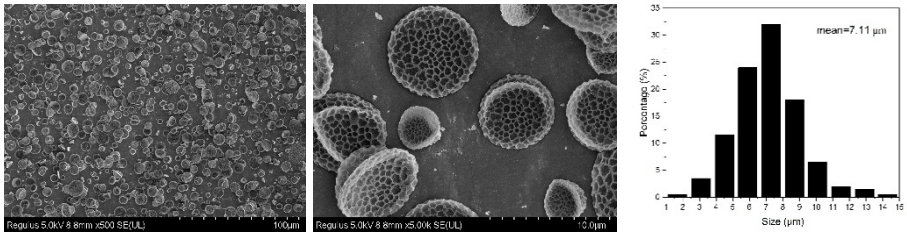
Morphology

Figure 1 shows representative SEM (Hitachi Regulus 8100) images of EC/Rif composites with different mass ratios of EC and Rif prepared by electrospray. Particle size distributions were obtained through the Nano Measurer software by counting 200 particles randomly. As shown in Figure 2, all EC/Rif particles exhibited circular surface with dense surface voids. As illustrated in Figure 1, mean particle sizes of 7.11, 7.13, and 7.49 μm were obtained for EC:Rif 3:1, EC:Rif 2:1, and EC:Rif 1:1, respectively. The results suggested that no significant change was found for the average particle sizes of prepared different EC/Rif composites.

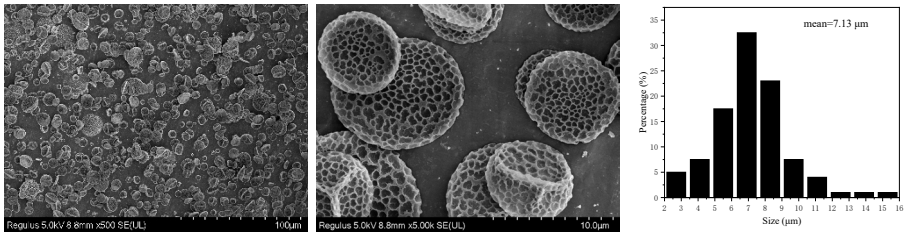
Structure analysis

Figure 2 shows the XRD (Rigaku Corporation SmartLab SE) and FTIR (Thermo Scientific Nicolet IS-10) measurements of EC, Rif, and EC/Rif composites. As shown in Fig. 2a, main peaks appeared approximately 7.5, 11.9, 13.8, 14.5, 16.3, 18.6, 20.5, 21.3, 22.3, 26.1, 29.4 and 36.7° in 2θ, indicating the crystalline nature of Rif [14]. There exited peaks at 2θ of 8.5° and 20.0° for pure EC with properties of semi-crystalline structures [12]. For EC/Rif composites prepared by electrospray, similar XRD patterns with EC and no peaks of Rif were found, which further confirming that the prepared composites are in amorphous state.

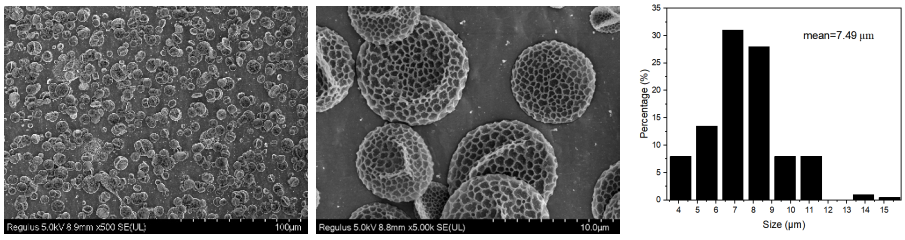
From the FTIR spectra of Rif in Figure 2b, characteristic peaks at 1725 cm^{-1} (–C=O stretching of ester bond), 1244 cm^{-1} (C–O–C), and 941 cm^{-1} (–NH rocking) were observed [15]. For EC, the absorption about 1378 and 1110 cm^{-1} were found and related to C–H bending and C–O–C stretching at low wavenumber, respectively. The peak about 3480 cm^{-1} was related to –OH stretching and the peaks at 2975 cm^{-1} and 2862 cm^{-1} were corresponding to diverse C–H stretching modes [16]. For the FTIR spectra of EC/Rif composites, the main characteristic peaks of Rif became weaker than pure Rif, and the spectra were found similar to EC, and no new characteristic peak was observed. The results suggested that no new compounds were generated during the preparation of EC/Rif composites.



