Chitosan from shrimp (Peneaus monodon) skin waste as natural coagulant to remove heavy metal Hg

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Chitosan from shrimp (Peneaus *monodon*) skin waste as natural coagulant to remove heavy metal Hg

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Abstract
This study aimed to isolate chitosan from waste of shrimp skin and applied as environmentally friendly natural coagulant. This study included the isolation, characterization and application of chitosan in the adsorption of heavy metal Hg. Chitosan was obtained from waste of shrimp (Peneaus monodon) skin via deproteination, demineralization, depigmentation and deacetylation.

Optimization of adsorbent mass was carried out by varying the adsorbent mass of 0, 0.3, 0.6, 0.9, 1.2 and 1.5 g. In addition, the effect of pH was conducted by performing the adsorption under different pH of 2, 6, 7, 8 and 9. The results showed that the use of 1.2 g of chitosan gave Hg(II) removal of 99.86% or 9.986 µg/L. It could be concluded that chitosan as natural coagulant could adsorb Hg(II) in water.

Keywords: Chitosan, Peneaus monodon, Mercury, Waste, Adsorbent.

Introduction

Mercury (Hg) is one of global pollutant which may give bad impacts to human health and ecosystem[4]. Several techniques have been applied to remove this heavy metal such as ion exchange, solvent extraction, ultra-filtration, adsorption and coagulation[19].

Coagulation is one of effective methods in minimizing the heavy metal concentration in waste water[3]. One of widely employed natural coagulant is chitosan (β-(1-4)-2-amino-2-dioxy-D-glucose). Chitin and chitosan could be abundantly found from crustaceans like shrimp and cram[7].

Natural coagulants have several advantageous comparing to commercial ones such as availability of the raw material, cheap, environmentally friendly and biodegradable[6]. Both chitin and chitosan are not toxic and biodegradable[5,16]. Lukum et al[11,12] reported that chitosan obtained from Gorontalo shrimp skin wastes has deacetylation degree of 80% and was able to adsorb Pb(II) from sugar factory Tolanghua, Gorontalo. Several reports demonstrated that chitosan was effective to reduce COD level and turbidity of textile industry liquid waste as much as 72.5% and 94.9%. The efficiency of chitosan in reducing the turbidity of seawater was higher than ferro sulfate and was similar with that of alum[2].

Chitosan and its derivatives displayed good adsorption capacities toward arsenic[17]. Adsorption of Hg(II) onto chitosan probably occurred via single or mixture interaction: coordination with amino group or combination with vicinal hydroxyl group, electrostatic in acidic media or ion exchange with protonated amino group[14].

According to Lertsutthiwong et al[10], chitosan could be obtained from chitin via deacetylation process. It has free amino group which might be able to bind metal ions. It has been employed to remove heavy metal ion from the effluent. Chitosan and its derivatives are cheap and effective as heavy metal adsorbent[23]. Shrimp is abundant natural resources particularly in Gorontalo Province. In several traditional markets in Gorontalo, it was observed that the shrimp skin was discarded and was left to rod without any further treatment and may lead to environmental pollution and damage environment. These problems might be solved by applying the shrimp waste as the source of chitosan. Several reports showed that chitosan displayed good activities in the adsorption of Hg(II)[18] and Pb(II)[20] ions.

This research aimed to prepare chitosan from the shrimp skin waste and to apply it as environmentally friendly natural coagulant. The produced chitosan would be applied in the adsorption of Hg(II) ions. In addition, the effect of pH and mass adsorbent would be evaluated.

Material and Methods

Materials: The shrimp skin was obtained from Gorontalo. The chemicals employed for this study were standard solution of $Hg(NO_3)_2$, HCl, NaOH, hydrogen peroxide, acetic acid, ammonia, sodium sulfate, nitric acid, aquadest, filtered paper and universal indicator.

Tools: The used instruments in this study were laboratory glassware, oven, magnetic stirrer, hotplate, stirrer, centrifuge, sieve (90 mesh), desiccator, furnace, atomic absorption spectrometer (AAS, AA240FS VARIAN), infrared spectrometer (FTIR), analytical balance, mortar and pestle.

Isolation of chitosan from shrimp *(Peneaus monodon)* skin: Shrimp skin was washed and dried on the open air. It was then grinded by using mortar and sieved to give 90 mesh size. Isolation of chitosan[11] was carried out with the

following steps: deproteination, demineralization, depigmentation and deacetylation.

