Chemistry and chemical technology

by Jayanudin Jayanudin

Submission date: 22-Sep-2020 06:23AM (UTC+0700)
Submission ID: 1393339642
File name: Manuscripts_revision__reviewed_Final.docx (522.5K)
Word count: 4682
Character count: 26952

THE DEVELOPMENT, EVALUATION, AND ANTIOXIDANT ACTIVITY ANALYSIS OF CHITOSAN MICROCAPSULES CONTAINING RED GINGER OLEORESIN ROSS-LINKED WITH SODIUM TRIPOLYPHOSPHATE USING EMULSION CROSS-LINKING TECHNIQUE

Jayanudin[1,2], Moh. Fahrurrozi[1], Sang Kompiang Wirawan[1], Rochmadi[1*]
[1]Chemical Engineering Department, Faculty of Engineering, Universitas Gadjah Mada
Jl. Grafika No. 2 Yogyakarta-Indonesia
[1,2]Chemical Engineering Department, Faculty of Engineering, Universitas Sultan Ageng Tirtayasa
Jl. Jenderal Sudirman km.3 Cilegon-Indonesia
Corresponding author: rochmadi@ugm.ac.id

Abstract

Drug delivery systems could improve the stability, effectiveness, and bioavailability of the drug. Encapsulation delivery systems were needed to overcome the disadvantages of red ginger oleoresins as phytopharmaceuticals that might fail to reach the target tissue and maintain therapeutic levels. This study aims to determine encapsulation efficiency, particle size, and antioxidant activity as a result of the influence of the concentration of chitosan and TPP solutions as well as pH. This study, chitosan-based carrier of red ginger oleoresin, was prepared using the emulsion cross-linking technique with sodium tripolyphosphate (TPP) as a cross-linking agent. Some parameters such as the concentration of chitosan and TPP solution and pH could influence the encapsulation efficiency, particle size, characterization of chitosan microcapsule, and analysis of antioxidant activity. Chitosan microcapsules containing red ginger oleoresins have been successfully produced although with non-smooth surfaces. Preparation of chitosan microspheres generated encapsulation efficiency (0.73 ± 0.04 - 0.92 ± 0.02%), average diameter size (35.71 ± 6.6 - 63.95 ± 7.5 μm), and antioxidant activity (54.59 ± 1.49% - 83.63 ± 1.52%)

Keywords: Antioxidant activity, red ginger oleoresin, chitosan microcapsule, emulsion cross-linking, sodium tripolyphosphate

1. Introduction

Modern drug delivery systems use polymers as carriers of drugs to control the drug release. The advantages of the controlled release include increased efficacy, reduced toxicity, and improved patient compliance and comfort [1]. Chitosan is a biocompatible, biodegradable and non-toxic biopolymer. The high nature of bioadhesive properties of chitosan have more advantages compared to other natural polymers such as cellulose, xanthan gum and starch [2]. This nature has made chitosan as a very suitable drug carrier. Chitosan microcapsules are a form of drug delivery system that can be prepared by physical methods such as spray drying, extrusion, fluidized bed, and others. In additon to the physical methods, there are chemical methods which include coacervation, in situ polymerization, crosslinking emulsions, and interfacial polymerization. The method used in this study was emulsion crosslinking with sodium tripolyphosphate as a crosslinking agent. Emulsion crosslinking method was developed using glutaraldehyde or glutaraldehyde saturated toluene (GST) as crosslinking agent. The microcapsules produced by the emulsion crosslinking method have good spherical geometry with a smooth surface and have been successfully carried out by Jayanudin et al. [3], Jameela and Jayakrishnan [4], and Ofokansi et al [5]. These studies used glutaraldehyde which might have side effects if used for drug delivery. Although a study reported by Campos et al [6] concluded that no adverse effects on cell viability due to cross-reactions which caused toxicity levels to be minimal, a safe crosslinking agent to replace glutaraldehyde is also necessary.

