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Review

Chitosan: An option for development of essential oil delivery systems for oral cavity care?

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ABSTRACT

Microencapsulation of bioactive compounds has received increased attention in the last decade. Among the polymers used for developing microparticulated systems, chitosan has been widely cited. Obtained by deacetylation of chitin, chitosan is a natural, biodegradable, biocompatible and mucoadhesive polymer with permeability enhancement properties. These data justify its use for overcoming the reduced efficacy of conventional treatments of oral diseases. Various tests simulating the buccal environment have described controlled drug release profile and significant activity against buccal pathogens by chitosan microparticles entrapping antimicrobial agents. Considering the increasing microbial resistance to conventional antibiotics, essential oils have shown to be an important option against these pathogens. For sustained stability and prolonged release of essential oils from pharmaceutical formulations, some authors have studied the association of chitosan to them. This review disserts about the application of chitosan and essential oils on oral cavity care pointing out their association may be an interesting option.

1. Introduction

The development of microencapsulation technology has received increased attention generating a large range of applications in several sectors, such as industry, agriculture, medicine, pharmacy and biotechnology. In biomedical field various formulations are based in microparticles, aiming the masking of unpleasant tastes and odors, controlled release of drugs, protection of drugs from aggressive body fluids, such as gastric juice, isolation of cells and development of immunoassays (Madene, Jacquot, Scher, & Desobry, 2006; Peniche, Argüelles-Monal, Peniche, & Acost, 2003). Different kinds of microparticles are usually manufactured by using polymers as the shell or matrix materials where those biodegradable and biocompatible are the polymers of choice (Munday & Cox, 2000). These systems further present the versatility of allowing the incorporation of suitable amounts of drugs and improving the bioavailability of degradable drugs and the permeation of hydrophilic substances across epithelial layers (Dhawan, Singla, & Sinha, 2004). Microparticles also show the advantages of providing a large surface area, easier estimation of mass transfer behavior and precise kinetics modeling and controlled release of drugs to the body fluid (Lin, Chang, Chen, Chou, & Kuo, 2006).

Chitosan, a natural linear biopolyaminosaccharide, is usually obtained by alkaline deacetylation of chitin. Chitin is the second most abundant polymer in the nature after cellulose, being the principal component of exoskeleton of crustaceans such as crabs, shrimps, prawns, lobsters and cell walls of some fungi such as *Aspergillus, Zygomicetes* and *Mucor* (Sinha et al., 2004). The production of chitosan is economically feasible, especially if it includes the recovery of carotenoids and proteins. Chitin is found naturally as the conjugated form with proteins which after extracting process can be recovered for other uses, such as animal feed. Furthermore, the crustacean shells contain considerable quantities of astaxanthin, a carotenoid that has so far not been synthesized, and which is marketed as a fish food additive in aquaculture, especially for salmon (Rao, Munoz, & Stevens, 2000; Ravi Kumar, 2000).

Chemically, as shown in Fig. 1, chitosan is a copolymer of glucosamine and *N*-acetyl-glucosamine with one primary amino and two free hydroxyl groups for each C6 building unit. These free amino groups attribute to chitosan a positive charge that allows its reaction with negatively charged surfaces and anionic polymers and also undergoes chelation with transition and post-transition metal ions and thus its application on metal capture (Ravi Kumar, 2000; Sinha et al., 2004). Nearly all aqueous acids can dissolve chitosan, of which the most commonly used are formic acid and acetic acid, which can convert the glucosamine units into a soluble form $R–NH3^+$. In chitosan the degree of deacetylation (DD) ranges from 40% to 98% and the molecular weight (MW) ranges between 5×10^4 Da and 2×10^6 Da. These parameters are very important

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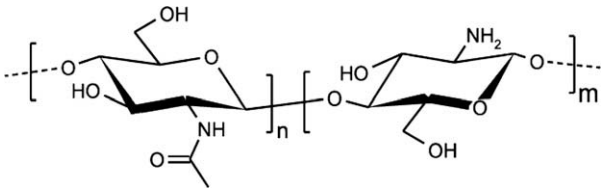

Fig. 1. Chemical structure of chitosan.

for predicting the application of the polymer (Mourya & Inamdar, 2008). Specifically for pharmaceutical applications it has been used as a vehicle for directly compressed tablets (Knapczyk, 1993), as a binder (Upadrashta, Katikaneni, & Nuessle, 1992), as a disintegrant, for the production of controlled release solid forms (Nunthanid et al., 2004) or for improvement of drug dissolution (Illum, 1998).

2. Chitosan mucoadhesive properties and absorption enhancement

Mucoadhesive polymers are synthetic or natural macromolecules capable of attaching to mucosal surfaces (Grabovac, Guggi, & Bernkop-Schnürch, 2005). Mucoadhesive polymers may fulfill the desirable features of a prolonged residence time at the site of drug absorption owing to increased contact with the absorbing mucosa, resulting in a steep concentration gradient to favor drug absorption, and localization in specified regions to improve the bioavailability of drugs (Dhawan et al., 2004).

The basic components of mucus are mucin glycoproteins which form an unstirred gel layer over the epithelial cells of the mucosa. For optimum mucoadhesion, there has to be an intimate contact between the adhesive and the substrate and interpenetration of the polymer chains with the mucin glycoprotein network. Chitosan interacts with mucin by multiple modes, namely due to molecular attractive forces formed by electrostatic interaction between positively charged chitosan and negatively charged mucosal surfaces. These properties may be attributed to strong hydrogen bonding groups like –OH, –COOH, strong charges, high MW, sufficient chain flexibility and to surface energy properties favoring spreading into mucus (Qaqish & Amiji, 1999).

Factors such as cross-linking status and ionic modification can significantly influence the mucoadhesivity of polymeric micropaticles. Dhawan et al. (2004) showed that the microspheres prepared by emulsification followed by ionotropic gelation were found to be more mucoadhesive as compared with those obtained by other methods. This can be explained by the fact that the extent of adsorption of mucin is dependent on the charge of chitosan microspheres. The chitosan microspheres cross-linked by thermal and chemical (glutaraldehyde) process showed the lowest charge value, whereas the highest charge values were obtained from ionotropic gelated microparticles. He, Davis, and Illum (1998) also demonstrated the relationship between the charge of chitosan microspheres, and negative charge of mucus glycoprotein and mucoadhesion extent. Thus the electrostatic attraction between the positively charged mucoadhesive chitosan microspheres and negatively charged mucus glycoprotein plays an important role in the adsorption of chitosan microspheres on mucin.

The exceptional mucoadhesive properties of chitosan, in comparison to other polymers like polycarbophil, used as a reference substance, were found for the first time employing chitosan films in pig intestinal mucosa (Lehr, Bouwstra, Schacht, & Junginger, 1992). Grabovac et al. (2005) investigated the mucoadhesive properties of several polymers by measurement of time of mucoadhesion on porcine small intestinal mucosa and of total work of adhesion by tensile studies. It was showed that the mucoadhesive profile of the precipitated and lyophilized form of chitosan was pH dependent. At acid medium (pH 3.0) chitosan was immediately disintegrated. The best results of chitosan occurred for lyophilized form, at higher pH values (pH 6.5 and 7.0). Takeuchi, Matsui, Yamamoto, and Kawashima (2003) compared the mucoadhesive properties of carbopol and chitosan-coated liposomes using intestines isolated from male Wistar rats. The mucoadhesive properties of carbopol-coated liposomes were comparable with that of chitosan-coated liposomes and both formulations improved the enteric absorption of the encapsulated peptide.

