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Development of Polyvinyl Alcohol-Sodium Alginate Beads as Immobilization Matrices of 1,5–Diphenylcarbazide for the Detection of Cr(VI)

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Abstract

Polyvinyl alcohol–sodium alginate beads as a sensor for the detection of Cr(VI) in aqueous solution were developed by immobilizing 1,5–diphenylcarbazide. In the presence of Cr(VI), the color of beads sensor changed from yellowish-colorless into purplish-pink. Beads were prepared using polyvinyl alcohol–boric acid method. The resulted beads were characterized by FTIR spectrophotometer, and the absorbance measurement was carried out at a wavelength of 547 nm. The optimum response of the beads sensing was obtained at pH 2, for 8 min sensing. The linearity of the calibration curve was obtained with a limit of detection for 25 mL sample was 0.11 mg L^{-1}. The beads sensor had good precision, accuracy and also exhibited good stability for 30 d. Subsequently, the beads sensor was able to detect the presence of Cr(VI) in the water samples, proved by the color change of the beads sensor relative to the visual standards.

1. Introduction

In the last few years, there has been an increasing amount of heavy metal pollutants into the environment and ecosystem caused by anthropogenic activities, especially in the industrial field. Chromium is one of the heavy metal pollutants that are interesting, due to the carcinogenic properties and the high toxicity, even at low concentrations. In the natural environment, chromium exists as Cr(VI) and Cr(III). These species differ significantly in their toxicity and mobility. Chromium(III) is an essential nutrient needed by the body to improves insulin efficiency and generally precipitates onto mineral surfaces, so it is immobile in the environments, whereas Cr(VI) is highly soluble and approximately 100 times more toxic than Cr(III) [1]. The extensive use of chromium in the industrial processes, such as electroplating, leather tanning, dyeing, and metal finishing, increases the concentration of chromium in the environment. Indonesia's Ministry of Environment regulated the maximum concentration of chromium in the effluent leather tanning industry is 0.6 mg L^{-1}. Hence, continuous monitoring of Cr(VI) in the environment is essential.

Several analytical methods have been used for chromium determination, such as inductively coupled plasma emission spectrometry (ICP-AES) [2], atomic absorption spectroscopy (AAS) [3], X-Ray fluorescence [4], and liquid chromatography [5]. Although these methods offer high sensitivity and accuracy, they do not distinguish the oxidation state of chromium, and only total concentrations are measured. These conventional methods also have several disadvantages, such as time-consuming, costly, required sophisticated instruments, skilled operators, and cannot be employed for in-situ detection [6]. To overcome these problems, a new method has evolved to provide simple, cheap, and fast for in-situ detection of chromium species [6].

The optical sensor is a promising method for in-situ detection — most of these methods, based on immobilization of a color reagent in a suitable material. The use of a specific color reagent could improve the selectivity of the sensor. Since the 1,5-diphenylcarbazide (DPC) method was a reference method for the speciation analysis of Cr(VI); this method was used as the basis of the development of the Cr(VI) optical sensor. Several studies for the determination of Cr(VI) based on immobilization of 1,5–diphenylcarbazide (DPC) have been reported. Determination of Cr(VI) by immobilizing of DPC in sol-gel modified silanes have been reported [7]. The optical sensor was developed based on the immobilization of DPC in a plasticized cellulose triacetate matrix [8]. The other optical sensor for the determination of Cr(VI) in synthetic wastewater was developed based on the immobilization of DPC in a cellulosic paper strip [9].

The other solid matrices that have been widely used for the immobilization matrix are polyvinyl alcohol (PVA) and sodium alginate beads. PVA is a synthetic polymer, which is cheap, non-toxic, durable and stable, but very hydrophilic [10]. To reduce the solubility and increases the physical properties of PVA, a cross-linker and natural polymer such as sodium alginate were added to the solution [11]. The economical and straightforward method for cross-linked PVA is the boric acid method [12]. This method produced elastic, strong, and durable beads, but the reaction rate of PVA-boric acid beads was very slow so that PVA tends to agglomerate. This problem is overcome with the addition of a small amount of sodium alginate. To obtain the excellent mechanical properties of sodium alginate gel, a low concentration of calcium chloride was added to the boric acid for the cross-linker of sodium alginate. The combination of PVA-sodium alginate beads was developed as the immobilization matrix of lipase [13]. The PVA-sodium alginate beads were used as the immobilization matrix of Acidithiobacillus ferro-oxidans, and the bio-oxidation capacity of this material was increased [14]. In the other fields, γ–Fe_2O_3 encapsulated in PVA-sodium alginate beads were developed for the photocatalytic reduction Cr(VI). The result showed that PVA-sodium alginate beads have macroporous structures so that the Cr(VI) could easily diffuse from the solution into the beads pore, and interact with the γ–Fe_2O_3 inside the beads [15].