Sodium tripolyphosphate (TPP) is the most widely used crosslinking agent because it is non-toxic and multivalent to prevent possible damage to drugs [7]. The use of TPP as a crosslinking agent in the research consists of two ways. First, TPP was added dropwise to a mixture of polymers and bioactive. This way has been reported by Jarudilokkul et al [8] to encapsulate protein and Alqahtani et al [9] to prepare diclofenac-containing chitosan nanoparticles. The second way is the mixture of polymer and bioactive were added dropwise to the TPP solution as practiced in the research conducted by Csaba et al [10] for the delivery of oligonucleotide and plasmid DNA and encapsulation of curcumin loaded in chitosan as drug delivery carrier as reported by Akolade et al [7]. Ionic gelation/ionotropic gelation methods using TPP as a crosslinking agent produce microspheres/nanospheres chitosan, where the bioactive is more dispersed over the carrier material. The matrix type of encapsulate has a disadvantage as there is a possibility of an active agent found on the particle surface, so it is not effective for the active agent that is sensitive to environmental influences. This method is not suitable for encapsulation of red ginger oleoresins which are sensitive to changes in heat, oxygen, and microorganisms. The emulsion cross-linking technique makes the red ginger oleoresin coated in chitosan solution because the first step is to form an emulsion of oil in water (red ginger oleoresin coated by

chitosan solution) and then added that emulsion to vegetable oil to form an emulsion of oil in water in oil (O/W/O). The use of vegetable oil aims to stabilize the emulsion even without using an emulsifier. The final product is microcapsules where the red ginger oleoresin is inside the core which is protected by chitosan as wall material of microcapsules (red ginger oleoresin encapsulated by chitosan).

Red ginger oleoresin contains many active components which are useful for antioxidant, antimicrobial, and anti-inflammatory [11], but has weaknesses that are sensitive to heat, light, and oxygen [12]. Other disadvantages are poor solubility, and low bioavailability. The chitosan-based red ginger oleoresin delivery carrier can improve stability, solubility and bioavailability. In this study, chitosan containing red ginger oleoresin as a carrier was prepared by the emulsion crosslinking method with sodium tripolyphosphate (TPP) as a crosslinking agent. One of the the aims of this study was to find out the effect of concentration of chitosan solution, pH, and concentration of TPP solution against on encapsulation efficiency, particle size, characterization of microcapsules and release profile of red ginger oleoresin. Another aim was to conduct ananalysis of the antioxidant activity of red ginger oleoresin microcapsules.

2. Materials and methods

2.1. Materials

Red ginger oleoresin was obtained from Lansida group Ltd. Sodium tripolyphosphate was gained from Sigma-Aldrich. These materials were then used as crosslinking agents. Then, glacial acetic acid was purchased from Merck, and toluene technical grade was provided by Tri Jaya Dinamika Ltd. Other materials such as petroleum ether, n-hexane, and methanol were all supplied by Labora Ltd. In addition, chitosan with 87.2% degree of deacetylation (DD) was provided by Biotech Surindo Ltd., and corn oil was produced by Surya Agung Ltd.

2.2. Preparation of chitosan microcapsules containing red ginger oleoresin

Emulsion crosslinking technique used to preparare red ginger oleoresin containing chitosan microsphere was a modification from Jayanudin et al [13] and Jarudilokkul et al [8]. Chitosan was dissolved with 1% (v/v) glacial acetic acid to generate 1%, 2%, 3%, and 4% (w/v) chitosan concentration. 4 g red ginger oleoresin was added to 40 mL chitosan solution, and then stirred using IKA-Werk Ultra-Turrax for 30 minutes to prepare an oil-in-water (O/W) emulsion. The first emulsion was added in corn oil and stirred again for 1 hour to get oil in water in oil (O/W/O) emulsion. Sodium tripolyphosphate (TPP) solution was added dropwise to the emulsion. After TPP was added, the pH of the mixture was adjusted to 5 by adding 2% acetic acid and the mixture was still stirred for 3 hours. Red ginger oleoresin loaded in chitosan microcapsules were separated with a centrifuge and then washed using petroleum ether and followed by hexane. The final stage was that chitosan microcapsules were dried in an oven at 75°C.

2.3. Encapsulation efficiency

Encapsulation efficiency was determined based on the method reported by Jayanudin et al [3] and Tan et al [14]. Surface oil was determined by adding 1 g of dried microcapsules in hexane and stirring for 1 minute, after which they were filtered, dried, and weighed. Total oil was determined by extracting 1 g of microcapsules in 200 mL of methanol for 6 hours in a Soxhlet extractor. After completion, the microcapsules were dried and weighed. The encapsulation efficiency was calculated using equations 1-3

$$\text{Surface oil } (S_O) = w_i - w_f \quad (1)$$

$$\text{Total oil } (T_O) = w_i - w_{extracted} \quad (2)$$

Where w_i refers to initial weight of microcapsules, w_f is final weight of microcapsules, and $w_{extracted}$ is weight of microcapsules after extracted by soxhlet

$$\%\ \text{Encapsulation efficiency } (EE) = \frac{T_O - S_O}{T_O} \times 100 \quad (3)$$

2.4. Scanning Electron Microscope (SEM)

Morphological analysis of red ginger oleoresin containing chitosan microcapsules was conducted using a scanning electron microscope (SEM) with JSM 6510LA type. Resolution: high vacuum mode: 3.0 nm (30 kV) and low vacuum mode: 4.0 nm (30 kV), acceleration voltage: 0.5 to 30 kV. Chitosan microcapsules were coated with platinum.

