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Fabrication and Characterization of Glucosamine Hydrochloride from Chitin of Horseshoe Crab Shell (*Tachypleus gigas*)

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Abstract. Preparation of glucosamine hydrochloride from the chitin of horseshoe crab shells using the chemical hydrolysis method has been done using HCl concentration variation ratios of 7%, 9%, 11%,14 % with a ratio of 9:1 (v/w) for 4 hours at a temperature of 90ºC. Determination of glucosamine hydrochloride characteristics was characterized using Fourier transform-infra red (FT-IR) spectroscopy, in which the characteristics of glucosamine hydrochloride obtained in the OH group of glucosamine hydrochloride were 3446 cm^{-1} (s), 3448 cm^{-1} (s), 3450 cm^{-1} (s), 3448 cm^{-1} (s), respectively. For NH group of glucosamine hydrochloride were 1557 cm^{-1} (s), 1559 cm^{-1} (s), 1556 cm^{-1} (s), 1560 cm^{-1} (s), respectively. For CN group of glucosamine hydrochloride were 1379 cm^{-1} (m), 1379 cm^{-1} (m), 1379 cm^{-1} (m), 1379 cm^{-1} (m), respectively. While the glycoside bond of glucosamine hydrochloride 1073 cm^{-1} (w), 1074 cm^{-1} (w), 1074 cm^{-1} (w), 1074 cm^{-1} (w), respectively. Determination of the concentration of glucosamine hydrochloride with Ultraviolet Spectrophotometer analysis at a maximum wavelength of 197 nm with a standard solution of N-acetyl glucosamine in a solution of phosphate acid 0.005%, in which obtained the concentration of glucosamine hydrochloride 7% = 33.67 ppm, 9% = 36.35 ppm, 11% = 40.16 ppm, 14% = 43.97 ppm.

Keywords: Glucosamine Hydrochloride, Chitin Shells of Horseshoe Crab, Analysis FT-IR

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1 Introduction

Glucosamine is an important building block for cartilage, which acts as a cushion at the ends of bones and prevents bones from cracking during movement. Glucosamine in the body keeps cartilage from calcifying faster. In addition, glucosamine is also useful for stimulating the formation and restoration of damaged cartilage tissue and helps reduce joint pain. Bones need calcium, while joints need glucosamine to be free to move and avoid joint pain. Bones that contain an adhesive substance and a little lime keep the hard bones from colliding with each other so that they can cause joint pain.

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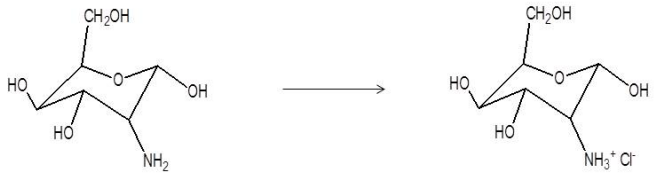
Normally, joint cartilage has a smooth surface and usually, there are small tears that can then be repaired by the body itself. But with age, these tears can no longer be repaired by the body because the ability to synthesize glucosamine decreases. In addition, with age, collagen cells also decrease.

The body's ability to synthesize glucosamine decreases with age and this can lead to osteoarthritis and joint pain. Therefore, in order to keep the joint cartilage well lubricated, it is recommended to add glucosamine with external intake. This has caused the worldwide consumption of glucosamine to increase enormously, so reliable methods have been optimized for preparing high quality glucosamine products.

Glucosamine (2-amino-2-deoxyglucose, chitosamine) is an amino sugar obtained from the hydrolysis of chitin. Chitin is mainly produced from the outer shell of the *crustaceans*, *arthropods*, and fungi. In industry, glucosamine is produced by hydrolysis of the exoskeleton of *crustaceans* such as shrimp and crabs. Glucosamine products for people with arthritis are usually formulated as hydrochloride salts of glucosamine sulfate.