PVA and sodium alginate are hydrophilic. Either PVA or sodium alginate has functional groups, which are active for the hydrogen bonding. These properties could be used for the immobilization of DPC into the beads. To our knowledge, the development of PVA-sodium alginate beads for the immobilization matrix of DPC as the optical sensor for Cr(VI) determination never been reported. In this study, the optical sensor of PVA-sodium alginate for the detection of Cr(VI) in aqueous solution based on immobilization of DPC was synthesized. The optimum conditions of sensing and the validity parameter were studied, and compared to the previously reported optical Cr(VI) sensor. The standard visuals were prepared by the sensing process of the beads sensor in the various concentration of potassium dichromate, and the resulted color of beads was analyzed with chromameter. Furthermore, the beads sensor was applied to detect the presence of Cr(VI) in wastewater samples, and the results were then compared to the reference method.

2. Material and Methods

2.1. Reagents and chemicals

Polyvinyl alcohol (PVA) was obtained from Bratachem, Indonesia, sodium alginate in analytical grade was obtained from Sigma-Aldrich, 1,5–diphenylcarbazide (DPC, $C_{13}H_{14}N_4O$), potassium dichromate ($K_2Cr_2O_7$), sulfuric acid (H_2SO_4), boric acid (H_3BO_3), and calcium chloride ($CaCl_2.2H_2O$) were purchased from Merck. All aqueous solutions were prepared using distilled water.

2.2. Preparation of the beads sensor

PVA-sodium alginate beads were prepared by using the PVA-boric acid method, with the addition of calcium chloride as a crosslinker of sodium alginate. Initially, 10 g of PVA was stirred in 89 g of distilled water at 90 °C for about 5 h and continued for the other 19 h at room temperature. Subsequently, 1 g sodium alginate was added to the PVA solution and stirred for 24 h to obtain the total concentration of PVA, and sodium alginate was 10 and 1 %wt., respectively. Then, 5 mL of 1% DPC solution was added to the 95 mL of PVA-sodium alginate solution. The mixed solution was then stirred for 24 h in a dark condition. After that, the mixed solution was dropped into the 5% of boric acid and 2% of calcium chloride (1:1) using the 10 mL syringe to form the spherical beads with the average diameter ±3 mm. The beads were then kept in the boric acid-calcium chloride solution for 24 h to complete the crosslinking reaction and gelation process. The beads were then washed several times with distilled water to ensure the removal of borate ions in the surface of beads before using the beads as beads sensor. The resulted beads were characterized using the spectrometer FTIR Shimadzu Prestige 21.

2.3. Measurement procedure

Measurement of beads sensor was carried out by immersing the beads in a 25 mL solution of Cr(VI) for 8 min at pH 2. Wastewater samples were obtained from the effluent of the leather tanning industry located in Bantul, Yogyakarta, Indonesia. The collected wastewater samples were filtered by using Whatman filter paper 42 pore size and stored in plastic bottles at 4 °C. The resulted measurement with the beads sensor compared to the DPC method, as a reference method. DPC method was performed to determine the concentration of Cr(VI) in the wastewater samples. This measurement was conducted at pH 2, by adjusting the water samples with 0.5 M of sulfuric acid. Then, 0.3 mL of 1% DPC was added into the 25 mL volumetric flask containing 24.7 mL sample at pH 2. The mixture was then shaken for about 15-30 s and allowed to stand for about 10 min to complete the color development process. The absorbance of the solution was then measured by using the spectrophotometer UV-Vis GBC Cintra at a wavelength of 543 nm.

3. Results and discussion

The formation of the Cr(VI)-DPC complex is due to the reaction of Cr(VI) with DPC that involves 2 steps. The first step is the reduction of Cr(VI) to Cr(III) and the oxidation of DPC to DPCO, subsequent with the formation of a colored complex between Cr(III)-DPCO, as shown in Figure 1 [8]. The exact structure of this complex is unknown, but it appears that the complex Cr(VI)-DPC is a cationic complex. This colored complex is specific to Cr(VI), and have been extensively used for Cr(VI) determination in aqueous solution by the spectrophotometric method [8]. The reaction between Cr(VI) and DPC is shown in Figure 1.