2.5. Thermal gravimetric analysis (TGA)

Thermal gravimetric analysis (TGA) with DTG-60 Shimadzu type was used to analyze thermal stability using DTG-60 detector. The samples were heated from 30 to 300°C at a rate of 10°C/min, and nitrogen flow rate was 30 mL/min.

2.6. Fourier-transform infrared spectroscopy (FTIR)

Interaction analysis between components in microcapsules used Fourier transform infrared spectroscopy (FTIR) using KBr pellets, in a Shimadzu IR spectrophotometer, which operated between 500 cm^{-1} and 4000 cm^{-1}.

2.7. Analysis of particle size

The diameter of microcapsules was determined by a digital microscope with 500x magnifications referring to the study reported by Jayanudin, et al [15]. Microcapsule size calibration was done by comparing the real diameter size of the wire fibers with the size of wire fibers using a digital microscope, and then made as a correction factor. The diameter size of microcapsules measured by a digital microscope was multiplied by a correction factor to obtain the real diameter size of microcapsules. The average diameter was calculated by observing 100 microcapsules. The average diameter of microcapsule was determined by equation 4.

$$\bar{d} = \sum_{i=n}^{n} \frac{d_i}{N} \quad 4)$$

Where, $\bar{d}$ is average diameter, d_i is each droplet diameter, and N is the total number of calculated droplet.

2.8. Analysis of antioxidant activity

Antioxidant of red ginger oleoresins in chitosan microspheres could be determined using DPPH assay. This method was modified from Jayanudin et al [16] and Gbadegesin and Odunola [17]. The reaction mixture consisted of 1 ml sample, 6 mL ethanol and 0.6 mL DPPH solution (0.5 mM in ethanol). After 30 minutes a reaction occurred between DPPH and the antioxidant compound causing a color change and was read by GENESYS 10S UV/VIS Spectrophotometer with a wavelength of 517 nm. Determination of blank absorbance was determined by ethanol mixture with samples. The percentage of antioxidant activity was calculated by equation (5)

$$AA\% = 100 - \left[\frac{\left(Abs_{control} - Abs_{sample}\right) x\ 100}{Abs_{control}}\right] \quad 5)$$

Where $Abs_{control}$ is the control reaction of absorbance (only absorbance of DPPH) and Abs_{sample} is absorbance in the presence of a sample (absorbance of DPPH along with concentrations of sample).

3. Results and discussion

3.1. Encapsulation efficiency

Encapsulation efficiency determines the effectiveness of the encapsulation process of red ginger oleoresin using emulsion crosslinking with TPP as a crosslinking agent. Table 1 shows the effect of concentration of chitosan solution, concentration TPP solution, and pH against the encapsulation efficiency of red ginger oleoresin microcapsules. The value of the encapsulation efficiency produced from this study was between 83.25±0.04 to 91.64±0.02%. Minimum encapsulation efficiency was generated from 4% chitosan concentration and pH 4. While, the maximum value of encapsulation efficiency was obtained from 4% chitosan concentration, and pH 5.

Table 1. Encapsulation efficiency and particle size of chitosan microcapsules filled with red ginger oleoresins and formulated based on the effect of chitosan solution concentration and pH.

Parameter				
Chitosan (% w/v)	**Sodium tripolyphosphate (% w/v)**	**pH**	**[a,c]Encapsulation efficiency (%)**	**[a,d]Particle size (µm)**
1	5	5	87.81±0.03	35.71±6.6
2			89.40±0.07	36.95±5.4
3			90.18±0.05	38.52±4.9
4			91.64±0.02	41.43±8.3
4	5	4	83.25±0.04	47.35±9.1
		5	91.64±0.02	41.43±8.3
		6	91.42±0.01	48.11±9.1

