Chitosan particles as new essential oil carrier for antimicrobial application

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Introduction

Natural products and their derivatives represent more than 50% of all the drugs in clinical use in the world (Gurib-Farim, 2006). Known for their fragrance and medicinal properties, such as antimicrobial activity, the essential oils are volatile, natural, complex mixture of compounds formed by aromatic plants as secondary metabolites (Bakkali, 2008). However essential oils undergo undesirable deterioration reactions in the presence of oxygen from the air. Oxidation reactions may form allergenic products and/or products with less biological activity than the original compounds (Neumann and Garcia, 1882; Hammer, 2006). Concerning to these data, encapsulation technology seems to be a suitable option for improving the stability and efficacy of essential oil-based formulations.

Based on the traditional medicine of some Brazilian regions, the *Zanthoxylum tingoassuiba* (Rutaceae) was found as an anti-inflammatory, analgesic, antibacterial and antifungal agent. (-)-α-bisabolol, a sesquiterpene with known anti-inflammatory and anti-cancer activity, is one of the main compounds of the *Z. tingoassuiba* essential oil (Albuquerque, 2007). Properties such as biodegradability and low toxicity make chitosan, a natural polymer, suitable for use in biomedical and pharmaceutical formulations (Sinha, 2004). Furthermore, various studies have been showed a good mucoadhesive profile of chitosan (Martina, 2003; Kockisch, 2004). Then, in this work, essential oil-entrapped chitosan particles were prepared and characterized in function of the (-)-α-bisabolol content and release aiming the development of a new buccal drug delivery system. Besides the influence of some variables on the particles manufacturing were evaluated.

Material and Methods

The aerial parts of *Z. tingoassuiba* were distilled by using of a steam apparatus. The major components of the Z. tingoassuiba essential oil were determined by ^{13}C and ^{1}H NMR and mass spectrometry. The chitosan particles were prepared by ionotropic gelation with sodium tripolyphosphate (TPP). Different amounts of chitosan was dissolved in 1%(v/v) acetic acid and Tween 80 (2% v/v) was added into the solutions as a surfactant. *Z. tingoassuiba* essential oil was dissolved in dichloromethane (0.03:1). This oil phase was mixed with aqueous phase (1:10) by homogenizer at 8,000rpm for 1 min. The emulsion was dropped into crosslinking agent solution (TPP at pH 5.0), using a 23-gauge hypodermic syringe, under magnetic stirring. Different crosslinking times were applied. The obtained particles were filtered and dried under room temperature.

For quantifying the (-)-α-bisabolol content in the essential oil, a reversed-phase HPLC method was used where the mobile phase was a mixture of A, acetonitrile:water:phosphoric acid (19:80:1), and B, acetonitrile. The linear gradient elution program was from A:B (50:50) to 100% B in 25min, and

returning to A:B (50:50) in 5min. The eluent was pumped at a flow rate of 0.8mL/min, the injection volume was 20µL and the detection wavelength was 200nm.

Association efficiency (AE) to chitosan particles were determined indirectly by using of the following equation:

$$A.E. = \frac{\text{Total amount of (-)-á-bisabolol - (-)-á-bisabolol in supernatant}}{\text{Total amount of (-)-á-bisabolol}} X\,100$$

The total amount of (-)-α-bisabolol was determined based on the percentage of (-)-α-bisabolol present in the *Z. tinguoassuiba* essential oil that was determined by HPLC method as described above. In order to determine release profile of (-)-α-bisabolol from the particulate system, chitosan particles were collected into tests tubes containing 20mL of phosphate saline buffer pH 6.8 (120min/100rpm). Samples (400µL) were periodically removed and the volume of each sample was replaced by the same volume of fresh medium. The amount of (-)-α-bisabolol released from the particles were evaluated by HPLC as previously described. All the parameters were determined in triplicate.

Results and Discussion

Several works have been studied the entrapping of essential oils in polymeric matrices, such as gelatin capsules (Passino, 2004; Maji, 2007), sodium alginate beads (Lai, 2007) and chitosan films (Zivanovic, 2005). However the most of these formulations use glutaraldehyde as a chemical cross-linker for improving the mechanical strength of the particulate system. Considering the toxicity of chemical cross-linkers, drug delivery systems developed without these substances are more attractive for biomedical applications (W. Fürst and A. Banerjee, 2005). This fact justifies the use of the ionotropic gelation method for production of the particulate system of this work.

Distillation of Z. tingoassuiba leaves gave a yellow essential oil in good yield (0.6 ± 0.2%). The analysis of essencial oil by ^{13}C and ^{1}H NMR and mass spectrometry showed that oil composition is a mixture of mono and sesquiterpenoids. The main constituents are methyl N-methyl anthranylate and α-bisabolol. These compounds represent more than 60% of mass of the essential oil.

