The thickness of the microcapsule layers of the SPI nanofibrils

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Abstract. Multilayer microcapsules are micro-sized capsules composed of several layers. Multilayer microcascules can be made from Soy Protein Isolate (SPI) nanofibrils using the Layer by Layer (LbL) method. The thickness of the microcapsule layer is one of the physical characteristics that need to be studied for the application of multilayer microcapsules in the fields of health, food, industry and agriculture. The thickness of the microcapsule layer can be determined based on the diameter of the microcapsule layer. This study examines the thickness of each layer of multilayer microcapsule made from SPI nanofibrils. The diameter of the microcapsules can be measured using a zetasizer. The measurement results of the microcapsule layer diameter showed that the diameter of the second, fifth, and seventh layers were 9,703 nm, 12,450 nm, and 12,890 nm, respectively. The equation of the microcapsule layer diameter to the number of microcapsule layers was $y = 658.73x + 8{,}603.9$ with the value of $R^2 = 0.92$. The average thickness of the microcapsule layer is 604.45 nm.

1. Introduction

Microencapsulation is a process in which tiny particles or droplets are coated to give small capsules with many unique properties. One of the purposes of the capsules is to protect and to release their contents in response to specific triggers at certain controlled rate; the microcapsule is illustrated in Figure 1. The design of the shell is one of the determining factors for how the microcapsule behaves. There are two types of microcapsules, mono-layered and multi-layered microcapsules. The advantages of designing multi-layered microcapsules are control over the thickness of shell and therefore the release can be controlled. The microcapsules can carry different active materials either in between the layers or in the core. Microencapsulation (the process of making microcapsules) can be used as a means of controlling the release of material wrapped in special parts of the body. This microencapsulation is applied to medicine [1] and the food industry [2] specially to protect the bio-availability of probiotic bacteria. in the small intestine [3], avoiding spoilage of food taste [4] and the crunchiness of food, formulating new product concepts with new sensory experiences and healthier foods [5], formulating food packaging materials, enhancing food safety, or protecting sensitive functional ingredients. The study of microcapsules in the field of medicine and food includes several aspects, including materials that can be used as the encapsulant, such as hydrogel, sugar alcohol [6], starch [7], or hydro colloids;

encapsulation techniques such as spray chilling [6,8], complexation [9], colloidosomes [10], layer by layer deposition [11–13]; control the release of a content [14] and so on.
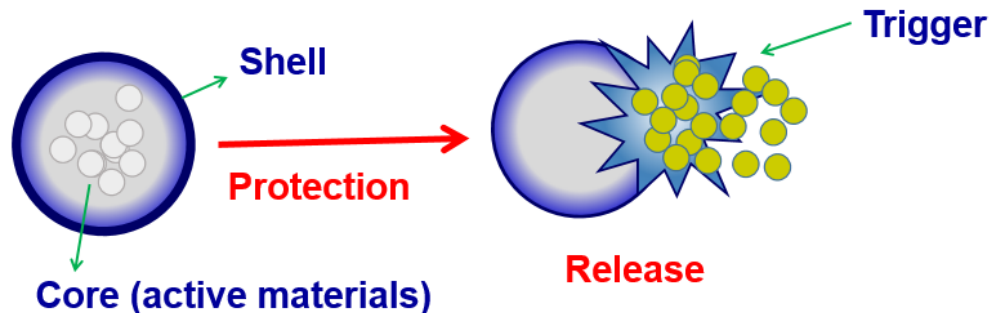

Figure 1. Illustration of microcapsule

An important factor in the manufacture of the microcapsule as a release control device is that the capsule must be able to protect and release its contents in response to a particular stimulus at a controlled rate. These special properties are influenced by the mechanical, physical, and chemical properties of the microcapsules. The mechanical strength, stability of the capsule material against physical and chemical exposure, as well as its release properties are important factors that must be considered. Mono-dispersity size and core design are important factors for determining the properties of the microcapsules at the specific release rate and sensory perception of a capsule by consumers [5].
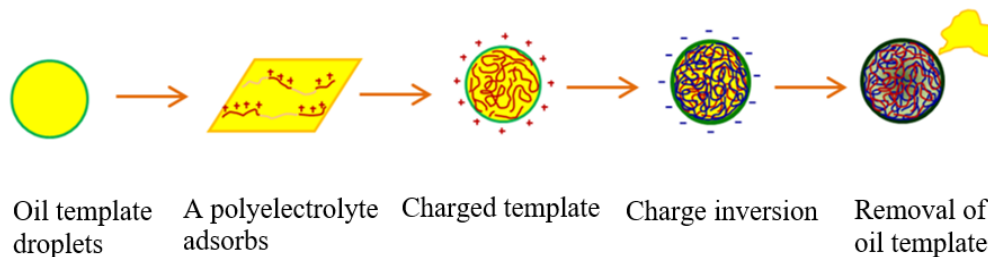

