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Characteristics of eugenol loaded chitosan-tripolyphosphate particles as affected by initial content of eugenol and their in-vitro release characteristic

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Abstract: The aim of this research was to determine encapsulation efficiency, loading capacity and controlled release of eugenol loaded chitosan-tpp products which prepared by coaservation method. The characteristic of eugenol-loaded chitosan showed that %EE and % LC increased by increasing the initial eugenol content. The optimum of %EE (72.63%) and %LC (43.96%) were obtained at the ratio of chitosan to eugenol of 1:1.5. The FTIR spectrum showed the characteristic peaks of eugenol appearing on spectrum of eugenol encapsulated and blue-shift in the hydroxyl band from 3425.58 cm^{-1} in chitosan-tpp to 3417.86 cm^{-1} and 3394.72 cm^{-1} in eugenol loaded chitosan-tpp indicating that eugenol was successfully encapsulated. The surface morphologies of freeze-dried particles with the optimum %EE showed that more surface roughness and porosity than plain particles. Furthermore, the *in vitro* release of particles with minimum and optimum %EE were also investigated in acid (Simulated Gastric Fluid) and base (Simulated Intestinal Fluid) medium at ambient temperature.

1. Introduction

Eugenol ($C_{10}H_{12}O_2$), is phenolic compound with IUPAC name 4-allyl-2-methoxyphenol. Eugenol is the major component (76.23-85.3%) of clove oil (*Syzygium aromaticum*) [1],[2]. The eugenol has been reported with many advantages including antioxidant, antifungi, antiseptic and anticancer [3]. However, as other most plant-derived bioactive compounds, eugenol is volatile and less in bioavailability [4]. Therefore, a technique to overcome these problems is required. The encapsulation technique has been reported as an efficient method to reduce these limitations [4].

Encapsulation is a process by which a sensitive material is coated or entrapped within another material to protect the sensitive substance from the influences of external environment. Another advantage of encapsulation is, to mask the organoleptic properties like colour, taste, odour of the substance and to obtain controlled release of the drug susbtan[3] [5]. Encapsulation of eugenol have been reported in previous studies; there were eugen[3]loaded-β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin by inclusion method [4], eugenol loaded β-cyclodextrin grafted chitosan by inclusion complex [6]. Eugenol

loaded chitosan by coacervation method has been repoted by Woranuch and Yoksan [7], but the encapsullation efficiency was not more than 25%.

In this study, the eugenol encapsulation has been deeply studied. The encapsulation was done by using chitosan-NaTPP as coating and crosslinker by coacervation method. The in-vitro release characteristic of this encapsulation process is one of the significant findings of this research.

2. Material and method

2.1 Materials

Chitosan (CV. M&H Farm, Indonesia), molecular weight was 624.739 kDa, determined by intrinsic viscosity (Kasaai, 2007), degree of deacetylation (DD) was 64.2 % calculated using baseline method (Khan, 2002). Eugenol 99% (CV. Happy Green, Jakarta), sodium tripolyphosphate (Bratachem,Indonesia), buffer solution pH 7.4 (Flexylab) and buffer pH 1.2; acetic acid (Merck).

2.2 Preparation of eugenol-loaded chitosan particles by coacervation method.

Eugenol-loaded chitosan particles were prepared according to a modified method [8]. Chitosan solution (1.2% (w/v)) was prepared by agitating chitosan in an aqueous acetic acid solution (1% (v/v)) at ambient temperature until it reached homogenous solution. Tween 20 (0.3 g) was added to the chitosan solution (40 ml), and the mixture was stirreed for 30 min to obtain a homogeneous solution. Eugenol was gradually dropped into the stirred mixture, and agitation was carried out for 20 min. The concentration of eugenol was varied (25%, 50%, 75%, 100%, 125%, 150% and 175% (w/w of chitosan)) to obtain different weight ratios of chitosan to eugenol (1:0.25. 1:0.5. 1:0.75. 1:1. 1:1.25. 1:1.5 and 1:1.75. respectively). Tripolyphosphate (TPP) solution 0.5% (40 ml) was slowly dropped into an o/w emulsion while stirring at ambient temperature for 30 min. The formed particles were collected by centrifugation at 7000 rpm for 10 min and subsequently washed several times with deionized water. The susupensions were immediately freeze-dried at -100 ºC for 24 h.