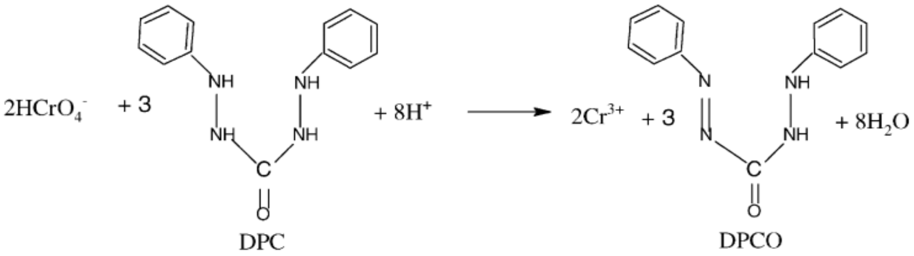

Figure 1: Reaction of Cr(VI) with DPC [8].

FTIR characterization

In this study, immobilization of DPC in the matrix PVA-sodium alginate beads as Cr(VI) colorimetric sensors was carried out. Polyvinyl alcohol and sodium alginate were chosen as matrices for immobilization because both polymers have functional groups that are possible to interact with DPC through hydrogen bonds. The characterization of the beads was conducted by using an FTIR spectrometer to determine the interactions that occur between PVA, sodium alginate, DPC, and Cr(VI)-DPC complex. The FTIR spectra of PVA-sodium alginate beads before and after the sensing process are shown in Figure 2. For PVA-sodium alginate-DPC beads, the characteristic peak of O–H stretching vibration of hydroxyl groups from PVA was observed at 3426 cm^{-1} [12,16], overlap with the stretching vibration of symmetric and asymmetric N–H from DPC. The band observed around 3426 cm^{-1} corresponds to the stretching of O–H from the intermolecular and intramolecular hydrogen bonds between PVA and sodium alginate [12]. The characteristic peak of sodium alginate was also seen at 1643 dan 1427 cm^{-1}, referred to the asymmetrical and symmetrical stretching vibration of COO$^-$ [12,16]. The observed peak at 2947 cm^{-1} showed the stretching vibration of alkyl and aromatic C–H.

After the sensing process to the Cr(VI) solution, the peaks of stretching vibration C–C and C–O have shifted to 1119 cm^{-1}. The O–H peaks have shifted to the lower wavenumber, which is 3410 cm^{-1}, proved the new formation of hydrogen bonding between Cr(VI)-DPC complex with the beads. The COO$^-$ peaks have also shifted to the lower wavenumber, 1635, and 1435 cm^{-1}, indicating less interaction between PVA and alginate due to the breaking of the –COO and the –OH bonds and the formation of new bonds resulting from hydrogen bonding between Cr(VI)-DPC complex with the beads [12]. The study revealed that PVA-alginate beads are microporous, having larger pore diameters [12]. Therefore, in the sensing process, the Cr(VI) from the aqueous solution would diffuse into the beads through the pores. Thus, the Cr(VI) would interact with immobilized DPC in the matrix system through N-H and N=N functional groups, resulting in the purplish-pink complex of Cr(VI)-DPC. This complex of Cr(VI)-DPC then also interacted with the PVA-alginate through the hydrogen bonds, making the complex of Cr(VI)-DPC was completely entrapped and did not release from the matrix, as confirmed by no purplish-pink color observed in the aqueous Cr(VI) solution.