| Oil template droplets | A polyelectrolyte adsorbs | Charged template | Charge inversion | Removal of oil template |

Figure 2. LbL method (Adapted from Rossier et al. [5])

The method that can be applied to make microcapsules with these properties is the layer by layer (LbL) adsorption technique. This method uses the principle of self-assembly generated by chemical-physical phenomena naturally [5]. The oil granules act as a microcapsule template and as a container for the encapsulated active ingredients [5]. The flow chart for making microcapsules using the LbL method is presented in Figure 2.

The thickness of the microcapsule layer is one of the physical characteristics that need to be studied for the application of multilayer microcapsules in the fields of health, food, industry and agriculture. The thickness of the microcapsule layer can be determined based on the diameter of the microcapsule layer. This study examines the thickness of each multilayer microcapsule layer made of SPI nanofibrils.

2. Materials and methods

2.1. Materials and chemicals
The materials and chemicals are nanofibrils obtained from SPI [12,13,15–17], High methoxyl pectin (HMP), n-hexadecane and HCl 37%, and double distilled water was used to disperse the proteins.

2.2. Microcapsules preparation
Microcapsules made of protein nanofibrils as one of the building blocks were prepared following the method of Warji et al [12] and Purwanti et al [13]. The method was adopted to prepare the microcapsules

from SPI nanofibrils. At the beginning, SPI and HMP solutions were prepared by dissolving 0.1% w/w WPI, SPI or HMP in 25 mM sodium chloride solution at pH 3.5. The solution was centrifuged for 30 min at 925 xg to precipitate undissolved materials and then, filtered through 0.45 μm cellulose acetate syringe filter. The capsule template was made by emulsifying 1% w/w hexadecane in unheated WPI or SPI solution using a rotor-stator dispersion tool (T25 Ultra-Turrax®, Ika, Germany) equipped with S25N – 25F dispersing element at 9,500 rpm for 1 min. The result was emulsion droplets that had positive charges because pH of the protein was below its isoelectric point. The droplets were separated from the remaining protein solution using centrifugation at 70 xg to avoid any interactions between non-adsorbed proteins and the biopolymer in the next layer. The droplets were then dispersed in HMP solution that was set at pH 3.5. At this pH, the HMP solution had negative charges; therefore, it formed a layer on top of positively charged emulsion droplets. These bi-layered droplets were harvested by centrifugation and then dispersed in positively charge solution of protein fibrils. The protein nanofibrils deposited onto the droplets as the third layer. Subsequently, additional layers of HMP and protein nanofibrils can be deposited onto the droplets by repeating the same procedures until the desired number of layers achieved. This research prepared microcapsules with 7 layers.

2.3. Measurement of the microcapsule's diameter
The cream of two-layers microcapsules, five-layer microcapsules, and seven-layer microcapsules were diluted to 0.01% in a pH 3.5 format buffer solution and then measured using ZetaSizer NanoZS.

3. Results and discussions
The measurement results of the microcapsule layer diameter showed that the diameter of the second, fifth, and seventh layer are 9,703 nm, 12,450 nm, and 12,890 nm, respectively. The equation of the microcapsule layer diameter to the number of microcapsule layers is $y = 658.73x + 8,603.9$ with a value of $R^2 = 0.92$. This equation can be used to calculate or predict the diameter of other microcapsule layers.

