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Release behavior of *trans,trans*-farnesol entrapped in amorphous silica capsules

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ABSTRACT

Farnesol, a compound widely found in several agro-food by-products, is an important bioactive agent that can be exploited in cosmetics and pharmaceutics but the direct bioapplication of this compound is limited by its volatility. Here the entrapment of farnesol in silica capsules was investigated to control the release of this bioactive compound in the vapor phase and in ethanol solutions. The preparation of silica capsules with oil cores was obtained by employing a sol–gel method using O/W/O multiple emulsions. Two distinct chemical vehicles for farnesol have been investigated, retinol and oleic acid, that afterwards have been emulsified as internal oil phases. The volatile release of farnesol from the as-prepared SiO_2 capsules was investigated by headspace solid phase microextraction (HS-SPME) followed by gas chromatographic analysis (GC), and the release to ethanol was carried out by direct injection of the ethanolic fraction into the GC system. It is demonstrated that these capsules are efficient for the long controlled release of farnesol and that the respective profiles depend on the chemical parameters employed in the synthesis of the capsules.

1. Introduction

Over the past decade, a major trend in the emerging area of encapsulation technology has been the design of increasingly sophisticated capsules for controlled release of bioactive molecules [1,2]. These materials find many applications in a wide spectrum of fields such as medicine, pharmaceutics, food and paint industries [3]. In addition, it is known that the encapsulation of materials using inorganic particles and organic polymers can alter the surface characteristics of the cores and enhance the storage stability of the entrapped materials [1]. Diverse nanocarriers for drug delivery applications have been investigated, these include liposomes [4], cyclodextrines [5], colloidosomes [6], silica microcapsules [1–3] and metal-organic frameworks [7].

In particular, there has been an increasing interest in the development of mesoporous and hollow SiO_2 materials for controlled drug delivery due to their attractive features [8–11]. In fact, owing to their chemical robustness and biocompatibility, silica capsules offer an interesting alternative to pure organic based delivery systems, which generally show lower drug loading capability and rapid drug release. Several methods for the preparation of silica capsules have been developed, these include Pickering emulsions [12,13], water-in-oil-in-water multiple emulsion templating using sodium silicate as precursor [14], water-in-oil (W/O) or oil-in-water (O/W) emulsions using tetraethylorthosilicate (TEOS) as silica precursor [9,15–17]. Among these systems, multiple emulsions, both of oil-in-water-in-oil (O/W/O) or water-in-oil-in-water (W/O/W) type, have been used as a tool to drug delivery in specific body targets, by prolonging the release of drugs with a short biological half-life [1]. Although less investigated, O/W/O multiple emulsions are good candidates for the controlled release and stabilization of lipophilic drugs [18]. In this context, the entrapment and *in vitro* release of Vitamin A (retinol) in silica particles has been previously reported [1,19]. Additionally, a comparative study for the stability of retinol in three types of emulsions: O/W, W/O and O/W/O, has shown the highest stability when the O/W/O emulsion was used [20].

Farnesol, a natural sesquiterpenoid (C_{15}) occurs in many essential oils, mainly in rose and orange blossoms. Farnesol is a fragrance ingredient widely used in cosmetics, fine fragrances, shampoos, and toilet soaps as well as in non-cosmetic products such as household cleaners [21]. Also, recent studies have shown that farnesol affects the growth of a number of bacteria and fungi, pointing to a potential role as an antimicrobial agent [22,23]. For example, farnesol has been incorporated into microcapsules that can be degraded by the action of bacteria. These microcapsules, composed of natural proteins, were filled with two active compounds that can be released upon reaching targeted temperatures, allowing the delivery in perspiration conditions [24].

Despite the relevance of farnesol in diverse applications, its use has also been limited due to the volatility of this compound, leading to unnecessary losses. This research describes for the first time the entrapment of *trans,trans*-farnesol in SiO_2 capsules using O/W/O multiple emulsions. The emulsions act as soft organic templates to produce amorphous SiO_2 capsules by hydrolysis and condensation reactions using TEOS as the sol/gel precursor. Due to their chemical and thermal stability and biocompatibility, silica capsules are an

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advantageous alternative to conventional pure organic based delivery systems (e.g. micelles, liposomes, and polymer particles), which generally also show lower drug loading capability and rapid drug release. It will be shown that oleic acid can be used as an efficient drug vehicle thus presenting economical advantages in relation to the use of the more expensive vehicle retinol. In addition, with the method here reported the surface of these SiO_2 capsules can be easily chemical functionalized in order to meet the demands in terms of the release profile of active substances. In principle, this process can be adapted to the production of amorphous SiO_2 capsules containing other volatile bioactive cores, but here this will be demonstrated using SPME-GC-MS monitoring for farnesol releasing behavior.

2. Experimental

2.1. Materials

Tetraethyl orthosilicate (TEOS, 98%), polyoxyethylene sorbitan monolaurate (Tween20), sorbitan monoleate (Span 80) N-decyl alcohol (>98%) were purchased from Sigma-Aldrich. Triblock copolymer pluronic P123 (EO20PO70EO20, Mw. 5800), polyvinylpyridinone, hydroxypropyl-cellulose (Mw. 100,000), polyethylene glycol, retinol (95%) and oleic acid (90%) were also purchased from Aldrich Chemical Company. Ammonia (25%, Merck), ethanol (Riedel-de Haën) and *trans,trans*-farnesol (95%) Fluka (for sake of simplicity the term farnesol will be used therein). All the reagents were of analytical grade and used without further purification.

