Zein nanoparticles as a carrier system for terpinen-4-ol

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The terpinen-4-ol (T4OL) is the major component of the Tea Tree Oil (TTO), which is extracted from leaves of *Melaleuca alternifolia*, a native plant to Australia. T4OL has shown anti-inflammatory effect, antibacterial, antifungal and an anticancer activity in human melanoma cell lines (M14) [1] and lung cancer cells have also been showed in recent studies [2]. Due to its intrinsic properties, T4OL can cause allergic reactions when applied directly in the skin, limiting its use. The main challenge of developing new systems containing TTO or its pure components to the treatment of human body is that the system must be effective and safe. In this sense, encapsulation can be used alternatively to reduce this topical irritation. Nanoparticle delivery has been an approach widely used to optimize skin local therapies [3]. Nanoparticles prepared from biopolymers represent an interesting alternative due to its high biocompatibility and biodegradability. Some proteins can be used as wall material, since they have the characteristic of being amphiphilic, which is a major driving force for the self-assembly, essential to the formation of nanoparticles. Given that zein, a prolamin fraction of corn protein, has long been recognized for its coating ability for the encapsulation of bioactive compounds [4], the main objective of this work was to evaluate the its potential as a carrier for T4OL, aiming an topical application in skin. Zein nanoparticles were obtained by the antisolvent precipitation process (dessolvation). Zein was solubilized in a binary system ethanol/water (87:13), and then T4OL was added in the solution and they were mixed for 30 min. For each sample, 3 mL of zein solution containing T4OL was dropped at a constant rate of 6 mL/h in 9 mL of an aqueous solution, containing the selected amount of surfactant (tetradecyl-trimethyl-ammonium bromide – TTA), under magnetic stirring at 1000 rpm. The organic solvent was eliminated by evaporation under reduced pressure. The freshly prepared nanoparticle dispersions were submitted to particle size, zeta potential by photon correlation spectroscopy and laser-Doppler anemometry, respectively, using a Zetasizer Nano Series (Malvern Instruments, Worcestershire, UK), and morphologic analysis (Transmittion electron microscopy – TEM). The resulting suspensions were centrifuged at 4000 rpm for 30 min in Amicon ultra-filter (cellulose regenerated membrane with a molecular weight cut of 100 kDA) to calculate the efficiency of encapsulation (EE). The supernatants were removed, diluted in 87% v/v ethanol and the T4OL content was analyzed by HPLC. The EE was calculated as the amount of T4OL added in the zein solution in relation to that present in the supernatant (did not encapsulate). Since many factors influence the obtention of nanoparticles, the first step of this study included a fractional factorial experimental design (3/1/9) in that three factors were varied (concentrations of the protein, the active compound and surfactant), as shown in Table 1. The experimental design and the statistical analysis (ANOVA) were performed using Statistica Software ® version 7.0 to determine the significance of the factors studied. The responses analyzed were the particle size, polydispersity (PDI), zeta potential (mV) and EE. The response surface methodology was used to analyse the effect of the three factors on encapsulation efficiency. All experiments were performed in random order and duplicate. Zein nanoparticles showed diameters ranging from 59.5 to 95.5 nm and PDI in the range of 0.09 to 0.32 and zeta values rangin from 33.5 mV to 57.7 mV. No significant effect in the analyzed factors (concentration of zein, T4OL and TTA) on the size and polydispersity of nanoparticles was observed. This result can be explained in terms of the polymer chain lenght, since zein used in this study comprises a family of proteins composed by α, β, and γ- zein, with molecular weights ranging from 10-25 kDa.

Table 1: Factors evaluated in the experimental design and obtained results*

Experiment	Zein (mg/mL)	T4OL (% w/w zein)	TTA (% aqueous phase)	Size (nm)	PDI	Zeta (mV)	Encapsulation Efficiency (%)
1	20	10	0.1	91.96 ± 1.93	0.15 ± 0.01	33.5	63.7
2	20	20	0.5	87.62 ± 5.79	0.32 ± 0.04	57.7	60.8
3	20	30	0.3	59.53 ± 1.27	0.18 ± 0.02	48.5	85.8
4	35	10	0.5	67.13 ± 0.48	0.22 ± 0.01	55.1	82.0
5	35	20	0.3	85.96 ± 0.80	0.11 ± 0.01	46.1	78.1
6	35	30	0.1	84.39 ± 1.03	0.15 ± 0.01	45.7	85.0
7	50	10	0.3	92.31 ± 0.74	0.09 ± 0.03	50.8	93.2
8	50	20	0.1	89.52 ± 0.70	0.12 ± 0.01	48.5	85.7
9	50	30	0.5	95.53 ± 2.15	0.09 ± 0.03	45.0	93.1

*T4OL: Terpinen-4-ol; TTA: Tetradecyl trimethyl ammonium bromide; PDI: polydispersity

The morphology of empty zein nanoparticles is shown in Figure 1a. Nanoparticles with or without T4OL present a compact spherical structure. Concerning the encapsulation efficiency (EE), the Pareto chart showed that only the zein concentration affected the EE significantly (ANOVA, $p < 0.05$) (Figura1b), TTA and T4OL did not affected EE significantly in the concentration range studied. Figure 1c shows the influence of TTA/Zein proportion in EE fitted by the linear regression model expressed in Equation 1. The model is limited to the studied concentration range.

$$EE\ (\%) = \underset{(\pm 7.33)}{58.86} + \underset{(\pm 0.198)}{0.641}\ [zein] \quad R^2\ 0.60012 \qquad (1)$$

Although zein has increased the EE in a concentration-dependent manner, for concentrations higher than 50 mg/mL, an aggregation of this protein was observed. Thus, it is not possible to carried out experiments with zein in high concentrations.

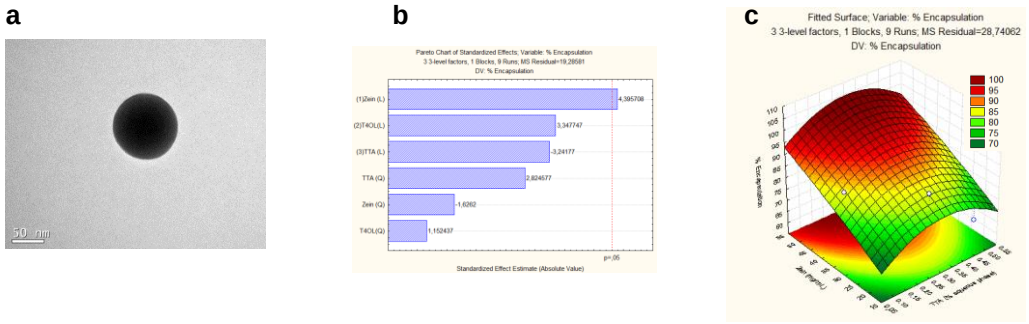

Figure 1: a) Morphology of an empty zein nanoparticle; b) Pareto chart showing the influence of factors on the EE of T4OL in zein particles and c) Response surfaces for the EE of T4OL (30% w/w zein) as a function of the concentration of the TTA and zein.

References

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