FORMULATION OF ARTESUNATE-LOADED EUDRAGIT^{R} RS PO-BASED NANOPARTICLES BY EMULSION EVAPORATION METHOD

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Abstract

Background: The aim of this study was to formulate artesunate (ART)-loaded Eudragit^{R} RS PO (Eudragit)-based nanoparticles (NPs) as well as to evaluate their physiochemical characteristics; ***Materials and methods:*** The NPs were fabricated by the emulsion evaporation method. The effects of several formulation parameters on the physicochemical properties of NPs were investigated including ART content, Eudragit concentration, the ratio of oil phase and water phase (O/W), and Tween 80 concentration (%, w/v). The size distribution of NPs was measured by means of dynamic light scattering (DLS). The morphology of NPs was investigated by scanning electron microscopy (SEM). The *in vitro* drug release was studied by a dialysis method; ***Results:*** The best formulation showed a spherical shape with the size of about 170 nm, PDI of 0.240 and zeta potential of 53.6 mV. Encapsulation efficiency and loading capacity of NPs were 88.56% and 18.64%, respectively. The drug release profile in phosphate buffer of pH 7.4 was characterized by a biphasic pattern with initial burst release followed by a sustained release; **Conclusion:** The Eudragit NPs could be potential as carriers in the cancer therapy of ART.

Keywords: artesunate, nanoparticle, Eudragit^{R} RS PO, emulsion, evaporation

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1. INTRODUCTION

Artesunate (ART) is a semi-synthetic derivative of artemisinin, which is the active principle of the traditional herb *Artemisia annua*, is one of potential antimalarial treatments (1). Due to its strong cytotoxicity, ART has recently been the subject for various studies about its effects on cancer cell lines. It is shown that ART plays the important role against leukemia, melanoma, non-small cell lung cancer, colon, renal, ovarian, prostate, central nervous system, prostate, breast cancer (2, 3).

However, ART is not very stable, most probably by the opening of the lactone ring, due to its unusual peroxy group and poor aqueous solubility (4). Therefore, the development of a drug delivery carrier that can maintain a sustained release profile and avoid rapid degradation is essential for effective therapy of ART (5).

In this study, Eudragit^{R} RS PO (Eudragit), an ammonio methacrylate copolymer, which is the common polymer used to form water-insoluble film coats for sustained-release products, is now applied for the fabrication of the nanoparticles (NPs).

Moreover, the emulsion evaporation method is one of the popular methods for preparing polymeric NPs due to its technological advantages compared to other processes. However, the physicochemical characteristics of NPs are often influenced by a variety of formulation parameters so that it is necessary to investigate and optimize these parameters.

Objectives

- To fabricate Eudragit^{R} RS PO nanoparticles containing artesunate by the emulsion evaporation method and to investigate the physicochemical characteristics of these nanoparticles.

2. MATERIALS AND METHODS

Materials

Artesunate (ART, purity: 99.8%) was provided by Sao Kim Pharma (Hanoi, Vietnam). Eudragit^{R} RS PO was purchased from Evonik Industries AG, Germany. Acetonitrile, potassium dihydrophosphate were of HPLC grade. All other chemicals were of analytical grade and were used without further purification.

Methods

Preparation of Eudragit nanoparticles

Eudragit nanoparticles containing ART were prepared using a single emulsion solvent evaporation method (6). Briefly, ART and Eudragit were dissolved in 5 mL of dichloromethane (DCM) and this solution was added drop-wise to aqueous solution containing Tween 80 emulsifier (1 mL per minute). The oil-in-water emulsion was fabricated

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by homogenization using high-intensity probe sonicator, Vibracell VCX130 (Sonics & Materials, Newtown, CT, USA) at 100 W for 5 minutes in ice cold water (5–10°C). The formed emulsion was stirred for 4 h at 1,000 rpm at room temperature to evaporate the organic solvent. The NPs were washed and collected by ultrafiltration. The influence of several formulation parameters on the physicochemical properties of NPs were investigated including ART content (mg), Eudragit concentration (%, w/v), the ratio of oil phase to water phase (O/W), and Tween 80 concentration (%, w/v) (Table 1).