2.2. Preparation of multiple emulsions

O/W/O multiple emulsions were prepared through a two-step emulsification process. In a first step, the primary O/W emulsion was prepared. Tween 20 as high HLB surfactant (1 wt%) was added to an aqueous solution containing a stabilizing polymer. The use of three types of polymers (PEG, PVP and P123) was investigated. Farnesol was added either to retinol or to oleic acid, and then dispersed in the water phase. After 30 min of stirring, NH_4OH (2 wt%) was added to the water phase. In a second step the primary emulsion was slowly added to *n*-decyl alcohol as external oil phase containing Span 80 as low HLB surfactant (2 wt%) and 0.8 wt% HPC. The weight ratio of the O/W primary emulsion in the external oil phase was kept at 1:9, and the resulting O/W/O emulsion was stirred at low shear for 30 min. All the experimental compositions are shown in Table 1.

2.3. Fabrication of silica capsules entrapping farnesol

To prepare silica capsules by the sol–gel method, an amount of TEOS equivalent to the molar ratio $H_2O/TEOS = 4$ was gently added to the multiple emulsions. The mixture was then stirred with a magnetic stirrer for 7 h at room temperature. After the reaction was completed, the sample was centrifuged at 3000 rpm for 15 min; in order to remove non-reacted chemicals the as prepared particles were washed twice with ethanol.

2.4. Characterization

The droplet size and morphology of the multiple emulsions were investigated by optical microscopy using an Olympus BX51 microscope. The FT-IR spectra of KBr pellets of the samples were recorded by using a Mattson 7000 spectrometer, at 64 scans at a resolution of 4 cm^{-1}. Transmission electron microscopy (TEM) was carried out on a Hitachi H-9000 microscope operating at 300 kV. To prepare the TEM samples, a drop of the diluted ethanolic solutions of the samples were deposited on a carbon-coated copper grid, and the solvent was left to evaporate. Scanning electron microscopy (SEM) images were carried out using a Hitachi SU-70 and average sizes for the sample have been estimated directly from the images.

2.5. Farnesol controlled release

In order to evaluate the release of farnesol from SiO_2 capsules prepared in a O/W/O multiple emulsion, two assays were performed for 500 h: the release to the vapor phase was evaluated using headspace solid phase microextraction (HS-SPME) followed by gas chromatographic analysis (GC), and the release to ethanol was carried out by direct injection of the ethanolic fraction into the GC system. For headspace sampling, *ca* 23.8–26.8 mg of the SiO_2 capsules with 1 mL ethanol were introduced into a 2 mL glass vial. The vial was capped with a PTFE septum and a cap (Chromacol, Hertfordshire, UK), and was stored at room temperature for 500 h. At each sampling moment, the SPME fiber was introduced for 10 min into the vial to promote the transfer of the farnesol from the headspace to the coating fiber. The SPME device included a fused silica fibre coating partially cross-linked with 50/30 μm divinylbenzene-carboxen-poly(dimethylsiloxane). For ethanol release assay, *ca* 39.5–41.1 mg of the SiO_2 capsules and 2 mL of ethanol were introduced into a 2 mL glass vial. At each sampling moment, 5 μL of each ethanolic solution was injected into a gas chromatograph. A PerkinElmer Clarus 400 gas chromatograph with split injector and a flame ionization detector (FID) was used to performed both analysis (SPME and direct injection of solutions), equipped with a 30 m × 0.32 mm (i.d.), 0.25 μm film thickness DB-FFAP fused silica capillary column (J&W Scientific Inc., Folsom, CA, USA). The oven temperature was programmed from 100 to 200 °C at 20 °C/min (hold 1 min at 200 °C). The injector and detector temperatures were 250 °C. The flow rate of the carrier gas (H_2) was set at 2.6 mL/min. The injection port was lined with a 0.75 mm (i.d.) glass liner, in the case of SPME analysis. Split injection mode was used (154 mL/min). The GC area data were used as an approach to estimate the relative content of farnesol.

3. Results and discussion

In this study, O/W/O multiple emulsions have been investigated using various chemical compositions, which include the use of retinol or oleic acid as vehicles for farnesol encapsulation. The prepared multiple emulsions were then employed as soft organic templates to prepare silica capsules by a sol–gel method involving the hydrolysis and condensation of silane oligomers derived from TEOS used as precursor. The hydrolysis and condensation reactions take place in the water phase though TEOS was previously added to the oil phase (*n*-decyl alcohol). This is because vigorous stirring of the external oil phase facilitate the penetration of TEOS through the surfactant layer surrounding the water phase in which the hydrolysis occur. As the hydrolysis proceed, the water-soluble silica oligomers are kept inside the aqueous droplet [1,8,9]. Thus the aqueous phase acts as space-limiting micro-reactors for the hydrolysis process, and the internal oil droplets serve as templates for cores.

The use of multiple emulsions in materials synthesis requires judicious control over several experimental parameters in order to achieve emulsion stability. In order to obtain suitable emulsions for the encapsulation of farnesol, several concentrations for Tween 20 and Span 80 were investigated and here results are presented among those that result in the more morphological uniform droplets as evaluated by optical microscopy. Moreover, the droplets average size and size distribution have a major role in the emulsion stability in a way that emulsions with precisely controlled droplet size exhibit better stability. As the interfacial curvature of the internal droplets is tensed due to the small size of the droplets, the addition of surfactant in the external phase will help the formation of a hole in the external film when the internal drops are close to the surface. This enables a decrease of the curvature tension that becomes more positive and