Chitosan solutions, gels, films, sponges, micro and nanoparticles have demonstrated to promote absorption of small polar molecules and peptide/protein drugs through ocular (Majumbar, Hippalgaonkar, & Repka, 2008; Sahoo, Dilnawaz & Krishnakumar, 2008; Yuan et al., 2008), nasal (Costantino, Illum, Brandt, Johnson, & Quay, 2007; Gavini et al., 2006), pulmonary (Grenha et al., 2007; Ventura et al., 2008), oral (Portero, Teijeiro-Osorio, Alonso, & Remunan-Lopez, 2007; Thanou, Verhoef, & Junginger, 2001) and intestinal mucosa (Borchard et al., 1996; Thanou et al., 2001; Şenel & Hincal, 2001) by using animal models and human volunteers. The absorption enhancement of the peptide analog buserelin was studied after transduodenal co-administration with chitosan gels (pH 6.7) in rats (Borchard et al., 1996). Chitosan substantially increased the bioavailability of the peptide in comparison to control (no polymer) or carbopol 934P® containing formulations. Chitosan was also found to exert marked permeabilization effect on buccal mucosa for TGF-β by *in vitro* test in porcine oral mucosa treated with chitosan gels at 2% concentration entrapping TGF-β (Şenel et al., 2000).

As already mentioned, the mechanism of chitosan absorption enhancement has been suggested to be a combination of mucoadhesion and an effect on the gating properties of tight junctions from epithelium cells (Lehr et al., 1992). Dodane, Amin Khan, and Merwin (1999) studied the effects of chitosan on Caco-2 cells and observed, by confocal microscopy, a decrease of staining of tight junction proteins on cells that received chitosan treatment. Furthermore, chitosan induced redistribution of F-actin. Because actin has been shown to be important in regulating paracellular flow across cultured intestinal epithelia, the above effects of chitosan on epithelial barrier function might be due to a partial alteration of the cytoskeleton. The increased paracellular permeability was not accompanied by apparent changes in the junctional morphology. All these effects were reversible, indicating that chitosan had a transient effect on the cellular barrier. All these data suggest that chitosan could be used as a permeability enhancer without causing membrane perturbations such as the effect described after treatment with sodium dodecyl sulfate. Chitosan causes relatively mild and reversible effects on epithelial morphology which makes it an advantageous absorption enhancing compound for mucosal delivery of drugs (Dodane et al., 1999).

On another work, Schipper, Varum, and Artursson (1996) established that chitosan with high DD is effective as permeation enhancer at low and high MW, and also showed clear dose-dependent toxicity, whereas chitosan of low DD is effective at only high MW and showed low toxicity.

3. Chitosan antimicrobial properties

The antimicrobial activity of chitosan has been considered in a wide variety of fungi and bacteria (Hernández-Lauzardo et al., 2008; Ikinci et al., 2002; No, Park, Lee, & Meyers, 2002; Qin et al., 2006). Taking into account these data, the ability of chitosan to extend the storage life of fruits and vegetables have been demonstrated (Campaniello, Bevilacqua, Sinigaglia, & Corbo, 2008; Fernandez-Saiz, Lagaron, & Ocio, 2009; Sebti, Martial-Gros, Carnet-Pantiez, Grelier, & Coma, 2005).

For achieving necessary mechanical strength and viscosity for modeling drug delivery systems, chitosans with DD higher than 50% are preferred (Hejazi & Amiji, 2003). Due to the poor water solubility of these chitosans, their dissolution is usually done in acetic acid, which antimicrobial activity is widely known. No et al. (2002) observed that the antibacterial activity of chitosan was enhanced by acetic, formic or lactic acid into the medium. Liu et al. (2006) showed that acetic acid solutions over 200 ppm have a marked biocide response to *Escherichia coli*. However, it was also demonstrated that chitosan samples from 50 ppm exceeded the action of acetic acid.

Qin et al. (2006) correlated the water solubility of chitosan and its antimicrobial activity. Water soluble chitosans (<50% DD) did not show any antimicrobial activity. In opposing, the water insoluble chitosans dissolved in acid medium showed to be microbicide. Due to the physiological pH in the cell is around neutral, the water insoluble samples can precipitate, forming an impervious layer around the cell, blocking channels, which are crucial for living cells. Water soluble samples could not form this layer. Other important concept highlighted by Qin et al. (2006) is when the chitosan molecules are too large, the chitosan layer may be not very compact, helping to understand the great antimicrobial activity of low MW chitosans in several works (Baytukalov et al., 2005; Hernández-Lauzardo et al., 2008; Liu et al., 2006; Tikhonov et al., 2006).

It has been postulated that the antimicrobial chitosan occurs as a result of several mechanisms. The interaction between positively charged chitosan molecules and negatively charged microbial cell membranes could lead to their disruption and leakage of essential intracellular constituents (Helander, Nurmiaho-Lassila, Ahvenainen, Rhoades, & Roller, 2001; Ikinci et al., 2002; Kong et al., 2008). Dissociated chitosan molecule in solution, with low MW, could bind with DNA and inhibit synthesis of mRNA through penetration toward the nuclei of the microorganisms (Kong et al., 2008; Sebti et al., 2005) while with higher MW, chitosan could act as a chelating agent that selectively binds trace metals, which are essential for toxin production or microbial growth.

4. Drug delivery profile on oral cavity

The efficacy of conventional treatments of oral diseases, e.g. dental caries or fungal infections, is often reduced by the limited retention of the applied formulations within the oral cavity (Kockisch, Rees, Tsibouklis, & Smart, 2005). The short residence time of formulations at their intended site of action may result in reduced bioavailability (Kockisch, Rees, Young, Tsibouklis, & Smart, 2003). A large number of studies has documented the use of buccal delivery systems for controlled release of drugs, such as fentanyl (Diaz del Consuelo, Falson, Guy, & Jacques, 2007), denbufylline (Martin, Wilson, Koosha, & Uchegbu, 2003), zinc sulfate (Diarra, Pourroy, Muster, Zingraff, & Boymond, 1998), chlorhexidine (Medlicott, Holborow, Rathbone, Jones, & Tucker, 1999) and theophylline (Geresh et al., 2004). The desirable attributes of these drug delivery systems are high drug loading capacity, good mucoadhesion, non-irritancy, tastelessness and sustained drug delivery. An erodible formulation has the added advantage of not requiring retrieval after delivery of the dose (Martin et al., 2003).