[a]Mean±SD, [b]n = 3, [c]n = 3,[d]n = 100

At a lower pH, it allows the chitosan molecule with confirmation of extension caused by a strong charge repulsion because most amino groups from chitosan were protonated. The lower charging density of the molecule was caused by the protonation of TPP molecules, so that chitosan molecules cannot be adequately cross-linked by TPP to form stable particles [18]. This possibility caused pH 4 to produce lower encapsulation efficiency compared to pH 5. While decreasing encapsulation efficiency at pH 6 was due to

approaching the isoelectric point of chitosan (pKa = 6.3), the deprotonation process could occur and cause aggregation by decreasing the repulsion force between particles [19]. The aggregation process between particles makes a small portion of red ginger oleoresin can diffuse out during the process of compaction of chitosan microcapsules. Similar results occurred in the study by Patil et al [20], increasing pH caused a decrease in encapsulation efficiency.

The encapsulation efficiency of this study obtained high values from the comparison of studies that have been reported with ionic gelation methods such as a study by Hasheminejad et al [21] which obtained encapsulation efficiency at 45.77 ± 1.25%, Alqahtani et al [9] was 31.1 ± 3.1%, and Tripathy et al [22] was 54.72 ± 1.342%.

3.2. Particle size

Determination of particle size was done by observing a hundred particles using a digital microscope before the average diameter was defined. The effect of the parameters carried out in this study on the average particle size can be seen in Table 1. The average particle size was produced between 35.71 ± 6.6 µm to 63.95 ± 7.5µm. The particle size of chitosan microcapsules containing red ginger oleoresin increased when chitosan concentration also increased. The lower viscosity of the chitosan solution had a better solubility for the efficiency of the gelation process that caused the particle size became smaller. The lower concentration of chitosan solution would affect the smaller particle size and vice versa. The lower molecular weight of chitosan could reduceparticle size because it has a shorter polymer chain that supports dissolution and interaction with TPP [23]. Other causes of higher concentrations of chitosan solution make the walls of the microcapsules more rigid and compact [24], and the walls of the microcapsules are also thicker so the diameter of the microcapsules becomes larger.

Particle size is also influenced by pH value. Table 1 shows that the particle size was increased when pH increased from 4 to 5, but it decreased at pH 6. At pH below 5, the protonation degree of chitosan was less influenced. The interaction of charge between two molecules becomes stronger and more stable, resulting in a relatively larger size [18]. The highest particle size occurs at pH 6. This was likely because the crosslinking was reduced due to deprotonation domination.

The effect of the concentration of chitosan solution and pH was an important parameter to determine the particle size of chitosan microcapsules. The highest average particle size in this study was 48.11±9.1µm obtained from 4% (w/v) concentration of chitosan solution and pH 6. Meanwhile, the smallest particle size was 35.71±6.6 µm which was obtained from 1% (w/v) concentration of chitosan solution and pH 5. When compared to the particle size produced by the ionic gelation method, the particle size in this study was larger than the study conducted by Hasheminejad et al [21] which produced a particle size from 22.45 ± 0.90 to 1287.70 ± 0.60 nm, and Alqahtani et al [9] which reported that the particle size produced was from 295.33 ± 3.01 nm to 336 ± 22.1 nm.

3.3. Characterization of red ginger oleoresin containing chitosan microcapsules

3.3.1. Fourier-transform infrared spectroscopy (FTIR)

FTIR analysis for chitosan and chitosan-TPP microcapsules can be seen in Figure 1. FTIR analysis shows that the absorption bands for chitosan and chitosan-TPP microcapsules were at 3425.58 cm^{-1} and 3387 cm^{-1} were assigned to N-H stretch vibration mode and O-H stretch vibration.

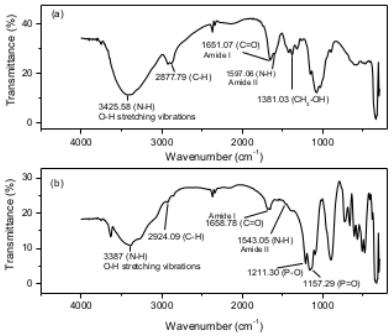

Figure 1. FTIR spectrum of (a) chitosan and (b) chitosan/TPP. Chitosan microcapsules used in FTIR analysis was without red ginger oleoresin.

