



Applied Chemistry Project

Project title Effects of acids and ZSM-5 as catalyst for chitosan
depolymerization process

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by

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In Partial Fulfillment for the Degree of Bachelor of Science
Program in Applied Chemistry (International Program)
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Abstract

Chitosan (CS) is a linear polysaccharide derived from chitin which is the second most abundant polysaccharide in the world. With its biocompatibility and non-toxic property, it has been used in various fields such as agriculture field, industrial field, biomedical field, or even chemical field. However, chitosan itself is a large molecule that is insoluble in water and this fact limits lots of their applications. To utilize this chitosan for the best results, there becomes to be more and more research on the conversion of chitosan into chitosan oligomers with expected molecular weights less than 3,900 Da or about 24 mer which have high anti-biomedical property that is suitable for advanced biomedical application. In this report, it carried out about chitosan depolymerization using two different kinds of acid including HCl and CH₃COOH along with ZSM-5 as a catalyst to achieve chitosan oligomers. The result showed that HCl showed greater results than CH₃COOH as about 30% of their depolymerized products are chitosan oligomer with molecular weight of 3800 Da which are in the range that preferred. While the appearance of ZSM-5 provided wider molecular weight distribution in both HCl and CH₃COOH condition.

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1. Introduction

1.1 Background

1.1.1 Chitin, chitosan and chitosan oligomers

Chitin is a linear polysaccharide consisting of β -(1 \rightarrow 4)-linked N-acetyl-D-glucosamine. It is the second abundant polysaccharide after cellulose and is also insoluble in water and acidic aqueous solution. Chitin can be found in the cell walls of fungi, yeasts and mainly in exoskeleton of insect and marine arthropods such as crabs and shrimp.

Chitosan (CS) is a linear polysaccharide derived from chitin. Chitosan's structure is composed of randomly distributed β -(1 \rightarrow 4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). Like chitin, chitosan is also insoluble in water however it is soluble in acidic aqueous solution [1].

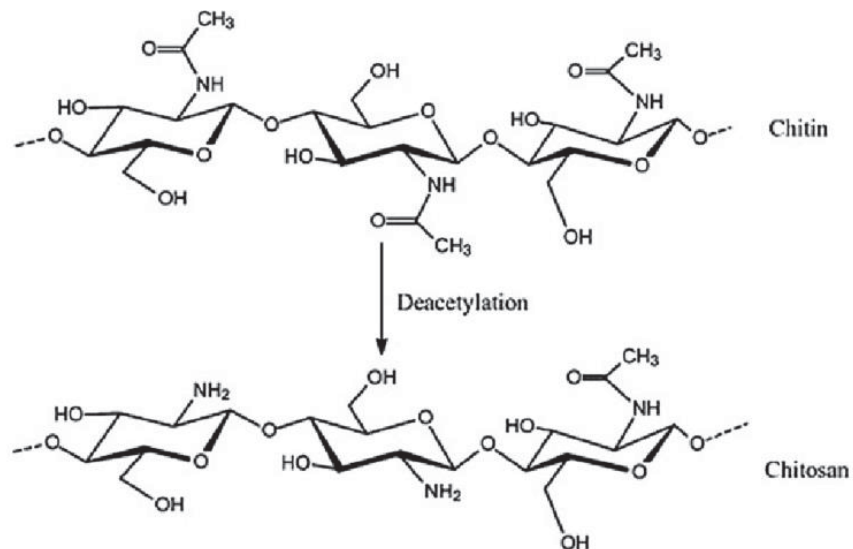


Figure1.1 Structure of chitin and chitosan

On the other hand, chitosan oligomers which have been converted from chitosan until can be soluble in water usually has their average molecular weight around less than 3,900 Da. There are many methods to convert chitosan into chitosan oligomers including acid-hydrolysis, ball-milling treatment, or enzymatic hydrolysis.

1.1.2 Application of chitin, chitosan and chitosan oligomers

Due to their difference in solubility, chitin, chitosan and chitosan oligomers have been used in different purposes. Chitin which are insoluble in both water and acidic solution usually found in technique and agricultural applications such as protein purification, fat and heavy metal binder, antifungal plant protection agent and fertilizer additive. On the other hand, chitosan which have more solubility property and strong anti-biomedical effect can be found in basic medical and food applications including antibacterial and microbial effects, anti-inflammatory and nutrient encapsulation. Last but not least, chitosan oligomers which have low molecular weight less than 3,900 Da can be soluble in both water and acidic aqueous solution, normally used in cosmetic and advanced medical applications such as anti-oxidant agent, gene therapy and drug delivery [2],[3].

