Synthesis of 1,5-diphenylcarbazide-immobilized alginate/pectin films for colorimetric detection of Cr(VI)
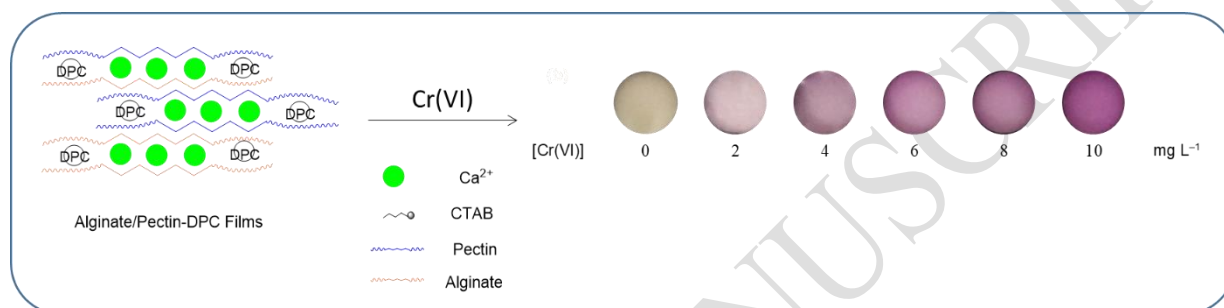
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Graphical Abstract

ABSTRACT

An optical sensor for colorimetric detection of Cr(VI) was synthesized by immobilizing 1, 5-diphenylcarbazide (DPC) into alginate/pectin films. The effectiveness of DPC immobilization was increased through the addition of cation surfactant (CTAB) to form hydrophobic-ion pairs. At optimum condition, the films have good selectivity for Cr(VI) compare to other interference metals. The formation of violet color in the films showed its potential application for visual colorimetric detection of Cr(VI). Furthermore, the films developed were shown to be a simple, fast, and sensitive sensors for the direct determination of Cr(VI) with a detection limit as low as 0.233 mg L^{-1} and time response of 8 minutes.

Keywords: 1,5-diphenylcarbazide, alginate/pectin films, colorimetric detection of Cr(VI)

1. Introduction

Hexavalent chromium or Cr(VI) is a toxic heavy metal with non-biodegradable properties (Deep, Sharma, Tuteja, & Paul, 2014). It is produced by industrial activity, such as catalytic converters for automobiles, ore refining, cement-producing plants, and leather tanneries (Bayramoğlu & Yakup Arica, 2008). In addition, it is soluble, carcinogenic, and mutagenic (Kim & Om, 2013), and due to the toxicity, the US Environmental Protection Agency recommends that the concentration in drinking water should be less than 0.1 mg L^{-1} (US Environmental Protection Agent, 2010).

The amount of Cr(VI) in environmental samples was determined using some analytical methods, such as UV-Visible spectrophotometry (Ndung'u, Djane, Malcus, & Mathiasson, 1999) and

atomic absorption spectrometry (Güell, Fontàs, Salvadó, & Anticó, 2007). However, these methods are affected by several limitations, such as complicated protocols, time and cost consuming, requiring specific method and skill, and yielding information on total Cr concentration only. Therefore, many studies give interest in developing a rapid detection method for Cr(VI) in wastewater. Also, the colorimetric method was considered as a trustworthy assay and has been applied to various samples. It offers some advantages such as easy readability, rapid, cost-effective, and no requirement of any sophisticated instrumentation (Zevin, Reisfeld, Oehme, & Wolfbeis, 1997).

The DPC reagent was successfully immobilized into the silica matrix using the sol-gel technique for the detection of chromate ions (CrO_4^{2-}). Meanwhile, cetyltrimethylammonium Bromide (CTAB) as quaternary ammonium salt was used to extract CrO_4^{2-} from solutions and prevent other cation to diffuse into the membrane. This method has good sensitivity since the limit of detection (LOD) was 1 ppb (Zevin *et al.*, 1997). Optical sensors for Cr(VI) analysis was developed using cellulose triacetate (CTA) matrix immobilized DPC as chromogenic reagent (Scindia, Pandey, Reddy, & Manohar, 2004). In addition, the membrane was modified with anion exchanger (aliquat-336) to increase the effectiveness of DPC immobilization and stability of Cr(VI)-DPC complex. However, these methods take a long time response to form a stable complex, and a simple optical sensor was successfully developed using a cellulose-based paper strip as an immobilization matrix of DPC reagent. Cellulose paper is a biopolymer with good physical properties, and it shortens the response time due to its high porosity. This optical sensor provides high selectivity from other anionic species such as SO_4^{2-}, Cl^-, NO_3^-, Br^-, I^- (Kong & Ni, 2009).

Alginate is a hydrophilic polysaccharide composed of β-D-mannuronic (M) and α-L-guluronate acid (G) copolymers (Sriamornsak & Kennedy, 2008). In contrast, pectin has a linear polymer structure of D-galacturonic acid and it is connected by α-1.4-glycosidic bonds (Bierhalz, Da Silva, De Sousa, Braga, & Kieckbusch, 2013). The gelation process between alginate and pectin originates from the specific interactions between Ca^{2+} ions and galacturonic residue from pectin and guluronic from alginates [12-13]. Their combination provides unique properties that are strong and resistant to water, making them widely used in several fields as a carrier matrix [12,14].

In this study, a new film sensor for Cr(VI) determination was developed by immobilizing DPC as a chromogenic reagent into alginate/pectin films. The use of a hydrophilic biopolymer to accelerate the diffusion of Cr(VI) into a matrix resulting in a rapid detection method. Furthermore, the cationic surfactants (CTAB) were used to retain and stabilize the complex in the membranes. This optical membrane provides a rapid, sensitive, and simple alternative method to determine Cr(VI) in an aqueous sample.

