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Preparation of polyamide nanocapsules of *Elaeagnus angustifolia* L. delivery with *in vivo* studies

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ABSTRACT

The purpose of this study is to prepare polyamide nanocapsules containing *Elaeagnus angustifolia* L. using an emulsion diffusion technique with *in vivo* studies. Diethylenetriamine (DETA) was used as the encapsulating polymer in acetone ethyl acetate (EA) and dimethyl sulfoxide (DMSO) as the organic solvents, Tween 80, Tween 60, Tween 20, Gelatin, and sodium lauryl sulfate (SLS), in water as the stabilizers. Two ratios of organic to aqueous phases were used with each solvent and stabilizer. The nanocapsule *E. angustifolia* were obtained with a mean diameter of 60–180 nm, zeta potential of −11.3 mV, Particles Dispersion Index (PDI) of 0.2, and a high encapsulation efficiency of 0.98%. Acetone was superior to EA and DMSO, and Tween 20 was superior to Tween 80, Tween 60, Gelatin and SLS in obtaining smaller nanocapsules. An organic of aqueous phase ratio of 1:5 was shown to be more suitable for the smaller nanocapsules. Finally, nanocapsules containing *E. angustifolia* and calcium carbonate as well as calcium tablets available on the market, were administered to rats for 3 weeks, and the calcium levels were measured in their blood as well as compared to a normal control group. The results showed that the *E. angustifolia* group has a higher level of calcium in their blood.

1. Introduction

The dried powder of the fruit of *Elaeagnus angustifolia* mixed with milk was used as a remedy for rheumatoid arthritis and joint pain. *E. angustifolia* has many potential applications in drug manufacturing and thus is an excellent candidate for an investigation into its delivery in nanocapsule form. *E. angustifolia* is a calcium-rich herb of the *Elaeagnaceae* family that has been found to have a variety of therapeutic effects. Anti-inflammatory, antipyretic and other effective treatment of diseases have been the subject of study of the plant (Taheri et al., 2010). The volatile oils with based extract in the plant are also wide applications in the industry for commercial product like drugs, detergents, perfumes, and herbal teas (Esmaeili et al., 2011). Although, the discovery of new drug molecules enables the treatment of patients with minimal side effects and thus this material in particular, is compatible with human natural requirements. In previous reviews, drug delivery with based extract plant has shown to be a suitable choice for drug containers (Khafagy et al., 2007). In nanotechnology, the production and application of drugs in the nano-scales can be used. In drugs with nanocapsule form bioactive characteristics, various sensory attributes and water solubility are also viably used. Thus, one of the beneficial forms of drugs are nanocapsules, which are found useful in the pharmaceutical industries as they have been used for targeted delivery, minimized toxicity, enhanced bio-distribution and higher cell uptake of some drugs and nutraceuticals (Habib et al., 2012). Esmaeili et al. (2013) have recently improved the nanocapsulation of *Crataegus azarolus* a drug with hypotensive effects that also acts as a direct and mild heart tonic. Fragrance and flavor an important role and have been widely products, such as perfume, soap, cream, food, cigarette, etc. Encapsulation is one of important methods to solve these problems. Encapsulation techniques are used in the food and cosmetic industries to control the release of entrapped materials and to protect against surrounding environments. However, fragrances and flavors are complex mixtures of comparatively volatile substances and labile components of which the sensory perception can be changed as a result of heating, oxidation, chemical interactions or volatilization. Microencapsulation technology is an effective method to minimize the harm of these problems. Encapsulation of fragrances or flavors has been attempted using various methods (Esmaeili et al., 2013). Nanoencapsulation has also been investigated to protect palmitate structures against photo-degradation UV radiation (Huang et al., 2010; Mishra et al., 2010; Sane and Limtrakul, 2009) therefore, the nanocapsulation of *E. angustifolia* and *in vivo* studies are considered a novel method for protecting the delivery of various drugs; hence, there is need to study the

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conditions that influence the characteristics of the nanocapsules of *E. angustifolia* in order to use them as functional ingredients in foods. In this study, the effect of calcium and *E. angustifolia* nanocapsules were studied on rats in the form of a drug and has been shown that calcium levels increased after a period of 21 days. Therefore, the purpose of this research is the use of new technologies in the manufacture of nanocapsules to deliver calcium ions to all body tissues where it is needed.

2. Experimental

2.1. Chemicals

Seboycolchloride (SC), diethyltriamine (DETA), olive oil, *Elaeagnus angustifolia* L. extract, Acetone, ethyl acetate (EA), dimethyl sulfur oxide (DMSO), Span, Tween 80, Tween 60, Tween 20, sodium lauryl sulfate (SLS), Gelatin and pure water were used in this study.