Deproteination was conducted by immersing the dry shrimp skin powder into NaOH 3.5% (ratio 1:10) into a beaker under stirring condition at 90 °C for 4h. The solid was filtered and washed with aquadest until neutral pH was obtained. The sample was dried in oven at 60°C for 24h.

Demineralization was carried out by immersing the sample into HCl 1N (ratio 1:7) at room temperature under stirring condition for 1h. The solid was filtered and washed with aquadest until neutral pH was obtained. The sample was dried in oven at 60°C for 24h.

Depigmentation was performed by immersing the material into H_2O_2 2% (ratio 1:10) at room temperature under stirring condition for 5 min. The solid was filtered and washed with aquadest until neutral pH was obtained. The sample was dried in oven at 60°C for 24h.

Deacetylation was done by immersing the sample into NaOH 50% (ratio 1:10) under stirring condition at 120 °C for 1h. The solid was filtered and washed with aquadest until neutral pH was obtained. The sample was dried in oven at 60°C for 4h.

The properties of chitosan were characterized such as the determination of water content, ash content, solubility test and determination of acetylation degree by using FTIR. The degree of acetylation was determined using baseline method from FTIR using the following equation[8]:

$$\%\,DD = 100 - \left[\left(\frac{A_{1655}}{A_{3450}}\right) \times 115\right]$$

where A (Absorbance) = $\log(P_0/P)$; A_{1655} = Absorbance at wavenumber 1655 cm^{-1} for the absorption of amide/acetamide (CH_3CONH) and A_{3450} = Absorbance at wavenumber 3450 cm^{-1} for the absorption of hhydroxyl (-OH) group.

Preparation of standard solution and calibration curve: Stock solution of Hg(II) 100 ppb was prepared by dissolving 5 mL of solution of Hg(II) 1 ppm into volumetric flask 50 mL with solution of HNO_3-HCl 5% (v/v). From this stock solution, 2.5, 5, 7.5, 10 and 12.5 mL were taken and was diluted to 50 mL to give standard solution 5, 10, 15, 20 and 25 ppb. Calibration curve was prepared by determining the absorbance of all standard solution together with the blank solution at 253.7 nm.

Determination of adsorption performance of mercury ion on chitosan under different pH and mass adsorbent: Determination of optimum mass of chitosan on the adsorption of Hg(II) was carried out by mixing 100 mL of Hg(II) solution (10 ppb) with chitosan in various mass (0, 0.3, 0.6, 0.9, 1.2 and 1.5 g) at pH 2. The mixture was stirred at high rate for 1 min and followed with medium rate for 30 min. The mixture was allowed to stand for 5 min to obtain the separation. The upper layer was separated and centrifuged for 15 min to give sediment and filtrate. The filtrate was then analyzed by using AAS. The number of removed Hg(II) was obtained from the difference between the concentration of metal ion before and after the adsorption process.

The effect of pH on the adsorption of Hg(II) on chitosan was carried out by mixing 100 mL of Hg(II) solution (10 ppb) and chitosan (optimum mass) in Erlenmeyer flask. The adsorption was conducted at different pH of 2, 6, 7, 8 and 9. The pH value was adjusted by HCl and NaOH solution. The mixture was stirred at high rate for 1 min and followed with medium rate for 30 min. The mixture was allowed to stand for 5 min to obtain the separation. The upper layer was separated and centrifuged for 15 min to give sediment and filtrate. The filtrate was then analyzed by using AAS.

Determination of Hg(II) concentration: Solution of Hg(II) ion was carried out using AAS with the same condition as the standard solution. The obtained absorbance was fitted in the equation y = a + bx to obtain the concentration of Hg(II).

Data analysis: The performance of chitosan on the adsorption of Hg(II) was determined by submitting the calculation of filtrate of chitosan on various mass and pH to the equation y = a + bx.

Results and Discussion

Isolation of chitosan from Gorontalo skin shrimp (*Peneaus Monodon)* waste: The first step of chitosan isolation from skin shrimp was by washing the skin to eliminate the impurities. The sample was then dried at room temperature to remove the water. The sample was grinded and sieved to obtain the molecular size of 90 mesh.

The next step was deproteination to eliminate the protein by breaking the bond between chitin and protein on the shrimp skin. The deproteination was carried out by heating the sample with NaOH 3.5% (ratio 1:1à) at 90 °C for 4h. The mixture was filtered. The filtrate contained Na-proteiase where the sodium ion bonded the negatively-charged protein chain. The protein on the skin shrimp will be soluble on the basic solution and the chitin would be separated[1]. The deproteination reaction was depicted in figure 1. The deproteination product was light brown powder in 51.72% yield. It indicated that the number of protein bonded to sodium ion was 48.28%.