2. Material and Methods

2.1. Materials and instrumentation

Alginate and pectin were obtained from Sigma Aldrich, while ethanol, Cetyltrimethylammonium Bromide (CTAB), and 1, 5-diphenylcarbazide (DPC) were purchased from Merck. Furthermore, the stock solution (1000 mg L^{-1}) of chromium was prepared from the $K_2Cr_2O_7$ solid (Merck), and the standard solution of Cu^{2+}, Zn^{2+}, Co^{2+}, Fe^{3+}, Cr^{3+}, Ni^{2+} (Merck) were used for the selectivity study and prepared using distilled water from CV Progo Mulyo. All analytical grade chemicals were used without further purification.

Determination of the film's absorbance and characterization was conducted using UV-Vis (Simadzu UV-1800) and Fourier Transform-Infrared Spectrophotometer (Shimadzu IR Prestige) while Scanning Electron Microscope (SEM, JSM-6510LA) was used to analyze the morphology of surface membranes. In addition, Metler Toledo SevenCompact was used to determine the pH value.

2.2. Synthesis of alginate/pectin films immobilized DPC

Alginate (0.05) g and pectin (0.20 g) were dissolved in 10 mL of distilled water then stirred until homogeneous. The solutions were poured into a petri dish (d = 60 mm) and dried at 55 °C for 15 hours. Furthermore, the dried films were then cross-linked with a 2% $CaCl_2$ solution for 24 hours. After the cross-link process, they were rinsed by distilled water and immobilized with DPC. The immobilization process was conducted by immersing the alginate/pectin films in an ethanol solution containing 0.3% DPC and 1% CTAB for 24 hours. Thereafter, the films were air-dried and sealed in a plastic bag for further testing.

2.3. Colorimetric detection of Cr(VI) with alginate/pectin-DPC films

Colorimetric detection of Cr(VI) using alginate/pectin-DPC films was conducted by immersing the films in dichromate solution (3 mL) for 8 minutes. Various concentration of dichromate solution (2-10 mg L^{-1}) was adjusted at pH = 5 using 0.1M HCl and 0.1M NaOH solutions. In addition, the absorbance of the complex was measured at 545 nm using a UV-Visible spectrophotometer. The selectivity of alginate/pectin-DPC films was also investigated by performing the absorbance of the films into interference metal solutions (Cu^{2+}, Zn^{2+}, Co^{2+}, Fe^{3+}, Cr^{3+}, Ni^{2+}). The effect of interference metal was further studied by measuring the absorbance of the films in a mixture solution containing Cr(VI) and other metals in the same ratio.

3. Result And Discussion

3.1. IR Spectroscopy

Determination of Cr(VI) using the colorimetric method is based on the formation of a colored complex between Cr(VI) and DPC as a chromogenic reagent. The formation involved two steps: (i) redox reaction between Cr(VI) and DPC to form Cr(III) and 1,5-diphenylcarbazone (DPCO); (ii) formation of Cr(III)-DPCO complex which is characterized by the appearance of violet color (Pflaum & Howick, 1954)(Willems, Blaton, Peeters, & Ranter, 1977). The developed film sensor was

synthesized by immobilizing DPC into the alginate/pectin matrix in this study. Figure 1 shows the FTIR spectra of alginate/pectin-DPC.
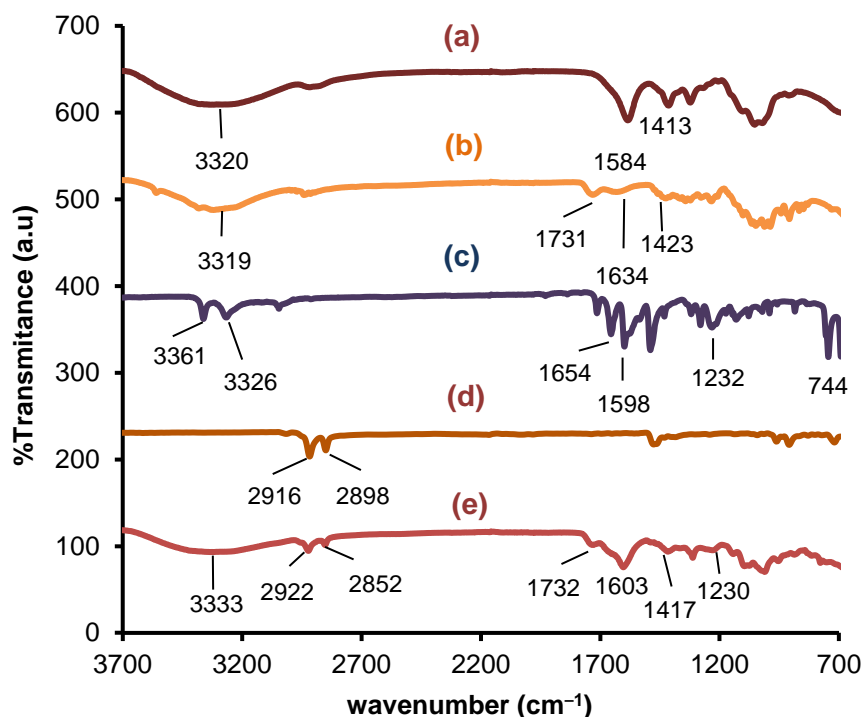

Figure 1 FTIR spectra of (a) sodium alginate, (b) pectin, (c) DPC, (d) CTAB, and (e) alginate/pectin-DPC films.

