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Oil-in-water emulsions stabilized by sodium phosphorylated chitosan

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ABSTRACT

Oil-in-water (O/W) emulsions with sodium phosphorylated chitosan (PCTS) were obtained via simple emulsification. PCTS in aqueous solution was amphiphilic with a hydrophilic–lipophilic balance (HLB) of 19 and a critical aggregation concentration (CAC) of 0.13% w/v. The emulsifying efficiency and emulsion stability of PCTS over oil droplets were evaluated in terms of the droplet size, droplet size distribution and microscopic observation using confocal laser scanning microscopy. PCTS preferred to cover oil droplets to produce an O/W emulsion and formed long term stable particles (90 days storage at room temperature) when using PCTS concentrations from above the CAC to 3% w/v. However, emulsions formed from PCTS concentrations below the CAC or over 3% w/v were unstable with particle agglomeration by flocculation after only 7 days storage, although they reverted to individual droplets that retained their integrity in acidic conditions. Overall, PCTS forms effective stable O/W encapsulated particles with potential applications in lipophilic drug encapsulation via a simple emulsion system.

1. Introduction

Encapsulation is a vesicular system in which the active substances (payload) are loaded into an inner core cavity surrounded by a polymeric membrane shell. The shell of the encapsulated particles can serve as an active substance or just as a payload carrier. Several methods had been developed for the production of encapsulated particles (Mora-Huertas, Fessi, & Elaissari, 2010) including polymer-coating, layer by layer, precipitation methods and so on. Among these various methods, the formation of emulsions, a heterogeneous system in which two typically immiscible liquids of different polarities are dispersed one into the other in the form of droplets, is a simple and interesting system for encapsulation since the emulsion provides either lipophilic or hydrophilic cavities where lipophilic or hydrophilic components, respectively, can be encapsulated. However, the emulsion possesses a low stability because it is thermodynamically unstable owing to the unfavorable contact between the lipophilic and hydrophilic phases (such as oil and water) that typically results in phase separation (Dickinson, 1992a, 1992b). Therefore, emulsifiers or surfactants are added into the immiscible mixture and these are important in the formation and stabilization of emulsions (Dickinson, 1992a, 1992b). Small molecular emulsifiers, so-called surfactants, function to decrease the surface tension between the immiscible phases. However, surfactants often cause irritation to the human skin (Gajjar & Benford, 1990) and respiratory pathway (Sukhapan & Brimblecombe, 2002), making them somewhat unsuitable for use in medical and cosmetic applications. Macromolecular emulsifiers not only decrease the surface tension, generate and stabilize emulsions, but they also function as an encapsulator or protective barrier for the dispersed droplets and any substances inside them, because of their alignment at the interface between the oil and water phases.

Owing to these reasons, polymers are currently widely used as emulsifiers in diverse emulsion systems, such as polyethyleneglycol stearates (Li et al., 2008), acrylate and its hydrophobically modified derivatives (Buraczewska, Berne, Linberg, Törmä, & Lodén, 2007). However, attention has also shifted to the utilization of natural polymers or biopolymers as emulsifiers instead of synthetic polymers because of their greater biological compatibility (Garti, 1999). Some natural and water soluble emulsifiers have been used to emulsify and stabilize food emulsions, including proteins

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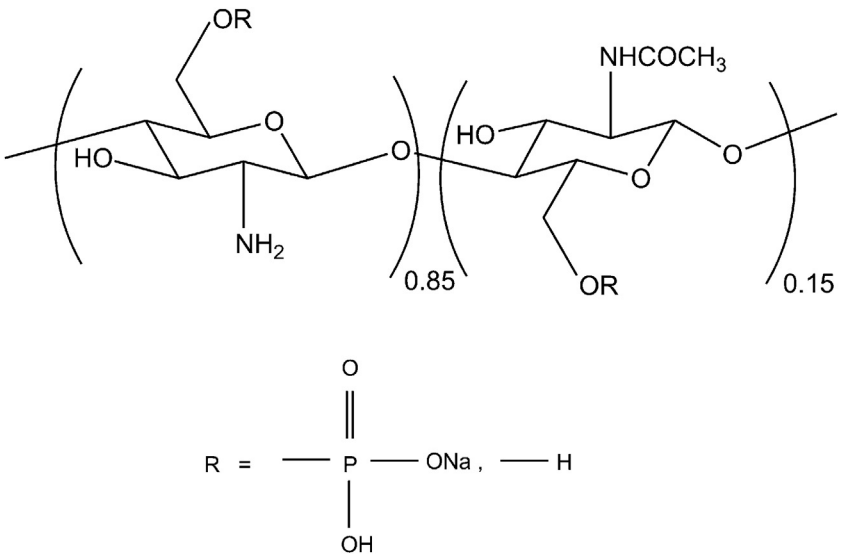

Scheme 1. Schematic structure of PCTS. The proportion of R sites that are phosphate or hydrogen moieties depends upon the degree of substitution (DS) of phosphate. The DS of the PCTS used in this study was 0.04.