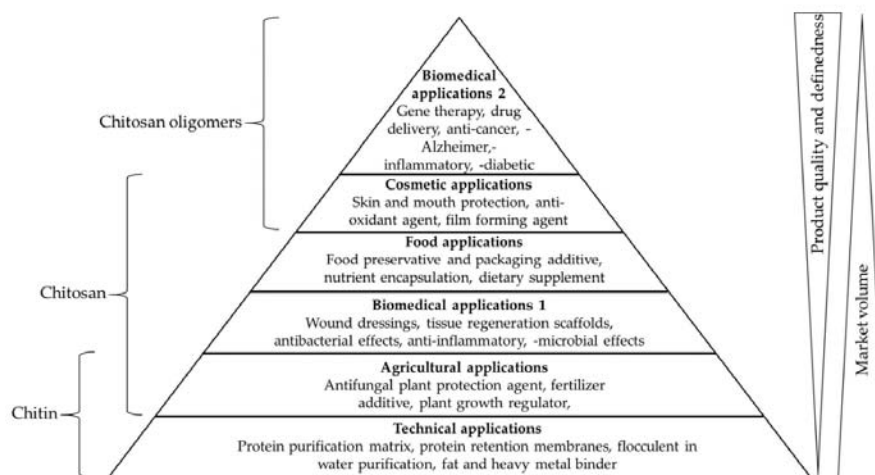


Figure1.2 Application of chitin, chitosan and chitosan oligomers

1.2 Literature reviews

1.2.1 Dissolution of chitosan in different solvents

Due to its molecular structure and pKa, chitosan is insoluble in water and other neutral solution. However, it is soluble in organic acidic solution such as diluted acetic acid (1%). Acetic acid became a popular solvent to prepare chitosan solution before it has been depolymerized by acid-hydrolysis or enzymatic-hydrolysis [4].

Solvent	Chitosan
H ₂ O	Insoluble
NaOH (1%)	Insoluble
HCl (1%)	Swelling
Acetic acid (1%)	Soluble
Dimethyl acetamide	Swelling
Dimethylformamide	Swelling
Dimethylsulfoxide	Insoluble
Pyridine	Swelling
Acetone	Insoluble
Ethanol	Insoluble
Dichloromethane	Insoluble

Table1.1 Solubility of chitosan in different solvents [4]

1.2.2 Acid hydrolysis of chitosan

Acid hydrolysis is the most common chemical method to depolymerize chitosan since it is good for the mass production, low costs, and simplicity. Acid hydrolysis method also help increasing in deacetylation degree of chitosan, which improve reactivity of chitosan with anionic species. The main process of acid hydrolysis is to protonate glycosidic linkage. From studying the interaction between chitosan and water of the acid hydrolysis it showed good results in improving solubility of chitosan in water [5].

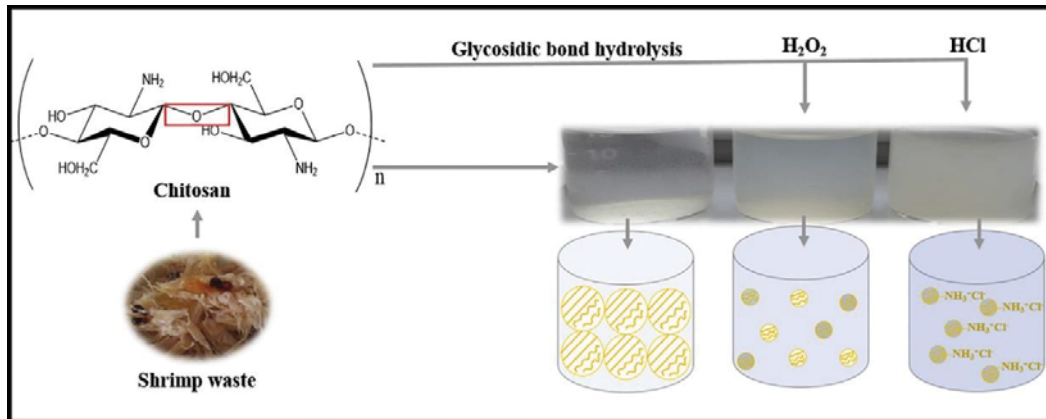


Figure 1.3 Depolymerization of chitosan by oxidative and acid pathways

According to Aljbour, N. D. Beg, M. D. H., and Gimbun, J., they used 2M HCl to depolymerize high molecular weight chitosan (HMWC) to low molecular weight chitosan (LMWC) the experiment showed the great results with the average yield of hydrolyzed product is around 87% which is high when compared with the result that got from the experiment that used more concentrate HCl. However, one of the drawbacks of acid hydrolysis method is it cannot control the desired molecular weight product well due to excessive degradation into monosaccharide and several purification processes are required when used high concentration acid [6].