2.3 Determination of encapsulation efficiency and loading capacity.

The content of eugenol-loaded in chitosan particles was determined by UV-vis spectrophotometry [9]. Eugenol-loaded particles (±20 mg) samples were taken into methanol (20 ml) and stirrer for 1 h. The supernatant was collected and the eugenol concentration was measured using UV-vis spectrophotometer at wavelenght of 282.5 nm. The amount of eugenol was calculated by appropiate calibration curve of free eugenol in methanol. The encapsulation efficiency (EE) and loading capacity (LC) of eugenol were calculated from Eqs. (1) and (2) respectively :

$$\text{EE (\%)} = \frac{Total\ amount\ of\ loaded\ eugenol}{initial\ amount\ of\ eugenol} x\ 100 \qquad (1)$$

$$\text{LC (\%)} = \frac{Total\ amount\ of\ loaded\ eugenol}{weight\ of\ particles\ after\ freeze\ drying} x\ 100 \qquad (2)$$

2.4 Instrumental analyses.

FTIR ananlyses for pure chitosan, chitosan-TPP, pure eugenol and eugenol loaded particles were recorded from wavenumber 400-4000 cm^{-1}. Samples were prepared by grinding the dried nanoparticles with KBr and pressing them to form disks. The morphology of the freeze dried particles was studied by scanning electron microscopy (SEM).

2.5 In vitro release studies.

In vitro release was conducted by dissolving 20 mg freeze-dried particles in 20 ml solution containing 60% buffer solution and 40% methanol under gentle agitation. The buffer solution was prepared at pH 1.2 (Simulated Gastric Fluid) and pH 7.4 (Simulated Intestinal Fluid). At specific time intervals, a specific volume of supernatant was sucked out for analysis, and was replaced with an equivalent volume of fresh media. To calculate the total cumulative amount of eugenol release loaded chitosan particles, eugenol concentration (ppm) in the release medium was measured at sampling time intervals by a UV-

vis spectrophotometer at 281 nm [10]. Cumulative percentage of eugenol released was obtained by dividing the cumulative amount of eugenol release at each sampling time point to the initial weight of the eugenol-loaded in the sample:

$$\%\text{Release} = \frac{Eugenol\ released\ at\ that\ time}{intial\ weight\ of\ eugenol - loaded\ in\ sample} x\ 100\%$$

3. Results and discussion

3.1 Encapsulation efficiency and loading capacity

The percentages of EE and LC of different formulation were demonstrated in Figure 1. The amount of loaded eugenol was determined using UV-vis spectrophotometry from its absorbance at 282.5 nm. The EE (%) tends to increase by increasing initial eugenol content. The maximum of EE (72.63%) and maximum of LC (43.96%) were obtained for the sample prepared using a weight ratio of chitosan to eugenol of 1:1.5. This result was higher than a study conducted by Woranuch and Yoksan [7]. A decrease of EE at ratio of 1.75 was found due to the loading limitation. These findings are in accordance with previous studies [8], the EE and LC maximum do not necessarily occur at the same point.

