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Encapsulation Rutin with Chitosan-NATPP Using Coaservation Method

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Abstract

This study aims to make microparticles of Rutin, a flavonoid compound, at various concentrations with NaTPP-chitosan as matrix. Encapsulation is done by coaservation method. The success of encapsulation was identified by UV-Vis spectrophotometry, Fourier Transform Infra-Red (FTIR) spectroscopy and Scanning electron microscopy (SEM). Based on UV-Vis spectrophotometry analysis, the efficiency of encapsulation (EE) and loading capacity (LC) was obtained at 62,43-94,36 and 1,73-32,1%, at Rutin concentration 0,625-11,25 mM. The success of rutin encapsulation is demonstrated by the rutin characteristics of the product seen with the presence of peaks of aromatic rings, indicating the presence of Rutin contained in the chitosan matrix. SEM analysis shows rough and porous surface morphology in microcapsules. The Rutin release profile of the microcapsules is described as two phase processes, burst release at the initial discharge in the first 30 min followed by slow release. At the highest LC (11.25 mM concentration), it provides greater discharge rates for both synthetic fluid simulations $77.53 \pm 3.59\%$ and $78.76 \pm 4.00\%$ after 3 h of discharge. The controlled release data showed high discharge rates at acidic pH than alkaline pH.

Keywords: citosan; coaservation; encapsulation; rutin

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INTRODUCTION

The rutin (quercetin-3-O-rutinoside, Figure 1) is a flavonoid compound belongs to the flavonol glycoside group consists of a quercetin aglicon and a rutin disaccharide with the molecular formula $C_{27}H_{30}O_{16}$ (Jantrawut *et al.*, 2013). These compounds have some pharmacological activity, such as anti-inflammatory, antitumor, and antibacterial. It has been reported that this compound has a low oral bioavailability (Almeida *et al.*, 2010). Therefore, this compound is necessary to develop a formula that can

overcome these problems, one of methods is by using encapsulation system (Almeida *et al.*, 2010).

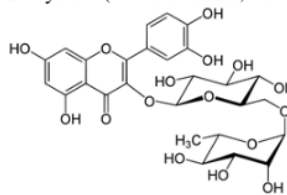


Figure1. Structure of Rutin

Encapsulation is a technique of packaging, adsorption, or coating of active substances in both solid, liquid and gas form in a given matrix (Krasakoopt *et al.*, 2004). The advantage of encapsulation techniques is to protect and control the release of active ingredients under certain conditions at a controlled level so as to enable side effects to be minimized. Various microencapsulation technologies such as spray drying, coacervation, extrusion, and inclusion complexation have been used to encapsulate bioactive compounds. Jiang *et al.* (2013) have performed rutin encapsulation using coacervation complex method using chitosan-alginate with the acquisition of %EE and %loading capacity (LC) optimum, 89.94% and 46.87% respectively. Encapsulation rutin using phosphatidylcholine-cholesterol obtained an EE% of 80.4% (Hooresfand *et al.*, 2015). The preparation and characterization of quercetin and rutin used inclusion complexation method using cyclodextrine has been reported by Sri *et al.* (2007).

Several encapsulation methods have been reported. The coacervation is one of encapsulation methods by utilizing macromolecular properties containing cation and anion charge to interact electrostatically to form capsule (Yeo *et al.*, 2005). The advantage of this method included a relatively simple and light preparation, without the use of heating which can damage the active ingredient so it can be used for drugs with less stable categories (Mohammadpour *et al.*, 2012). Chitosan is commonly used in drug delivery systems because of its biodegradability, low-toxicity (Zhang and Kosaraju, 2007). The use of biodegradable polymers to produce porous matrices is expected to form a suitable system for the release of bioactive compounds over a period of time (Natalia *et al.*, 2017). Therefore, chitosan can be used as a support material for the release of bioactive compounds. Sodium tripoliphosphat is selected as a crosslinking agent. It was reported by Zeng *et al.* (2009) that tripoliphosphate was considered as the best crosslinking agent which has a high negative charge density so it can crosslink with polycationic of chitosan polymers which would be greater than other polyanionic, such as citrate and sulfate.

This study aims to produce rutin-chitosan encapsulation with the method of coacervation. The variation of rutin concentration in the matrix was evaluated for its encapsulation efficiency and loading capacity as well as their controlled released under acid and base conditions.

