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Chitosan-alginate nanocapsules for encapsulation of turmeric oil

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Turmeric oil is widely used in pharmaceutical and cosmetic applications because of its antibacterial, antifungal, antioxidant, and insect-repellent properties. However, turmeric oil is volatile, insoluble in water and unstable in certain environments, which causes difficulties with formulation development and stability of new products. One approach to overcome these problems is to encapsulate turmeric oil in carriers formed from naturally occurring polysaccharides. Among such polysaccharides, chitosan and alginate have been widely used as particulate carriers for encapsulation and controlled release of bioactive compounds. The potential for size reduction of the carriers to the nanometer scale is of particular interest for delivery systems. In this review, we provide an overview of the versatile properties of turmeric oil and discuss the use of alginate and chitosan for capsule formation and encapsulation of turmeric oil in chitosan-alginate nanocapsules. We also discuss the *in vitro* skin permeation of turmeric oil from nanocapsules.

1. Introduction

Turmeric (*Curcuma longa* L., Zingiberaceae) has long been used as a condiment and folk medicine in Southern Asia. The rhizome of turmeric contains curcuminoid compounds, turmeric oil, oleoresin and other components (Leung 1980). Turmeric oil is an essential oil commonly used in food, cosmetic and pharmaceutical applications due to its antimicrobial, anti-inflammatory, antioxidant and insect repellent properties (Jayaprakasha et al. 2002; Negi et al. 1999; Roth et al. 1998). However, turmeric oil is unstable, volatile and insoluble in water, and this has restricted its use as a therapeutic agent. Nanoparticle technology could serve as a potential approach for protecting turmeric oil from environmental effects through encapsulation or entrapment of the oil in nanoparticles. This approach is also attractive for delivery systems, since a particle size on the nanometer scale is effective for targeting of organs.

Nanoparticles including nanospheres and nanocapsules vary in size from 10 to 1000 nm (Hamidi et al. 2008). Nanocapsules are vesicular systems in which the drug is maintained within a cavity surrounded by a polymer membrane. The most common method of nanocapsule formation is by interfacial deposition of polymers with subsequent solvent displacement (Fessi et al. 1989). Polymers for nanocapsule formation are required to be biodegradable, biocompatible and safe in humans, and naturally occurring polysaccharides such as alginate and chitosan meet these requirements.

In this review, we provide an overview of the properties of turmeric oil, the use of alginate and chitosan for encapsulation of turmeric oil, and a discussion of *in vitro* skin permeation by turmeric oil from nanocapsules.

2. Turmeric oil

Turmeric oil can be extracted from the dried rhizome of turmeric by steam distillation followed by extraction with volatile solvents (Manzan et al. 2003), supercritical carbon dioxide (SC-CO_2) extraction (Chang et al. 2006), or SC-CO_2 combined with co-solvent addition (Braga et al. 2003). Using GC-MS, He et al. (1998) identified the major constituents of turmeric oil as ar-turmerone, α-turmerone and β-turmerone. The structures of these compounds are shown in Fig. 1. We have determined the ar-turmerone content in commercially available turmeric oil using a reverse-phase HPLC method (Lertsutthiwong et al. 2008) with some modifications from Chang et al. (2006). Chromatography was performed using an Altima C18 column (4.6 mm × 150 mm, i.d., 5 μm) at 33 °C with isocratic elution of acetonitrile and water (65:35, v/v) at a flow rate of 1.0 ml/min. UV detection was set at 254 nm and the injection volume was 20 μl. The ar-turmerone was eluted at a retention time of 11.5 min in the chromatogram of commercial turmeric oil, consistent with the retention time of standard ar-turmerone (Fig. 2).