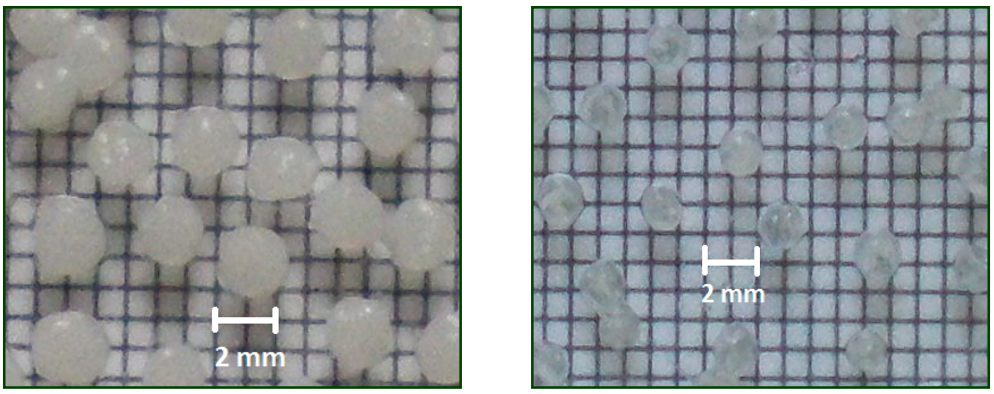

Figure 1. Fresh (left) and dried (right) chitosan particles containing *Z. tingoassuiba* essential oil

In all the formulations the chitosan particles showed spherical shape and variable mechanical strength. It was observed that as minor the chitosan concentration, the crosslinking time and the TPP concentration were, minor the strength of particles to handling was (data not showed). It may

be result from the formation of relatively strong walls of microparticles upon high chitosan concentration and interaction with TPP (Ko, 2002).

Particle Size

Chemically, chitosan is a copolymer of glucosamine and *N*-acetyl-glucosamine, having one primary amino and two free hydroxyl groups for each C6 building unit (Sinha, 2004). Due to these groups, chitosan is a hydrophilic polymer that in contact with water, it becomes swollen. This explains the difference between wet and dried particles. The table 1 shows that as the crosslinking time increased, the particle size decreased. Spending more time in contact with TPP, more cross-linkages among chitosan chains were formed, producing a tighter cross-linked matrix, justifying the smaller size. This same effect was observed in formulations with the highest concentration of TPP.

Variables		Association Efficiency (%)	Particle Size (mm)	
			Wet	Dried
Chitosan concentration (%w/v)	1	88.86±0.86	2.27±0.05	1.43±0.12
	1.5	98.04±0.14	2.06±0.05	1.14±0.06
	2	98.61±0.22	2.19±0.05	1.12±0.05
Crosslinking time (min)	30	98.53±0.11	2.03±0.01	1.07±0.07
	60	98.61±0.22	2.19±0.05	1.12±0.05
	120	98.63±0.03	1.98±0.01	1.02±0.01
TPP concentration (%w/v)	5	95.98±0.78	2.09±0.02	1.04±0.02
	10	98.61±0.22	2.19±0.05	1.12±0.05
	20	98.96±0.26	2.05±0.04	1.01±0.03

Table 1. Association efficiency and particle size of dried and wet chitosan particles (data shown are the mean ± standard deviation, n=3).

Association Efficiency

The (-)-α-bisabolol, as the rest of other compounds into the essential oil, is highly volatile. In order to avoid the losses by evaporation of the sesquiterpene, the time and speed for homogenizing the emulsion and the crosslinking times were carefully selected. As described in table 1, generally, the AE values were more than 80%. Therefore, the losses of (-)-α-bisabolol from chitosan particles were minimal. The lowest chitosan concentration provided the lowest AE. At a low concentration there are less chitosan chains than other formulations, establishing less cross-links, releasing easier the (-)-α-bisabolol content. At the lowest cross-linking time and TPP concentration, the AE decreased. These data demonstrate that these parameters may be linked with the porous size of the polymeric matrix, interfering on the retention of the essential oil into the particles.

The release profile of the (-)-α-bisabolol content of the essential oil from the chitosan particles up to 120 minutes at 37°C was obtained. The particles showed a desired controlled release profile. Present studies are being conducting regarding the antimicrobiological activity and buccal mucoadhesivity.

Conclusions

These results demonstrated that chitosan particles ionically cross-linked can be a suitable option for entrapping essential oils. Parameters like chitosan and TPP concentration and the cross-linking time are very important for the particle size and the association efficiency of the system. The highest values of these parameters showed the best results. Other parameters of manufacturing of these particles and their influence in the biological activity of the essential oil will be presented.

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