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Chitosan beads loaded with essential oils in cosmetic formulations

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Synopsis

The aim of this work is to evaluate the stability and release of chitosan beads loaded with volatile molecules of *Mentha piperita* essential oil (E.O.) in a cosmetic formulation. The ability of the beads to quickly release *Mentha piperita* E.O. during use of a cosmetic formulation such as a bath foam is also assessed. The chitosan beads were produced with three different chitosan dispersions gelled with two different gelling solutions: (a) a 10% solution of sodium hydroxide (NaOH) and (b) a 4% solution of sodium tripolyphosphate (TPP). A few properties of six bead samples loaded with *Mentha piperita* E.O. are assessed. The properties are morphology, size, swelling ability, encapsulation efficiency, stability in time, and fast release of *Mentha piperita* E.O. during the use phase of the cosmetic formulation.

INTRODUCTION

Essential oils are the highly concentrated, volatile, aromatic products of natural plants that accumulate in specialized structures such as oil cells, glandular trichomes, and oil or resin ducts. In some cases they seem to be a part of the plant's immune system. In others they may simply be the end products of metabolism (1). Essential oils, therefore, contain the true essence (or "soul" or life force) of the plant from which they derive, and are found in a few of the plant's elements, such as the flowers, leaves, stems, roots, or other parts, depending on the plant (2).

Chemically speaking, essential oils contain hundreds of organic constituents, including hormones, vitamins, and other natural elements that work at many different levels. In aromatic plants, the essential oils are mostly volatile compounds that give them their distinctive aroma (3).

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The chemical composition and aroma of essential oils can provide valuable psychological and physical-therapeutic benefits. These are usually obtained by methods that include inhalation or the application of the diluted essential oil to the skin. As they are often very concentrated, essential oils should never be used undiluted on the skin, and they are often diluted with carrier oils (such as sweet almond oil, apricot kernel oil, olive oil, etc). On applying these blends by rubbing, the tiny molecules of the essential oils are absorbed through the skin. They then dissolve in the body's natural fats and fluids that flow through the lymphatic and blood system, causing it to relax, stimulate, detoxify, and regenerate (4).

Careful inhalation of essential oils can also provide therapeutic benefits; the aromas are perceived by the olfactory system, which passes on signals to the limbic system in the brain. The brain then responds to a particular scent by affecting our emotions and our chemical balance. Moreover, essential oil molecules enter the lungs and are absorbed into the bloodstream (5). It is for these reasons that essential oils are used in aromatherapy, cosmeticology, pharmaceutics, and in many other applications.

In these work we have chosen *Mentha piperita* essential oil (*Mentha piperita* E.O.), which is considered one of the oldest and most highly considered herbs for its remarkable properties. *Mentha piperita* E.O. has a considerable number of therapeutic properties: it is an analgesic, an antiseptic, an anti-spasmodic, an expectorant, a stimulant, and a vasoconstrictor, besides possessing other properties (6,7). It can be very useful against dermatitis, acne, and scabies, and can relieve itching, sunburn, and inflammations of the skin. In addition to these properties, *Mentha piperita* E.O. is also widely used in cosmeticology for its cooling effect and because it helps to ease tension and stress. For these reasons, *Mentha piperita* E.O. is frequently used in cosmetic cleansing formulations such as soaps, foam baths, dentifrices, mouthwashes, and foot creams.

The volatile constituents of all essential oils and therefore also of *Mentha piperita* E.O. are a complex mixture of organic compounds, some of which are less stable, and may therefore undergo chemical alteration in the presence of light or under high temperatures. The aim of this study was to evaluate the ability of particle polymeric systems, such as chitosan beads, to load, protect, and keep for an adequate storage time the volatile molecules of *Mentha piperita* E.O. Additionally, when beads are formulated into a cosmetic formulation (such as a bath foam), the rate of release during conditions of use was determined.

