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High loading fragrance encapsulation based on a polymer-blend: Preparation and release behavior

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ABSTRACT

The six fragrances, camphor, citronellal, eucalyptol, limonene, menthol and 4-*tert*-butylcyclohexyl acetate, which represent different chemical functionalities, were encapsulated with a polymer-blend of ethylcellulose (EC), hydroxypropyl methylcellulose (HPMC) and poly(vinyl alcohol) (PV(OH)) using solvent displacement (ethanol displaced by water). The process gave ≥40% fragrance loading capacity with ≥80% encapsulation efficiency at the fragrance to polymer weight ratio of 1:1 and at initial polymer concentrations of 2000–16,000 ppm and the obtained fragrance-encapsulated spheres showed hydrodynamic diameters of less than 450 nm. The release profile of the encapsulated fragrances, evaluated by both thermal gravimetric and electronic nose techniques, indicated different release characteristics amongst the six encapsulated fragrances. Limonene showed the fastest release with essentially no retention by the nanoparticles, while eucalyptol and menthol showed the slowest release.

1. Introduction

Components in essential oils are secondary metabolites with unique odors. They are used worldwide not only in folk medicine, spa, cosmetics and toiletries, but also in many scented household and occupational products. Scientific studies on the biological actions of these essential oils have already started to accumulate (Fukumoto et al., 2006; Moretti et al., 2002; Morita et al., 2003). These unique odorous molecules are being synthesized or isolated from natural sources and used as fragrance components in various industries. However, many of these fragrance molecules are unstable due to their reactive functionalities, such as aldehyde, ketone and terpenes. Degradation not only causes changes in their sensory characteristics, but also, in many cases, creates allergenic products (Karlberg et al., 1992; Matura et al., 2005, 2006). It has been known that control of the volatilization rate and degradation is the heart of prolonging the sensory characteristics of fragrance materials. One way of doing so is encapsulation, which provides both stabilization and a controlled release of the entrapped materials. Other benefits of encapsulation include ease of handling (e.g. a stable solid encapsulated product instead of an unstable volatile liquid), improved safety (e.g. reduced flammability) and an increased applicability to various products (e.g. water dispersible essential oil-encapsulated spheres can be easily applied in water based formulations). However, the fragrance release properties are the key issue in selecting a particular encapsulation technology.

The existing fragrance encapsulation technologies includes double emulsion preparation (Edris and Bergnståhl, 2001), molecular inclusion into a host, such as cyclodextrin (Wang and Chen, 2005), incorporation into solid lipid nanoparticles using appropriate lipids and surfactants (Lai et al., 2006), coacervation with various carbohydrates with and without the use of crosslinking agents (Soper et al., 2000; Chang and Dobashi, 2003), interfacial polymerization based on various polymers, such as polyurethane-urea (PUU) and phenol–formaldehydes (Ouall and Lahoussine, 2006; Scarfato et al., 2007; Hwang et al., 2006a,b; Rodrigues et al., 2008) and *in situ* polymerization, such as the synthesis of fragrance-encapsulated mesoporous silica spheres (Wang et al., 2008). Amongst these, interfacial polymerization and complex coacervation are the two most popular choices.

Partial solubility in water of many essential oils' components usually causes instability in the microencapsulation by interfacial reactions because of the change in the hydrolytic stability of the particle during polymerization reaction. Moreover, side reactions between the monomers with several reactive functionalities of the essential oil's components can lead to some alteration of the

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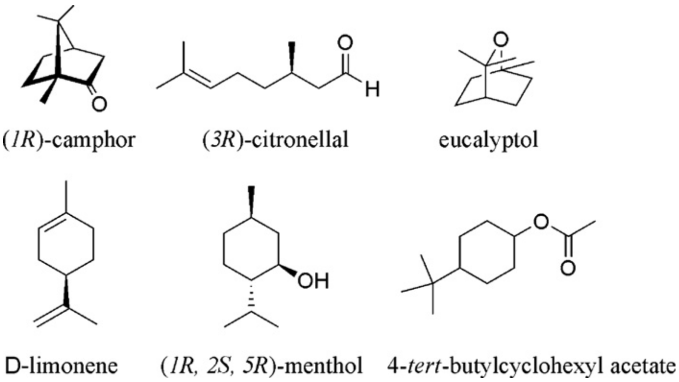

Fig. 1. The chemical structure of the six fragrance molecules.

encapsulated products, especially in the PUU system. To avoid such drawbacks, the environmental unfriendly phenol–formaldehyde (melamine–formaldehyde) has been used. The system is neither biocompatible nor biodegradable which limits its applications.

Complex coacervation has been reported and patented by several authors as a method for fragrance prolongation (Soper et al., 2000; Bachtsi and Kiparissides, 1996; Jean-Pierre et al., 1998; Markus and Linder, 2004). However, the coacervated products generally have a weak mechanical resistance, due to water solubility of the polymer, making them inappropriate for many applications where a long shelf-life and a good mechanical strength are required. The use of crosslinking agents to improve the stability, although it has been demonstrated, possesses the drawbacks of side reactions between the encapsulated material and the residues of the crosslinking agents.

For fragrance-encapsulated polymeric microspheres, the release of the fragrance component(s) incorporated in the polymer is controlled by the initial loading of the fragrance(s) in the polymer and the ability of the fragrance molecules to diffuse through the polymeric barrier into the surrounding environment. Interactions between fragrance molecules and the polymer matrix, together with the vapor pressure of the volatile substances on each side of the matrix, are the major driving forces influencing diffusion. The characteristics of the encapsulated fragrance-blend are the result of the release rates of each component. Thus, it is essential to understand the release profile of each component in order to predict the change of odorous note throughout the application.