MATERIALS AND METHODS

Chitosan (CV. M&H Farm, Indonesia), molecular weight 624.739 kDa, deacetylation degree 64.2% (Khan *et al.*, 2002). Rutin (Merck), sodium tripolyphosphate (Brataco, Indonesia), buffer solution pH 7.4 (Flexylab) and buffer solution pH 1.2; glacial acetic acid (Merck, Jerman), tween 20. Chitosan

solution (2%, w/v) was prepared by dissolving chitosan in acetic acid solution (1%, v/v) at room temperature until homogen

Encapsulation Process

Chitosan 2% (w/v) was dissolved using 1% acetic acid using stirrer until homogeneous. Added 0.3 grams of tween to 20-40 mL of chitosan solution. The rutin is made with a concentration variation of 0.625; 1.25; 3.75; 6.25; 8.75; and 11.25 mM were added to 2% (w/v) chitosan solution followed by stirrer mixture for 30 min. 2% (b/v) sodium tripolyphosphate solution of 40 ml was added to chitosan solution dropwise with constant stirring using stirrer (Zeng *et al.*, 2009). Furthermore, the centrifugation was carried out at a speed of 7000 rpm for 10 minutes. The pellet (precipitate) was then washed with the aquades continued freeze drying (Natalia *et al.*, 2017). A 1000 ppm stock solution was prepared by dissolving 10 mg of rutin in 10 mL of methanol, then diluting from 1000 ppm to 100 ppm. Furthermore, from 100 ppm, standard solutions of 4, 8, 12, 16, 20 and 24 ppm were prepared. Then the absorbance of the solution was measured at a maximum wavelength of 257.5 nm rutin.

Encapsulation Efficiency Test and Loading Capacity.

The efficiency of the encapsulation was carried out in a direct manner in which the pellet (precipitate) was dissolved into methanol followed by stirring using stirrer for 1 h, filtered and filtrated, and the absorbance was measured by UV spectrophotometer at maximum wavelength (λ) 257.5 nm. The absorbances obtained were used to determine the number of encapsulated rutins by using standard curve equations (Ibezim *et al.* 2011). Encapsulation efficiency (EE) and loading capacity (LC) were calculated from Eqs. (1) and (2), respectively y (Keawchaon and Yoksan, 2011):

$$\%EE = \left(\frac{\text{weight of loaded rutin}}{\text{weight of initial rutin}} \right) \times 100\% \quad (1)$$

$$\%LC = \left(\frac{\text{weight of loaded rutin}}{\text{weight of sampel}} \right) \times 100\% \quad (2)$$

Controlled Release Test

The release test on the synthetic fluid was performed to measure the microencapsulation capability as a carrier of bioactive compound, this result was then analyzed by using UV-Vis spectrophotometer at λ_{max} (Parize *et al.*, 2012). The microencapsulated of rutin (10 mg) was introduced into 50 mL of synthetic fluid medium consisting of buffer solution with the addition of methanol for 3 hours. Every 30 minute, 4 mL sample was taken and replaced with 4 mL of new buffer media solution. The sample was measured using UV-Vis spectrophotometer at λ_{max} on each medium to determine the concentration of detached rutin. Each

synthetic fluid medium was performed twice as many repetitions (duplo).

FTIR dan SEM Analysis

Characterization of Encapsulation Products. Rutin spectra FTIR, chitosan, CS-TPP microcapsules, and CS-TPP load rutin products were characterized by FTIR (Shimadzu FTIR Prestige 21) using a KBr plate with a frequency range of 400 cm^{-1} - 4000 cm^{-1} . Characterization of the surface morphology of the encapsulation product was performed using the instrument Scanning Electron Microscope (SEM).

RESULTS AND DISCUSSION

Encapsulation Rutin in chitosan-TPP

The rutin is physically in yellow powder. After encapsulation and drying process using freeze drying, CS-TPP load rutin microenkapsulan product is a fine white to yellowish powder (Figure 2).

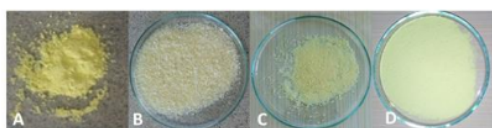


Figure 2. Morphology of rutin (a) CS (b) CS-TPP (c) and rutin load CS-TPP (d)

Efficiency Encapsulation and Loading Capacity of Rutin load CS-TPP

The efficiency of encapsulation (%EE) and loading capacity (%LC) increased by the increase of the amount of active compound used in the encapsulation, as shown in Figure 3. Increasing rutin from 0.625 to 11.25 mM resulted %EE from 60 to 90 and %LC from 1.73 to 32.1%.

