

EFFECT OF THE CHITOSAN CONCENTRATION ON THE STRUCTURE OF CHITOSAN BEADS FORMED IN NaOH and NaOH.SiO₂

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Abstract

This study aimed to investigate the effect of chitosan concentration (2–5%) and chitosan/SiO₂ ratio (4:1, 3:2, 3:1, 2:3, w/w) on the properties as well as the structure of chitosan beads (CS) and chitosan-SiO₂ beads (CS-SiO₂) formed in the NaOH and NaOH.SiO₂ solutions. The CS and CS-SiO₂ beads were produced by inject the mixture between the chitosan powder (various concentration) as well as the chitosan-SiO₂ mixture (different ratio) and acetic acid solution (5%) into NaOH (4%). Through tensile strength testing and SEM analysis, it is showed that the dimension of CS increased with the increase of chitosan concentration and reached the highest diameter of 3.1mm and weight of 400mg for CS bead (5% chitosan – 2CD). Whereas, the average diameter and weight of dry CS-SiO₂ beads reduced disproportionally with the SiO₂ amount, with the smallest diameter of 1.96 mm and weight of 250mg (3:1). In addition, the SEM images showed that the structure, hardness as well as the color of the CS-SiO₂ beads changed with the amount of SiO₂ in the bead. When the amount of SiO₂ increased, the diameter and number of pores of the CS-SiO₂ beads increased, while the hardness decreased. The CS beads at 5% had the largest breaking strength and elongation with 10N and 2mm, respectively; whereas, the CS-SiO₂ beads at 3:2 had the smallest breaking strength of 5 N and elongation of 1.5mm.

Keywords: chitosan, SiO₂, bead, hardness, CS-SiO₂

Introduction

Chitosan or poly-(1→4)-2-amino-2-deoxy-b-D-glucose is a biopolymer that can be chemically expressed as nontoxic, heterogeneous, linear, cationic and biodegradable polysaccharide with high molecular weight [3]. Usually, chitosan is prepared by deacetylation of chitin produced from shrimp and crab wastes with alkaline (deacetylation degree >75%) [12]. Chitosan, in comparison with other polysaccharides, has got more advantages in industries, agriculture, medicine and wastewater remediation because of presence of its large number of –NH₂ and –OH groups and its removing or recovering of organic substances ability such as proteins, polysaccharides, fatty acids, and bile acids from wastewater [2, 7, 8, 12]. In the wastewater treatment field, chitosan with various types such as: powder, solution or film has been studied in many countries, particularly in Vietnam. Among the different types of chitosan formation, chitosan beads are preferred because of its higher surface area and porosity. The study of Wu et. al (2000) showed that chitosan in bead-type has more efficiency in dye adsorption than that in flake-type [11]. Another researchers such as: Chang and Juang (2005), Guibal et. al (2003) also have an agreement with Wu et. al (2000, 2010) about the higher adsorption capacity of chitosan beads in comparison with chitosan flake [1, 4, 11, 12]. However, the bead formation research and its application are still limited. Theoretically, the smaller the diameter of adsorption substances is, the bigger the adsorption efficiency is due to the increase of total surface area of adsorption substance [3]. For the application of chitosan beads in reality, it is essential to enhance the surface area, the porosity of chitosan bead through determining and analyzing the physical and durable properties as well as the effect level of chitosan concentration of chitosan beads or chitosan-SiO₂ beads formed in NaOH solution [3]. Therefore, this research conducted the experiments to investigate the impact of the chitosan and SiO₂ concentration on the bead structure by using the differently physical analysis methods for the environmental treatment purposes

Materials and Methods

Materials

Chitosan powder (deacetylation degree 95% and molecular weight 1,12 million Dalton) is supported by Thien Nguyen Biomedical Co., Ltd, Ho Chi Minh city. The another chemical substances such as: acetic acid (CH₃COOH) 99,5 % and NaOH 96 % are manufactured in China and these meet the analysis standard. SiO₂ is supported from Chemistry Department, Hue Science University.

Methods

Preparation of Chitosan beads (CS) and Chitosan-SiO₂ (CS-SiO₂) beads

Preparation of Chitosan beads (CS)

The volume of 100ml chitosan solution with different concentration (2%; 2,5%; 3%; 3,5%; 4% and 5%) is prepared by mixing chitosan powder with acetic acid solution (5%) during 18 hours by grinding machine. Then, the aqueous solution is injected into the syringe and broken up into mono-dispersed droplets (1 droplet/second) by the shear force of the continuous flow in NaOH solution (4%) (figure 1). After solidification process, chitosan beads (CS) are washed several times with distilled water until the pH=7 [3, 12].