To the best of our knowledge, biocompatible polymeric nanocarriers which can encapsulate fragrance molecules effectively without the use of crosslinking agents and also give highly stable encapsulated products with a satisfying long lasting controlled release property, are very rare, if ever reported. In this paper, we, therefore, demonstrate the fragrance encapsulation process using a blend of non-toxic, biocompatible and biodegradable polymers. Six common fragrance molecules of different chemical functionalities, camphor (ketone), citronellal (aldehyde), eucalyptol (ether), limonene (unsaturated hydrocarbon), menthol (alcohol) and 4-*tert*-butylcyclohexyl acetate (ester) (Fig. 1), were encapsulated and their release profiles were investigated.

2. Materials and methods

Hydroxypropyl methylcellulose (HPMC; Mn 90,000; D.S. methoxy 1.10–1.60; M.S. propylene oxide 0.10–0.30), poly(vinyl alcohol) (PV(OH); Mw 124,000–186,000, 87–89% deacetylated), ethylcellulose (EC; Mn 175,000; ethoxy content 48%), 4-*tert*-butylcyclohexyl acetate or Vertenex, and (1*R*)-(+)-camphor or (*R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one were purchased from Aldrich (Steinheim, Germany). (3*R*)-citronellal or (*R*)-3,7-dimethyloct-6-en-1-al, D-limonene or (*R*)-4-isopropenyl-1-methylcyclohexene (1*R*, 2*S*, 5*R*)-menthol or (1*R*, 2*S*, 5*R*)-2-(2-propyl)-5-methylcyclohexanol, and eucalyptol or 1,3,3-trimethyl-2-oxabicyclo[2,2,2]octane were purchased from Acros Organics (Geel, Belgium). Dialysis tubing cellulose membranes (MWCO) 12,400 Da, size 76 mm × 49 mm were purchased from Sigma–Aldrich (Steinheim, Germany).

^{1}H nuclear magnetic resonance (NMR) analyses were performed using a Varian Mercury spectrometer, which operated at 400.00 MHz for ^{1}H in deuterated chloroform ($CDCl_3$) with tetramethylsilane (TMS) as an internal standard (Varian Company, Palo Alto, CA, USA). Centrifugation was performed on Allegra 64R et Avanti 30 (Beckman Coulter, Inc., Fullerton, CA, USA). Thermal gravimetric analysis (TGA) was carried out on a simultaneous thermal analysis Model STA490C (NETZSCH, Selb, Germany).

2.1. *Self-assembling of the polymer-blend*

Self-assembling of polymer-blend into particles was induced by solvent displacement (water displacement of ethanol). A solution of the polymer-blend (HPMC:PV(OH):EC at a w/w/w ratio of 1:1:6) was prepared at 70 °C using 75% (v/v) aqueous ethanol to obtain the solution of the appropriate concentration. The mixture (20 mL) was then placed into a dialysis bag and dialyzed against water (5 × 1000 mL). The final volume of the obtained suspension was adjusted to 50 mL by adding water. This self-assembly of the polymer-blend into nanoparticles was carried out at initial polymer concentrations of 2000, 4000, 8000, 12,000, 16,000, 18,000, 24,000 and 28,000 ppm. The final volume of the suspension usually doubled the volume of the polymer solution used at the beginning so the final concentrations of the polymer in the obtained suspension were adjusted to 1000, 2000, 4000, 6000, 8000, 9000, 12,000 and 14,000 ppm, respectively, by adding water. The particle size distribution and zeta potential of the obtained suspensions were acquired by dynamic light scattering (DLS) using a Mastersizer S and Zetasizer nanoseries (Mulvern Instruments, Worcestershire, UK) equipped with a He–Ne laser beam at 632.8 nm (scattering angle of 173°). Each measurement was carried out in triplicate and an average value is reported.

Morphology and approximate sizes were characterized using scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM). The photographs of SEM were obtained using JSM-6400 (JEOL, Ltd., Japan). A drop of the nanoparticle suspension was placed on a glass slide and dried overnight. The sample was coated with a gold layer under vacuum at 15 mA for 90 s. The coated sample was then mounted on an SEM stud for visualization. The accelerating voltage used was 15 kV. TEM photographs were obtained using JEM-2100 (JEOL, Ltd., Japan) with an accelerating voltage of 120 kV in conjunction with selected area electron diffraction (SAED). The AFM photographs were acquired with Nano Scope IV (Veeco Metrology Group, CA, USA). A glass slide was dipped into the obtained suspension to gain the dried smooth film of nanoparticles on surface of the glass slide.

2.2. *Fragrance encapsulation*

2.2.1. *Particles with various fragrance contents*

Camphor was encapsulated into the polymer-blend at camphor to polymer weight ratios of 1:1 and 2:1. For each composition, 80 mg of the polymer-blend were dissolved in 20 mL of 75% (v/v) aqueous ethanol at 70 °C. Then the desired amount of camphor (80 and 160 mg) was added to the cool solution and the mixture was placed into a dialysis bag and dialyzed against distilled water (5 × 500 mL) in a tightly closed container with no void volume between the dialysate and the lid. The final volume of the resulting colloidal suspension (suspension in the dialysis bag) was adjusted to 40 mL with water. Ten milliliters of the obtained suspension were filtering centrifuged (centrifugal-filtering devices with MWCO 100000, Amicon Ultra-15, Millipore, Ireland) and the obtained solid was dissolved in 5 mL ethanol. The ethanol solution was quantified for camphor using headspace analysis in combination with gas chromatography/mass spectroscopic detector (headspace-GC/MS) with the aid of a calibration standard curve. Calibration standards were freshly prepared in ethanol with the final volume of 5 mL. The encapsulation efficiency percentage (% EE), loading capacity (% loading) and yield of the process (% yield) were each determined as follows:

$$\%\ \mathrm{EE} = \left[\frac{\text{weight of encapsulated fragrance}}{\text{weight of fragrance used initially}}\right] \times 100 \tag{1}$$

$$\%\,\mathrm{loading} = \left[\frac{\text{weight of encapsulated fragrance}}{\text{weight of fragrance} - \text{encapsulated particles}}\right] \times 100 \tag{2}$$

$$\%\,\mathrm{yield} = \left[\frac{\text{weight of fragrance} - \text{encapsulated particles}}{\text{weight of polymer and fragrance used initially}}\right] \times 100 \tag{3}$$