Turmeric oil has several biological properties that can be exploited pharmaceutically. For example, turmeric oil inhibits the growth of pathogenic molds and dermatophytes *in vitro*, but it is not irritable to the skin (Apisariyakul et al. 1995). Negi et al. (1999) showed that turmeric oil has antibacterial activity against gram positive bacteria (*Bacillus cereus, Bacillus coagulans, Bacillus subtilis*, *Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). Turmeric oil also possesses mosquitocidal (Roth et al. 1998), antioxidant (Jayaprakasha et al. 2002; Sacchetti et al. 2005) and anticarcinogenic (Aratanechemuge et al. 2002)

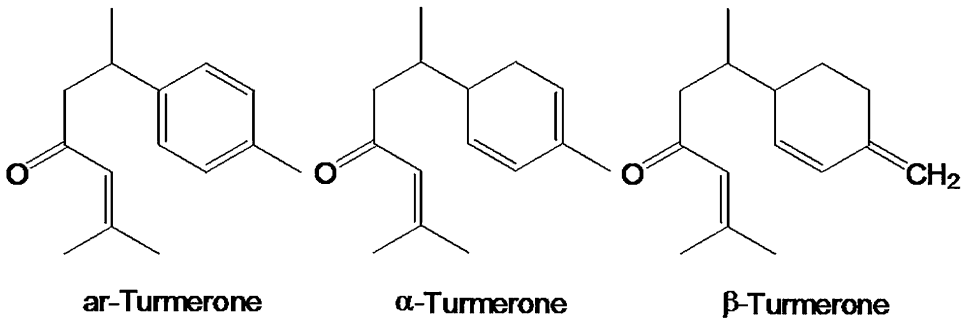


Fig. 1: Major constituents of turmeric oil

properties, and has a cytotoxic effect against many animal and human pathogens. This effect is probably due to penetration of the oil through the cytoplasmic membrane, with destruction of pathogen structures through subsequent leakage of fluid from the cell and resultant cell death (Bakkali et al. 2008).

3. Alginate

Alginate is a versatile biopolymer with a wide range of applications. It has been used as a matrix for bioactive compounds, an excipient in pharmaceutical preparations for local administration, and a rate-controlling excipient in drug delivery systems (Tønnesen and Karlsen 2002). Alginate is an anionic biopolymer that is mainly isolated from marine brown algae as a family of linear polysaccharides consisting of α-L-guluronic acid (G) and β-D-mannuronic acid (M) residues joined by 1,4-glycosidic linkages (Fig. 3) (George and Abraham 2006). The G and M segments are arranged in a pattern of blocks along the polymer chain, which varies in composition and sequence (Gombotz and Wee 1998).

Alginate is of interest in drug delivery systems due to its non-toxicity, biocompatibility, mucoadhesion and gelation (Tønnesen and Karlsen 2002). Alginate also has other unique properties that have enabled its use as a matrix for encapsulation and delivery of active compounds. These properties include a high gel porosity controlled by simple coating procedures, the absence of a requirement for organic solvents during the encapsulation process, and biodegradation under physiological conditions (Gombotz and Wee 1998).

Alginate forms a hydrogel with divalent or multivalent cations such as Ca^{2+} and Ba^{2+}. Due to the stronger affinity of divalent ions for G residues compared to M residues, the gel produced from G-rich alginate is more rigid and porous because of the orientation of the residues within an "egg-box" structure, whereas M-rich gels are weak and less porous because of their randomly packed structure (Smidsrød and Skjåk-Bræk 1990). Alginate hydrogels have many advantages for the development of nanoparticles, including those for encapsulation, controlled release and delivery of bioactive compounds to target organs. However, low capsule stability and loss of encapsulated active compounds have been observed with nanocapsules formed by an alginate polymer, and these problems may be solved by the use of cationic polymers such as chitosan as a stabilizer or crosslinking agent (Chandy et al. 1998).