MATERIALS AND METHODS

MATERIALS

High-molecular-weight [2.000.000 (hC)] >75% deacetylation chitosan and medium-molecular-weight [750.000 (mC)] 75–85% deacetylation chitosan, sodium tripolyphosphate (TPP), and sodium hydroxide (NaOH) were purchased from Aldrich (Milan, Italy). Glycolic acid, *Mentha piperita* E.O., and base bath foam (BBF composition: water, sodium lauryl sulfate, cocamidopropyl betaine, cocamide DEA, sodium chloride, and bromo-2-nitropane-1,3-diol) were supplied by Galeno (Prato, Italy). Distilled water was further purified with a Milli-Q® System (Millipore, Bedford, MA). All solvents used were analytical grade.

PREPARATION OF CHITOSAN BEADS LOADED WITH *MENTHA PIPERITA* E.O.

Three different chitosan-based polymeric dispersions were prepared for the beads using glycolic acid as an anionic system, as described in a previous study (8). Briefly, a preweighed amount (1% w/w) of different types of chitosan (hC, mC) or a mixture of chitosans (hC/mC 1:1 w/w) were slowly dissolved in a water solution containing glycolic acid (1% w/w) under magnetic stirring for two hours. *Mentha piperita* E.O. (1%) was then added to the dispersions while stirring gently.

The corresponding beads were prepared using two different gelling solutions:

(a) by dropping the bubble-free polymeric dispersion (~5 g) through a disposable syringe onto a polyanionic solution of 5% TPP (~50 ml) as an ionically crosslinking agent (TPP-bead batches A_1, B_1, and C_1).

(b) by dropping the bubble-free polymeric dispersion (~5 g) through a disposable syringe onto an alkaline solution of 10% NaOH (~50 ml) as a coacerving agent (NaOH-bead batches A, B, and C).

The formation time of the beads may change, depending on the different type of chitosan dispersion (molecular weight, degree of deacetylation, apparent viscosity, etc.) and the different gelling solutions (a or b) used. Generally, formation time may vary from 30 minutes to 60 minutes when the system is kept under gentle magnetic stirring with a speed of not more than 50 rpm. After this time, the gelled beads are separated, rinsed with distilled water, and then air-dried in a dryer to a constant weight.

OPTICAL MICROSCOPE ANALYSIS

A Carl Zeiss Axiostar Plus (transmitted-light) optical microscope with three magnifications (5×, 10×, 40×), equipped with a Sony DSC-575/585 camera connected to a computer program to process images, was used to evaluate the size and morphology of the dry beads. The size was assessed using the 5× magnification on an average of about twenty dried beads. The size of the fresh and swelled beads was assessed with reference to a standard measure (10 mm).

UV SPECTROPHOTOMETER ANALYSIS

The absorbance of *Mentha piperita* E.O. was measured at a wavelength of 273 nm with a Hitachi U-2000 UV spectrophotometer. A calibration curve was developed using methanol solutions of *Mentha piperita* E.O. at concentrations ranging from 1.70 to 11 μg/ml).

EVALUATION OF THE ENCAPSULATION OF *MENTHA PIPERITA* E.O.

A preweighed amount of each batch of beads was suspended in methanol (10 ml). The beads were next subjected to vigorous mechanical shaking with a vortex mixer for one minute and subsequently sonicated for one hour with a Branson 1200 ultrasound bath. The filter solutions were analyzed at a wavelength of 273 nm. All loaded determinations were run in triplicate and the mean values were reported.

SWELLING RATIO MEASUREMENTS

The swelling ratio or water uptake was determined gravimetrically. The weight of the completely dried bead samples was measured directly (W_0). The beads were then introduced in bottles containing 50 ml of the swelling medium (0.9% NaCl) and stirred at 50 rpm at 37°C. At predetermined times (30, 60, and 120 min), the beads were removed from the medium, blotted to remove excess water, and immediately weighed. This procedure was repeated until the beads reached a constant weight (W_t) (equilibrium water uptake).