(1) F1

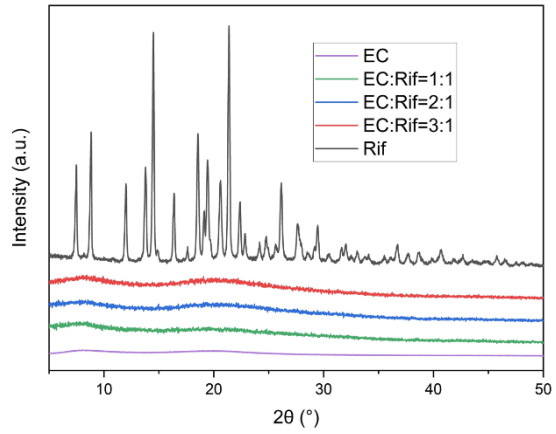


(2) F2

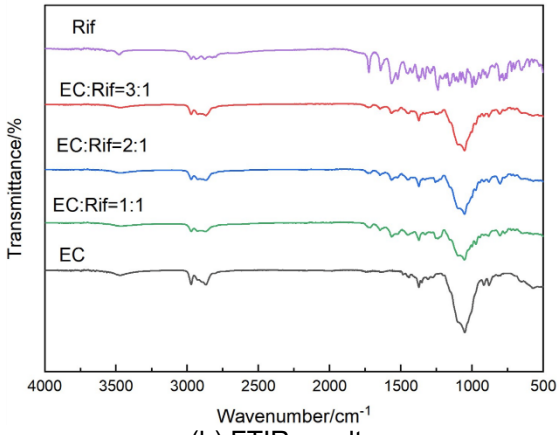


(3) F3

Figure 1. SEM results and particle size distribution of prepared EC/Rif composites



(a) XRD records



(b) FTIR results

Figure 2. XRD and FTIR records of pure EC, Rif, and EC/Rif composites

Thermal properties

Figure 3 shows the thermal behaviors of pure EC, Rif, and EC/Rif composites at different ratios measured by differential scanning calorimeter (DSC) (Mettler-Toledo DSC823e) and thermogravimetric (TG) analysis (Mettler-Toledo TGA/SDTA851e) with a heating rate of 10℃/min and N_2 flow rate of 50 ml/min. As shown in Figure 3a, there exhibits an obvious exothermic process [17] at 263.6 ℃, and slighter lighter than the value of 240 ℃ reported in the literature [18]. Perhaps some of the other 3 % within the used Rif with 97 % purity might cause a slow increase of the exothermic temperature. For EC, no obvious melting and exothermic process were found. For EC/Rif composites,

as the content of EC increases, the exothermic event belonging to Rif weakens significantly and even almost disappeared. The absence of a peak can be the indicator of complete conversion into an amorphous state [19]. The thermal events from DSC curves of the EC/Rif composites were similar to that of pure EC, indicating that Rif incorporated into EC in amorphous state, especially at higher EC content.

