STUDY OF BIODEGRADATION AND IN VITRO CONTROLLED RELEASE BEHAVIOR OF ARTESUNATE-LOADED CHITOSAN NANOPARTICLES

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Tran Dai Lam, Nguyen Thi Thuy Nga, Vu Dinh Hoang

Faculty of Chemical Technology, Hanoi University of Technology

Tran Viet Hung

Institute of Drug Control, Ministry of Health

TÓM TẮT

NGHIÊN CỨU SỰ PHÂN HỦY SINH HỌC VÀ QUÁ TRÌNH GIẢI PHÓNG THUỐC *IN VITRO* TỪ CHẤT MANG NANO CHITOSAN GẮN HOẠT CHẤT ARTESUNAT

Trong công trình này, polime tương thích sinh học chitosan cấu trúc nanô đã được điều chế sử dụng làm chất dẫn thuốc chữa sốt rét artesunat. Đã tiến hành các nghiên cứu đặc trưng hóa lí và sinh học đối với các sản phẩm thuốc gắn trên chất mang chitosan tổng hợp được. Kết quả bước đầu cho thấy quá trình giải phóng thuốc này kéo dài hơn so với thuốc không có chất mang, trong môi trường sinh lí giả ruột và giả dạ dày. Nghiên cứu này tạo tiền đề cho việc chế tạo các chất mang nano chitosan giúp phân giải chậm thuốc nhằm kéo dài tác dụng của thuốc trong cơ thể, tăng hiệu quả điều trị của các loại thuốc có thời gian bán phân hủy trong cơ thể ngắn như các dẫn xuất của artemisinin và nhiều loại thuốc khác.

I. INTRODUCTION

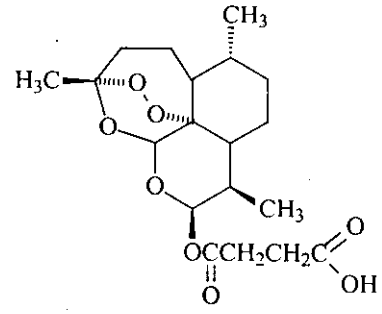
In recent years, biodegradable and biocompatible polymeric micro/nanoparticles have attracted a considerable attention as potential carriers for the controlled and site-specific delivery of drugs [1–3]. Chitosan (CS), a deacetylated derivative of chitin, is a naturally occurring

polysaccharide found abundantly in marine crustaceans, insects and fungi. Due to its unique polymeric cationic character, CS has been extensively examined for the development of drug delivery systems in the pharmaceutical industry [4-5]. Up to now, drug delivery formulations based on CS (films beads, microspheres, etc.)have been usually prepared by chemical cross-linking agents like glutaraldehyde. However, these chemical cross-linking agents could induce toxicity and other undesirable effects. To overcome this disadvantage, reversible physical cross-linking agents like low molecular weight anions such as citrate, tripolyphosphate (TPP) were applied in the formulation preparation via electrostatic interactions.

An important advantage of formulation preparation at nanoscale is that biocompatible and biodegradable polymer based nanoparticles could serve as drug carriers for controlled release and site-specific targeting of drug.

In the previous paper [6] we have reported the synthesis and characterization of CS nanoparticles. Here we described the preparation of the CS nanoparticles loaded with an antimalarial drug artesunate (ART), and the study of their biodegradation and *in vitro* controlled release behaviour.

Artesunate is a new effective derivative of artemisinin now used broadly in antimalarial treatment in many countries.



Artesunic acid
(Artesunate)

II. MATERIALS AND METHODS

II.1. Materials

CS was medical grade (MW = 200.000, determined by viscometry measurements; DA = 70 %, determined by IR analysis [6]). TPP (Merck, Germany), CH_3COOH (China), were of analytical grade. Glucosamine (used as reference in HPLC experiments); antibiotics: pennicilline, streptomycine and ketoconazone (used for avoiding bacterial contamination) were supplied by Institute of drug control.

