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Original Article

Formulation of essential oil-loaded chitosan–alginate nanocapsules

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ABSTRACT

Naturally occurring polymers such as alginate (AL) and chitosan (CS) are widely used in biomedical and pharmaceutical fields in various forms such as nanoparticles, capsules, and emulsions. These polymers have attractive applications in drug delivery because of their biodegradability, biocompatibility, and nontoxic nature. The pharmaceutical applications of essential oils such as turmeric oil and lemongrass oil are well-known, and their active components, ar-turmerone and citral, respectively, are known for their antibacterial, antifungal, antioxidant, antimutagenic, and anticarcinogenic properties. However, these essential oils are unstable, volatile, and insoluble in water, which limits their use for new formulations. Therefore, this study focuses on developing a CS–AL nanocarrier for the encapsulation of essential oils. The effects of process parameters such as the effect of heat and the concentrations of AL and CS were investigated. Various physicochemical characterization techniques such as scanning electron microscopy, Fourier transform infrared spectroscopy, and ultraviolet–visible spectroscopy were performed. Results of characterization studies showed that 0.3 mg/mL AL and 0.6 mg/mL CS produced minimum-sized particles (<300 nm) with good stability. It was also confirmed that the oil-loaded nanocapsules were hemocompatible, suggesting their use for future biomedical and pharmaceutical applications. Furthermore, the antiproliferative activity of turmeric oil- and lemongrass oil-loaded nanocapsules was estimated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay in A549 cell lines and it was found that both the nanoformulations had significant antiproliferative properties than the bare oil.

1. Introduction

Nanotechnology, a boon to health care, involves advances in medicine, robotics, cosmetics, communication, and genomics [1–3]. Applications of nanoscience and nanotechnology in health care led to the genesis of a new field called *nanomedicine* [4,5]. At present, nanomedicine makes use of nanomaterials such as nanoshell, nanobiosensor, nanovaccines, nanorobots, and nanocapsules for various applications such as diagnosis,

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nervous system tracking, magnetic resonance imaging contrast enhancers, detection of pathogens, and other biomedical applications [6]. Nanotechnology in drug-delivery system provides an opportunity to deliver drugs for a prolonged time with natural affinity [7]. Nanoparticulate systems, dendrimers, micelles, liposomes, and nanoemulsions are some of the nanocarrier systems widely used in drug delivery. These nanocarrier systems help in improving biodistribution of drugs and solubility of hydrophobic compounds, as well as increasing the bioavailability, reducing the number of doses, improving drug targeting, and minimizing toxicity, etc. Among the many nanocarriers, alginate (AL) and chitosan (CS) have gained more attention due to their biocompatibility, biodegradability, mucoadhesiveness, and longer *in vivo* circulation time [8–10]. AL is a negatively charged, Food and Drug Administration-approved, naturally occurring polysaccharide consisting of D-mannuronic acid and L-guluronic acid arranged as blocks in the polymeric chain. They are known to undergo proton-catalyzed hydrolysis that is dependent on pH and temperature [11]. It is known that at low pH, alginic acid forms an insoluble AL skin, which prevents the release of encapsulated material. However, at higher pH, it becomes a soluble viscous layer, releasing the encapsulated material [12]. By contrast, CS is a positively charged linear copolymer polysaccharide consisting of D-glucosamine and *N*-acetyl-D-glucosamine with reactive amino and hydroxyl groups [13]. CS is prepared from chitin by deacetylation of chitin using alkaline solutions [14]. It is soluble at low pH but insoluble at high pH [15–17] and has a unique property called the *permeation enhancing effect*, which makes it a good carrier system. It increases permeation by opening epithelial tight junctions, which is important for the movement of hydrophilic compounds [12]. CS being cationic and AL being anionic make a good polyelectrolyte complex (PEC) [18]. The formation of PEC is known to be influenced by various factors such as pH, molecular weight of the polymers, and the ratio of AL to CS [19,20]. Positive aspects of CS–AL PEC have favored its use as a drug-carrier system [8,21,22].