Similar processes were repeated for the other five fragrances which included citronellal, eucalyptol, limonene, menthol and 4-*tert*-butylcyclohexyl acetate. The aqueous suspensions of fragrance-encapsulated nanoparticles so obtained were subjected to a morphological analysis by SEM, TEM, AFM and DLS.

Part of the fresh aqueous suspension was centrifuged at 45,000 × *g* for 30 min and the obtained white solid was air-dried at room temperature (overnight). The freshly dried particles were dissolved in deuterated chloroform and subjected to ^{1}H NMR analysis in order to confirm the presence of the encapsulated fragrance. The freshly dry samples were subjected to a controlled release study using the e-nose and TGA.

2.2.2. Headspace-gas chromatography in conjunction with mass spectroscopic detector (headspace-GC/MS)

Headspace-GC/MS analysis was carried out on the Agilent 6890N/5975i GC/MS equipped with a headspace oven of Agilent 7694 (Agilent Technologies, Wilmington, DE, USA). The headspace sample (ethanol solutions of the centrifuged fragrance-encapsulated spheres and the calibration standards) was loaded in a 20 mL capped flat bottom headspace vial with headspace aluminum crimp caps with PTFE/silicone septa (Agilent technologies, USA), and heated in a headspace oven at 85 °C for 20 min before the headspace gas was automatically transferred into the GC system. The transfer line was held at 110 °C for 0.4 min, before being flushed into a GC system for 2 min. A Restex 10624 Stabilwax column (30 m × 0.32 mm ID × 0.25 μm) (Restex Corp., Pennsylvania, USA) was used. The carrier gas was helium under a constant flow rate of 1 mL/min. The initial GC oven temperature was 40 °C which was held for 3 min and was then ramped by 6 °C/min to 220 °C and held for 2 min. The inlet temperature was 260 °C. The mass spectrometer was operated in an electron impact (EI) mode, using the total ion scan mode with scanning from mass 50 to 500 Da. One mL of the headspace gas from each sample was injected with the auto-sampler into the above GC/MS.

2.2.3. Citronellal-encapsulated particles prepared at various concentrations

The appropriate amount of polymer-blend (80, 120, 160, 320 and 640 mg) was dissolved in 20 mL of 75% (v/v) aqueous ethanol. The mixture was then stirred and heated at 70 °C until a clear solution was obtained and citronellal (same amount to the polymer-blend) was added. The mixture was then placed into a dialysis bag and dialyzed against water. The final volume of the suspension was adjusted to 40 mL with water. The % EE, % loading and % yield were determined as described above. The obtained citronellal-encapsulated particles were subjected to morphological analysis by SEM and DLS.

2.3. Determination of the release profiles

The release profiles of the centrifuged-air-dried particles were acquired by quantitating the amount of fragrances remained in samples which had been left uncovered for specified times. Quantification was carried out using the electronic nose and thermal gravimetric analysis.

2.3.1. Electronic nose (e-nose)

Electronic nose analysis was carried out using a home-made instrument (Fig. 2) consisting an array of eight commercial gas sensors which respond to various kinds of volatile organic compounds such as alcohol, aldehyde, ketone acid and hydrocarbon. This range of chemical sensitivity should be sufficient to detect most volatile components in aroma oil. Seven 50 mg-samples were left in open containers (20 mL flat bottom headspace vial, O.D. × H. 20 mm × 70 mm) at 30 °C. At the specified collection time, each vial was capped and subjected to e-nose analysis. Release profile was compared to the corresponding unencapsulated fragrance sample.

Unencapsulated camphor sample was prepared by adding 20.5 mg of camphor into a vial and the polymer-blend (in this case 29.5 mg) was added to make the total weight of 50 mg. The sample was thoroughly mixed. The other five unencapsulated fragrance samples were prepared similarly but the amounts of fragrance added into the vials were 19.9, 22.1, 8.0, 21.0 and 21.0 mg for citronellal, eucalyptol, limonene, menthol and 4-*tert*-butylcyclohexyl acetate samples, respectively. Amounts of polymer-blend added were adjusted accordingly, as to get the final weight of 50 mg. Seven vials were prepared for each fragrance and they were left opened at 30 °C. At the specified collection time, each vial was capped and subjected to e-nose analysis.

Analysis was carried out by putting the sample vial into the chamber for analysis, with a blank vial (containing only polymer-blend) in the reference chamber. It is necessary that during the measurement, switching between a reference and a sample line must be performed. Four electrically controlled solenoid valves were used for this system (Fig. 2). When a pair of two solenoid valves is open, another pair will be automatically closed. This guarantees there is no mixing of the gas from the reference and sample

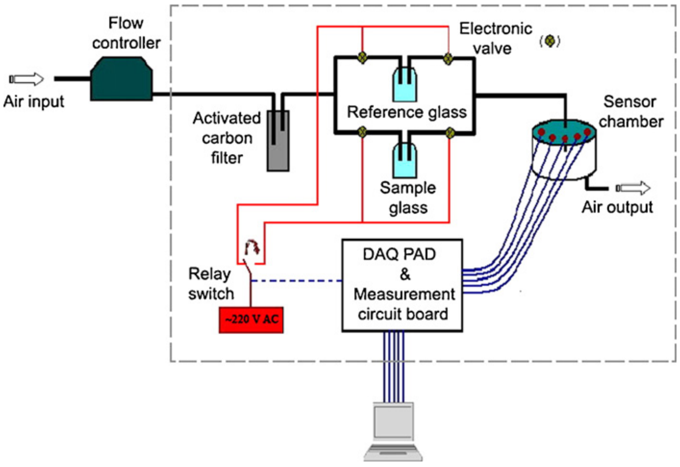

Fig. 2. Diagram showing the working principle of the e-nose system.