Apart from HCl, other concentrated acid such as H_2SO_4 and H_3PO_4 were used to be studied in chitosan depolymerization as well. To find new way of production of low molecular weight chitosan, Zamani, A., Edebo, L., Niklasson, C., & Taherzadeh, M. J. performed a study by treated 1,388 kDa chitosan with 72mM, 216 mM and 360 mM H_2SO_4 at 120°C. By treating high molecular weight chitosan with 72 mM H_2SO_4 for 30 min the molecular weight decreased to 174 kDa with 82% recovery and by increasing time of reaction to 240 min the final molecular decreased to 24 kDa with 54% recovery. The experiment also performed with 216 mM and 360 mM H_2SO_4 with the result showed in higher degree depolymerization but decrease in percent recovery. This result

showed that too high concentration acid is not a good condition to obtain high-yield low molecular weight chitosan [7].

Hasegawa, M., Isogai, A., & Onabe, F. also performed an experiment about production of low molecular weight chitosan by using 85% H_3PO_4 to solve the problem of too strong reaction that limit the production of low molecular weight chitosan with degree of polymerization more than 6 without by-product. They obtained effective result as the product from treating chitosan with 85% H_3PO_4 gave low molecular weight chitosan with high yield, narrow degree of polymerization and weight-average degree of polymerization between 7-17 [8].

From many studies about acid hydrolysis of chitosan, it suggested that the optimum condition for obtain low molecular weight chitosan with high yield and narrow degree of polymerization without forming by product is not to use too high concentration acid.

1.2.3 Zeolite as a support for acid-hydrolysis

Nowadays the idea of applying zeolite in acid-hydrolysis process of chitosan is growing to catch an interest of scientists in many fields. As zeolite are aluminosilicate that have a porous structure that can accommodate a wide variety of cations. It also known as the molecular sieve which referred to its ability to selectively sort molecules based primarily on a size exclusion process. According to their property, zeolite is commonly used as commercial adsorbents and heterogenous catalysts in many reactions such as in hydrocarbon isomerization reactions or even for gas separation [9]. In 2015, *Ibrahim, K.* performed a study on acid hydrolysis of chitosan with zeolite as adsorbent, in this study three types of zeolite with different pore sizes (3 Å, 5 Å, 10 Å) have been used as an adsorbent material to help accelerate the equilibrium of the acid hydrolysis reaction during the chitosan oligomer formation which the results showed that the presence of zeolite causes the shift in hydrolysis equilibrium of chitosan forward and increased

the yield of the hydrolyzed products. However, different pore sizes did not affect property of the obtained chitosan that much. Nevertheless HZSM-5 with pore size 5 Å reported to be the most preferred zeolite since it is very stable in both acidic solution and hot water, it also did not decompose into solution like the other two zeolite that decomposed and caused the solution to change a color [10],[11].

According to this fact, ZSM-5 becomes to be an interesting zeolite to be used in further study on chitosan depolymerization.

1.3 Objective

In order to solve the problem of chitosan applications that due to the poor solubility of chitosan, so in this project we aim to find the efficient acid hydrolysis method to convert insoluble chitosan into the valuable chitosan oligomer with expected molecular weight less than 3,900 Da that can be soluble in water and apply in many useful applications. To meet that process, we need to success of this following objective:

1. To study the effect of different acids and ZSM-5 in depolymerization process of chitosan

2. Experimental

2.1 Chemicals

1. Raw chitosan flakes (60 kDa)
2. HCl 37%
3. CH₃COOH 100%
4. NaOH 10 M
5. EtOH
6. MeOH
7. Acetone
8. ZSM-5
9. Acetonitrile
10. Milli Q water
11. Distilled water
12. Water
13. Charcoal powder

2.2 Equipment and instrument

1. Beaker 25 ml
2. Beaker 50 ml
3. Beaker 100 ml
4. Beaker 250 ml
5. Beaker 500 ml
6. Beaker 1000 ml
7. Viral glass tube
8. Centrifuge tube 15 ml
9. Centrifuge tube 85 ml
10. Vortex mixer
11. Centrifuge machine
12. Glass watch
13. Micropipette 1 ml
14. Micropipette 5 ml
15. Glass pipette 1 ml
16. Glass pipette 5 ml
17. Plastic dropper 5 ml
18. Glass dropper + rubber
19. Glass rod
20. Spatula
21. Syringe 5 ml + syringe filter 0.45um
22. Cylinder 10 ml