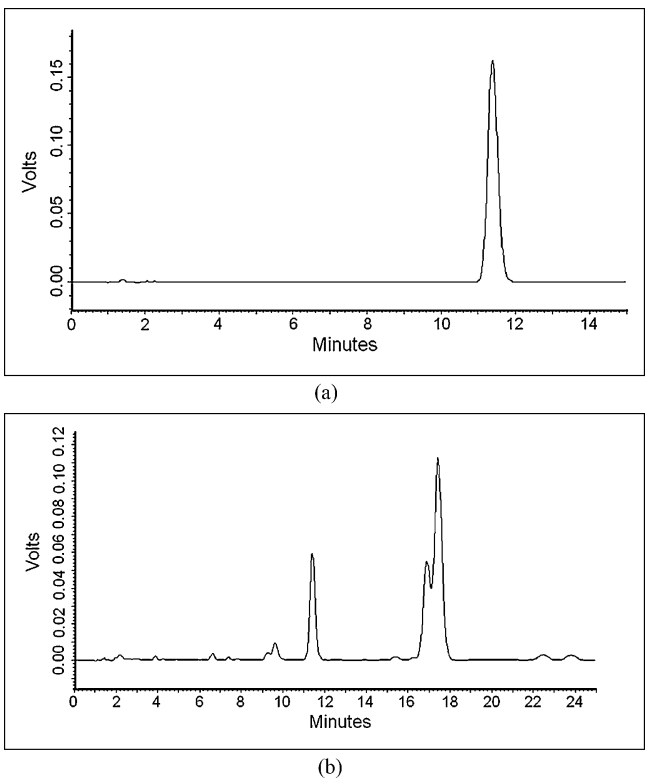


Fig. 2: HPLC chromatograms of standard ar-turmerone (a) and commercially available turmeric oil (b)

4. Chitosan

Chitosan is a naturally occurring polysaccharide that is mainly produced by alkaline deacetylation of chitin (Lertsutthiwong et al. 2002; Weska et al. 2007; Youn et al. 2007). Chitin is the second most abundant polymer in nature after cellulose, being the principal component of exoskeleton of crustaceans and cell walls of some fungi (Roberts 1992). In commercial chitosan production, shells of shrimp, crab or squid pen are deproteinated with diluted alkaline and demineralized with diluted acid, followed by deacetylation with a concentrated alkaline solution (Lertsutthiwong et al. 2002).

Structurally, chitosan is a linear cationic polymer consisting of D-glucosamine and *N*-acetyl-D-glucosamine units coupled by β-(1–4)-glycosidic linkages, as shown in Fig. 4 (Roberts 1992). This structure is similar to that of cellulose, except that the C2-hydroxyl group of cellulose is replaced by an acetamido or amino group. The relative proportions of D-glucosamine and N-acetyl-D-glucosamine residues account for the solubility, biological activity and biodegradability of chitosan (George and Abraham 2006). Under slightly acidic conditions, chitosan has a cationic character that allows it to interact with negatively charged surfaces and anionic polymers.

Chitosan is extensively used in drug delivery systems because of its non-toxic, biocompatible, biodegradable and mucoadhesive properties. Chitosan forms hydrogel particles from large to nanometer scale under relatively mild gelation conditions, and thus can be used for encapsulation of bioactive compounds (Anal et al. 2006; Borges et al. 2005; De and Robinson 2003; Sarmento et al. 2007). For example, Hsieh et al. (2006) pre-

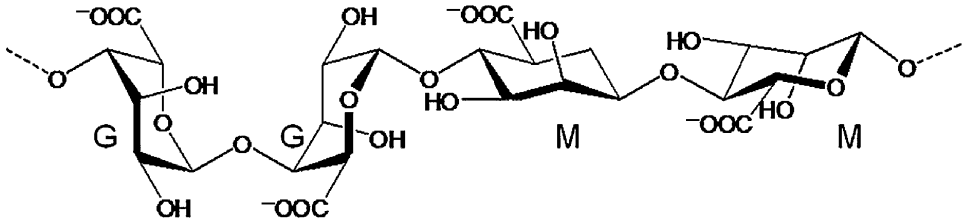


Fig. 3: Molecular structure of alginate

pared chitosan microparticles ranging in size from 11 to 225 μm with entrapped citronella oil using a modified emulsification technique. Smaller microparticles release the oil at a faster rate because of their larger specific surface area. Chitosan-alginate systems have been widely studied for nanoparticle formation for drug delivery, but only a few studies have examined encapsulation of oily compounds.