The spectrum of sodium alginate (**Figure 1a**) has an absorption band at 3320, 1584, and 1413 cm^{-1} which were attributed to stretching vibration of –OH, asymmetric, and symmetrical stretching vibration of –COO$^-$, respectively (L. Hua, Chan, Wu, & Wu, 2009). Conversely, pectin has a typical absorption band of polysaccharides as possessed by alginate at 3319, 1634, 1423 cm^{-1} as shown in Fig 1b. The band at 1731 cm^{-1} originates from the methyl ester group (–COOCH$_3$) and non-dissociated carboxylic acid (–COOH) which is consistent with the study of (Maciel, Yoshida, & Franco, 2015).

Figure 1e showed FTIR spectra after the immobilization process, and the absorption band at 1230 cm^{-1} was attributed to stretching vibrations of C–N from DPC. Furthermore, the shift of –COO$^-$ asymmetric and symmetric stretching vibrations to smaller wavenumbers indicates the crosslinking process between –COO$^-$ and Ca^{2+} to form "egg-box structure" in the films (Braccini & Pérez, 2001; S. Hua, Ma, Li, Yang, & Wang, 2010). The peak at 2922 and 2852 cm^{-1} is due to the stretching and bending vibrations of the methylene (–CH$_2$–) and methyl (–CH$_3$) groups of CTAB. The shifted peak from 3320 to 3333 cm^{-1} indicates an overlap between stretching vibrations of OH groups in alginate/pectin polymers with N–H from DPC. However, there is no shifting at 1731 cm^{-1} since the methyl ester group is not involved in interactions with Ca^{2+} (Nešić *et al.*, 2017).

According to the FTIR spectra of alginate/pectin-DPC films, the proposed interactions of the DPC immobilization process were described in **Figure 2**. Before this process, the interactions between the hydrophobic groups of CTAB and DPC occurred in ethanol. The hydrophobic group of CTAB surrounded the DPC reagent to form hydrophobic-ion pair (Kong & Ni, 2009). Furthermore, there was an interaction between the positive and negative charges obtained from CTAB and the non-crosslink carboxyl groups of the alginate/pectin matrix. The DPC in the film was stabilized by the electrostatic force generated from their interaction.
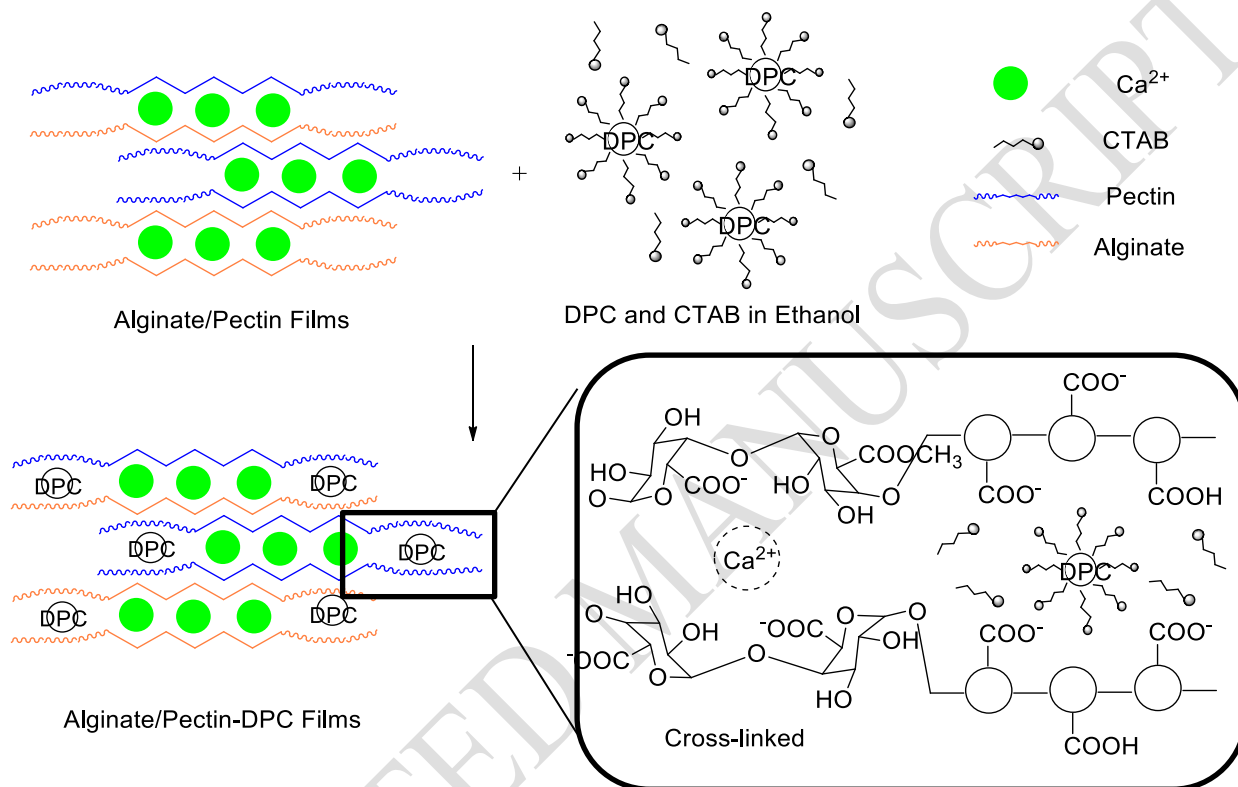

Figure 2 Proposed interaction between alginate/pectin matrix and DPC in the presence of CTAB

