Immobilization of Dithizone onto Chitin Isolated from Prawn Seawater Shells (*P. merguensis*) and its Preliminary Study for the Adsorption of Cd(II) Ion

Mudasir*, Ginanjar Raharjo, Iqmal Tahir and Endang Tri Wahyuni

Chemistry Department, Faculty of Mathematics and Natural Sciences, Gadjah Mada University, Sekip Utara, P.O. Box Bls. 21, Yogyakarta 55281, Indonesia

*Corresponding author: mudasir@ugm.ac.id

Abstract: *Immobilization of dithizone onto biopolymer chitin isolated from prawn seawater shells* (P. merguensis) *to enhance the selectivity and ability of chitin in adsorbing heavy metal cadmium (Cd) has been conducted. The study includes isolation of chitin from the prawn seawater, immobilization of dithizone onto chitin and adsorption of Cd(II) ions. Several parameters influencing immobilization as well as Cd(II) adsorption were optimized. Results of the study showed that high purity chitin polymer can be isolated from the prawn seawater shells* (P. merguensis). *The best immobilization conditions of dithizone onto chitin are achieved when the reaction is carried out for 6 h at 70^{o}C in toluene medium. In general, the ability of chitin polymer in adsorbing Cd(II) ion increases after immobilization of dithizone onto chitin. The optimum conditions for Cd(II) adsorption are at pH 6 for chitin-dithizone and pH 7 for chitin using 0.3 g of adsorbent.*

Keywords: immobilization, dithizone, chitin, cadmium(II), adsorption

1. INTRODUCTION

Aqueous effluents emanating from many industries usually contain dissolved heavy metals such as Cd, lead (Pb), copper (Cu) and mercury (Hg).[1] If these industrial liquid wastes are discharged without prior-treatment, they may have an adverse impact on the environment.[2] Higher awareness of the ecological effects of toxic metals and their accummulation through food chains has prompted a demand for purification of industrial wastewaters prior to their discharge into the natural water bodies and thus increasing interest has been shown in the removal of heavy metals. Conventional methods for removing metals from industrial waste solutions, which include chemical precipitation, chemical oxidation or reduction, filtration, ion exchange, electrochemical treatment, application of membrane technology and evaporation recovery are sometime ineffective or extremely expensive, especially when the metals dissolved are in large volumes of solution and at relatively low concentrations (around 1–100 ppm).[3] The newly discovered metal sequestering properties of certain types of biomass of selected bacteria, fungi, yeast, algae, higher plants, and products derived from these organisms, offer considerable promise.[4–6] The

general term 'biosorption' has been used to describe a property of microorganisms to retain toxic heavy metals from aqueous solutions.[7] The degree of removal of heavy metals from wastewater by biosorption depends on the multimetal competitive interactions in solution with the sorbent material.[8]

Chitin is the structural polysaccharide in the exoskeleton of animals. It is the polymer of *N*-acetylglucosamine, where generally <50% of the acetyl groups has been lost. Chitin is an amide of acetic acid available in large amounts from the shells of arthropods.[9] Its chemical structure is shown in Figure 1(a). Chitin stoichiometry is $(C_8H_{13}NO_5)_n$ and contains 6.9% nitrogen. Chitin is a high molecular weight biopolymer of glucosamine and *N*-acetylglucosamine. The applicability of this chitinous material is large considering their chemical, physical and biological properties.[10] The chitinous materials can be used in different forms as: flakes, powders, solutions, gels, membranes, fibres, pellets or capsules.[11–12] One of the most important properties of chitin is its ability to remove metal ions.[13] Their structure allows excellent complexation capacity with metal ions, particularly transition and post-transition metals.[14] It was supposed that the chelation of a single metal ion by several –NH− or $–NHCOCH_3$ groups effectively isolates each metal ion from its neighbors.[15] Consequently, chitin may be used in wastewater treatment for the removal of cations such as Cd ions.[9] However, the adsorption of chitin towards metal ions is not selective, especially when alkali and alkali-earth metal ions are also available in the solution in high level of concentrations. Therefore, modification of the chitin surface should be carried out using a specific and sensitive ligand for heavy metal ions.[16–17] Dithizone (diphenylthiocarbazone) is a suitable ligand for such purposes because it contains many N donor atoms, –NH as well as –SH groups which is very specific for heavy metal ions such as Pb, Cd, Cu and Hg.[18]

The purpose of this study was to immobilize an organic ligand, dithizone [Fig. 1(b)] onto the surface of natural chitin in order to enhance the selectivity and adsorption capacity of dithizone-immobilized natural chitin towards heavy metal ions. The modified bioadsorbents are intended to be used for the adsorption of heavy metals in industrial liquid waste as well as the supporting material in the solid-phase extraction process for pre-concentration of heavy metals. Dithizone is selected as the organic ligand in this study because it is considered to be very selective for Hg, Cd and Pb.[18–19] The immobilization of dithizone onto the surface of polymer[16] and silica gel[17] has been reported and successfully used for the removal and selective pre-concentration of heavy metals. In this study, natural biopolymer chitin was used as a supporting material for the immobilization of dithizone, which was easily isolated from prawn seawater shells and cheaper than synthetic polymer or silica gel.