2.2. Preparation of the nanocapsule

Approximately 1 mg of SC, 2.5 mg of olive oil, and 1.5 mg of span were dissolved in 18, 15, 10, 5 ml of organic solvent (acetone, EA, DMSO), and 2.5 mg supplemental *E. angustifolia* were added and dissolved in 2, 5, 10, 15 ml of methanol. The organic phase was then added to 100 ml of water drop by drop. Thereafter, 1.5 mg of surfactant (Tween 80, Tween 60, Tween 20, Gelatin, SLS) 4 mg of monomer DETA and 1.5 mg of glycerin, were added at room temperature then acetone is removed under vacuum and water is removed by freeze drying using a rotary evaporator, and the cycle was repeated four times, and four times with deionized water to dilute.

2.3. Characterization of nanocapsule

2.3.1. Particle size determination

The sizes of the nanoparticles were measured as mean intensity diameter using the dynamic light scattering technique and the particle size analysis (PSA) report model PSAN12 1-872. The reference solvent was distilled water (index of refraction = 1.33), and the reference soluble was polystyrene (index of refraction = 1.55). The nanocapsules were considered as light-absorbing objects. However, the final particle diameter was calculated on the basis of an average of three or more readings.

2.3.2. Zeta potential determination

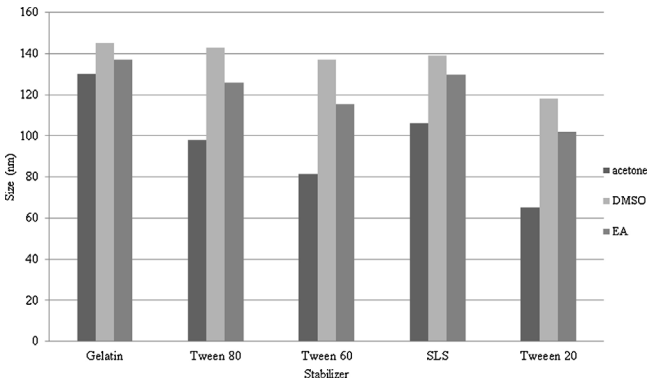
Zeta potential was estimated based on electrophoretic mobility under an electric field using a zeta potential particle size analyzer. The relation between the zeta potential and mobility is given by the Smoluchowski equation:

$$\xi = \frac{\mu\eta}{\varepsilon}$$

where ξ = zeta potential, μ = mobility, η = viscosity, ε = dielectric constant. The zeta potential of the nanocapsule dispersions was measured under a 5 V/cm electric field and a dielectric constant of 79 (of water) at 25 °C.

2.3.3. Scanning electron microscopy

The appearance of the nanocapsule populations was visualized by scanning electron microscopy (SEM). A drop of the nanocapsule suspension was placed on a glass plate and dried at lump UV after that acetone was removed. The sample was then coated under vacuum with gold and palladium by cathode sputtering. Each sample was analyzed with an SEM voltage of 15 kV.



Scheme 1. The effect of the type of solvent on the size of nanocapsules prepared with different types of stabilizers.

2.4. Plant material extraction and isolation

Dried, finely powdered aerial parts of *E. angustifolia* (83.25 g) were dissolved with methanol in a Soxhlet extractor for 2 days. After the filtration and evaporation of the solvent, the crude residue (28.30 g) remained (Esmaeili et al., 2012).

2.5. Atomic absorption

Calcium content was measured by absorption atomic method. The calcium content of the standard sample and *E. angustifolia* nanocapsules were measured and compared with each other. After the absorption of calcium in the *E. angustifolia* nanocapsules, the new samples were again measured and compared.

2.6. Determination of encapsulation efficiency

The percentage of *E. angustifolia* incorporated during nanoparticle preparation was determined by estimating both the capsulated and the free (noncapsulated) *E. angustifolia* by using a validated absorption atomic method described below. Encapsulation efficiency of the technique was calculated according to the following equations:

$$\text{Capsulation } E.\ angustifolia = \text{Total } E.\ angustifolia - \text{Free } E.\ angustifolia \text{ in the dispersion}$$

$$\text{Capsulation efficiency} = \frac{\text{Capsulated } E.\ angustifolia}{\text{Total } E.\ angustifolia} \times 100\%$$

2.7. Statistical analysis

All statistical analysis was carried out using SPSS 12 for Windows. The statistical significance of differences among values was assessed using the one-way ANOVA test. Error bars are indicated wherever necessary.

3. Results

To compare the role of the stabilizer used in the preparation of nanocapsules, the data obtained were gathered in Scheme 1. Statistical analysis indicates that the solvent generally has a highly significant ($P \leq 0.01$) effect on the size of the nanoparticles (Table 1).

Systems with two solvents were investigated and have been shown in Table 2. It was observed that the ratio of acetone to methanol is lower in the smaller particle sizes which can be due to the reduction of the boiling point (338 K).