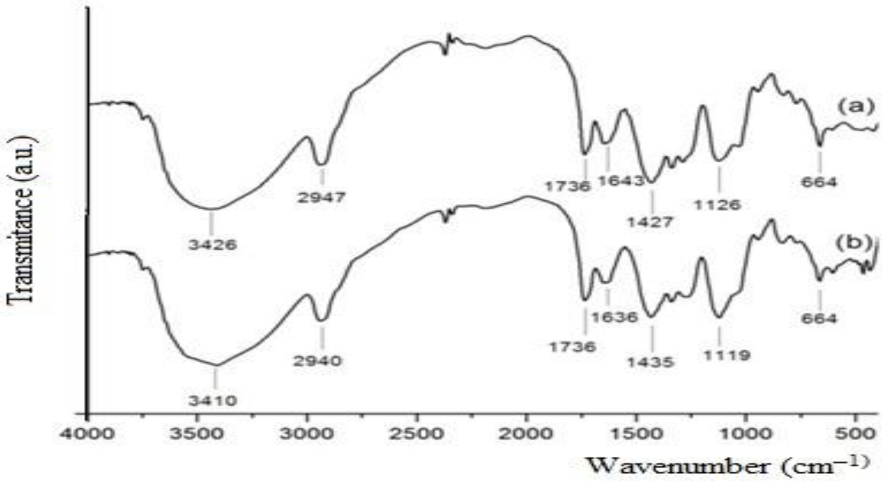

Figure 2: FTIR spectrum of (a) PVA-sodium alginate-DPC beads and (b) PVA-sodium alginate-DPC-Cr(VI) beads.

Determination of the optimum condition of beads sensing

PVA-sodium alginate beads were used as a supporting matrix for DPC because both PVA and sodium alginate have functional groups that could interact with DPC through hydrogen bonding. PVA-sodium alginate beads are also hydrophilic material with macroporous structure so the Cr(VI) could easily diffuse into the beads pore, and the complex formed between DPC and Cr(VI) could be retained in the beads pore well. In the presence of Cr(VI), the color of the beads sensor changed to purplish-pink, which proved that the reaction of Cr(VI)-DPC occurred in a solid phase. In this study, the determination of the optimum condition of beads sensing was carried out, including the determination of maximum wavelength, determination of optimum DPC concentration, the effect of pH at the sensing process, and response time of beads sensor.

Determination of maximum wavelength

The maximum wavelength is characteristic of each compound. Measurement made of a compound at its maximum wavelength will ensure the highest sensitivity and minimize deviation from Beer's Law. Therefore, the absorbance of Cr(VI)-DPC complex in the beads was measured spectrophotometrically to find the maximum wavelength, and the absorption spectra are shown in Figure 3. From figure 3, it can be seen that the maximum absorbance was obtained at a wavelength of 547 nm. It is slightly shifted from the aqueous phase, which is 543 nm. This difference might be because of the different phases measured, the measured absorbance of the beads sensor was analyzed at a solid phase, while the conventional aqueous complex of Cr(VI)-DPC was carried out at a liquid or aqueous phase.

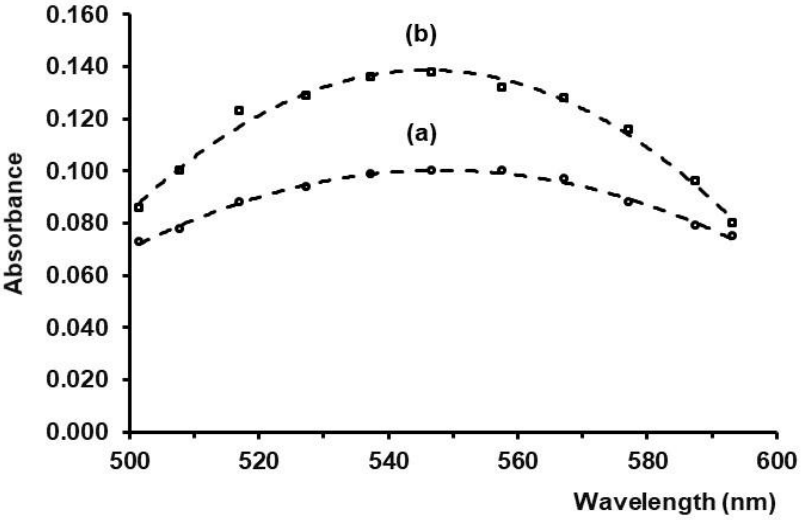

Figure 3: The absorbance spectra of Cr(VI)-DPC complex in the beads sensor at the concentration of Cr(VI) 6 (a) and 8(b) mg L^{-1}

Determination of optimum DPC concentration

Determination of optimum DPC concentration in beads was conducted with the variation of DPC concentration, i.e., 0; 2.27; 4.53; 6.77; 9.01 and 11.24 mg DPC g^{-1} beads. Figure 4 shows the optimum DPC concentration immobilized in the beads sensor. The maximum absorbance was obtained at the DPC concentration of 4.53 mg DPC g $beads^{-1}$. At a higher DPC concentration, the measured absorbance of the beads does not increase, because all the Cr(VI) in the aqueous solution had reacted with the DPC immobilized in the beads.