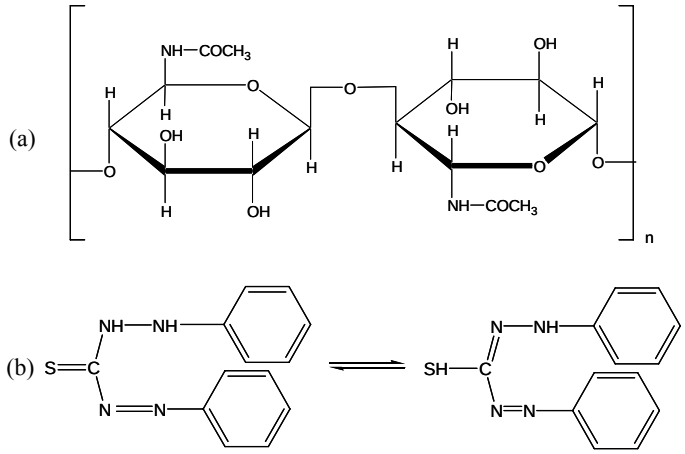


Figure 1: Chemical structure of (a) chitin and (b) dithizone.

2. EXPERIMENTAL

2.1 Reagents and Materials

Metal salts of analytical grade and dithizone (1,5-diphenylthiocarbazone) of reagent grade, were all purchased from Merck, Germany. Natural chitin was obtained by isolating it from prawn seawater shells. Organic solvents were of reagent grade and used as received. For all solutions, double distilled water was used and the buffer solutions were prepared from sodium hydrogen phosphate to which different volumes of hydrochloric acid were added, and the pH value of the resulting solution was adjusted with the use of a pH meter.

2.2 Instrumentations

The pH measurements were carried out by a TOA pH meter model HM-5B calibrated against two standard buffer solutions of pH 4.0 and 9.2. Infrared spectra of biopolymer chitin and dithizone-immobilized chitin were measured from KBr pellets by a Shimadzu FT-IR/8201 PC spectrophotometer. Metal ion analyses were performed with a Perkin Elmer 3110 flame atomic absorption spectrometer (AAS). X-ray diffraction analyses of chitin and dithizone-immobilized chitin were recorded on Phillips model PW 3710 BASED X-ray diffraction (XRD) spectrophotometer (Shimadzu 6000X, radiation source: Cu, K-lambda 1.5402 nm).

2.3 Procedures

2.3.1 Isolation of chitin

In this research, prawn seawater shells were obtained from seafood restaurant waste around Yogyakarta region, Indonesia. Prawn shells, which have been boiled for 1 h and separated from its meat, were washed and depigmented with caporite solution (4%, technical grade) for 24 h and were then dried at room temperature (27^{o}C).

Chitin was isolated from the shell of prawns using modified method[20] of No et al.[21] consisting of deproteination and demineralization processes. Dried prawn shells were ground and filtered through a 100 mesh filter. Deproteination of the prawn shells was carried out by refluxing 50 g of the filtered prawn shells with 500 ml 3.5% NaOH (w/v) for 2 h at 65ºC. The mixture was cooled and the obtained residues were washed with water until the filtrate was neutral, which was done by checking the filtrate with pH paper indicator. For the purpose of demineralization, 30 g of the residues were mixed with 450 ml 1.0 M HCl and stirred for 30 min at room temperature. The mixture was then filtered and the solid obtained was washed with water as described above and dried at 60ºC to yield chitin powder.

2.3.2 Characterization of biopolymer chitin

The contents of ash and total carbon in chitin have been determined by gravimetric and volumetric methods, respectively. The nitrogen content was determined using the Kjeldahl method. Further characterization was performed by infrared spectroscopy for functional groups and XRD for the crystalinity using the instrumentations as mentioned in section 2.2.

2.3.3 Immobilization of dithizone on biopolymer chitin

In order to prepare dithizone-immobilized chitin, the following procedure was applied: 4.0 g biopolymer chitin was added to 80 ml toluene and mixed with 1.0311 g dithizone in a 500 ml flask. The mixture was refluxed and stirred for 2, 4, and 6 h at 70^{o}C. The product was filtered and washed consecutively with toluene, ethanol and water several times until the filtrate showed no characteristic color of dithizone. The dithizone-immobilized chitin was then dried in an oven at 60^{o}C for 12 h and filtered through 200 mesh filter. The dithizone-immobilized chitin obtained was brown in color and subjected for characterization and adsorption study.

