รายงานวิจัยฉบับสมบูรณ์

โครงการพัฒนาอนุภาคขนาดไมโคร/นาโนเมตรสำหรับนำส่งยาสู่ลำไส้ใหญ่ที่เตรียมด้วยเทคนิคอิเลคโทรไฮโดรไดนามิคอะตอมไมเซชัน

โดย ภก.ดร. กัมปนาท หวลบุตตา

สิงหาคม พ.ศ. 2558

สัญญาเลขที่MRG5680086

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ภก.ดร. กัมปนาท หวลบุตตา
คณะเภสัชศาสตร์ มหาวิทยาลัยบูรพา

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย
(ความเห็นในรายงานนี้เป็นของผู้วิจัย
สกว. ไม่จำเป็นต้องเห็นด้วยเสมอไป)

เอกสารแนบหมายเลข 2

รูปแบบ Abstract (บทคัดย่อ)

Project Code : MRG5680086

(รหัสโครงการ) MRG5680086

Project Title : Development of micro/nanoparticles for colonic drug delivery using electrohydrodynamic atomization technique

(ชื่อโครงการ) การพัฒนาอนุภาคขนาดไมโคร/นาโนเมตรสำหรับนำส่งยาสู่ลำไส้ใหญ่ที่เตรียมด้วยเทคนิคอิเลคโทรไฮโดรไดนามิคอะตอมไมเซชัน

Investigator : Kampanart Huanbutta, Faculty of Pharmaceutical Sciences, Burapha University

(ชื่อนักวิจัย) ภก.ดร. กัมปนาท หวลบุตตา, คณะเภสัชศาสตร์ มหาวิทยาลัยบูรพา

E-mail Address : kampanart@buu.ac.th

Project Period : 2 years

(ระยะเวลาโครงการ) 2ปี

วัตถุประสงค์ (Objectives)

1. To optimize nanoparticles formulation factors and the instrument parameters for colonic drug delivery purpose
2. To develop nanoparticles for colonic drug delivery using EHDA technique

บทคัดย่อ

ปัจจุบันมีการศึกษาและพัฒนาระบบนำส่งยาสู่ลำไส้ใหญ่ที่เตรียมจากอนุภาคขยาดนาโนเมตรเนื่องจากระบบนำส่งดังกล่าวสามารถนำส่งยาได้เฉพาะที่ เกิดการสะสมของอนุภาคบริเวณที่เกิดการอักเสบในลำไส้ใหญ่ (ของผู้ป่วยลำไส้ใหญ่อักเสบ) นอกจากนี้ช่วยให้ยาอยู่ในระบบทางเดินอาหารนานขึ้น งานวิจัยนี้มีจุดประสงค์เพื่อหาสูตรตำรับและสภาวะในการเตรียมอนุภาคขนาดนาโนเมตรเพื่อใช้ในการนำส่งยาสู่ลำไส้ใหญ่ด้วยเทคนิคอิเลคโทรไฮโดรไดนามิคอะตอมไมเซชัน โดยยาต้นแบบที่ใช้คือยาเพรดนิโซโลนและโพลิเมอร์ที่ใช้เตรียมอนุภาคคือยูดราจิตเอส 100 (Eudragit® S100) ในการเตรียมสารละลายโพลิเมอร์ยูดราจิตเอส 100 ถูกละลายในตัวทำละลายต่างๆ ประกอบด้วย เมทานอล เอทานอล ไอโซโพรพานอล บูทานอล และสารละลายเอทานอล สำหรับตำรับที่มีการบรรจุยาจะมีการแปรผันอัตราส่วน (โดยน้ำหนัก) ระหว่างโพลิเมอร์และยา นำสารละลายที่เตรียมด้าพ่นด้วยเครื่องอิเลคโทรไฮโดรไดนามิคอะตอมไมเซชัน พารามิเตอร์ต่างๆของเครื่องอันประกอบด้วย ความต่างศักย์ไฟฟ้า ระยะทางการพ่น อัตราการพ่นและความเร็วของการหมุนของส่วนเก็บสาร ถูกปรับอย่างละเอียดเพื่อให้ได้อนุภาคเปล่าและอนุภาคที่บรรจุยา สารละลายโพลิเมอร์ที่เตรียมจากเมทานอลให้ค่าการนำไฟฟ้าสูง (93.51 μs) แต่ความหนืดต่ำ (4.48 Cp) ด้วยคุณสมบัตินี้ส่งผลให้อนุภาคที่เตรียมจากเมทานอลมีขนาดเล็ก (422 nm) และมีลักษณะเป็นทรงกลม ส่วนผลิตภัณฑ์ที่เตรียมจากสารละลายไอโซโพรพานอล บูทานอล มีรูปร่างไม่แน่นอนและเกิดการหดตัวของโพลิเมอร์ ผลิตภัณฑ์ที่ได้จะเปลี่ยนจากทรงกลมเป็นเส้นใยเมื่อใช้ความเข้มข้นของยูดราจิตเอส 100 สูงและใช้ค่าความต่างศักย์สูง ช่วงขนาดของอนุภาคที่บรรจุยาที่ความเข้มข้นต่างๆ (0.5%-3.5%) อยู่ระหว่าง 448.60 ถึง 660.58 นาโนเมตร ระบบสามารถบรรจุยาได้สูงสุดร้อยละ 91.50 จากผลการทดลองทั้งหมดแสดงให้เห็นถึงความเป็นไปได้ที่สามารถนำระบบดังกล่าวในการนำส่งยาสู่ลำไส้ใหญ่

Abstract

Nowadays, nanoparticles (NPs) for colon-specific drug delivery system have been further studied due to their specific accumulation in the inflamed tissue in the colon and prolong drug staying in gastrointestinal tract for the treatment of inflammatory bowel disease (IBD). The present study was aimed at finding suitable formulation and fabricating condition to prepare NPs for colonic drug delivery via electrohydrodynamic atomization (EHDA). The NPs were prepared by dissolving prednisolone (model drug) and Eudragit® S100 (EDS100) in different solvents including, methanol, ethanol, isopropanol, butanol and mixtures of ethanol with purified water in various drug/polymer weight ratio and EDS100 concentrations. Then, the prepared solution was sprayed using the EHDA machine. The preparation condition and the instrument parameters such as applied voltage, injected distance, feed rate, and drum collector rolling rate were delicately adjusted again to obtain blank and drug loaded NPs. The polymer

solution prepared from methanol offered high conductivity (93.51 μs) with low viscosity (4.48 Cp). These make the NPs papered from methanol were spherical and small, diameter size of 422 nm. The products of isopropanol and butanol were irregular shrinkage particles. The sprayed products were changed from particle to fiber when using high concentration of EDS100. More fiber product was obtained when high applied voltage (20kV) was utilized. Size range of the NPs loaded prednisolone at different concentrations (0.5%-3.5%) was from 448.60 to 660.58 nm. The maximum drug encapsulation was 91.50%. From the preliminary result, this nanoparticulate system expresses possibility to fabricate specific colonic drug delivery.

บทนำและความสำคัญของปัญหา (Introduction)

Electrohydrodynamic atomization (EHDA) has gained attention in the field of drug delivery, and it has been used to fabricate drug carriers including polymeric microparticles, nanoparticles (NPs) and nanofibers used in various pharmaceutical applications [1, 2]. Theoretically, EHDA is a process where a liquid breaks up into spray droplets under the drive of an electrical field. Various modes of EHDA were reported, of which the stable cone-jet is of specific interest [3]. Under such mode, liquid flowing through a nozzle maintained at high potential is subjected to an electric field (Fig. 1), which leads to elongation of the meniscus to a form of jet and subsequently the jet deforms and breaks up into fine droplets. The small jet diameter allows rapid evaporation of the solvent and, as a result, particles are deposited on the collector. Applied voltage, feed rate, distance from the nozzle to collector, drum collector rolling rate, charge and viscosity of sprayed solution are vital parameters to determine product characteristics [3]. The principal advantages of this technique in drug delivery system are (i) to offer opportunity of heat sensitive drugs loading, (ii) the relative rapid and simple way to prepare a several materials into NPs, and (iii) controllable operation factors.

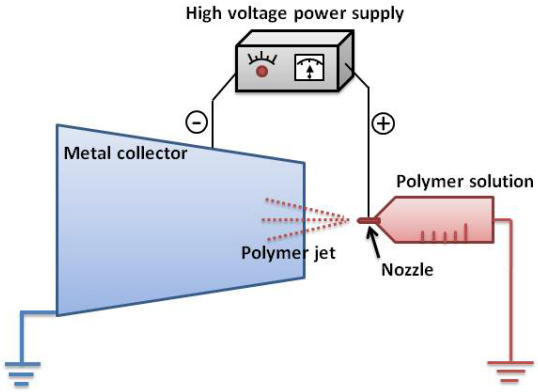


Fig. 1. Schematic diagram to show nanoparticles fabrication by EHDA.

Inflammatory bowel diseases (IBD) are relapsing and chronic inflammatory disorders of the intestinal mucosa, with an organ-susceptibility for the colon [4]. Conventional drug delivery systems are not completely successful when applied for the treatment of IBD [5]. Aside from incidences where therapy fails due to insufficient drug deposition at the site of action, adverse drug effects have also limited therapeutic outcome. These adverse effects are thought to be related to the lack of selective drug release at the inflamed colon. Nanoparticulate systems for colonic drug delivery exhibit that size-dependency of particles impacts their epithelial uptake and a preferential accumulation in the inflamed colon has been found [6]. Lamprecht and co-workers [7] demonstrated that high binding to the inflamed tissue of Lewis rat colon was found for 100-nm particles. This is because thicker mucus layer produced in the ulcerated regions increasing adherence of the small particles.

Prednisolone is a synthetic corticosteroid drug that is particularly effective as immunosuppressant and anti-inflammatory drug. It have been used to treat a number of different conditions such as inflammatory bowel disease (IBD), inflammation (swelling), severe allergies, adrenal problems, arthritis and asthma [8, 9]. For treatment of IBD, administration of prednisolone at a large and frequent dose for a long period causes significant and prolonged absorption of the drugs from the small intestine, often leading to toxic side effects [10]. Therefore, the specific delivery of drugs to diseased parts should be developed. Furthermore, prednisolone is a class II substance according to the Biopharmaceutics Classification System. It is a poor water soluble agent [11]. Consequently, improvement of the dissolution rate of prednisolone such as solid dispersion, nanoparticle preparation and liquisolid compacts can obtain more rapid and complete absorption [12-14].

To extensively target drug to the colon, nanocarriers made of pH-dependent polymer have been applied. In the gastro-intestinal tract, from the stomach to the colon, intraluminal pH varies progressively up to 7. In order to obtain pH-sensitive NPs, Eudragit® S, a polymer of methacrylic acid and methyl methacrylate, which dissolves when the pH is above 7, was utilized to control the release of entrapped drug [15, 16]. Therefore, the aim of this study was to design and optimize formulation and processing factors influencing the characteristics of NPs prepared by EHDA technique.

วิธีทดลอง (Materials and methods)

1. Materials

Eudragit® S100 (EDS) (lot number B041005026) was a gift from Evonik Degussa (Thailand) Co., Ltd., Thailand. Methanol (lot number 14080041) was purchased from RCI Labscan Ltd., Thailand. Ethanol (lot number J32T04) was obtained from Mallinckrodt Baker, Malaysia. Butanol (lot number K35977190619) was purchased from Merck, Germany. Isopropanol (lot number V1A707131A) was received from Carlo Erba Reagents, France. Prednisolone (lot number R25301) was a gift from Bangkok Lab & Cosmetic Co., Ltd., Thailand. All other chemicals were of reagent grade and used without further purification.

2. Characterization of EDS solution

The prepared EDS solutions were characterized by several techniques to reveal the effect of the polymer solution properties on the NPs. The conductivity of each EDS solution was measured by conductivity meter (model ECtestr11+, Eutech Instruments Pte Ltd., Singapore). The viscosity was examined by viscometer (model DV-III Ultra, Brookfield, USA). The surface tension was measured by sessile drop method using a drop shape instrument (model FTA 1000, First Ten Angstroms, USA). All experiments were performed in triplicate.

3. Preparation of NPs

To prepare the blank NPs, various concentrations of EDS were dissolved in several solvents, including methanol, ethanol, isopropanol, butanol and mixtures of ethanol/water. The EDS solution was then sprayed using EHDA machine (Bangkok Cryptography, Thailand). For the drug-loaded NPs, prednisolone was dissolved in the EDS solution before spraying. The drug:EDS weight ratios were varied at 0.5:5, 1.5:5, 2.5:5 and 3.5:5. As presented in Table 1, the processing parameters such as applied voltage, injected distance, feed rate, and drum collector rolling rate were carefully

adjusted to obtain blank and drug-loaded NPs. The products were kept in desiccator for further investigation.

Table 1. The processing parameters of EHDA studied.

Applied voltage (kV)	Injected distance (cm)	Feed rate (mL/min)	Drum collector rolling rate (rpm)
5-20	10-25	0.5-1.5	30-60

4. Morphology and particle size

Surface morphology of the prepared NPs was carried out using scanning electron microscope (SEM; model Maxim 2000S, CamScan Analytical, UK). Sample powders for SEM analyses were sprinkled on a stub and sputter-coated with gold to increase their conductance. Particle size and size distribution were analyzed by J MicroVision software (version 1.2.7).

5. Zeta potential determination

Zeta potential of the blank and drug-loaded NPs was monitored by zeta potential analyzer (model ZetaPlus, Brookhaven, USA) to evaluate the coating efficiency. The samples were diluted to 0.5% w/v using deionized water as a solvent and then agitated for 3 min before measuring. The average and standard deviation of the measurement of three batches of samples were reported.

6. Drug content and encapsulation efficiency

Drug content was determined by dispersing prednisolone-loaded NPs (2 mg) in methanol (10 mL), stirring for 24 h and then sonicating for 2 h before the dispersion was filtered through 0.45-μm cellulose acetate membrane. The amount of prednisolone in the filtrate was quantified by high performance liquid chromatography (HPLC; model JASCO PU-2089plus quaternary gradient inert pump, and JASCO UV-2070plus multiwavelength UV–vis detector, Jasco, Japan) (1). The analytical column used was a reverse phase Kinetex™ column (C18, 250×4.6 mm, pore size 5 μm, Phenomenex, USA). The mobile phase was a mixture of methanol, tetrahydrofuran and water (at a ratio of 3:25:72). The column effluent was detected at 254 nm with a UV/vis detector. The calculation equation is as follows:

$$\text{Drug content (\%)} = \frac{\text{Amount of drug in nanoparticles}}{\text{Weight of nanoparticles}} \times 100 \qquad (1)$$

The encapsulation efficiency was defined as the ratio of actual and original amount of prednisolone encapsulated in NPs. The encapsulation efficiency was calculated using the following equation:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Actual amount of drug-loaded in nanoparticles}}{\text{Initial amount of drug-loaded in nanoparticles}} \times 100 \quad (2)$$

7. Fourier transform infrared (FTIR) spectroscopy

To investigate the effect of the EHDA processing parameters on the drug-polymer interaction, prednisolone, EDS, their physical mixtures and prednisolone-loaded NPs were pulverized and blended with KBr and compressed. The measurement was carried out using a FTIR spectrophotometer (model Magna-IR system 750, Nicolet Biomedical Inc., USA).