The next step was demineralization of mineral contained in the skin shrimp such as carbonate de calcium ($CaCO_3$) et phosphate de calcium [$Ca_3(PO_4)_2$] by using HCl 2N (ratio 1:7)[22]. The demineralization product was dark brown solid as 24.98% which indicated that the amount of mineral salt was 75.02%.

After the demineralization process, the product underwent the depigmentation process to eliminate the carotenoid dyes such as red-orangeastaxanthin[21]. This process was carried out by adding H_2O_2 2% (ratio 1:10) at room temperature for 5 min. The chitin obtained after the depigmentation process was light brown solid with 22.71% yield.

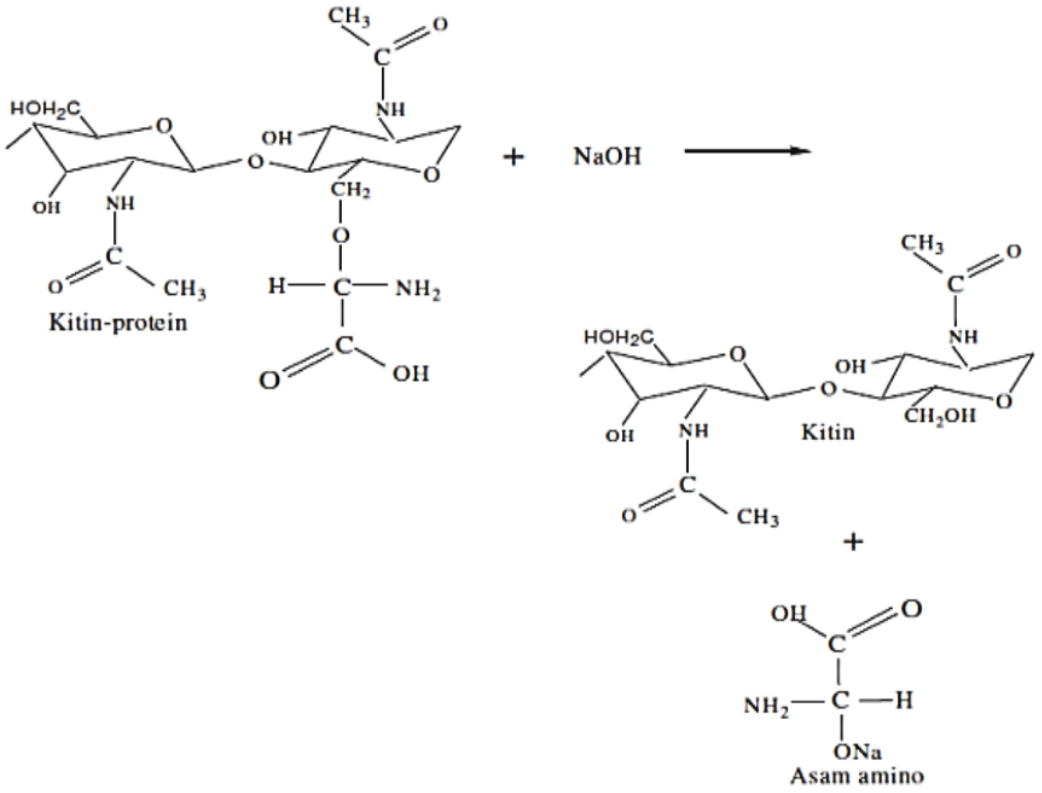

Figure 1: Reaction of protein and base

The deacetylation process was carried out to transform chitin into chitosan. It was conducted by immersing the sample in NaOH 50% (ratio 1:10) at 120 °C for 1h. The sample was then washed with aquadest until neutral pH was reached. The solid was dried at 60 °C for 4h. The obtained chitosan was then analyzed to determine the degree of deacetylation by using FTIR. The deacetylation process gave chitosan as brownish white solid with deacetylation degree of 73.88% which met the quality standard of commercial chitosan i.e. 60%[18] with the yield of 17.73%.

Figure 2 described the acetylation process of chitin by using strong base of NaOH. The FTIR data of chitosan isolated from the waste of shrimp skin is presented in table 1. Then, several chitosan properties were characterized such as water content, ash content and solubility. The chemical analysis data of chitosan is presented in table 2.