The swelling ratio of the bead samples was calculated according to the following equation:

$$\text{Swelling ratio} = W_t/W_0$$

where W_0 and W_t are the weights of the dry and swollen beads, respectively, measured at time t with constant weight. All determinations were run in triplicate and the obtained mean values were reported.

BEAD STABILITY IN BBF

A predetermined amount of each bead sample (~10) was introduced in bottles containing 50 ml of BBF. The amount of unchanged beads was visually assessed at predetermined times (five days for the first month and then every month) up to six months at room temperature.

A temperature stability assay (9) was performed: A predetermined amount of each bead sample (~10), in 50 ml of BBF, was treated in freeze-thaw cycle cabinets (−10°C to +42°C, two cycles every 24 hours) for two weeks. Daily checks were performed to visually assess the amount of unchanged beads. All determinations were run in triplicate.

BEAD BREAKAGE ASSAY IN BBF

A predetermined amount of each bead sample (~10) was introduced in bottles containing 20 ml of BBF and 5 ml of distilled water. The next step was to submit this blend to a five-second vigorous mechanical shaking in a vortex mixer three times. Each time the number of the broken beads were assessed. The percent of broken beads was defined as:

$$\frac{\text{Number of broken beads}}{\text{Given number of beads}} \times 100$$

All determinations were run in triplicate and the mean values were reported.

RESULTS AND DISCUSSION

In this work six chitosan bead samples with different molecular weights were produced. Thanks to the presence of cationic and free hydroxide groups (10) in chitosan, it is possible to obtain polymeric systems capable of loading different types of molecules of pharmaceutical and cosmetic interest (11,12).

We chose chitosan dispersions as a polymeric system not only for their pharmaceutical properties (13), but also for their chemical-physical properties, as studied previously (8). One of these is apparent viscosity, which is an important physical property during the first step in the production of beads, which are obtained by dripping the chitosan dispersions into a gelling solution with an adjustable constant flow rate. Moreover, chitosan dispersions are capable of emulsifying the lipophilic *Mentha piperita* E.O. into a stable homogeneous blend.

Gelification of chitosan dispersions was obtained with TPP and NaOH solutions, which are, respectively, an ionically crosslinking agent and a coacervating agent. During the ionically crosslinking process, the counter-ions diffuse into the polymeric structure; thus, the positive amino groups in the chitosan chains react with the negative groups of TPP, forming either intermolecular or intramolecular bonds (14). During the coacervating process the salting-out effect occurs.

This study investigates the effects of the different chitosan dispersions and of the crosslinking or coacervating agents used on the properties of beads loaded with *Mentha piperita* E.O. Table I reports the six different bead batches obtained from the chitosan dispersions and gelling solutions used.

A particle size and morphology analysis (Table II) was carried out on the wet and dry samples of beads loaded with *Mentha piperita* E.O. Statistically, particle size values show that wet TPP-bead batches (A_1, B_1, and C_1) were slightly smaller than wet NaOH-bead batches (A, B, and C), while this difference in size was not observed in the corresponding dry beads, this difference, on the contrary, in some cases being larger for the dry TPP beads (A_1, B_1, and C_1) (Table II). This is probably due to the a greater water uptake ability of NaOH beads (A, B, and C) during the bead formation step. This hypothesis is confirmed by bead yield, which is about 60% higher. The resulting water loss during the drying step reduces the size of all bead batches to practically similar values.

The characteristic spherical shape of the beads in the wet state was usually lost after the drying step and developed in an uneven shape with decreased volume. Optical microscope analyses of the dry beads showed a mat dense mass for TPP beads (A_1, B_1, and C_1) compared to the more transparent brighter mass of NaOH beads (A, B, and C) (Figure 1A and Figure 1B respectively).