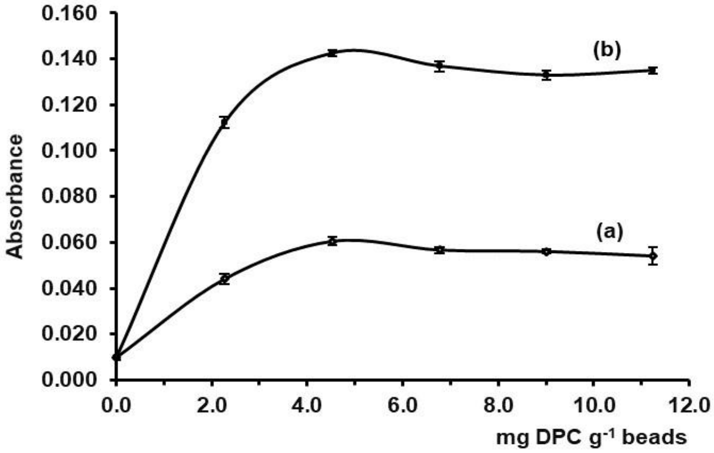

Figure 4: The absorbance spectra of the beads sensor at various concentrations of DPC on the concentration of Cr(VI) (a) 2 and (b) 8 mg L^{-1}.

The decrease of the measured absorbance after the optimum DPC concentration may be due to the complexation reaction between Cr(VI)-DPC occurred in the beads surface, so the Cr(VI)-DPC complex did not well entrap and easily leached to the aqueous solution. This was observed by the appearance of a purplish-pink color with very low intensity in a solution of Cr(VI) at higher DPC concentrations. The high concentration of DPC immobilized in beads allows DPC not only to be entrapped inside the beads pore but also attached to the surface of the beads. This can cause complex Cr(VI)-DPC do not only occur inside the pore of beads through the diffusion process but also to the surface of the beads. Complex Cr(VI)-DPC formed on the surface of beads are relatively more easily leached because they are not well entrapped in the beads.

The effect of pH at the sensing process

pH condition is essential to the reaction of the formation of a complex between Cr(VI) and DPC. In addition, since the existence of Cr(VI) species is pH-dependent, the effect of pH on the sensing process is important to determine. The effect of pH on the response beads sensing was examined at the pH range of 1-5 (with the addition of 0.5 M of sulfuric acid) by measuring the absorbance of the beads after sensing in a solution of Cr(VI) at the concentration 2 and 8 mg L^{-1}. The results of the pH effect are shown in Figure 5.

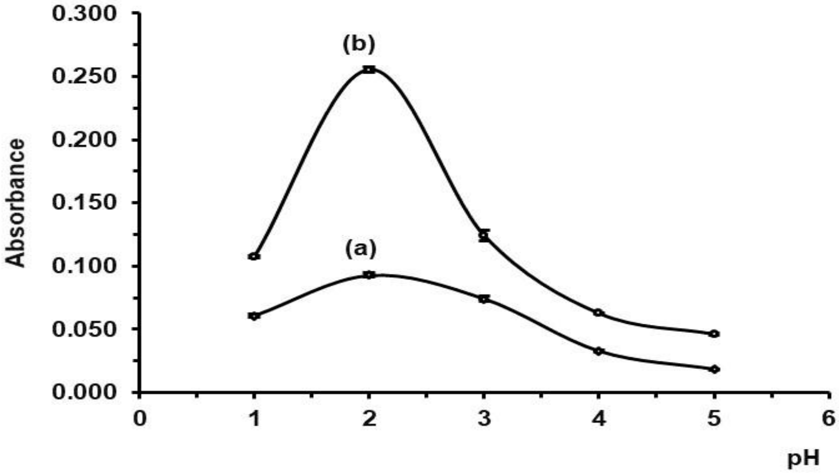

Figure 5: Absorbance spectra of beads sensor at different pH conditions on the concentration of Cr(VI) 2 (a); 8 (b) mg L^{-1}

Figure 5 shows that the maximum absorbance of the beads sensor was obtained at pH 2. The optimum pH sensing conditions are related to the equilibrium of Cr(VI) species present in solution. Hexavalent chromium exists primarily as 7 forms depending upon the pH of the solution: chromate CrO_4^{2-}, dichromate $Cr_2O_7^{2-}$, hydrogen chromate $HCrO_4^-$, dihydrogen chromate H_2CrO_4, hydrogen dichromate $HCr_2O_7^-$, tetrachromate $Cr_4O_{13}^{2-}$, trichromate $Cr_3O_{10}^{2-}$. The last three ions do not exist in solutions of pH higher than 0 or at chromium concentrations lower than 1 mol L^{-1}. According to pH and concentration ranges, three ions and one molecule have to be considered: CrO_4^{2-}, $Cr_2O_7^{2-}$, $HCrO_4^-$ and H_2CrO_4 [17,18].

