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# Novel oral administrated ellagic acid nanoparticles for enhancing oral bioavailability and anti-inflammatory efficacy

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ABSTRACT

Ellagic acid (EA), a naturally occurring polyphenolic compound, is commonly known for its anti-inflammatory properties. The low bioavailability greatly limits the clinical applications of EA. In this study, a biodegradable hollow zein nanoparticle with an average diameter of about 70 nm was developed to mediate oral delivery of EA. The inner core of the nanoparticle consists of EA/sodium carbonate (EA/$Na_2CO_3$) prepared by coprecipitation, which was further encapsulated in hollow zein nanoparticles with triethyl citrate as a natural plasticizer. The optimized ellagic acid-hollow plasticized zein nanoparticles (EA-HTZN) exhibited a small dimension of 72 nm with a PDI of 0.131, a drug loading capacity as high as 326 mg $g^{-1}$ at an equilibrium concentration of 5.0 mg $mL^{-1}$. EA-HTZN had high drug loading and prevented their precipitation at simulated physiological environment. The EA-HTZN significantly improved permeation ability in vitro. Oral administration of EA-HTZN showed effective against inflammation related to suppression of pro-inflammatory cytokines (TNFα, IL1 β) overproduction in carrageenan-induced mouse paw edema model. Pharmacokinetic parameters of optimized formulation revealed 3.6- and 2.1-fold increase in bioavailability as compared to EA suspension and EA solid nanoparticle, respectively. Together, these results demonstrated the successful formulation of EA-HTZN and their potential to improve oral delivery through high drug loadings and good stability.

## 1. Introduction

Inflammation is the body response against invading pathogens. More reports have provided evidence that inflammation is involved in the pathogenesis of life-threatening and debilitating disorders including cancer, aging, cardiovascular dysfunction, and other diseases. Ellagic acid(EA), a natural polyphenolic compound presented in fruits and nuts such as raspberries, strawberries, pomegranates and other plant foods, is related to anti-inflammatory [1],antioxidant, anti hepatocar-cinogenic and antiviral properties [2–4]. EA bioavailability is low and classified as class IV drug in biopharmaceutical classification system (BCS) because it has poor solubility(less than 10 μg ml) and permeability ($0.13 \times 10^{-6}$) [5]. EA is sensitive to microorganism environments and irreversibly bound to macromolecules as well [6].

Oral administration is the preferred route for drug delivery. Various delivery systems have been designed, e.g. hydrogel [7], dendrimers [8], liposome [9], and micro/nano-particles [10]. Zein is a group of alcohol-soluble proteins extracted from corn gluten meal. Being prolamins, the surface of zein molecules has more than 50% hydrophobic amino acid residues making it water insoluble [11]. Due to its inherent hydrophobic property, zein can be easily converted to spherical colloidal nanoparticles by the method of anitsolvent coprecipitation for drug encapsulation [12]. Solid and hollow nanoparticles are two of the most common types of nanoparticles [13]. The latter have the potential to load more drugs but are difficult in preparation which required thermal or other treatments. Recently, a novel method was reported to prepare hollow zein nanoparticles (HZN) with sodium carbonate as a sacrifice template [14,15]. Drug encapsulated in HZN showed high drug loading and sustained profile than that in solid zein nanoparticles (SZN) [16]. When crossing the intestinal epithelium, nanopartical stability is the critical factor with remarkably high efficiency [17]. As a fact, zein films have poor moisture barrier effect and weak mechanical properties due to the hydrophilic nature of their amino acids. Zein nanoparticles without any modification were rapidly hydrolyzed or aggregated resulting in a burst release of encapsulated drug [18,19]. Great research efforts have been focused to modify the film in a purpose to improve properties of biopolymer films, particularly their mechanical [20–22]. Zein coating with a proper plasticizer showed promising visual and

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mechanical characteristics [20]. Poly (lactic acid) and cellulose acetates plasticized with citrate esters improved processability and decreased the degradation rate [23–26]. As a nontoxic and biodegradable plasticizer, triethyl citrate(TEC) was used to plasticize zein film [27,28]. TEC, derived from citric acid, is a hydrophobic plasticizer with nontoxicity and biodegradation. TEC is better than other plasticizers in maintaining the film strength while significantly improving film flexibility as well as water resistance [29]. Rigid zein film used on nanocarrier may easily cross the intestinal epithelium to reach the blood circulation [17].

In this study, The EA-loaded oral delivery system was developed using a plasticizer consisting of zein hollow nanoparticles. It is hypothesized that high amounts of EA were entrapped in hollowand plastic zein and protected from aggregating with biomacromolecule. The small nanoparticles formed of modified zein was capable of permeation across the intestinal epithelium without binding to DNA and protein leading to high anti-inflammatory effects. The pharmacokinetic efficacy highlighted the potential of this oral delivery system to enhance drug bioavailability following oral delivery of EA-HTZN to mice.

## 2. Material and methods

### 2.1. Material and annimal

Zein, sodium carbonate ($Na_2CO_3$, purity 99.0%), triethyl citrate (TEC, ≥97%), Ellagic acid (EA, ≥99.2%). The other chemicals and reagents were of analytical grade and obtained commercially. Double distilled water was produced by a Millipore water purification system (Millipore Corporation, Bedford, MA, USA).