3.2. Morphology of alginate/pectin-DPC films

The analysis of films using SEM was used to characterize the morphology of alginate/pectin-DPC films before and after sensing with dichromate ion.
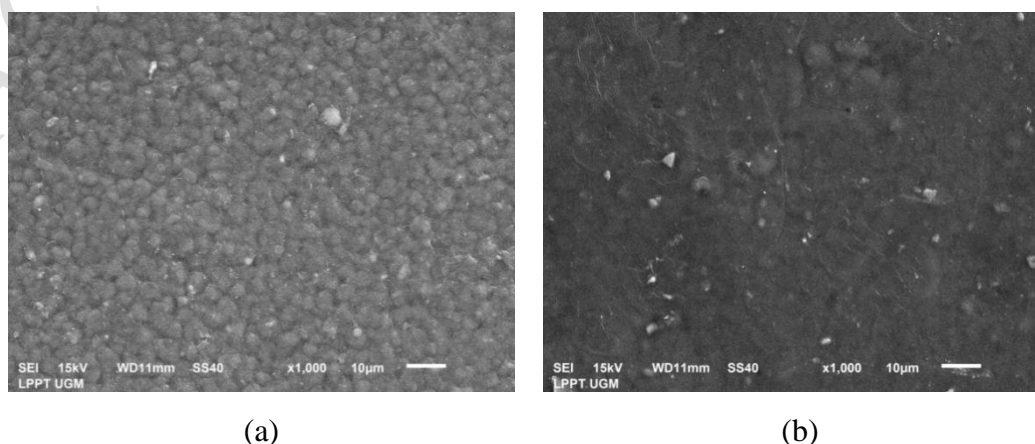

(a)   (b)

Figure 3 SEM image of alginate/pectin-DPC films (a) before and (b) after sensing with Cr(VI)

Figure 3a shows discontinuous and heterogeneous surfaces before sensing. Some holes were observed due to the crosslinking process that does not occur in all polymer chains and the sudden release of water during drying can make holes like pores (Aguilar *et al.*, 2015; Nešić *et al.*, 2017). Furthermore, the morphology of films is more continuous and homogenous (**Figure 3b**), while the formation of DPCO-Cr(III) complex after absorption of dichromate ion may cover the pores and result in a homogeneous appearance (Aguilar *et al.*, 2015).

3.3. Optimization of detection condition

The maximum absorption of the Cr(III)-DPCO complex was found at 545 nm, while the wavelength obtained was similar to previous study using sol gel-based membrane, CTA film, and cellulose paper which occurred at 540, 548, and 550 nm, respectively (Zevin *et al.*, 1997; Scindia *et al.*, 2004; Kong & Ni, 2009; Habibah, Hapsari, Suratman, & Siswanta, 2020).

The formation of Cr(III)-DPCO complex was at acidic pH (Ashley, Howe, & Nygren, 2003) shown in Figure **4**, and the optimum absorbance was obtained at pH 5. The low absorbance was at pH 4 due to leaching complex from the films into the solution. In a higher pH than 5, the decrease in absorbance was due to decreasing the amount of H^+ ions in the solution which affects the redox reaction between DPC and Cr(VI). The increase changed the dichromate ($Cr_2O_7^{2-}$) species to chromate (CrO_4^{2-}) and lowered reduction potential standard ($E^\circ\ CrO_4^{2-}/Cr^{3+} = 1.19$ V $< E^\circ\ Cr_2O_7^{2-}/Cr^{3+} = 1.33$ V).
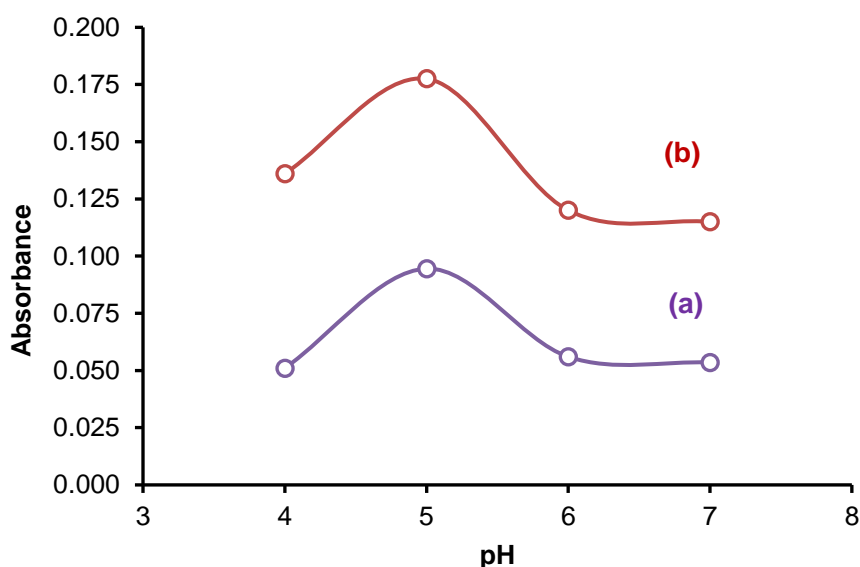

Figure 4 The effect of pH on the absorbance of alginate/pectin-DPC films at concentrations of Cr(VI) (a) 4 and (b) 8 mg L^{-1}