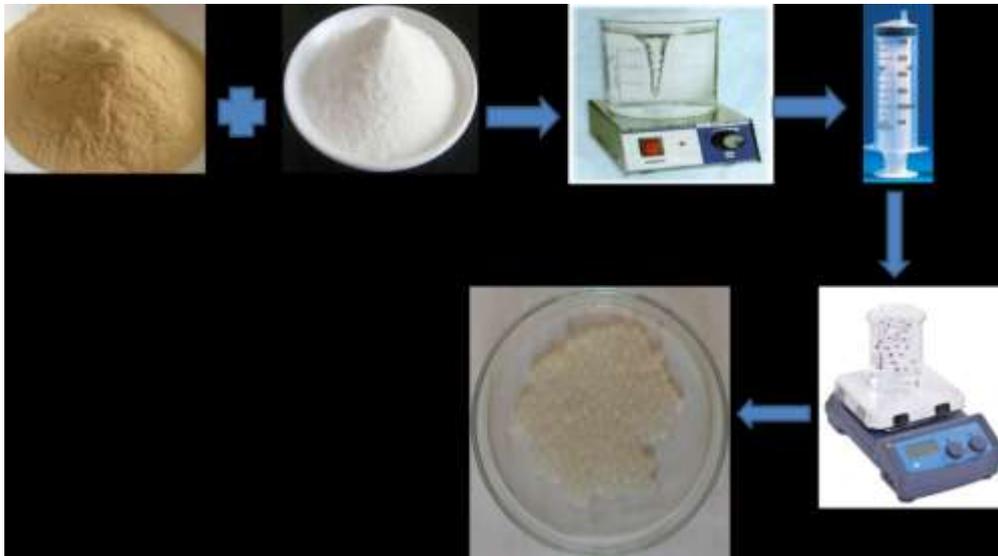


Figure 1. Chitosan beads (CS) and Chitosan-SiO₂ (CS-SiO₂) beads formation process

Preparation of Chitosan-SiO₂ beads (CS-SiO₂)

To prepare CS-SiO₂ beads, the mixture between chitosan and SiO₂ with the weight ratio (4:1; 3:2; 3:1 and 2:3) (gram) is dissolved in the acetic acid (5%) by using grinding machine during 18 hours. The process of CS-SiO₂ bead formation was the same as the CS bead formation.

Determination methods of bead dimensions

CS and CS-SiO₂ beads are dried at -50°C by using lyophilized method during 8 hours. The dimensions of beads before and after drying process are measured by electronic micrometer. The average value of each measurement is calculated based on the dimensions of five beads before and after drying process.

Determination method of bonding durability in the beads

The bonding durability of CS and CS-SiO₂ strings in wet and dry state is tested by tensile strength device (Model 334E52) and Bluehill lite software. The ultimate tensile strength is determined use Equation 1:

$$G_{ic} = \frac{3P\delta}{2b(a = |\Delta|)} \times \frac{F}{N} \text{ or } G_{ic} = \frac{3P^2C^{2/3}}{2A_1bh} \quad (1)$$

Where:

- G_{ic} : the ultimate tensile strength at the time the crack appeared, J/mm²
- P : tensile force, (N)
- δ : displacement in tensile measurement, mm
- a : crack length, mm
- Δ : adjustment factor of crack length,
- b : sample width, mm
- F : adjustment factor for δ
- N : adjustment factor for weight
- $C = \delta/P$
- A_1 : the slope of line illustrating the relationship between a/h and $C^{\frac{1}{3}}$

Fatigue strength is determined according to ASTM D3479-96 (2007) standard on American MPS 810 device (Material Test System 810), the tension force was 70% of the tensile strength of material at the vibration frequency with 2 Hz.

The deformation factor of string is calculated by the formulation 2:

$$\varepsilon = \frac{\Delta L_0}{L_0}$$

$$\varepsilon (\%) = 100 \times \frac{\Delta L_0}{L_0} \quad (2)$$

Where:

- ε : the deformation factor illustrated by nondimensional ratio or %
- L_0 : sample length, mm
- ΔL_0 : the increase of sample length, mm

The nominal value of tensile deformation is calculated by Equation 3:

$$\varepsilon = \frac{\Delta L}{L}$$

$$\varepsilon (\%) = 100 \times \frac{\Delta L}{L} \quad (3)$$

Where:

- ε : the nominally tensile deformation illustrated by no dimensional ratio or %
- L : the initial length between the two grips, mm
- ΔL : the length increase between the two grips, mm.

Determination method of CS and CS-SiO₂ bead structure

To evaluate the surface structure of CS and CS-SiO₂ beads, the scanning electron microscope method (SEM) is used by using Jeol-JMS 6490 device at various wavelengths from 200nm to 10 μ m

Statistic analysis methods

Data collection is processed by statistic method by using Microsoft Excel and SPSS 21 softwares. In addition, Oneway- ANOVA method is implemented to evaluate the diameter deviation of CS and CS-SiO₂ beads before and after drying process with the statistical significance $p < 0,05$.