5. Process development for encapsulation of turmeric oil

Development of new formulations of essential oils has been limited because of their volatility, instability and poor water solubility. One approach to overcome these problems is to encapsulate the essential oils in biopolymeric matrices of sizes varying from large to nanometer in scale (Chang and Dobashi 2003; Lai et al. 2007; Mora-Huertas et al. 2010; Motwani et al. 2008; You et al. 2005). Several methods have been proposed for encapsulation of essential oils, including melt emulsification (Zhao et al. 2010, 2011), emulsification-diffusion, double emulsification, polymer-coating, layer-by-layer (Mora-Huertas et al. 2010), coacervation, ionotropic gelation (Pedro et al. 2009). We have recently reported the preparation of nanocapsules containing turmeric oil by emulsification using alginate and chitosan as wall materials (Lertsutthiwong et al. 2009). The preparation and characteristics of these turmeric oil-loaded nanocapsules are described below.

5.1. Preparation of turmeric oil-loaded nanocapsules

The preparation of nanocapsules for encapsulation of turmeric oil was performed using a simple protocol consisting of three steps: o/w emulsification, gelification and solvent removal. Briefly, the o/w emulsion was made by dispersion of an ethanolic solution of turmeric oil into appropriate volumes of alginate solution containing Tween 80®, with subsequent sonication. The emulsion was added dropwise to a suitable amount of calcium chloride and chitosan, and the mixture was stirred for an additional 30 min and equilibrated overnight before removal of the solvent under reduced pressure. Factors affecting the characteristics of the turmeric oil-loaded nanocapsules were investigated during the protocol development, with the following results.

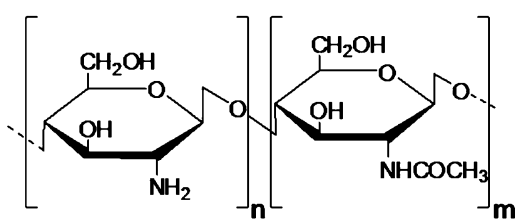
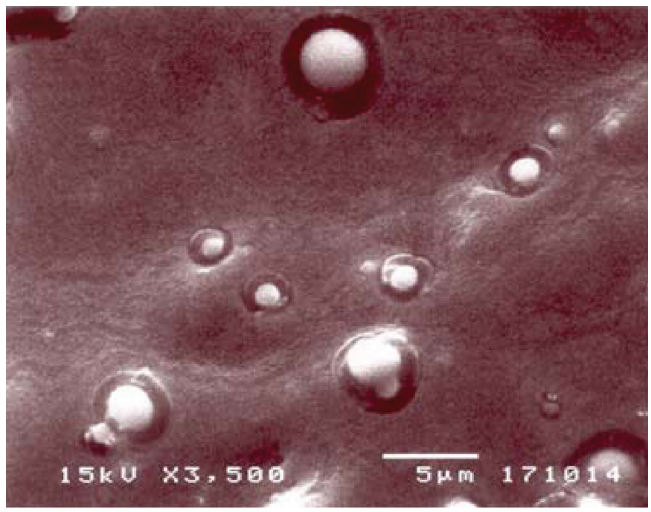