The relative merits of the potential usefulness of carbopol 974P NF, polycarbophil Noveon AA-1, chitosan (low MW) and Gantrez MS-255 microspheres as formulation tools for the enhanced retention of a therapeutic entity within the oral mucosa were evaluated by Kockisch et al. (2003) by *in vitro* mucoadhesion tests. The chitosan microspheres had the highest retention times on a 30° inclined porcine esophageal mucosa at saliva flow rates from 0.5 to 7 mL/min (chosen to represent the resting and the maximum stimulated saliva flow in the human oral cavity, respectively). The comparatively short residence times for carbopol and polycarbophil may be attributed to their swelling characteristics. These poly(acrylic acid) polymers can hydrate more readily than chitosan or Gantrez (both of which exhibit limited swelling) to form a nonadhesive mucilage and therefore be flushed. In addition to their swelling behavior, chitosan microparticles may owe their promising mucoadhesive performance to their cationic nature. These results correlate with those obtained on another work carried out by the same group (Kockisch, Rees, Young, Tsibouklis, & Smart, 2004). It has been suggested that within aqueous environment, cationic materials display a mechanism of mucoadhesion in which not only hydrogen bonding but also salt-bridge effects, involving the positively charged chitosan microparticles and the negatively charged mucus glycoproteins, are of importance.

On another work, Kockisch et al. (2005) evaluated the release profile of the hydrophobic drug, triclosan, from different polymeric microspheres, prepared by double emulsion method, focusing efforts on development of oral care formulations, specifically dental pastes. The drug loading efficiencies for carbopol, polycarbophil and Gantrez were of the order of 30% whereas that for chitosan was 70%. Triclosan-loaded chitosan microspheres have been shown to exhibit controlled-release behavior over extended time periods in both aqueous (with sodium lauryl sulfate, a surfactant present in many toothpaste formulations) and glycerol based medium (a constituent of many toothpaste formulations).

One of the great challenges for applying pharmaceutical formulations based on chitosan in oral cavity is the presence of lysozyme, an enzyme widely distributed in mammalian, present in different fluids as saliva or vaginal fluid, and also in cells like macrophages (Bernardo, Blanco, Sastre, Teijón, & Teijón, 2003; Cölfen, Harding, Varum, & Winzor, 1996). The literature has described the hydrolytic activity of lysozyme on chitosan. Bernardo et al. (2003) demonstrated that the presence of lysozyme into dissolution medium of chitosan microspheres entrapping bupivacaine, increased the total amount of the drug released from the particles and decreased the time of degradation of the particles, however, the system showed a sustained and controlled drug release profile. By using different dissolution media with different enzymes (pepsin, lysozyme and pancreatin), Anal, Stevens, and Remunan-Lopez (2006) showed that ampicillin-loaded chitosan microspheres treated with sodium tripolyphosphate (TPP) presented more stability when compared to those obtained by the spray drying technique.

Some works have analyzed the relationship between the DD of chitosan and the resistance to degradation by lysozyme. *In vitro* and *in vivo* tests for investigation of degradation of chitosan films carried out by Tomihata and Ikada (1997) indicated chitosan films with DD less than 73.3% were more susceptible to degradation by lysozyme. This data correlate with the loss of activity of lysozyme on highly deacetylated chitosan, observed by Cölfen et al. (1996).

5. Application of chitosan on oral cavity treatment

Over 400 different types of oral cavity disorders exist, such as oral mucositis, gingivitis, periodontitis, dental caries and oral lesions which are usually treated by local therapy. However, several problems are associated with local treatment in the oral cavity. Many conventional mouth rinses and antimicrobials contain alcohol or astringents and may have an unpleasant taste, which exacerbates the condition. The flushing action of saliva and the consumption of foodstuffs also rapidly reduce the drug concentration, which should be maintained above the minimum inhibitory concentration throughout the course of therapy. Bioadhesive polymers appear to be particularly attractive for the development of drug delivery systems improving intraoral administration and reducing the frequency of application and the amount of drug

administered, allowing the effective treatment of a whole range of conditions (Aksungur et al., 2004; Patel, Smith, Grist, Barnett, & Smart, 1999).

Normally commensal in the oral cavity, *Candida albicans* in the suppressed or compromised patient can rapidly invade the oral tissues and spread to the lungs or esophagus. Terminally ill patients with cancer and/or AIDS will suffer from some or all of these conditions. Others will tend to suffer from a dry mouth and some degree of infection and so will be likely to have oral symptoms. In all cases, palliation represents the standard clinical management relief of symptoms and an attempt to check infections (Aksungur et al., 2004).

Aksungur et al. (2004) demonstrated by *in vitro* and *in vivo* studies that chitosan is an excellent candidate for development of drug delivery systems aiming the treatment of oral mucositis, offering not only the palliative effects of an occlusive dressing but also the potential for delivering therapeutic compounds. Topical application of both chitosan gel and suspension containing nystatin to the oral mucosa significantly reduced the severity and the incidence of oral mucositis, reduced weight loss and increased survival, and provided a significant healing.

Giunchedi, Juliano, Gavini, Cossu, and Sorrenti (2002) evaluated the efficacy of chlorexidine-loaded chitosan microspheres against *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *C. albicans*. The loading of chlorhexidine into chitosan is able to maintain or improve the antimicrobial activity of the drug. The improvement is particularly high against *C. albicans*. Unloaded microparticles have an antimicrobial activity due to the polymer itself. Some reports have described the capability of chitosan to inhibit the adhesion of *C. albicans* cells to human buccal cells and to prevent the development of mycosis (Aksungur et al., 2004; Azcurra et al., 2006).

Aiming to develop a drug delivery system for combating periodontal diseases, that are associated with anaerobic Gram-negative bacteria infections and affect teeth-supporting structures, El-Kamel, Ashri, and Alsarra (2007) tested the performance of pure chitosan and chitosan/poly(ε-caprolactone) microspheres entrapping metronidazole benzoate. Both delivery systems showed a prolonged drug release providing concentrations higher than MIC value of metronidazole against *Porphyromonas gingivalis*, a periodontal pathogen. *In vivo* bioadhesion studies carried out in healthy volunteers presented higher adhesion time for chitosan/poly(ε-caprolactone) because its bi-layer of chitosan. In addition, no discomfort, no severe salivation and an acceptable taste were reported by volunteers.

Ikinci et al. (2002) tested the antimicrobial activity of chitosan formulations either in gel or film forms against *P. gingivalis*. Chitosan showed to have antimicrobial activity against *P. gingivalis*, higher with high MW chitosan. The combination of chitosan with chlorhexidine gluconate showed higher activity when compared to drug alone, thus providing a chlorexidine gluconate application at lower concentrations avoiding its unwanted side-effects. Chitosan gels and films seem to be promising delivery systems for local therapy of periodontal diseases with its bioadhesive property and antimicrobial activity (Table 1).

6. Essential oils and their application on oral cavity care

During the last years, due to the increased multidrug resistance of many human pathogenic microorganisms as well as the appearance of undesirable side-effects of certain antibiotics, the investigation of the chemical compounds within traditional plants has become desirable. Antimicrobial properties have been reported more frequently in a wide range of extracts and natural products (Kumarasamy, Cox, Jaspars, Nahar, & Sarker, 2002; Rabanal, Arias, Prado, Hernández-Pérez, & Sánchez-Mateo, 2002; Rahman, Kühn, Rahman, Olsson-Liljequist, & Möllby, 2004).

Essential oils are natural complex mixtures of volatile, lipophilic substances obtained from different parts of plants by steam distillation, solvent extraction, or cold pressing, having a composition that can vary widely in relation to several factors (e.g., source, plant location, cultivation technique and season). They are usually characterized by a strong odor and are composed by secondary metabolites of aromatic plants with oxygenated structures such as alcohols, ketones, aldehydes, and esters, presenting therapeutic properties among which are antibacterial, antifungal and insecticidal activities (Bakkali, Averbeck, Averbeck, & Idaomar, 2008; Lai, Loy, Manconi, Manca, & Fadda, 2007).