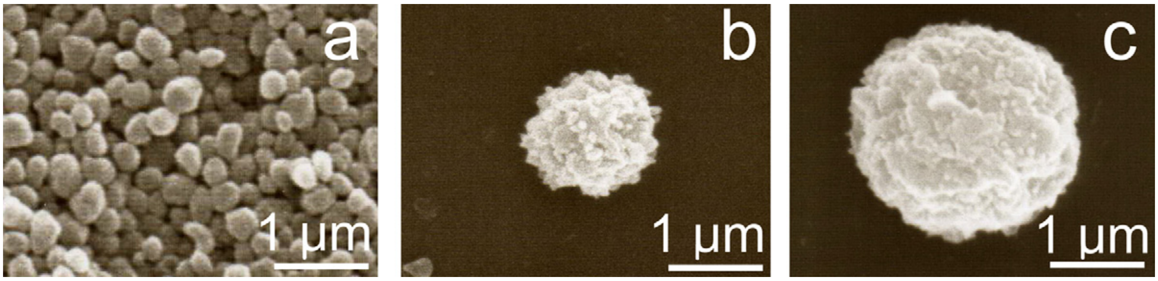

Fig. 3. Representative SEM and TEM images of spheres obtained with the starting polymer concentrations of (a) 2000–16,000 ppm, (b) 18,000 ppm and (c) 24,000–28,000 ppm. The figures are representative of products from at least three independent preparations.

lines. Then, the gas from the reference or sample flows to the sensor chamber. In the experiment, the reference lines were measured for 5 min in the first stage. Then, the gas from headspace of the sample vial was presented into the sensor chamber for 1 min. In each experiment, the measurement of reference and sample was iterated at least five times. The data acquisition was carried out on an USB-DAQ-Card National Instruments NI USB-6008 and the measurement program was written using LabVIEW version 8.0.29. The $\Delta R/R0$ values of the sensors were recorded for each combination of above variations. The sensors response (sensitivity of sensors) was plotted by % ($R/R0$) with time.

2.3.2. Thermal gravimetric analysis (TGA)

TGA was used to determine the weight loss related to the release of fragrance from the sample. The centrifuged and air-dried samples (or unencapsulated fragrance samples prepared similarly to those used in the e-nose experiment) were kept in open containers at 30 °C and a specific amount (30 mg) was taken to TGA analysis at appropriate times. For particles encapsulated with citronellal, limonene and 4-tert-butylcyclohexyl acetate, the thermal programs were started with heating from room temperature to 100 °C at a constant heating rate of 50 °C/min and the temperature was held at 100 °C for 30 min in order to evaporate the water molecules out from the samples. Then, the temperature was increased at a heating rate of 5 °C/min from 100 °C to 200 °C and held at 200 °C for 40 min before being ramped at the same heating rate to 300 °C. The thermal programs for particles encapsulated with camphor, eucalyptol menthol and fresh powder, were started by ramping from ambient temperature to 100 °C at heating rate of 50 °C/min and held for 15 min as above, and then increasing from 100 °C to 150 °C at a heating rate of 5 °C/min and held for 20 min before being increased from 150 °C to 300 °C at a heating rate of 10 °C/min.

3. Results and discussion

3.1. Self-assembly of the polymer-blend

When ethanol in the polymer-blend solution was displaced by water, the formation of milky white aqueous particle suspension was observed. SEM analyses of the dry particles and the aqueous suspension revealed similar results of spherical particles in which their sizes varied with the initial concentration of the polymer solution used. At starting polymer concentrations of 2000–16,000 ppm, smooth surface-spherical particles with diameters less than 400 nm were obtained (Fig. 3a), while at starting polymer concentrations of 18,000 ppm, jagged spherical particles with diameters ∼1 μm were observed (Fig. 3b). Increasing the initial polymer concentration to 24,000–28,000 ppm resulted in ∼2 μm particles being created (Fig. 3c). Higher still polymer concentrations (32,000 ppm) resulted in the significant formation of precipitates.

Amongst the three polymers used in the blend (EC, HPMC and PV(OH)), EC is the only polymer that is insoluble in water, thus upon slow displacement of ethanol by water, EC chains self-assembled into spheres with probably most of the hydrophobic ethoxyl moieties oriented away from the surrounding water medium and with most of the hydroxyl groups of the sugar units oriented outwards to have a maximum interaction with water molecules. During such self-assembling process, some PV(OH) and HPMC chains could be trapped along with the EC molecules through entanglements, thus making the spherical walls being composed of the polymer-blend. Some of the PV(OH) and HPMC chains must also be left in the aqueous medium because of their good water solubility. In this stage they act as stabilizers for the polymer-blend-spheres. Upon drying, these two polymers also cover up the surface of the spheres, making them readily dispersible in water.

All the obtained particles showed no morphological change after either heat treatment at 100 °C for 30 min or strong mechanical agitation by stirring at 2400 rpm, indicating both the relative thermal and the mechanical stabilities of the polymer-blend-particles. Although some settling of the microspheres in suspensions prepared from initial polymer concentrations of ≥20,000 ppm was observed, they could easily be redispersed by agitation.