2.3.4 Preliminary biosorption study of Cd(II) ion

Adsorption of Cd(II) ion from a single metal aqueous solution was investigated in batch adsorption-equilibrium experiments. Aqueous metal ion solutions of 25 ml containing 10 μg/ml Cd(II) ions was mixed with 0.2 g of chitin or dithizone-immobilized chitin at room temperature and the pH of the solution was varied in the range 3.0–8.0. The reaction mixture was mechanically shaken for 3 h and the adsorbents were then separated from the adsorption medium. The concentration of Cd(II) ions in the solution was determined by AAS and the amount of adsorbed metal ions was calculated by difference using Equation (1):[16]

$$Q = [(C_o - C_A)\, V]/m \quad (1)$$

where Q is the amount of metal ions adsorbed onto unit amount of the adsorbents (mg/g), C_o and C_A are the initial and final concentrations of metal ions (μg/ml), respectively, V is the volume of the aqueous phase (ml), and m is the weight of the chitin or chitin-dithizone adsorbents.

The effect of adsorbent mass on the amount of adsorbed Cd(II) ion was investigated using the same procedure, but the weight of adsorbent used with 10 μg/ml Cd(II) ion solution was varied in the range of 0.05–0.5 g and the pH of the solution was kept constant at 6.0.

3. RESULTS AND DISCUSSION

3.1 Isolation and Characterization of Chitin

Isolation of chitin from prawn seawater shells consisting of depigmented, deproteinized and demineralized steps. Depigmentation step is intended to remove the odor and to bleach the product so that the chitin obtained is white in color. To avoid evaporation of water from the solution, the deproteinizing step is carried out by refluxing the prawn shell powder in NaOH solution. If reaction mixture is heated in an opened system, from time to time water will be evaporated from the solution and NaOH in the solution is concentrated, resulting in the formation of chitosan due to the deacylation process, which may occur in the solution.

The purpose of the demineralizing step is to remove any absorbed minerals from the surface of the chitin. This is a crucial step because the chitin obtained will be further utilized for the adsorption of metal ions. This step is conducted by mixing prawn shell powder with HCl solution and stirring it at room temperature. According to Muzzarelli,[9] $Ca_3(PO_4)_2$ and $CaCO_3$ are the most

common minerals found as impurities in chitin. By adding HCl, these minerals would be leached from the surface of the chitin biopolymer in accordance with the following reaction steps:

$$HCl(aq) \longrightarrow H^+(aq) + Cl^-(aq) \quad (1)$$

$$H^+(aq) + H_2O \rightleftharpoons H_3O^+(aq) \quad (2)$$

$$Ca_3(PO_4)_2(s) + 2\ H_3O^+(aq) \longrightarrow 3\ Ca^{2+}(aq) + 2\ H_3PO_4(aq) + O_2(g) \quad (3)$$

$$CaCO_3(s) + 2\ H_3O^+(aq) \longrightarrow Ca^{2+}(aq) + CO_2(g) + 3\ H_2O(l) \quad (4)$$

The reaction was done at room temperature to avoid depolymerization of chitin biopolymer into its monomers.

Figure 2 gives the IR spectra of the reference and isolated chitin from prawn seawater shells (*P. merguensis*). It is clearly seen from Figure 2 that both spectra have similar absorption patterns suggesting that good quality of chitin biopolymers has been obtained. Detailed examination of the spectra reveals that both spectra have characteristic bands for chitin. Absorption peak at 3448.5 cm^{-1} indicates the stretching vibration of aliphatic O-H, those observed at 3271 cm^{-1} and 3109 cm^{-1} each belongs to asymmetric and symmetric stretching vibration of N-H group from acetamide ($-NHCOCH_3$), respectively.[20] Absorption peak at 2931.6 cm^{-1} is from −C-H stretching vibration of $-CH_3$, which is supported by the existence of the absorption at 1380.9 cm^{-1}, characteristic for the bending vibration of $-CH_3$. Absorption band at 1658.7 cm^{-1} represents the stretching vibration of the carbonyl group, C=O from acetamide ($-NHCOCH_3$). Other characteristic absorptions for chitin are at 1558.4 cm^{-1} and 1311.5 cm^{-1}, indicating the bending vibration of –NH and stretching vibration of –CN from acetamide group, respectively.[22] Absorption at 1157.2 cm^{-1} belongs to the –C-O vibration of polysaccharide and that observed at 1026.1 cm^{-1} is the stretching vibration for –C-O-C– of the glucosamine ring.