Determination of the optimum concentration of DPC was conducted to determine DPC concentration, which may be immobilized into the alginate/pectin matrix. Figure 5, shows that the maximum absorbance was obtained at the concentration of DPC 0.3%. The increasing absorbance from concentration 0.1-0.3% of DPC indicates that there are still negative charges remaining in the alginate/pectin matrix which did not interact with the positive charge of CTAB/DPC hydrophobic-ion pairs (Aguilar *et al.*, 2015). Furthermore, the absorbance has reached a constant value at a concentration of 0.3-0.5% due to the limitation of negative charge from the matrix that can be bound with DPC. The optimum concentration of DPC that can be immobilized in the alginate/pectin film is greater than in the cellulose paper matrix (Kong & Ni, 2009).
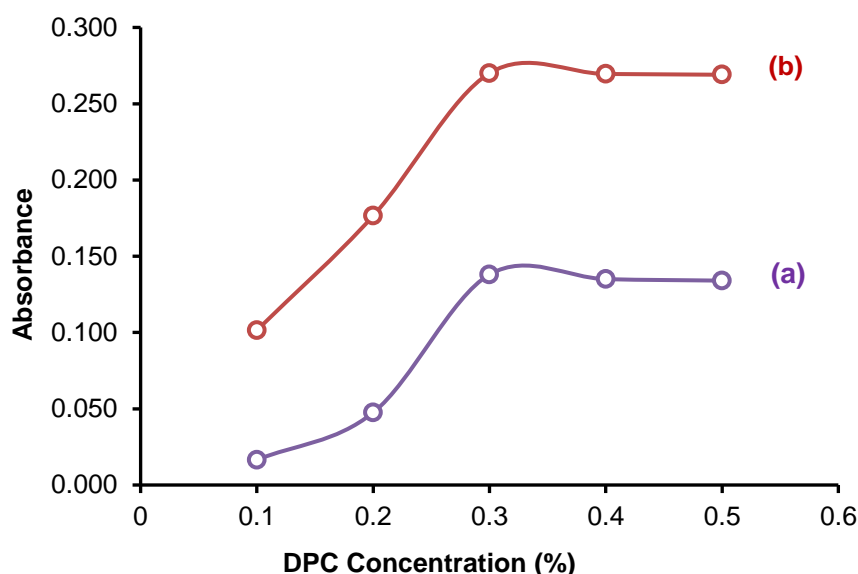

Figure 5 The effect of DPC concentration on the absorbance of alginate/pectin-DPC films at concentrations of Cr(VI) (a) (a) 4 and (b) 8 mg L^{-1}
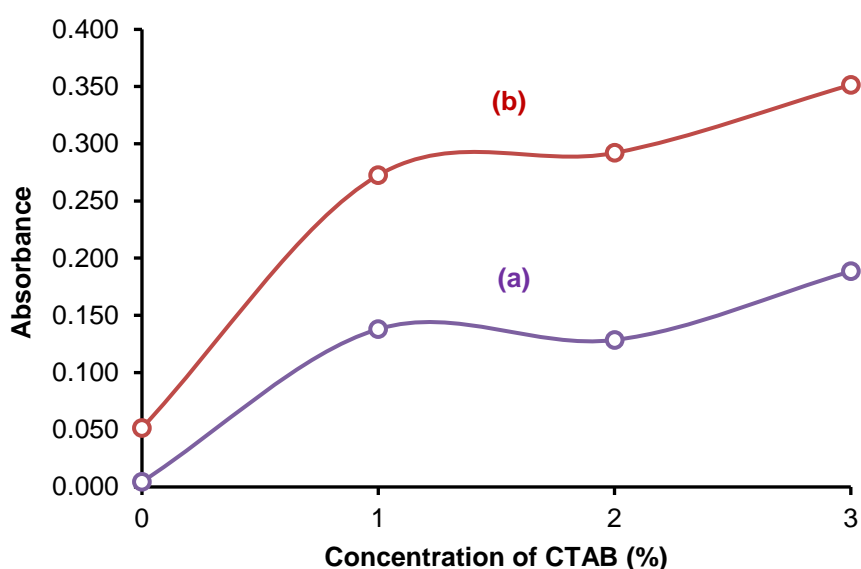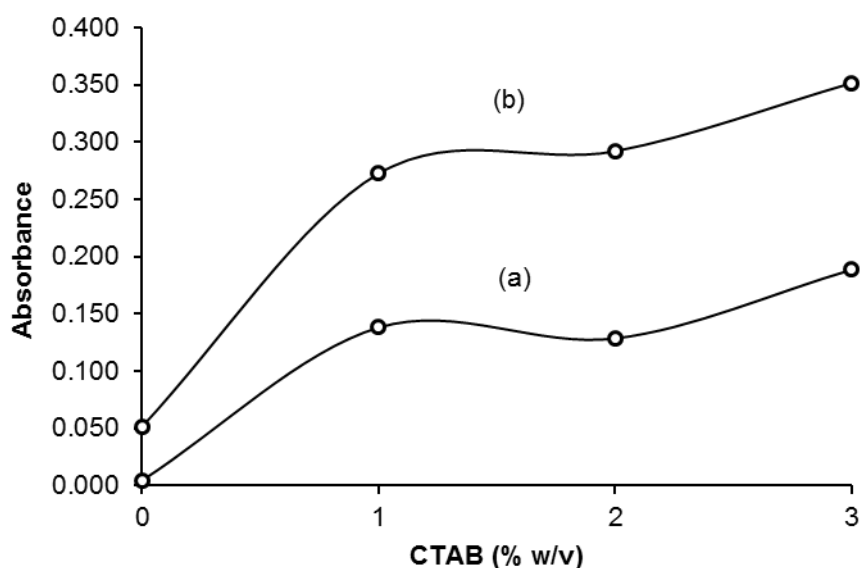
Figure 6 The effect of CTAB concentration on the absorbance of alginate/pectin-DPC films at concentrations of Cr(VI) (a) (a) 4 and (b) 8 mg L^{-1}

The effect of CTAB concentration in solution was performed in **Figure 6**. From the result, the low absorbance at 0% indicates the small number of complexes formed in the films, and under this condition, the DPC may only be trapped in the holes. However, there was a significant increase in absorbance when the concentration of CTAB was increased. It is due to the formation of DPC-CTAB hydrophobic ion-pairs that can interact with the $-COO^-$ group from alginate and pectin which does not contribute to the crosslink process but increase the amount of DPC immobilized in the matrix (Kong & Ni, 2009).