II.2. Preparation of nano-CS (NCS)

Nano-CS was formed by ionic gelation according to the procedure in [6]. The powders were then characterized by different methods like IR, TEM, TG.

II.3. Preparation of simulated media

Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared in accordance with USP 22. The SGF was prepared by dissolving 2 g sodium chloride and 3,2 g pepsin in 7 ml HCl and the volume was adjusted to 1 l by

water to obtain a pH about 1,2. The SIF was prepared by dissolving 6,8 g of NaH_2PO_4 in 250 ml of water. The solution was mixed and 190 ml of 0,2 N NaOH and 400 ml of water and 10 g of pancreatin (Sigma) were added. The pH was then adjusted with 0,2 N NaOH to 7,5 ± 0,1 and the volume was completed to 1 l by water.

II.4. Methods of characterization (FTIR, TG/DTA, TEM/SEM)

FTIR spectra were recorded at FTIR-IMPACT 400 Spectrometer with KBr discs.

Thermal analyses (TG/DTA) were performed on NETZSCH STA 409 PC/PG equipment, in N_2 atmosphere. The temperature range was 30- 800°C. The heating rate is 5°C/min.

UV-vis measurements were carried out at UV-vis Agilent 8453 spectrophotometer in the range of 300-800 nm.

Particle size and the morphology was observed by TEM (EM-125K, Voltage: 100 kV). A droplet of CS dispersion was placed onto a copper grid covered with carbon and let to dry.

II.5. ART-loading procedure on nano-CS particles

CS nanoparticles was added to water to give mixture **A** and artesunate was dissolved in ethanol 96% to give solution **B**. Solution B was added slowly to mixture A at 50-60°C during 5-6 h to give mixture **C**. After evaporation of solvent part from C, the rest part was kept at 80°C during 2 h. The product in form of CS nanoparticles loaded with artesunate was filtered and washed with ethanol.

II.6. In vitro biodegradation study of CS nanoparticles and in vitro drug release of ART from ART-loaded CS in simulated media

a) In vitro biodegradation study of CS by HPLC

In vitro biodegradation of CS was determined using a dissolution apparatus (Dissolution tester UDC-804, LOGAN, USA) at the temperature of 37°C ± 0,5°C. An aliquot of release medium was taken through a sampling syringe attached with a 0,45 μm membrane filter (Millipore) at predetermined time intervals ((2, 4, 6, 8, 16, 30 days) and was analyzed by HPLC with the following parameters: Column: RP 18 (250 x 4mm, 10μm); Mobile phase: H_2O –MeOH (30:70); Detector: UV -- 240 nm; Flow rate: 1ml/min; Injection volume: 20 μl.

b) In vitro drug release of ART from ART-loaded CS by HPLC

Column: RP 18 (250x4mm, 5μm). Mobile phase $NaCH_3COO$- CH_3CN (0,49 g $NaCH_3COO$ in 600 ml H_2O, adjusted by CF_3COOH to have a pH 3,7. Detector: UV-220nm. Flow rate: 1,5 ml/min. Injection volume: 20 μl.

III. RESULTS AND DISCUSSIONS

III.1. IR analysis of ART- loaded CS-TPP nanoparticles

FTIR spectra of nano CS, TPP and ART- loaded CS nanoparticles were recorded. The presence of the P=O and P-O groups at the frequency of 1180 cm^{-1} and 1250 cm^{-1} respectively; the band shifts (from 1650 cm^{-1} and 1595 cm^{-1}, corresponding to C-O and N-H stretching respectively in pure CS, to 1636 cm^{-1} and 1539 cm^{-1} for nano CS) on the spectrum of nano CS (CS-TPP) (fig.1, dash line) clearly indicated the interaction between CS and TPP in nano CS. On the spectrum of CS-ART: the vibrations of –O-O- group (at 942 and 879cm^{-1}), C=O (1646 cm^{-1}) and N-H (1559 cm^{-1}) in amide linkage, formed between CS and ART also clearly demonstrated covalent binding of ART onto CS nanoparticles ((fig.1, solid line).