Several studies have been conducted on the hydrolysis of glucosamine. The Rotta Research Laboratory (1978) reported that glucosamine sulfate has been successfully prepared by immersing chitin in sulfuric acid solution, but this reaction produced a small amount of yield. In order to avoid this problem, Mojarrad, *et al* . (2006) stated that glucosamine sulfate can be prepared first by refluxing chitin with a hydrochloric acid solution to produce glucosamine hydrochloride



Glucosamine **Glucosamine Hydrochloride**

2 Materials and Methods

2.1 Equipments

In this study, the equipments used were glassware, Fourier transform-infra-red (FT-IR), UV-Vis spectroscopy, beaker glass, measuring cup, Erlenmeyer, volumetric pipette, volumetric flask, triple beaker, water bath, analytical balance, funnel, filter paper, dropper, rubber ball, spatula, and centrifugation.

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2.2 Materials

The materials used were horseshoe crab shells, hydrochloric acid (HCl), sodium hydroxide (NaOH), phosphoric acid (H_3PO_4), formic acid, ethanol, N-acetylglucosamine, and aquadest.

2.3 Preparation of Chitin

Preparation of chitin was conducted based on Agusnar, H . (2006). Firstly, shells were washed and then added 0.5% of NaOH for 24 hours. Next, washed with distilled water until the pH reached 6.7. A solubility test was carried out with 85% phosphoric acid. Demineralized with 5% of HCl solution for 24 hours, washed with H_2O to pH was 6.7. A solubility test was also performed with 37% of formic acid and dried at room temperature. Then mashed and sieved through an 80 mesh sieve.

2.4 The process of Hydrolysis of Glucosamine Hydrochloride

It was conducted using modified chemical hydrolysis from Mojarrad et al (2006). As much as 2.5 g of chitin powder was soaked in a hydrochloric acid solution with the same treatment at different concentrations of 7%, 9%, 11%, and 14%, with a ratio of 9:1 for 4 hours at 90ºC. Hydrolysis was continued by centrifugation of glucosamine hydrochloride slurry at 10,000 rpm for 15 minutes. The precipitate obtained was washed with ethanol, then centrifuged again at 10,000 rpm for 15 minutes. Furthermore, the precipitate obtained was dried in an oven at 40ºC for 4 hours. The glucosamine hydrochloride result was then analyzed for its characteristics, such as Fourier transform infrared (FT-IR) spectroscopy, and the concentration level was determined by Ultraviolet-Visible (UV) spectrophotometer analysis.

2.5 Analysis of glucosamine hydrochloride by FTIR

The glucosamine hydrochloride (7%, 9%, 11%, 14%) obtained and standard glucosamine were mixed with KBr in a ratio of 1:100 and then ground until smooth using a mortar. This mixture was placed in a press and pressed at a pressure of 800 kg. The pressed pieces were measured for absorbance through FTIR. The scanning range used is between 450 cm^{-1} to 4000 cm^{-1}.

2.6 Analysis of Glucosamine Hydrochloride with UV-Visible Spectrophotometer.

For phosphoric acid solution.

Phosphoric acid solution was made at 0.002%; 0.005%; 0.008%; and 0.012%, then the absorbance was measured at 197 nm in which distilled water was used as a blank.

For N-acetylglucosamine.

Standard solutions of N-acetylglucosamine were 10, 20, 30, 40, 50, 60, and 70 mg/L prepared in 0.005% phosphoric acid solution, then the absorbance was measured at 197 nm.

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For Glucosamine Hydrochloride.

Solutions of glucosamine hydrochloride were (20%, 25%, 30%, and 37%) and standard glucosamine was prepared in 0.005% of phosphoric acid and then the absorbance was measured at 197 nm.

3 RESULT AND DISCUSSION

3.1 Chitin Preparation

Preparation of chitin was conducted based on Agusnar, H . (2006), and the result of chitin obtained was 30.8%.

3.2 Determination of Glucosamine Hydrochloride Characteristics by FTIR

The characteristics of glucosamine hydrochloride can be determined using FTIR analysis, which was shown in Table 1.