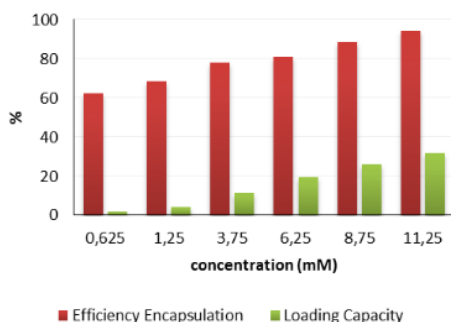


Figure 3. Block diagram of %EE and %LC against the increase of rutin concentration

Controlled Release Test

Controlled release tests were performed for encapsulation products at optimum LC value (concentration of 11.25 mM) and a minimum (0.63 mM concentration). Figure 4 showed the release profile of encapsulation rutin on medium simulated

gastric fluid at pH 1.2. At a minimum LC (concentration 0.63 mM), the initial release in the first 30 min was $56.26 \pm 2.79\%$ and after 1 hour increased to $58.67 \pm 1.76\%$, which continuously increase until 3 hrs with the release percentage of $71.82 \pm 3.79\%$. At the optimum LC (concentration of 11.25 mM), initial release in the first 30 min was $51.93 \pm 1.37\%$. The release after 1 hr increased to $63.44 \pm 0.43\%$, then the release continued to increase until the 180th minute with the release percentage of $77.53 \pm 3.59\%$.

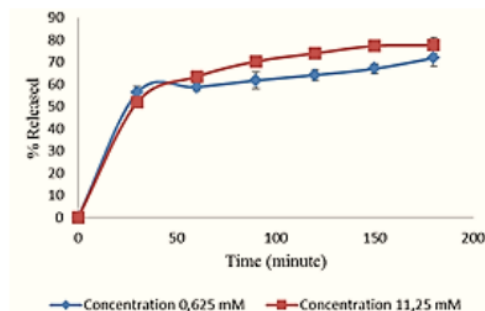


Figure 4. Release profile encapsulation rutin on medium simulated gastric fluid at pH 1,2

A second release test is performed on an intestinal fluid simulation (base pH). The release results are shown in Figure 5.

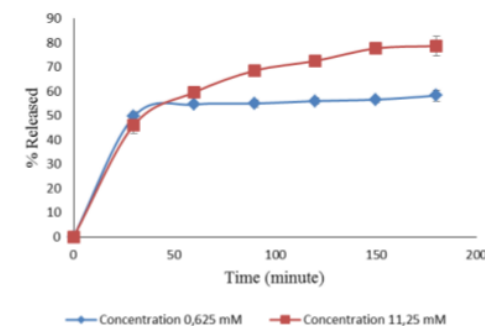


Figure 5. Release profile encapsulation rutin on PBS medium at pH 7,4

At the lowest LC (concentration 0.625 mM), the initial release in the first 30 min was $49.74 \pm 1.98\%$. The release increase significantly from 1 to 3 hrs of test and achieved $58.28 \pm 2.25\%$. While at the highest LC (concentration 11.25 mM), the initial release in the first 30 minutes amounted to $46.16 \pm 3.32\%$ and continue to increase to $59.69 \pm 2.41\%$. After 3 hrs of release test, the release $78.76 \pm 4.00\%$. The rutin release profile of the microparticles is described as two phase processes, ie burst releases at initial discharge in the first 30 minutes and followed by a slow release at the first hour to the third hour of test (concentration 11.25 mM) provides a greater release rate than the minimum LC in both synthetic

fluid simulations of $77.53 \pm 3.59\%$ and $78.76 \pm 4.00\%$ after 3 h release. In addition, the rutin release mechanism of the carrier system is strongly influenced by pH. The above data shows the release of rutin compounds in the gastric fluid higher than in the simulated intestinal fluid. The greater release of the rutin in the acid medium is probably due to the swelling of the microencapsulation caused by ionic repercussions between free amino groups protonated on a chain chain with its neighboring chains, thus increasing the availability of space for entry of the buffer medium into the matrix. (Lee *et al.*, 2012; Keawchaon and Yoksan, 2011).