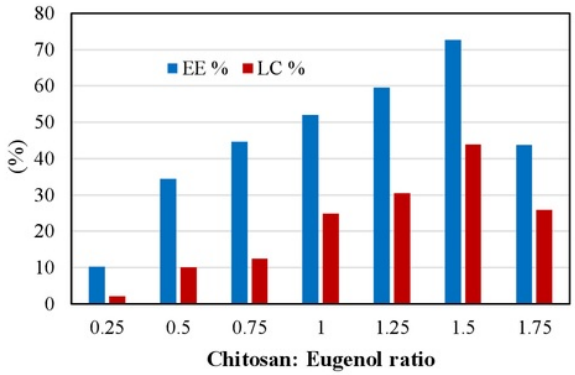

Figure 1. Encapsulation efficiency and loading capacity of eugenol encapsulate

3.2 FTIR characterization

Figure 2 shows FTIR spectra of chitosan powder, chitosan-TPP, eugenol and eugenol-loaded chitosan particles (1:0.25; 1:0.75 and 1:1.5). In general, chitosan powder show characteristic peaks at 3425.58 cm^{-1} (-OH and $-NH_2$ stretching), 2877.79 cm^{-1} (-C stretching), 1651.07 cm^{-1} (amide I/C=O stretching), 1597.06 cm^{-1} (amide II/-NH bending), 1072.42 cm^{-1} (C-O-C stretching) and 894.97 cm^{-1} (pyranoside ring stretching vibration) [8],[9]. For chitosan-TPP the peak of hydoxyl group is already broader after crosslinked with NaTPP. The peak of amide I (C=O) 1651.07 cm^{-1} and amide II (-NH) 1597.06 cm^{-1} shifted to 1635.64 cm^{-1} and 1543.05 cm^{-1} respectively,and new peaks appeared at 1219.01 cm^{-1} (P=O), implying the complex formation via electrostatic interaction between NH^{3+} groups of chitosan and phosphoric groups of TPP within the particles [8] (N *et al.*, 2012; Yoksan *et al.*,2010). Pure eugenol spectra shows sharp characteristic peaks at 3518.16 cm^{-1} (-OH stretching), 3070.68 cm^{-1} (-CH stretching/sp^2), 2738.92-2908. cm^{-1} (-CH stretching/ sp^3), 1464. cm^{-1} (-CH bending) and 1512.19-1604.77 cm^{-1} (C=C aromatic ring) [7].

The FTIR spectrum of eugenol loaded chitosan (1:0.75 and 1:1.5) show characteristic peaks of eugenol at 1435.08-1458.18 cm^{-1} ($-CH_3$ bending). The spectrum of product showed the shifted wavenumber in

the –OH stretching to 3417. 86 $cm^{-1.}$ 3417.86 cm^{-1} and 3394.72 $cm^{-1.}$ respectively, which showed the possible interaction of eugenol with chitosan-TPP.

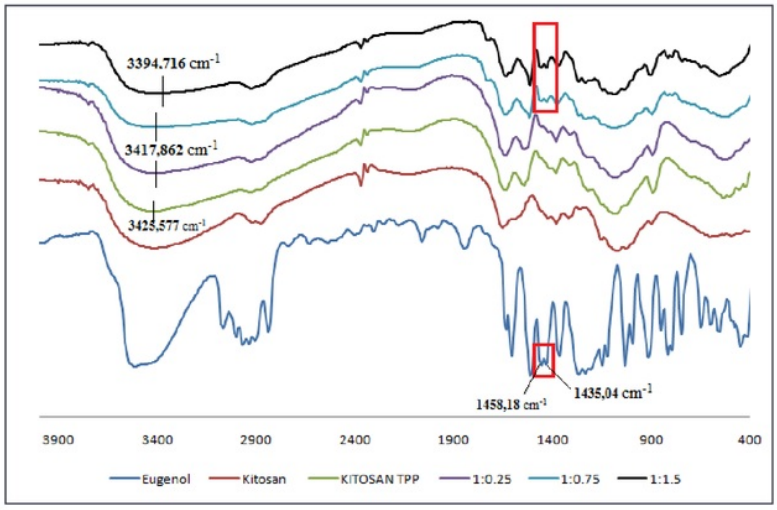

Figure 2. FTIR spectra of eugenol, chitosan , chitosan-TPP and eugenol-loaded chitosan particles 1:0.25; 1:0.75 and 1:1.5

3.3 Scanning Electrom Microscopy (SEM)

The morphology of the particles was observed by using SEM. The SEM images of the chitosan particles and eugenol-loaded chitosan particles (1:1.5). At 100 x magnification, it appears that chitosan-TPP and encapsulated eugenol was flaky and not uniform, this result is similiar to previous study by Kaasgaard and Keller [10] on freeze dried particles. The clumping of particles occured due to effect of centrifugation and freeze drying which cause the agglomerated particles. The freeze drying results of the colloidal suspension produce a porous surface, the pores being generated from water crystals formed during freezing, which are subsequently removed by sublimation [11]. The surface morphology of the eugenol-loaded particles, at 1000 x magnification was rough and more pores and hollow form than the chitosan-TPP.