Table III reports the swelling ratio and the mean diameter values at time t for all bead batches. These values confirm that the gelling agents used also affect swelling behavior. In fact, NaOH beads (A, B, and C) show a greater water uptake capability compared to TPP beads (A_1, B_1, and C_1) (Figure 2). This is probably due to a greater water perme-

Table I

Composition of Chitosan Beads Obtained with Two Gelling Solutions

Bead batches	Chitosan dispersions	*Menhta piperita* E.O. (% weight)	Gelling solutions
A	hc	0.3	NaOH
A_1	hc	0.3	TPP
B	mc	0.3	NaOH
B_1	mc	0.3	TPP
C	hc/mc (1:1)	0.3	NaOH
C_1	hc/mc (1:1)	0.3	TPP

Table II
Size Values of Wet and Dry Bead Samples

Bead batches	Yield (%)	Wet beads (mm)	Dry beads (mm)
A	50	3.94 ± 0.32	1.58 ± 0.05
A_1	32	3.34 ± 0.28	1.47 ± 0.04
B	69	3.00 ± 0.21	1.24 ± 0.01
B_1	36	2.98 ± 0.19	1.37 ± 0.03
C	43	3.10 ± 0.22	1.15 ± 0.02
C_1	33	3.09 ± 0.21	1.37 ± 0.02

±S.D. on the mean of three measurements.

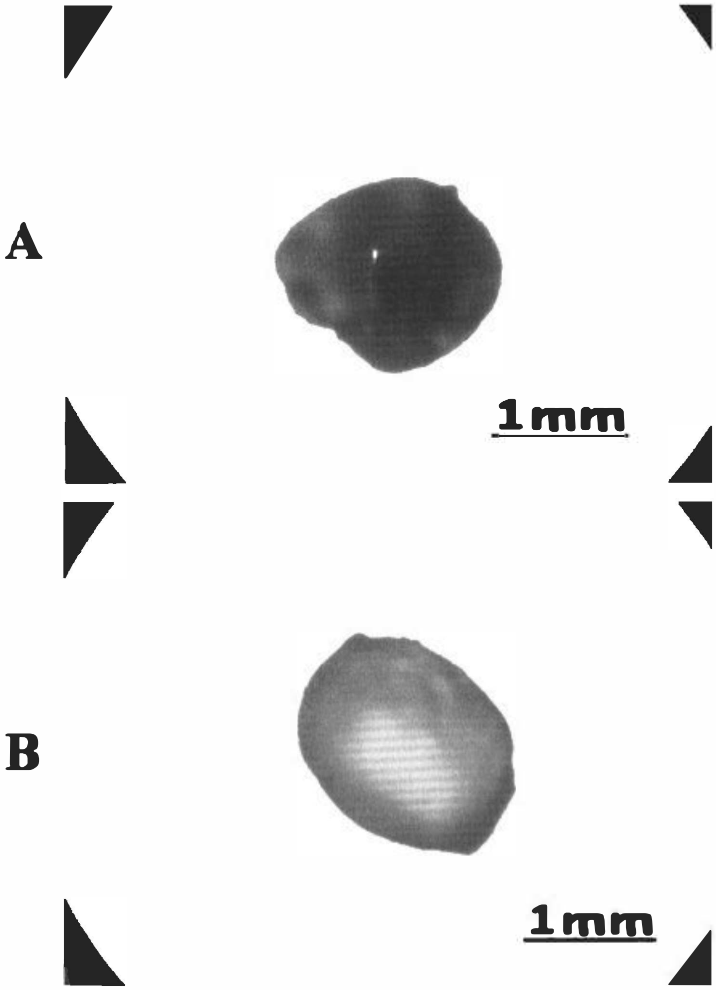

Figure 1. Optical microscope digital photo of: (A) sample A_1 dry bead and (B) sample A dry bead.

Table III
Swelling Ratio (w/w) and Mean Diameter ($\overline{\Sigma}$) (mm) at Time t Values.