Results and discussions

Chitosan beads (CS)

Chitosan beads at various concentrations (2-5%) formed in 4% NaOH solution are showed in Figure 2. Through organoleptic estimation, the hardness of chitosan beads tends to increase when the chitosan concentration rises. This result also agree with Zhao et.al [13] where they pointed out that the porosity of chitosan beads decreased when the chitosan concentration increased. At 2-2,5% chitosan concentration, the dimensions of chitosan beads are quite homogeneous, while these hardness is smaller and easy to crack. This also indicated in Long *et al.* (2011) study, the chitosan beads at 2,5% chitosan concentration was not round and cracked easily as well as bead formation in 4% NaOH solution [6].

There is an increase trend in the diameter of chitosan beads at chitosan concentration being larger than

2,5%, while these dimensions are inhomogeneous. The results from Ping He et al. also showed the similar trend. It can be explained that the size of the beads increase because of the presence of higher amount of chitosan in dropped beads [5]. Moreover, this bead formation tends to be string shape and the homogeneous state of these is quite low when the chitosan concentration rises to 4% or 5%. These results also coincide with the research of Long et al. (2011) and Vakili [6, 10]. The reason for this case can be explained by the increase of viscosity of chitosan solution as well as unequal shear force on syringe, it is hard to inject the high chitosan solution and this affects on the bead formation process.

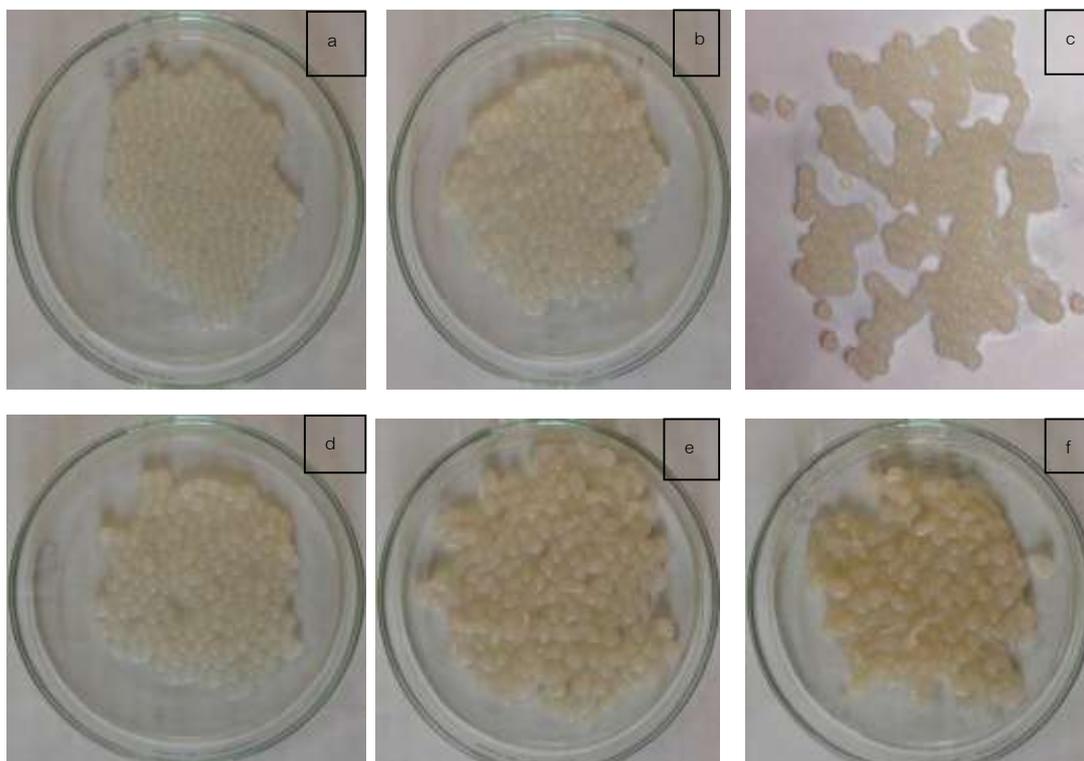


Figure 2. Chitosan beads (CS) at different chitosan concentration

a. chitosan 2%; b. chitosan 2,5%; c. chitosan 3%; d. chitosan 3,5%; e. chitosan 4%; f. chitosan 5%

Chitosan-SiO₂ beads (CS-SiO₂)