At basic pH (9, 10 and 13.3) only chromate ions exist in the solution [17]. At neutral pH, above pH 6, the principal species in the solution are CrO_4^{2-} (75%) and $HCrO_4^-$ (25%) [17, 18, 19]. At acid pH (4.2, 4.6, 6) the principal species are CrO_4^{2-} (0.5% to 23%), $HCrO_4^-$ (95% to 25%) and $Cr_2O_7^{2-}$ (5% to 1%) [17, 18]. At pH equal to 2, $HCrO_4^-$ (90%), $Cr_2O_7^{2-}$ (5%) and H_2CrO_4 (5%) are present [17], while at pH ~1 (pH < 0.7), H_2CrO_4 predominates [20]. The complex of Cr(VI)-DPC, as shown in Figure 1 [8], is formed between DPC and Cr(VI) in the form of $HCrO_4^-$. In pH 2, the dominant species of Cr(VI) is $HCrO_4^-$, which is up to 90% [17], so that the increase in $HCrO_4^-$ species could increase the Cr(III) and DPCO through the reduction-oxidation between Cr(VI) and DPC, leading to the increase in purplish-pink complex of Cr(VI)-DPC. Therefore, the measured absorbance at pH 2 is the highest compared to the other pH condition.

Response time of beads sensor

Figure 6 shows the response time of the beads sensor on the different concentrations of Cr(VI) aqueous solution. Based on Figure 6, it appears that in the early minutes, there is an increase in the absorbance of the beads. This is possible because the formation of Cr(VI)-DPC complex still continues so that the absorbance of the beads still increases and stays constant after 8 minutes. The different phenomena observed in a solution of 8 mg L^{-1} Cr(VI). The absorbance of the beads increased, then steady at 4-8 minutes, and then decreased continuously after 8 minutes. This is due to the DPCO oxidize to the higher oxidation product, diphenylcarbadiazone (DPCDO), by the excess of Cr(VI) in the solution, as shown in figure 7 [8]. The resulted DPCDO does not react both with Cr(III) and Cr(VI) [21]. Consequently, the absorbance of the beads decreased. Based on the result of the absorbance measurement, the sensing process was carried out for 8 min to avoid a decrease in the absorbance of the beads.

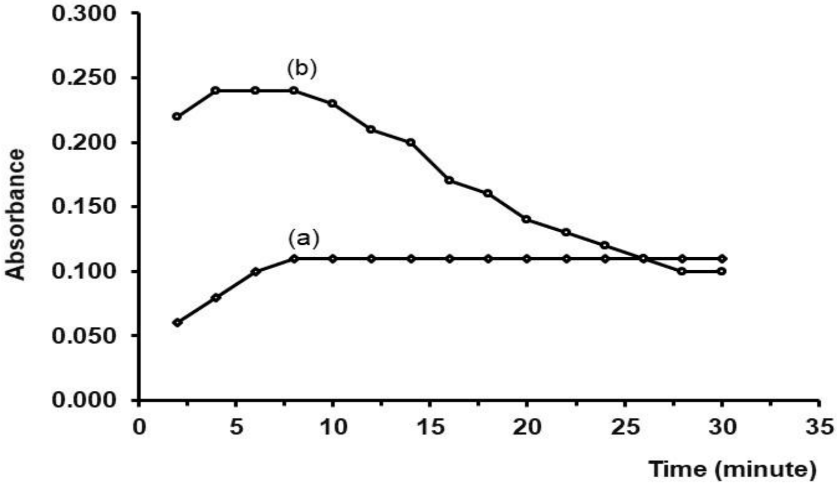

Figure 6: The response time of beads sensor at different concentration of Cr(VI) aqueous solution 2 (a) and 8 mg L^{-1} (b).