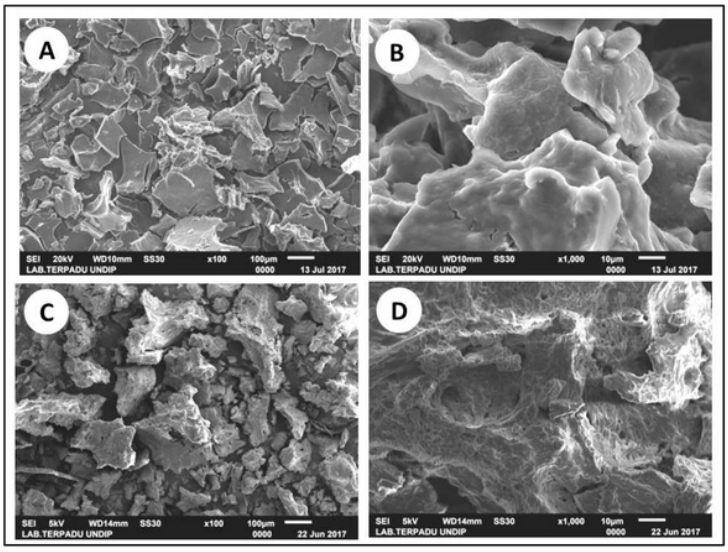

Figure 3. Surface morphology of chitosan-TPP (A) 100x, (B) 1000x and eugenol loaded chitosan particles (C) 100 x and (D) 1000 x

3.4 In vitro release study

The in vitro release profiles of eugenol from particles, prepared using different weight ratio of chitosan to eugenol (1:0.25 and 1:1.5) were shown in Fig 4. The amount of eugenol released at different times was measured at 281 nm. At low concentration of eugenol (25% w/w chitosan) in Simulated Gastric Fluid (SGF) brust effect was 99.3 %, occured within 15 min while at concentration of eugenol 150% was about 86.34%. While at the same concentrations, Eugenol released in Simulated Intestinal Fluid (SIF) wasabout 92.65% and 85.04%, respectively. This initial burst release was attributed to the eugenol molecules adsorbed on the surface of the particles and oil entrapped near the surface [12]. In addition, the very early release of eugenol was due to the presence of methanol in the release medium, wherein eugenol has good solubility in organic solvent. This profil show that the initial burst effect of low concentration of eugenol (25%) was lower than higher concentration of eugenol (150%) in particles. These findings is in accordance with previous studies [13].

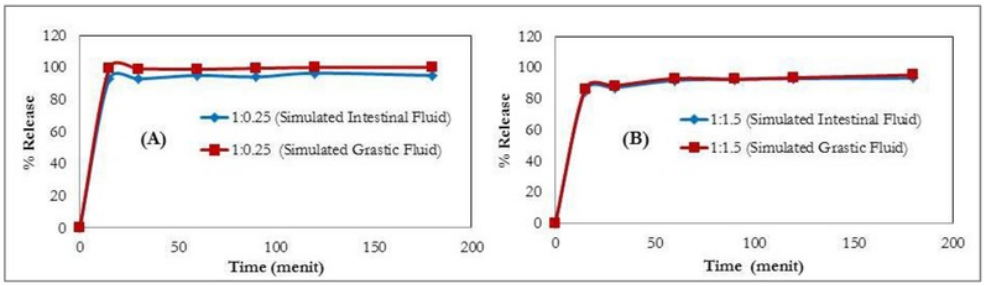

Figure 4. In vitro release profiles of eugenol from chitosan particles prepared using different weight ratio of chitosan to eugenol 1:0.25 (A) and 1:1.5 (B). Values were expressed as mean (n = 2).

In the second stage, the release rate was relatively slow, this might be due to the diffusion of the eugenol dispersed into the polymer matrix as the dominant mechanism. The diffusion occurs in three stages, the first step is penetration of the buffer solution into the particles, causing the polymer swell/ the second step is the change of glassy polymer to rubber matrix then the third stage is the diffusion of the compound from the swollen rubber matrix [14]. The release of eugenol in SGF was faster than SIF due to in acid state (pH <4) almost all free amine groups in chitosan will be protonated, so there will be repulsions between the groups with another amino group on neighboring chain. This causes swelling of the chitosan and the elution of the compound more quickly [15].

4. Conclusion

We found that %EE and %LD of eugenol on citosan-NaTPP encapsulation were higher than previous studied conducted by other researchers. Howeever, in vitro release of eugenol in this encapsulation product has been nearly complete both in the stomach and in the intestinein

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