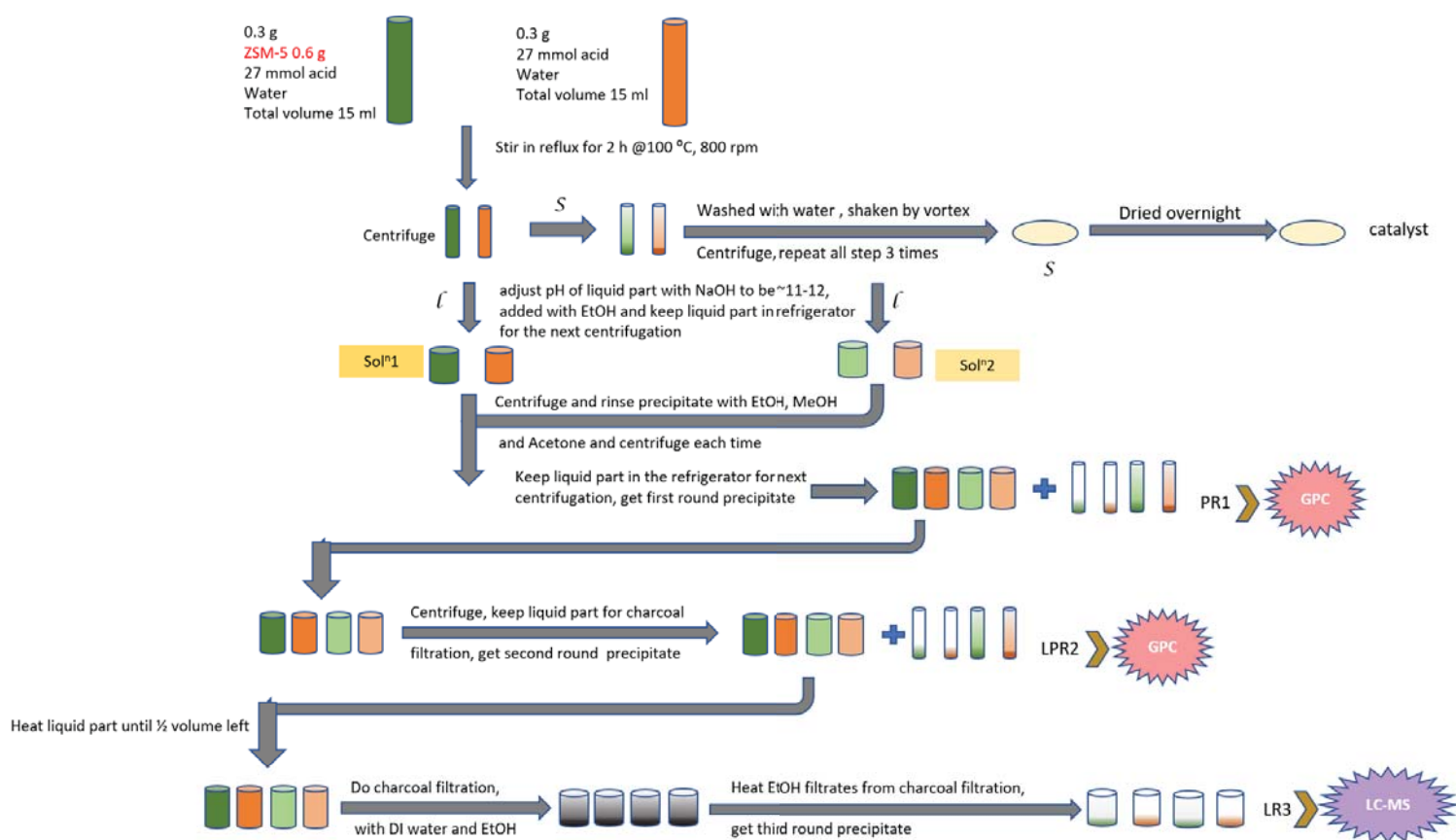
23. Evaporating dish
24. Suction flask
25. Buchner funnel
26. Rubber bung
27. Vacuum filtration
28. Hotplate
29. Filter funnel
30. Analytical balance
31. Precision balance