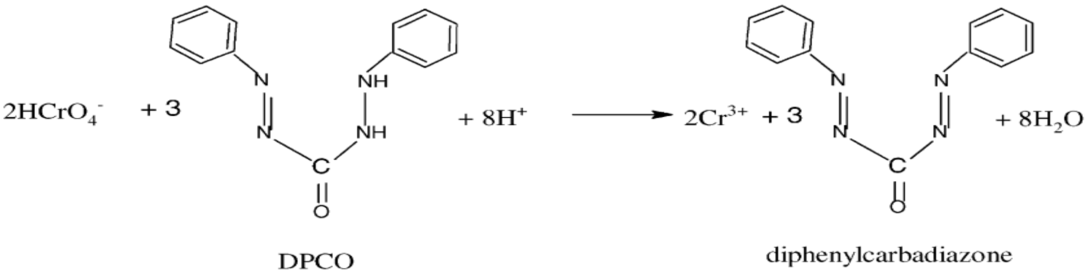

Figure 7: The oxidation of DPCO to diphenylcarbadiazone (DPCDO) in the Cr(III)–DPCO complex by the excess of Cr(VI) [8].

Interference study

The response of beads sensor to the co-existing ion was examined at an optimum condition for Cr(VI) by immersing the beads sensor in a solution of Cr(VI) mixed with the investigated ion at a concentration ratio 1:1, 1:2, and 1:4 to the concentration of the investigated ion. The absorbance of the beads sensor was then measured at 547 nm. The concentration of Cr(VI) was 5 mg L^{-1}. The results of the interference study were summarized in Table 1. The concentration of the different ion considered as interference ion if the measured absorbance of the beads sensor changes more than ± 5% [22]. Hg(II) with a concentration of more than 20 mg L^{-1} considered as the interference ion. Hg(II) could react with DPC through the same mechanism with Cr(VI). Hg(II) is an oxidizing ion so that the DPC could be oxidized [23]. Subsequently, Hg(I), which is resulting from the redox reaction, will interact with DPCO forming a purple complex

[23] so that the absorbance measured increased. Therefore, removal of the interference ion, especially Hg(II), should be considered before the sensing process with the beads sensor.

Table 1: Determination of interference ion

Ion	Concentration (mg L^{-1})	% Error	Ion	Concentration (mg L^{-1})	% Error
Cu(II)	5	+0.543	Mn(II)	5	–0.543
	10	+0.543		10	–1.087
	20	+2.717		20	–2.174
Co(II)	5	–2.174	Zn(II)	5	–0.543
	10	–2.174		10	–1.087
	20	–1.087		20	–1.630
Pb(II)	5	–2.717	Ni(II)	5	–3.261
	10	–4.348		10	–3.261
	20	–4.348		20	–3.804
Cr(III)	5	+1.087	Hg(II)	5	+1.630
	10	+1.630		10	+2.717
	20	+2.174		20	+5.435
Fe(III)	5	–1.630			
	10	–2.174			
	20	–4.348			

Calibration

The linearity of the beads sensor was determined by immersing the beads sensor in various concentrations of Cr(VI) aqueous solution. The color of the beads sensor changed after immersing in various concentrations of Cr(VI) aqueous solution from yellowish-colorless to purplish-pink as shown in figure 8.

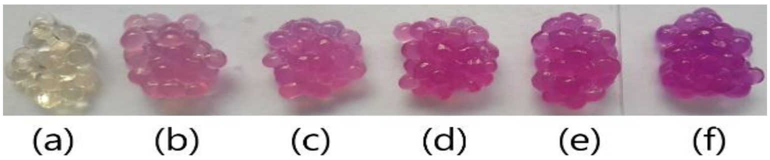

Figure 8: The color change of the beads sensor after immersing in various concentrations of Cr(VI) aqueous solution. 0 mg L^{-1} (a), 2 mg L^{-1} (b), 4 mg L^{-1} (c), 5 mg L^{-1} (d), 6 mg L^{-1} (e), and 8 mg L^{-1} (f).

The absorbance of the beads sensor after immersing in various concentrations of Cr(VI) was then measured spectrophotometrically. The calibration curve was obtained from the measured absorbance, and a linear relationship (figure 9) between the absorbance of the beads and concentration of Cr(VI) solution was also obtained in a concentration range 2-8 mg L^{-1} at a solution volume of 25 mL. The linear equation is $y = 0.0279 + 0.0202$, with R^2 value of 0.999. The linearity obtained in this study is relatively greater when compared to the linearity obtained in previous works which had an R^2 value of 0.9971 [9], and 0.9872, 0.9978 [24]. Limit of detection and limit of quantification of this developed method was measured based on the standard deviation of the beads blank signal, which was 0.11 and 0.34 mg L^{-1}, respectively. A lot of methods to determine Cr(VI) have been developed, one of which is the development of cellulosic paper-based test strips [9], microfluidic detection systems [24], and the utilization of nanoparticles [25]. The cellulosic paper-based test strips had a linear relationship in the range of 0.20-21.0 mg L^{-1} [9], while the microfluidic detection system obtained good linearity in the range between 0.03–3.00 mg L^{-1}, with the detection limit and quantitation limit, were found to be 0.023 and 0.076 mg L^{-1}, respectively [24]. Visual detection of Cr(VI) ions using silver-coated gold nanorods