The diameters of the first, third, fourth and sixth layers are obtained from predictions using this equation. The diameter of the single-layer or droplet microcapsules is 9,263.27 nm, the three-layer and six-layer microcapsules are 10,582 nm and 12,560.1 nm.
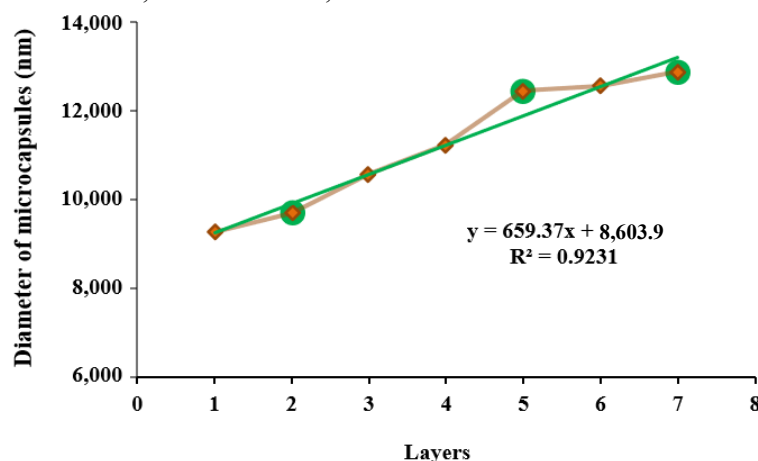

The equation shown on the chart: $y = 659.37x + 8,603.9$, $R^2 = 0.9231$

Figure 3. Diameter of microcapsules. Measurement (●) and prediction diameter (◆)

SPI microcapsules are spherical as in the SEM image (Figure 4a). The thickness of each layer is predicted based on this spherical shape as shown in Figure 4b. The droplet radius is 4,631.64 nm, the thickness of the second, third, fourth, fifth and seventh layers were 219.86 nm, 439.50 nm, 329.68 nm, 604.31 nm, 55.06 nm and 164.94 nm, respectively. The thickness of the second layer is obtained from the results of subtracting the diameter of the two-layer microcapsule with the droplet diameter then dividing it in half. The thickness of the third layer is obtained from subtracting the diameter of the three-layer microcapsule layer from the diameter of the two-layer microcapsule then dividing it in half. The thickness of the fourth, fifth, sixth and seventh layers is calculated the same as the third layer.
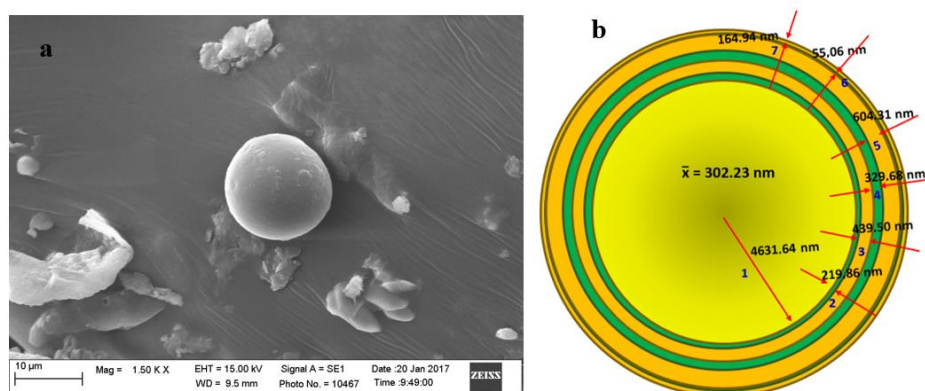
Figure 4. SEM image of SPI microcapsule (a) and layers of microcapsule (b)

The average thickness of the seven microcapsule layers without including the droplet thickness was 302.23 nm. The thickness of the seven layers of microcapsules is 1,813.37 nm; this thickness is similar to the thickness of the seven layers multilayer microcapsules produced by Purwanti et al [13]; wherein the seven-layer microcapsule thickness of the same material is 1.42 µm or 1,420 nm. The thickness of this microcapsule is thicker than the seven-layer microcapsule of WPI material; which is about 250 nm thick as reported by Rossier et al [5]. The thickness of each layer and the thickness of the microcapsule walls can provide information about the size of the microcapsules. This information makes it easier to apply microcapsules in the fields of agriculture, food, medicine and industry.

4. Conclusions

Some conclusions of this research contribute to availability of scientific knowledge on the thickness layers of multilayered-microcapsules from SPI using LbL adsorption method. There are some conclusions as follows: diameter of the first layer (droplet) was 9,263.27 nm and the seventh layer was 12,890 nm. The average thickness of the microcapsule layer is 302.23 nm and the thickness of the seven layers of microcapsules was 1,813.37 nm.

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