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Modification of polyvinyl chloride membranes for mycotoxins detection

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ABSTRACT

In this work, we investigated modification and characterizations of modified-Polyvinyl Chloride (PVC) membranes for mycotoxin detections. The modification of PVC is a successfully nucleophilic substitution of Chloride atoms on the PVC membranes. The chemical compositions and characteristics of modified-PVC were characterized by Raman Microscope, NMR, GPC, DSC and FE-SEM. Sulfo-N-hydroxysuccinimide (Sulfo-NHS) and Carbodiimide EDC were used as an active ester functionalities with amino groups of modified-PVC membranes for covalent bonding of antibody. The antibody and mycotoxin interactions can be transduced directly by measuring the Differential Pulse Voltammetry (DPV). It was found that the zearalenone (ZEN) detections can be accomplished with these performances: sensitivity 14.1 ± 3 μA/μg, linear range 10^{-2} - 10^{-5} μg/ml and linearity 0.999.

1. Introduction

Mycotoxins are toxic group of secondary metabolites produced by various fungi such as Fusarium, Penicillium and Aspergillus [1]. These mycotoxins are found commonly in food and agriculture products including aflatoxins (AFs), ochratoxin A (OTA), fumonisins (FUM), deoxynivalenol and zearalenone (ZEN) [2–4]. Mycotoxins can enter human body through food chain which can cause serious hazards such as teratogenic and mutagenic. Mycotoxin pollution is not only caused huge economic losses but also became a major hidden danger to human health and safety. Therefore, strengthening prevention and control of mycotoxins have become urgent needs to ensure food safety and environmental safety worldwide.

Zearalenone (ZEN) is a white crystal produced by Fusarium species. ZEN has been classified as a category 2A carcinogen by The International Agency for Research and Cancer (IRAC). Contaminants in foodstuffs were limited by the maximum concentration of mycotoxin utilized by Commission regulation (EC) NO 1881/2006. The European Food Safety states the mean level daily intake of ZEN should be below 250 μg/kg [5]. Many methods to quantify mycotoxin including HPLC [6], LC-MS [7,8], GC–MS [9] and thin-layer chromatography [10] have been reported. Although these published works show splendid method performances, these methods still require expensive equipment and complicated sample preparations. To improve the limitation of ZEN detection, The direct enzyme-linked immunosorbent assay (ELISA) technique was developed by this study as an electrochemical immunosensor to assay ZEN. An Electrochemical immunosensors are based on the principle that antigen-antibody interaction is performed in an apparatus of at least two electrodes electrochemical cell: one is the working electrode and the other is a reference electrode. The redox reaction of the analyst takes place on the working electrode which can be constructed from various materials including Platinum, Gold, Silver and Palladium. This modern electroanalytical technique combining the sensitivity and specificity of the antigen-antibody binding interaction gaining a high sensitivity and relative simplicity technique leads to the production of electrochemical immunosensor promising for clinical diagnostics [11,12], mycotoxins [13–16], foods [17] and pesticides [18].

Electrochemical immunosensor was prepared by coating Amino-PVC membrane on the gold electrode. EDC and sulfo-NHS react with amino group of Amino-PVC membrane for covalent bonding of antibody and detect antibody interaction due to measurement of ZEN using Differential Pulse Voltammetry (DPV). Modification of polymers was used to enhance sensitivity such as formaldehyde gas sensors [19], Nitrate-ISFET sensors [20–22], Covalent β- Galactosidase [23], Biomaterial

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[24], and anion-exchange membrane [25].

In this study, to develop an electrochemical immunosensor to assay ZEN, Amino-PVC was modified by nucleophilic substitution with ethylenediamine. Raman Microscope, Nuclear Magnetic Resonance (NMR), Differential Scanning Calorimeter (DSC), Field Emission Scanning Electron Microscope (FE-SEM) and Gel Permeation Chromatography (GPC) were used to characterize The chemical compositions and characteristics of Amino-PVC. Finally, Differential Pulse Voltammetry (DPV) was utilized to detect ZEN. The study found that ZEN detection can be accomplished these performances: sensitivity 14.1 μA/μg, linear range 10^{-2} - 10^{-5} μg/ml and linearity 0.999.

2. Experimental

2.1. Chemicals and immunochemical

Polyvinyl Chloride (Selectophore™), 2-Nitrophenyloctylether (2-NPOE), thylenediamine.

(EDA), Potassium Ferrocyanide ($K_4Fe(CN)_6$), Tetrahydrofuran (THF), Potassium Ferricyanide ($K_3Fe(CN)_6$), 1-Ethyl-3(3-dimethylaminopropyl) carbodiimide (EDC), Potassium Chloride (KCl) and Sulfo-N-hydroxy-succinimide (Sulfo-NHS) came from Sigma-Aldrich. ZEN standard solution (1 mg/ml) and antibody were supplied by Abcam.

