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CHARACTERIZATION OF ELECTROSPRAYED CHITOSAN/PLA-PEG-PLA (COPOLYMER) NANOPARTICLES FOR ENCAPSULATION OF HYDROPHILIC DRUG

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Abstract. Hydrophilic drug encapsulation efficiency of nanoparticles has recently received attention from the field of medicine delivery. In this work, chitosan/PLA-PEG-PLA copolymer (CS/CP) nanoparticles containing paracetamol were produced by an electrospraying method. Interactions between functional groups of chitosan, the copolymer, and the drug in their structures were demonstrated by Fourier-transform infrared spectroscopy (FTIR) and X-ray powder diffraction (XRD). The morphology and size of the nanoparticles formed at different ratios of CS/CP were evaluated by scanning electron microscopy (SEM). As a result of the optimal conditions, the obtained CS/CP-drug nanoparticles were spherical shapes with average diameters of around 220 nm. Importantly, the nanoparticles possessed a good encapsulation efficiency of 60.25 %. These studies suggest that electrosprayed nanoparticles become compact by linkages between the active sites of the material and hydrophilic drugs, and that can significantly improve the encapsulation of therapeutic molecules.

Keywords: nanoparticles, electrospraying, encapsulation, chitosan.

Classification numbers: 2.4.3, 2.7.1.

1. INTRODUCTION

Nanotechnology has been developed rapidly and applied to widespread fields of life. In the pharmaceutical area, it can be considered as one of the strategies in the growth of novel drug delivery systems including disease diagnosis, treatment, and prevention. In particular, biopolymer-based drug carrier systems with the nano-size range have been researched in recent

years with different kinds such as micelles, hydrogels, and nanoparticles. Especially, polymeric nanoparticles have received a lot of attention due to their stability and ease of surface modification. Owning two important properties including the nanoscale and the use of biodegradable materials make it possible for nanoparticles to provide targeted delivery of drugs, improve bioavailability, prolong drug release or solubilize drugs [1].

Chitosan is a natural polymer that possesses good physical properties such as biocompatibility, low toxicity, and biodegradability. Besides, it owns some functional groups like hydroxyl and amine, so it is easy to make chemical modifications in its structure to obtain different characteristics used in various applications. That is exhibited by electrostatic interaction between the protonated amine groups NH_3^+ of chitosan and the negative charge of environmental components, the chain flexibility, or hydrogen bond formation with bonding groups [2-5]. As a result, chitosan has been widely used as a carrier of different therapeutic agents. Particularly, chitosan nanoparticles have been illustrated as a vehicle of several medicines (insulin, BSA, doxorubicin, ampicillin) with a variety of administrations (transdermal, inhaled, oral, parenteral) [5].

However, the obstacle of pure chitosan nanoparticles as a drug delivery system is the undesirable drug burst effect (particularly hydrophilic drugs) resulting from its fragile nature, low mechanical strength, and high swelling tendency in the aqueous environment. Therefore, improving the mechanical strength of chitosan and increasing the affinity of drugs with the material can limit this problem, and also obtain high encapsulation efficiency. There are some suggestions to overcome this problem such as creating polymer/layered silicate composites, coating of chitosan nanoparticles by another polymer that can attach a therapeutic agent, or interacting physically or chemically between drugs and chitosan [4,5]. Among those, the idea that blending chitosan with other polymers to change its matrix structure with the aim of reinforcing stability, increasing drug entrapment efficiency, as well as reducing burst release of the incorporated therapeutic agent is considered a good solution.

In this study, the PLA-PEG-PLA copolymer was chosen to mix with chitosan to prepare electrosprayed nanoparticles with the expectation of high encapsulation efficiency of paracetamol as a model drug representing a type of hydrophilic drug. There are some reasons to choose PLA-PEG-PLA copolymer. First of all, PLA and PEG are biodegradable polymers with excellent biocompatibility and nontoxicity. Secondly, the copolymer was an amphiphilic molecule with the PLA as hydrophobic part and the PEG as hydrophilic part, so it can load the hydrophilic drug [6-9]. The last important thing, the functional groups of chitosan and the copolymer can bond together by ester or hydrogen linkage to form a compact matrix structure, even they can attach to the active sites of paracetamol like hydroxyl and amide groups [10,11]. It is hoped that these interactions can improve the limitation of pure chitosan nanoparticles.