Figure 1 shows that there were differences in the peak between chitosan (a) and chitosan-TPP microcapsules (b). The differences were the peak of 1211.30 cm^{-1} (P-O) and 1157.29 cm^{-1} (P = O)

3.3.2. Thermogravimetric analysis (TGA) and Differential thermal analysis (DTA)

Thermogravimetric analysis (TGA) is a type of test carried out to determine weight loss in a sample due to temperature changes. TGA is used to characterize materials such as polymers for determining the degradation of temperature, decomposition of organic and inorganic materials, and solvent residues. TGA analysis is usually simultaneous with DTA analysis, which is used to analyze material changes as a temperature function. DTA is used to study thermal properties and phase changes due to enthalpy changes from the material. Figure 2 shows the results of TGA/DTA analysis for chitosan microcapsules containing red ginger oleoresins based on changes of chitosan concentration.

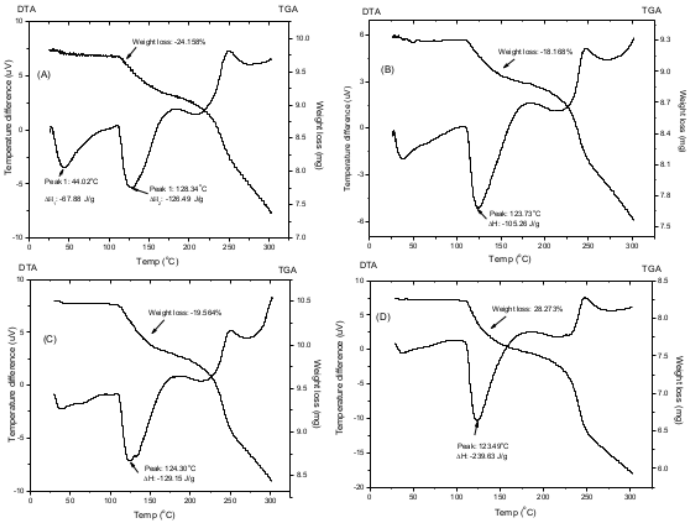

Figure 2. TGA and DTA Analysis of chitosan microcapsules for several concentration of chitosan solution: (A) 1% (w/v), (B) 2% (w/v), (C) 3% (w/v), and (D) 4% (w/v). The chitosan microcapsules containing red ginger oleoresin was made by 5% TPP concentration and pH 5.

TGA analysis in Figure 2 starts from temperatures of 30°C to 300°C. The weight loss occurred in two stages. The first stage occurred at a temperature from 30°C to 112°C and the second stage occurred at a temperature from 112°C to 300°C. The weight loss in the first stage occurred due to evaporation of water in chitosan microcapsules. After that, chitosan microsphere melted and decomposed at 112°C -300°C. At this stage, the weight of chitosan microsphere lost drastically because of decomposition. The acetylated and deacetylated units of the polymer could trigger depolymerization and decomposition. The saccharide rings could also dehydrate at a temperature of 190-330°C [25]. The weight loss started from 30-300°C; they were 24,158% (1% (w/v) concentration of chitosan solution), 18,168% (2% (w/v) concentration of chitosan solution chitosan), 19,564% (3% (w/v) concentration of chitosan solution chitosan), and 28,273% (4% (w/v) concentration of chitosan solution chitosan).

Figure 2 also shows the DTA analysis. It can be seen that chitosan microcapsules from all concentration of chitosan solution had different endothermic peaks. The endothermic peak shown in Figure 2 was the melting point of the chitosan microcapsules from different concentrations of chitosan solution. The melting point (endothermic peak) for 1% (w/v) concentration of chitosan solution occurred at 128.34°C with enthalpy ($\Delta\widehat{H}$) = -126.49 J/g, 2% (w/v) concentration of chitosan solution at 123.73°C with enthalpy ($\Delta\widehat{H}$) = -105.26 J/g, 3 % (w/v) concentration of chitosan solution at 124.30 °C with enthalpy ($\Delta\widehat{H}$) = -129.15 J/g, and 4% (w/v) concentration of chitosan solution at a temperature of 123.49°C with enthalpy ($\Delta\widehat{H}$) = -239.63 J/g.

3.3.3. Scanning Electron Microscopy (SEM)

The SEM analysis was used to determine the surface morphology and shape of chitosan microcapsules. Figure 3 shows the surface morphology of microcapsules from chitosan microcapsules cross-linked with TPP at various magnifications.