Table 1. The formulation parameters studied in the fabrication of nanoparticles

Formulation	ART content (mg)	Eudragit concentration (%)	O/W ratio	Tween 80 concentration (%)
FM1	40	3.0	1:10	1.5
FM2	50	3.0	1:10	1.5
FM3	60	3.0	1:10	1.5
FM4	40	2.5	1:10	1.5
FM5	40	3.5	1:10	1.5
FM6	40	3.0	1:10	1.0
FM7	40	3.0	1:10	2.0
FM8	40	3.0	1:5	1.5
FM9	40	3.0	1:15	1.5

Measurement of particle size and morphology

Particle size, size distribution and zeta potential of nanoparticles were measured by using Zetasizer Nano ZS90 (Malvern Instruments Ltd., Worcestershire, UK). All the samples for the analysis were prepared by redispersing nanoparticles in distilled water. The intensity of scattered light was detected at 90° to an incident. All the data analysis was performed in automatic mode with triplicate measurements within each sample.

The morphology of nanoparticles was evaluated by using a scanning electron microscope (SEM – JEOL JSM-7600F, JEOL Ltd., Japan). A drop of ART-loaded nanosuspension was placed on an aluminium foil and then was dried at room temperature prior to the observation (7).

Encapsulation efficiency and loading capacity

To determine the total drug content, a suspension of ART loaded Eudragit nanoparticles (1 mL) was placed in an ultrafiltration tube (MWCO 10000, Millipore, Billerica, MA, USA) and was centrifuged at 4,500 rpm for 15 min (7). Then the filtrate was analysed for free drug using HPLC. Encapsulation efficiency (EE) and loading capacity (LC) were calculated using equations as follows:

$$\text{"EE" (\%)} = \frac{(\text{"Total ART"} - \text{"Free ART"})}{\text{"Total ART"}} \times 100\%$$

$$\text{"LC " (\%)} = \frac{\text{"Weight of ART in nanoparticles"}}{\text{"Weight ofART/PLGA"-"CS nanoparticles"}} \times 100\%$$

In vitro drug release

Drug release studies were carried out in a dialysis bag (molecular weight cut off 10 kDa, Membrane-Cel, Chicago, IL, USA) containing 3 mL of ART loaded nanosuspension. The dialysis bag was placed in a 50 mL tube containing 10 mL of phosphate buffer pH 7.4 (PBS) as a release media. The tube was capped and placed on a shaking water bath (Julabo, Germany) rotating at 100 rpm and maintained at 37°C. At predetermined time points, 1 mL of sample was collected and replaced with a fresh media after sampling. The ART assay was carried out on a Shimadzu HPLC system (Japan) equipped with a LC 20AD HPLC pump, conjugated with an on-line degassing unit (DGU-20A), an autosampler (SIL-20A) and a PDA detector (SPD-M20A). The data was collected and analysed by Shimadzu's LC solution software. Chromatographic separations were performed on an analytical column HiQ Sil C18 (250 x 4.6 mm, 3µm) manufactured by KYA technology (Japan). The mobile phase was the mixture of acetonitrile and phosphate buffer solution with pH 3.0 (48:52, volume per volume ratio). The UV absorbance was measured at wavelength of 216 nm with 1.0 mL/min of the flow rate and 50 µL of the injection volume (6).

Statistical analysis

All data are expressed as mean ± Standard Deviation (S.D.) from triplicates.

3. RESULTS

The preparation of Eudragit nanoparticles

During the preparation of NPs, to obtain NPs with the proper size as well as high loading capacity, the effects of several parameters of the formulation and the process such as ART content, Eudragit concentration, the ratio of oil phase to water phase, and emulsifier concentration were evaluated.