As has been known, there are some procedures to make chitosan or modified chitosan nanoparticles such as coacervation/ionic gelation, emulsion, drying techniques, spray drying, and solvent evaporation. In those methods, the electrospraying technique is a good method for preparing nanoparticles because of the superior advantage of using less solvent [12-14]. This has great significance for the drug delivery system because solvents can denature the polymers and become toxic substances in the mechanism.

For the purpose of researching an injectable drug carrier, this article presents electrosprayed nanoparticles from PLA-PEG-PLA copolymer and chitosan which meet the requirements including spherical shape and diameter in a range of nano-size (< 500 nm) analyzed by SEM. The structure and crystallinity of the polymer were evaluated by FTIR, XRD. The drug loading

capacity and effective encapsulation of the nanoparticles were determined by high-performance liquid chromatography (HPLC).

2. MATERIALS AND METHODS

2.1. Materials

Chitosan (Mw = 150000 g mol^{-1}), PBS buffer, Poly (ethylene glycol) (PEG) (Mn = 1750) and Stannous octoate [$Sn(Oct)_2$] were purchased from Sigma-Aldrich. D,L-Lactide was supplied from Tokyo Chemical Industry. Acid acetic was bought from Xilong. Paracetamol was obtained from the Institute of Drugs Quality Control, Ho Chi Minh City (Viet Nam).

2.2. Methods

Electrospraying experiment

The electrospraying apparatus to form chitosan/copolymer nanoparticles operated at a voltage of 12 kV, a flow rate of 0.1 mL h^{-1}, an 18G needle (inner diameter 0.838 mm), and a distance of 12 cm between the nozzle of the needle and the collector. The spraying liquid contained 0.2 % chitosan in 80 wt.% acetic acid concentration. They were stirred to obtain a homogenous solution before adding the copolymer into the solution. Then, these mixtures were stirred for 1 hour before spraying. The principle of this technique was described as the previous procedure [13]. Under the working conditions of the apparatus, the nanoparticles were deposited in alumina foil (the collector). They were dried in a vacuum at room temperature before scanning electron microscopy (SEM) was performed. The experiments were conducted at different ratios of CS to CP including 7/3, 8/2, and 9/1 to evaluate the effect of the copolymer on the characterization of chitosan.

A drug content of 10 wt.% based on the weight of chitosan was chosen for the test as this concentration is likely to have a negligible impact on the morphology of CS/CP nanoparticles as well as to be suitable for the drug loading capacity of CS/CP nanoparticles. The drug was directly dissolved in the CS/CP solution. Then CS/CP-drug is prepared by electrospraying to make the nanoparticles.

Synthesis of PLA-PEG-PLA copolymer

PLA-PEG-PLA copolymer was synthesized according to a previously described procedure [8, 9]. The polymerization occurred by ring-opening of D,L-lactide in the presence of stannous octoate catalyst and PEG as an initiator.

2.3. Analytical methods

Characterization of the CS/CP nanoparticles

The morphologies of particle surfaces were studied by scanning electron microscopy (SEM - S4800 HITACHI). Size distributions of the nanoparticles were determined by ImageJ analysis and Minitab software.

Structures of the polymers were analyzed by Fourier-transform infrared spectroscopy (FTIR) on a Shimadzu FT IR-8400 S spectrophotometer. The materials were mixed with KBr and pressed to a plate for measurement.

The crystalline phase of the materials was measured by X-ray diffraction (XRD). The spectrometry was obtained using a powder diffraction meter with Cu-Ka radiation in the range 5-70°(2θ).

Characterization of the copolymer

The composition of the copolymer was determined by ^{1}H NMR (proton nuclear magnetic resonance) from a Bruker Advance machine at 500 MHz with $CDCl_3$ containing 0.03 % (v/v) Tetramethylsilane (TMS) as a solvent signal. Based on the spectrum, the polymerization degree (DP) of each PLA block and molar weight of the synthesized copolymer were specified as in the previous document [8].