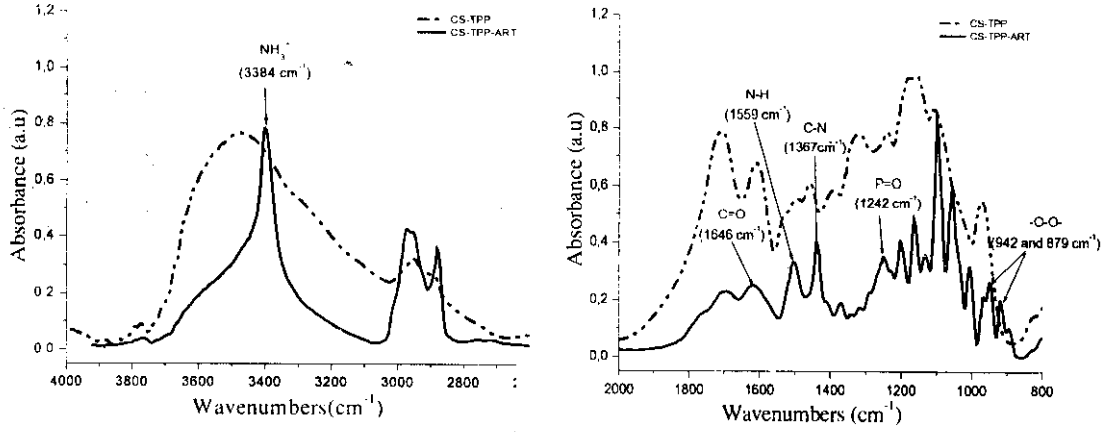
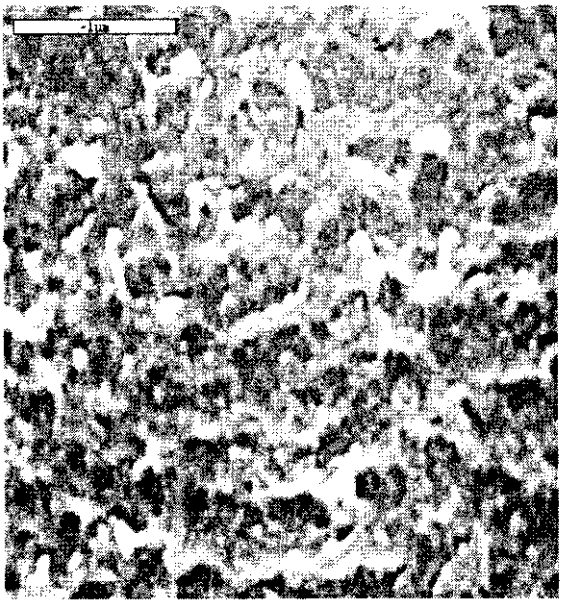


Fig.1: *FTIR spectra of nano CS (dash line) and ART loaded on nano CS (solid line): 4000-2500 cm^{-1} range (a); 2000-800 cm^{-1} range (b)*

Fig.2: *TEM photographs of non-loaded CS (left) and ART-loaded CS (right)*

III.2. TEM and SEM analyses of ART-loaded CS-TPP particles

It can be seen that ART-loaded CS particles are more irregular in shape and have rougher surfaces than non loaded CS particles. Encapsulated of ART inside a polymer matrix of CS made the average size of ART-loaded CS particles bigger than that of non- loaded CS ones: about 200-300 nm (ART-loaded CS) vs. 100-150 nm (non-loaded CS) (fig.2).