8. Powder X ray diffraction

Powder X-ray diffraction patterns of the stating materials, their physical mixtures and the NPs were analyzed by using a powder X-ray diffractometer (model D8, Bruker, Germany) under the following conditions: graphite monochromatized Cu Kα radiation, voltage 45 kV, electric current 40 mA, slit: DS1°, SS1°, RS, 0.15 nm, and scanning ratio $2\theta = 5^{\circ}\ \text{min}^{-1}$.

9. Differential scanning calorimetry (DSC)

DSC thermograms of the stating materials, their physical mixtures and the NPs were determined by a differential scanning calorimeter (model Sapphire, Perkin Elmer, USA) using indium as a standard. Each sample, 2–3 mg, was accurately weighed into a close aluminum solid pan. The scanning rate was run at 10°C/min from 20 to 300°C under nitrogen purge.

10. Statistical analysis

Analysis of variance (ANOVA) and Levene's test for homogeneity of variance were performed using SPSS version 10.0 for Windows (SPSS Inc., USA). *Post hoc* testing ($p < 0.05$) of the multiple comparisons was performed by either the Scheffé or Games-Howell test depending on whether Levene's test was insignificant or significant, respectively

ผลการทดลอง และวิจารณ์ทดลอง (Result and discussion)

1. Characterization of EDS solution

The conductivity, viscosity and interfacial tension of 5% w/v EDS in methanol, ethanol, 2-propanol or t-butanol are given in Table 2. EDS did not completely dissolve in t-butanol; therefore, characterization of the solution of EDS in t-butanol could not be conducted. In methanol, the viscosity of 5% w/v EDS was the lowest (4.48 cP) while the conductivity and interfacial tension were the highest, which were 93.51 μS and 22.82 mN/m, respectively. This might be because methanol contains higher amount of hydroxyl group, at the same weight, compared to other alcohols. Therefore, high hydroxyl groups can increase conductivity of the solution (2). Furthermore, earlier studies have shown that viscosity affects conductivity; a decrease in viscosity results in the increase in the movement of the ions, so the conductance of the electrolyte increases from 2-propanol to methanol (3).

The interfacial tension is one of the crucial parameter to determine properties of the sprayed products (4). High surface tension value of the solution can decrease size of the particles. The interfacial tension values all of the EDS solutions were slightly different ranging from 20.46-22.82 mN/m. EDS solution prepared from methanol gave the highest interfacial tension. This result is in agreement with the previous study (5), which explained that the surface tension decreases with the length of the hydrocarbon tail.

Table 2. Conductivity, viscosity and interfacial tension of 5% w/v EDS in different solvents.

Solvent	Conductivity (μS)	Viscosity (cP)	Interfacial tension (mN/m)
Methanol	93.51 ± 2.56	4.48 ± 1.10	22.82 ± 0.35
Ethanol	31.64 ± 0.74	5.76 ± 0.37	21.71 ± 0.19
2-Propanol	9.13 ± 0.09	10.88 ± 0.37	20.46 ± 0.25
t-Butanol	N/A*	N/A*	N/A*

* EDS did not completely dissolve in t-butanol.

The conductivity of drug-loaded EDS solution slightly decreased when the drug loading was higher (Table 3). This is because prednisolone is nonpolar drug which can lowering conductivity of the solution (6). For Interfacial tension value, there were not different amount several drug concentrations.

Table 3 Conductivity, viscosity and interfacial tension values of predinolone-loaded EDS solution using methanol as dissolving solvent.

The EDS solution loaded with different prednisolone concentrations	Conductivity (μS)	Viscosity (cP)	Interfacial tension (mN/m)
0.5% prednisolone	97.03 ± 1.26	2.99 ± 0.37	21.60 ± 0.87
1.5% prednisolone	94.70 ± 2.86	4.05 ± 0.32	22.20 ± 0.17
2.5% prednisolone	92.41 ± 0.54	4.27 ± 0.37	22.28 ± 0.30
3.5% prednisolone	92.02 ± 0.54	4.91 ± 0.36	21.48 ± 0.23

3.2 Morphology and particle size

The characterized blank and drug dissolved solutions were sprayed by EHDA to reveal the effect of the solution properties on appearance of the sprayed products. As shown in Fig. 2, the NPs prepared from EDS (5% w/v in methanol) offered spherical particles with diameter of 447.9 nm. The NPs prepared from EDS in ethanol and the mixture between ethanol and water (70:30) were spherical and fibrous as illustrated in Figs. 2b and 2c, respectively. The electrosprayed products of EDS in isopropanol and t-butanol were collapsed particles (Figs. 2d and 2e). The proposed fabrication mechanism of the NPs by EHDA is presented in Fig. 3. The boiling point of solvents may influence the evaporation of solvents. The boiling point of isopropanol and butanol are higher than those of methanol and ethanol; therefore, solvent evaporation may not complete during spraying. After that, the wet droplets hit the collector causing irregular and collapsed particles (7). This phenomenon can be explained by Rayleigh's equation as shown in Equations (3) and (4).

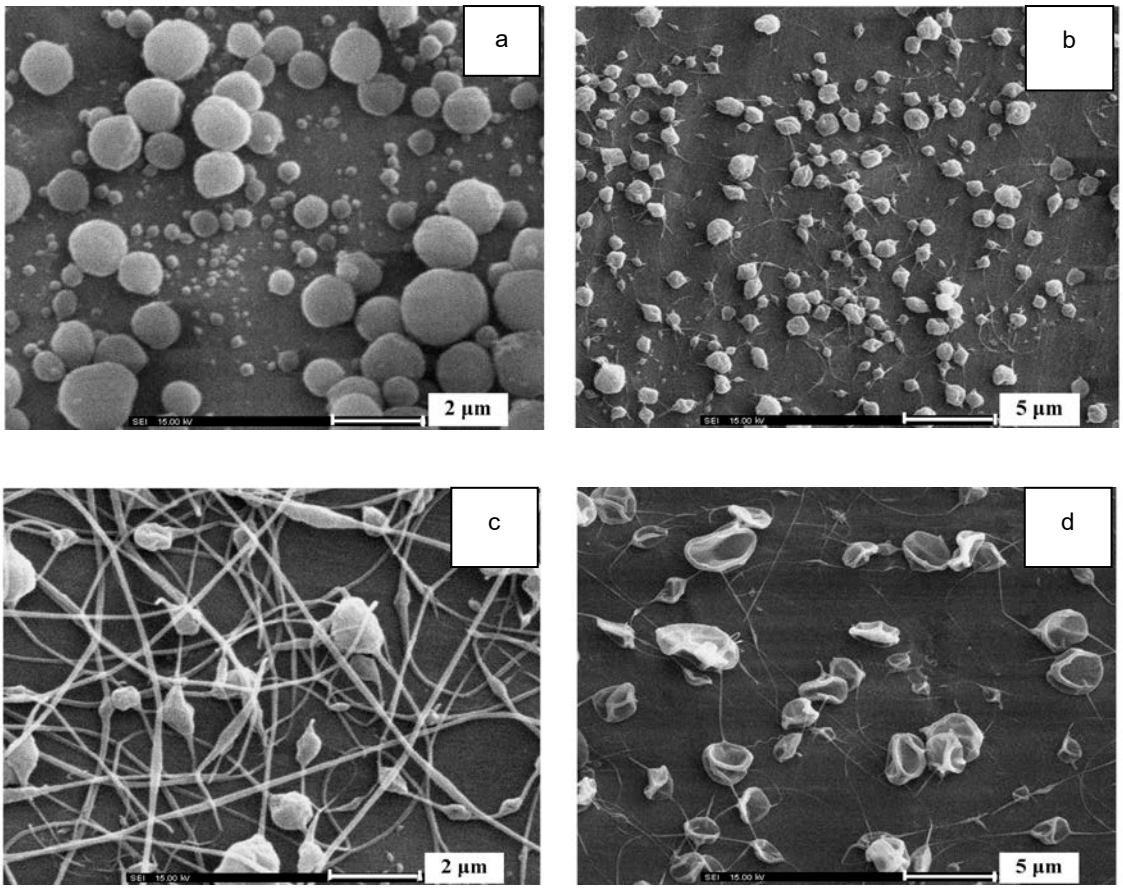
$$\mathrm{X} = \frac{CE}{2SE} = q^2(64\pi^2\varepsilon\gamma R^3) \quad (3)$$

where X is Rayleigh limit, CE is Coulomb energy, SE is surface free energy, q is the surface charge of the droplet, ε is the permittivity of the medium surrounding the droplet, $\gamma$is the surface tension of the liquid, and R is the radius of a droplet (8, 9).

$$\mathrm{d}_d = \propto \left(\frac{Q^3\varepsilon\rho}{\pi^4\sigma\gamma}\right)^{\frac{1}{6}} \quad (4)$$

where d_d is droplet size of neat liquids, $\propto$ is a constant depending on liquid permittivity, Q is the liquid flow rate, ε is the dielectric constant in a vacuum, ρ is the density of the liquid, γ is the surface tension of the liquid, and σ is the conductivity of the liquid (10).

From the characterization of the EDS solution results shown in Table 1, the EDS dissolved in methanol provided higher conductivity and surface tension with low viscosity, resulting in a high Rayleigh limit and low droplet size of neat liquids. Consequently, there is high possibility that the droplet breakup occurred, causing small spherical particles without fiber (11). On the other hand, EDS dissolved in ethanol and 2-propanol offered lower conductivity and surface tension with high viscosity. These make high Rayleigh limit value, causing connected spraying liquid and connected fiber products.



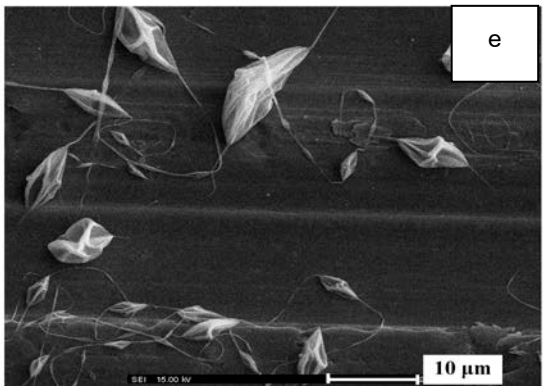


Fig. 2. SEM images of the NPs prepared from EDS (5% w/v) dissolved with different media; (a) methanol, (b) ethanol, (c) ethanol/water (70:30), (d) 2-propanol and (e) t-butanol.

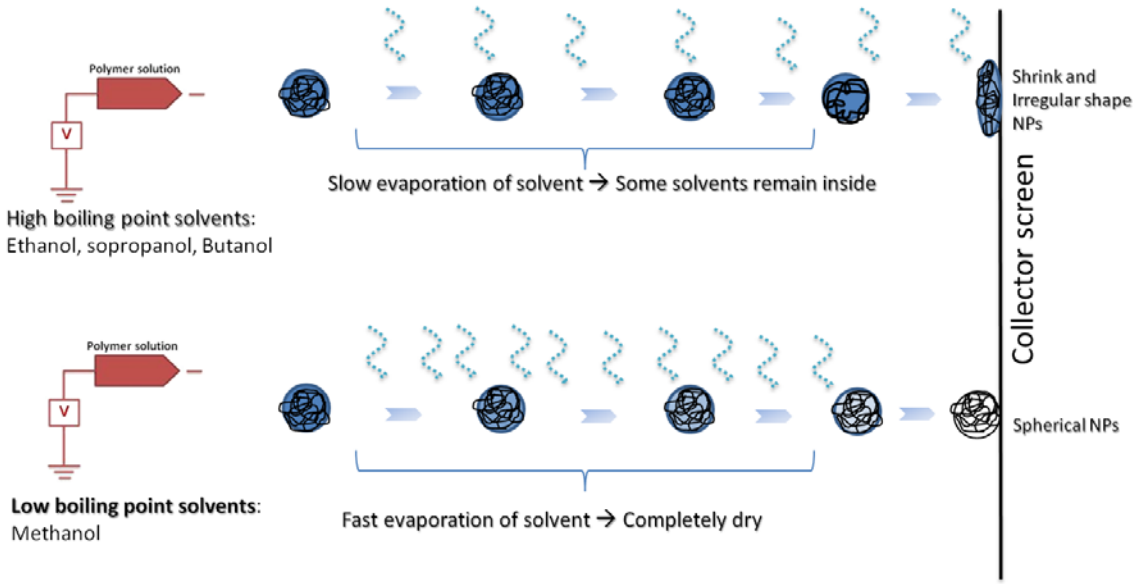


Fig. 3 Proposed fabrication mechanism of the NPs by EHDA

The electrosprayed products were changed from spherical particles to fibers when the spraying solution prepared from high concentration of EDS (Fig. 4). As explained in the Equation (3), a higher viscosity and lower surface tension of 10% w/v EDS (data not shown) resulted in the formation of smooth filaments or fiber. Figs. 4a, 5a and 5b illustrated the electrosprayed products fabricated by 10, 15 and 20 kV of applied voltages, respectively. More fibrous products were obtained and the product

size is smaller when applying higher voltages (15 kV and 20 kV). This is in agreement with previous research that the size of particle slightly decreases when voltage is increased (12, 13). Moreover, Shenoy et al. (14, 15) stated that, since the voltage is increased, morphology changes from spherical particle to elongated particles or bended fiber to eventually only fiber. This might be due to the fact that high voltage accelerates the sprayed drop to elongate, resulting in connected fiber (16).