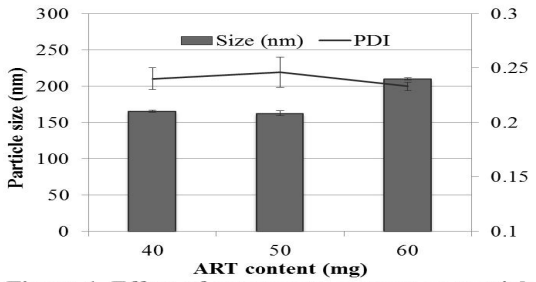

Figure 1. Effect of artesunate content on particle size, polydispersity index (PDI) (n=3)

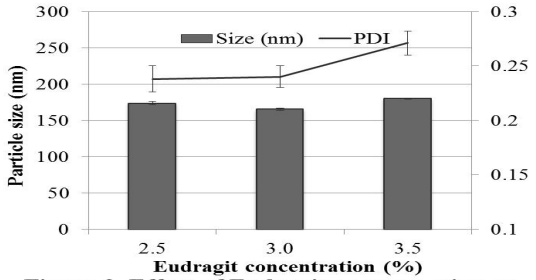

Figure 2. Effect of Eudragit concentration on particle size, polydispersity index (PDI) (n=3)

ART content from 40 mg to 60 mg was evaluated to understand its effect on the size of NPs. The result in Figure 1 showed that Eudragit nanoparticles with the uniform size distribution were formed in the range of 160–210 nm. The increase of ART content led to the increase of the particle size, however, all the polydispersity index values (PDI) were maintained below 0.300.

In the preparation of the colloidal system, the polymer concentration in the dispersed phase is another important factor. For this evaluation, Eudragit concentrations were varied from 2.5 to 3.5 % (w/v). The result in Figure 2 indicated that the nanoparticle size was increased with the increase of the polymer concentration, especially PDI was increased remarkably at high Eudragit concentration. High polymer concentration led to the increase of the viscosity of the organic phase (polymer solution), which resulted in a poorer dispersibility of the organic phase into the aqueous phase. In addition, the resistance of high viscous solution hinders the nanoparticle formation and enhances aggregation in more concentrated solution, thereby forming bigger emulsion droplets (8).

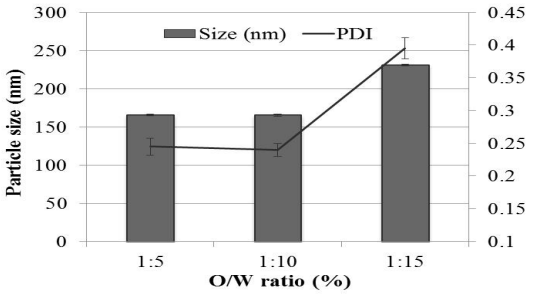

Figure 3. Effect of oil/water (O/W) ratio on particle size, polydispersity index (PDI) (n=3)

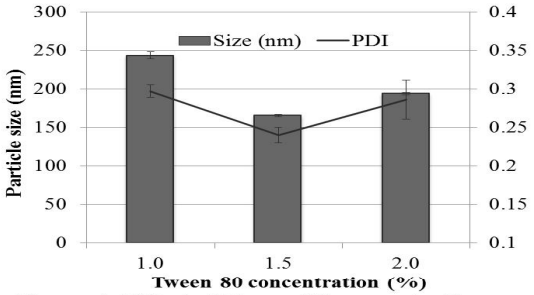

Figure 4. Effect of Tween 80 concentration on particle size, polydispersity index (PDI) (n=3)

The next key factor in the process of fabricating NPs is the ratio of oil phase to water phase. Figure 3 showed that the increase of the aqueous phase volume slightly decreased the particle size and PDI. However, so high water volume led to big particle size (> 200 nm) and large distribution with PDI > 0.3. This could be explained by the aqueous phase not fully exerted by the probe sonication.