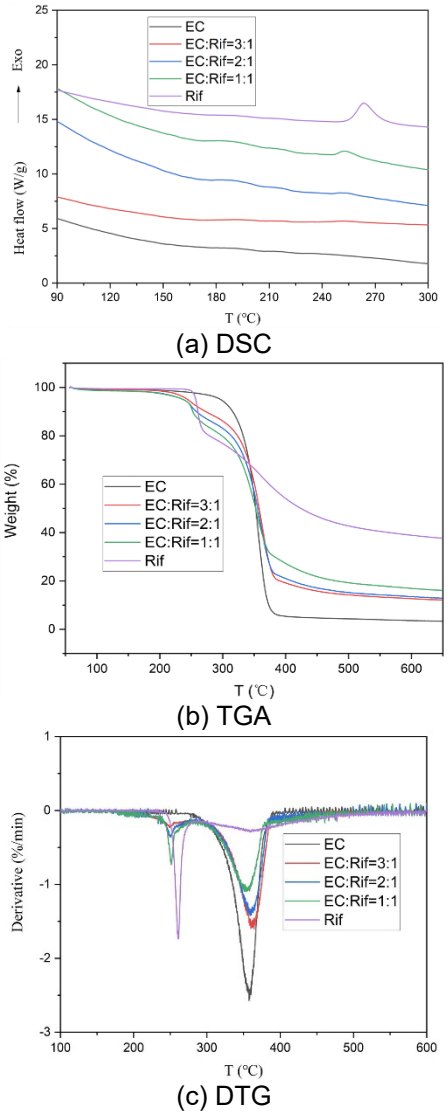


Figure 3. DSC, TG/DTG thermograms of pure EC, Rif, and EC/Rif composites

The TG and derived thermogravimetry (DTG) profiles of pure EC, Rif and EC/Rif formulations are shown in Figure 3b and Figure 3c, respectively. The TG and DTG curves indicated that pure Rif was thermally stable until 240 °C, and two stages occurred for the thermal decomposition process. The first stage occurred rapidly between 240 and 280 °C with a mass loss about 20%, while the second occurred slowly between 280 and 550 °C with mass loss of 40%. Pure EC was found thermally stable up to 280 °C and the thermal decomposition event occurred in one sharp step in the ranges of 280~400 °C with mass loss of 95%. EC/Rif formulations presents two weight loss events, which could be corresponding to the thermal decomposition of the Rif, Rif and EC, respectively. The weight loss values for EC:Rif 3:1, EC:Rif 2:1, and EC:Rif 1:1 were about 11%, 14% and 17% for a temperature range of 200–280 °C, respectively. The first thermal decomposition event of the EC/Rif composites was advanced and became slow compared to that of pure Rif obtained from TG/DTG analysis. The results could be due to the presence of the dilution of Rif in the prepared EC/Rif formulations powders in the present work and corroborate with the characteristics presented by the literature [20]. The second weight loss event for EC:Rif 3:1, EC:Rif 2:1, and EC:Rif 1:1 were about 75%, 72% and 65% for a temperature range of 280–550 °C, respectively. These thermal analysis corroborating with the XRD and FTIR results of the EC/Rif composites evidenced that Rif was molecularly dispersed in EC matrix.

In vitro *release behavior*

In vitro drug release profiles of EC/Rif composites with different Rif contents are illustrated in Figure 4. Figure 4 showed that all EC/Rif composites exhibited a relatively fast release of Rif within 8 h, followed by a slow and sustained release as the time increased. As for EC:Rif 3:1 composite, nearly 12.8% of Rif was released at 8 h and 15.6% at 24h. The sample EC:Rif 2:1 showed about 17.2% Rif released at 8 h and 24.4% at 24h. In contrast, sample EC:Rif 1:1 showed the greatest burst release with approximately 54.1% of Rif over a 8 h period, and the highest cumulative release value with 64.6% at the end of 24h. Compared to about 63% of the Rif released after only 30 min from equal mass ratio of EC/Rif composites through supercritical anti-solvent process [21], the release behavior of the prepared EC/Rif composites by electrospray has a significant advantage.

The release profiles of Rif from the EC:Rif 1:1 microparticles for pH 7.4 and 4.4 buffer are presented in Figure 5. As seen, compared to pH 7.4, the Rif release value from the EC:Rif 1:1 particle was substantially reduced to 16.3 % at 8h and 24.8% at 24h at pH 4.0, respectively. Under acidic conditions,