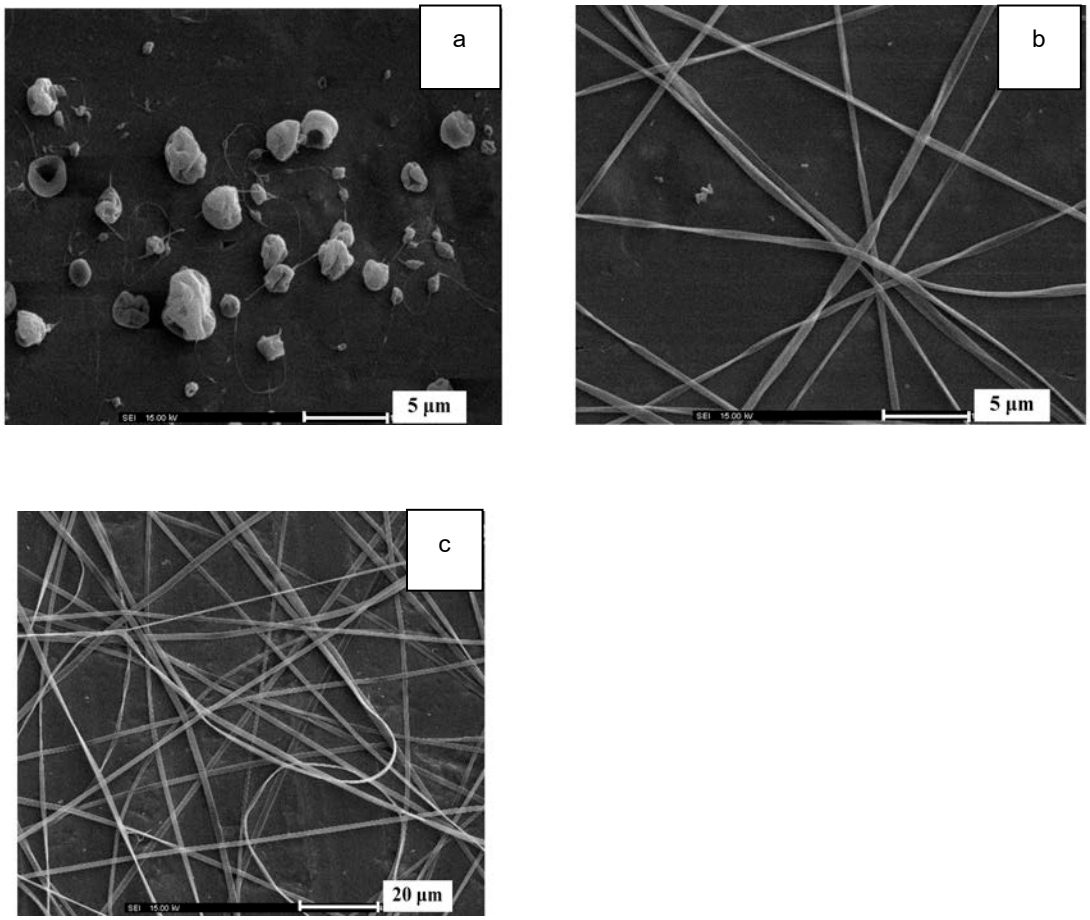


Fig. 4. SEM images of the products prepared from (a) 5% w/v, (b) 7.5% w/v and (c) 10% w/v EDS dissolved in ethanol under applied voltages of 10 kV.

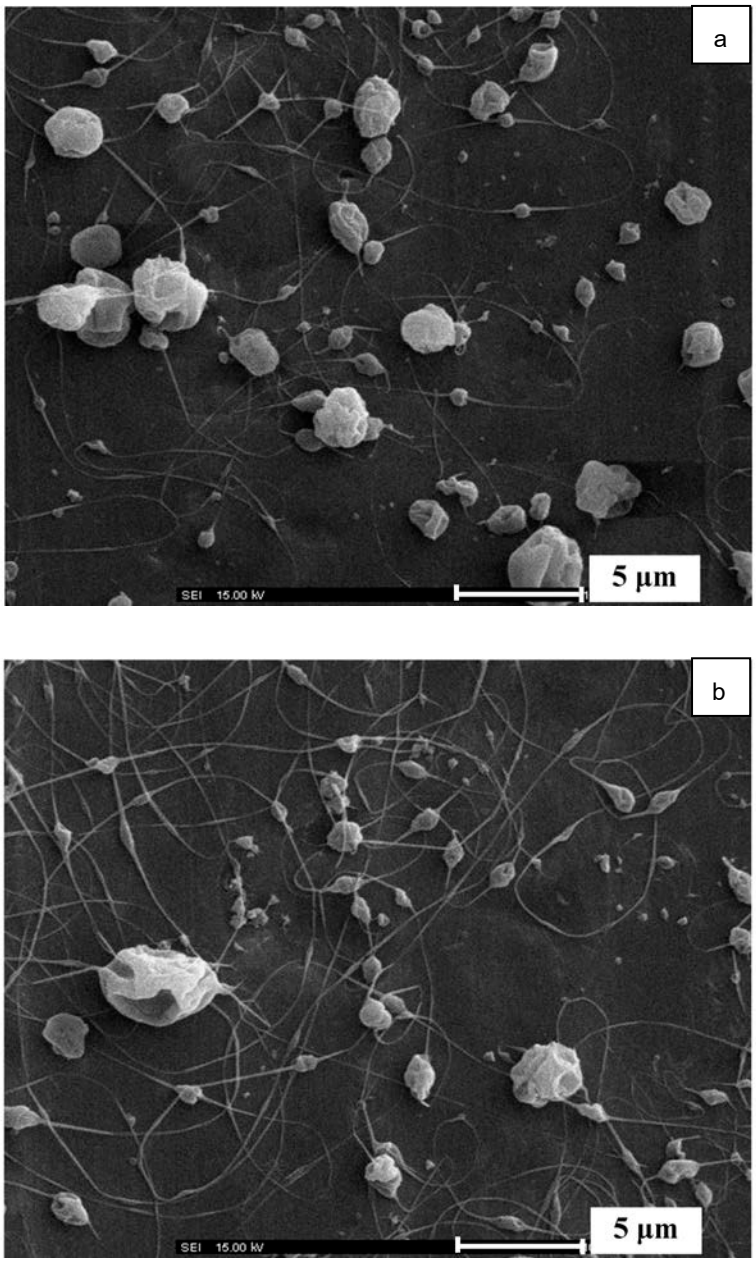


Fig. 5. SEM images of the products prepared from 5% w/v EDS dissolved in ethanol under applied voltages of (a) 15 kV and (b) 20 kV.

Particle morphology of prednisolone and the NPs loaded with different concentrations of prednisolone is demonstrated in Fig. 6. It is observed that sprayed prednisolone without EDS was irregular in shape with the size ranged from 5 to 10 µm (Fig. 6a). All of the NPs (Figs. 6b-e) were spherical in shape with average size ranged from 448.60 to 660.98 nm (see Table 3). The particle size was not significantly different among the NPS containing different drug concentrations. This result could be explained by the conductivity, viscosity and interfacial tension of EDS solutions loaded with different prednisolone concentrations (Table 2), which were slightly different, resulting in

a slight difference in size and shape (10). Furthermore, the irregular shaped particles were not found for all prednisolone-loaded NPs. This could confirm that all prednisolone was loaded in the NPs (17).

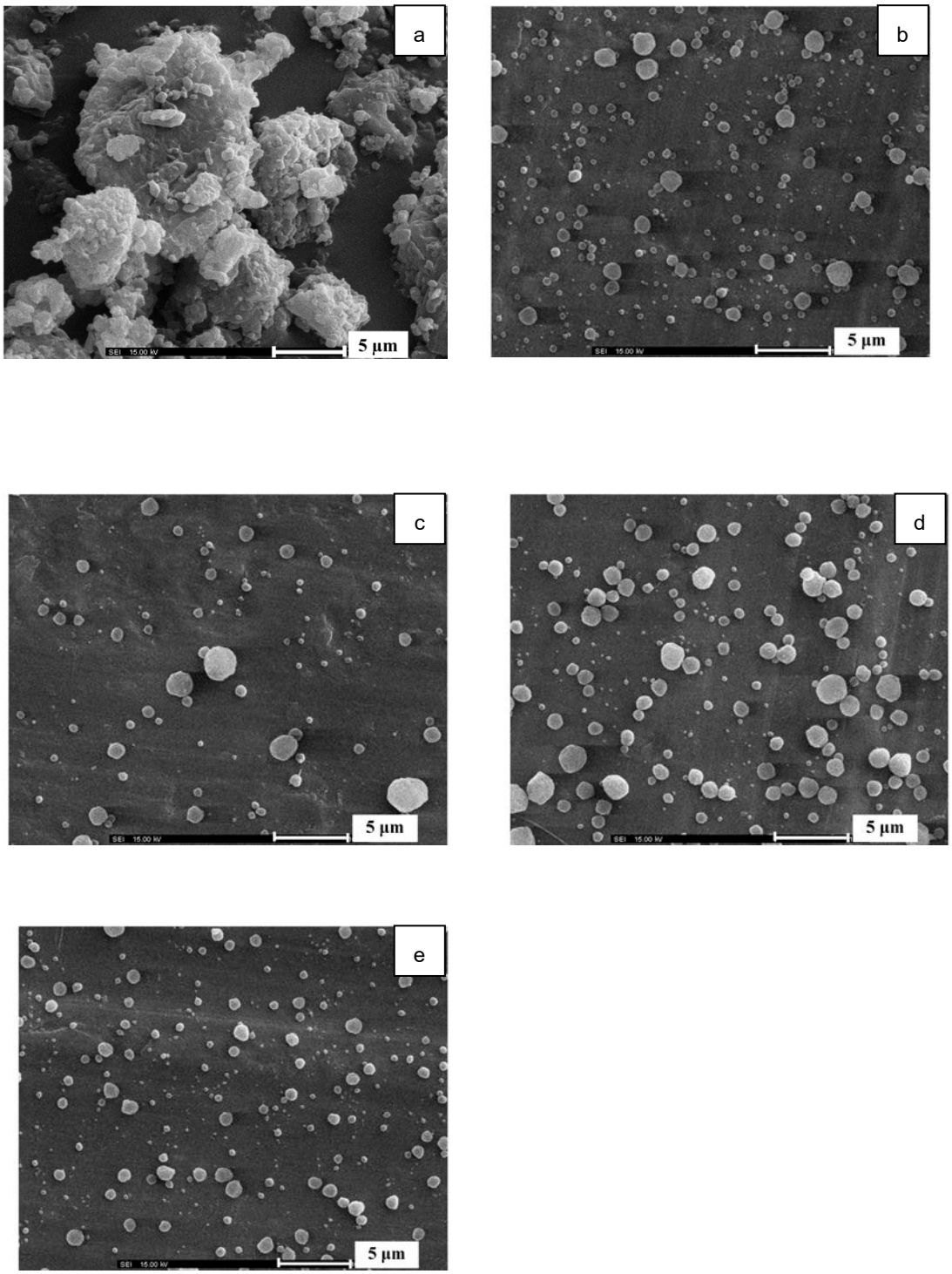


Fig. 6. SEM images of the (a) sprayed prednisolone, and prednisolone-loaded NPs containing (a) 0.5% w/v, (b) 1.5% w/v, (c) 2.5% w/v and (d) 3.5% w/v prednisolone, using 5% w/v EDS in methanol under applied voltage of 10 kV.

Table 4 Particle size and zeta-potential value of NPs containing different concentrations of prednisolone.

	Concentration of prednisolone in NPs			
	0.5% w/v	1.5% w/v	2.5% w/v	3.5% w/v
Particle size (nm)	448.6±65.2	492.4±69.9	661.0±65.7	671.1±59.3
Zeta-potential (mV)	-16.08±0.24	-33.20±0.46	-25.71±0.78	-23.64±0.76

3.3. Zeta-potential determination

Table 3 shows the particle size and zeta-potential value of NPs containing different concentrations of prednisolone. Particle size of the NPs ranged from 448.6 to 671.1 nm. The zeta potential value of all NPs was negative, resulting from the negative charge of carboxylic moiety in the EDS structure (17). The results indicated the encapsulation of prednisolone in EDS.

3.4. Drug loading and encapsulation efficiency

Drug loading and encapsulation efficiency of NPs containing different concentrations of prednisolone are shown in Table 4. The percentage of drug loading ranged from 6.58% to 37.68% and the encapsulation efficiency ranged from 72.35% to 92.65%. The encapsulation efficiency of the NPs depended on concentration of prednisolone in the NPs. High portion of prednisolone (2.5% and 3.5% prednisolone in 5% w/v EDS) provided high drug loading and encapsulation efficiency. Such an effect on encapsulation of drug is quite commonly reported. The increase in encapsulated drug might rise the viscosity of the EDS solution. This makes the EHDA product larger resulting in higher drug encapsulation.

Table 5**.** Drug loading and encapsulation efficiency of NPs containing different concentrations of prednisolone.

The NPs loaded with different prednisolone concentrations	Drug loading (%)	Encapsulation efficiency (%)

0.5%	6.58±0.06	72.35±0.63
1.5%	17.27±0.28	74.84±1.23
2.5%	30.86±0.54	92.65±1.61
3.5%	37.68±0.85	91.50±2.07

3.5. Fourier transform infrared (FTIR) spectroscopy

FITR spectra of the starting materials (EDS and prednisolone), their physical mixture and the NPs loaded with 2.5% prednisolone are illustrated in Fig. 7. The spectrum of prednisolone showed the characteristic stretching vibration of C=O at 1700 cm^{-1} and C=C from cyclohexadiene at 1660 cm^{-1}. The FTIR spectrum of EDS exhibited peaks at 1731 and 1159 cm^{-1} due to C=O stretching of carboxylic acid and C-O stretching of ester, respectively. The spectra of physical mixture of EDS and prednisolone showed peaks at similar wave number as the starting materials. However, strong peak of prednisolone at 1700 cm^{-1} was covered by broad peak of EDS. The IR absorption of NPs showed the same pattern as prednisolone and EDS but peaks at 1605 and 891 cm^{-1} were weaker, which could be attributed to the encapsulation a drug molecule inside the polymer and led to the low IR absorption of prednisone (18).