Healthy Wistar rats (200 ± 20 g) were purchased from Dashuo Biotechnology (Chengdu, China) and maintained under standard housing conditions. All animal procedures were supervised by Institutional Animal Care and ethics committee of Guiyang University of Chinese Medicine. The European Community guidelines were accomplished as accepted principles for the use of experimental animals. The animals were familiarized to the customary laboratory conditions in cross aerated animal house at temperatures 25 ± 2 °C/75% relative humidity and light and dark cycles of 12:12 h, fed with regular pallet nutrition.

### 2.2. Farication of TEC- zein film (TZ)

Zein and TEC solutions were prepared by dissolving the corresponding amount of TEC and zein in 20 ml 70% v/v aqueous ethanol to a concentration of 2 g of total substances. In the total substances, the mass ratio of TEC was 2%–12%. To make plasticized zein solutions, the solutions were magnetically stirred for 12 h. Then the solutions were heated at 90°Cfor 20min in a water bath and cooled to room temperature. Hot-cool cycle was repeated 3 times.

### 2.3. Characterization of film properties

The physical and thermal properties of protein-coated films were measured. Zein films were obtained by casting TZ solutions onto polystyrene petri dishes (9.0 cm diameter). The solution was then dried in an ovenset at 25 inchHg and 40 °C for 48 h. Fourier Transform Infrared Spectroscopy (FTIR, BRUKER IFS-55, Switzerland) was used to detect TEC in the zein matrix. The samples were pelletized with potassium bromide (IR grade, Merck, USA) and the spectra were recorded by scanning between 400 and 4000 $cm^{-1}$averaging 20 scans at a resolution of 4 $cm^{-1}$. The glass transition temperature of the sample was determined using a differential scanning calorimeter (Mettler DSC 30 S, Mettler Toledo, UK). Approximately 10 mg of the samples were loaded into aluminum pans with lid that also served as the reference. Analysis was performed in a nitrogen atmosphere at a heating rate of 10 °C/min from −50°Cto 250 °C.

### 2.4. Fabrication of hollow plasticized zein nanoparticles (HTZN)

HTZN was prepared by using modified anti-solvent precipitation method with $Na_2CO_3$ as sacrificing template [30]. The core of sacrifice template was formed by pouring 1.4 ml of pure ethanol into 0.6 mL deionized water containing carbonate (1.0 wt%). TZ (2 mL) was mixed with the core template solution, followed by pouring the mixture into distilled water at a volume ratio of 1:500. Zein molecules precipitated and wrapped onto the core form nanoparticles [14]. Particles were stirred for another 5 h for stabilization. The $Na_2CO_3$ cores dissolved in water and diffused outwards, leaving hollow interior in the particles. HTZN was dialyzed for 3 days with dialysis membrane to reduce the basicity due to $Na_2CO_3$. The final pH value of the HTZN solution was around 7.2. This ratio of $Na_2CO_3$/TZ was selected based on preliminary trials using mass ratios of 3:1, 1:1,2:3, and 1:3, among which the dispersion prepared at a mass ratio of 2:3 was the most stable and had the lowest polydispersity. Solid nanoparticle with plasticized zein (STZN) and native zein (SZN) were prepared as controls. TZ and zein solution (70% v/v aqueous ethanol) were precipitated in water to produce solid nanoparticles [5],respectively. In addition to freshly prepared dispersions, sample after freeze-drying and storage at −20°Cwere characterized as well.

DiD nanoparticle were prepared using the same method as HTZN nanoparticle and used as fluorescent probes for the studies.

### 2.5. Nanoparticles characterizations

The average particle size and zeta potential of the two kinds of nanoparticles were measured without dilution by dynamic light scattering (DLS) and electrophoretic light scattering(ELS) with photon correlation spectroscopy (Malvern Nano ZS90, UK). The size distribution was evaluated by polydispersity index (PDI). Additionally, the nanoparticles were 200-fold diluted and conducted for transmission electron microscopy (TEM) (HITCHI H-7650 II, Hitachi Ltd., Japan) analysis.

### 2.6. Drug loading of hollow and solid nanoparticle

It was easy to prepare EA-$Na_2CO_3$ cores by coprecipitation due to the limited quantity of EA and $Na_2CO_3$ in aqueous ethanol. EA (0.5–10.0 mg $ml^{-1}$) was dissolved into 0.1 M NaOH and then added into $Na_2CO_3$ aqueous solution as a part of cores at a volume ratio of 1:1. EA-$Na_2CO_3$ cores were formed in 70% ethanol. ZT (zein 1.0 mg·ml-1) was mixed with the core solution, followed by pouring the mixture into distilled water at a volume ratio of 1:500. Particles were stirred for 5 h for stabilization. Nanoparticle was dialyzed for 3 days to remove $Na_2CO_3$ and unloaded EA. The drug loading was calculated from the amount difference of drug in the bath before and after drug sorption by HPLC. The calculation for the encapsulation efficiency was below:

Drug loading % = amount of sorbed drug/ amount of initially added drug × 100%

To make a fair comparison, EA-SZN and EA-STZN were prepared by precipitating in aqueous ethanol [5], respectively. Approximately equal amounts of EA in 0.1 M NaOH was prepared and added into native or plasticized zein solution slowly, and then stirred for 5 h. Sample were stored after freeze-drying.

### 2.7. Nanoparticles stability and release studies in vitro

The stability of the nanoparticles was evaluated using the reported method [31,32] with slight modification. Briefly, EA-HTZN, EA-STZN and EA-SZN were incubated in 9 mL of medium of simulated gastric fluid (SGF, containing pepsin, pH 1–3) and simulated intestinal fluid (SIF, containing pancreatin, pH 5–7) with different pH with adjusted by