Fine-tuning of the particles' size could be achieved through adjusting the starting polymer concentration (Table 1). All particles of sizes 250–360 nm showed a similar zeta potential of approximately −30 mV, indicating acceptable stability. Thus, it was concluded that a likely stable aqueous suspensions of the polymer-

Table 1
Particle sizes and zeta potential of the nanoparticles prepared at different initial polymer concentrations.

Starting polymer concentration (ppm)	Hydrodynamic diameter of the empty particles (nm)	Zeta potential of the empty particles (mV)
2000	253.0 ± 1.3 nm (PDI = 0.08)	−30.11
4000	267.7 ± 2.8 nm (PDI = 0.14)	−33.24
6000	362.9 ± 3.7 nm (PDI = 0.20)	−26.32
8000	357.9 ± 0.7 nm (PDI = 1.48)	−32.87
12,000	363 ± 4.3 nm (PDI = 1.68)	−26.53
16,000	358 ± 3.9 nm (PDI = 1.83)	−27.19

Data are shown as the mean + S.D. and are derived from 3 repeats.

Table 2
The encapsulation efficiency (at a fragrance:polymer weight ratio of 1:1) and the properties of the encapsulated fragrances prepared from a starting polymer concentration of 4000 ppm.

Fragrance	Vapor pressure of the fragrance (mm Hg at 25 °C)	Boiling point of the fragrance (°C)	% EE (suspension)	% Loading (suspension)	% Loading (after centrifuged and air-dried)	Diameter of the fragrance-encapsulated spheres (nm)
Camphor	0.65	204	85.7 ± 0.8	46.2 ± 0.2	41.1 ± 4.3	287 ± 3.1
Citronellal	0.28	203	92.3 ± 1.6	48.0 ± 0.4	39.7 ± 4.9	278 ± 3.6
Eucalyptol	1.65	177	84.0 ± 2.1	45.6 ± 0.6	44.3 ± 3.2	295 ± 2.6
Limonene	1.98	176	79.7 ± 3.4	44.4 ± 1.1	16.2 ± 5.2	280 ± 2.9
Menthol	0.0323	212	85.2 ± 1.1	46.0 ± 0.3	41.8 ± 4.4	279 ± 3.2
4-*tert*-butylcyclohexyl acetate	0.0498	229	84.2 ± 1.5	45.7 ± 0.4	42.3 ± 4.7	298 ± 3.1

blend-particles could be prepared up to a maximum concentration of 8000 ppm (starting polymer concentration of 16,000 ppm).

3.2. Encapsulation

Six fragrances were carefully selected for encapsulation so as to encompass molecules of different chemical functionalities. They consisted of camphor (ketone), citronellal (aldehyde), eucalyptol (ether), menthol (alcohol), limonene (unsaturated hydrocarbon) and 4-*tert*-butylcyclohexyl acetate (ester).

First, the encapsulation was carried out at a starting polymer concentration of 4000 ppm using two weight ratios between fragrance and polymer, i.e., 1:1 and 2:1. It should be mentioned here that a special closed container was used during dialysis in which neither air nor void volume was allowed in the system in order to avoid partition of fragrance molecules into the void space. It was found that dialysis in this air-free condition gave a suspension with no precipitation, while in contrast a significant amount of precipitate was found (especially at high polymer concentrations) when there was an air-contact to the surface of the dialysis bag (data not shown). We speculate that the air-contact interfered with the self-assembly through solvent evaporation and favoring precipitate formation at the air-interface. Encapsulation efficiency and loading capacity were then obtained through analysis of the obtained particle suspensions. Encapsulation efficiency was ≥80% at 1:1 fragrance to polymer ratio (Table 2). However, the 2:1 fragrance to polymer ratio resulted in observable non-dispersible floating fragranced gum which complicated the determination of loading capacity and encapsulation efficiency. Thus, the maximum fragrance to polymer ratio was limited to 1:1 in all further studies. Satisfyingly, all six fragrances could be loaded into the nanospheres at a fragrance:polymer ratio up to 1:1 and the obtained spheres possessed fragrance loading of ≥44%.

SEM images indicated a similar spherical shape amongst the six fragrance-encapsulated polymeric spheres (see an example of menthol-encapsulated spheres in Fig. 4a). TEM pictures showed particles with obvious core (Fig. 4b), while the AFM photographs of the encapsulated nanoparticles indicated soft and not rigid polymeric spheres (Fig. 4c).

When ethanol was displaced by water, self-assembling of the water-insoluble EC, likely with some entanglements with HPMC and PV(OH) occurred as described previously. With the presence of fragrance molecules, self-assembly occurred together with the entrapment of the water-insoluble fragrance molecules. The stability of the fragrance-loaded spheres is then a result of the hydrophobic interactions between the fragrance molecules and the ethoxy moieties of the EC chains. The amount of HPMC and PV(OH) was probably more prominent towards the rim of the sphere. All the six fragrances could be entrapped well into the spheres, indicating a wide window of encapsulation capability of the polymer-blend-spheres.

Confirmation of the successful encapsulation was carried out by sonicating the dry fragrance-encapsulated spheres in $CDCl_3$ (5 min at 40 kHz, 25 °C) and then subjected the resulting solution to ^{1}H NMR analysis. Resonance of protons from fragrance molecules could be observed clearly for all encapsulated products (data not shown), confirming the presence of fragrances in the spheres. In addition, no degradation was observed for all six fragrances.