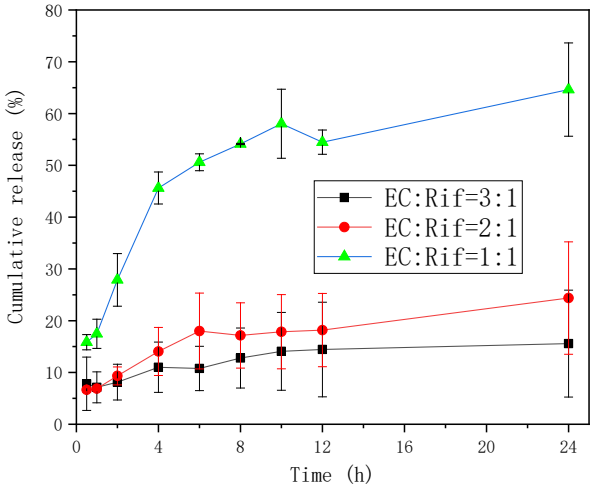


Figure 4. Rif release profile from EC/Rif composites

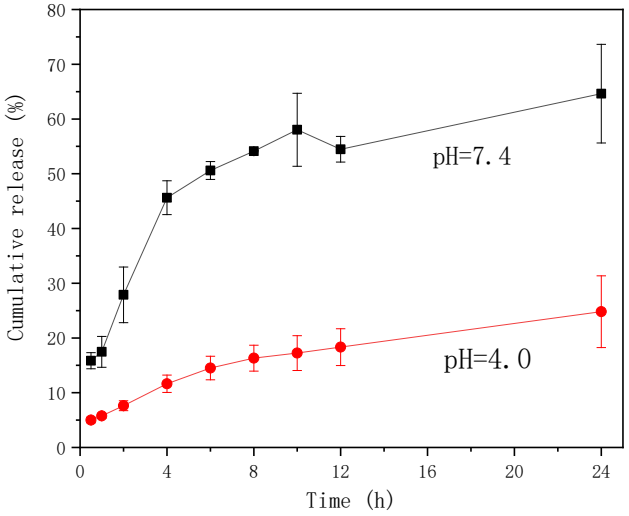


Figure 5 Comparison of Rif release profile from EC: Rif=1:1 formulation at pH with 7.4 and 4.0

the higher retention of Rif in the EC structure was pronounced. It was noticeable that all EC/Rif formulations behaved incomplete release after 24 h. The Rif release from EC/Rif matrice is proposed to be driven by the diffusion of Rif through the semicrystalline structure of EC at pH 7.4 [22]. The higher dissolution rate could be partly attributed to the increased amount of Rif located in the outer surface of EC/Rif composites [23] and the partial amorphous conversion of Rif at higher content, as confirmed by DSC. In addition, the increasing Rif content in the EC/Rif systems could result in a large concentration gradient, then the effective driving strength for diffusion increases, leading to an increase in Rif release rate and cumulative release [24]. While under the acidic condition of pH 4.0, the prevailing factors for the Rif release could be regarded as the solubilization of the EC matrix and the diffusion mechanism become less important [25].

CONCLUSIONS

In this research, we focused on improving the bioavailability and therapeutic efficacy of rifampin, also known as rifampicin, a first-line drug (antibiotic) used in tuberculosis treatment, by developing a novel drug delivery system using ethyl cellulose as a matrix, fabricated using electrospray technology, resulting in disk-like rifampin-loaded ethyl cellulose composites at micro-nano scale. The study suggests that this newly fabricated composite offer potential for controlled the antibiotic release, improving tuberculosis therapy by prolonging drug release and responding to varying pH conditions, thus enhancing rifampin's bioavailability. Notably, the study has limitations or potential challenges, such as scalability, interaction mechanism, and/or biocompatibility, which are crucial for translating these findings to real world applications (e.g. pharmaceutical products conditioning, dosage forms). Future researches will focus on the exploration of testing in *in vivo* models or investigating specific polymers other than cellulose.

EXPERIMENTAL SECTION

Materials

EC with chemically purity and Rif with 97% were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. China. Dichloromethane (DCM) with analytical grade was bought from Sinopharm Chemical Reagent Co., Ltd, China. Phosphate buffer solution (PBS) with pH = 7.4 was produced by Beijing Labgic Technology Co., Ltd. PBS with pH = 4.0 was bought from by Shanghai Yifen Scientific Instrument Co., Ltd.

Sample preparations

As shown in Figure 6, the EC/Rif composites was prepared by electrospray method, and the preparation process are as follows. Total mass of 0.3 g mixture with EC and Rif were weighted forming the mass ratio of 3:1, 2:1 and 1:1, respectively, and fully dissolved in 10 mL DCM. Magnetic stirring was applied to form uniform solution and named EC:Rif 3:1, EC:Rif 2:1, and EC:Rif 1:1, respectively. The prepared solution was pumped by a syringe pump (TYD01-02, Baoding Leifu Fluid Technology Co., Ltd) with 10-mL plastic syringe of 0.6 mm inner diameter stainless needle. A high-voltage power source (DW-P303-1, TianJin Dongwen High Voltage Power supply Corp) was applied to generate the electric field between the stainless nozzle and the collector with aluminum foil. Other electrospray parameters of prepared EC/Rif composites were as follows: flow rate was 1.5 ml/h, voltage value was 18 kV, collection distance was 15 cm. The EC/Rif composites were collected from the aluminum foil and then dried in a 50 ℃ oven for 2~3 hours before further use.