Rahayu and Purnavita[18] reported that the deacetylation degree of chitosan might affect its adsorption properties. The higher is the deacetylation degree, the higher is amount of free amine (-NH_2) group which was present in the polymer. Therefore, it might increase the affinity of chitosan toward the metal ion. Comparing to chitin, chitosan is more effective in term of adsorption performance since the later contains higher amount of free amine groups than the former. The isolated chitosan was then applied in the adsorption of Hg(II) under the various adsorbent mass and pH value.

Adsorption study of mercury ion onto chitosan

Preparation of calibration curve: Analysis of mercury ion was carried out by using AAS based on SNI 01.1754:-6-2006 and was measured at the wavelength of 253.7 nm. The concentration of Hg(II) was determined by submitting the value of absorbance into equation:

$y = 0.0448x + 0.0802$ ($r^2 = 0.99794789$)

Concentration of Hg(II) before the adsorption process was 10 µg/L.

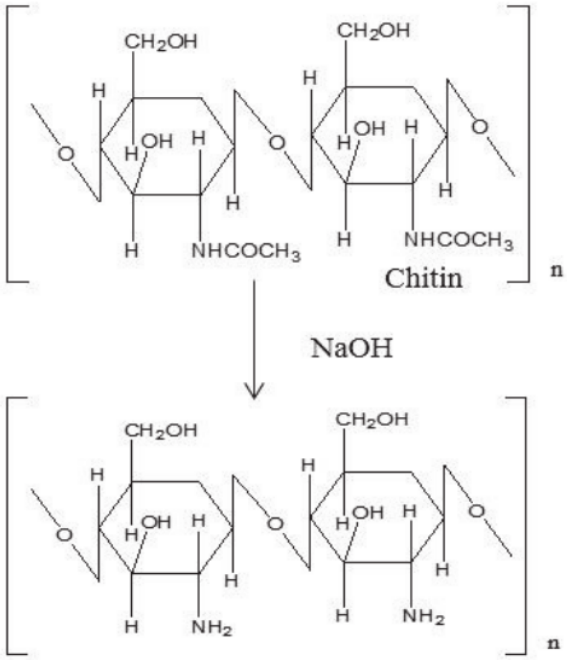

Figure 2: Deacetylation of chitin to give chitosan

Table 1
FTIR data of chitosan

Functional group	Bond (vibration type)	wavenumber (cm^{-1}) literature	wavenumber (cm^{-1}) experimental
Hydroxyl	O-H *stretching*	3450, 3340	3423.41
Alkyl	CH (-CH_2-) *stretching asym*	2864	2881.45
Amide II	*Mainly*-NH *in plane deformation*	1650	1656.74
Amine	NH *stretching*	1580	1593.09
Alkyl	CH (-CH_2-) *bending asym*	1418	1421.44
Alkyl	CH (-CH_2-) *bending sym*	1377	1379.01
Carbonyl	C-O (-C-O-C-) *stretching asym*	1082	1081.99
Carbonyl	C-O (-C-O-C-) *stretching sym*	1033	1033.77

Table 2
Chemical analysis data of chitosan isolated from waste of shrimp *(Peneaus monodon)* skin

Parameter	Standard chitosan	Chitosan (experimental)
Water content	≤ 10 %	6,48 %
Ash content	≤ 3 %	0,40 %
Deacetylation degree	≥ 60 %	73,88 %
Solubility:		
- Water	Not soluble	Not soluble
- Concentrated HCl	Slightly soluble	Slightly soluble
- HNO_3	Slightly soluble	Slightly soluble
- CH_3COOH 1%	Soluble	Soluble
- Concentrated NH_3	Not soluble	Not soluble
- Na_2SO_4 2%	Not soluble	Not soluble

Source: Rahayu and Purnavita[18]; Lukum and Usman[1]

Effect of adsorbent mass: The determination of optimum adsorbent mass in the adsorption of Hg(II) on chitosan was carried out by mixing 100 mL of Hg solution (10 ppb) with chitosan with various mass (0, 0.3, 0.6, 0.9, 1.2 and 1.5 g). The adsorption was performed at pH 2. The mixture was stirred at high rate for 1 min and followed with medium rate for 30 min. The mixture was allowed to stand for 5 min to obtain the separation. The filtrate was then analyzed by using AAS. The number of removed Hg(II) was obtained from the difference between the concentration of metal ion before and after the adsorption process. The effect of adsorbent mass on the adsorption of Hg(II) onto chitosan was presented in figure 4.