To investigate if fragrance-encapsulated spheres could be prepared at other concentrations, encapsulation at a citronellal:polymer weight ratio of 1:1 was evaluated using starting polymer concentrations of 2000, 4000, 6000, 8000, 12,000, 16,000 and 32,000 ppm. SEM and TEM images of the products indicated that citronellal-encapsulated nanospheres (diameter between 200 and 450 nm) were formed at starting polymer concentrations of ≤16,000 ppm while at 32,000 ppm citronellal-encapsulated microparticles (diameter around 1–2 μm) were obtained together with some non-dispersible polymeric masses mixed with citronellal oil (gum-like material). Quantitative analysis of the encapsulated products indicated 88 ± 1.8, 85 ± 1.6, 84 ± 2.2, 86 ± 2.1, 83 ± 2.0 and 80 ± 2.8% EE for particles prepared at the starting polymer concentrations of 2000, 4000, 6000, 8000, 12,000 and 16,000 ppm, respectively. However, at the starting polymer concentration of 32,000 ppm, only 51 ± 5.6% EE was obtained. The

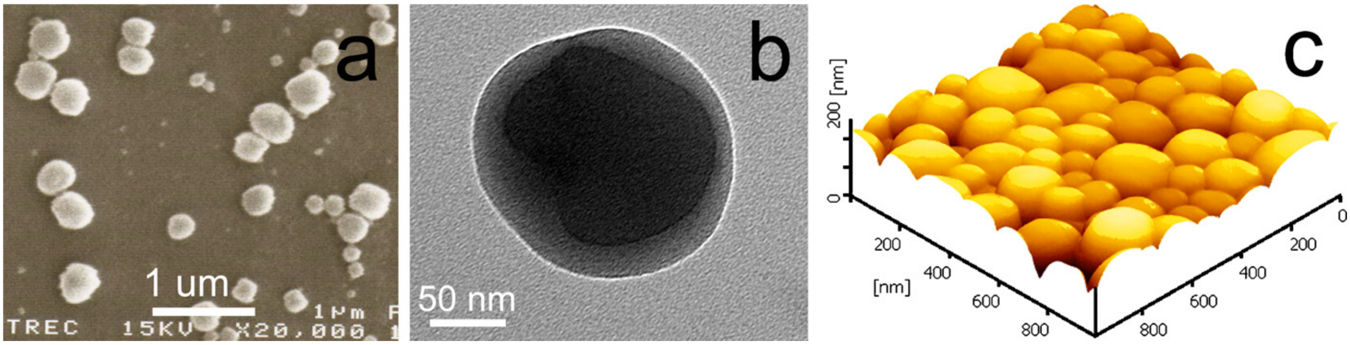

Fig. 4. Representative (a) SEM, (b) TEM and (c) AFM images of menthol-encapsulated polymeric nanoparticle suspension prepared at the starting polymer concentration of 4000 ppm and with a 1:1 menthol to polymer weight ratio. Images are representative of 5 independent samples.

low encapsulation efficiency at high polymer concentration agreed well with a significant formation of a non-dispersible polymeric mass observed.

All the above results clearly indicated that the polymer-blend of HPMC:PV(OH):EC at a (w/w/w) ratio of 1:1:6 could effectively nanoencapsulate all six fragrances with different chemical functionalities through solvent displacement of the ethanol by water and gave fragrance-encapsulated nanospheres with excellent water dispersibility. The process could be carried out at initial polymer concentrations of up to 16,000 ppm (with a similar concentration of fragrance) to give a % EE of ≥80%.

3.3. *Release behavior*

In this work dry fragrance-encapsulated particles was obtained through centrifugation and air-drying. Fragrance lost during the drying process was noticed. Ethanol dissolution of the dry fragrance-encapsulated particles was carried out and the fragrance contents were determined using headspace-GC–MS analysis. Results indicated that the lost varied with drying time (data not shown). Also different fragrance gave different lost. Although the six suspensions (camphor, citronellal, eucalyptol, limonene, menthol and 4-*tert*-butylcyclohexyl acetate) initially contained comparable fragrance loading (Table 2), the dry particles contained different fragrance loading, i.e., 41 ± 4.3, 40 ± 4.9, 44 ± 3.2, 16 ± 5.2, 42 ± 4.4 and 42 ± 4.7% loading for camphor, citronellal,

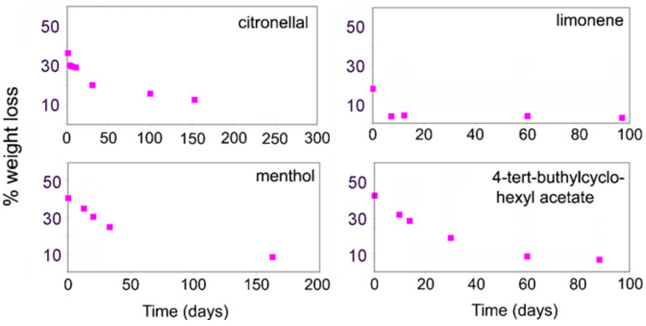

Fig. 5. The release of various fragrances from the dry polymeric nanospheres (prepared at an initial polymer concentration of 4000 ppm and a fragrance:polymer weight ratio of 1:1) as analyzed by TGA. Each point is the average value of two data points that are within 10% of each other. Initial fragrance loading of the dry samples was 40 ± 4.9, 16 ± 5.2, 42 ± 4.4 and 42 ± 4.7% for citronellal, limonene, menthol and 4-*tert*-butylcyclohexyl acetate-encapsulated particles, respectively.