Besides the eco-friendly and biodegradable nature, the specific advantage of essential oils appears to be in synergistic effects of their compounds as evidenced in greater activity when applied as natural essential oil compared with summary of the effects of the individual substances. It is possible that the activity of the main components could be modulated by other minor molecules (Bakkali et al., 2008). Concerning to this, Jaki et al. (in press) through purity–activity relationship studies showed that ursolic acid, a triterpene, with higher purity presented less activity against *Mycobacterium tuberculosis*. Generally, polyphenols and terpenes are major contributors to antimicrobial effects of essential oils. A number of reports established a good correlation between strong antibacterial activity and the presence of monoterpenes, eugenol, cinnamaldehyde, carvacrol, and thymol in essential oil (Bakkali et al., 2008; Chami, Bennis, Chami, Aboussekhra, & Remmal, 2005; Zivanovic, Chi, & Draughon, 2005). Brehm-Stecher and

Table 1
Drug delivery systems based on chitosan for oral cavity treatment.

Formulation	Drug	Application	*In vitro* and *in vivo* results
Chitosan films, gels	Nystatin	Oral mucositis treatment	• Chitosan alone showed significant higher reduction of mucositis than films and gels with nystatin in hamsters; • In the oral cavity of healthy volunteers, nystatin concentration was maintained above MIC for *C. albicans* for 90 min at the saliva of the application site of gels and 45 min at contralateral site;
Chitosan/ε-caprolactone (CH/PCL) and chitosan films	Metronidazole benzoate (MET)	Periodontal diseases treatment	• CH/PCL films at proportion of 1:0.625 had the best tensile properties and lowest MET release; • In the oral cavity of healthy volunteers, 1:0.625 CH/PCL films had the highest residence time and a concentration of MET above MIC against *Porphyromonas gingivalis* was maintained in saliva over 6 h
Chitosan microspheres	Chlorhexidine	Buccal infection treatment	• The MIC of chlorhexidine loaded in chitosan microspheres against *E. coli, C. albicans, S. aureus* and *Pseudomonas aeruginosa* was reduced or maintained • Tablets prepared by direct compression with mannitol and sodium alginate released were able to maintain detectable concentration of chlorhexidine in saliva of healthy volunteers for more than 3 h

Johnson (2003) showed that the sesquiterpenoids bisabolol, nerolidol, farnesol and apritone enhanced the sensitization of *E. coli* and *S. aureus* strains to traditional antibiotics by affecting the membrane permeability of these bacteria.

Several works have demonstrated the antimicrobial activity of essential oils against cariogenic and periodontopathic bacteria, such as *P. gingivalis, Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum, Streptococcus mutans*, and *Streptococcus sobrinus* which are present in dental plaque as biofilms and also against oral fungi, such as *C. albicans* (Chami et al., 2005; Hammer et al., 2003; Shapiro, Meier, & Guggenheim, 1994; Takarada et al., 2004; Vagionas, Graikou, Ngassapa, Runyoro, & Chinou, 2007). Phenolic compounds obtained from the *Artocarpus heterophyllus* (Moraceae, heart wood) methanolic extract, by Sato et al. (1996), showed intensive activity against cariogenic bacteria. Serial chromatographic purifications offered two active compounds which were identified as 6-(3-methyl-l-butenyl)-5,2′,4′-trihydroxy-3-isoprenyl-7-methoxyflavone and 5,7,2′,4′-tetrahydroxy-6-isoprenylflavone. Both compounds completely inhibited the growth of primary cariogenic bacteria at 3.13–12.5 μg/mL. They also exhibited the growth inhibitory effects on plaque-forming streptococci.

Concerning to the antimicrobial activity and biocompatibility of the essential oils, its use in mouthwashes can be a beneficial and safe component of daily oral health routines (Claffey, 2003; Gunsolley, 2006). The literature has shown the good efficacy of essential oil-based formulations against halitosis derived from tongue microflora (Roldán, Herrera, & Sanz, 2003), supragingival plaque and gingivitis (Charles, Pan, Sturdivant, & Vincent, 2000; Charles et al., 2001; Fine et al., 2007; Sharma et al., 2004). Considering these data, the use of essential oils in formulations for oral cavity care has demonstrated to be very suitable.

7. Chitosan particles entrapping essential oils

Essential oils undergo undesirable deterioration reactions in the presence of oxygen from the air, familiar to us as the phenomena of rancidity, with accompanying off-flavors and smells. Oxidation reactions may form allergenic products and/or products with less biological activity than the original compounds (Hammer, Carson, Riley, & Nielsen, 2006; Neumann & Garcia, 1882). Previous studies on *d*-limonene, a monoterpene obtained from several essential oils, show that this compound oxidizes on air exposure (autoxidation) and that strong allergenic oxidation products are formed (Sköld, Börje, Matur, & Karlberg, 2002). Hammer et al. (2006) summarized studies that have suggested that the products formed by oxidation of terpenes from tea tree oil, during storage, are strongly allergenic. Thus, for sustaining the stability and biological activity of labile products, such as essential oils, their association with polymeric matrices has been studied (Bustos, Romo, Yáñez, Díaz, & Romo, 2003). Table 2 summarizes the works that studied the association between chitosan and essential oils.

Table 2
Association of essential oils with chitosan.

Essential Oil	Formulation	Application	Year of publication
Anise, basil, coriander and oregano oils	Chitosan films	Food preservation	2005
Citronella oil	Chitosan microcapsules	Study of method	2006
Oregano oil	Chitosan films	Food preservation	2006
Mentha piperita	Chitosan beads	Cosmetics	2006
Zanthoxylum limonella	Chitosan–gelatin microcapsules	Mosquito repellent	2008

Popa, Aelenei, Popa, and Andrei (2000) evaluated the interaction between chitosan and polyphenols separated from spruce wood bark and demonstrated that the chitosan and the polyphenols formed a stable complex and the release of the polyphenols occurred only in an alkaline medium (pH 9). Analysis of the release of the polyphenols showed that, in the initial stage, the diffusion occurs according to the Fick's law, while in the last stage, the process develops according to zero-order kinetics. Hence, the association between chitosan and essential oils may be suitable for further enhancing the antimicrobial properties of chitosan.

Zivanovic et al. (2005) associated various essential oils to chitosan films for studding their application on food conservation. By using an *in vitro* assay, the authors demonstrated that the intensity of antimicrobial efficacy of essential oils against *Listeria monocytogenes* and *E. coli* was in the following order: oregano >> coriander > basil > anise. The chitosan films and chitosan–oregano essential oil films were applied on inoculated bologna samples and stored 5 days at 10 °C. Pure chitosan films reduced the pathogens counts by 2 logs, whereas the films with 1% and 2% oregano essential oil decreased the numbers of *L. monocytogenes* by 3.6–4 logs, respectively, and *E. coli* by 3 logs. The other essential oils presented a weak antimicrobial activity when associated with the films. The same group showed the carvacrol is the dominant compound in oregano essential oil with 59.57% of total volatiles detected. It was also demonstrated that the oregano oil associated with chitosan films is suitable for meat preservation by fat and moisture absorption and sustained release of the antimicrobial compound, carvacrol, into the product (Chami et al., 2005; Chi, Zivanovic, & Penfield, 2006).