Emulsifier (Tween 80) concentration is another critical parameter in this process which affects the interfacial tension and the viscosity of the external phase. The particle size was decreased with the increase of the Tween 80 concentration, however, not decreased anymore at high concentration (2%). This could be attributed to the balance between the interfacial tension and the viscosity of the external phase (8).

A nanosized drug delivery system is preferentially and selectively accumulated to solid tumour tissues due to enhanced permeability and retention (EPR) effect. EPR effect is dependent on the size of particles. The smaller the particle size, the easier the penetration into the tumour through leaky vasculature. An average size of approximately 200 nm or less is ideal for the EPR effect (9). Therefore, FM1 was chosen as the best formulation including 40 mg of ART, 3 % (w/v) of Eudragit, 1.5 % (w/v) of Tween 80 and the oil/water ratio of 1:10. This formulation was further evaluated for other physicochemical properties.

The evaluation of physicochemical properties

Scanning electron microscopy image of Eudragit NPs obtained from the best formulation is shown in Figure 5. The NPs are spherical in shape and polydispersed with the size range of about 170 nm which is consistent with the DLS data (165.3 ± 1.556 nm). Zeta potential of NPs was 53.6 ± 2.54 mV, which helps to maintain the stability of the nanosuspension because it was reported that a value of below −30 mV (or above +30 mV) indicates a stable colloidal dispersion (10). In addition, EE and LC of these NPs were 88.56% and 18.64%, respectively.

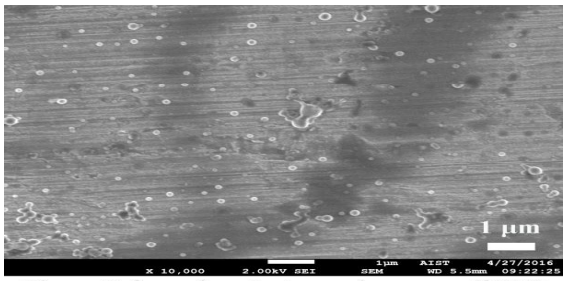

Figure 5. Scanning electron microscopy (SEM) micrograph of ART loaded Eudragit NPs (FM1)

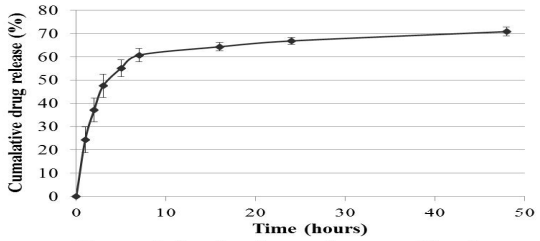

Figure 6. *In vitro* drug release profile of ART-loaded Eudragit NPs (FM1) (n= 3)

Figure 6 showed the cumulative release curve of ART from the NPs. After 48 hours, about 70% of ART which was loaded in NPs was released. The release profile was characterized by a biphasic pattern with a period of rapid initial drug release followed by a continuous period of slow release after 24 hours due to the diffusion or the erosion of the PLGA matrix (11). In details, the cumulative drug release from Eudragit NPs at 2h and 24h was 37.19% and 66.80%, respectively. This could promote the EPR effect which is affected by the circulation time of the drug nanocarrier. The controlled release pattern of ART from NPs was expected to release their payloads at targeted organelles so that the carrier will be safe on normal cells as well as express the highest activity on cancer cells (9).

4. CONCLUSION AND RECOMMENDATIONS

In this study, the best formulation of Eudragit NPs containing ART was obtained by evaluating the effect of several formulation parameters on the size and PDI of NPs. The NPs have the spherical shape with the size, PDI and zeta potential of about 170 nm, 0.240 and 53.6 mV, respectively. EE and LC of NPs were 88.56% and 18.64%, respectively. The present NPs showed a biphasic *in vitro* drug release pattern with an initial rapid drug release followed by an extended drug release phase. These NPs can be used for the further *in vitro* cytotoxicity studies and potentially as the carrier system for anticancer drugs.

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