2.3 Methodology

2.3.1 Hydrolysis of chitosan and chitosan oligomer formation

Two conditions of solution were prepared; first condition is without catalyst (ZSM-5) and other condition is with catalyst. A 0.3 g of 60kDa chitosan flakes was mixed with 0.6 g of ZSM-5 (in with catalyst condition) ,27 mmol of acid solution (HCl and CH₃COOH), and water to make the total volume become 15 ml. Then this solution went under hydrolysis process during the reflux reaction at 100 °C and stir at 800 rpm for 2 hours. After finished the reaction, the solution then left in the room temperature for 30 minutes and in the cold water for 15 minutes before did the centrifugation for 10 minutes. The liquid part, remarked as solution1, was then performed the adjustment of the pH to be about 11-12 with NaOH while the solid part was added with 10 ml of milliQ water, was shaken with vortex for 5 minutes as there might have some chitosan left on the ZSM-5 and then was centrifuged again for 10 minutes, this session was repeated for 3 times. Then the liquid from the solid part which assigned to be solution2 was also been adjusted the pH as well. For the left solid part, it was put in the oven overnight to become catalyst. The adjusted pH solutions were added with 15 or 30 ml of EtOH depend on their condition, for the solution1, 15 mL of EtOH was added while in case of solution2, 30 mL of EtOH was added and kept in the refrigerator overnight to let the solution precipitates and did centrifuge again in the next day. During the centrifugation, the precipitates were rinsed with EtOH, MeOH and acetone which precipitates were later kept in the refrigerator and waited for the freeze dry to become the first-round precipitates which will be later analyzed by GPC, while the liquid part were also been kept in the refrigerator for centrifugation in the next day as there may be some small oligomer chitosan left in the solution and did not precipitates in the first-round. The liquid part from the centrifugation was then heated until the volume was half left before performed the charcoal

filtration with distilled water and EtOH which the EtOH filtrates was later kept in the refrigerator for heating in the next part. As the same time, the solid part was kept in the refrigerator and waited for the freeze dry to become the second-round precipitates and analyzed with GPC. Lastly the EtOH filtrates from charcoal filtration were heated until there are only precipitates left which is the third-time precipitates that will be later analyzed by LC-MS.



Scheme2.1 Protocol of chitosan hydrolysis and chitosan oligomer formation

2.3.2 Analysis of chitosan oligomer

2.3.2.1 Gel Permeation Chromatography (GPC)

In this study GPC was used to find the molecular weight of chitosan oligomer that obtained from the precipitates round one and two.

2.3.2.2 Liquid chromatography–mass spectrometry (LC-MS)

In the LC-MS case, it was used to find the molecular weight of chitosan oligomer that has been obtained from the precipitates round three that came from heating the liquids part and is the one with the smallest size among the three precipitates samples.

2.3.2.2.1 Prepare sample for LC-MS analysis

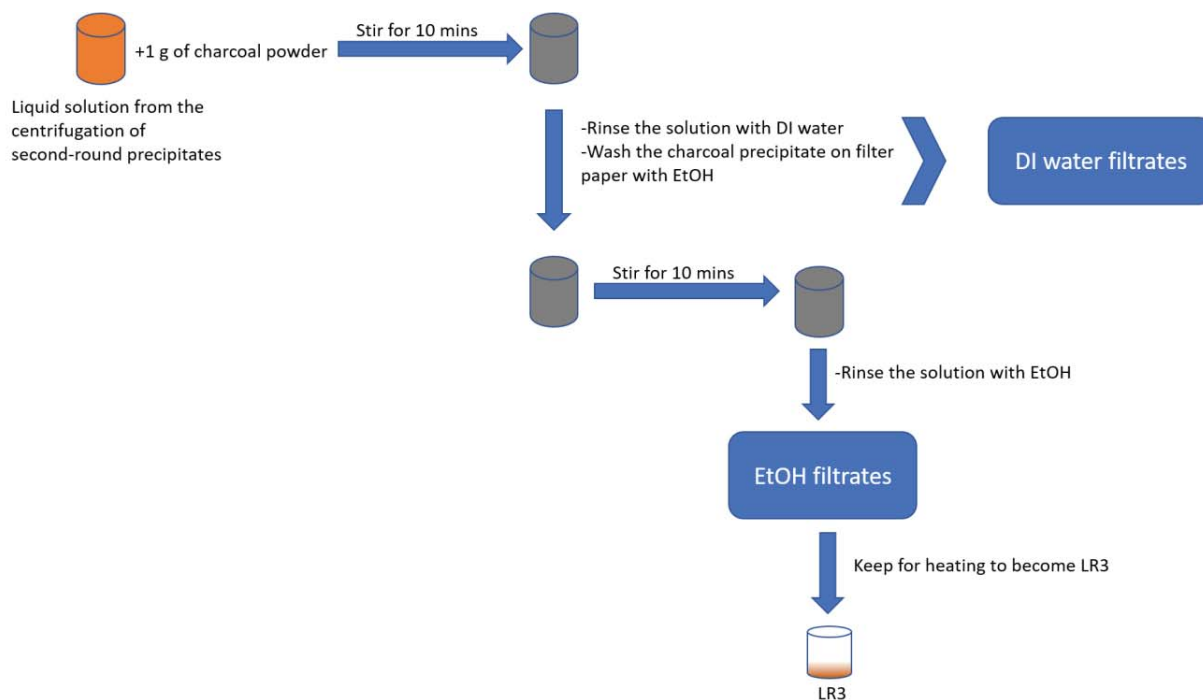
1.5 ml of total sample for analyze with LC-MS was prepared by weighted 1.2 ml of chitosan oligomer sample then added with 1.042 ml of Acetonitrile and 0.446 ml of water. For the blank sample, 1.5 ml of mixture of 70% acetonitrile and 30% water was prepared.