III.3. TG analysis of ART-loaded CS particles

TGA experiments were carried out on nano CS, pure ART and ART-loaded CS. For CS nanoparticles, the loss of weight appeared in the TG response from 197°C to 300°C. These TG data showed some decrease of thermal stability of CS-TPP nanoparticles compared to pure CS (data not shown). For ART-loaded CS, a significant weight loss was observed earlier than un loaded CS nanoparticles, indicating some structural change in ART-loaded CS.

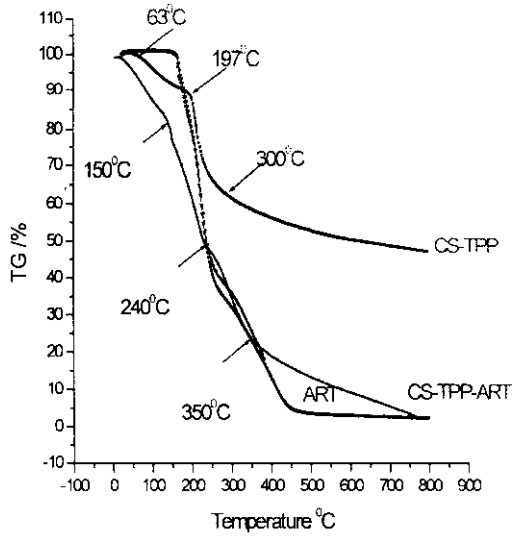


Fig.3: SEM photograph of non-loaded CS (left) and TG of ART-loaded CS (right)

III.4. In vitro biodegradation study of CS nanoparticles and in vitro drug release of ART from ART-loaded CS in simulated media

III.4.1. In vitro biodegradation study of CS by HPLC

Conventional drug delivery system has the following limitations: i) Pharmacokinetic: the drug is cleared from the body too quickly or the drug does not distribute in a sufficient amount at the right site; ii) toxicological: The drug has to avoid some tissues; iii) poor intracellular penetration; iv) poor sub-cellular distribution. Therefore, one of the most important problem to be solved is to

improve the delivery of drugs to the target sites while minimizing side effects. Several strategies have been explored so far in the development of controlled and targeted drug delivery, among which the design of biodegradable nanoparticulate systems have drawn considerable interest thanks to their numerous advantages compared to conventional ones, for example, improved efficacy and selectivity (site specific targeting), reduced side effects and toxicity.

In the first step of this study, the degradation of CS (polymeric carrier of the drug) was investigated by HPLC. Polymer degradation is the chain scission process that breaks polymer chain down to oligomers and into monomers (In this case glucosamine (GA) is the degradation product of CS). This experiment was carried out in two simulated media of SIF (Simulated Intestinal Fluid, pH 7,5) and SGF (Simulated Gastric Fluid, pH 1,2), prepared in accordance with USP 22 (US Pharmacopeia). (see Experimental section II.3). We found that released Glucosamine (a degradation product of CS) reached the maximum value after 7-10 days and this concentration was higher in SGF than that in SIF, indicating the influence of pH solution on the degradation rate of the polymer (fig.5).