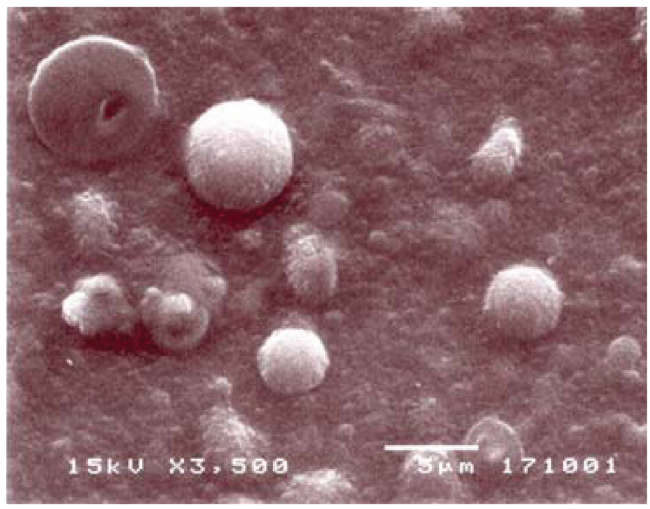
Fig. 4: Molecular structure of chitosan

5.2. Effect of Tween 80® on nanocapsule size

Firstly, the effect of Tween 80® on the morphology of turmeric oil-loaded nanocapsules prepared as above but without sonication and chitosan addition were investigated. SEM images of nanocapsules produced with or without Tween 80® are shown in Fig. 5. The images suggest that Tween 80® is required to produce a small capsule size. Zhao et al. (2010) also demonstrated that increasing Tween 80® concentration reduced the droplet size. It is of note that a wide range of sizes was observed when only mechanical stirring was used, resulting in nanocapsules of non-uniform size (Fig. 5). Sonication produced more uniform capsules with sizes on the nanometer scale (Lertsutthiwong et al. 2008). The optimal process for encapsulation of turmeric oil in nanocapsules included use of Tween 80® and sonication for 15 min after dispersion of turmeric oil in the alginate solution.



(a)



(b)

Fig. 5: SEM images of turmeric oil-loaded nanocapsules (oil/alginate/chitosan ratio, 1:1:0) produced using a stirring technique (a) with and (b) without Tween 80®

5.3. Effects of chitosan on the size and stability of nanocapsules

We recently investigated the effects of chitosan on the characteristics of turmeric oil-loaded alginate nanocapsules (Lertsutthiwong et al. 2009). Chitosan addition after calcium chloride produced significantly larger nanocapsules compared with the those prepared without chitosan (about 150 nm). Chitosan with an average molecular weight of $\sim 2.0 \times 10^5$ and $\sim 5.0 \times 10^5$ Dalton produced nanocapsule sizes of about 500 and 700 nm, respectively. This result is consistent with findings by Lu et al. (1999) that high-molecular weight chitosan generated larger nanocapsules compared with use of low-molecular weight chitosan. In addition, increasing the amount of chitosan in the formulation produced bigger nanocapsules. Prego et al. (2006) suggested that an increase in particle size was an indication of attachment of the polymers to the surface of the oil cores. Therefore, the increased size of nanocapsules containing chitosan may indicate a location of chitosan on the surface of the alginate polymer, mainly due to electrostatic interactions, and this may be useful for stabilization of the oil core. The turmeric oil-loaded chitosan alginate nanocapsules were stable for up to 4 months at 4 °C and 25 °C (Lertsutthiwong et al. 2009). This may be due to strong ionic interactions between alginate and chitosan resulting in formation of a polyelectrolyte complex, with consequent stabilization and reduction of the porosity of the alginate particles (Smidsrød and Skjåk-Bræk 1990).

5.4. Effect of the order of chitosan addition on nanocapsule size

Rajaonarivony et al. (1993) suggested that the order of addition of crosslinking agents during preparation of alginate nanocapsules had an effect on the size of the nanocapsules, and that the formation of alginate nanoparticles was strongly influenced by the nature of the first compound added into the alginate nanocapsule formulation. Therefore, we investigated the effects of the order of chitosan addition (before or after calcium chloride) on the preparation of turmeric oil-loaded nanocapsules using a fixed ratio of oil/alginate/chitosan at 1:1:0.1 (Lertsutthiwong et al. 2009). Interestingly, regardless of the molecular weight of chitosan, addition of chitosan after calcium chloride produced smaller nanocapsules compared to those obtained with chitosan added before calcium chloride. This may be due to the structure of complexes formed between alginate and either calcium or chitosan, as suggested by Sarmento et al. (2006). When calcium chloride is added as the first compound into the alginate solution, the interaction between calcium ions and alginate occurs at oligopolyguluronic sequences, and compact egg-box structures are formed. The addition of chitosan as the second compound then stabilizes this system to give small nanocapsules. On the other hand, when chitosan is added to the alginate as the first compound, the protonated amino groups of chitosan interact with alginate at oligopolymannuronic and random mannuronic-guluronic sequences, resulting in a randomly packed structure. Consequently, the size of nanocapsules formed by adding chitosan prior to calcium chloride was larger than for those produced by the opposite order of addition.