2.3.3 Charcoal Filtration

In this study charcoal filtration was used in order to remove salts that occur from the acid and NaOH that used in adjusting pH of the chitosan solution and as the result clear chitosan solution were obtained and heated to become third-round precipitates.

1 g of charcoal powder was added to liquid solution from the centrifugation of the second-round precipitates, then stirred the charcoal solution for 10 minutes. After 10 minutes passed the solution was filtrated in the vacuum with distilled water after finished the filtration the leftover charcoal on the filter paper was washed with EtOH and poured into the new beaker, stirred the

solution for 10 minutes preformed filtration similar to the process above but change the rinsing solvent from distilled water to EtOH. The filtrates from charcoal filtration with EtOH was heated to become third-round precipitates.



Scheme2.2 Charcoal filtration

3. Results and discussion

3.1 Qualitative analysis by Gel Permeation Chromatography (GPC)

Gel permeation chromatography (GPC) is one type of size exclusion chromatography that has ability to separate the analyte based on their size, the separation occurs in the porous column. The concept of the GPC is that the retention time of the analyte will inversely proportional to the size of analytes as the smaller analytes can enter the pores more easily and therefore spend more time in these pores and elute last, so their retention time will increase. In contrast, the larger analyte will spend shorter time in the pores and elute first [12].

3.1.1 Effect of acid and ZSM-5

In this study, two different acids which are HCl and CH₃COOH have been used to hydrolyze chitosan along with ZSM-5 as a catalyst. The reaction condition was fixed to 15 mL of chitosan solution, 0.3 g of chitosan flakes (60 kDa), 27 mmol of acid and in with catalyst condition, 0.6 g of ZSM-5 was used. This condition act at 100 °C for 2 h of reaction time.

Table3.1 Summary results from GPC analysis

Condition	Molecular weight (Da(mer))						Sample weight (g)
RT	89,638 Da (557 mer)						0.3096
100°C	5,575 Da (35 mer)	62,204 Da (386 mer)					0.3023
ZSM-5	27,474 Da (171 mer)	59,690 Da (370 mer)	69,301 Da (430 mer)				0.3408
HCl	815 Da (5 mer)	3,800 Da (24 mer)	15,092 Da (94 mer)				0.2546
HCl+ZSM-5	815 Da (5 mer)	862 Da (5 mer)	864 Da (5 mer)	6,541 Da (41 mer)	13,819 Da (86 mer)	18,639 Da (116 mer)	0.2776
CH ₃ COOH	34,815 Da (216 mer)	54,338 Da (338 mer)					0.2960
CH ₃ COOH +ZSM-5	6,936 Da (43 mer)	22,527 Da (140 mer)	52,965 Da (328 mer)	64,751 Da (402 mer)			0.3320

Reaction conditions: 0.3 g Chitosan, 15 ml of 1wt% CH₃COOH, total volume = 15 mL of chitosan solution (Flakes chitosan as a substrate) at 100°C for 2h, Starting chitosan 60kDa (372 mer)

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Reaction conditions: 0.3 g Chitosan, 0.6 g ZSM-5 (S/C=0.5), 27 mmol of acid, 15 mL of chitosan solution (Flakes chitosan as a substrate) at 100°C for 2h, Starting chitosan 60kDa (372 mer)

Table3.1 showed molecular weight of chitosan sample from first and second-round precipitates after got depolymerized by different condition. From the starting chitosan with molecular weight 60,000 Da (372 mer), HCl showed acceptable results as some depolymerized products were in range of 815-3,800 Da (5-24 mer) which assumed to give better results when compared to the reaction at room temperature and 100°C without addition of acid or ZSM-5. While for CH₃COOH condition in both with and without catalyst condition, no expected molecular weight product was formed. These trends could be suggested that the strength of the acid affect depolymerization process effectively as it can be observed from the result that CH₃COOH which is a weak acid so it cannot depolymerize chitosan as well as HCl which is a very strong acid. In addition, the result showed that ZSM-5 has significant effect in both HCl and CH₃COOH condition as it helped in cracking the chitosan into smaller chitosan oligomer than in without the use of