By using a modified emulsification technique, Hsieh, Chang, and Gao (2006) produced chitosan microparticles entrapping citronella oil, which evidenced different particle sizes, ranging from 11 ± 3 μm to 225 ± 24 μm. The smallest microparticles showed the biggest release rate, probably because they had a larger specific surface area, causing the oil release rate to be faster. It was observed that the formulation with the lowest chitosan concentration (0.2% wt) showed the lowest association efficiency, due to the great amount of citronella oil floating on the top layer of the emulsion. The low chitosan concentration provided too thin wall membranes for microparticles avoiding the oil cover. At highest chitosan concentration (1.5% wt), no sign of citronella oil was detected on the surface of the emulsion. Besides, this work showed that if the microparticles were pretreated in 80 °C, the chitosan wall membrane would shrink, leading to difficulties in the oil release.

For cosmetic application Anchisi, Meloni, and Maccioni (2006) produced, by coacervation and ionotropic gelation techniques, chitosan beads loaded with *Mentha piperita* essential oil. The bead batches showed encapsulation efficiency in the 65–70% range, with a significant difference in the size of dried and wet beads because the swelling properties of chitosan in aqueous media. The average size ranged from 1.15 ± 0.02 to 3.94 ± 0.32. Chitosan beads gelled with TPP were more stable and resistant than those produced by coacervation in cosmetic formulation.

The *Zanthoxylum limonella* essential oil was encapsulated by Hussain and Maji (2008), by using a natural and non-toxic chemical cross-linker, genipin (Yuan et al., 2007) after complex coacervation between chitosan and gelatin. Complex microcapsules were obtained with encapsulation efficiency ranging from 48.36 ± 1.52% to 51.60 ± 3.47%. The evaluation of chitosan–gelatin ratio showed that the release rate of oil from the microcapsules was dependent of the percentage of chitosan. The higher chitosan percentage provided the lower release rate. The study also showed interactions between chitosan and gelatin, but no interaction with the oil.

The data discussed above and summarized in Table 2 reveal the reduced number of works on association of essential oils to chito-

san or its derivates, which were not developed for biomedical applications. Furthermore, the other polymeric matrices such as gelatin (Maji, Baruah, Dube, & Hussain, 2007; Passino, Bazzoni, & Moretti, 2004), β-cyclodextrin (Ayala-Zavala et al., 2008) and starch (Jeon, Vasanthan, Temelli, & Song, 2003), applied for essential oil encapsulation, are usually designed for insect pest control and food preservation. A few number of these formulations has therapeutic proposes (Chang, Leung, Lin, & Hsu, 2006; Lai et al., 2007), among which the glutaraldehyde use, for chemical crosslinking, is frequently reported. Glutaraldehyde is classified as toxic substance, and repeated exposure causes irritation of eyes, nose, throat or skin resulting in dermatitis and asthma (Fürst & Banerjee, 2005). Although FDA suggested 1% glutaraldehyde solution for crosslinking the gels for biomedical applications, the results obtained from *in vitro* cytotoxity tests by Rathna (2008) indicated that with the increase of incubation time, a significant number of live cells tend to decrease.

8. Conclusion

The reduced efficacy of actual medicines in treating infections in oral cavity is a relevant challenge, mainly in cases of immunossupression. Concerning to this, chitosan has been showed as an interesting option for entrapping antimicrobial agents for oral cavity care. Chitosan is a natural, biocompatible and biodegradable polymer with a suitable mucoadhesive profile for combating the flushing effect of saliva and mastication. Furthermore, several studies have indicated antimicrobial activity of chitosan.

The increasing number of resistant bacteria and fungi to synthetic and natural purified drugs justifies the research for sources of new drugs. Thus, essential oils have shown great importance, due to the large range of substances with antibacterial and antifungal activity obtained from them. However, the development of pharmaceutical formulations for achieving stability and efficacy of essential oils has been another challenge. To overcome these limitations, the encapsulation technology can be a good option to guarantee protection to the essential oils avoiding oxidation reactions and more efficacy for establishing their sustained and controlled release. However, there are few studies with the association of chitosan with essential oils. Therefore, more studies have to be performed for developing and characterizing new formulations taking benefit of the potential of chitosan for essential oil entrapment.

References

Aksungur, P., Sungur, A., Ünal, S., Iskit, A. B., Squier, C. A., & Senel, S. (2004). Chitosan delivery systems for the treatment of oral mucositis: *In vitro* and *in vivo* studies. *Journal of Controlled Release, 98*, 269–279.

Anal, A. K., Stevens, W. F., & Remunan-Lopez, C. (2006). Ionotropic cross-linked chitosan microspheres for controlled release of ampicillin. *International Journal of Pharmaceutics, 312*, 166–173.

Anchisi, C., Meloni, M. C., & Maccioni, A. M. (2006). Chitosan beads loaded with essential oils in cosmetic formulations. *Journal of Cosmetic Science, 57*, 205–214.

Ayala-Zavala, J. F., Soto-Vaeldez, H., González-León, A., Álvarez-Parrilla, E., Martín-Belloso, O., & González-Aguilar, G. A. (2008). Microencapsulation of cinnamon leaf (*Cinnamum zeylanicum*) and garlic (*Allium sativum*) oils in β-cyclodextrin. *Journal of Inclusion Phenomena and Macrocyclic Chemistry, 60*, 359–368.

Azcurra, A. I., Barembaum, S. R., Bojanich, M. A., Calamari, S. E., Aguilar, J., Battellino, L. J., et al. (2006). Effect of the high molecular weight chitosan and sodium alginate on *Candida albicans* hydrophobicity and adhesion to cells. *Medicina Oral Patologia Oral y Cirurgía Bucal, 11*, E120–E125.

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils – A review. *Food and Chemical Toxicology, 46*, 446–475.

Baytukalov, T. A., Bogoslovskaya, O. A., Olkhovskaya, I. P., Glushchenko, N. N., Ovsyannikova, M. N., Lopatin, S. A., et al. (2005). Regenerating activity and antibacterial effect of low molecular weight chitosan. *Biology Bulletin, 32*, 545–548.

Bernardo, M. V., Blanco, M. D., Sastre, R. L., Teijón, C., & Teijón, J. M. (2003). Sustained release of bupivacaine from devices based on chitosan. *Il Farmaco, 58*, 1187–1191.

Borchard, G., Lueβen, H. L., de Boer, A. G., Verhoef, J. C., Lehr, C.-M., & Junginger, H. E. (1996). The potential of mucoadhesive polymers in enhancing intestinal peptide drug absorption. III: Effects of chitosan–glutamate and carbomer on epithelial tight junctions *in vitro*. *Journal of Controlled Release, 39*, 131–138.

Brehm-Stecher, B. F., & Johnson, E. A. (2003). Sensitization of *Staphylococcus aureus* and *Escherichia coli* to antibiotics by the sesquiterpenoids nerolidol, farnesol, bisabolol, and apritone. *Antimicrobial Agents and Chemotherapy, 47*, 3357–3360.