Table 1 gives the results of ash and total nitrogen contents in chitin isolated from prawn shell. These results together with IR spectra data confirmed that the isolated material was chitin biopolymer of high purity.[21]

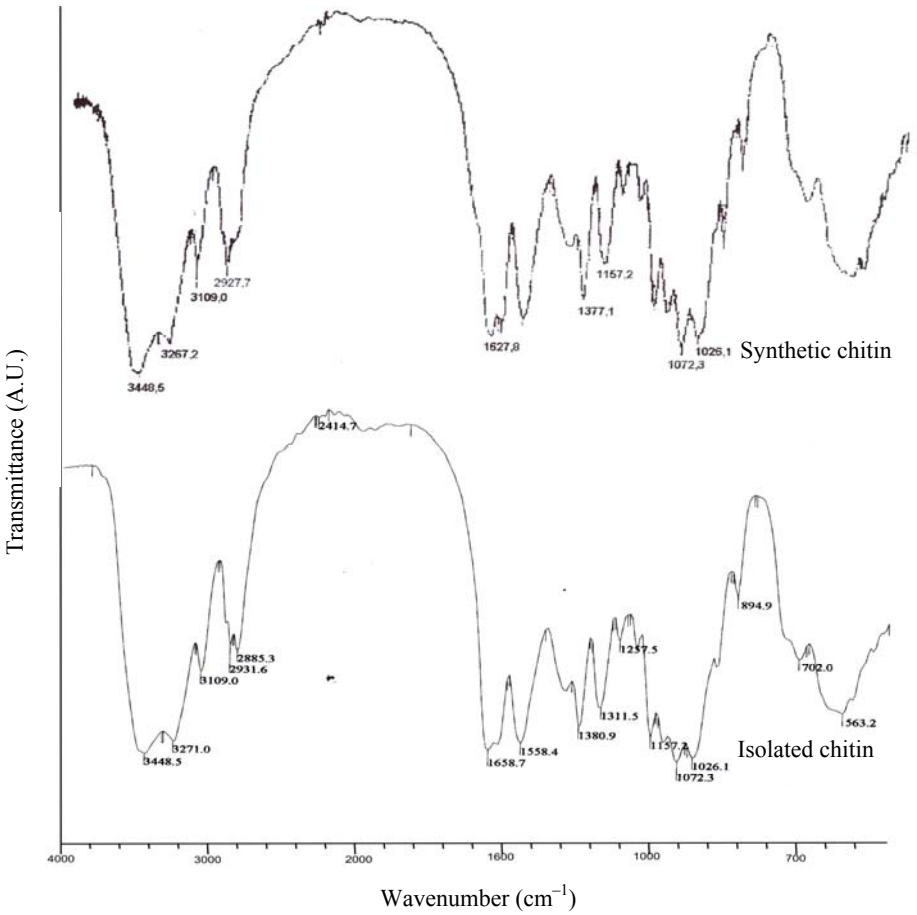


Figure 2: IR spectra of (a) reference and (b) isolated chitin.

Table 1: Ash and total nitrogen contents of isolated chitin from prawn shell.

Parameters	Content (% wt)
Total nitrogen	3.9
Ash content	0.59

3.2 Immobilization of Dithizone on Chitin

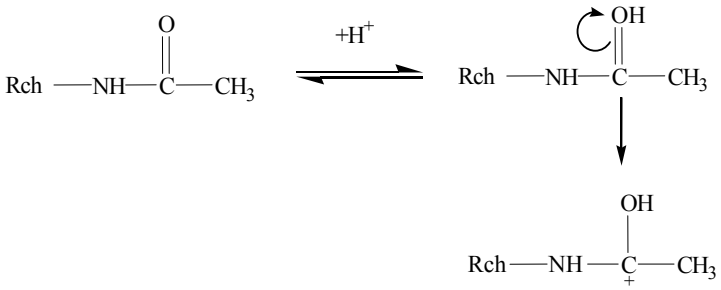
Immobilization of dithizone on the surface of chitin was done by impregnation and physical adsorption methods. The adsorbent was prepared by refluxing chitin with organic ligand, dithizone in toluene medium. Toluene was selected as a solvent instead of water in order to avoid the coverage of active sites of chitin surface by water molecules. After completion of the reaction, the product was washed consecutively with toluene, ethanol and water to remove the

excess dithizone, and other polar and non-polar impurities. This can be achieved by observing the filtrate after washing which shows no trace of dithizone. The product was finally dried in the oven at 70^{o}C to evaporate any water molecules adsorbed from the atmosphere which in turn could reduce the metal adsorption capacity of the adsorbents.