CS-SiO₂ beads with the weight ratio between chitosan and SiO₂ at 4:1, 3:2; 3:1 and 2:3 are formed. The experimental results show that the CS-SiO₂ (2:3) bead formation by syringe is easy. However, the CS-SiO₂ (2:3) beads are not coagulated in NaOH solution. These results can be explained by the use of low concentrations of chitosan to produce gel, the chitosan molecules shrinking together, occupying a small volume; results in the weight and size of gel particles forming has been too small. Another reason is poor network closely of gel forming when using low concentrations of chitosan gel to produce of beads and result in increasing pore size of chitosan bead. It is estimated that due to the unsuitable weight ratio between chitosan and SiO₂, the bonding between chitosan and SiO₂ molecular is weak during the coagulation process. Finally, the CS-SiO₂ (2:3) beads are not formed in NaOH solution. On the other hand, the coagulation process takes place in CS-SiO₂ solutions at (4:1, 3:1, 3:2) weight ratio showed in figure 3. Through the organoleptic estimation, the color of CS-SiO₂ beads changes from yellow to white when the amount of SiO₂ in CS-SiO₂ solution increases. The reason for this situation is due to the color of SiO₂ substance affecting on the color property of CS-SiO₂ beads. From the experimental results, thanks to the amount of SiO₂, it is easier to form CS-SiO₂ beads because of the

decrease of the viscosity of the CS-SiO₂ solution. Nevertheless, the structure of CS-SiO₂ beads changes and this leads to the decrease in hardness of CS-SiO₂ beads.

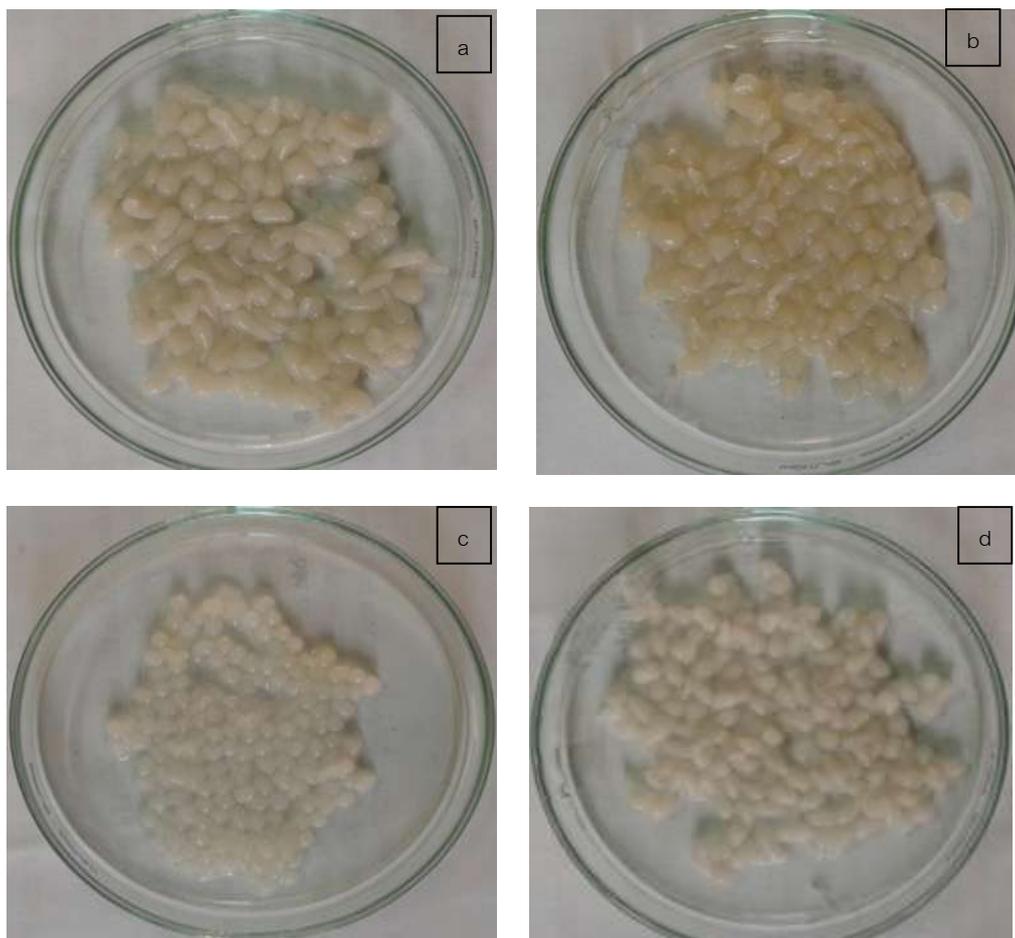


Figure 3. Chitosan (CS) and Chitosan-SiO₂ (CS-SiO₂) beads

a. CS-SiO₂ (3:1); b. CS (4%); c. CS-SiO₂ (3:2); d. CS-SiO₂ (4:1)