ZSM-5 condition and when using ZSM-5 to depolymerize chitosan alone, it also helped distributing molecular weight of chitosan oligomer product as showed in **Table3.1**. For HCl without ZSM-5 condition, three set of molecular weight which are 815 Da, 3,800 Da and 15,092 Da were observed. While in the condition of pure ZSM-5, no preferred product was obtained as only chitosan product with molecular weight of 27,474 Da, 59,690 Da and 69,301 Da were observed. In contrast when combine ZSM-5 in HCl reaction, the distribution of chitosan oligomer molecular weight become wider and more product with preferable molecular weight were obtained as chitosan product with molecular weight of 815 Da, 862 Da, and 864 Da were obtained along with higher molecular weight chitosan product of 6,541 Da, 13,819 Da and 18,639 Da. The same trends went for CH₃COOH condition as well, in the reaction of CH₃COOH without ZSM-5, only product with molecular weight of 34,815 Da and 54,338 Da were formed while in with ZSM-5 condition more of smaller chitosan product such as 6,936 Da and 22,527 Da were obtained along with high molecular weight product of 52,965 Da and 64,751 Da.

3.2 Qualitative analysis by Liquid chromatography–mass spectrometry (LC-MS)

Liquid chromatography–mass spectrometry is an analytical chemistry technique that combines the usage of liquid chromatography with mass spectrometry technique, so this method gains both physical separation and mass analysis capabilities which lead to the high analytical ability for chemical [13].

3.2.1 Effect of acid and ZSM-5

Two different acids with ZSM-5 were used in this study, **Table3.3** showed percent area of third-round precipitates chitosan in small mer. According to **Table3.3**, majority of oligomer product from room temperature, 100°C and both solution1and 2 of ZSM-5 condition contribute

to be dimer which are 46.9%, 45.7%, 54.5%(for solution1) and 55.8%(for solution2) respectively. While most of the precipitate from HCl condition contribute to be monomer (26.6% for solution1 and 24.3% for solution2), and dimer for the HCl with ZSM-5 (29.5% for solution1 and 25% for solution2). In case of CH₃COOH, 40.5% of precipitates belong to monomer while for CH₃COOH with ZSM-5 condition 25% of precipitates are trimer in solution1 and 23.6% are dimer in solution2.

Table3.2 Summary results from LC-MS analysis

Samples Third-round precipitate	% Area							Sample weight (g)
	Mono	Di	Tri	Tetra	Penta	Hexa	Hepta	
RT	0.7	46.9	13.8	1.5	21	0.0	0.0	0.0025
100°C	0.0	45.7	14.7	21.3	18.3	0.0	0.0	0.0018
Sol ⁿ 1 ZSM-5	0.6	54.5	18.7	21.3	4.8	0.0	0.0	0.0013
Sol ⁿ 2 ZSM-5	0.0	55.8	14.8	14.2	13.9	0.0	1.3	0.0020
Sol ⁿ 1HCl	26.6	16.7	20.8	12.7	22	1.2	0.0	0.0025
Sol ⁿ 2HCl	24.3	19.7	20.2	13.7	22.1	0.0	0.0	0.0034
Sol ⁿ 1HCl+ZSM-5	23.4	29.6	15.8	10.5	20.8	0.0	0.0	0.0023
Sol ⁿ 2HCl-ZSM-5	19.9	25	21.3	13.3	20.5	0.0	0.0	0.0027
Sol ⁿ 1CH ₃ COOH	40.5	26.4	12.8	10.8	8.4	1.2	0.0	0.0082
Sol ⁿ 1CH ₃ COOH+ZSM-5	16.9	20.1	25	15	21.8	1.2	0.0	0.0022
Sol ⁿ 2CH ₃ COOH-ZSM-5	17.9	23.6	21.7	15.1	21.1	0.7	0.0	0.0020

Reaction conditions: 0.3 g Chitosan, 15 ml of 1wt% CH₃COOH, total volume = 15 mL of chitosan solution (Flakes chitosan as a substrate) at 100°C for 2h, Starting chitosan 60kDa (372 mer)

Reaction conditions: 0.3 g Chitosan, 0.6 g ZSM-5 15 ml of 1wt% CH₃COOH, total volume = 15 mL of chitosan solution (Flakes chitosan as a substrate) at 100°C for 2h, Starting chitosan 60kDa (372 mer)

Reaction conditions: 0.3 g Chitosan, 0.6 g ZSM-5 (S/C=0.5), 27 mmol of acid, 15 mL of chitosan solution (Flakes chitosan as a substrate) at 100°C for 2h, Starting chitosan 60kDa (372 mer)

However, when calculate for mer percentage of all precipitate ,the precipitate from the third-round did not show significant effect at all due to its low sample weight when compare to the sample weight of precipitate from the first and second round that were analyzed by GPC as presented in Table3.3.