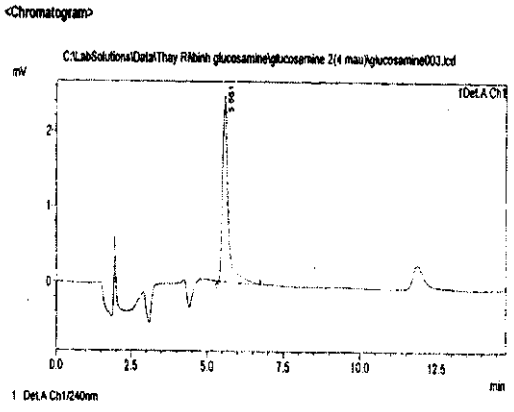


Fig.4: *Typical chromatogram of standard GA (t_R=5,56 min.)*

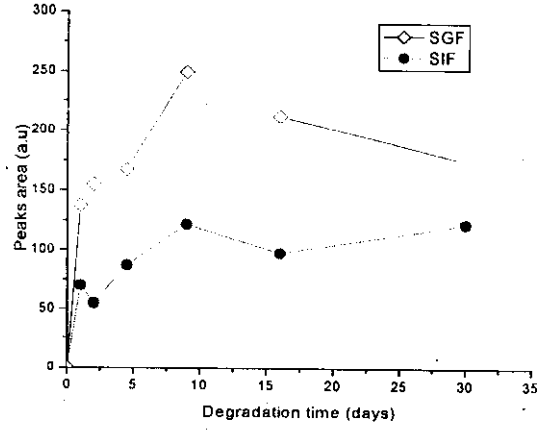


Fig.5: *Accumulative release of GA (CS degradation) in SGF and SIF*

III.4.2. Monitoring release of ART in simulated media by HPLC

In general, drug release from polymeric carriers is made by erosion of polymeric backborne and drug diffusion throughout the polymer membrane. Typical chromatogram of standard ART (with the retention time t_R= 12,20 min) was first recorded (figure not shown). The chromatograms of ART release from CS-TPP particles in SGF (pH 1,2) after 8 hours (t_R=12,35 min.), ART release from CS-TPP particles in SIF (pH 7,5) after 8 hours (t_R=12,19 min.) were presented in fig.5.

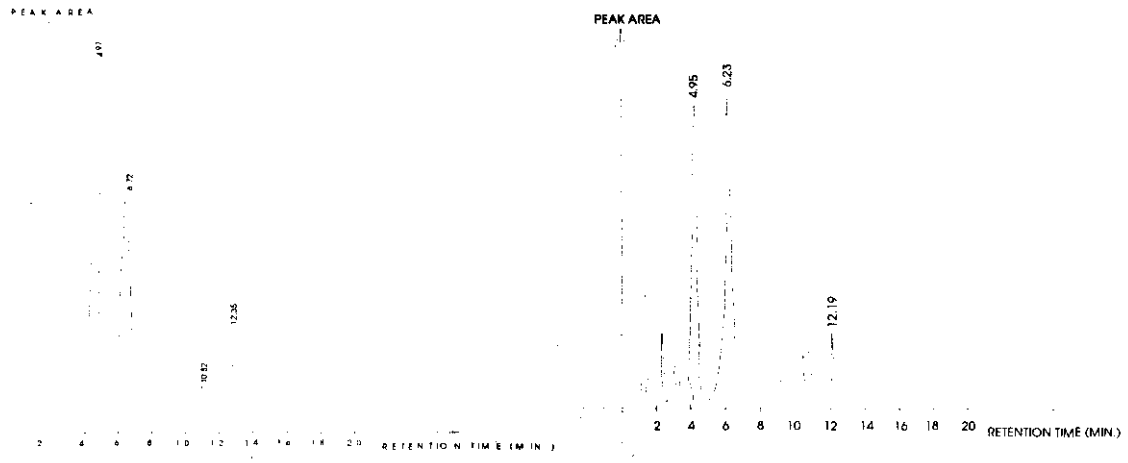


Fig.5: Typical chromatograms of ART release in SGF (left) and SIF (right) after 8 hours

III.4.3. Accumulative release of free (non-encapsulated) ART in simulated media

From the HPLC data it can be seen that the very short time of release was recorded for free ART in both media (7-8 h in SGF, 30 min in SIF). Especially, in SIF medium (fig.6, right), due to the acid-base neutral reaction between ART (acid) and the medium (base) ART concentration decreased rapidly upon the time.