The properties of CS and CS-SiO₂ beads

The dimension of CS and CS-SiO₂ beads before and after drying process

Results in Table 1 and Table 2 indicated that the diameters of CS and CS-SiO₂ beads depended on the concentration of chitosan and chitosan-SiO₂ solution and significant different ($p < 0,05$). The largest diameter of CS bead occurred in 5% chitosan concentration with 3,98mm while the smallest of diameter is for CS-SiO₂(3:1) bead with 2,17mm ($p < 0,05$). Furthermore, there is a profound change in the diameter of CS and CS-SiO₂ beads before and after drying process where the largest difference in diameter is in CS-SiO₂ (3:2) bead. This diameter reduced from 3,08 mm (before drying process) to 2,02 mm (after drying process). A more detailed of bead structure look at the SEM images reveals that the density and the dimension of porosity of the bead structure increases due to the amount of SiO₂, therefore the amount of water evaporating from the CS-SiO₂ beads is much more than CS beads. This leads to the great change in diameter of CS-SiO₂ beads after drying process. In addition, CS-SiO₂ beads are considered as potential substance for toxic treatment not only in wastewater field but also in another industrial one because of the increase of total surface area [9, 14]. For CS beads, these dimensions tends to increase when the chitosan concentration is larger than 3% and CS beads turn into string shape at 5% chitosan concentration.

Table 1. The diameters of CS and CS-SiO₂ beads before drying process

Type of bead	Average diameter (mm)	Minimum diameter (mm)	Maximum diameter (mm)
2CD	2,56 ±0,04 ^a	2,52	2,62
3CD	3,99 ±0,14 ^b	3,85	4,21
4CD	3,08 ±0,10 ^c	2,97	3,19
5CD	2,17 ±0,02 ^c	2,14	2,20
7CD	3,16 ±0,08 ^c	3,09	3,29

Noted:

- 2CD (CS): Chitosan 4% + CH₃COOH 5% + NaOH 4%; 3CD (CS): Chitosan 5% + CH₃COOH 5% + NaOH 4%; 4CD (CS-SiO₂): Chitosan 3% + 2g SiO₂ + CH₃COOH 5% + NaOH 4%; 5CD (CS-SiO₂): Chitosan 3% + 1g SiO₂ + CH₃COOH 5% + NaOH 4% and 7CD (CS-SiO₂): Chitosan 4% + 1g SiO₂ + CH₃COOH 5% + NaOH 4%

- The values in the same row that have a different superscript are significantly different at P<0.05

Table 2. The diameters of CS and CS-SiO₂ beads after drying process

Type of bead	Average diameter (mm)	Minimum diameter (mm)	Maximum diameter (mm)
2CD	2,10 ±0,01 ^a	2,08	2,13
3CD	3,10 ±0,02 ^b	3,08	3,14
4CD	2,02 ±0,03 ^c	1,99	2,05
5CD	1,96 ±0,02 ^c	1,93	1,99
7CD	2,98 ±0,03 ^c	2,95	3,01

Noted:

- 2CD (CS): Chitosan 4% + CH₃COOH 5% + NaOH 4%; 3CD (CS): Chitosan 5% + CH₃COOH 5% + NaOH 4%; 4CD (CS-SiO₂): Chitosan 3% + 2g SiO₂ + CH₃COOH 5% + NaOH 4%; 5CD (CS-SiO₂): Chitosan 3% + 1g SiO₂ + CH₃COOH 5% + NaOH 4% and 7CD (CS-SiO₂): Chitosan 4% + 1g SiO₂ + CH₃COOH 5% + NaOH 4%

- The values in the same row that have a different superscript are significantly different at P<0.05

The weight of CS and CS-SiO₂ beads before and after drying process

The weight results of CS and CS-SiO₂ beads before and after drying process are show in Table 3. The experimental results indicate that the weight of CS bead (5%) is considerably higher than that of CS (4%) and the weight of the former after drying process decreases significantly. For the CS-SiO₂ bead, the amount of SiO₂ can contribute into the increase of CS-SiO₂ wet weight, however, the change in the weight of this bead is much higher than that of CS bead due to the above reasons mentioned in Table 3.

Table 3. The weight of CS and CS-SiO₂ beads after drying process

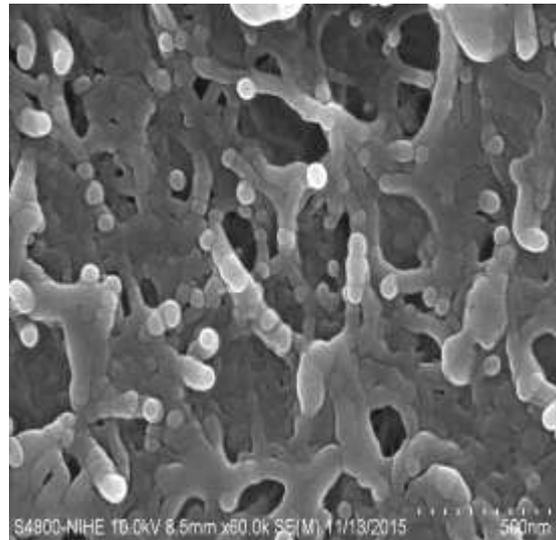
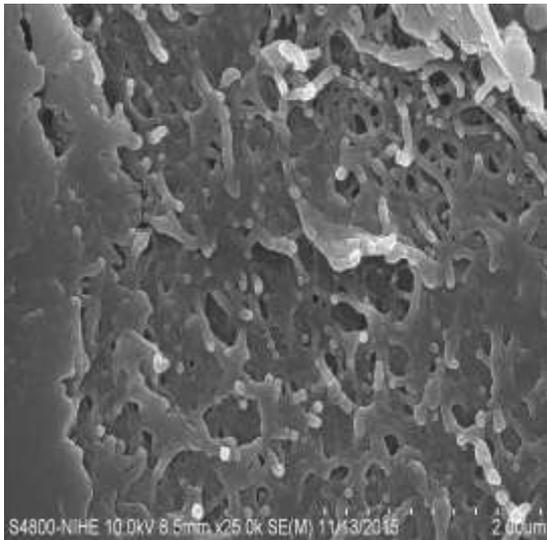
Unit: gram (g)