Tabel 1. Data Characteristics of Glucosamine HCl with FT-IR

Sample	OH (cm^{-1})	NH (cm^{-1})	CN (cm^{-1})	Glycoside Bonds (cm^{-1})
Glucosamine HCl standard	3295 (s)	1539 (s)	1379 (m)	1033.50 (s)
Glucosamine HCl 7%	3446 (s)	1557 (m)	1379 (m)	1073.57 (w)
Glucosamine HCl 9%	3448 (s)	1559 (m)	1379 (m)	1074.46 (w)
Glucosamine HCl 11%	3450 (s)	1556 (m)	1379 (m)	1074.85 (w)
Glucosamine HCl 14%	3448 (s)	1560 (m)	1334 (m)	1074.54 (w)
Glucosamine HCl according to Mojarrad, *et al.*	3350 (s)	1535 (s)	1394 (m)	1034 (s)

Based on Table 1 shows that glucosamine hydrochloride from the hydrolysis of shellfish chitin with a ratio of variations in HCl concentration of 7%, 9%, 11%, and 14% produced an absorption band that was almost following the standard glucosamine hydrochloride. Mojarrad et al reported that it can be happened due to the influence of HCl concentration, temperature, and hydrolysis time so the glucosamine hydrochloride formed was still in a small concentration. This can also be influenced by differences in chitin raw materials, because the chitin raw materials used were different, so resulting in different chitin content.

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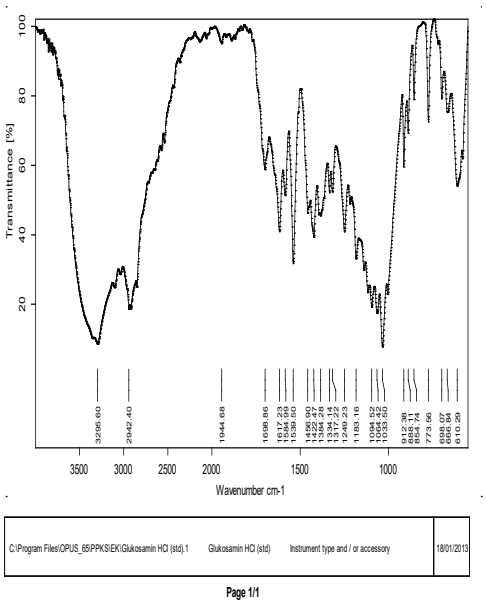

Figure 1. FT-IR spectrum of standard glucosamine hydrochloride

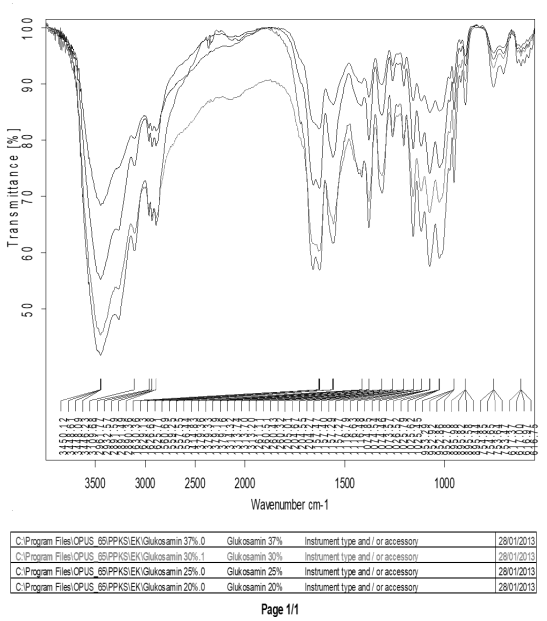

Figure 2. FT-IR spectra of glucosamine hydrochloride of 7%, 9%, 11%,14 %

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3.3 Determination of The Concentration of Glucosamine Hydrochloride By UV-Visible Spectrophotometer

Muzzarelli (1978) said that a good solvent for horseshoe crab was phosphoric acid, so phosphoric acid was used as a solvent in this study. The measurement of phosphoric acid absorbance can be seen in Table 2.

