Food-grade Zein Nanoparticles for Oral Delivery of Epigallocatechin-3-gallate (EGCG)

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Statement of Purpose: Epigallocatechin-3-gallate (EGCG) is a major polyphenol in green tea and has strong anti-oxidant properties. It has been shown to have health benefits in cardiovascular diseases, cancer and obesity [1, 2]. However, its oral bioavailability is very low due to its chemical instability and poor permeability. To this end, the main objective of this study is to develop an effective oral delivery system for EGCG using food-grade biopolymers. Zein, a water insoluble corn protein was used to prepare nanoparticles. The goal of this study was to develop an optimal method for encapsulation of EGCG in zein nanoparticles and evaluate the chemical stability and in-vitro release in gastrointestinal fluids.

Methods: The nanoparticles were prepared by phase separation method using hydro alcoholic solution and aqueous buffer (pH 3). The particle size, zeta potential and encapsulation efficiency of the nanoparticles were characterized. Vitamin C was added as an anti-oxidant to prevent the chemical degradation of EGCG during the preparation of nanoparticles. The nanoparticles were separated by ultracentrifugation and lyophilized. The particle size, polydispersity index, zeta potential were characterized using particle size and zeta analyzer. The encapsulation and loading efficiency were characterized using HPLC method. The effect of polymer/EGCG ratio, alcohol concentration and the effect of dispersing EGCG in hydroalcoholic phase vs aqueous phase on the nanoparticle characteristics were studied. The release of EGCG from the nanoparticles was determined in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). The stability of Zein-EGCG nanoparticles in SGF and SIF were studied using HPLC.

Results: Factorial design was used to optimize the different parameters for the preparation of EGCG loaded zein nanoparticles. The optimal parameters were found to be zein:EGCG ratio of 10:1, 62% alcohol and a phase volume ratio of 1:2.25. These conditions produced nanoparticles with the highest encapsulation efficiency (Table 1).

Table 1. Optimized Zein- EGCG NPs. Particle characteristics at Zein/EGCG ratio of 10:1, Alcohol Concentration 62% and Phase volume 1:2.5 (Alcohol: Aqueous)

Size (nm)	PDI	Zeta Potential (mV)	Encapsulation efficiency (%)
343.63± 6.47	0.349±0.02	34.86± 1.15	90.89±1.32

When the nanoparticles were incubated in SGF, approximately 6% and 16% of EGCG was released was observed after 2 and 24h respectively.

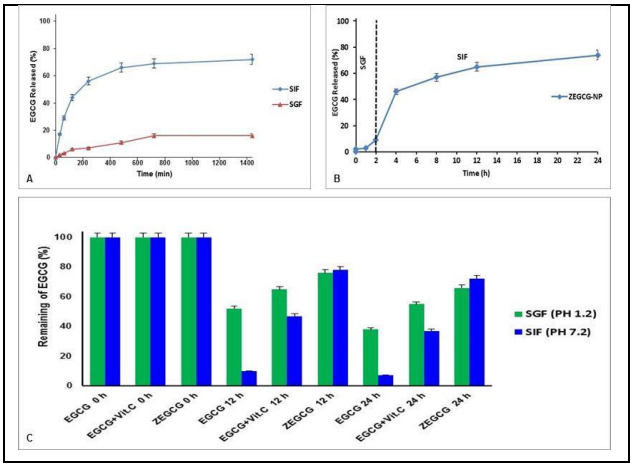

Figure 1. **(A)** The *in vitro* release curves of EGCG from zein-EGCG nanoparticles in SGF and SIF, respectively; **(B)** EGCG release profile from Zein nanoparticles after incubation in simulated gastric fluid (SGF, 0 - 2 h) and intestinal fluid (SIF, 2-24 h) under sink conditions; **(C)** Amount of EGCG remaining in buffer, SGF and SIF after 12 and 24 h respectively. Data is expressed as the mean ±SD, n=3.

On the contrary, when nanoparticles were incubated in SIF, approximately 44% of the loaded EGCG was released after 2h and the remaining EGCG was released in a sustained manner for 24 hrs (Fig.1a). To simulate the gastrointestinal conditions, the nanoparticles were initially incubated in SGF for 2 hours followed by incubation in SIF till 24 hours. As shown in Fig. 1B, around 20% of EGCG was released in first 2hrs in SGF followed by sustained release of EGCG in SIF for 24 hrs. It was found that the zein nanoparticles protected and ascorbinc acid protected EGCG from chemical degradation in SGF and SIF. More than 60-70% of EGCG remained stable in the nanoparticles compared to 10-30% of free EGCG remaining at the end of 24 hrs (Fig. 1c)

Conclusions: EGCG was successfully encapsulated in zein nanoparticles. The EGCG/zein ratio and alcohol concentration influenced the particle size and encapsulation efficiency of EGCG in zein nanoparticles. The zein nanoparticles sustained the release of EGCG in simulated gastrointestinal fluids. Furthermore, the nanoparticles enhanced the stability of EGCG. Future studies will focus on further optimization of the formulation and in-vitro permeability of ECGC nanoparticles will be tested in Caco-2 cells. The findings from this study can be used to develop stable and orally bioavailable EGCG formulation for incorporation in nutritional products.

References:

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