Bead batches	Swelling ratio ($W_t/W_\bullet$)	$\overline{\Sigma}$ ϕ (mm) at time t
A	1.40 ± 0.03	4.24 ± 0.36
A_1	1.06 ± 0.01	3.36 ± 0.27
B	1.05 ± 0.02	2.99 ± 0.25
B_1	1.03 ± 0.01	2.85 ± 0.19
C	1.87 ± 0.04	3.15 ± 0.22
C_1	1.40 ± 0.03	2.67 ± 0.14

± S.D. on the mean of three measurements.

ability of coacervated polymers compared to ionically crosslinked polymers, where the electrostatic interactions between anions and positive chitosan charges increase the mechanical strength of the bead structures while reducing their water permeability and therefore also their swelling.

All bead batches show good *Mentha piperita* E.O. encapsulation efficiency in the 65–70% range, with slightly higher values for NaOH beads (A, B, and C). No significant differences in *Mentha piperita* E.O. encapsulation efficiency were observed when different chitosan dispersions were used.

At this point one of the most important objectives was to evaluate the bead disintegration time within the chosen cosmetic formulation (BBF) for the release of *Mentha piperita* E.O. and also for the stability of the beads during an estimated storage time. Figure 3 shows the results obtained from the bead-breakage assay in BBF. As can be observed, only NaOH-bead batches (A,B, and C) showed positive results to this stress test, and particularly the beads in batch B showed the highest breakage percentage, at

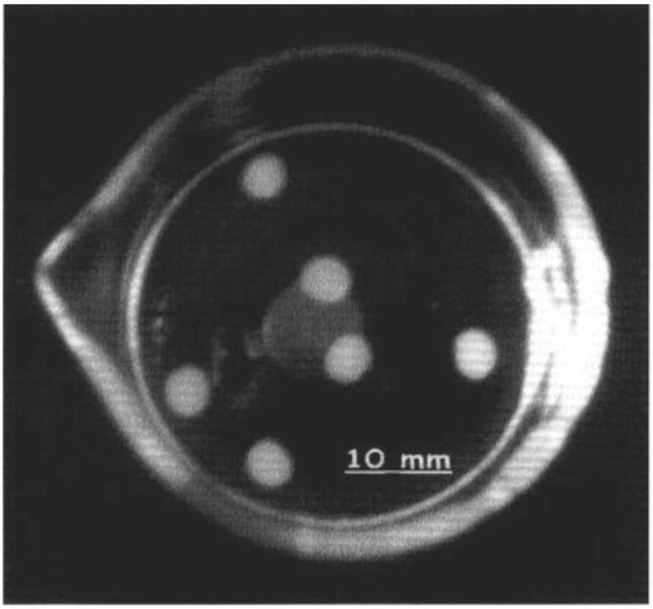

Figure 2. Swelling behavior of NaOH beads (sample B) at time t.