Determination of encapsulation efficiency and loading capacity of paracetamol

The drug encapsulation efficiency (EF) and loading capacity (LC) were determined by dissolving 15 mg of CS/CP-drug nanoparticles in 5 mL of 0.2 M acetic acid [15]. The solution was centrifuged at 6000 rpm for 30 min. Then, the supernatant was analyzed for total paracetamol content by UV/VIS spectrometer (V-730 Jasco) at 243 nm [16].

The encapsulation efficiency was calculated from the following expression:

$$\mathrm{EE}(\%) = \frac{\text{total drug in the particles}}{\text{total initital drug}} \times 100 \quad (1)$$

The paracetamol loading capacity (LC) was calculated by the following equation:

$$\mathrm{LC}(\%) = \frac{\text{amount of drug in the particles}}{\text{total mass of the particles}} \times 100 \quad (2)$$

3. RESULTS AND DISCUSSION

3.1. Synthesis of PLA-PEG-PLA copolymer

The obtained copolymers were characterized by ^{1}H NMR spectroscopy. Fig. 1 shows the proton spectrum corresponding to the PLA-PEG-PLA copolymer dissolved in $CdCl_3$. Signals at 5.2 ppm and 1.6 ppm are attributed to the methine and methyl protons of PLA, and the signal at 3.6 ppm is presented for the methylene protons of PEG. The polymerization degree (DP) of the PLA block and average molecular weight of the copolymer were calculated by comparing the intensity of the PLA characteristic resonance at 5.2 ppm with that of PEG at 3.6 ppm.

According to [8], the DP_{PLA} and molecular weight of the copolymer were determined by following equations 3 and 4, respectively.

$$\mathbf{DP_{PLA}} = \frac{\mathbf{1}}{\mathbf{2}} \frac{\mathbf{DP_{PEG}}}{\frac{\boldsymbol{EG}}{\boldsymbol{LA}}} \quad (3)$$

$$M_{n(\text{triblock copolymer})} = 2*(DP_{PLA} *72) + M_{nPEG} \quad (4)$$

where EG/LA is the ratio of ethylene oxide and lactyl units calculated from the ^{1}H NMR spectrum.

Consequently, the PLA_{1960}-PEG_{1750}-PLA_{1960} was successfully synthesized, where the numbers 1960 and 1750 represent the average molecular weights (Mn) of the PEG and PLA blocks, respectively.

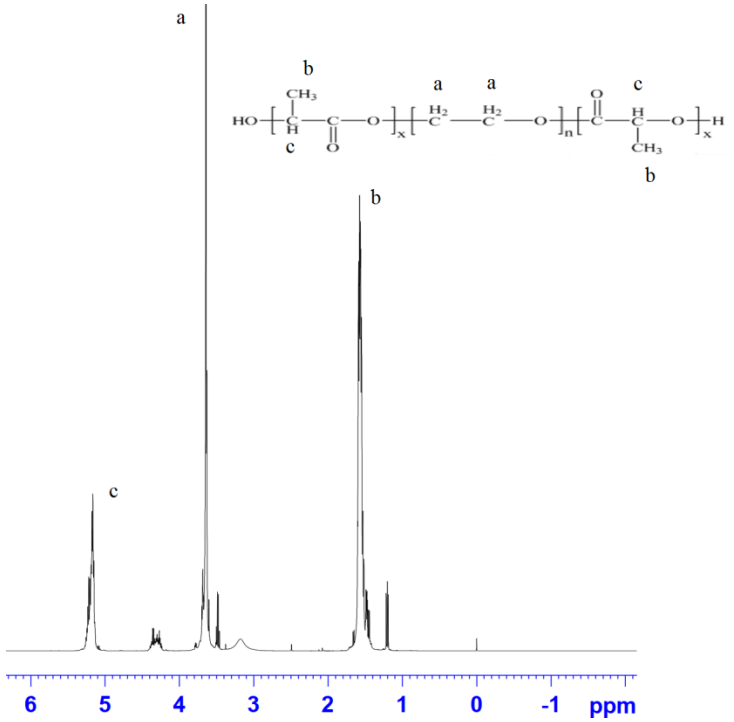

Figure 1. NMR spectrum of PLA_{1960}-PEG_{1750}-PLA_{1960}.