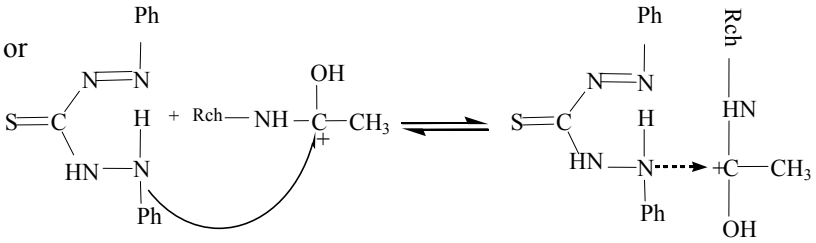
The basic structure of chitin consists of glucosamine ring bearing –OH group. Since the binding and steric hindrance between –OH group and glucosamine ring is quite strong, the direct binding of –NH group of dithizone to glucosamine ring by substituting the –OH group is unlikely to happen. Therefore, the interaction of dithizone and chitin most likely occurs via lone-pair electron attack of N in dithizone to the –OH group of glucosamine ring. Chitin also posses nucleophilic acetamide group (–$NHCOCH_3$) containing carbonyl –C=O group, which can be protonated to give partial positive charge on the carbon atom in the carbonyl group. This protonated carbonyl group, –C=O may undergo electrostatic interaction with lone-pair electron of N atom in dithizone ligand. The proposed possible interaction between dithizone and chitin biopolymers is given in following scheme:

Acetamide group of chitin is first protonated:

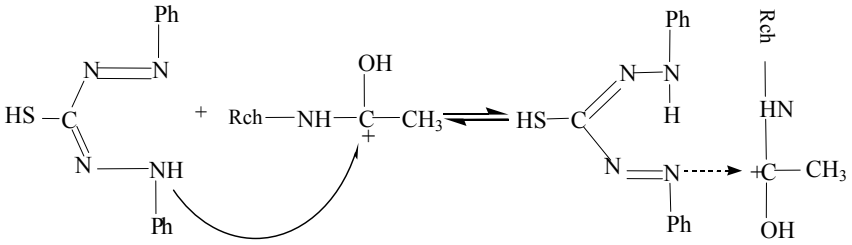
(i)



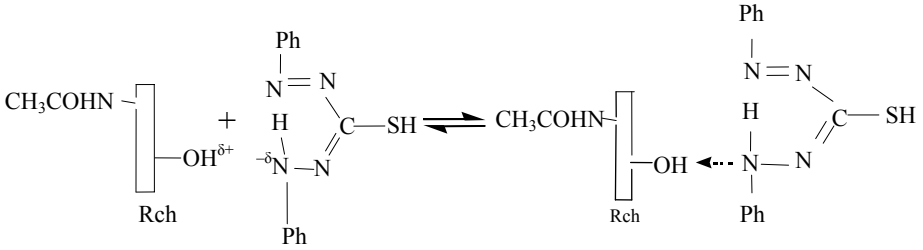
Possible interaction :



Scheme 1: Possible interaction between dithizone and chitin biopolymer (Rch = glucosamine ring).



involving –OH group (ii)



Scheme 1: *(continued)*

The IR spectra of dithizone (upper) and dithizone-immobilized chitin (lower) are given in Figure 3. A closer observation of Figure 3 reveals that in addition to the common bands of chitin biopolymer, which is slightly shifted due to the interaction with dithizone, the IR spectra of dithizone-immobilized chitin also contains several bands characteristics of dithizone. The vibration at 3448.5 cm^{-1}, characteristic of aliphatic –OH decreases its intensity and becomes sharper, indicating that the hydrogen bond between –OH on the glucosamine ring and water molecules are reduced due to the binding of the –OH group to –NH groups of dithizone. The weak band at 2893.0 cm^{-1} indicates stretching C-H of olefin and the one at 1589.5 cm^{-1} is assigned to –NH bending. Other important vibrations are at 1589.2 cm^{-1} due to C=C of aromatic skeleton of phenylic groups, supported by the bands at 894.9 and 756.0 cm^{-1} attributed to out of plane vibrations of aromatic C-H, a weak band at 2500 cm^{-1} suggests the existence of –SH, while the one at 2279 cm^{-1} indicates the existence of C=N. All of the mentioned vibrations indicated that dithizone has been successfully immobilized onto the surface of chitin.