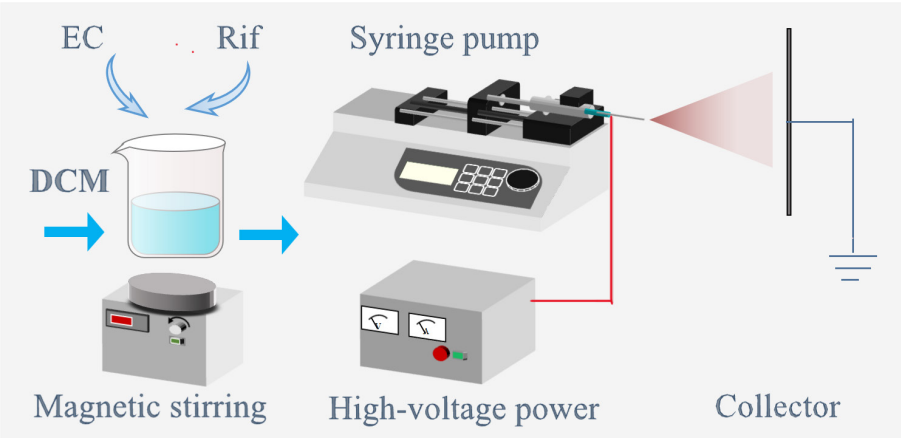


Figure 6. The schematic diagram of the EC/Rif preparation process

Drug release

Standard curve determination

Pure Rif was weighed in PBS solutions and fully dissolved to prepare a series of concentrations (5, 10, 15, 20, 30, and 50 mg/L). Absorbance was determined by a UV–Vis spectrophotometer (U3900 Hitachi, Japan) at 477 nm with pure PBS solution as reference. The equation of the standard curve of Rif was fitted and given below between the Rif concentration in the PBS solution

against the absorbance record (Figure 7): C=0.03442×Abs-0.07768. The correlation coefficient R^2 was 0.9992, and the linear relation met well with the Beer–Lambert law.

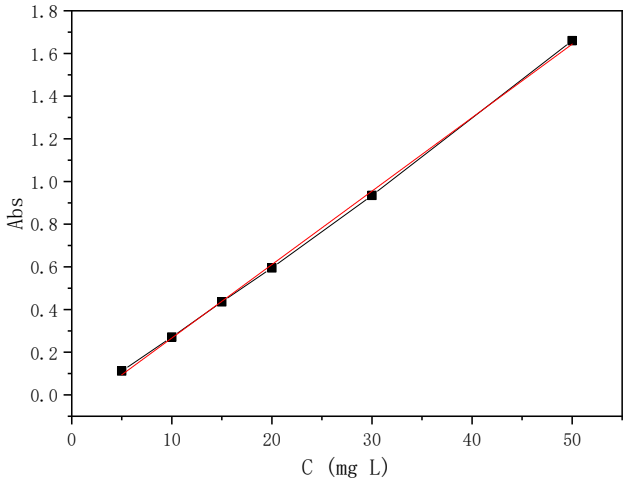


Figure 7. Standard calibration curve of Rif at 477 nm

In vitro *drug release behavior*

About 30 mg EC/Rif composites with three replicate samples were fully submerged in 100 mL of PBS buffer solution at a temperature of 37±1°C and 100 rpm using a desktop constant temperature oscillator (TH2-312, Shanghai Jinghong Experimental Equipment Co., Ltd, China). Fluid samples were obtained at 0.5 h, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, and 24 h. Equal fresh PBS medium was added after each sampling time to maintain the volume of buffer. The absorbance of the fluid samples was measured by UV-vis spectrophotometer and then the released Rif content could be computed based on the standard curve of Rif.

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