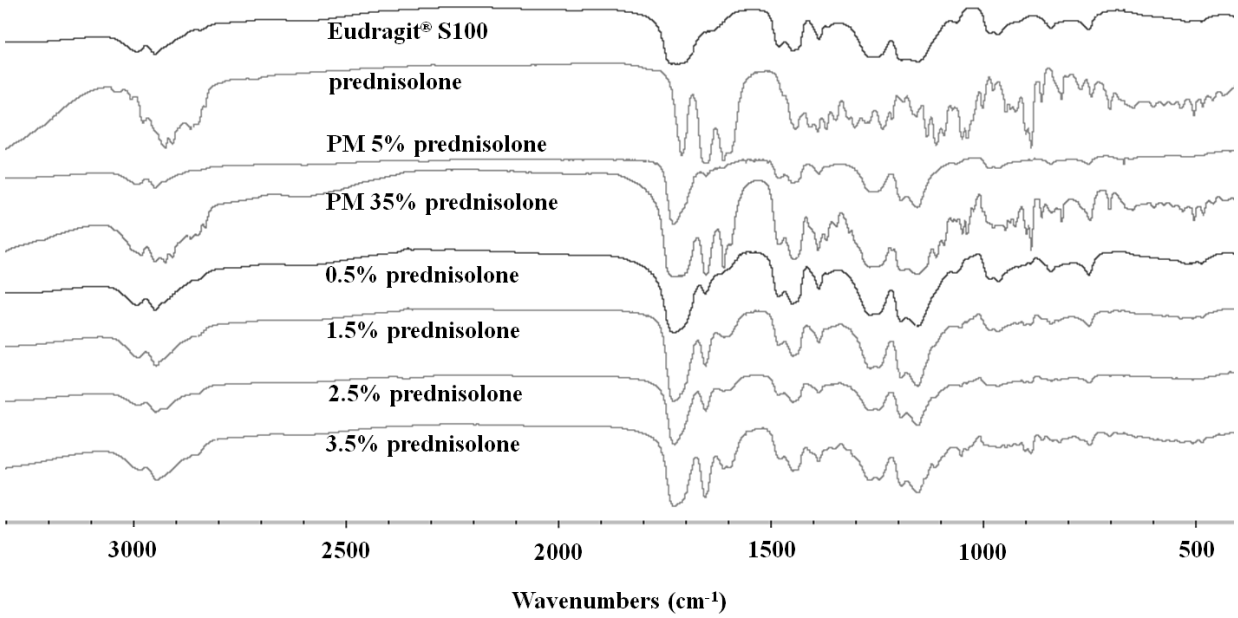


Fig. 7 FTIR spectra of prednisolone, EDS, physical mixture of prednisolone and EDS (PM) and prednisolone-loaded NPs.

3.6. Powder X ray diffraction

Fig. 8 illustrated the powder X-ray diffractograms of the starting materials (EDS100 and prednisolone), their physical mixture and the NPs loaded with 2.5% prednisolone. The powder X-ray diffraction pattern of EDS exhibited typical halo patterns of amorphous structure while prednisolone revealed sharp crystalline peaks (19, 20). The NPs demonstrated broad peaks at 14.82° due to the EDS while PM expressed sharp peaks at 7.72°, 10.36°, 13.74°, 15.22°, 17.74° and 21.00°, which appeared at the same positions as prednisolone. This indicated that prednisolone dispersed molecularly in EDS.

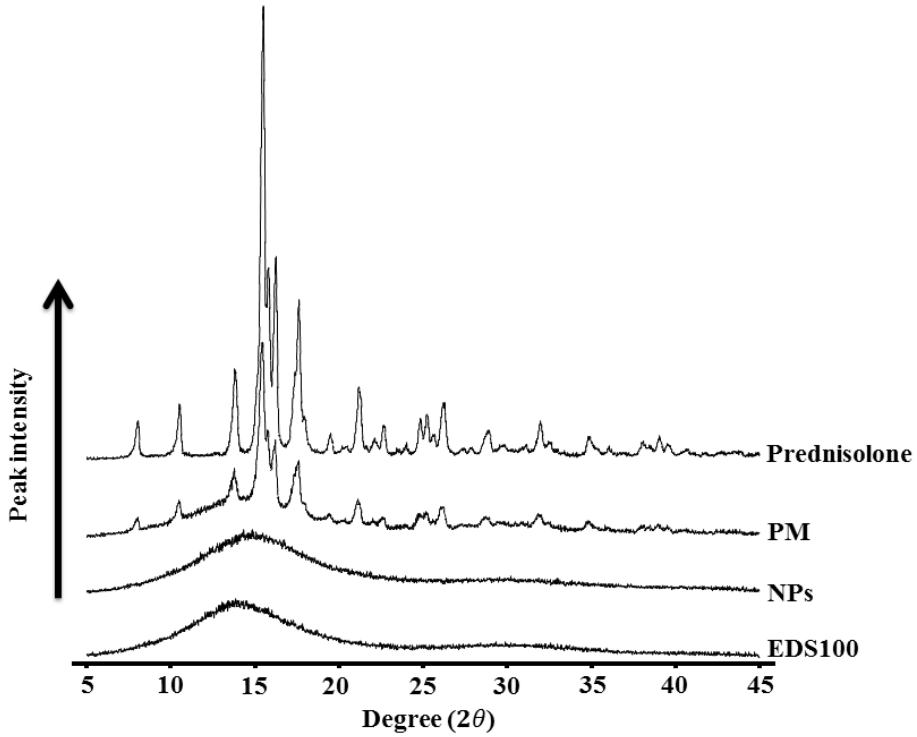


Fig. 8 Powder X-ray diffractograms of prednisolone, EDS, physical mixture of prednisolone and EDS (PM) and prednisolone-loaded NPs. The PM was prepared at the same drug:polymer ratio as NPs.

3.7. Differential scanning calorimetry (DSC)

The DSC thermograms of prednisolone, EDS, their physical mixture and the NPs loaded with 2.5% prednisolone are shown in Fig. 9. The thermogram of EDS showed a broad endothermic peak representing amorphous state of the polymer (19). The DSC thermogram of prednisolone expressed an endothermic characteristic peak at 240ºC assigned to its melting point (20). The DSC thermogram of physical mixture of prednisolone:EDS (1:2) showed that the endothermic peak of the drug existed at lower temperature compared to prednisolone alone and lost its distinct sharpened appearance. This observation suggested the presence of an interaction between

prednisolone and the polymer at high temperature. For the NPs, no melting peak of prednisolone was found. It is indicated that prednisolone has been highly dispersed in the NPs by EHDA fabrication technique (21).

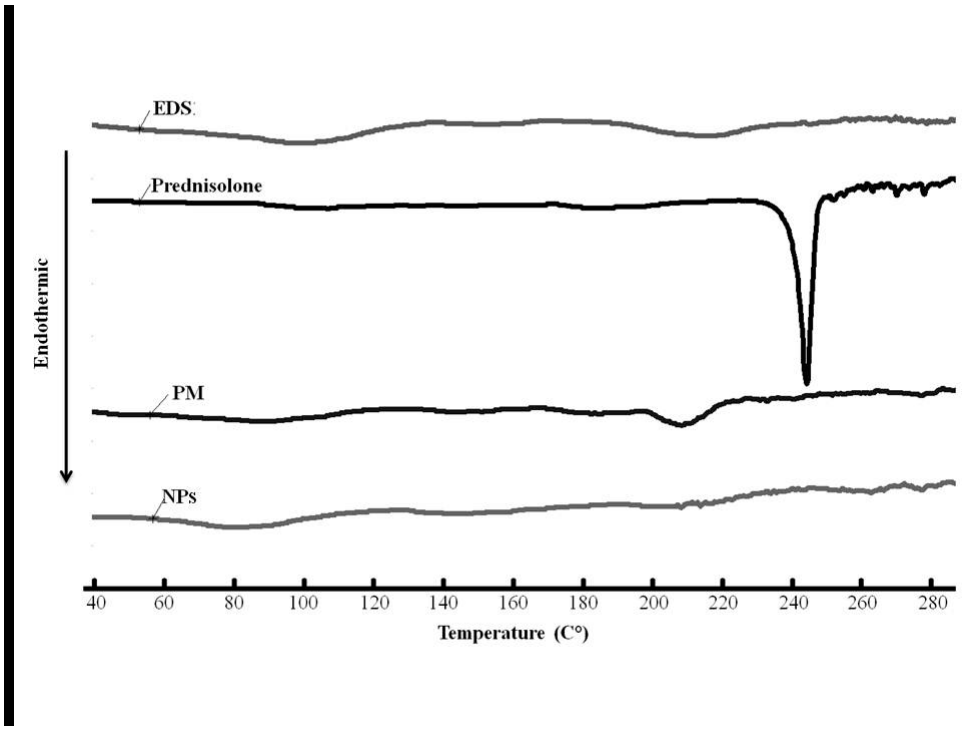


Fig. 9 DSC thermograms of prednisolone, EDS, physical mixture of prednisolone and EDS (PM) and prednisolone-loaded NPs. The PM was prepared at the same drug:polymer ratio as NPs.

สรุปผลการทดลอง (Conclusion)

EHDA technology can be applied for the fabrication of EDS-based NPs containing prednisolone. Properties of the sprayed solution are crucial factors to determine characteristic of the EHDA products. The spherical NPs were obtained from 5% w/v EDS dissolved in methanol under conditions of 10 kV applied voltage. Moreover, prednisolone can be loaded in the NPs with high encapsulation efficiency. This nanoparticulate system expresses possibility to fabricate colon-specific drug delivery system.

ข้อเสนอแนะสำหรับงานวิจัยในอนาคต

- พัฒนาให้สามารถกักเก็บยาได้นานขึ้นในระบบทางเดินอาหารส่วนต้น
- ทดสอบความเป็นพิษของระบบนำส่งยาในเซลล์ Caco-2 และสัตว์ทดลอง
- ทดสอบประสิทธิภาพระบบนำส่งยาระดับ in-vivo

Keywords : Electrohydrodynamic atomization, nanoparticles, prednisolone
(คำหลัก) Electrohydrodynamic atomization, nanoparticles, prednisolone

เอกสารแนบหมายเลข 3

Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ (ระบุชื่อผู้แต่ง ชื่อเรื่อง ชื่อวารสาร ปี เล่มที่ เลขที่ และหน้า) หรือผลงานตามที่คาดไว้ในสัญญาโครงการ
2. Kampanart Huanbutta, Tanikan Sangnim, Sontaya Limmatvapirat, Jurairat Nunthanid and Pornsak Sriamornsak, Advanced Materials Research, Vol. 1060 (2015) หน้าที่ 103-106
3. การนำผลงานวิจัยไปใช้ประโยชน์
   - เชิงพาณิชย์ (มีการนำไปผลิต/ขาย/ก่อให้เกิดรายได้ หรือมีการนำไปประยุกต์ใช้โดยภาคธุรกิจ/บุคคลทั่วไป)
   - เชิงนโยบาย (มีการกำหนดนโยบายอิงงานวิจัย/เกิดมาตรการใหม่/เปลี่ยนแปลงระเบียบข้อบังคับหรือวิธีทำงาน)
   - เชิงสาธารณะ (มีเครือข่ายความร่วมมือ/สร้างกระแสความสนใจในวงกว้าง)
   - เชิงวิชาการ (มีการพัฒนาการเรียนการสอน/สร้างนักวิจัยใหม่)
4. อื่นๆ (เช่น ผลงานตีพิมพ์ในวารสารวิชาการในประเทศ การเสนอผลงานในที่ประชุมวิชาการ หนังสือ การจดสิทธิบัตร)
   - งานประชุม The 3rd International Conference and Exhibition on Pharmaceutical, Nutraceutical and Cosmeceutical Technology 2014 (PharmaTech 2014) ณ ศูนย์ประชุมแห่งชาติสิริกิติ์ ระหว่างวันที่ 1-2 ธันวาคม 2558

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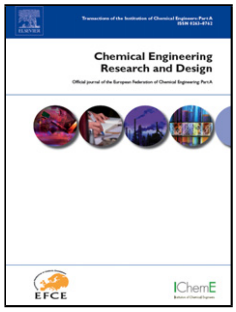

Title: Design and Characterization of Prednisolone-loaded Nanoparticles Fabricated by Eletrohydrodynamic Atomization Technique

Author: Kampanart Huanbutta Tanikan Sangnim Sontaya Limmatvapirat Jurairat Nunthanid Pornsak Sriamornsak

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Design and Characterization of Prednisolone-loaded Nanoparticles Fabricated by Eletrohydrodynamic Atomization Technique

Kampanart Huanbutta[1,*], Tanikan Sangnim[2,3], Sontaya Limmatvapirat[2,3], Jurairat Nunthanid[2,3], Pornsak Sriamornsak[2,3]

[1]Faculty of Pharmaceutical Sciences, Burapha University, Chonburi 20131, Thailand

[2]Department of Pharmaceutical Technology, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand

[3]Pharmaceutical Biopolymer Group (PBiG), Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand

Keywords: Electrohydrodynamic atomization, nanoparticles, prednisolone

*Corresponding author

Kampanart Huanbutta, Faculty of Pharmaceutical Sciences, Burapha University, Chonburi, 20131, Thailand

Phone number +66 38 102610, Fax number +66 38 102610,

E-mail: kampanart@buu.ac.th

ABSTRACT

Nanoparticles (NPs) have been used for colon-specific drug delivery system due to their specific accumulation in the inflamed tissue in the colon and prolong presence in colon for the treatment of inflammatory bowel disease. The present study aimed to design NPs for colon-specific drug delivery via electrohydrodynamic atomization (EHDA). The NPs were prepared by dissolving prednisolone and Eudragit® S100 (EDS) in different solvents using various EDS concentrations and prednisolone/EDS weight ratios. Then, the prepared solution was sprayed using the EHDA machine. The process parameters such as applied voltage, injected distance, feed rate, and drum collector rolling rate were carefully adjusted to obtain blank and drug-loaded NPs. The EDS solution prepared from methanol offered high conductivity (93.51 μs) with low viscosity (4.48 cP), resulting in spherical and small NPs with average diameter of 422 nm. The NPs prepared by using isopropanol and butanol as a solvent were irregular in shape. The sprayed products were changed from particle to fiber when using high concentration of EDS. High-fiber product was obtained when high applied voltage (20kV) was applied. Size range of the NPs loaded with prednisolone at different concentrations (0.5%-3.5%) was 448-660 nm. The maximum drug encapsulation was 91.50%. From the obtained results, the NPs were successfully fabricated by EHDA technique and this showed a promising potential for further drug delivery system development.

