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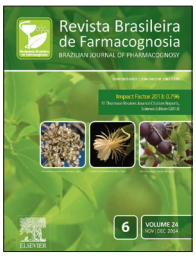

Original article

Properties and controlled release of chitosan microencapsulated limonene oil

Jefferson M. Souza[a], *Artemísia L. Caldas*[a], *Shafagh D. Tohidi*[b], *Javier Molina*[c], *António P. Souto*[b], *Raul Fangueiro*[b], *Andrea Zille*[b,*]

[a]*Departamento de Moda, Design e Estilismo, Universidade Federal do Piauí, Campus Ministro Petronio Portela, Teresina, PI, Brazil*
[b]*Centro de Ciência e Tecnologia Têxtil, Universidade do Minho, Campus de Azurém, Guimarães, Portugal*
[c]*Departamento de Ingeniería Textil y Papelera, EPS de Alcoy, Universitat Politècnica de València, Alcoy, Spain*

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ABSTRACT

Chitosan microcapsules containing limonene essential oil as active ingredient were prepared by coacervation using three different concentrations of NaOH (0.50, 1.00, 1.45 wt%) and fixed concentrations of chitosan and surfactant of 0.50 wt%. The produced microcapsules were fully characterized in their morphology and chemical composition, and the kinetic release analysis of the active ingredient was evaluated after deposition in a non-woven cellulose fabric. The concentration of 1.00 and 1.45 wt% clearly show the best results in terms of dimension and shape of the microcapsules as well as in the volatility results. However, at the concentration of 1 wt% a higher number of microcapsules were produced as confirmed by FTIR and EDS analysis. Free microcapsules are spherical in size with disperse diameters between 2 and 12 μm. Immobilized microcapsules showed sizes from 4 to 7 μm, a rough surface and loss of spherical shape with pore formation in the chitosan walls. SEM analysis confirms that at higher NaOH concentrations, the larger the size of the microcapsules. This technique shows that by tuning NaOH concentration it is possible to efficiently control the release rate of encapsulated active agents demonstrating great potential as insect repellent for textiles.

Introduction

The microencapsulation of substances has as principle the preparation of an emulsion, which involves the compound to be encapsulated (a solid, liquid or gaseous product) in order to protect it and to preserve its potential (Mahdavi et al., 2014). The encapsulation process also involves merger, absorption or dispersion of the combinations of solid, liquid or gaseous encapsulated bioactive materials. The main objective is the protection against degradation promoted by the external environment and the controlled release of specific substances (Greay and Hammer, 2011). An important factor for the encapsulation of those materials is their protection against degradation and the improvement of their stability and solubility, such as: solubility of hydrophobic components in hydrophobic arrays and vice versa (Pirvu et al., 2010; Shateri-Khalilabad and Yazdanshenas, 2013). Microcapsules are applied to

* *Corresponding author.*
E-mail: azille@2c2t.uminho.pt (A. Zille).
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improve the efficiency of encapsulated materials or to create new applications, including functions in textile products, thus allowing them repellent, odorous, moisturizing or antimicrobial properties, among others. They can be used in a wide range of clothing, such as pants, socks, underwear and gloves (Nelson, 2002; Nazzaro et al., 2012). Flavours have a large range of applications in the industry. However, some of them are very sensitive to environmental or industrial process conditions. The flavour loss may reach values of 90% in free form due to their extreme volatility and reactivity with other components. The release kinetics of the active elements within the microcapsules depends directly on the processes and formulation parameters. They are specifically designed to release components when subjected to certain parameters. The active ingredient can be released through two methods: forced and controlled release. The forced release is obtained by rupturing the microcapsule membrane under thermal and/or mechanical conditions, such as friction. The controlled release is based on the diffusion of the encapsulated active element through the membrane or its degradation (Jamekhorshid et al., 2014). With respect to the materials used in the production of microcapsules, polysaccharides, such as alginate, starch and cellulose; and proteins as collagen and gelatin are widely used due to their ability to bind to flavour compounds, plus their biodegradability and low cost. They are used for the production of those materials in the food and pharmaceutical area (Can Karaca et al., 2013; Soliman, 2013). Researchers have been recently exploring the use of chitosan as an encapsulating agent (Peng et al., 2010a; 2010b; Estevinho et al., 2013a; Nuisin et al., 2013). Chitosan, the N-deacetylated derivative of chitin, is a cationic polyelectrolyte due to the presence of amino groups, one of the few occurring in nature. This gives chitosan singular chemical and biological characteristics, such as: biocompatibility, antibacterial properties, heavy metal ion chelation ability, gel-forming properties and hydrophilicity (Santos et al., 2013). Due to its chemical configuration and to features like abundance, low toxicity, hydrophobicity, biodegradability, biocompatibility and antimicrobial activity, chitosan is employed for the preparation of films, gels, microspheres and microcapsules. It has been used in various areas such as biotechnology, cosmetics, food and pharmaceuticals, as a way to release active compounds, among others (Kong et al., 2010; Cruz-Romero et al., 2013). The use of chitosan in protein and drug delivery systems is being actively researched and reported in the literature (Chen et al., 2013). Chitosan has one important advantage over other encapsulating agents, which is the possibility to establish covalent or ionic bonds with the crosslinking agents, building a network of sorts, in which the active substance is retained. In consequence, these chemical bonds carry advantages in terms of controlled release (Estevinho et al., 2013b).