The effect of time was also investigated to determine the optimum response of the alginate/pectin-DPC films to form a complex by varying time between 2-20 minutes. The result shows

that the optimum time to give the maximum absorbance was 8 minutes, as shown in **Figure 7**. It is faster than the time required by DPC to form a stable complex in the CTA membrane which takes 60 minutes (Scindia *et al.*, 2004). Furthermore, Alginate/pectin was a hydrophilic matrix, while CTA was hydrophobic, and in polar solution, the hydrophilic is most suitable.
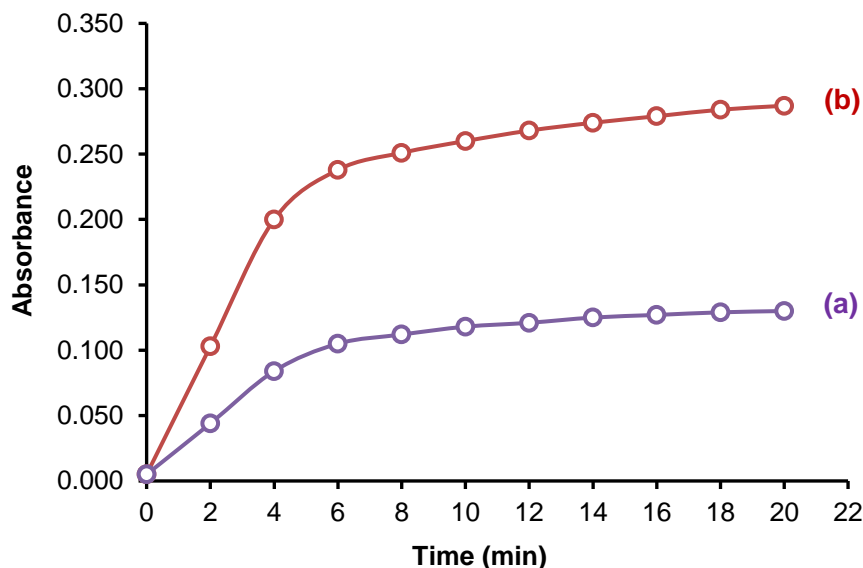

Figure 7. The response time profile of the sensor film to Cr(VI) solution at concentrations of (a) 4 and (b) 8 mg L^{-1}

3.4. Selectivity of alginate/pectin-DPC films against interferent metals

Some metal ions may form a color complex with DPC such as Cu(II), Cd(II), Hg(I) and (II), Fe(III) (Gohil, 2010; Bagheri *et al.*, 2012; Behbahani, Aliakbari, Amini, Behbahani, & Omidi, 2015). In optimum condition, it has good selectivity with Cr(VI) as shown in **Figure 8**. The alginate/pectin-DPC films have good selectivity based on the low absorbance of films in the interference ion metals solution. However, the addition of interferent metals at concentrations up to 10 mg L^{-1} did not influence the absorbance of films compared to the solution without the metals. This is due to the different mechanism from interferent metals to form colored complexes between DPC and Cr(VI).
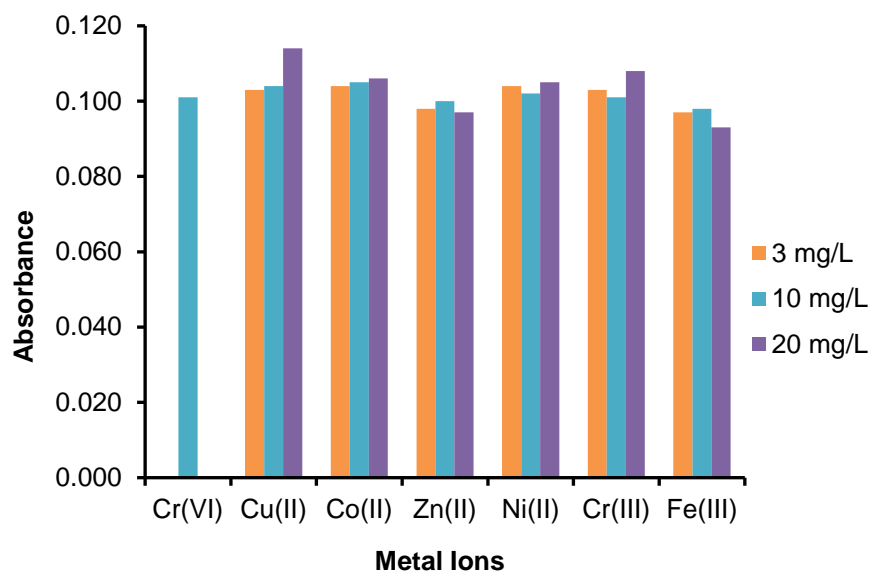
Figure 8 The colorimetric response of alginate/pectin-DPC film at optimum conditions to 3 mg L^{-1} Cr(VI) solution containing interferent metals at concentrations of 3, 10 and 20 mg L^{-1}