1. Introduction

Electrohydrodynamic atomization (EHDA) has gained attention in the field of drug delivery in the past decade; it has been used to fabricate drug carriers including polymeric microparticles, nanoparticles (NPs) and nanofibers used in various pharmaceutical applications (Kenawy et al., 2002; Shen et al., 2011). Theoretically, EHDA is a process where a liquid breaks up into spray droplets under the drive of an electrical field. Various modes of EHDA were reported, of which the stable cone-jet is of specific interest (Xie et al., 2014). Under such mode, liquid flowing through a nozzle maintained at high potential is subjected to an electric field (Fig. 1), which leads to elongation of the meniscus to a form of jet and subsequently the jet deforms and breaks up into fine droplets. The small jet diameter allows rapid evaporation of the solvent and, as a result, particles are deposited on the collector. Applied voltage, feed rate, distance from the nozzle to collector, drum collector rolling rate, charge and viscosity of sprayed solution are vital parameters to determine product characteristics (Xie et al., 2014). The principal advantages of this technique in drug delivery system are (i) to offer opportunity of heat sensitive drug loading, (ii) the relative rapid and simple way to prepare a several materials into NPs, and (iii) controllable operation factors.

Inflammatory bowel diseases (IBD) are relapsing and chronic inflammatory disorders of the intestinal mucosa, with an organ-susceptibility for the colon (Mowat et al., 2011). Conventional drug delivery systems are not completely successful when applied for the treatment of IBD (Friend, 2005). Aside from incidences where therapy fails due to insufficient drug deposition at the site of action, adverse drug effects have also limited therapeutic outcome. These adverse effects are thought to be related to the lack of selective drug release at the inflamed

colon. Nanoparticulate systems for colonic drug delivery exhibit that size-dependency of particles impacts their epithelial uptake and a preferential accumulation in the inflamed colon has been found (Lamprecht, 2009). Lamprecht and co-workers (Lamprecht et al., 2001) demonstrated that high binding to the inflamed tissue of Lewis rat colon was found for 100-nm particles. This is because thicker mucus layer produced in the ulcerated regions increasing adherence of the small particles.

Prednisolone is a synthetic corticosteroid drug that is particularly effective as immunosuppressant and anti-inflammatory drug. It have been used to treat a number of different conditions such as IBD, inflammation (swelling), severe allergies, adrenal problems, arthritis and asthma (Gionchetti et al., 2002; Porter, 2011). For treatment of IBD, administration of prednisolone at a large and frequent dose for a long period causes significant and prolonged absorption of the drugs from the small intestine, often leading to toxic side effects (Campieri et al., 1997). Therefore, the specific delivery of drugs to diseased parts should be developed. Furthermore, prednisolone is a class II substance according to the Biopharmaceutics Classification System; it is a poorly water-soluble drug (Vogt et al., 2007). Consequently, the improvement of the dissolution rate of prednisolone by different techniques such as solid dispersion, nanoparticle preparation and liquisolid compacts can offer more rapid and complete absorption (Chen et al., 2015; Spireas and Sadu, 1998; Zakeri-Milani et al., 2011).

To extensively target drug to the colon, nanocarriers made of pH-dependent polymer have been applied. In the gastrointestinal tract, from the stomach to the colon, intraluminal pH varies progressively up to 7. In order to obtain pH-sensitive NPs, Eudragit® S, a polymer of methacrylic acid and methyl methacrylate, which dissolves when the pH is above 7, was used to control the release of entrapped drug (Damge et al., 2010; Makhlof et al., 2009). Therefore, the

aim of this study was to design and optimize formulation and processing factors influencing the characteristics of NPs prepared by EHDA technique.

2. Materials and methods

2.1. Materials

Eudragit® S100 (EDS) (lot number B041005026) was a gift from Evonik Degussa (Thailand) Co., Ltd., Thailand. Methanol (lot number 14080041) was purchased from RCI Labscan Ltd., Thailand. Ethanol (lot number J32T04) was obtained from Mallinckrodt Baker, Malaysia. Butanol (lot number K35977190619) was purchased from Merck, Germany. Isopropanol (lot number V1A707131A) was received from Carlo Erba Reagents, France. Prednisolone (lot number R25301) was a gift from Bangkok Lab & Cosmetic Co., Ltd., Thailand. All other chemicals were of reagent grade and used without further purification.

2.2. Characterization of EDS solution

The prepared EDS solutions were characterized by several techniques to reveal the effect of the polymer solution properties on the NPs. The conductivity of each EDS solution was measured by conductivity meter (model ECtestr11+, Eutech Instruments Pte Ltd., Singapore). The viscosity was examined by viscometer (model DV-III Ultra, Brookfield, USA). The surface tension was measured by sessile drop method using a drop shape instrument (model FTA 1000, First Ten Angstroms, USA). All experiments were performed in triplicate.

2.3. Preparation of NPs

To prepare the blank NPs, various concentrations of EDS were dissolved in several solvents, including methanol, ethanol, isopropanol, butanol and mixtures of ethanol/water. The EDS solution was then sprayed using EHDA machine (Bangkok Cryptography, Thailand). For

the drug-loaded NPs, prednisolone was dissolved in the EDS solution before spraying. The drug:EDS weight ratios were varied at 0.5:5, 1.5:5, 2.5:5 and 3.5:5. As presented in Table 1, the processing parameters such as applied voltage, injected distance, feed rate, and drum collector rolling rate were carefully adjusted to obtain blank and drug-loaded NPs. The products were kept in desiccator for further investigation.

2.4. Morphology and particle size

Surface morphology of the prepared NPs was carried out using scanning electron microscope (SEM; model Maxim 2000S, CamScan Analytical, UK). Sample powders for SEM analyses were sprinkled on a stub and sputter-coated with gold to increase their conductance. Particle size and size distribution were analyzed by J MicroVision software (version 1.2.7).

2.5. Zeta potential determination

Zeta potential of the blank and drug-loaded NPs was monitored by zeta potential analyzer (model ZetaPlus, Brookhaven, USA) to evaluate the coating efficiency. The samples were diluted to 0.5% w/v using deionized water as a solvent and then agitated for 3 min before measuring. The average and standard deviation of the measurement of three batches of samples were reported.

2.6. Drug content and encapsulation efficiency

Drug content was determined by dispersing prednisolone-loaded NPs (2 mg) in methanol (10 mL), stirring for 24 h and then sonicating for 2 h before the dispersion was filtered through 0.45-μm cellulose acetate membrane. The amount of prednisolone in the filtrate was quantified by high performance liquid chromatography (HPLC; model JASCO PU-2089plus quaternary gradient inert pump, and JASCO UV-2070plus multiwavelength UV–vis detector, Jasco, Japan) (Tobita et al., 2002). The analytical column used was a reverse phase Kinetex^{TM} column (C18,

250×4.6 mm, pore size 5 μm, Phenomenex, USA). The mobile phase was a mixture of methanol, tetrahydrofuran and water (at a ratio of 3:25:72). The column effluent was detected at 254 nm with a UV/vis detector. The calculation equation is as follows:

$$\text{Drug content (\%)} = \frac{\text{Amount of drug in nanoparticles}}{\text{Weight of nanoparticles}} \times 100 \quad (1)$$

The encapsulation efficiency was defined as the ratio of actual and original amount of prednisolone encapsulated in NPs. The encapsulation efficiency was calculated using the following equation:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Actual amount of drug-loaded in nanoparticles}}{\text{Initial amount of drug-loaded in nanoparticles}} \times 100 \quad (2)$$

2.7. Fourier transform infrared (FTIR) spectroscopy

To investigate the effect of the EHDA processing parameters on the drug-polymer interaction, prednisolone, EDS, their physical mixtures and prednisolone-loaded NPs were pulverized and blended with KBr and compressed. The measurement was carried out using a FTIR spectrophotometer (model Magna-IR system 750, Nicolet Biomedical Inc., USA).

2.8. Powder X ray diffraction

Powder X-ray diffraction patterns of the stating materials, their physical mixtures and the NPs were analyzed by using a powder X-ray diffractometer (model D8, Bruker, Germany) under the following conditions: graphite monochromatized Cu Kα radiation, voltage 45 kV, electric current 40 mA, slit: DS1°, SS1°, RS, 0.15 nm, and scanning ratio $2\theta = 5^{o}\,\text{min}^{-1}$.

2.9. Differential scanning calorimetry (DSC)

DSC thermograms of the stating materials, their physical mixtures and the NPs were determined by a differential scanning calorimeter (model Sapphire, Perkin Elmer, USA) using indium as a standard. Each sample, 2–3 mg, was accurately weighed into a close aluminum solid pan. The scanning rate was run at 10°C/min from 20 to 300°C under nitrogen purge.

2.10. Statistical analysis

Analysis of variance (ANOVA) and Levene's test for homogeneity of variance were performed using SPSS version 11.5 for Windows (SPSS Inc., USA). *Post hoc* testing ($p < 0.05$) of the multiple comparisons was performed by either the Scheffé or Games-Howell test depending on whether Levene's test was insignificant or significant, respectively.

3. Result and discussion

3.1. Characterization of EDS solution

The conductivity, viscosity and interfacial tension of 5% w/v EDS in methanol, ethanol, 2-propanol or t-butanol are given in Table 2. EDS did not completely dissolve in t-butanol; therefore, characterization of the solution of EDS in t-butanol could not be conducted. In methanol, the viscosity of 5% w/v EDS was the lowest (4.48 cP) while the conductivity and interfacial tension were the highest, which were 93.51 µS and 22.82 mN/m, respectively. This might be because methanol contains higher amount of hydroxyl group, at the same weight, compared to other alcohols. Therefore, high hydroxyl groups can increase conductivity of the solution (Sonkar et al., 2012). Furthermore, earlier studies have shown that viscosity affects conductivity; a decrease in viscosity results in the increase in the movement of the ions, so the conductance of the electrolyte increases from 2-propanol to methanol (Asif, 2009).

The interfacial tension is one of the crucial parameter to determine properties of the sprayed products (Ciach et al., 2003). High surface tension value of the solution can decrease size of the particles. The interfacial tension values all of the EDS solutions were slightly different ranging from 20.46-22.82 mN/m. EDS solution prepared from methanol gave the highest

interfacial tension. This result is in agreement with the previous study (Ghahremani et al., 2012), which explained that the surface tension decreases with the length of the hydrocarbon tail.

The conductivity of drug-loaded EDS solution slightly decreased when the drug loading was higher (Table 3). This is because prednisolone is nonpolar drug which can lowering conductivity of the solution (Rub et al., 2014). For Interfacial tension value, there were not different amount several drug concentrations.

3.2 Morphology and particle size

The characterized blank and drug dissolved solutions were sprayed by EHDA to reveal the effect of the solution properties on appearance of the sprayed products. As shown in Fig. 2, the NPs prepared from EDS (5% w/v in methanol) offered spherical particles with diameter of 447.9 nm. The NPs prepared from EDS in ethanol and the mixture between ethanol and water (70:30) were spherical and fibrous as illustrated in Figs. 2b and 2c, respectively. The electrosprayed products of EDS in isopropanol and t-butanol were collapsed particles (Figs. 2d and 2e). The proposed fabrication mechanism of the NPs by EHDA is presented in Fig. 3. The boiling point of solvents may influence the evaporation of solvents. The boiling point of isopropanol and butanol are higher than those of methanol and ethanol; therefore, solvent evaporation may not complete during spraying. After that, the wet droplets hit the collector causing irregular and collapsed particles (United States Pharmacopeial, 2006). This phenomenon can be explained by Rayleigh's equation as shown in Equations (3) and (4).

$$X = \frac{CE}{2SE} = q^2(64\pi^2\varepsilon\gamma R^3) \quad (3)$$

where X is Rayleigh limit, CE is Coulomb energy, SE is surface free energy, q is the surface charge of the droplet, ε is the permittivity of the medium surrounding the droplet, γ is the surface tension of the liquid, and R is the radius of a droplet (Rayleigh, 1882; Taflin et al., 1989).

$$d_d = \propto \left(\frac{Q^3 \varepsilon \rho}{\pi^4 \sigma \gamma}\right)^{\frac{1}{6}} \quad (4)$$

where d_d is droplet size of neat liquids, $\propto$ is a constant depending on liquid permittivity, Q is the liquid flow rate, ε is the dielectric constant in a vacuum, ρ is the density of the liquid, γ is the surface tension of the liquid, and σ is the conductivity of the liquid (Gañán-Calvo, 1999).

From the characterization of the EDS solution results shown in Table 1, the EDS dissolved in methanol provided higher conductivity and surface tension with low viscosity, resulting in a high Rayleigh limit and low droplet size of neat liquids. Consequently, there is high possibility that the droplet breakup occurred, causing small spherical particles without fiber (Festag et al., 1997). On the other hand, EDS dissolved in ethanol and 2-propanol offered lower conductivity and surface tension with high viscosity. These make high Rayleigh limit value, causing connected spraying liquid and connected fiber products.

The electrosprayed products were changed from spherical particles to fibers when the spraying solution prepared from high concentration of EDS (Fig. 4). As explained in the Equation (3), a higher viscosity and lower surface tension of 10% w/v EDS (data not shown) resulted in the formation of smooth filaments or fiber. Figs. 4a, 5a and 5b illustrated the electrosprayed products fabricated by 10, 15 and 20 kV of applied voltages, respectively. More fibrous products were obtained and the product size is smaller when applying higher voltages (15 kV and 20 kV). This is in agreement with previous research that the size of particle slightly decreases when voltage is increased (Bock et al., 2012; Hong et al., 2008). Moreover, Shenoy et al. (Gupta et al., 2005; Huang et al., 2003) stated that, since the voltage is increased, morphology changes from spherical particle to elongated particles or bended fiber to eventually only fiber.

This might be due to the fact that high voltage accelerates the sprayed drop to elongate, resulting in connected fiber (Thammachat et al., 2010).

Particle morphology of prednisolone and the NPs loaded with different concentrations of prednisolone is demonstrated in Fig. 6. It is observed that sprayed prednisolone without EDS was irregular in shape with the size ranged from 5 to 10 μm (Fig. 6a). All of the NPs (Figs. 6b-e) were spherical in shape with average size ranged from 448.60 to 660.98 nm (see Table 3). The particle size was not significantly different among the NPS containing different drug concentrations. This result could be explained by the conductivity, viscosity and interfacial tension of EDS solutions loaded with different prednisolone concentrations (Table 2), which were slightly different, resulting in a slight difference in size and shape (Gañán-Calvo, 1999). Furthermore, the irregular shaped particles were not found for all prednisolone-loaded NPs. This could confirm that all prednisolone was loaded in the NPs (Huanbutta et al., 2013).

3.3. Zeta-potential determination

Table 3 shows the particle size and zeta-potential value of NPs containing different concentrations of prednisolone. Particle size of the NPs ranged from 448.6 to 671.1 nm. The zeta potential value of all NPs was negative, resulting from the negative charge of carboxylic moiety in the EDS structure (Huanbutta et al., 2013). The results indicated the encapsulation of prednisolone in EDS.