Type of bead	Weiight of beads	
	Before drying	After drying
2CD	6,66	0,36
3CD	8,39	0,4
	CS-SiO ₂ beads	
4CD	9,9	0,6
5CD	4,85	0,25
7CD	7,33	0,39

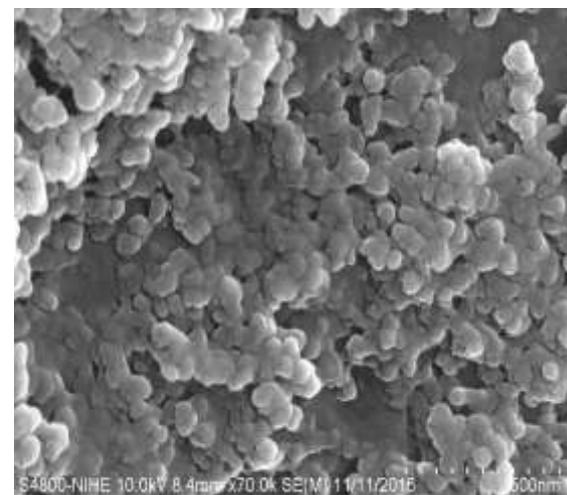
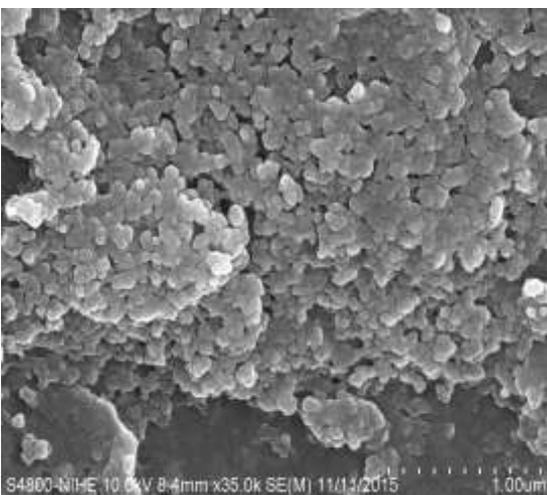
The porosity of CS and CS-SiO₂ beads

To investigate the effect of chitosan and SiO₂ concentration on the surface structure of CS and CS-SiO₂ beads, SEM image analysis method was applied to identify of 5 type beads with the wave lengths change from 200nm to 10 μm. SEM image analysis result of 5 types CS and CS-SiO₂ beads are shown in Figure 4 a, b, c, d and e. Figure 4 showed that the surface structure of CS-SiO₂ and CS beads are significantly different. Surface structure of CS-SiO₂ bead have pore network of distribution more than CS beads. On the other hand, the concentrations of SiO₂ in the chitosan gel increase resulting in increasing of pore network in the surface structure (Figure 4 c, d, e). However, the pore size (capillaries) in the bead structure is different and heterogeneity. In addition, the pore structure of the distribution network on the surface of CS-SiO₂ beads (mass ratio CS/SiO₂ at 3: 1 and 3:2) is more ambiguous than the pore structure of the distribution network on the surface of the CS and CS-SiO₂ 4% or 4: 1 (Figure 4 a, e). The pore size of CS and CS-SiO₂ beads ranges from 500nm-2μm in which the pore size from 500-700nm accounted for the largest number. Results from SEM image analysis and experimental measurement indicated that CS-SiO₂ bead have more pore distribution than CS bead, but the hardness is decrease if SiO₂ concentration increasing. This can be explained by the presence of SiO₂ in the contact between amine and aldehyde group and it took a long time for silica transfer to silica gel. It resulted in lowering in solidification velocity. Thus, the CS-SiO₂ beads would be formed spongy pores and they have larger specific surface area than that in CS beads [14]. In addition, the uniformity in mixing has reduced the links and dissection of SiO₂ in NaOH incomplete as results in increasing of pore size in the bead structures. Therefore, time and speed as well as the method to mix of SiO₂ through dissection of NaOH environmental to be further research to assess the completeness of the surface structure of CS and CS-SiO₂ beads.