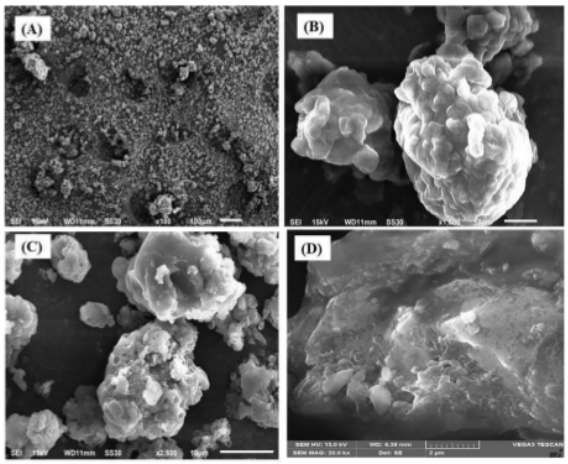

Figure 3. Surface morphology analysis of chitosan microcapsules containing red ginger oleoresin. (A). 100x magnification, (B) 1,500x magnification, (C) 2,500x magnification, and (D) 20,000x magnification. This form of microcapsules was made from 4% (w/v) concentration of chitosan solution, 5% (w/v) concentration of TPP solution, and pH 5.

Figure 3 shows the surface shape of chitosan microcapsules cross-linked with TPP generated a non-smooth surface and did not exhibit good spherical geometry. Many chitosan flakes were attached on the microcapsules surface. At the initial process of solidification, the microcapsule wall layer was gel-shaped because it was cross-linked with TPP. Then, it dissolveed due to the addition of acetic acid to adjust the pH. The chitosan solution was hardened again during the crosslinking process. This phenomenon was very likely caused by the number of chitosan flakes attached to the surface of the microcapsules, as shown in Figure 3C and D. The uneven surface shape and did not exhibit good spherical geometry might be caused by non-uniform crosslinking reaction of chitosan-TPP as shown in Figure 3B. While, Figure 3D shows the surface of the chitosan microcapsules was enlarged to 20,000x. The solid surface was seen, even though many flakes of chitosan was attached on the chitosan microcapsule surface.

3.4. Antioxidant activity of red ginger oleoresin containing chitosan microsphere

DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was used to analysis the antioxidant activity of chitosan microcapsules containing red ginger oleoresin. This method was used to predict antioxidant activity by the mechanisms in which antioxidants in a substance act to inhibit lipid oxidation by scavenging radical DPPH . The use of DPPH because of free radicals is stable in methanol or aqueous solutions and accepts electrons or radical hydrogen to turn into stable diamagnetic molecules [26]. The results of the antioxidant activity of red ginger oleoresin containing chitosan microsphere can be seen in Table 3.

Table 3. Antioxidant activity of red ginger oleoresin microcapsules

Samples tested	Antioxidant activity (%)
Chitosan concentration (%)	
1	58.65±1.54
2	67.48±1.43
3	64.22±1.29
4	83.63±1.52
Changes of pH	
4	54.59±1.49
5	83.63±1.52
6	77.01±1.16

Mean±SD, n = 3

In general, it can be said that antioxidant activity increased as concentration of chitosan increased,. Although at 2% (w/v) concentration of chitosan solution had higher antioxidant activity than 3% (w/v) concentration of chitosan solution, the difference was not significant. Antioxidant activity at pH 6 was lower than pH 5 and higher than pH 4. This was likely related to the encapsulation efficiency produced, as shown in Table 1. The high encapsulation efficiency had higher oleoresin content, so the antioxidant activity was also higher. The value of antioxidant activity produced from chitosan microcapsules containing red ginger oleoresins was quite high from 54.59 ± 1.49% to 83.63 ± 1.52%. When compared to the study reported by Eleazu et al [27] that produced antioxidant activity from 45% to 75%, the antioxidant activity produced by this study was higher. It was even higher than the synthetic antioxidants. As reported by Gbadegesin and Odunola [17], the antioxidant activity of butylated hydroxyanisole (BHT) and butylated hydroxytoluene (BHA) was 48%-60% and 50-69%. The value of this antioxidant activity was higher than the antioxidant activity produced from ginger oleoresin resulting from ginger extraction which was 40-76% [28]. The same case happened to the study reported by Bellik [29], that the antioxidant activity of oleoresin which was calculated by 2,2´-azino-bis (3-ethylbenzenthiazoline-6-sulphonic) acid (ABS) method produced 9.17 ± 0.94% to 50.58 ± 2.86%. The high value of the antioxidant activity of red ginger oleoresin containing chitosan microcapsules showed one of the success parameters of the encapsulation process by emulsion crosslinking technique using sodium tripolyphosphate as a crosslinking agent.