3.2. Characterization of electrosprayed CS/CP nanoparticles

Three samples named M1, M2, and M3 were prepared with a weight ratio of chitosan to PLA_{1960}-PEG_{1750}-PLA_{1960} copolymer of 7:3, 8:2, and 9:1, respectively. These samples were then sprayed to generate CS/CP nanoparticles. In fact, the operating conditions of the electrospraying process to obtain a good chitosan nanoparticle shape at 12 kV voltage, 12 cm distance, and 0.1mL h^{-1} flow rate were optimized from many previous experiments [17]. The SEM results at various contents of the copolymer in chitosan under the same spraying conditions can be observed in Fig. 2. The morphology of the M1 nanoparticles is spherical, similar to the SEM images of pure chitosan particles, while the other CS/CP samples (M2, M3) do not have a good round shape, some particles still have tails. In addition, the particles of M1 are located separately without aggregation, in contrast to the particles of M2, M3, which are stacked.

Based on the results of the size distribution of the M1 nanoparticles (Fig. 1a), it shows an average size of 236 nm. Note that this diameter is smaller than that of pure chitosan nanoparticles of 367 nm (Fig. 1d). It can be assumed that there is an interaction between chitosan and the copolymer, causing the particles to be compacted and reduced in size. With more advantages, M1 was chosen for the next steps of the experiment.

The FTIR spectra of chitosan, PLA-PEG-PLA copolymer, and CS/CP are shown in Fig. 3 displaying the characteristic groups of polymers and their interactions. All FTIR spectra indicate the -OH functional group at the band of 3400-3600 cm^{-1} with different appearances. The copolymer has the sharpest peak attributed to the -OH groups of PLA, chitosan gives a broadband corresponding to both $-NH_2$ and -OH groups, while CS/CP demonstrates a great wide-stretching band with low absorption intensity. Additionally, the band at 2700 - 3000 cm^{-1} in the chitosan spectrum, which is a characteristic region for the -CH group, gives a higher

intensity absorption band than that of the chitosan sample adding to the copolymer. It can be implied that the hydrogen-bonding interaction is attributed to the chitosan and terminal hydroxyl groups in the PLA. Besides, the specific peak of the -C=O group for the ester process between PLA and PEG at 1750 cm^{-1} is decreased with the attendance of chitosan. Meanwhile, the -C=O group of the amide at 1650 cm^{-1} and the -NH group at 1562 cm^{-1} shown on the chitosan sample are overlapped by multiple bands and shifted to lower wavenumber at 1627cm^{-1} with higher intensity exhibited on the CS/CP spectrum. Furthermore, the absorption peaks of the -C-O group in chitosan and CS/CP spectra are different both in position and shape band. It is found that the copolymer incorporation with chitosan leads to a narrower peak, higher intensity, and is shifted to a higher wavenumber of 1091 cm^{-1} instead of being placed at 1066 cm^{-1} in chitosan data. All of these spectra demonstrate the interaction between chitosan and PLA-PEG-PLA copolymer.