Tabel 2. Absorbance Measurement Data for Phosphoric Acid at λ = 197 nm

Concentration Phosphoric Acid (%)	Absorbance (Ho)
0.000	0.00
0.002	0.53
0.005	1.18
0.008	1.82
0.012	2.67

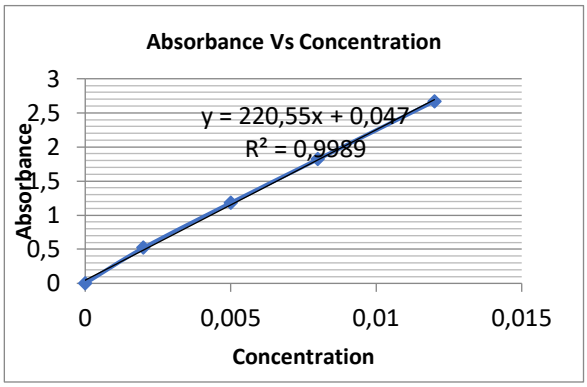

Figure 3. Phosphoric acid calibration curve

Measurement of the absorbance of N-acetylglucosamine in phosphoric acid was shown in Table 3. Table 3. shows that the concentration of phosphoric acid used was 0.005%. Muzzarelli (1978), the solubility of chitin was good at 0.005% of phosphoric acid, so 0.005% of phosphoric acid was used in this study.

The absorbance measurement of H_3PO_4 (Table 2) shows an absorbance value was 1.18 of 0.005% of H_3PO_4. Thus, the results of all measurements of the absorbance of N-acetylglucosamine and samples of glucosamine hydrochloride dissolved in 0.005% of H_3PO_4 must be reduced by 1.18 to obtain the true absorbance of the two compounds.

Tabel 3. Absorbance measurement data for N-acetylglucosamine in 0.005% of phosphoric acid at λ_{max} = 197 nm

Concentration N-acetylglucosamine (mg/L)	Absorbance (H) for N-Acetylglucosamine in	Absorbance (H) N-acetylglucosamine

Phosphoric Acid 0.005%		
0	1.18	0.00
10	2.08	0.90
20	3.13	1.95
30	3.98	2.80
40	4.98	3.80
50	5.88	4.70
60	6.93	5.75
70	8.13	6.95

The results of the absorbance measurement of the standard N-acetylglucosamine in Table 3. were plotted in a calibration curve so obtained in the form of a linear line. The regression line equation for this calibration curve can be derived using the least square method.

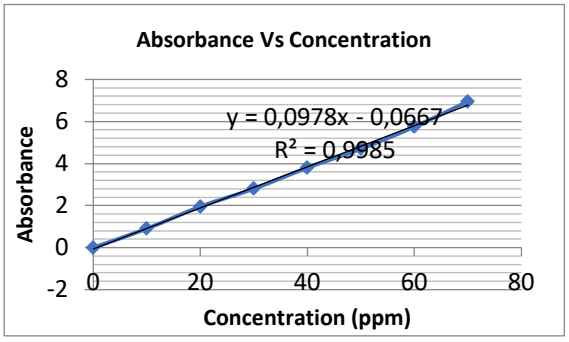

Figure 4. N-acetylglucosamine calibration curve

By using the least square method, the concentration of glucosamine hydrochloride from chitin of horseshoe crab shells were 7% = 33.67 ppm, 9% = 36.35 ppm, 11% = 40.16 ppm, 14 % = 43.97 ppm and standard glucosamine = 64.59 ppm. The results obtained show that the concentration of glucosamine hydrochloride from the hydrolysis of chitin of horseshoe crab shells increased with the increase in the concentration of hydrochloric acid, with the best results was at 14% of HCl.

4 Conclusion

Based on the data obtained in this study, it can be concluded that glucosamine hydrochloride has been made from hydrolyzed chitin of horseshoe crab shells. Glucosamine hydrochloride exhibited almost the same characteristics as standard glucosamine hydrochloride. The FT-IR spectrum of glucosamine hydrochloride obtained almost corresponds to the spectrum of standard glucosamine hydrochloride. The concentration level of glucosamine hydrochloride increased as the HCl concentration increased. It was recommended for further researchers to

hydrolyze glucosamine hydrochloride into glucosamine sulfate, and tested its feasibility so it can be consumed by humans.

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