5.5. Effect of the order of chitosan addition on oil recovery

Use of chitosan in the process of nanocapsule formation gave better recovery of turmeric oil in the nanocapsule suspension compared to the process without chitosan, suggesting that chitosan prevented oil loss during nanocapsule preparation (Lertsutthiwong et al. 2009). The prevention of oil loss by addition of chitosan may be due to membrane formation by chitosan through ionic interactions between the positively charged amino groups of chitosan and the carboxylic residues of the alginate. The nanocapsules prepared with and without chitosan had similar loading capacities of turmeric oil, and the molecular weight and order of addition of chitosan had no significant effect on this parameter.

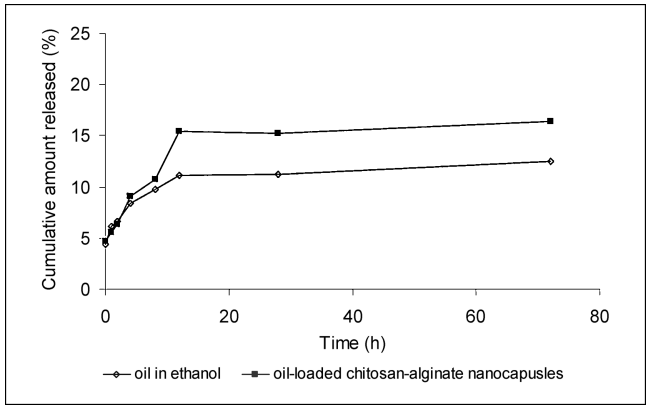


Fig. 6: Release profile of turmeric oil from chitosan-alginate nanocapsules permeating through shed snake skin compared with control (oil in ethanol)

6. *In vitro* skin permeation

The skin permeation of turmeric oil from turmeric oil-loaded chitosan-alginate nanocapsules was evaluated using a Franz diffusion cell with an effective diffusional area of 1.8 cm^2 mounted with cobra shed snake skin (*Naja kaouthia*). One ml of nanocapsule suspension containing about 500 μg of turmeric oil was added to the donor compartment of the Franz cell, and the receptor compartment was filled with phosphate-buffered saline (PBS) at pH 7.4 containing 0.25% sodium lauryl sulfate (SLS). The receptor medium was maintained at 37 °C with magnetic stirring. The medium (0.5 ml) was sampled at intervals and fresh medium was immediately replaced in the receptor compartment to keep the volume constant. The amount of turmeric oil in the withdrawn sample was analyzed using a UV spectrophotometer at 238 nm. The permeation data were plotted as the cumulative amount of turmeric oil that penetrated the membrane over time. Turmeric oil is insoluble in water, and therefore Sodium lauryl sulfate was used as a surfactant in the receptor medium to ensure pseudo-sink conditions. The amount of turmeric oil in the nanocapsule formulation that permeated through shed snake skin increased with time. About 15% of the turmeric oil in the nanocapsule suspension crossed the membrane over 12 h, whereas only 10% of oil from a control ethanolic solution permeated through the membrane (Fig. 6). However, the permeability of the turmeric oil from the nanocapsules was still relatively low. This may be due to the hydrophobicity of the oil, which may have caused it to be trapped in the shed snake skin membrane, with accumulation in the inner lipid-rich alpha layer rather than diffusion across the membrane (Itoh et al. 1990). However, nanocapsules formulated with alginate and chitosan still enhanced skin permeation of turmeric oil compared with that from an ethanolic solution. Therefore, the nanocapsules provide a new approach for transdermal delivery of turmeric oil, and optimization of the formulation may further improve the permeability characteristics.

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References

Anal AK, Stevens WF, Remuñán-López C (2006) Ionotropic cross-linked chitosan microspheres for controlled release of ampicillin. Int J Pharm 312: 166–173.

Apisariyakul A, Vanittanakom N, Buddhasukh D (1995) Antifungal activity of turmeric oil extracted from Curcuma longa (Zingiberaceae). J Ethnopharmacol 49: 163–169.

Aratanechemuge Y, Komiya T, Moteki H, Katsuzaki H, Imai K, Hibasami H (2002) Selective induction of apoptosis by ar-turmerone isolated from turmeric (*Curcuma longa L*) in two human leukemia cell lines, but not in human stomach cancer cell line. Int J Mol Med 9: 481–484.