Bustos, R., Romo, L., Yáñez, K., Díaz, G., & Romo, C. (2003). Oxidative stability of carotenoid pigments and polyunsaturated fatty acids in microparticulate diets containing krill oil for nutrition of marine fish larvae. *Journal of Food Engineering, 56*, 289–293.

Campaniello, D., Bevilacqua, A., Sinigaglia, M., & Corbo, M. R. (2008). Chitosan: Antimicrobial activity and potential applications for preserving minimally processed strawberries. *Food Microbiology, 25*, 992–1000.

Chami, N., Bennis, S., Chami, F., Aboussekhra, A., & Remmal, A. (2005). Study of anticandidal activity of carvacrol and eugenol *in vitro* and *in vivo*. *Oral Microbiology Immunology, 20*, 106–111.

Chang, C. P., Leung, T. K., Lin, S. M., & Hsu, C. C. (2006). Release properties on gelatin-gum arabic microcapsules containing camphor oil with added polystyrene. *Colloids and Surfaces B: Biointerfaces, 50*, 136–140.

Charles, C. H., Pan, P. C., Sturdivant, L., & Vincent, J. W. (2000). *In vivo* antimicrobial activity of an essential oil-containing mouthrinse on interproximal plaque bacteria. *Journal of Clinical Dentistry, 11*, 94–97.

Charles, C. H., Sharma, N. C., Galustians, H. J., Qaqish, J., McGuire, J. A., & Vincent, J. W. (2001). Comparative efficacy of an antiseptic mouthrinse and an antiplaque/antigingivitis dentifrice. A six-month clinical trial. *Journal of the American Dental Association, 132*, 670–675.

Chi, S., Zivanovic, S., & Penfield, M. P. (2006). Application of chitosan films enriched with oregano essential oil on bologna – active compounds and sensory attributes. *Food Science and Technology International, 12*, 111–117.

Claffey, N. (2003). Essential oil mouthwashes: A key component in oral health management. *Journal of Clinical Periondotology, 30*, 22–24.

Cölfen, H., Harding, S. E., Varum, K. M., & Winzor, D. J. (1996). A study by analytical ultracentrifugation on the interaction between lysozyme and extensively deacetylated chitin (chitosan). *Carbohydrate Polymers, 30*, 45–63.

Costantino, H. R., Illum, L., Brandt, G., Johnson, P. H., & Quay, S. C. (2007). Intranasal delivery: Physicochemical and therapeutic aspects. *International Journal of Pharmaceutics, 337*, 1–24.

Dhawan, S., Singla, A. K., & Sinha, V. R. (2004). Evaluation of mucoadhesive properties of chitosan microspheres prepared by different methods. *AAPS PharmSciTech, 5*, 1–7.

Diarra, M., Pourroy, G., Muster, D., Zingraff, M., & Boymond, C. (1998). Elaboration and evaluation of an intraoral controlled release delivering system. *Biomaterials, 19*, 1523–1527.

Diaz del Consuelo, I., Falson, F., Guy, R. H., & Jacques, Y. (2007). *Ex vivo* evaluation of bioadhesive films for buccal delivery of fentanyl. *Journal of Controlled Release, 122*, 135–140.

Dodane, V., Amin Khan, M., & Merwin, J. R. (1999). Effect of chitosan on epithelial permeability and structure. *International Journal of Pharmaceutics, 182*, 21–32.

El-Kamel, A. H., Ashri, L. Y., & Alsarra, I. A. (2007). Micromatricial metronidazole benzoate film as a local mucoadhesive delivery system for treatment of periodontal diseases. *AAPS PharmSciTech, 8*, E3–E11.

Fernandez-Saiz, P., Lagaron, J. M., & Ocio, M. J. (2009). Optimization of the biocide properties of chitosan for its application in the design of active films of interest in the food area. *Food Hydrocolloids, 23*, 913–921.

Fine, D. H., Markowitz, K., Furgang, D., Goldsmith, D., Charles, C. H., Lisante, T. A., et al. (2007). Effect of an essential oil-containing antimicrobial mouthrinse on specific plaque bacteria *in vivo*. *Journal of Clinical Periodontology, 34*, 652–657.

Fürst, W., & Banerjee, A. (2005). Release of glutaraldehyde from an albumin–glutaraldehyde tissue adhesive causes significant *in vitro* and *in vivo* toxicity. *The Annals of Thoracic Surgery, 79*, 1522–1528.

Gavini, E., Hegge, A. B., Rassu, G., Sanna, V., Testa, C., Pirisino, G., et al. (2006). Nasal administration of carbamazepine using chitosan microspheres: *In vitro*/*in vivo* studies. *International Journal of Pharmaceutics, 307*, 9–15.

Geresh, S., Gdalevsky, G. Y., Gilboa, I., Voorspoels, J., Remon, J. P., & Kost, J. (2004). Bioadhesive grafted starch copolymers as platforms for peroral drug delivery: A study of theophylline release. *Journal of Controlled Release, 94*, 391–399.

Giunchedi, P., Juliano, C., Gavini, E., Cossu, M., & Sorrenti, M. (2002). Formulation and *in vivo* evaluation of chlorhexidine buccal tablets prepared using drug-loaded chitosan microspheres. *European Journal of Pharmaceutics and Biopharmaceutics, 53*, 233–239.

Grabovac, V., Guggi, D., & Bernkop-Schnürch, A. (2005). Comparison of the mucoadhesive properties of various polymers. *Advanced Drug Delivery Reviews, 57*, 1713–1723.

Grenha, A., Grainger, C. I., Dailey, L. A., Seijo, B., Martin, G. P., Remunan-Lopez, C., et al. (2007). Chitosan nanoparticles are compatibles with respiratory epithelial cells *in vitro*. *European Journal of Pharmaceutical Sciences, 31*, 73–84.

Gunsolley, J. C. (2006). A meta-analysis of six-month studies of antiplaque and antigingivitis agents. *Journal of the American Dental Association, 137*, 1649–1657.

Hammer, K. A., Carson, C. F., Riley, T. V., & Nielsen, J. B. (2006). A review of the toxicity of *Melaleuca alternifolia* (tea tree) oil. *Food and Chemical Toxicology, 44*, 616–625.

Hammer, K. A., Dry, L., Johnson, M., Michalak, E. M., Carson, C. F., & Riley, T. V. (2003). Susceptibility of oral bacteria to *Melaleuca alternifolia* (tea tree) oil *in vitro*. *Oral Microbiology Immunology, 18*, 389–392.

He, P., Davis, S. S., & Illum, L. (1998). *In vitro* evaluation of the mucoadhesive properties of chitosan microspheres. *International Journal of Pharmaceutics, 166*, 75–88.

Hejazi, R., & Amiji, M. (2003). Chitosan-based gatrointestinal delivery systems. *Journal of Controlled Release, 89*, 151–165.

Helander, I. M., Nurmiaho-Lassila, E. L., Ahvenainen, R., Rhoades, J., & Roller, S. (2001). Chitosan disrupts the barrier properties of the outer membrane of Gram-negative bacteria. *International Journal of Food Microbiology, 71*, 235–244.

Hernández-Lauzardo, A. N., Bautista-Baños, S., Velázquez-del Valle, M. G., Méndez-Montealvo, M. G., Sánchez-Rivera, M. M., & Bello-Pérez, L. A. (2008). Antifungal effects of chitosan with different molecular weights on *in vitro* development of *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill. *Carbohydrate Polymers, 73*, 541–547.