3.5. Calibration curve and sensitivity

The performance of alginate/pectin-DPC films were investigated by measuring the absorbance with various concentration of Cr(VI) in optimum condition. According to **Figure 9**, the films have good linearity in the concentration range of 0-10 mg L^{-1} ($R^2 = 0.9978$). Meanwhile, the lowest concentration of Cr(VI) that can be detected was 0.233 mg L^{-1}.
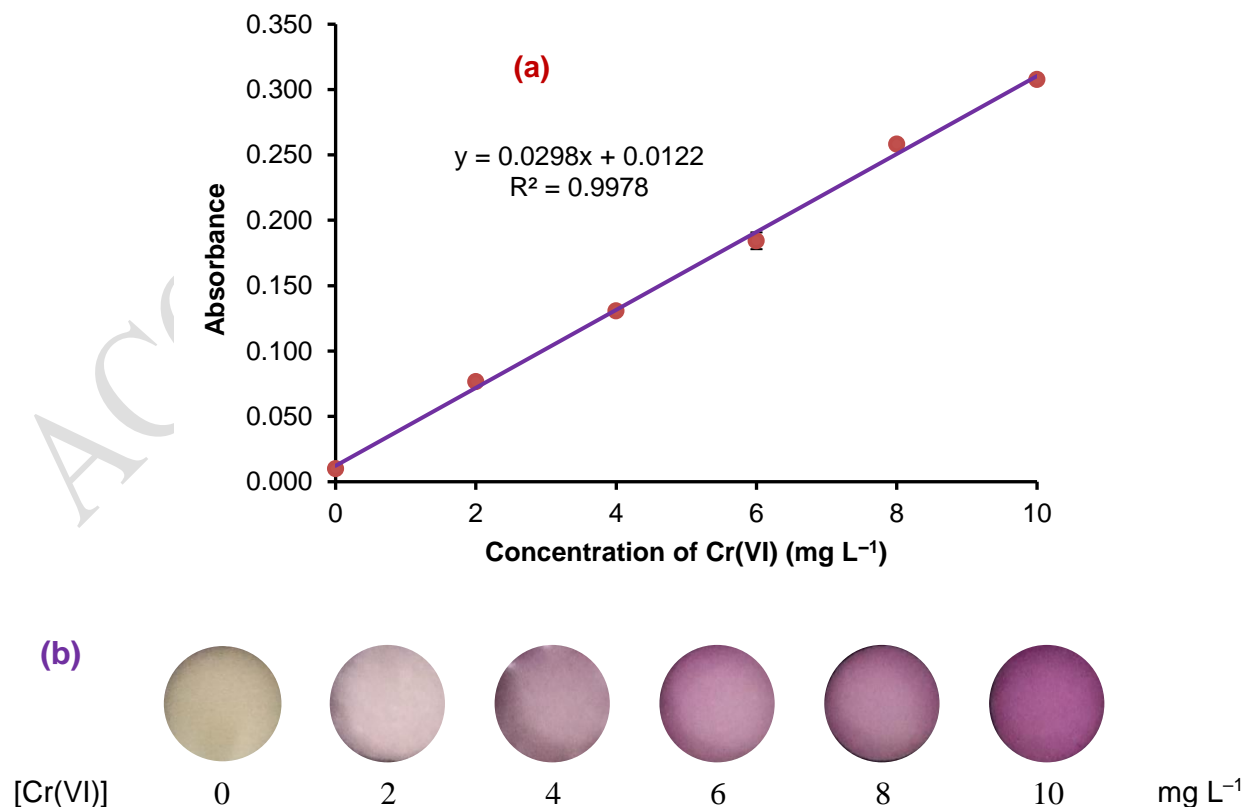

$$y = 0.0298x + 0.0122$$
$$R^2 = 0.9978$$

Figure 9 (a) Calibration curve of alginate/pectin-DPC films in Cr(VI) solution at various concentration (0, 2; 4; 6; 8; and 10 mg L^{-1}) and (a) the color response of alginate/pectin-DPC films

The precision of the film was evaluated by testing the repeatability of the developed sensor films. Furthermore, the colorimetric response was measured with a 3 repetition test in different batches. Based on the statistical method, the relative standard deviation (RSD) of the different batches was between 0.82–3.42%, and they show that the proposed film has good precision.

3.7. Detection of Cr(VI) in simulated electroplating wastewater

The applicability of the film sensor was performed by determining the Cr(VI) in simulated electroplating wastewater. Furthermore, two different samples of simulated electroplating wastewater before (sample A) and after (sample B) treatment process were spiked with 3, 5, and 8 mg L^{-1} Cr(VI) then measured with UV-Vis Spectrophotometric in maximum wavenumber. Table 1 showed that good recoveries between 91.36-106.59% were obtained for Cr(VI) from samples, therefore, the developed sensor has a good accuracy.

Table 1 Determination of Cr(VI) in simulated electroplating wastewater using alginate/pectin-DPC film in optimum condition

Sample	Cr(VI) added (mg L^{-1})	Cr(VI) (mg L^{-1})	% Recovery
A	0	1.092	-
	3	4.171	102.62
	5	5.743	93.02
	8	9.619	106.59
B	0	0.427	-
	3	3.285	95.24
	5	5.854	108.53
	8	7.736	91.36

4. Conclusion

Optical sensors for Cr(VI) ions have been successfully developed by immobilizing DPC into alginate/pectin films. Immobilization was conducted in the presence of cationic surfactants CTAB which can form hydrophobic interaction with DPC and electrostatic interaction with polysaccharide membranes. Subsequently, the optimum condition was obtained at pH 5 after 8 minutes sensing, and the limit of detection was observed at 0.233 mg L^{-1}. The optical sensors have a good accuracy and

provide a simple, fast, and selective method for Cr(VI) detection in the aqueous solution for daily analysis.

References

Aguilar, K. C., Tello, F., Bierhalz, A. C. K., Garnica Romo, M. G., Martínez Flores, H. E., & Grosso, C. R. F. (2015) Protein Adsorption onto Alginate-Pectin Microparticles and Films Produced by Ionic Gelation, *Journal of Food Engineering*, **154**(March 2018), 17–24.

Ashley, K., Howe, A. M., & Nygren, O. (2003) Sampling and Analysis Considerations for The Determination of Hexavalent Chromium in Workplace Air, 707–716.

Bagheri, A., Behbahani, M., Amini, M. M., Sadeghi, O., Taghizade, M., Baghayi, L., & Salarian, M. (2012) Simultaneous Separation and Determination of Trace Amounts of Cd(II) and Cu(II) in Environmental Samples Using Novel Diphenylcarbazide Modified Nanoporous Silica, *Talanta*, **89**, 455–461.