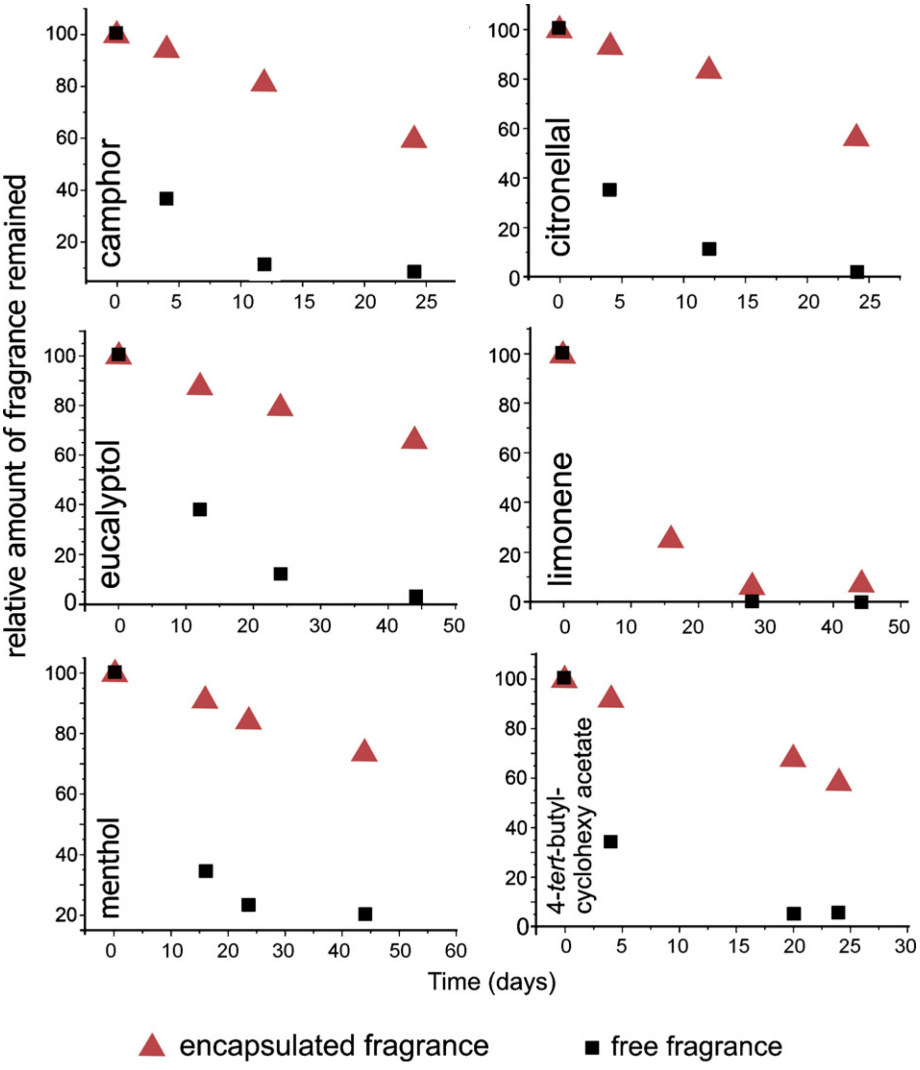

Fig. 6. The release profiles of the fragrance-encapsulated particles (prepared as a nanoparticle suspension with an initial polymer concentration of 4000 ppm and a fragrance:polymer weight ratio of 1:1 and subjected to centrifugation and air-drying), compared to the unencapsulated fragrances. The profiles are shown as amount of fragrance remained in the sample relative to amount of fragrance found in the sample on day 0 of the same sample. Initial fragrance loading of the dry samples was 41 ± 4.3, 40 ± 4.9, 44 ± 3.2, 16 ± 5.2, 42 ± 4.4 and 42 ± 4.7% for camphor, citronellal, eucalyptol, limonene, menthol and 4-*tert*-butylcyclohexyl acetate-encapsulated particles, respectively. Data are present as average values of two experimental data points which are within 15% of each other.

eucalyptol, limonene, menthol and 4-*tert*-butylcyclohexyl acetate-encapsulated particles, respectively.

For the centrifuged-air-dried samples, the TGA analysis, based upon the weight loss from the solid sample, where the reduction of the amount of fragrances remaining in the samples corresponds to the fragrance release behavior of the sample (Fig. 5), revealed that three (citronellal, menthol and 4-tert-buthylcyclo-hexyl acetate) of the four tested fragrances showed delayed release kinetics, but that limonene did not. Since the TGA analysis relies on getting the dry sample weight (initial weight after water evaporation), samples in which the fragrance significantly co-volatilizes out with water during the preheating period, such as camphor- and eucalyptol-encapsulated spheres, cannot be analyzed by this technique. However, the e-nose can be used to monitor the release of all six encapsulated products (Fig. 6). For the four common fragrances analyzed, the TGA and e-nose results agreed well and indicated that limonene-encapsulated particles gave the most significant burst at the beginning and only a very small percentage of limonene remained to be released at later times. Therefore, it was concluded that encapsulation of limonene into the polymer-blend-particles could not effectively help in prolonging the release of this fragrance. However, release behaviors of all other five fragrances could be prolonged by this polymeric encapsulation but to different extents. It should be pointed out here that each release profile of the unencapsulated fragrance shown in Fig. 5 was obtained from the mixture of fragrance and polymer-blend, not from the pure fragrance (see details in Section 2). Interactions between fragrance molecules and polymer matrix could delay the volatilization of the fragrance. It was noticed that after being encapsulated, the vapor pressure and boiling point of the fragrance were no longer the major factors determining the release rate. Rather the release characteristics are likely to be governed by the ability of the fragrance to diffuse through the polymer matrix, and different fragrance molecules possess different chemical interactions with the polymer-blend-shell, and thus diffusion rates.