Fourier Transform Infrared (FTIR)

In this research, Fourier Transform Infrared (FTIR) analyzes of rutin, chitosan, chitosan-TPP, and rutin contained in chitosan-TPP (Figure 6). In rutin FTIR spectra, the absorption bands at wave numbers 1651.07 cm^{-1} and 3410.15 cm^{-1} indicates C=O uptake in rutin flavononoid and vibration of O-H stretching (Patil and Jobanputra, 2015). Vibration C=C which is characterization of aromatic group of flavonoids is shown in absorption bands 1604.77 cm^{-1} and 1458.18 cm^{-1} (Natalia *et al.*, 2017). In the chitosan spectrum, there is a typical and major peak as shown in strong and broad band absorption of 3425.58 cm^{-1} waves indicating O-H vibration. In the wave numbers 2877.79 cm^{-1} and 1072.42 cm^{-1} indicate the presence of vibration C-H stretching and absorption by functional groups -C-O (Marques *et al.*, 2016). The absorption peaks at 1651.07 cm^{-1} and 1597.06 cm^{-1} refer to the -C=O group of the amide (Dudhani and Kosaraju, 2010)

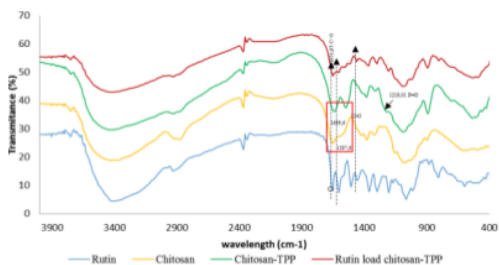


Figure 6. Spectra of rutin, CS, CS-TPP and rutin load CS-TPP microparticle using FTIR

The results of this study indicate that after the formation of microencapsulated vacancies, there appears to be a shift in absorption bands on the CS-TPP spectrum. The initial absorption peaks of 1651.07 cm^{-1} and 1597.06 cm^{-1} on chitosan shifted to wave numbers 1635.64 cm^{-1} and 1543.05 cm^{-1} and the new absorption bands at 1219.01 cm^{-1} which refers to band absorption band P=O stretching of tripolyphosphate (Dudhani and Kosaraju, 2010; Gierszewska and Ostrowska-czubenko, 2016). The presence of shifts in absorption bands and the emergence of new peaks is

associated with the possibility of amino groups involved cross-link (ionic crosslink) with phosphate groups of TPP (Dudhani and Kosaraju, 2010; Gierszewska and Ostrowska-czubenko, 2016). In the rutin CST TP-loaded FTIR spectrum, the success of rutin encapsulation is indicated by the presence of rutin characteristics in the encapsulation product. The emergence of new absorption bands such as 1604.77 cm^{-1} , 1458.18 cm^{-1} shows the aromatic C=C vibration of rutin flavonoids (Natalia *et al.*, 2017), absorption 1296.16 cm^{-1} indicates CO from the phenolic group and 1651.07 cm^{-1} indicate C=O uptake in rutin flavononoid (Patil and Jobanputra, 2015). The presence of absorbent bands in typical aromatic rings and other rutin absorption bands originally absent in the FTIR spectrum of chitosan and CS-TPP matrix indicates the presence of rutin compounds contained in CS-TPP microparticles.

Analysis using Scanning Electron Microscopy (SEM)

The CS-TPP microparticles (as controls) and rutins contained in the microparticles are visualized by using Scanning Electron Microscopy (SEM) to determine the surface morphology of the microencapsulation. The SEM results at 1000X magnification (Figure 7) show that the surface morphology of CS-TPP microparticles is coarse, coagulated and uneven, whereas for rutins contained in microscopic chitosan have a rough surface morphology and show more porosity. Such porous structures can induced swelling behavior, drug release and response to pH buffer medium. Loose and open structures with more porosity can be attributed to low cross-linking densities (Bhumkar and Pokharkar, 2006).

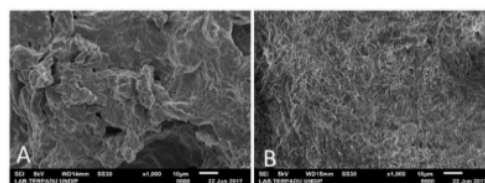


Figure 7. SEM image of microparticle CS-TPP (A) rutin load CS-TPP (B)

CONCLUSION

This study has successfully developed microencapsulated rutin under variation of chitosan-TPP as matrix. The efficiency and loading capacity of the encapsulation increased by increasing the matrix concentration. At the highest efficiency and loading capacity, the rutin was quickly released from the matrix in the acid or base environment. The FTIR analysis showed that crosslinking was developed between chitosan and tripolyphosphate (TPP) and rutin was shown by their absorbance band. The morphological analysis by SEM indicated that the

surface of encapsulated product was rough, porous with micro-sized particle and open structures.

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