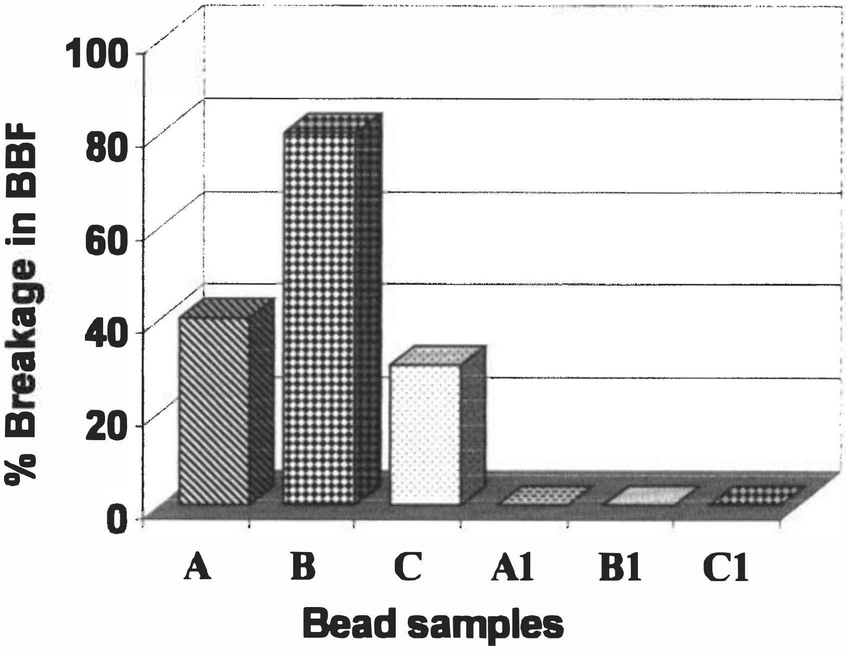

Figure 3. Bead breakage assay in BBF results.

about 60–80%. On the other hand, TPP-bead batches (A_1, B_1, and C_1) did not present marks of breakage, even in conditions of great stress.

All bead batches were stable for six months, estimated as storage time. As an example, Figure 4 shows a digital photo of beads in batch A in BBF at six months.

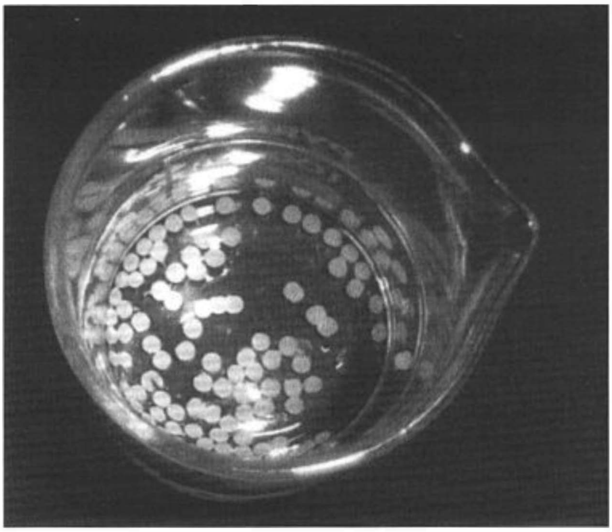

Figure 4. Digital photo of beads in batch A in BBF at six months.

CONCLUSIONS

In this paper we describe the feasibility of using chitosan beads as colloidal carriers for the encapsulation and delivery of volatile molecules such as *Mentha piperita* E.O. in a fast-release cosmetic formulation such as a bath foam. To this purpose, it is very important to assess the stability of the structure of the beads during the storage time of the cosmetic formulation and the breakage ability of the beads for the release of *Mentha piperita* E.O. during use.

The analyses carried out have shown that the different bead batches show different behaviors. Compared to TPP beads (A_1, B_1, and C_1), NaOH beads (A, B, and C) showed much better swelling properties and a greater release of *Mentha piperita* E.O., thanks to the fact that their structures are loose and therefore break more easily. This behavior is probably due to the chitosan gelling process in view of obtaining NaOH beads—in other words, coacervation, where the interactions between the chitosan chains are relatively weak. However, it has been observed that the structure of NaOH beads remains whole in the cosmetic formulation during the estimated storage time.

In-use testing has shown that beads A, B, and C break easily without leaving any annoying residues on the skin, since the chitosan polymeric matrix was homogenically distributed in the formulation and easily washes away. The sensory effect is a pleasing sensation of freshness due to the release of the *Mentha piperita* E.O. fragrance, which is particularly intense during the application of the bath foam on the skin. This effect has also been observed after six months of storage time, showing that a notable quantity of *Mentha piperita* E.O. remains encapsulated in the beads.

In contrast, TPP beads are more resistant, in agreement with the ionic crosslinking process where the electrostatic interactions between the TPP polyvalent anions and the positive chitosan charges give rise to a complete, dense structure with remarkable mechanical stability. Hence their shrinkage leads to a release of *Mentha piperita* E.O. that is too slow for the intended purpose. NaOH beads are therefore promising for the use of essential oils in the prompt release of cosmetic formulations.

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