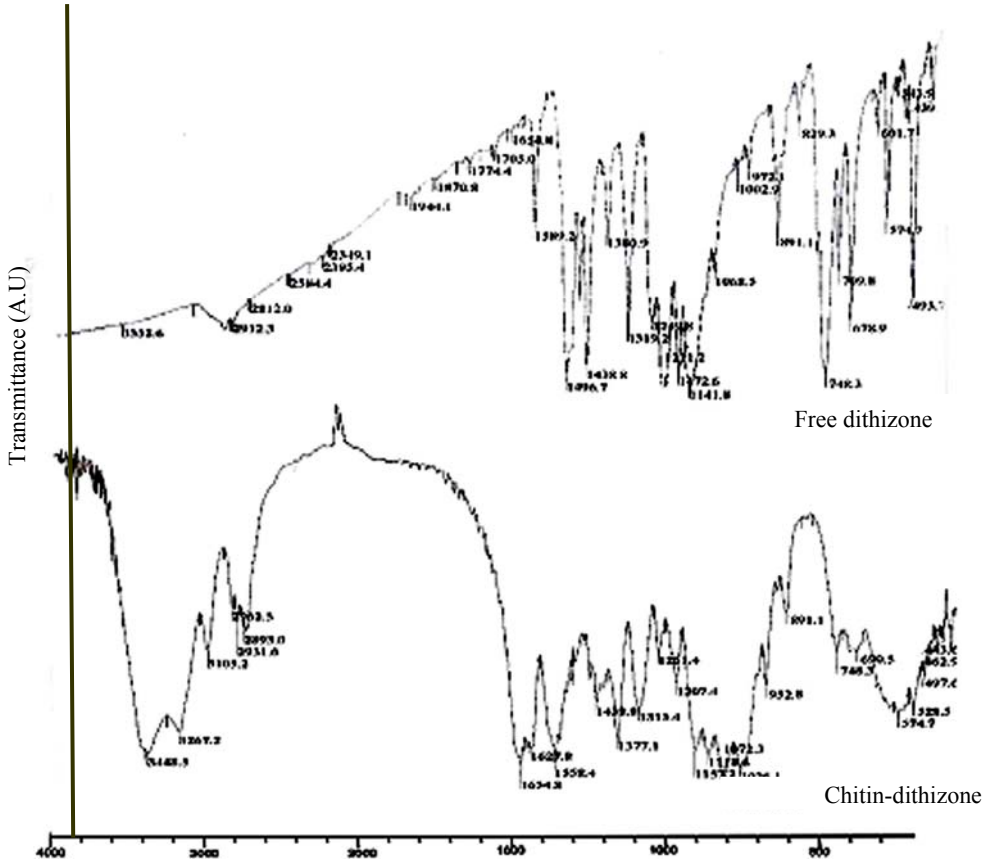


Figure 3: IR spectra of (a) free dithizone and (b) dithizone-immobilized chitin.

The XRD spectra and their d-spacing data of free dithizone and dithizone-immobilized chitin are in Figure 4 and Table 2, respectively. The XRD data confirmed the conclusion that dithizone has been successfully loaded onto the surface of chitin, indicated by the existence of band and d-spacing value at 2θ characteristics for dithizone.[23] Unfortunately, due to the small amounts of dithizone that can be immobilized onto the surface of chitin, the intensity of dithizone bands is considerably low as compared to those of chitin. The XRD also suggests that immobilization of dithizone onto the surface of chitin does not significantly affects the structure of the chitin as shown by the unchanging pattern of the diffractograms upon immobilization. Nevertheless it can be concluded from the results of XRD or IR analyses that dithizone has been successfully loaded onto the surface of chitin. This conclusion is also supported by the fact that the color of dithizone-immobilized chitin is brown, indicating the existence of dithizone, while unmodified chitin is white in color.[17]

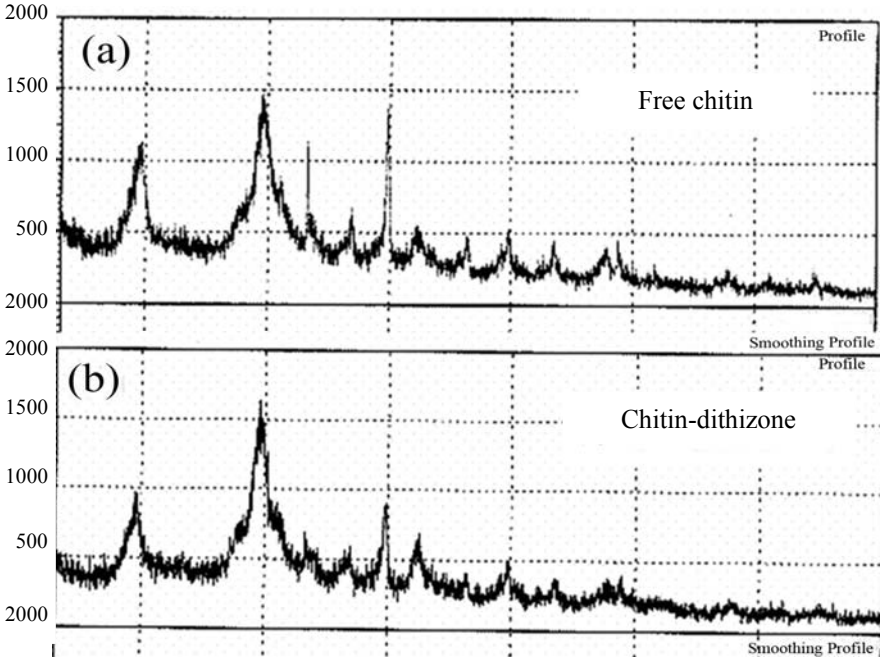


Figure 4: XRD spectra of (a) free chitin and (b) dithizone-immobilized chitin.