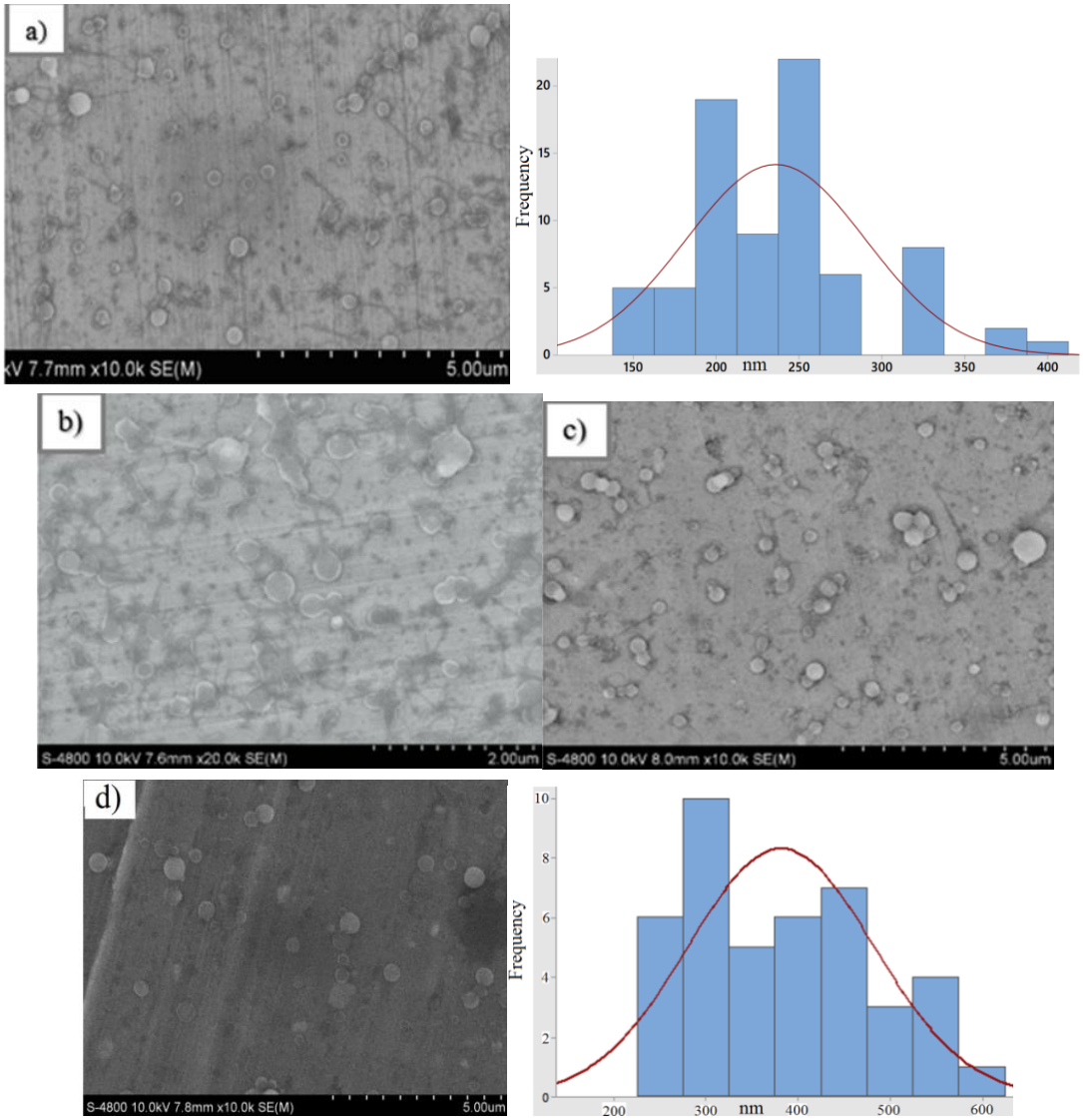

Figure 2. SEM images and size distributions of CS/CP with different weight ratios: a) 7/3, b) 8/2, c) 9/1 and d) chitosan.