Hsieh, W.-C., Chang, C.-P., & Gao, Y.-L. (2006). Controlled release properties of chitosan encapsulated volatile citronella oil microcapsules by thermal treatments. *Colloids and Surfaces B: Biointerfaces, 53*, 209–214.

Hussain, M. R., & Maji, T. K. (2008). Preparation of genipin cross-linked chitosan-gelatin microcapsules for encapsulation of *Zanthoxylum limonella* oil (ZLO) using salting-out method. *Journal of Microencapsulation*, 1–7.

Ikinci, G., Senel, S., Akincibay, H., Kas, S., Ercis, S., Wilson, C. G., et al. (2002). Effect of chitosan on a periodontal pathogen *Porphyromonas gingivalis*. *International Journal of Pharmaceutics, 235*, 121–127.

Illum, L. (1998). Chitosan and its use as a pharmaceutical excipient. *Pharmaceutical Research, 15*, 1326–1331.

Jaki, B. U., Franzblau, S. G., Chadwick, L. R., Lankin, D. C., Zhang, F., Wang, Y. & Pauli, G. F. (in press). Purity–activity relationships of natural products: The case of anti-TB active ursolic acid. *Journal of Natural Products*.

Jeon, Y. J., Vasanthan, T., Temelli, F., & Song, B. K. (2003). The suitability of barley and corn starches in their native and chemically modified forms for volatile meat flavor encapsulation. *Food Research International, 36*, 349–355.

Knapczyk, J. (1993). Excipient ability of chitosan for direct tableting. *International Journal of Pharmaceutics, 89*, 1–7.

Kockisch, S., Rees, G. D., Tsibouklis, J., & Smart, J. D. (2005). Mucoadhesive, triclosan-loaded polymer microspheres for application to the oral cavity: Preparation and controlled release characteristics. *European Journal of Pharmaceutics and Biopharmaceutics, 59*, 207–216.

Kockisch, S., Rees, G. D., Young, S. A., Tsibouklis, J., & Smart, J. D. (2003). Polymeric microspheres for drug delivery to the oral cavity: An *in vitro* evaluation of mucoadhesive potential. *Journal of Pharmaceutical Sciences, 92*, 1614–1623.

Kockisch, S., Rees, G. D., Young, S. A., Tsibouklis, J., & Smart, J. D. (2004). *In situ* evaluation of drug-loaded microspheres on a mucosal surface under dynamic test conditions. *International Journal of Pharmaceutics, 276*, 51–58.

Kong, M., Chen, X. G., Liu, C. S., Liu, C. C., Meng, X. H., & Yu, L. J. (2008). Antibacterial mechanism of chitosan microspheres in a solid dispersing system against *E. coli*. *Colloids and Surfaces B: Biointerfaces, 65*, 197–202.

Kumarasamy, Y., Cox, P. J., Jaspars, M., Nahar, L., & Sarker, S. D. (2002). Screening seeds of Scottish plants for antibacterial activity. *Journal of Ethnopharmacology, 83*, 73–77.

Lai, F., Loy, G., Manconi, M., Manca, M. L., & Fadda, A. M. (2007). *Artemisia arborescens* L essential oil loaded beads: Preparation and characterization. *AAPS PharmSciTech, 8*, E1–E7.

Lehr, C. M., Bouwstra, J. A., Schacht, E. H., & Junginger, H. E. (1992). *In vitro* evaluation of mucoadhesive properties of chitosan and some other natural polymers. *International Journal of Pharmaceutics, 78*, 43–48.

Lin, L.-C., Chang, S. J., Chen, S. F., Chou, Y. J., & Kuo, S. M. (2006). Effects of pH $Na_5P_3O_{10}$/NaOH reaction solution on the properties of chitosan microspheres. *Biomedical Engineering Applications, Basis and Communications, 18*, 167–177.

Liu, N., Chen, X.-G., Park, H.-J., Liu, C.-G., Liu, C.-S., Meng, X.-H., et al. (2006). Effect of MW and concentration of chitosan on antibacterial activity of *Escherichia coli*. *Carbohydrate Polymers, 64*, 60–65.

Madene, A., Jacquot, M., Scher, J., & Desobry, S. (2006). Flavour encapsulation and controlled release – A review. *International Journal of Food Science and Technology, 41*, 1–21.

Maji, T. K., Baruah, I., Dube, S., & Hussain, M. R. (2007). Microencapsulation of *Zanthoxylum limonella* oil (ZLO) in glutaraldehyde crosslinked gelatin for mosquito repellent application. *Bioresource Technology, 98*, 840–844.

Majumbar, S., Hippalgaonkar, K., & Repka, M. A. (2008). Effect of chitosan, benzalkonium chloride and ethylenediaminetetraacetic acid on permeation of acyclovir across isolated rabbit cornea. *International Journal of Pharmaceutics, 348*, 175–178.

Martin, L., Wilson, C. G., Koosha, F., & Uchegbu, I. F. (2003). Sustained buccal delivery of the hydrophobic drug denbufylline using physically cross-linked palmitoyl glycol chitosan hydrogels. *European Journal of Pharmaceutics and Biopharmaceutics, 55*, 35–45.

Medlicott, N. J., Holborow, D. W., Rathbone, M. J., Jones, D. S., & Tucker, I. G. (1999). Local delivery of chlorhexidine using a tooth-bonded delivery system. *Journal of Controlled Release, 61*, 337–343.

Mourya, V. K., & Inamdar, N. N. (2008). Chitosan-modifications and applications: Opportunities galore. *Reactive and Functional Polymers, 68*, 1013–1051.

Munday, D. L., & Cox, P. J. (2000). Compressed xanthan and karaya gum matrices: Hydration, erosion and drug release mechanisms. *International Journal of Pharmaceutics, 203*, 179–192.

Neumann, M., & Garcia, N. A. (1882). Kinetics and mechanism of the light-induced deterioration of lemon oil. *Journal of Agricultural and Food Chemistry, 40*, 957–960.

No, H. K., Park, N. Y., Lee, S. H., & Meyers, S. P. (2002). Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. *International Journal of Food Microbiology, 74*, 65–72.

Nunthanid, J., Laungtana-anan, M., Sriamornsak, P., Limmatvapirat, S., Puttipipatkhachorn, S., Lim, L. Y., et al. (2004). Characterization of chitosan acetate as a binder for sustained release tablets. *Journal of Controlled Release, 99*, 15–26.

Passino, G. S., Bazzoni, E., & Moretti, M. D. L. (2004). Microencapsulated essential oils active against indianmeal moth. *Boletín de Sanidad Vegetal. Plagas, 30*, 125–132.

Patel, D., Smith, A. W., Grist, N., Barnett, P., & Smart, J. D. (1999). An *in vitro* mucosal model predictive of bioadhesive agents in the oral cavity. *Journal of Controlled Release, 61*, 175–183.

Peniche, C., Argüelles-Monal, W., Peniche, H., & Acost, N. (2003). Chitosan: An attractive biocompatible polymer for microencapsulation. *Macromolecular Bioscience, 3*, 511–520.

Popa, M.-I., Aelenei, N., Popa, V. I., & Andrei, D. (2000). Study of the interactions between polyphenolic compounds and chitosan. *Reactive and Functional Polymers, 45*, 35–43.