3.4. Drug loading and encapsulation efficiency

Drug loading and encapsulation efficiency of NPs containing different concentrations of prednisolone are shown in Table 4. The percentage of drug loading ranged from 6.58% to 37.68% and the encapsulation efficiency ranged from 72.35% to 92.65%. The encapsulation efficiency of the NPs depended on concentration of prednisolone in the NPs. High portion of

prednisolone (2.5% and 3.5% prednisolone in 5% w/v EDS) provided high drug loading and encapsulation efficiency. Such an effect on encapsulation of drug is quite commonly reported (Wischke and Schwendeman, 2008). The increase in encapsulated drug might rise the viscosity of the EDS solution. This makes the EHDA product larger resulting in higher drug encapsulation.

3.5. Fourier transform infrared (FTIR) spectroscopy

FITR spectra of the starting materials (EDS and prednisolone), their physical mixture and the NPs loaded with 2.5% prednisolone are illustrated in Fig. 7. The spectrum of prednisolone showed the characteristic stretching vibration of C=O at 1700 cm^{-1} and C=C from cyclohexadiene at 1660 cm^{-1}. The FTIR spectrum of EDS exhibited peaks at 1731 and 1159 cm^{-1} due to C=O stretching of carboxylic acid and C-O stretching of ester, respectively. The spectra of physical mixture of EDS and prednisolone showed peaks at similar wave number as the starting materials. However, strong peak of prednisolone at 1700 cm^{-1} was covered by broad peak of EDS. The IR absorption of NPs showed the same pattern as prednisolone and EDS but peaks at 1605 and 891 cm^{-1} were weaker, which could be attributed to the encapsulation a drug molecule inside the polymer and led to the low IR absorption of prednisone (Li et al., 2009).

3.6. Powder X ray diffraction

Fig. 8 illustrated the powder X-ray diffractograms of the starting materials (EDS100 and prednisolone), their physical mixture and the NPs loaded with 2.5% prednisolone. The powder X-ray diffraction pattern of EDS exhibited typical halo patterns of amorphous structure while prednisolone revealed sharp crystalline peaks (Auda et al., 2010; Sahin and Arslan, 2007). The NPs demonstrated broad peaks at 14.82° due to the EDS while PM expressed sharp peaks at

7.72°, 10.36°, 13.74°, 15.22°, 17.74° and 21.00°, which appeared at the same positions as prednisolone. This indicated that prednisolone dispersed molecularly in EDS.

3.7. Differential scanning calorimetry (DSC)

The DSC thermograms of prednisolone, EDS, their physical mixture and the NPs loaded with 2.5% prednisolone are shown in Fig. 9. The thermogram of EDS showed a broad endothermic peak representing amorphous state of the polymer (Auda et al., 2010). The DSC thermogram of prednisolone expressed an endothermic characteristic peak at 240ºC assigned to its melting point (Sahin and Arslan, 2007). The DSC thermogram of physical mixture of prednisolone:EDS (1:2) showed that the endothermic peak of the drug existed at lower temperature compared to prednisolone alone and lost its distinct sharpened appearance. This observation suggested the presence of an interaction between prednisolone and the polymer at high temperature. For the NPs, no melting peak of prednisolone was found. It is indicated that prednisolone has been highly dispersed in the NPs by EHDA fabrication technique (Maghsoodi, 2009).

4. Conclusion

EHDA technology can be applied for the fabrication of EDS-based NPs containing prednisolone. Properties of the sprayed solution are crucial factors to determine characteristic of the EHDA products. The spherical NPs were obtained from 5% w/v EDS dissolved in methanol under conditions of 10 kV applied voltage. Moreover, prednisolone can be loaded in the NPs with high encapsulation efficiency. This nanoparticulate system expresses possibility to fabricate colon-specific drug delivery system.

5. Acknowledgements

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Figure captions

Fig. 1. Schematic diagram showing nanoparticles fabrication by EHDA.

Fig. 2. SEM images of the NPs prepared from EDS (5% w/v) dissolved with different media; (a) methanol (5000X), (b) ethanol (2000X), (c) ethanol/water (70:30) (5000X), (d) 2-propanol (2000X) and (e) t-butanol (1000X).

Fig. 3 Proposed fabrication mechanism of the NPs by EHDA.

Fig. 4. SEM images of the products prepared from (a) 5% w/v (2000X), (b) 7.5% w/v (2000X) and (c) 10% w/v (500X) EDS dissolved in ethanol under applied voltages of 10 kV.

Fig. 5. SEM images of the products prepared from 5% w/v EDS dissolved in ethanol under applied voltages of (a) 15 kV and (b) 20 kV.

Fig. 6. SEM images at magnification of 2000X of the (a) sprayed prednisolone, and prednisolone-loaded NPs containing (a) 0.5% w/v, (b) 1.5% w/v, (c) 2.5% w/v and (d) 3.5% w/v prednisolone, using 5% w/v EDS in methanol under applied voltage of 10 kV.

Fig. 7 FTIR spectra of prednisolone, EDS, physical mixture of prednisolone and EDS (PM) and prednisolone-loaded NPs.

Fig. 8 Powder X-ray diffractograms of prednisolone, EDS, physical mixture of prednisolone and EDS (PM) and prednisolone-loaded NPs. The PM was prepared at the same drug:polymer ratio as NPs.

Fig. 9 DSC thermograms of prednisolone, EDS, physical mixture of prednisolone and EDS (PM) and prednisolone-loaded NPs. The PM was prepared at the same drug:polymer ratio as NPs.

Table captions

Table 1. The processing parameters of EHDA studied.

Table 2. Conductivity, viscosity and interfacial tension of 5% w/v EDS in different solvents.

Table 3 Conductivity, viscosity and interfacial tension values of predinolone-loaded EDS solution using methanol as dissolving solvent.

Table 4 Particle size and zeta-potential value of NPs containing different concentrations of prednisolone.

Table 5. Drug loading and encapsulation efficiency of NPs containing different concentrations of prednisolone.

420 **Table 1.** Conductivity, viscosity and interfacial tension of 5% w/v EDS in different solvents.

Solvent	Conductivity (μS)	Viscosity (cP)	Interfacial tension (mN/m)
methanol	93.51 ± 2.56	4.48 ± 1.10	22.82 ± 0.35
ethanol	31.64 ± 0.74	5.76 ± 0.37	21.71 ± 0.19
2-propanol	9.13 ± 0.09	10.88 ± 0.37	20.46 ± 0.25
t-butanol	N/A*	N/A*	N/A*

421 * EDS did not completely dissolve in t-butanol.

422

423

423 **Table 2** Conductivity, viscosity and interfacial tension values of predinolone-loaded EDS
424 solution using methanol as dissolving solvent.

The EDS solution loaded with different prednisolone concentrations	Conductivity (μS)	Viscosity (cP)	Interfacial tension (mN/m)
0.5% prednisolone	97.03 ± 1.26	2.99 ± 0.37	21.60 ± 0.87
1.5% prednisolone	94.70 ± 2.86	4.05 ± 0.32	22.20 ± 0.17
2.5% prednisolone	92.41 ± 0.54	4.27 ± 0.37	22.28 ± 0.30
3.5% prednisolone	92.02 ± 0.54	4.91 ± 0.36	21.48 ± 0.23

425
426
427

427 **Table 3** Particle size and zeta-potential value of NPs containing different concentrations of
428 prednisolone.

	Concentration of prednisolone in NPs			
	0.5% w/v	1.5% w/v	2.5% w/v	3.5% w/v
Particle size (nm)	448.6±65.2	492.4±69.9	661.0±65.7	471.1±59.3
Zeta-potential (mV)	-16.08±0.24	-33.20±0.46	-25.71±0.78	-23.64±0.76

429
430
431

Table 4. Drug loading and encapsulation efficiency of NPs containing different concentrations of prednisolone.

The NPs loaded with different prednisolone concentrations	Drug loading (%)	Encapsulation efficiency (%)
0.5%	6.58±0.06	72.35±0.63
1.5%	17.27±0.28	74.84±1.23
2.5%	30.86±0.54	92.65±1.61
3.5%	37.68±0.85	91.50±2.07

435 **Highlight**

436 - Using a novel technique, electrohydrodynamic atomization (EHDA), to the prepare polymeric

437 nanoparticles (NPs)

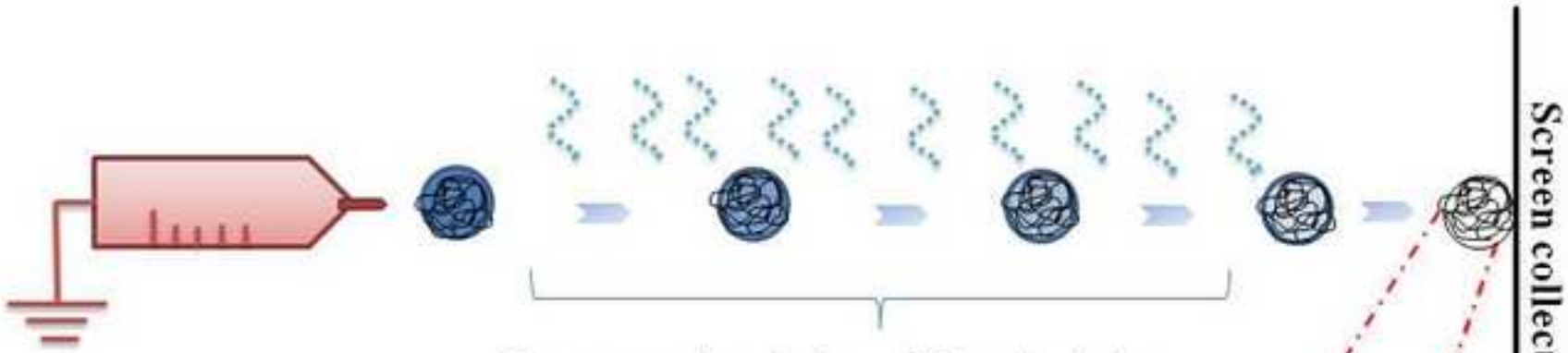
438 - High drug entrapment nanoparticulate system

439 - Several formulation and operating parameters were optimized and revealed

440 - The revealed condition and formulation can be used and developed with other drug