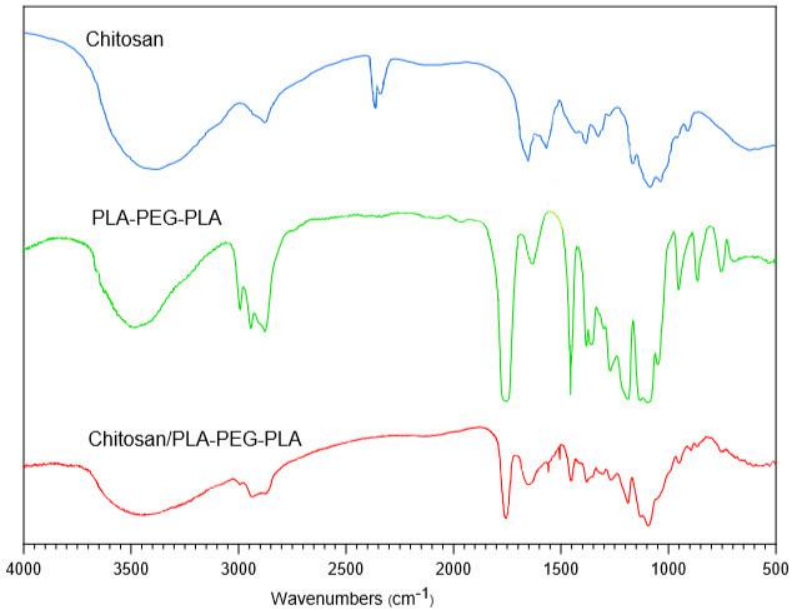

Figure 3. FT-IR results of the chitosan, PLA-PEG-PLA copolymer and CS/CP.

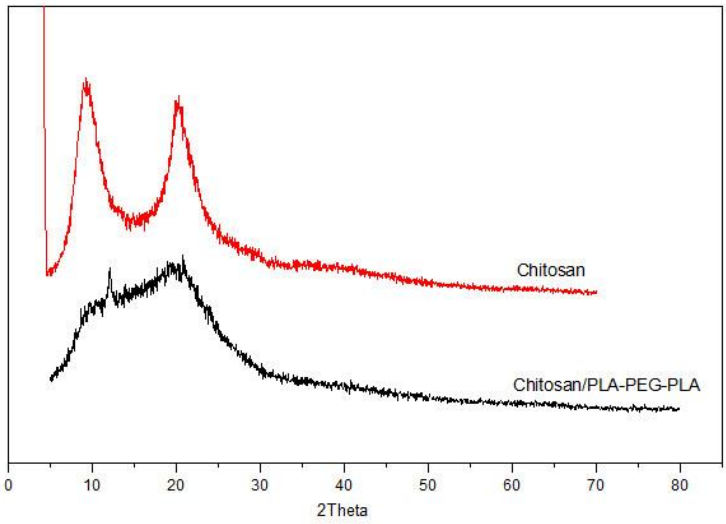

Figure 4. X-ray diffraction patterns of chitosan and CS/CP.

As shown in Fig. 4, chitosan exhibits the presence of two strong characteristic peaks at 2θ around 10.5^o (amine I "–N–CO–CH_3"), 20^o (amine II "–NH_2"), which correspond to the (020), (110) plane. Whereas, the XRD result of CS/CP shows only a broader, weaker peak of 20^o. It suggested that the reduction of intramolecular hydrogen bonds in the structure was the main reason for the disappearance of peak 10.5^o [18]. Therefore, chitosan containing PLA-PEG-PLA indicated that the original crystallinity of CS was destroyed and another state of its crystalline

structure was formed. Obviously, this confirms the bond formation between chitosan and PLA-PEG-PLA copolymer.

3.3. Preparation of CS/CP-paracetamol nanoparticles

Figure 5 shows the SEM image and particle size distribution of CS/CP-paracetamol nanoparticles prepared by electrospraying technique. With a drug-chitosan ratio of 10 wt.%, the CS/CP-paracetamol morphology is similar to that of the drug-free particles.. As observed, the particles are spherical with an average diameter of 220 nm.

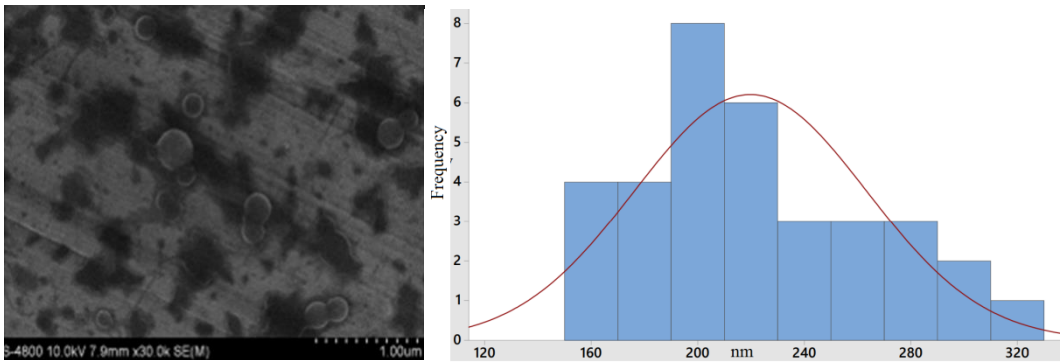

Figure 5. SEM image and particle size distribution of CS/CP-paracetamol particles.