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils – a review. Food Chem Toxicol 46: 446–475.

Borges O, Borchard G, Verhoef JC, de Sousa A, Junginger HE (2005) Preparation of coated nanoparticles for a new mucosal vaccine delivery system. Int J Pharm 299: 155–166.

Braga MEM, Leal PF, Carvalho JE, Meireles MAA (2003) Comparison of yield, composition, and antioxidant activity of turmeric (*Curcuma longa* L.) extracts obtained using various techniques. J Agri Food Chem 51: 6604–6611.

Chandy T, Mooradian DL, Rao GHR (1998) Chitosan/polyethyleneglycol-alginate microcapsules for oral deliver of hirudin. J Appl Polym Sci 70: 2143–2153.

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

Chang LH, Jong TT, Huang HS, Nien YF, Chang CMJ (2006) Supercritical carbon dioxide extraction of turmeric oil from *Curcuma longa* Linn and purification of turmerones. Sep Purif Technol 47: 119–125.

De S, Robinson D (2003) Polymer relationships during preparation of chitosan-alginate and poly-l-lysine-alginate nanospheres. J Control Release 89: 101–112.

Fessi H, Puisieux F, Devissaguet J Ph, Ammoury N, Benita S (1989) Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int J Pharm 55: R1–R4.

George M, Abraham TE (2006) Polyionic hydrocolloids for the intestinal delivery of protein drugs: alginate and chitosan-a review. J Control Release 114: 1–14.

Gombotz WR, Wee SF (1998) Protein release from alginate matrices. Adv Drug Delivery Rev 31: 267–285.

Hamidi M, Azadi A, Rafiei P (2008) Hydrogel nanoparticles in drug delivery. Adv Drug Delivery Rev 60: 1638–1649.

He XG, Lin LZ, Lian LZ, Lindenmaier M (1998) Liquid chromatography–electrospary mass spectrometric analysis of curcuminoids and sesquiterpenoids in turmeric (*Curcuma longa*). J Chromatogr A 818: 127–132.

Hsieh WC, Chang CP, Gao YL (2006) Controlled release properties of chitosan encapsulated volatile citronella oil microcapsules by thermal treatments. Colloids Surf B 53: 209–214.

Itoh T, Xia J, Magavi R, Nishihata T, Ritting JH (1990) Use of shed snake skin as a model membrane for *in vitro* percutaneous penetration studies: Comparison with human skin. Pharm Res 7: 1042–1047.

Jayaprakasha GK, Jena BS, Negi PS, Sakariah KK (2002) Evaluation of antioxidant activities and antimutagenicity of turmeric oil: a byproduct from curcumin production. Z Naturforsch [C] 57(9–10): 828–835.

Lai F, Loy G, Manconi M, Manca ML, Fadda AM (2007) Artemisia arborescens L essential oil loaded beads: preparation and characterization. AAPS PharmSciTech 8 (3): Article 67.

Lertsutthiwong P, How NC, Chandrkrachang S, Stevens WF (2002) Effect of chemical treatment on the characteristics of shrimp chitosan. J Met Mater Miner 12: 11–18.

Lertsutthiwong P, Noomun K, Jongaroonngamsang N, Rojsitthisak P, Nimmannit, U (2008) Preparation of alginate nanocapsules containing turmeric oil. Carbohydr Polym 74: 209–214.

Lertsutthiwong P, Rojsitthisak P, Nimmannit U (2009) Preparation of turmeric oil-loaded chitosan-alginate biopolymeric nanocapsules. Mater Sci Eng C 29: 856–860.

Leung AY (1980) Encyclopedia of common natural ingredients used in foods, drugs and cosmetics. New York, p. 313–314.

Lu Z, Bei J, Wang S (1999) A method for the preparation of polymeric nanocapsules without stabilizer. J Control Release 61: 107–112.

Manzan ACCM, Toniolo FS, Bredow E, Povh NP (2003) Extraction of essential oil and pigments from *Curcuma longa* [L.] by steam distillation and extraction with volatile solvents. J Agri Food Chem 51: 6802–6807.