441

442

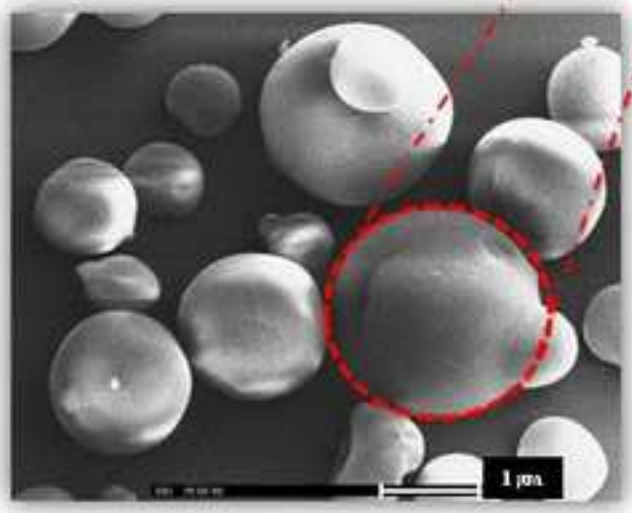


Formulation variation

- concentrations of EDS solution
- Solvents
- Drug concentration

Processing parameters variation

- applied voltage
- injected distance
- feed rate
- drum collector rolling



Spherical nanoparticles

Figure1

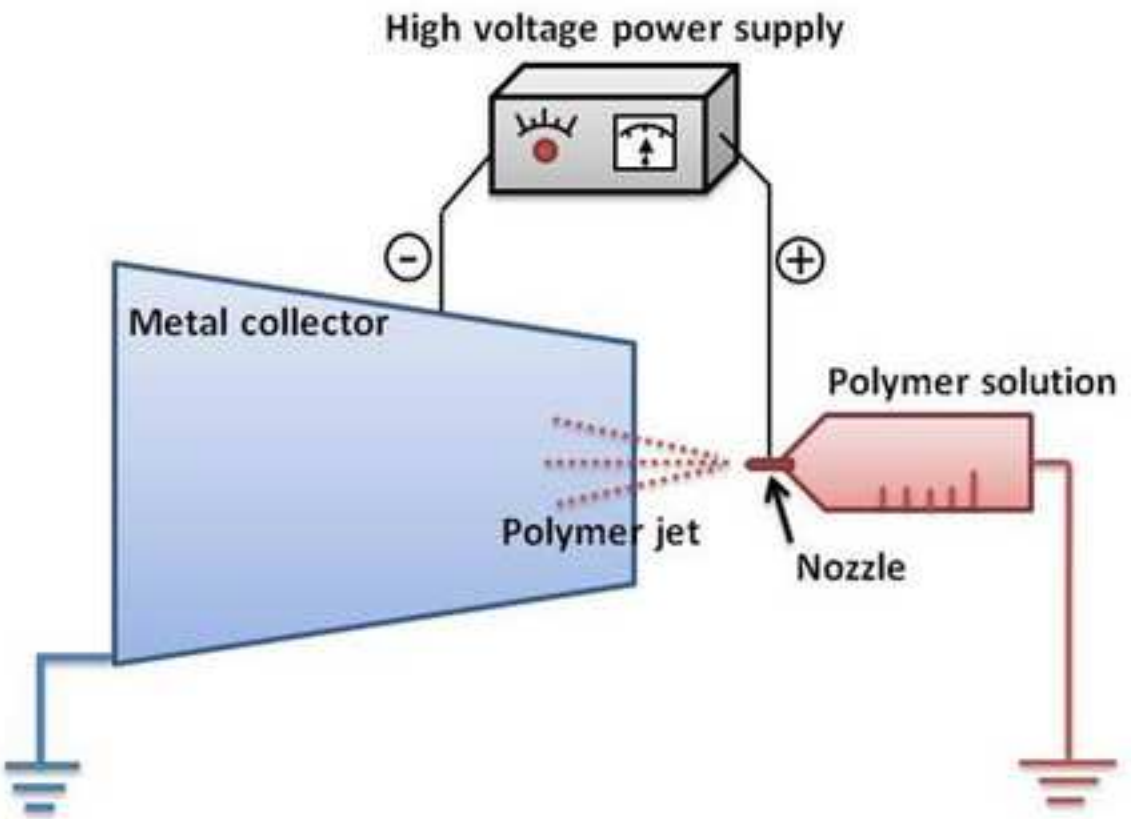

High voltage power supply
Metal collector
Polymer solution
Polymer jet
Nozzle

Figure2

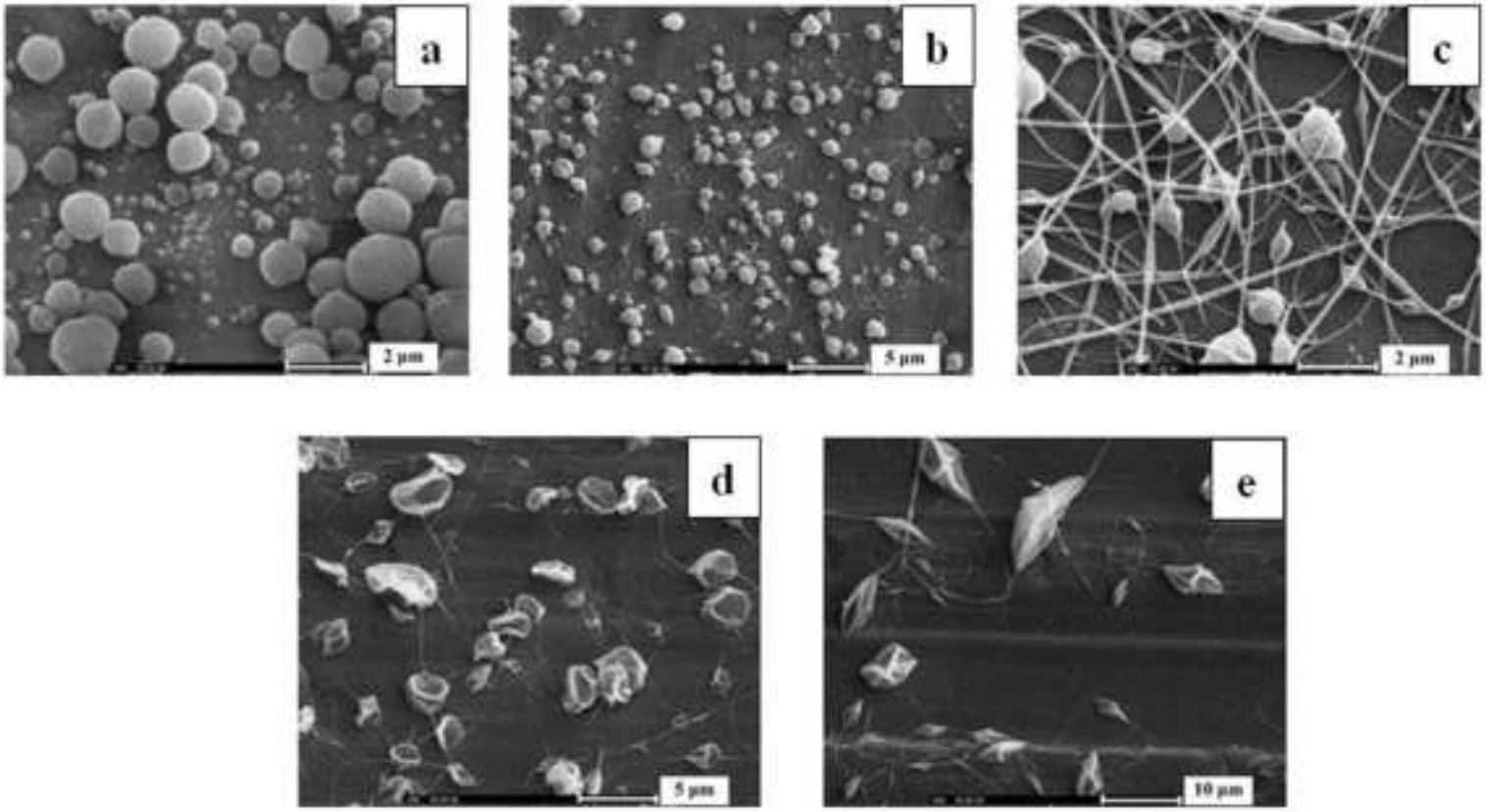


Figure3

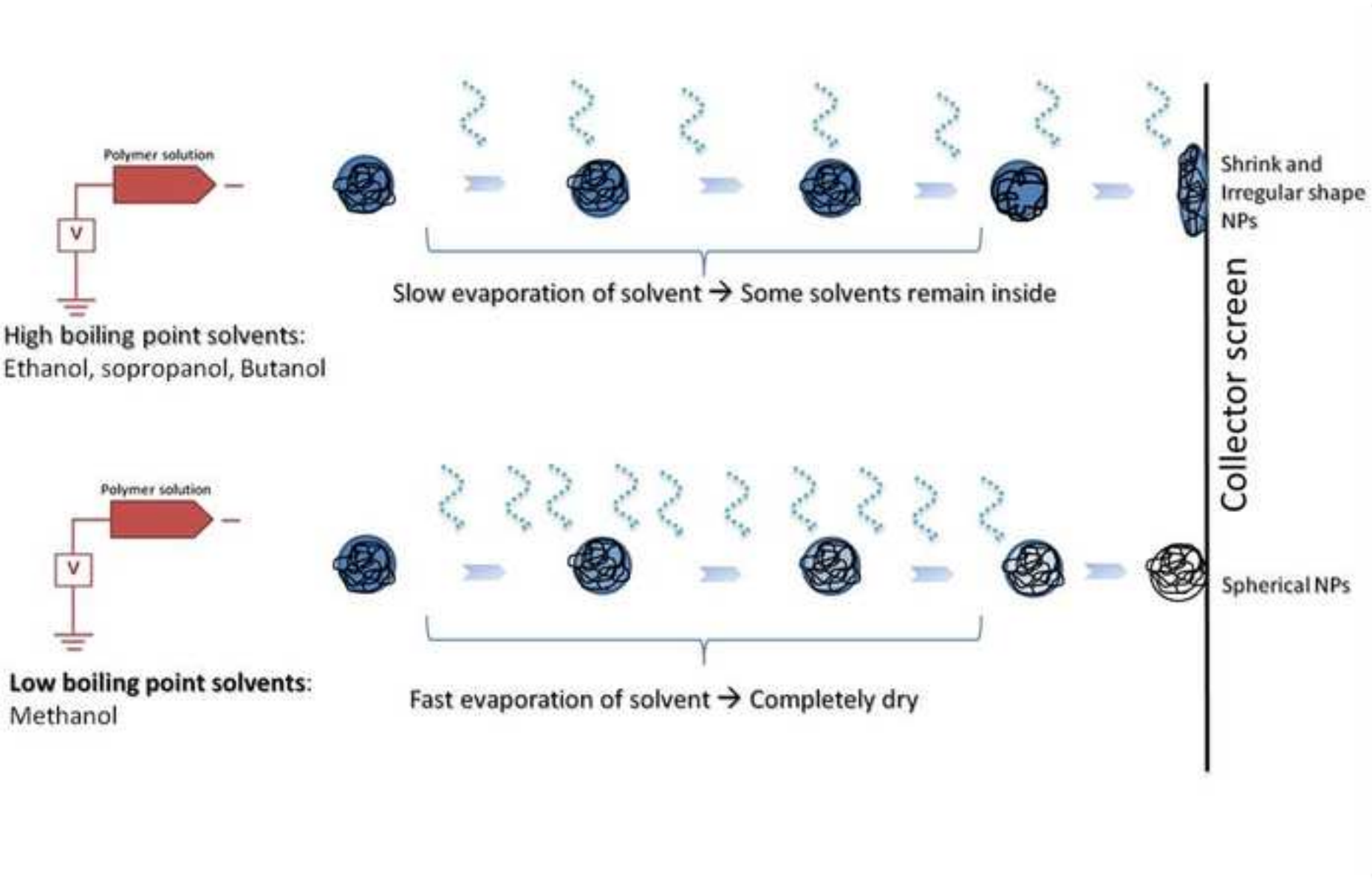

Polymer solution
V
Shrink and Irregular shape NPs
Slow evaporation of solvent → Some solvents remain inside
High boiling point solvents:
Ethanol, sopropanol, Butanol
Collector screen
Polymer solution
V
Spherical NPs
Low boiling point solvents:
Methanol
Fast evaporation of solvent → Completely dry