Table 2: Interpretation of 2θ and d-values along with relative intensity of XRD spectra for dithizone-immobilized chitin.

2θ	d	I/Io	Component
19.5374	4.53996	100	Chitin
9.3762	9.42476	37	Chitin
21.3400	4.16036	34	Dithizone
17.6400	4.96791	32	Dithizone
23.5200	3.77945	19	Dithizone
26.4600	3.36580	9	Dithizone
12.8000	6.91045	8	Chitin
39.2050	2.29603	7	Chitin
28.2200	3.15976	6	Chitin
15.1800	5.83192	4	Dithizone

Optimization of immobilization process has been carried out by varying the reaction/reflux time between dithizone and chitin biopolymer, i.e. 2, 4 and 6 h and each product (adsorbent) obtained is identified by IR spectroscopy (figures are not shown). It has been observed from the IR spectra that shorter reaction time gives incomplete immobilization, indicated by the absence of characteristic

absorption peaks of dithizone. On the other hand, although longer reaction time gives better immobilization, it seems that the active sites of the dithizone ligand bound to the surface of chitin undergo oxidation process due to the relatively long heating treatment, leading to relatively low intensity of absorption peaks which is characteristic for dithizone. Based on the IR spectra data, it is concluded that the best immobilization is achieved when the reflux process was conducted for 6 h as indicated by the existence of more intense peaks, characteristic of dithizone in the IR spectra.

3.3 Effect of pH on the Biosorption of Cd(II) Ions

Preliminary study of the heavy metal ion adsorption capability of dithizone-immobilized chitin is studied by employing the prepared adsorbent for the biosorption of Cd(II) ion in the solution. Typical Cd(II) adsorption by unmodified chitin and dithizone-immobilized chitin as a function of pH of the medium is presented in Figure 5. The equilibrium adsorption process has been done in batch system, e.g. an aqueous single metal ion solutions of 25 ml containing 10 μg/ml Cd(II) ions was interacted with 0.2 g adsorbent at room temperature and the reaction mixture was mechanically shaken for 3 h and the adsorbents were then separated from the adsorption medium. To eliminate interferences from the precipitation of Cd(II) ions as hydroxides as the pH of the medium is increased, a control solution containing Cd(II) ions at the same concentration as that used for adsorption equilibrium experiments is provided for each pH used in the experiment. Therefore the value of the adsorbed Cd(II) ions presented in Figure 5 is purely from the adsorption of dithizone-immobilized chitin.

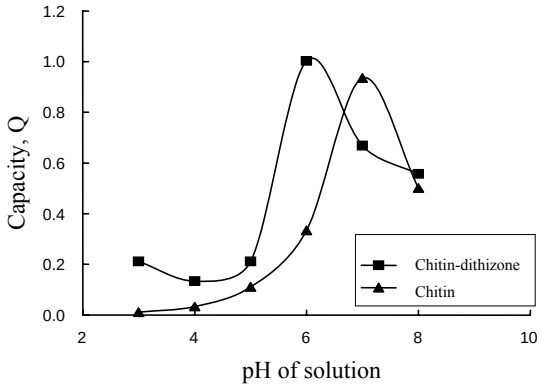


Figure 5: Effect of pH on the adsorption of Cd(II) ion by chitin and dithizone-immobilized chitin.