The results of the XRD pattern of CS/CP-paracetamol in Fig. 6 also exhibit a crystalline state at a broad peak of 20^{o} like the CS/CP sample but with higher intensity. All of these seem to indicate an interaction between the materials and paracetamol. This can be explained by increasing the functional groups in the chain of chitosan containing PLA-PEG-PLA, giving the opportunity to form hydrogen bonds between them and the -OH or -NH groups of paracetamol.

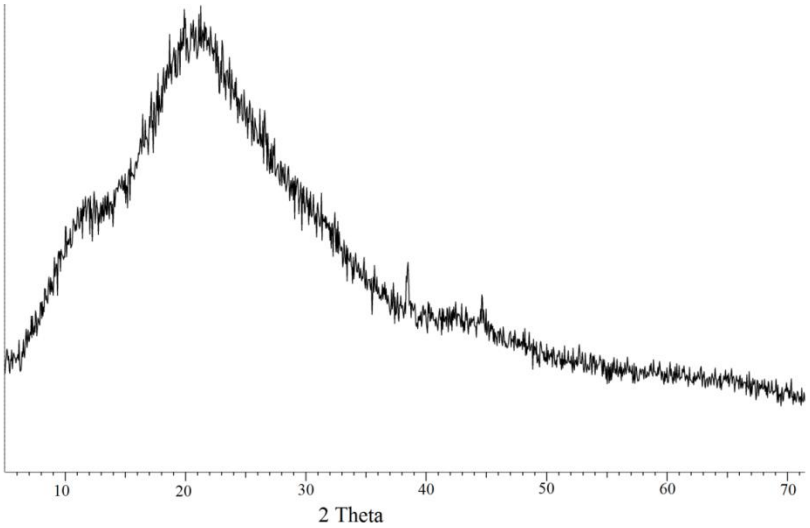

Figure 6. XRD of CS/CP-paracetamol.

The loading and encapsulation of paracetamol as a hydrophilic drug of CS/CP have a remarkable result in Table 1. The LC and EF were calculated to be 3.94 % and 60.25 %,

respectively. Besides, the drug-chitosan ratio (w/w) in the obtained nanoparticles was 6.02 wt.%. Compared with EF values of the electrosprayed polymeric nanoparticles that loaded hydrophilic drugs such as salbutamol sulfate (54 %) [19], bovine serum albumin (BSA) (54-79 %) [20], paracetamol (69 %) [16], the CS/CP nanoparticles show the same achievement. Clearly, this efficacious result is due to the interaction of chitosan and the copolymer with the drug. With high encapsulation and high crystallinity, it brings the hope that the drug burst effect can be controlled.

Table 1. LC and EF of paracetamol on CS/CP nanoparticles.

Sample	LC (%)	EF (%)
Chitosan/PLA_{1960}-PEG_{1750}-PLA_{1960}	3.94 ± 0.02	60.25 ± 0.25

4. CONCLUSION

The nanoparticles of chitosan blending PLA-PEG-PLA copolymer have been considered as a promising material for the development of hydrophilic drug delivery systems. The interaction between chitosan and the copolymer was indicated by FTIR and XRD. Under the same spraying conditions, the morphology of the CS/CP nanoparticles was good at a ratio of 7:3 (w/w) and had a similar shape to pure chitosan particles. Especially, the newly combined material from chitosan and PLA-PEG-PLA can increase linking between the characteristic group of hydrophilic drugs and the functional groups of the polymers. That was demonstrated by the great encapsulation of paracetamol of CS/CP. With these initial results, CS/CP nanoparticles generated by the electrospraying method will continue to be further investigated for the properties of a hydrophilic drug carrier for possible applications in the near future.

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CRediT authorship contribution statement. Author 1: Methodology, investigation, data curation, writing-review. Author 2: Formal analysis, data collection. Author 3: Visualization. Author 4: Supervision. Author 5: Validation, editing, supervision.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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