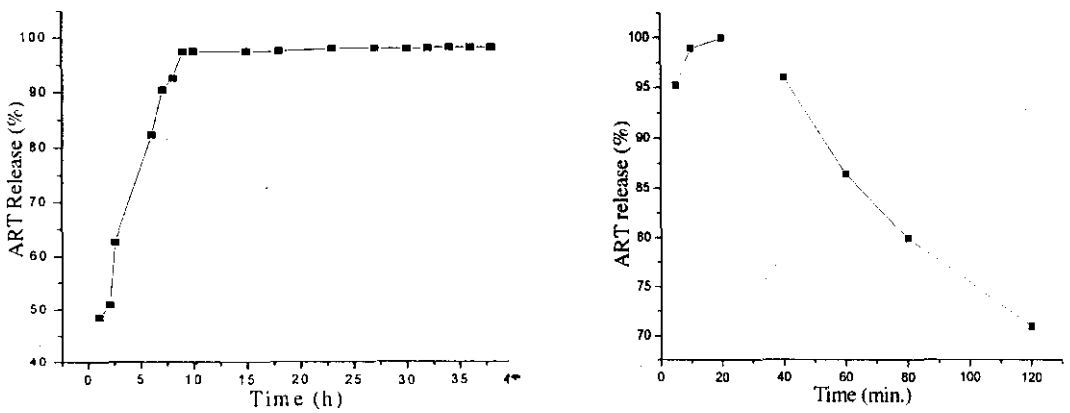


***Fig. 6:** Accumulative release of free (non-encapsulated) ART in SGF (left); in SIF(right)*

III.4.4. Accumulative release of encapsulated (loaded) ART from nano CS in simulated media

The release of ART was significantly prolonged when ART was loaded on CS-TPP nanoparticles carriers in both media. The average time of release was about 25-30 hours, depending on the composition of the drug (ratio of ART/CS), (fig.7). Compared with the release of free ART: it can be seen that the release of loaded ART from CS-TPP nanoparticles carriers had reached the most important objective of controlled and slowed drug release.

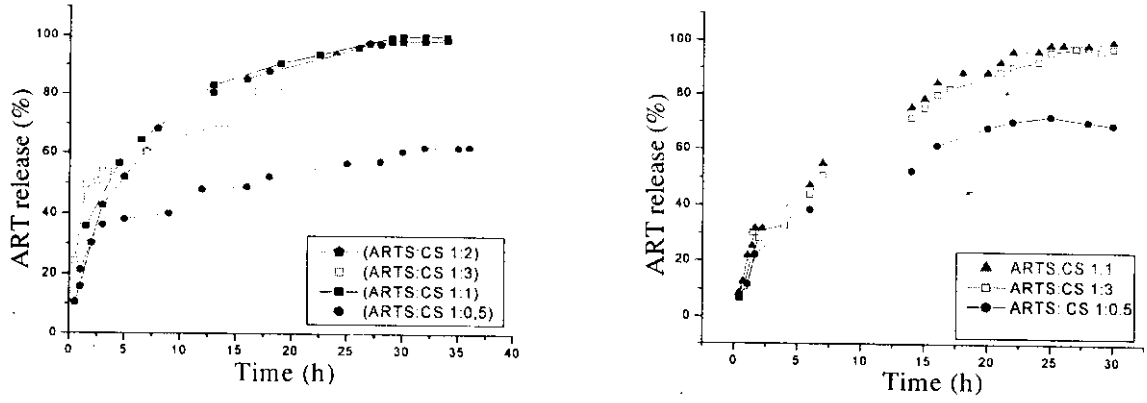


Fig.7: Accumulative release of ART from CS-TP-ART: in SGF (left); in SIF (right)

IV. CONCLUSIONS

- Successful and effective loading of ART as an effective antimalarial substance on CS-TPP nanoparticles carriers for bioassay tests have been demonstrated.

- *In vitro* biodegradation study of CS-TPP nanoparticles carriers in simulated media (SGF and SIF) was carried out.

- *In vitro* drug release of ART from ART-loaded CS in two simulated media was investigated. In comparison with the release of free ART, the release of ART from CS-TPP-ART nanoparticles had shown the capability of our system as an effective controlled and slowed drug release.

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