Portero, A., Teijeiro-Osorio, D., Alonso, M. J., & Remunan-Lopez, C. (2007). Development of chitosan sponges for buccal administration of insulin. *Carbohydrate Polymers, 68*, 617–625.

Qaqish, R. B., & Amiji, M. M. (1999). Synthesis of a fluorescent chitosan derivative and its application for the study of chitosan-mucin interactions. *Carbohydrate Polymers, 38*, 99–107.

Qin, C., Li, H., Xiao, Q., Liu, Y., Zhu, J., & Du, Y. (2006). Water-solubility of chitosan and its antimicrobial activity. *Carbohydrate Polymers, 63*, 367–374.

Rabanal, R. M., Arias, A., Prado, B., Hernández-Pérez, M., & Sánchez-Mateo, C. C. (2002). Antimicrobial studies on three species of *Hypericum* from the Canary Islands. *Journal of Ethnopharmacology, 81*, 287–292.

Rahman, M., Kühn, I., Rahman, M., Olsson-Liljequist, B., & Möllby, R. (2004). Evaluation of a scanner-assisted colorimetric MIC method for susceptibility testing of Gram-negative fermentative bacteria. *Applied and Environmental Microbiology, 70*, 2398–2403.

Rao, M. S., Munoz, J., & Stevens, W. F. (2000). Critical factors in chitin production by fermentation of shrimp biowaste. *Applied Microbiology and Biotechnology, 54*, 808–813.

Rathna, G. V. (2008). Gelatin hydrogels: Enhanced biocompatibility, drug release and cell viability. *Journal of Material Sciences: Materials and Medicine, 19*, 2351–2358.

Ravi Kumar, M. N. V. (2000). A review of chitin and chitosan applications. *Reactive and Functional Polymers, 46*, 1–27.

Roldán, S., Herrera, D., & Sanz, M. (2003). Biofilms and the tongue: Therapeutical approaches for the control of halitosis. *Clinical Oral Investigations, 7*, 189–197.

Sahoo, S. K., Dilnawaz, F. & Krishnakumar, S. (2008). Nanothecnology in ocular drug delivery. *Drug Discovery Today, 13*, 144–151..

Sato, M., Fujiwara, S., Tsuchiya, H., Fujii, T., Iinuma, M., Tosa, H., et al. (1996). Flavones with antibacterial activity against cariogenic bacteria. *Journal of Ethnopharmacology, 54*, 171–176.

Schipper, N. G. M., Varum, K. M., & Artursson, P. (1996). Chitosans as absorption enhancers of poorly absorbable drugs: Influence of molecular weight and degree of acetylation. *European Journal of Pharmaceutical Sciences, 4*, S153.

Sebti, I., Martial-Gros, A., Carnet-Pantiez, A., Grelier, S., & Coma, V. (2005). Chitosan polymer as bioactive coating and film against *Aspergillus niger* contamination. *Food Microbiology and Safety, 70*, M100–M104.

Şenel, S., & Hincal, A. A. (2001). Drug permeation enhancement via buccal route: Possibilities and limitations. *Journal of Controlled Release, 72*, 133–144.

Şenel, S., Kremer, M. J., Kaş, S., Wertz, P. W., Hincal, A. A., & Squier, C. A. (2000). Enhancing effect of chitosan on peptide drug delivery across buccal mucosa. *Biomaterials, 21*, 2067–2071.

Shapiro, S., Meier, A., & Guggenheim, B. (1994). The antimicrobial activity of essential oils and essential oil components towards oral bacteria. *Oral Microbiology Immunology, 9*, 202–208.

Sharma, N., Charles, C. H., Lynch, M. C., Qaqish, J., McGuire, J. A., Galustians, J. G., et al. (2004). Adjunctive benefit of an essential oil-containing mouthrinse in reducing plaque and gingivitis in patients who brush and floss regularly: A six-month study. *Journal of the American Dental Association, 135*, 496–504.

Sinha, V. R., Singla, A. K., Wadhawan, S., Kaushik, R., Kumria, R., Bansal, K., et al. (2004). Chitosan microspheres as a potential carrier for drugs. *International Journal of Pharmaceutics, 274*, 1–33.

Sköld, M., Börje, A., Matur, M., & Karlberg, A.-T. (2002). Studies on the autoxidation and sensitizing capacity of the fragrance chemical linalool, identifying a linalool hydroperoxide. *Contact Dermatitis, 46*, 267–272.

Takarada, K., Kimizuka, R., Takahashi, N., Honma, K., Okuda, K., & Kato, T. (2004). A comparison of the antibacterial efficacies of essential oils against oral pathogens. *Oral Microbiology Immunology, 19*, 61–64.

Takeuchi, H., Matsui, Y., Yamamoto, H., & Kawashima, Y. (2003). Mucoadhesive properties of carbopol or chitosan-coated liposomes and their effectiveness in the oral administration of calcitonin to rats. *Journal of Controlled Release, 86*, 235–242.

Thanou, M., Verhoef, J. C., & Junginger, H. E. (2001). Oral drug absorption enhancement by chitosan and its derivatives. *Advanced Drug Delivery Reviews, 52*, 117–126.

Tikhonov, V. E., Stepnova, E. A., Babak, V. G., Yamskov, I. A., Palma-Guerrero, J., Jansson, H. B., et al. (2006). Bactericidal and antifungal activities of a low

molecular weight chitosan and its N-/2(3)-(dodec-2-enyl)succinoyl/-derivates. *Carbohydrate Polymers, 64*, 66–72.

Tomihata, K., & Ikada, Y. (1997). *In vitro* and *in vivo* degradation of films of chitin and its deacetylated derivates. *Biomaterials, 18*, 567–575.

Upadrashta, S. M., Katikaneni, P. R., & Nuessle, N. O. (1992). Chitosan as a tablet binder. *Drug Development and Industry Pharmacy, 18*, 1701–1708.

Vagionas, K., Graikou, K., Ngassapa, O., Runyoro, D., & Chinou, I. (2007). Composition and antimicrobial activity of the essential oils of three *Satureja* species growing in Tanzania. *Food Chemistry, 103*, 319–324.

Ventura, C. A., Tommasini, S., Crupi, E., Giannone, I., Cardile, V., Musumeci, T., et al. (2008). Chitosan microspheres for intrapulmonary administration of moxifloxacin: Interaction with biomembrane models and *in vitro* permeation studies. *European Journal of Pharmaceutics and Biopharmaceutics, 68*, 235–244.

Yuan, Y., Chesnutt, B. M., Utturkar, G., Haggard, W. O., Yang, Y., Ong, J. L., et al. (2007). The effect of cross-linking chitosan microspheres with genipin on protein release. *Carbohydrate Polymers, 68*, 561–567.

Yuan, X. B., Yuan, Y. B., Jiang, W., Liu, J., Tian, E. J., Shun, H. M., et al. (2008). Preparation of rapamycin-loaded chitosan/PLA nanoparticles for immunossupression in corneal transplantation. *International Journal of Pharmaceutics, 349*, 241–248.

Zivanovic, S., Chi, S., & Draughon, A. F. (2005). Antimicrobial activity of chitosan films enriched with essential oils. *Journal of Food Science, 70*, M45–M51.