There is a variety of techniques used for encapsulating drugs, foods and cosmetics, such as: spray-drying, complex coacervation, atomization and liposomes (Gharsallaoui et al., 2007; Gou et al., 2013). When choosing the material to be used for this process, a number of factors must be taken into consideration, such as: physical and chemical properties of the core, porosity and solubility of the wall, viscosity, mechanical properties, film-forming ability and vitreous transition, compatibility of the core with the wall, and the wall material used must be insoluble and non-reactive with the core (Gouin, 2004).

The encapsulation of essential oils allows optimization of its functionality, a factor that enables a more prolonged action of its active principle, since essential oils are characterized by their high volatility. Essential oils can be extracted from different parts of the plants, such as: roots, leaves, rose petals, stems and fruits, as well as condiments. They have properties ranging from antimicrobial, healing, and odorants, among others (Silva et al., 2010). The essential oils with antimicrobial activity by its *anti-quorum-sensing* activity become an important mechanism for the reduction of virulence and pathogenicity of bacteria. Among the essential oils with antibacterial activity against the bacteria *E. coli* and *S. aureus*, the lemon essential oil (*Citrus limon* L.), with limonene, β-pinene, γ-terpinene and citral (neral and geranial) as main compounds showed interesting antimicrobial features (Kim and Morr, 1996; Rodrigues et al., 2008; Donsì et al., 2011).

In this study, chitosan microcapsules containing limonene essential oil (EO) as active ingredient were prepared by coacervation process by NaOH dripping technique. The produced microcapsules were fully characterized in their morphology and chemical composition by Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR), Energy Dispersive X-ray Spectroscopy (EDS), optical and scanning electron microscopy (SEM). Finally, the kinetic release analysis of the active ingredient was evaluated after deposition in a cellulose non-woven fabric for its potential as insect repellent.

Materials and methods

Materials

Chitosan (ChitoClear hq95-43000) was purchased from Primex (Iceland). The non-ionic surfactant was Lutensol ON 30 (BASF). All the other materials were purchased from Sigma-Aldrich and used without further purification.

Preparation of microcapsules

The process for the manufacturing of the microcapsules was as follows: 2 ml of limonene essential oil was added, in 20 ml of 0.5 wt% chitosan solution along with 0.5 wt% of surfactant. The solution was stirred at 700 rpm for 10 min. Then, the emulsion containing the essential oil was dripped into three different 100 ml solutions containing 0.5, 1 and 1.45 wt% of NaOH (0.12, 0.25, 0.36 M) stirred at 100 rpm. The solution was left on slow agitation for a period of 30 min after dripping. After the resting period, 1 ml of each microcapsule sample was removed for observation under an optical microscope. The solution was then filtered, washed three times with distilled water and dried at 30°C for a period of 15 h, in order to evaporate any remaining water from its surface.

Optical microscopy

The microcapsules distribution and morphology was recorded using an Olympus BH2 optical microscope, coupled to a JVC TK1280E camera and Micron Measurement video recorder capture software. The samples were observed separately for a period of 5 min using a magnification of 40 × 65.