Bayramoğlu, G., & Yakup Arica, M. (2008) Adsorption of Cr(VI) onto PEI Immobilized Acrylate-based Magnetic Beads: Isotherms, Kinetics and Thermodynamics Study, *Chemical Engineering Journal*, **139**(1), 20–28.

Behbahani, M., Aliakbari, A., Amini, M. M., Behbahani, A. S., & Omidi, F. (2015) Synthesis and Characterization of Diphenylcarbazide-Siliceous Mesocellular Foam and Its Application as A Novel Mesoporous Sorbent for Preconcentration and Trace Detection of Copper and Cadmium Ions, *RSC Advances*, **5**(84), 68500–68509.

Bierhalz, A. C. K., Da Silva, M. A., De Sousa, H. C., Braga, M. E. M., & Kieckbusch, T. G. (2013) Influence of Natamycin Loading Methods on The Physical Characteristics of Alginate Active Films, *Journal of Supercritical Fluids*, **76**, 74–82.

Braccini, I., & Pérez, S. (2001) Molecular Basis of Ca2+-Induced Gelation in Alginates and Pectins: The egg-box Model Revisited, *Biomacromolecules*, **2**(4), 1089–1096.

Deep, A., Sharma, A. L., Tuteja, S. K., & Paul, A. K. (2014) Phosphinic acid functionalized carbon nanotubes for sensitive and selective sensing of chromium(VI), *Journal of Hazardous Materials*, **278**, 559–565.

Fang, Y., Al-Assaf, S., Phillips, G. O., Nishinari, K., Funami, T., & Williams, P. A. (2008) Binding Behavior of Calcium to Polyuronates: Comparison of Pectin with Alginate, *Carbohydrate Polymers*, **72**(2), 334–341.

Gohil, R. . (2010) Synergestic Blends of Natural Polymers, Pectin and Sodium Alginate, *Journal of Applied Polymer Science*, **4**(120), 2324–2336.

Güell, R., Fontàs, C., Salvadó, V., & Anticó, E. (2007) Development of A Selective Optical Sensor for Cr(VI) Monitoring in Polluted Waters, *Analytica Chimica Acta*, **594**(2), 162–168.

Habibah, N., Hapsari, A. R., Suratman, A., & Siswanta, D. (2020) Development of Polyvinyl Alcohol-Sodium Alginate Beads as Immobilization Matrices of 1, 5--Diphenylcarbazide for the Detection of Cr (VI), *Journal of Materials and Environmental Sciences*, **11**(2), 176–186.

Hua, L., Chan, Y. C., Wu, Y. P., & Wu, B. Y. (2009) The Determination of Hexavalent Chromium (Cr6+) in Electronic and Eelectrical Components and Products to Comply with RoHS Regulations, *Journal of Hazardous Materials*, **163**(2–3), 1360–1368.

Hua, S., Ma, H., Li, X., Yang, H., & Wang, A. (2010) pH-Sensitive Sodium Alginate/Poly(vinyl alcohol) Hydrogel Beads Prepared by Combined Ca2+ Crosslinking and Freeze-thawing Cycles for Controlled Release of Diclofenac Sodium, *International Journal of Biological Macromolecules*, **46**(5), 517–523.

Kim, D., & Om, J. (2013) Direct Spectroscopic Determination of Aqueous Phase Hexavalent Chromium, *Univ. J. Eng. Sci.*, **1**(1), 1–4.

Kong, F., & Ni, Y. (2009) Development of cellulosic paper-based test strips for Cr(VI) determination, *BioResources*, **4**(3), 1088–1097.

Maciel, V. B. V., Yoshida, C. M. P., & Franco, T. T. (2015) Chitosan/pectin polyelectrolyte complex as a pH indicator, *Carbohydrate Polymers*, **132**, 537–545.

Ndung'u, K., Djane, N. K., Malcus, F., & Mathiasson, L. (1999) Ultrasonic Extraction of Hexavalent Chromium in Solid Samples Followed by Automated Analysis Using A Combination of Supported Liquid Membrane Extraction and UV Detection in A Flow System, *Analyst*, **124**(9), 1367–1372.

Nešić, A., Onjia, A., Davidović, S., Dimitrijević, S., Errico, M. E., Santagata, G., & Malinconico, M. (2017) Design of Pectin-Sodium Alginate Based Films for Potential Healthcare Application: Study of Chemico-Physical Interactions Between The Components of Films and Assessment of Their Antimicrobial Activity, *Carbohydrate Polymers*, **157**, 981–990.

Pflaum, R. T., & Howick, L. C. (1954) The Chromium-Diphenylcarbazide Reaction1, *J. Am. Chem. Soc*, **78**(9), 4862–4866.

Scindia, Y. M., Pandey, A. K., Reddy, A. V. R., & Manohar, S. B. (2004) Chemically selective membrane optode for Cr(VI) determination in aqueous samples, *Analytica Chimica Acta*, **515**(2), 311–321.

Sriamornsak, P., & Kennedy, R. A. (2008) Swelling and Diffusion Studies of Calcium Polysaccharide Gels Intended for Film Coating, *International Journal of Pharmaceutics*, **358**(1–2), 205–213.

US Environmental Protection Agent (2010) *Toxicological Review of Hexavalent Chromium (CAS No. 18540-29-9) Review Literature And Arts Of The Americas* (Washington D.C.).

Willems, G. J., Blaton, N. M., Peeters, O. M., & Ranter, C. J. (1977) The Interaction of Chromium(VI), Crhomium(III) and Chromium(II) with Diphenylcarbazide, Diphenylcarbazone

and Siphenylcarbadiazone, *Analytica Chimica Acta*, **88**, 345–352.

Zevin, M., Reisfeld, R., Oehme, I., & Wolfbeis, O. S. (1997) Sol-gel-derived optical coatings for determination of chromate, *Sensors and Actuators B: Chemical*, **39**(1–3), 235–238.