(Demetriades & McClements, 1998) and glycoproteins (proteins conjugated polysaccharides) (Fecher, Knoth, Scherze, & Muschiolik, 2007). However, common abundant biocompatible polysaccharides, such as cellulose and starch, are highly hydrophilic and so require hydrophobic modification to improve their amphiphilic properties. Chitosan is one type of natural, readily available, and sustainably renewable polysaccharide that has been used to produce an emulsion (Rodríguez, Albertengo, & Agulló, 2002). Nevertheless, chitosan does not dissolve in water, making it unsuitable for emulsion based encapsulation in many cosmeceutical, pharmaceutical and medical applications.

Therefore, in order to avoid organic solvents, we are interested in developing new bio-based water soluble emulsifier suitable for emulsification so as to be able to synthesize emulsion-based encapsulation of active agents (payloads). Sodium phosphorylated chitosan (PCTS), a water soluble phosphate derivative of chitosan, has been synthesized in our laboratory (Tachaboonyakiat, Netswasdi, Srakaew, & Opaprakasit, 2010), and the possible structure of PCTS is shown in Scheme 1. PCTS has been reported to be suitable for clinical applications, including pharmaceutical drug delivery, because of its cytocompatibility (Srakaew, Ruangsri, Suthin, Thunyakitpisal, & Tachaboonyakiat, 2011) and bioabsorbability (Zhu, Wang, Cui, Feng, & Groot, 2003). PCTS would, therefore, be expected to be a good emulsifier to form a thick adsorbed layer and so play a role as a protective layer. PCTS also has the advantage of that it, and the emulsions formed from it, are surfactant-free and so potentially suitable as an active encapsulating agent in pharmaceutical and personal care products.

In this research so as to evaluate the suitability of PCTS as a water soluble emulsifier, PCTS formed emulsion-based particles were synthesized and characterized. Since this is an emulsion system, the PCTS properties in aqueous solution were characterized in terms of the hydrophilic–lipophilic balance (HLB) and critical aggregation concentration (CAC). The emulsifying efficiency and emulsion stability were investigated in terms of the droplet size, droplet size distribution, zeta potential and microscopic appearance using zetasizer and confocal laser scanning microscopy (CLSM). Furthermore, the physical mechanism underlying the instability of the emulsions was also investigated to elucidate further details on the main reasons for emulsion instability. The PCTS emulsion was then evaluated for its drug encapsulation efficiency and drug release profile using naproxen as the model lipophilic payload drug.

2. Materials and methods

2.1. Materials

PCTS with a degree of substitution (DS) of phosphate of 0.04 and a molecular weight (M_W) of 58 kDa was synthesized as previously reported (Tachaboonyakiat et al., 2010). Briefly, chitosan was reacted with phosphorous pentoxide at 0.1 equivalent mole to chitosan residue in methanesulfonic acid at 0–5 °C for 3 h. The mixture was then precipitated in acetone and dried. In order to improve the water solubility of the phosphorylated chitosan, it was then dissolved in distilled water and dialyzed sequentially against distilled water and 0.1 M sodium hydroxide (NaOH), respectively. The dialyzed product was then neutralized with 0.1 M hydrochloric acid (HCl), and redialyzed against distilled water. The water soluble PCTS was then obtained after lyophilization.

Chitosan with a M_W of 250 kDa, as determined by high performance liquid chromatography (HPLC) (Shimadzu, LC-10ATVP, Japan), and a degree of deacetylation (DD) of 85, as determined by proton nuclear magnetic resonance (^{1}H NMR) (Varian, Unity Inova, US), was purchased from Seafresh Chitosan (Lab) Co., Ltd. (Bangkok, Thailand). Mineral oil (density 0.8 g/cm^3) was purchased from Witco (Houston, USA). Isopropyl myristate (IPM) was obtained from Merck (Darmstadt, Germany). Fluorescein isothiocyanate (FITC) was supplied from Fluka (Buchs, Switzerland). Dialysis tubing, CelluSep T2 (MWCO 6000–8000) was purchased from Membrane Filtration Products, Inc. (TX, USA). Naproxen was received from Aldrich (Steinheim, Germany). All chemicals and solvents were used as received.

2.2. Determination of the HLB of PCTS

The HLB value was determined according to the method of Becher (1960) by measuring the diameter of a toluene drop laid over known HLB emulsifier solutions. The experiment was performed in triplicate. The average diameter of the toluene drops in each of various HLB solutions were plotted to form the calibration curve (Supplementary information Fig. S1). In order to determine the HLB value of PCTS, the average diameter of toluene drops on the surface of a 1% w/v PCTS aqueous solution was measured at room temperature and the HLB value was calculated from the above calibration curve.