4. Conclusion

In summary, this study reveals that the concentration of chitosan, TPP concentration, and pH has an effect on the efficiency, particle size, thermal stability, and antioxidant activity. Based on the parameters studied, chitosan microspheres made from 4% chitosan concentration, 5% TPP concentration, and pH 5 produced the highest encapsulation efficiency and antioxidant activity: 91.64±0.02% and 83.63±1.52%. The particle size produced was from 35.71±6.6 μm to 63.95±7.5 μm. Although the surface was not smooth, chitosan microspheres have been successfully produced. The values of antioxidant activity in red ginger oleoresin microcapsules were from 54.59 ± 1.49% to 83.63 ± 1.52%.

Acknowledgments

We would like to express our gratitude to the Ministry of Research, Technology and Higher Education (Kemenristekdikti) of the Republic of Indonesia which has financially supported this research. Our thanks also go to the Chemical Engineering Department, Universitas Gadjah Mada for all research facilities that have been provided.

References

[1] Abu-Thabit NY., Makhlouf ASH., Historical development of drug delivery systems: From conventional macroscale to controlled, targeted, and responsive nanoscale systems. In: Makhlouf ASH and Abu-Thabit NY. (eds). Stimuli Responsive Polymeric Nanocarriers for Drug Delivery Applications, Woodhead Publishing, 2018, pp. 1-41.

[2] Kotze AF., Luessen HL., Thanou M., Verhoef JC., De boer AG., Junginger HE., Chitosan and Chitosan Derivatives as Absorption Enhancers for Peptide Drugs Across Mucosal Epithelia, in. Mathiowitz E., Chickering III DE., Lehr CM. (eds). Bioadhesive Drug Delivery Systems : Fundamentals, Novel Approaches, and Development. Chapter 13, 1999, pp.341–386.

[3] Jayanudin, Fahrurrozi M., Wirawan SK., Rochmadi, Preparation of Chitosan Microcapsules Containing Red Ginger Oleoresin Using Emulsion Crosslinking Method, J. Appl. Biomater. Funct. Mater., 2019, 17, 1–9.

[4] Jameela SR., Jayakrishnan A., Glutaraldehyde cross-linked chitosan microspheres as a long acting biodegradable drug delivery vehicle : studies on the in vitro release of mitoxantrone and in vivo degradation of microspheres in rat muscle, Bionmaterials., 1995, 16, 769–775.

[5] Ofokansi KC., Kenechukwu FC., Isah AB., Okigbo EL., Formulation and evaluation of glutaraldehyde-crosslinked chitosan microparticles for the delivery of ibuprofen, Trop. J. Pharm. Res., 2013, 12, 19–25.

[6] Campos E., Coimbra P., Gil MH., An improved method for preparing glutaraldehyde cross-linked chitosan-poly(vinyl alcohol) microparticles, Polym. Bull., 2013, 70, 549–561.

[7] Akolade JO., Oloyede HOB., Salawu MO., Amuzat AO., Ganiyu AI., Onyenekwe PC., Influence of formulation parameters on encapsulation and release characteristics of curcumin loaded in chitosan-based drug delivery carriers, J. Drug Deliv. Sci. Technol., 2018, 45, 11–19.

[8] Jarudilokkul S., Tongthammachat A., Boonamnuayvittaya V., Preparation of chitosan nanoparticles for encapsulation and release of protein, Korean J. Chem. Eng., 2011, 28, 1247–1251.

[9] Alqahtani FY., Aleanizy FS., El Tahir E., Alquadeib BT., Alsarra IA., Alanazi JS., Abdelhady HG.,