Mora-Huertas CE, Fessi H, Elaissari A (2010) Polymer-based nanocapsules for drug delivery. Int J Pharm 385: 113–142.

Motwani SW, Chopar S, Talegaonkar S, Kohli K, Ahmad FJ, Khar, RK (2008) Chitosan-sodium alginate nanoparticles as submicroscopic reservoirs for ocular delivery: Formulation, optimization and *in vitro* characterization. Eur J Pharm Biopharm 68: 513–525.

Negi PS, Jayaprakasha GK, Rao LJM, Sakariah KK (1999) Antibacterial activity of turmeric oil: a byproduct from curcumin manufacture. J Agri Food Chem 47: 4297–4300.

Pedro AS, Cabral-Albuquerque E, Ferreira D, Sarmento B (2009) Chitosan: an option for development of essential oil delivery systems for oral cavity care? Carbohydr Polym 76: 501–508.

Prego C, Torres D, Fernandez-Megia E, Novoa-Carballal R (2006) Chitosan-PEG nanocapsules as new carriers for oral peptide delivery: effect of chitosan pegylation degree. J Control Release 111: 299–308.

Rajaonarivony M, Vauthier C, Couarraze G, Puisieux F, Couvreur P (1993) Development of a new drug carrier made from alginate. J Pharm Sci 82: 912–917.

Roberts GAF (1992) Chitin Chemistry. London, The Macmillan Press.

Roth GN, Chandra A, Nair MG (1998) Novel bioactivities of *Curcuma longa* constituents. J Nat Prod 61: 542–545.

Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M, Bruni R (2005) Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chem 91: 621–632.

Sarmento B, Ferreira DC, Jorgensen L, van de Weert M (2007) Probing insulin's secondary structure after entrapment into alginate/chitosan nanoparticles. Eur J Pharm Biopharm 65: 10–17.

Sarmento B, Ferreira D, Veiga F, Ribeiro A (2006) Characterization of insulin-loaded alginate nanoparticles produced by ionotropic pre-gelation through DSC and FTIR studies. Carbohydr Polym 66: 1–7.

Smidsrød O, Skjåk-Bræk G (1990) Alginate as immobilization matrix for cells. Trends Biotechnol 8: 71–78.

Tønnesen HH, Karlsen J (2002) Alginate in drug delivery systems. Drug Dev Ind Pharm 28: 621–630.

Weska RF, Moura JM, Batista LM, Rizzi J, Pinto LAA (2007) Optimization of deacetylation in the production of chitosan from shrimp wastes: Use of response surface methodology. J Food Eng 80:749–753.

You J, Cui Fu-de, Li Qing-po, Han X, Yu Ying-wei, Yang Ming-shi (2005) A novel formulation design about water-insoluble oily drug: preparation of zedoary turmeric oil microspheres with self-emulsifying ability and evaluation in rabbits. Int J Pharm 288: 315–323.

Youn DK, No HK, Prinyawiwatkul W (2007) Physical characteristics of decolorized chitosan as affected by sun drying during chitosan preparation. Carbohydr Polym 69: 707–712.

Zhao Y, Wang C, Chow AHL, Ren K, Gong T, Zhang Z, Zheng Y (2010) Self-nanoemulsifying drug delivery system (SNEDDS) for oral delivery of Zedoary essential oil: Formulation and bioavailability studies. Int J Pharm 383: 170–177.

Zhao C, Liu Y, Fan T, Zhou D, Yang Y, Jin Y, Zhang Z, Huang, Y (2011) A novel strategy for encapsulating poorly soluble drug into nanostructured lipid carriers for intravenous administration. Pharm Dev Technol doi 10.3109/10837450.2010.546411.

Zhao Xiu-li, Yang Chun-rong, Yang Kai-liang, Li Ke-xin, Hu Hai-yang, Chen Da-wei (2010) Preparation and characterization of nanostructured lipid carriers loaded traditional Chinese medicine, zedoary turmeric oil. Drug Dev Ind Pharm 36: 773–780.