Essential oils are volatile, aromatic liquids obtained from plants with therapeutic activities. They are widely used in food flavoring agents, perfumes, and pharmaceutical drugs [23,24]. Of the many essential oils, turmeric oil and lemongrass oil are known for their high therapeutic potential. Turmeric belongs to the family Zingiberaceae. Traditionally, it is used for the treatment of sprains, superficial wounds, cough, and fungal infections. Both turmeric and its modified essential oil forms are known to have many therapeutic properties such as anticancer [23,25], antifungal [26], antioxidant [27], anti-inflammatory [28], and antimutagenic [29] effects. Ar-turmerone, a major ingredient of turmeric oil is well-known for its antimutagenic, antiplatelet, and antifungal properties [30,31]. Lemongrass belongs to the family Poaceae. It cools down body temperature, rheumatisms, muscle cramps, and many gastrointestinal disorders [32]. Some of the therapeutic properties of lemongrass oil include anti-inflammatory [33], antimicrobial [34], anticancer [35], and antiproliferative [36] actions. The major ingredient of lemongrass oil is citral, which is also known for its antifungal, anticancer, antiproliferative, and anticlastogenic properties [37]. However, turmeric oil and lemongrass oil have poor physical properties such as hydrophobicity, susceptible to degradation, and volatility, making them difficult to be used in pharmaceutical applications [30,31,38]. These disadvantages can be overcome to an extent by encapsulating these essential oils in a nanocarrier. There are several potential advantages of conjugating anticancer agents with nanoparticles, including targeted delivery and controlled release at diseased sites by changing their pharmacokinetic profile [39,40].

Therefore, in this work, formulation of CS–AL nanocapsules was carried out with the goal of enhancing the physical stability of both the essential oils by encapsulating them into the nanocapsules.

2. Materials and methods

2.1. Chemicals

Sodium AL and CS (degree of acetylation $\geq$ 75%) were purchased from HiMedia Laboratories (Mumbai, Maharashtra, India). Commercially available turmeric oil (*Curcuma longa*) and lemongrass oil (*Cymbopogon citratus*) were purchased from AOS Products Private Limited (New Delhi, India). Thiazolyl blue tetrazolium bromide was purchased from Sigma-Aldrich (Bangalore, Karnataka, India). Human lung adenocarcinoma epithelial cell line (A549) was obtained from the National Centre for Cell Science (Pune, Maharashtra, India). The F-12K 1× nutrient mixture (Kaighn's modified medium), fetal bovine serum, EDTA–trypsin 0.05%, Dulbecco's phosphate-buffered saline 1×, and penicillin–streptomycin were procured from Invitrogen (Bangalore, Karnataka, India). Calcium chloride and other solvents used in this study were of analytical grade. The experiments were performed using deionized distilled water and were carried out in triplicates.

2.2. Maintenance of cell culture

Human lung adenocarcinoma epithelial cells (A549) were cultured in F-12K (Kaighn's) medium containing 10% fetal bovine serum, 100 U/mL penicillin–streptomycin at 37°C in a 5% CO_2 humidified atmosphere.

2.3. Preparation of essential oil-loaded CS–AL nanocapsules

Emulsification processes are used generally in the preparation of nanocapsules as well as in other food, cosmetic, and chemical industries [41]. Essential oil-loaded CS–AL nanocapsules were prepared using the method suggested by Lertsutthiwong et al [30], but with some modifications. In brief, the oil in water nanoemulsion was prepared by adding 20 mL aqueous AL solution of various concentrations (0.3 and 0.6 mg/mL) to 1% (w/v) Tween-80. The pH of aqueous AL solution was maintained between 5 and 5.5 to obtain nano-sized capsules. Subsequently, 0.6 mL of ethanolic turmeric oil solution (20 mg/mL) was added dropwise into this mixture. Following 15 minutes of sonication, 4 mL of 0.67 mg/mL calcium chloride ($CaCl_2$) solution was added and the emulsion was stirred for 30 minutes. The emulsion was then combined with 4 mL of CS solution of various concentration [0.3 or 0.6 mg/mL in 1% (v/v) acetic acid] and stirred for the next 30 minutes. The pH of CS