Preparation, characterization, and antibacterial activity of diclofenac-loaded chitosan nanoparticles, Saudi Pharm. J., 2019, 27, 82–87.
[10] Csaba N., Köping-höggård M., Alonso MJ., Ionically crosslinked chitosan/tripolyphosphate nanoparticles for oligonucleotide and plasmid DNA delivery, Int. J. Pharm., 2009, 382, 205–214.
[11] El-Ghorab AH., Nauman M., Anjum FM., Hussain S., Nadeem M., A Comparative study on chemical composition and antioxidant activity of ginger (*Zingiber officinale*) and cumin (*Cuminum cyminum*), J. Agric. Food Chem., 2010, 58, 8231–8237.
[12] Vaidya S., Bhosale R., Singhal RS., Microencapsulation of Cinnamon Oleoresin by Spray Drying Using Different Wall Materials, Dry. Technol., 2006, 24, 983–992.
[13] Jayanudin, Fahrurrozi M., Wirawan SK., Rochmadi, Controlled Release Evaluation of Red Ginger Oleoresin Encapsulation using Simulated Gastric Fluid (SGF), Res. J. Pharm. Technol., 2018,11, 3431–3436.
[14] Tan LH., Chan LW., Heng PWS., Effect of oil loading on microspheres produced by spray drying., J. Microencapsul., 2005, 22, 253–259.
[15] Jayanudin, Fahrurrozi M., Wirawan SK., Rochmadi, Mathematical modeling of the red ginger oleoresin release from chitosan-based microcapsules using emulsion crosslinking method, Eng. Sci. Technol. an Int. J. (2018). doi:10.1016/j.jestch.2018.11.008.
[16] Jayanudin, Fahrurrozi M., Wirawan SK., Rochmadi, Antioxidant activity and controlled release analysis of red ginger oleoresin (*Zingiber officinale var rubrum*) encapsulated in chitosan cross-linked by glutaraldehyde saturated toluene, Sustain. Chem. Pharm., 2019, 12, 100132.
[17] Gbadegesin MA., Odunola OA., In vitro Antioxidant/Radical Scavenging Activities and Hepatoprotective Roles of Ethanolic Extract of Cassia occidentalis Leaves in Sodium Arsenite-Treated Male Wistar Rats, Br. J. Med. Med. Res., 2013, 3, 2141–2156.
[18] Liu H., Gao C., Preparation and properties of ionically cross-linked chitosan nanoparticles, Polym. Adv. Technol., 2009, 20, 613–619.
[19] Esquivel R., Juárez J., Almada M., Ibarra J., Valdez M.A., Synthesis and Characterization of New Thiolated Chitosan Nanoparticles Obtained by Ionic Gelation Method, Int. J. Polym. Sci., 2015, 1–5.
[20] Patil SS., Gupta VRM., Gupta KS., Doddayya H., Formulation and characterization of tpp cross-linked chitosan microspheres loaded with lornoxicam, J. Biomed. Pharm. Res., 2014, 3, 51–58.
[21] Hasheminejad N., Khodaiyan F., Safari M., Improving the antifungal activity of clove essential oil encapsulated by chitosan nanoparticles, Food Chem., 2019, 275, 113–122.
[22] Tripathy S., Das S., Prasad S., Kumar S., Pramanik P., Roy S., Synthesis , characterization of chitosan – tripolyphosphate conjugated chloroquine nanoparticle and its in vivo anti-malarial efficacy against rodent parasite : A dose and duration dependent approach, Int. J. Pharm., 2012, 434, 292–305.
[23] Omar Zaki SS., Ibrahim MN., Katas H., Particle size affects concentration-dependent cytotoxicity of chitosan nanoparticles towards mouse hematopoietic stem cells, J. Nanotechnol., 2015, 1–5.
[24] Ganesh S., Kumar DS., Kumar BS., Abhilash R., Bharadwaj PS., Prudhvi Raj KPS., Mohammed I., Pravalika T., Controlled Release Formulation and Evaluation of Idarubicin Microsphere Using Biodegradable Hydrophilic and Hydrophobic Polymer Mixtures, Asian J. Pharm. Clin. Res., 2010, 3, 179–182.
[25] Hosseini SF., Zandi M., Rezaei M., Farahmandghavi F., Two-step method for encapsulation of oregano essential oil in chitosan nanoparticles: Preparation, characterization and in vitro release study, Carbohydr. Polym., 2013, 95, 50–56.
[26] Kedare SB., Singh RP., Genesis and development of DPPH method of antioxidant assay, J. Food Sci. Technol., 2011, 48, 412–422.
[27] Eleazu C., Amadi C., Iwo G., Nwosu P., Ironua C., Chemical Composition and Free Radical Scavenging Activities of 10 Elite Accessions of Ginger (Zingiber officinale Roscoe), Clin. Toxicol., 2013, 3, 1–5.
[28] Murthy PS., Gautam R., Pura Naik J., Ginger Oleoresin Chemical Composition, Bioactivity and Application as Bio-Preservatives, J. Food Process. Preserv., 2015, 39, 1905–1912.
[29] Bellik Y., Total antioxidant activity and antimicrobial potency of the essential oil and oleoresin of Zingiber officinale Roscoe, Asian Pacific J. Trop. Dis., 2014, 4, 40–44.

Chemistry and chemical technology

ORIGINALITY REPORT

19%	13%	14%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

3%

★ www.mdpi.com

Internet Source

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	On		

Chemistry and chemical technology

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8