Figure 4 showed that the optimum adsorbent mass was 1.2 g. The results showed that chitosan displayed a good activity in the adsorption of Hg(II). Mekawati and Sumardjo[13] reported that chitosan might form a complex with heavy metal ion and transition metal ion particularly Cu(II), Ni(II) and Hg(II), but not with alkali and earth alkali metals. This study would be continued by investigating the effect of pH on the adsorption of Hg(II) onto chitosan.

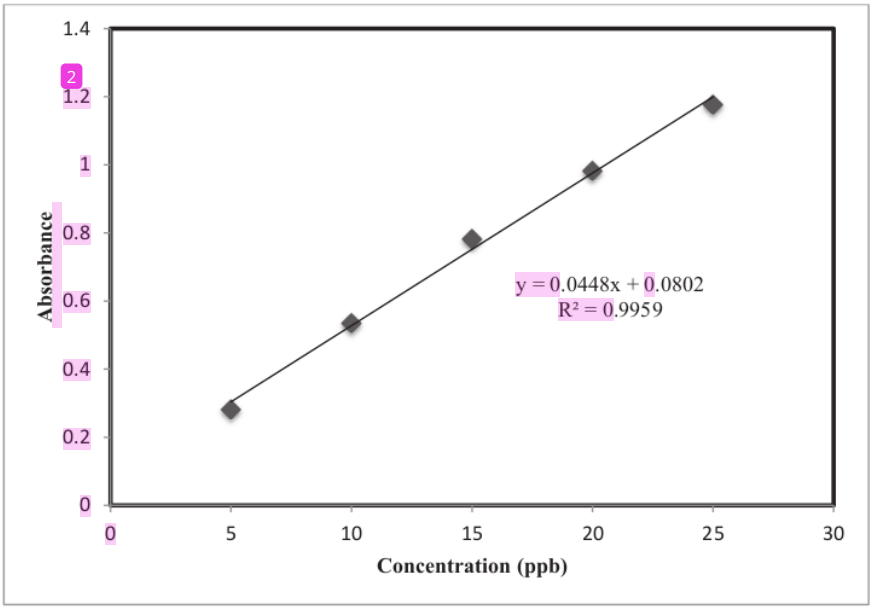

Figure 3: Calibration curve of Hg(II)

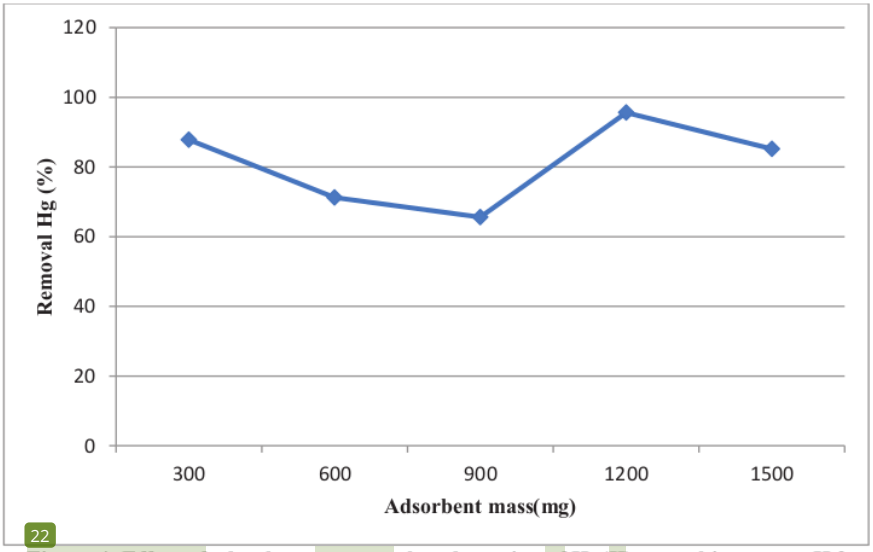

Figure 4: Effect of adsorbent mass on the adsorption of Hg(II) onto chitosan at pH 2

Effect of pH: The determination of optimum pH in the adsorption of Hg(II) on chitosan was carried out by mixing 100 mL of Hg solution (10 ppb) with chitosan (optimum mass under different pH values 2, 4, 7, 8 and 9). The mixture was stirred at high rate for 1 min and followed with medium rate for 30 min. The mixture was allowed to stand for 5 min to obtain the separation. The upper layer was separated and centrifuged for 15 min to give sediment and filtrate. The filtrate was then analyzed by using AAS. The number of removed Hg(II) was obtained from the difference between the concentration of metal ion before and after the adsorption process. The effect of pH on the adsorption of Hg(II) onto chitosan was presented in figure 5.