Table3.3 Summary result from LC-MS and GPC

No.	Condition	LC-MS (%) (Liquid part)					GPC (Solid part)			
		mono	di	tri	tetra	penta	mer (%)			
1	RT	0.0	0.4	0.1	0.1	0.2	557 mer (99.2%)			
2	100°C	0.0	0.3	0.1	0.1	0.1	35 mer (3.3%)	386 mer (96.2%)		
3	ZSM-5	0.0	0.5	0.2	0.2	0.1	171 mer (1.1%)	370 mer (6.3%)	430 mer (91.6%)	
4	HCl	0.6	0.4	0.5	0.3	0.5	5 mer (0.8%)	24 mer (31.8%)	94 mer (64.3%)	
5	HCl+ZSM-5	0.4	0.5	0.3	0.2	0.4	5 mer (20.8%)	41 mer (0.6%)	86 mer (61.5%)	116 mer (15.3%)
6	CH ₃ COOH	1.1	0.7	0.3	0.3	0.2	216 mer (3%)	338 mer (94.3%)		
7	CH ₃ COOH+ZSM-5	0.2	0.3	0.3	0.2	0.3	43 mer (2.6%)	140 mer (1%)	328 mer (89.4%)	402 mer (5.7%)

Reaction conditions: 0.3 g Chitosan, 15 ml of 1wt% CH₃COOH, total volume = 15 mL of chitosan solution (Flakes chitosan as a substrate) at 100°C for 2h, Starting chitosan 60kDa (372 mer)

Reaction conditions: 0.3 g Chitosan, 0.6 g ZSM-5 15 ml of 1wt% CH₃COOH, total volume = 15 mL of chitosan solution (Flakes chitosan as a substrate) at 100°C for 2h, Starting chitosan 60kDa (372 mer)

Reaction conditions: 0.3 g Chitosan, 0.6 g ZSM-5 (S/C=0.5), 27 mmol of acid, 15 mL of chitosan solution (Flakes chitosan as a substrate) at 100°C for 2h, Starting chitosan 60kDa (372 mer)

From Table3.3, it can be summarized that HCl is more effective acid than CH₃COOH when the purpose is to obtain chitosan oligomer with molecular less than 3,900 Da (24 mer) as almost 36% of the depolymerized chitosan product have molecular weight less than 24 mer in HCl with no ZSM-5 case and in with ZSM-5 case almost 23% are oligomer which are pentamer and smaller. On the other hand, only 2.6% and 1.3% of product are oligomer with expected molecular weight in CH₃COOH without and with ZSM-5 condition, respectively. In addition, the result showed that ZSM-5 has ability to help depolymerize chitosan into small oligomer and gave more distribution in molecular weight of chitosan oligomer product in both HCl and CH₃COOH condition when compared to the condition without ZSM-5 and when using ZSM-5 alone. Especially in HCl condition that ZSM-5 showed excellent supported-effects on depolymerize chitosan into low molecular weight chitosan as 22.6% of product contribute to be pentamer and smaller, compared with condition of pure ZSM-5 and HCl without ZSM-5 which only 1% and 2.3% of product are

smaller than hexamer. This trend could be a guide that the usage of ZSM-5 as acid-supported catalyst in acid depolymerization of chitosan provide more greater results than using only acid or zeolite as catalyst alone. Furthermore, as mentioned in literature that ZSM-5 is very stable in acidic solution and aqueous hot water, it also did not decompose in solution so in this study it was possible to separate ZSM-5 and chitosan by centrifugation and that made the left solution to be clear chitosan solution [11].

4. Conclusion

In conclusion, apart from the thermal effect, the usage of zeolite and different acids was found to have a remarkable effect on depolymerization of chitosan into chitosan oligomer with expected molecular weight of less than 3,900 Da (24 mer) as observed from GPC and LC-MS result. Strong acid like HCl showed great ability to depolymerize chitosan to an expected molecular weight than weak acid like CH₃COOH. For HCl condition, 28.9% of chitosan oligomer product has molecular weight 3,800 Da in without catalyst condition while in with catalyst condition, 22.6% of the product contribute to be pentamer and smaller which are an expected molecular weight range. In contrast, in both with and without catalyst of CH₃COOH condition no more than 3% of expected molecular weight of chitosan oligomer was formed. In addition, the combination of ZSM-5 with acid help distribute the range of molecular weight of chitosan oligomer and propose significant effect on depolymerize chitosan as the result could be seen in both HCl and CH₃COOH condition.

4.1 Suggestions for the future work

As it can be seen in this study that combine zeolite as catalyst with acid for depolymerization chitosan gave effective results so in the future, combination of more type of zeolite with higher acid property and strong acid such as H₂SO₄, HClO₄ and HNO₃ should be studied. In addition, more techniques to analyze chitosan product property such as viscosity, degree of deacetylation and percent yield should be studied.

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