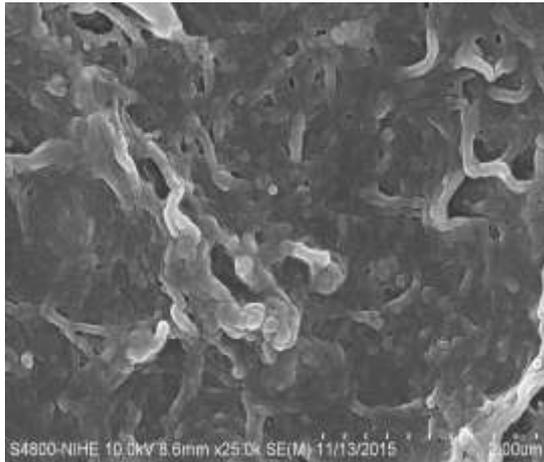
a. 2CD beads



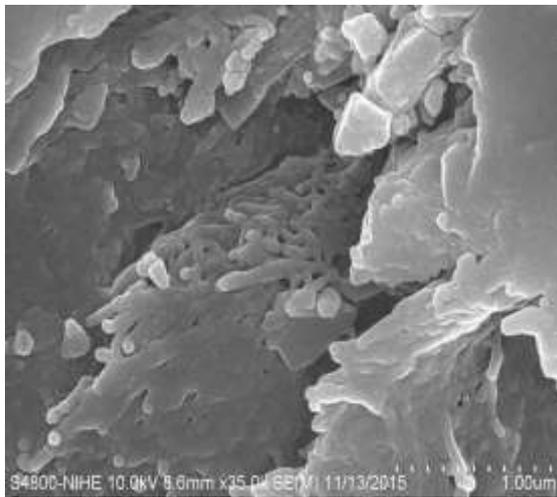
b. 3CD beads



c. 4CD beads



d. 5CD beads



e. 7CD beads

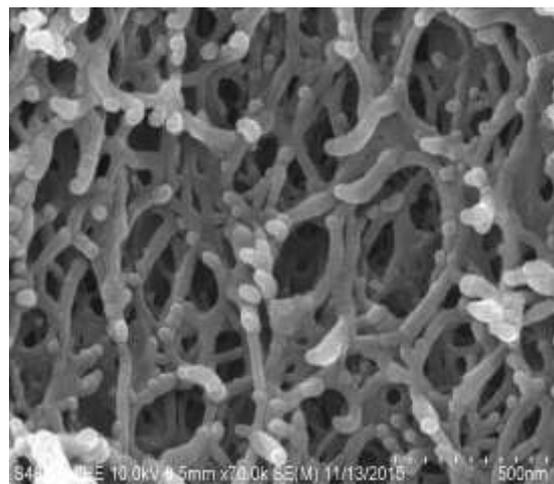
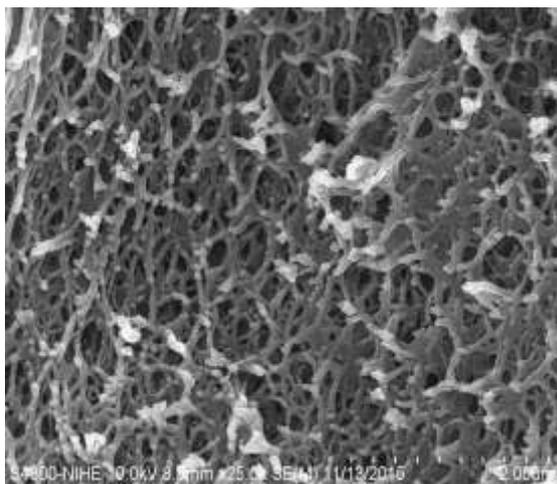


Figure 4. SEM image analysis the distribution of pores in CS and CS-SiO₂ bead structures
(a): 2CD (CS): Chitosan 4% + CH₃COOH 5% + NaOH 4%; (b) 3CD (CS): Chitosan 5% + CH₃COOH 5% + NaOH 4%; (c) 4CD (CS-SiO₂): Chitosan 3% + 2g SiO₂ + CH₃COOH 5% + NaOH 4%; (d) 5CD (CS-SiO₂): Chitosan 3% + 1g SiO₂ + CH₃COOH 5% + NaOH 4% and (e) 7CD (CS-SiO₂): Chitosan 4% + 1g SiO₂ + CH₃COOH 5% + NaOH 4%