4. Conclusion

We have successfully demonstrated that a blend of HPMC, PV(OH) and EC can be used to encapsulate five (out of six) diverse fragrances with different functional groups and act as fragrance reservoirs for a more sustained controlled release. Through solvent displacement (in this case, water displacing ethanol), fragrance encapsulation with a $\geq$80% EE was obtained at a fragrance:polymer ratio of 1:1 to give an aqueous suspension of the fragrance-encapsulated nanospheres (diameter of less than 450 nm) at a maximum concentration of 16,000 ppm or 1.6% (w/v) suspension. The process was applicable to all six tested fragrances, representative of compounds with different chemical functionalities, but one, limonene (an unsaturated hydrocarbon), showed no significant retention in the nanoparticles. Smaller particles could be prepared at lower polymer concentrations, while preparation at higher polymer concentrations (32,000 ppm) gave microparticles (diameter > 1000 nm), but at the encapsulation efficiency of $\sim$51%. Different fragrance molecules showed different release characteristic from the polymeric spheres, i.e., limonene showed the fastest release while eucalyptol and menthol showed the slowest release, and the release rate of the encapsulated fragrance was independent of the fragrance's vapor pressure.

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References

Bachtsi, A.R., Kiparissides, C., 1996. Synthesis and release studies of oil-containing poly(vinyl alcohol) microcapsules prepared by coacervation. J. Control. Release 38, 49–58.

Chang, C.P., Dobashi, T., 2003. Preparation of alginate complex capsules containing eucalyptus essential oil and its controlled release. Colloids Surf. B: Biointerfaces 32, 257–262.

Edris, A., Bergnståhl, B., 2001. Encapsulation of orange oil in a spray dried double emulsion. Nahrung 45, 133–137.

Fukumoto, S., Sawasaki, E., Okuyama, S., Miyake, Y., Yokogoshi, H., 2006. Flavor components of monoterpenes in citrus essential oils enhance the release of monoamines from rat brain slices. Nutr. Neurosci. 9, 73–80.

Hwang, J.S., Kim, J.N., Wee, Y.J., Yun, J.S., Jang, H.G., Kim, S.H., Ryu, H.W., 2006a. Preparation and characterization of melamine–formaldehyde resin microcapsules containing fragrant oil. Biotechnol. Bioprocess Eng. 11, 332–336.

Hwang, J.S., Kim, J.N., Wee, Y.J., Jang, H.G., Kim, S.H., Ryu, H.W., 2006b. Factors affecting the characteristics of melamine resin microcapsules containing fragrant oils. Biotechnol. Bioprocess Eng. 11, 391–395.

Jean-Pierre, B., Joel, R., Curtis, T., 1998. Method for preparing microcapsules of active substances coated with a polymer and novel microcapsules in particular resulting from the method. WO 98/13136 A1.

Karlberg, A.T., Magnusson, K., Nilsson, U., 1992. Air oxidation of D-limonene (the citrus solvent) creates potent allergens. Contact Dermatitis 26, 332–340.

Lai, F., Wissing, S.A., Müller, R.H., Fadda, A.M., 2006. Artemisia arborescens L essential oil-loaded solid lipid nanoparticles for potential agricultural application: preparation and characterization. AAPS PharmSciTech 7 (1), article no. 2.

Markus, A., Linder, C., 2004. Encapsulated essential oils. WO 2004/098767 A1.

Matura, M., Sköld, M., Börje, A., Andersen, K.E., Bruze, M., Frosch, P., Goossens, A., Johansen, J.D., Svedman, C., White, I.R., Karlberg, A.T., 2005. Selected oxidized fragrance terpenes are common contact allergens. Contact Dermatitis 52, 320–328.

Matura, M., Sköld, M., Börje, A., Andersen, K.E., Bruze, M., Frosch, P., Goossens, A., Johansen, J.D., Svedman, C., White, I.R., Karlberg, A.T., 2006. Not only oxidized R-(+)-but also S-(−)-limonene is a common cause of contact allergy in dermatitis patients in Europe. Contact Dermatitis 55, 274–279.

Moretti, M.D., Sanna-Passino, G., Demontis, S., Bazzoni, E., 2002. Essential oil formulations useful as a new tool for insect pest control. AAPS PharmSciTech 3 (2), article no. 13.

Morita, T., Jinno, K., Kawagishi, H., Arimoto, Y., Suganuma, H., Inakuma, T., Sugiyama, K., 2003. Hepatoprotective effect of myristicin from nutmeg (Myristica fragrans) on lipopolysaccharide/D-galactosamine-induced liver injury. J. Agric. Food Chem. 51, 1560–1565.

Ouall, L., Lahoussine, D., 2006. Process for production nano-capsules containing a fragrance. WO 2006/027664 A2.

Rodrigues, S.N., Fernandes, I., Martins, I.M., Mata, V.G., Barreiro, F., Rodrigues, A.E., 2008. Microencapsulation of limonene for textile application. Ind. Eng. Chem. Res. 47, 4142–4147.

Scarfato, P., Avallone, E., Iannelli, P., De Feo, V., Aderno, D., 2007. Synthesis and characterization of polyurea microcapsules containing essential oils with antigerminative activity. J. Appl. Polym. Sci. 105, 3568–3577.

Soper, J.C., Kim, Y.D., Thomas, M.T., 2000. Method of encapsulating flavours and fragances by controlled water transport into microcapsules. US Patent 6,106,875.

Wang, C.X., Chen, S.L., 2005. Fragrance-release property of β-cyclodextrin inclusion compounds and their application in aromatherapy. J. Ind. Text. 34, 157–166.

Wang, P., Zhu, Y., Yang, X., Chen, A., 2008. Prolonged-release performance of perfume encapsulated by tailoring mesoporous silica spheres. Flavour Frag. J. 23, 29–34.