(AuNR@Ag) as sensing probes had good linearity in the range of 10-60 µM (~0.52-3.12 mg L^{-1}) and the detection limit was measured to be 2 µM (~0.104 mg L^{-1}) [25]. Compared to those works and its simplicity, this bead has the potential to be developed as a colorimetric Cr(VI) sensor.

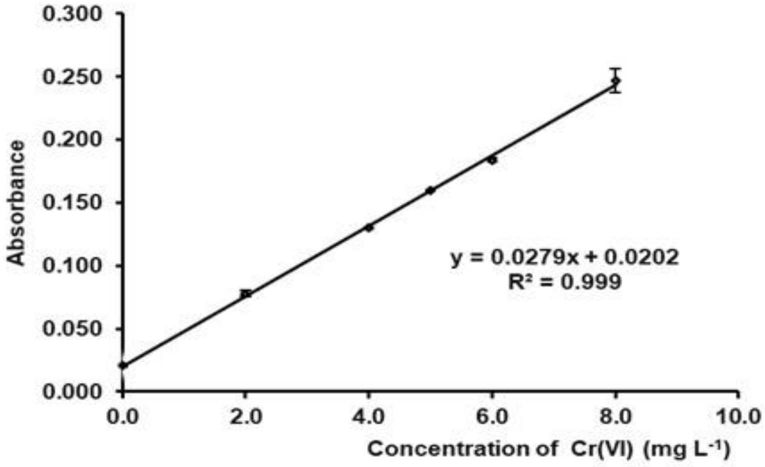

Figure 9: Calibration curve of the beads sensor.

Precision, accuracy and lifetime of the beads sensor

The precision of the beads sensor was evaluated by measuring the absorbance of 9 beads at a maximum wavelength of 547 nm. The precision of the beads sensor was examined with 1 series concentration of Cr(VI) aqueous solution. The calculated RSD of the bead sensor was less than 5%. The RSD value obtained in this study is similar to the RSD value obtained in the research development of strip paper for the detection of Cr (VI), which ranges from 2-4% [24]. The accuracy of the beads sensor was determined with the spiking method. Accuracy was expressed by % recovery. From the absorbance measured and calculated data, the % recovery value was obtained between 84-119%. The results showed that the beads sensor has good precision and accuracy. The lifetime of the beads sensor was also investigated by measuring the absorbance of the beads at a storage period of 10, 20, and 30 d. The result shows that the beads sensor has excellent stability for 30 d of storage period with the RSD value is less than 5%.

Sensing of Cr(VI) in a real wastewater sample

The beads sensor that has been qualified for the validation values was applied to detect the presence of Cr(VI) in the wastewater samples. The results are shown in Table 2. The concentration of Cr(VI) in the wastewater samples was measured by the beads sensor and compared with the reference method. The results proved that the beads sensor agreed with the reference method, with the accuracy value between 80-96%. In addition, the accuracy value obtained in this research was not much different from the accuracy value in the previous study, which ranged between 96-99% [9].

Table 2: Determination of Cr(VI) concentration in the wastewater samples.

Samples	Beads sensor method			Reference DPC method			% Error
	A ± SD	RSD (%)	[Cr(VI)] mg L^{-1}	A ± SD	RSD (%)	[Cr(VI)] mg L^{-1}	
1	0.0244±0.0006	1.374	0.149	0.108±0.001	0.998	0.156	–4.626
2	0.0258±0.0002	0.497	0.201	0.116±0.001	0.754	0.168	19.762
3	0.0237±0.0024	5.361	0.127	0.092±0.0003	0.310	0.132	–4.141

Conclusion

The development of the beads sensor for the detection of Cr(VI) has been conducted by immobilizing DPC into PVA-sodium alginate beads. The beads sensor exhibited good precision, accuracy, and stability over 30 d of storage period. The optimum response of the beads sensor was obtained at pH 2 for 8 min sensing. Based on the results, it is proved that the beads sensor could be used as an alternative method for Cr(VI) detection in aqueous samples.

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