The hardness of CS and CS- SiO₂ beads

To measure the hardness of beads, CS and CS-SiO₂ chains have been created and measured in dried and wet formation (Figure 5). The experimental results indicated that during the wet form, fixed chains with tensile force <1N was broken, so the process for determining the ability and strength of the string CS and CS-SiO₂ in wet gel was not performed. For dried CS and CS-SiO₂ chains, the experiment was conducted with the change of tensile force from 1 N to 10 N and the results were shown in Figure 6. The results in Figure 6 indicated that the displacement of CS and CS-SiO₂ chains are significant different according to SiO₂ concentration during the experimental process. The strength of CS string increases with increasing chitosan concentration, while the SiO₂ concentration increased resulting in durable reduction of the chain. Figure 6 also shows the CS string (5%) have the displacement (elongation) until the structure breaks is the largest with 2mm at tensile force 10 N, while the string of CS-SiO₂ (3:2) has the largest displacement with only 1.5mm and its structure breaks at 5N in tensile force. Therefore, the durability of string increase when chitosan concentration increasing, whereas the reliability of the CS-SiO₂ chain reduce when increasing of SiO₂ concentration, which shows that the durability of CS string is bigger than that of CS-SiO₂ string. For these results, it can be explained that due to CS-SiO₂ strings have more porous structure than that in CS strings , therefore their durability are smaller than CS’s durability [14]. Combination between physically evaluation and SEM image analysis confirmed that chitosan and SiO₂ concentration effect significantly on the durability and structure of CS and CS-SiO₂ bead and string. Therefore, the ratio of CS-SiO₂ beads during manufacturing process needs to be further research in order to create products with larger adsorption surface and higher reliability for industrial applications as well as in manufacturing materials for wastewater treatment.



Figure 5. CS and CS-SiO₂ chain before (a) and after (b) dry process

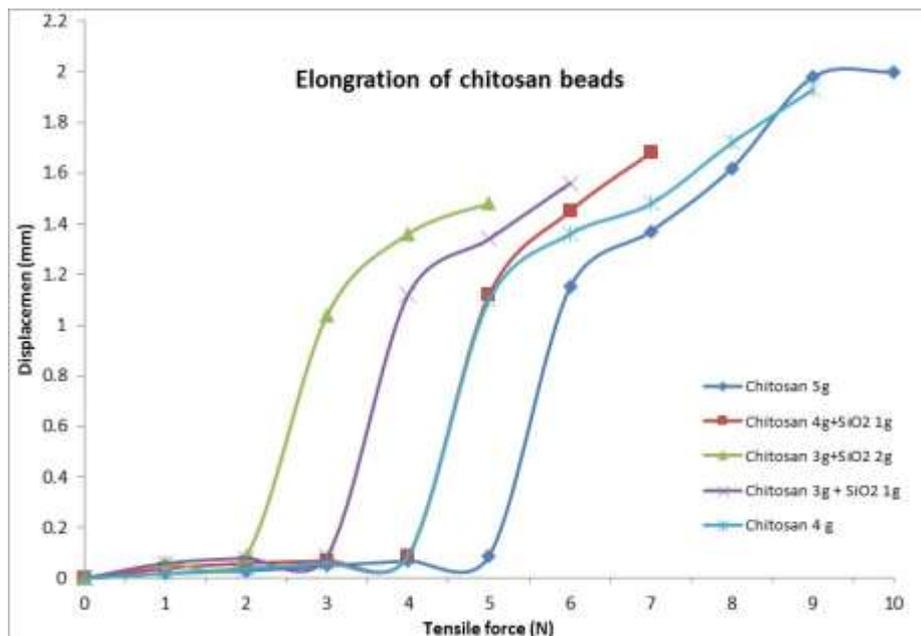


Figure 6. Tensile force and displacement in measurement of structural durability destroyed of CS and CS-SiO₂ dry beads

Conclusions

The experimental results indicated that the physical properties of the CS and CS-SiO₂ beads significantly different when chitosan and SiO₂ concentration changes in the structural during formulation process. Chitosan concentration increased in the structures then the diameter and weight of chitosan bead is increasing. While the presence of SiO₂ in the structure as result in reduces the size and weight of CS-SiO₂ bead after drying and significantly smaller than the size of the CS bead ($p < 0.05$). The hardness of CS bead at the concentration of 5% chitosan has biggest and structure is broken at 10N tensile force, while the durability of the CS-SiO₂ bead at ratio of 3: 2 have the lowest and the structural bead broken at tensile force of 5N. On the other hand, the changes of SiO₂ result in change of surface structure, hardness and color of the bead. If the SiO₂ concentration increases, the size and the number of pore in the bead surface structure increases (500nm-2 μ m) and the distribution unequally. In addition the hardness of the bead reducing and the surface color change from yellow to white. From the experimental results, it is believed that CS-SiO₂ beads will become potential substance for toxic treatment in both wastewater field and industrial one because of the increase of total surface area.

Acknowledgements

This research was supported by a Basic Science Research Program through the Hue University funded by the Ministry of Education (HUE2014-83)

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