Figure 5 showed that the adsorption of Hg(II) on chitosan was dependent on pH value[15]. The adsorption capacity increased from pH 2 and reached the maximum value at pH 8. It was observed that the adsorption value reduced at pH 9. According to Kovacevic and co-workers[9], mercury ion existed as Hg^{2+} under pH 7. In addition, the free amine groups in chitosan would be protonated and gave the positively-charged-ammonium salt under such pH value.

Therefore, there was an electrostatic repulsion between metal ion and protonated chitosan. Besides, the competition between proton and metal ion may lead the reduction of adsorption capacity. In this context, the adsorption of proton dominantly occurred at low pH (figure 6a). At pH range 6-8, $Hg(OH)^{+}$ was the dominant species in the solution. Additionally, increasing pH value might decrease the degree of protonation. As the consequence, the amount of free amine group would increase and could electrostatically interact with metal ion. Therefore, the adsorption capacity tended to increase at this pH range (figure 6b). At higher pH, the concentration of OH^{-} increased and led to the formation of $Hg(OH)_2$. It might reduce the electrostatic interaction (figure 6c).

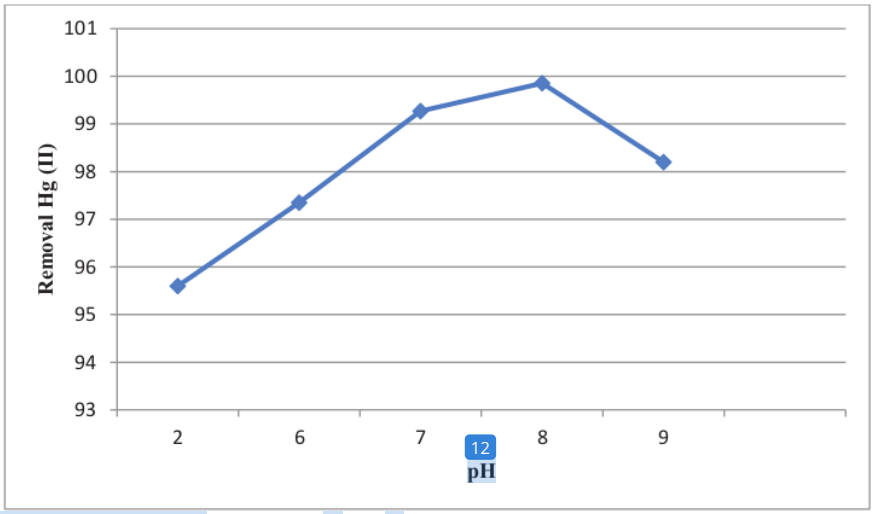

Figure 5: Effect of pH on the adsorption of Hg(II) onto chitosan with the optimum adsorbent mass (1.2 g)

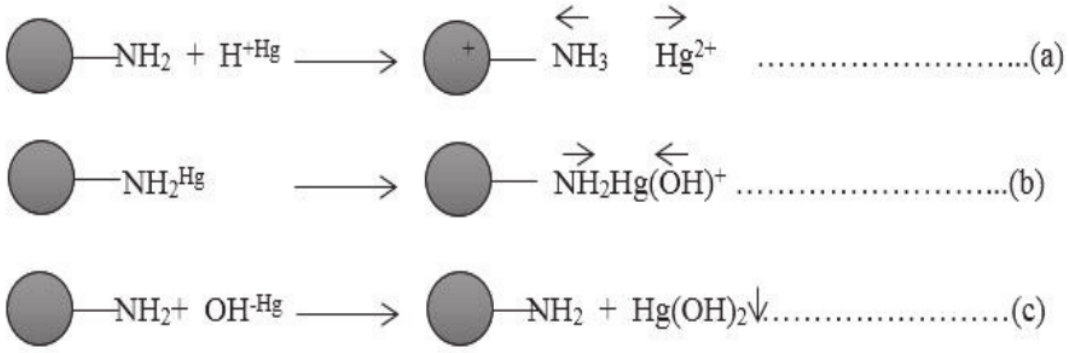

Figure 6: Interaction between chitosan and metal ion at (a) low pH, (b) medium pH and (c) high pH

Conclusion

Chitosan isolated from *Peneaus monodon* could be applied as environmentally friendly natural coagulant. It could be also applied as adsorbent of Hg(II) ion. The results showed that the optimum mass and pH values were 1.2 g and 8 respectively. Under the optimum condition, the percent removal of Hg(II) was 99.86%.

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