Figure4

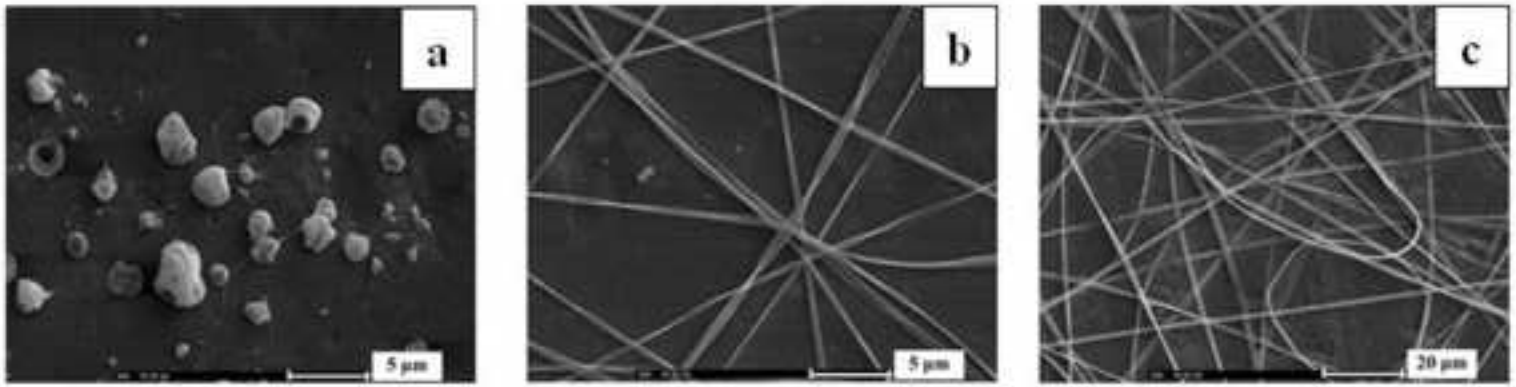


Figure5

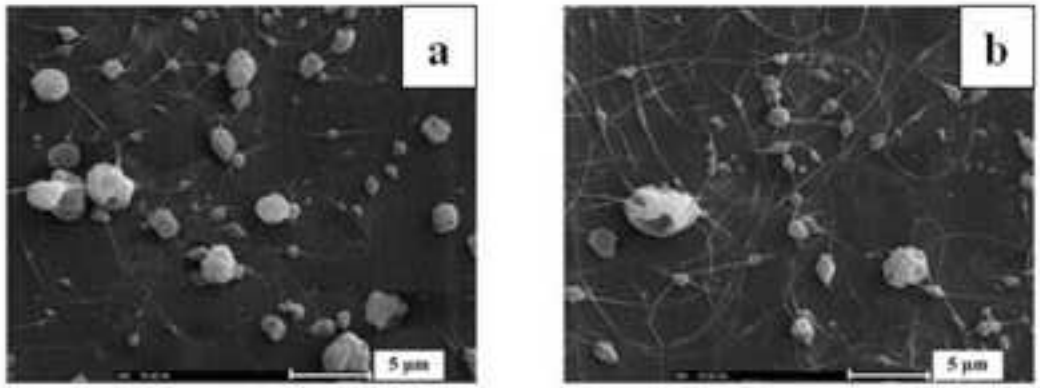


Figure6

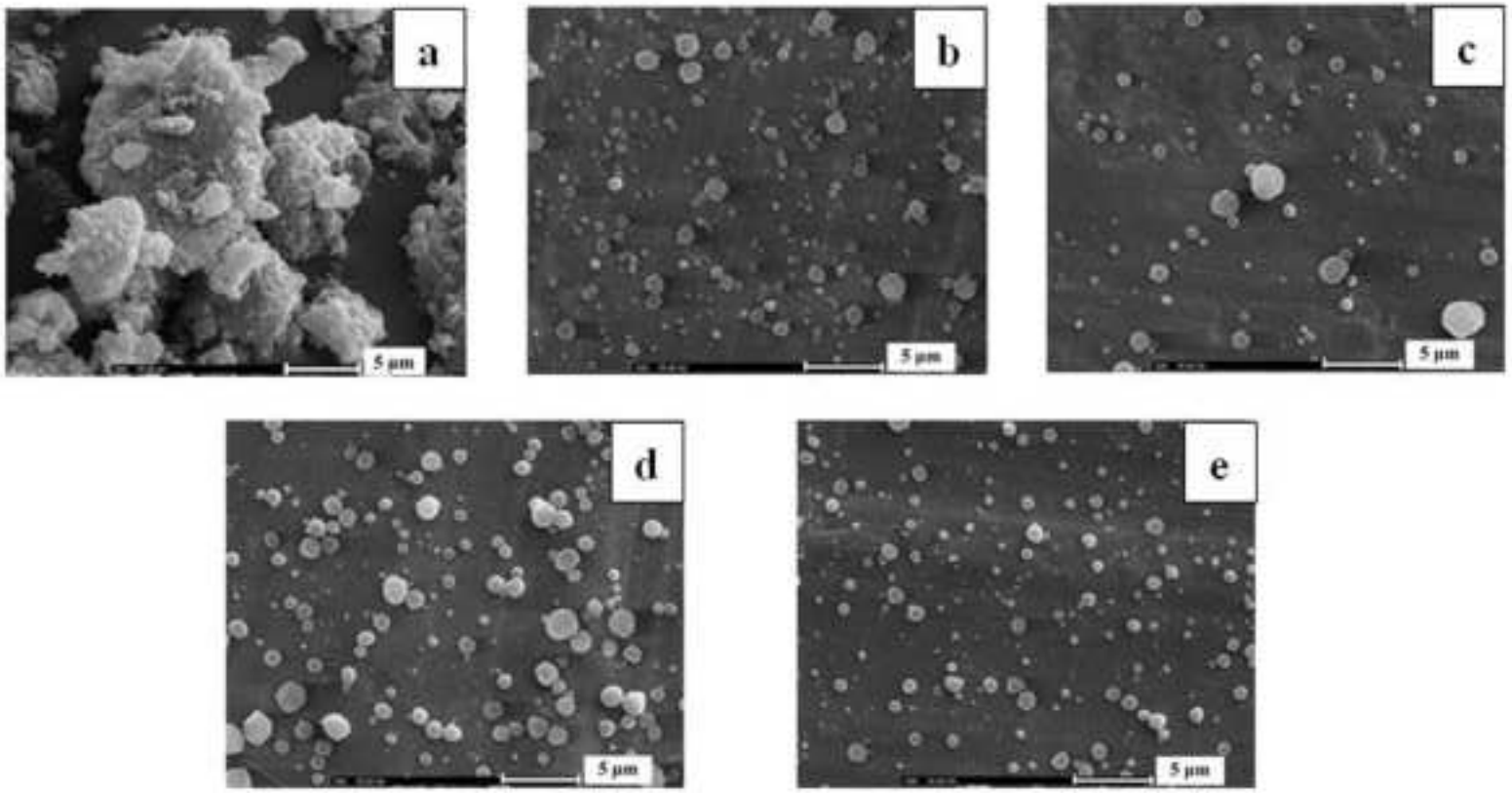


Figure7

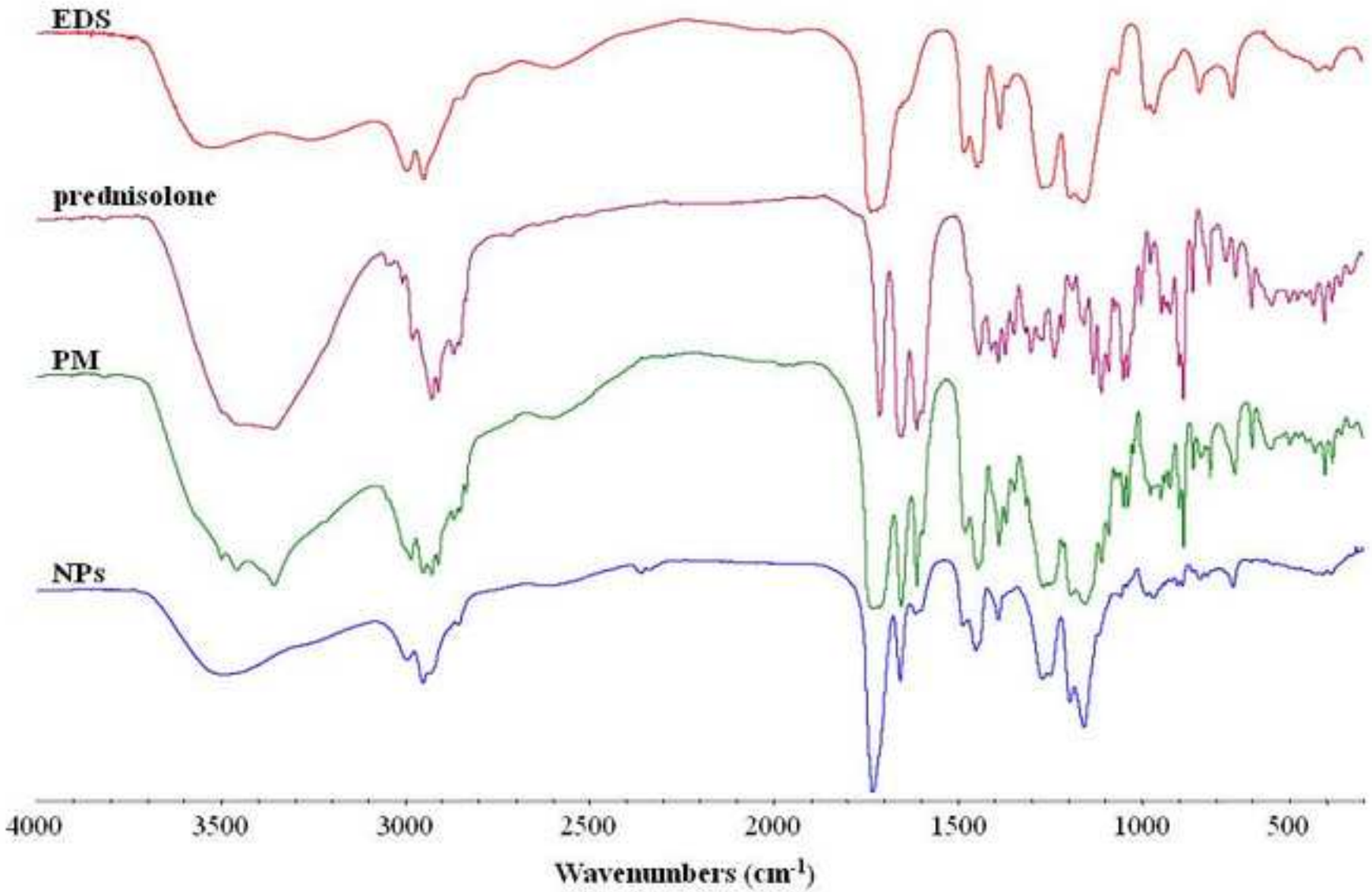

EDS
prednisolone
PM
NPs
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)

Figure8

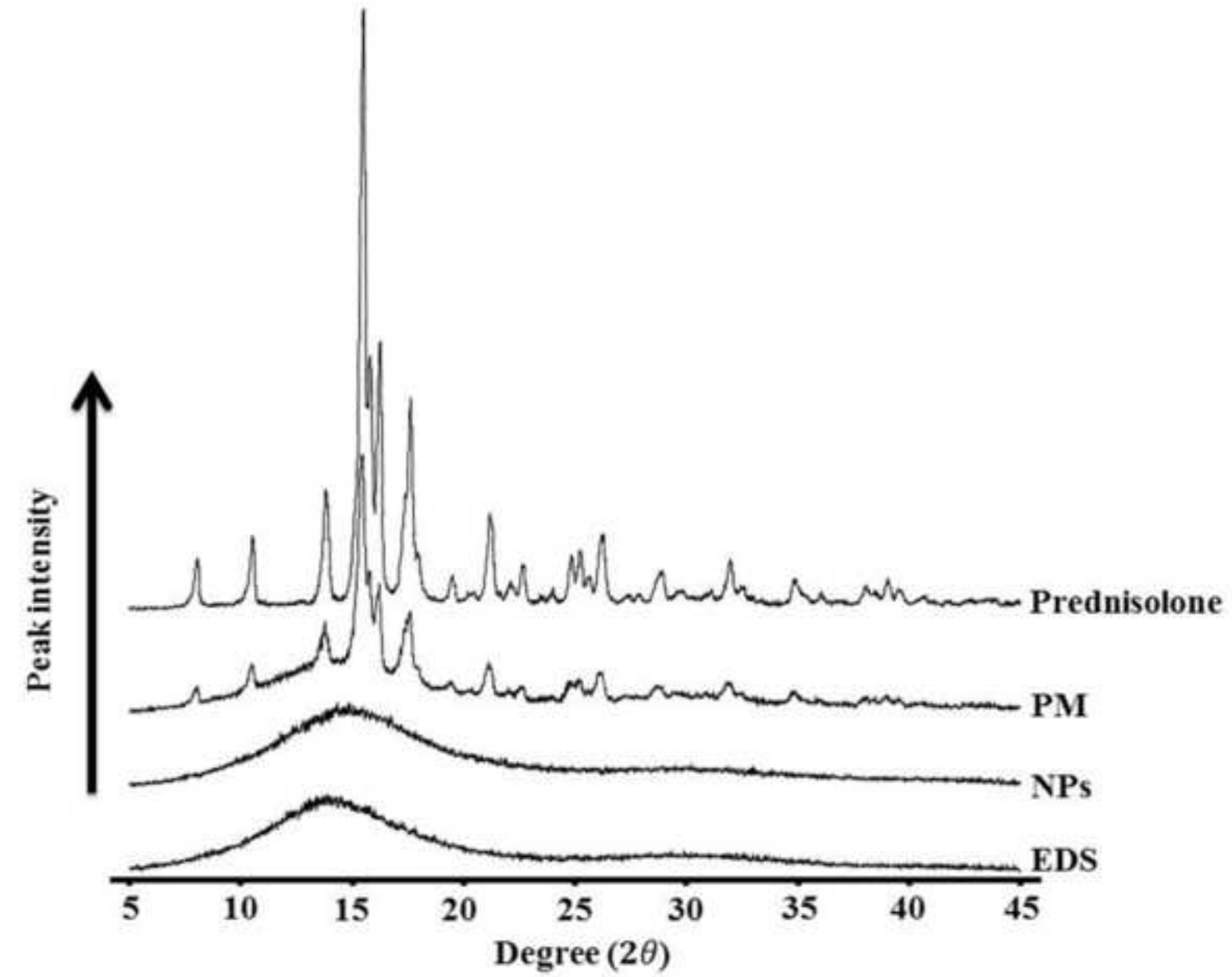

Peak intensity
Prednisolone
PM
NPs
EDS
5
10
15
20
25
30
35
40
45
Degree (2θ)

Figure9

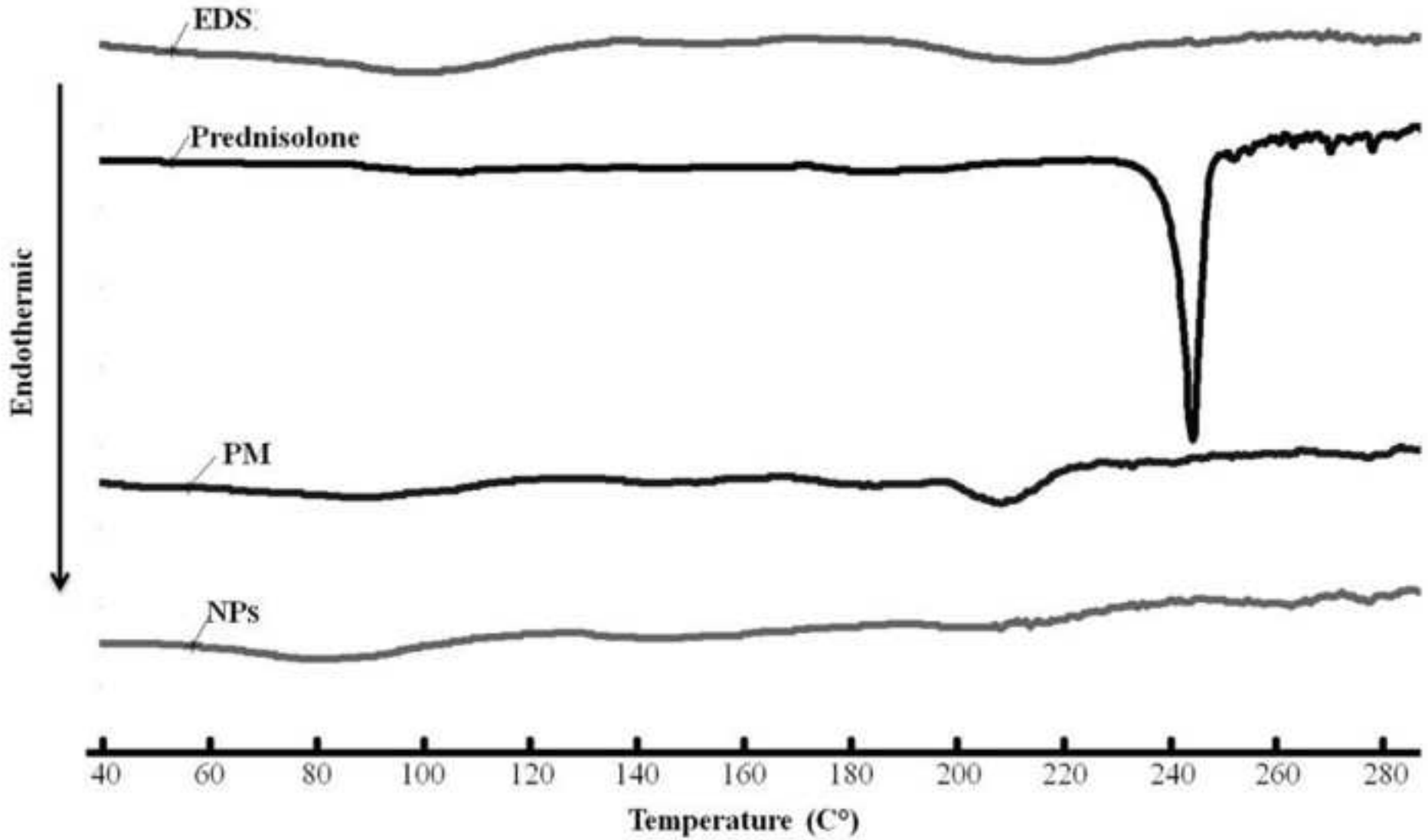

EDS
Prednisolone
PM
NPs
Endothermic
40
60
80
100
120
140
160
180
200
220
240
260
280
Temperature (C°)

Table 1. Conductivity, viscosity and interfacial tension of 5% w/v EDS in different solvents.

Solvent	Conductivity (μS)	Viscosity (cP)	Interfacial tension (mN/m)
methanol	93.51 ± 2.56	4.48 ± 1.10	22.82 ± 0.35
ethanol	31.64 ± 0.74	5.76 ± 0.37	21.71 ± 0.19
2-propanol	9.13 ± 0.09	10.88 ± 0.37	20.46 ± 0.25
t-butanol	N/A*	N/A*	N/A*

* EDS did not completely dissolve in t-butanol.

Table 2 Conductivity, viscosity and interfacial tension values of predinolone-loaded EDS solution using methanol as dissolving solvent.

The EDS solution loaded with different prednisolone concentrations	Conductivity (μS)	Viscosity (cP)	Interfacial tension (mN/m)
0.5% prednisolone	97.03 ± 1.26	2.99 ± 0.37	21.60 ± 0.87
1.5% prednisolone	94.70 ± 2.86	4.05 ± 0.32	22.20 ± 0.17
2.5% prednisolone	92.41 ± 0.54	4.27 ± 0.37	22.28 ± 0.30
3.5% prednisolone	92.02 ± 0.54	4.91 ± 0.36	21.48 ± 0.23

Table 3 Particle size and zeta-potential value of NPs containing different concentrations of prednisolone.

	Concentration of prednisolone in NPs			
	0.5% w/v	1.5% w/v	2.5% w/v	3.5% w/v
Particle size (nm)	448.6±65.2	492.4±69.9	661.0±65.7	471.1±59.3
Zeta-potential (mV)	-16.08±0.24	-33.20±0.46	-25.71±0.78	-23.64±0.76

Table 4. Drug loading and encapsulation efficiency of NPs containing different concentrations of prednisolone.

The NPs loaded with different prednisolone concentrations	Drug loading (%)	Encapsulation efficiency (%)
0.5%	6.58±0.06	72.35±0.63
1.5%	17.27±0.28	74.84±1.23
2.5%	30.86±0.54	92.65±1.61
3.5%	37.68±0.85	91.50±2.07