Figure 5 shows that the adsorption of Cd(II) ions by the two adsorbents significantly increases with the pH of the medium up to pH = 6 for dithizone-immobilized chitin and pH = 7 for unmodified chitin. This trend is easily understood because at very low pH, the active sites of the adsorbents are protonated by H^+ ion to yield partially positive charge of the sites, which is similar to charge of Cd(II) ions. As a result, adsorption of metal ions by both adsorbents is hindered. As the pH increase gradually, deprotonation of the active sites occurs resulting in favorable condition for the metal adsorption. Further increase in pH of the solution (> 7), however, gives rise to the decrease of the adsorbed Cd(II) ion, probably due to the precipitation of Cd(II) as hydroxide species (K_{sp} $Cd(OH)_2 = 2.5x10^{-14}$, thus at 10 µg/ml Cd(II), precipitation of Cd(II) hydroxide will occur at pH = 8.5) or the formation of other negative species (metal complexes) involving hydroxide ion. In addition, at higher pH, the active sites of the adsorbents are deprotonated and tend to posses partial negative charge. This condition electrostatically hinders the Cd(II) adsorption because at higher pH Cd(II) ions possibly also forms negatively charged species with hydroxide ion, $[Cd(OH)_3]^-$ or $[Cd(OH)_4]^{2-}$. Furthermore at a higher pH, there would be a competition between negatively charged active sites of the adsorbent and OH^- ion to attract metal ion. Hence, it is not surprising to observe the decrease of the adsorbed Cd(II) ions by both adsorbents as the pH of the solution is increased.

From Figure 5, it is also observed that dithizone-chitin adsorbs more Cd(II) ions as compared to those of unmodified chitin. This may be due to the addition of the various types of active sites (N, –NH and –SH) obtained from the immobilization of dithizone onto the surface of chitin which are very specific for soft and medium acid such as Cd(II) ion.[18,19]

3.4 Effect of Adsorbent Mass on the Biosorption of Cd(II) Ion

The effect of adsorbent mass on the biosorption of Cd(II) ion was examined by conducting adsorption experiments using fixed concentration of Cd(II) and various mass of chitin-dithizone adsorbent at its optimum pH and the results are compared with those of unmodified chitin as shown in Figure 6. As expected, the adsorbed Cd(II) ion increases with the increase in adsorbent mass. This trend is easily understood since increasing the adsorbent mass in the solution results in the increase of the amount of active sites on the surface of the adsorbent, giving greater chance of Cd(II) ion to be absorbed. However, when the adsorbent mass used in the solution is too large, less Cd(II) is absorbed; probably due to the problem of mass transfer or mobility of Cd(II) ions into the surface of the adsorbent. Another possibility is that the adsorbent undergoes sintering so that much of the active sites are hidden and could not be accessed freely by Cd(II) ions because these ions are hydrated by water molecules in the solution and therefore its hydrated complex size is somewhat bulky. For the solution

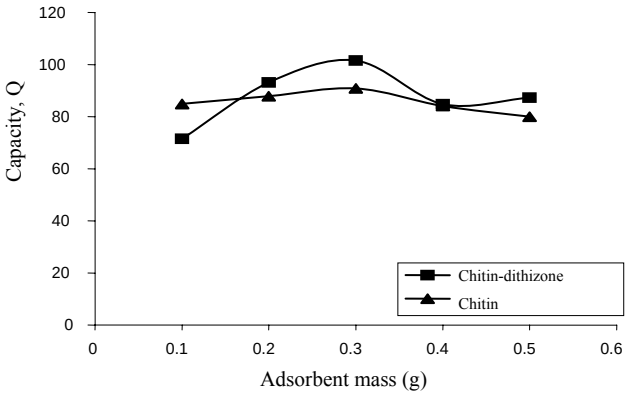


Figure 6: Effect of adsorbent mass on the adsorption of Cd(II) ion by chitin and dithizone-immobilized chitin.

containing 10 µg/ml of Cd(II) ion at pH = 6 and 25^{o}C, it is found that the adsorption of Cd(II) ions reaches its maximum value when 0.3 g adsorbent is used, resulting in the adsorption of Cd(II) ions on chitin-dithizone and unmodified chitin of 7.67 x 10^{-3} and 5.67 x 10^{-3} mol/g adsorbent, respectively.

4. CONCLUSION

Results of this research confirmed that high purity biopolymer chitin can be isolated from prawn seawater shells with good purity as indicated by its ash and total nitrogen contents, and degree of deacetylation as well as IR spectra. It has been demonstrated that organic ligand dithizone can be immobilized onto the surface of the isolated chitin by refluxing the two substances in the toluene. This dithizone immobilization on chitin gives rise to more selective and higher adsorption capacity for Cd(II) ions as compared to that of unmodified chitin. The adsorption of Cd(II) ions by chitin and dithizone-immobilized chitin reaches it maximum at pH = 7 for unmodified chitin and pH = 6 for dithizone-immobilized chitin. The adsorption of Cd(II) is also affected by adsorbent mass applied in the solution. For 25 ml solution containing 10 µg/ml of Cd(II) at pH = 6 and 25^{o}C, it is found that the adsorbent mass of 0.3 g gave the best adsorption results.

5. ACKNOWLEDGEMENT

This work is partially supported by Basic Research Incentive from State Ministry for Research and Technology (Insentif Riset Dasar-KMNRT), The Republic of Indonesia for fiscal year 2007–2008. The authors gratefully acknowledged the support.

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