2.2. Modification of PVC

The preparation of Amino-PVC was performed by treating it with large excess ethylenediamine solution and using pyridine as a catalyst. The PVC was functioned by replacing chloride atom with ethylenediamine to give the Amino-PVC a primary amino group. The excess ethylenediamine could prevent formation of crosslinking between different polymer chains as well as formation of cyclic derivatives. The reaction was performed at room temperature to avoid dehydrochlorination. After the reaction, the light yellowish colored Animate-PVC was filtered and washed with 10% methanol dilute to remove excess ethylenediamine. Pure Animate-PVC was obtained by recrystallizing from ethyl acetate methanol mixture.

2.3. Electrochemical immunosensor fabrication

Electrochemical immunosensor was fabricated on a structure of gold working electrodes by drop-casting Amino-PVC membrane then left it dry at room temperature. The Amino-PVC membrane solution, composed of 80 mg Amino-PVC and 450 mg 2-NPOE, was diluted in 2.5 ml THF. The gold working electrodes were applied with a drop of phosphate buffer solution and incubated overnight. After the gold working electrodes dried, were functionalized with EDC and NHS for 30 min. Then, immobilized with 10 μl of 20 μg/ml of antibody solution that was dropped on the gold working electrodes to create covalent bonding through EDC/Sulfo-NHS crosslinker then incubated for at least 3 h at room temperature. After antibody incubation, the gold working electrodes were rinsed with phosphate buffer solution to remove excess antibody. Finally, the gold working electrodes were incubated in 10 μl of various ZEN concentrations (10^{-2}–10^{-5} μg/ml) for 1 h at room temperature.

2.4. Characterization methodologies

Confocal Raman Microscope was used to characterize functional groups of Amino-PVC using excitation wavelength 785 nm, laser diode power 500 μW, spectral range from 200 to 3200 cm^{-1}, exposure time 20 s and resolution of 1 cm^{-1} (Renishaw inVia Reflex). The chemical structure was assured by Nuclear Magnetic Resonance (NMR) at the frequency of 600 MHz (Bruker Instrument Crop., Ascend 600, Germany) and molecular weight analysis was done by Gel Permeation Chromatography (GPC e2695, Waters Corp., USA).

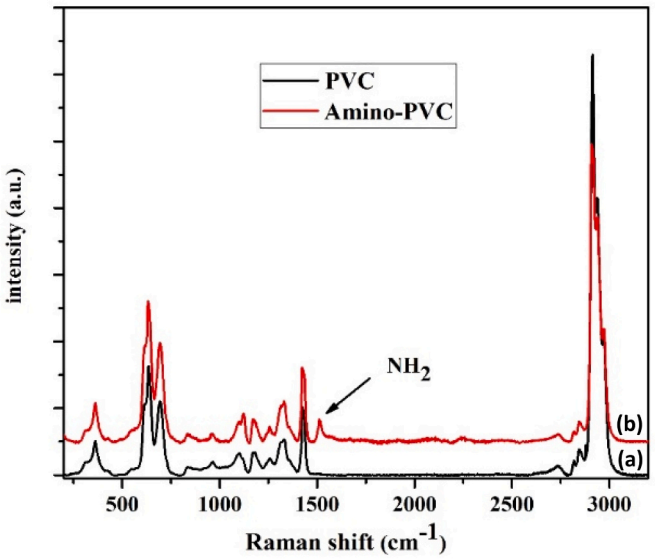

Fig. 2. Raman spectra of (a) PVC and (b) Amino-PVC.

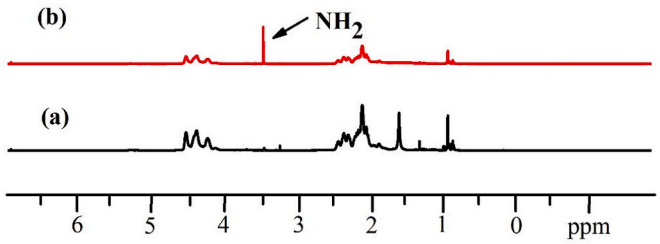

Fig. 3. NMR Spectra of (a) PVC and (b) Amino-PVC.

To study glass transition temperature (T_g) of PVC and Amino-PVC, Differential Scanning Calorimeter (DSC) was used with a heating rate of 20 °C/min in atmospheric Nitrogen and temperature range of 20 to 160 °C. A Schottky field-emission scanning electron microscope (FE-SEM), Hitachi SU-500, was used to capture the morphology of the immunosensor at 5 keV power.

Differential Pulse Voltammetry (DPV) was utilized to measure electrochemical characteristics (CHI660D, CHI Instruments, USA). Gold

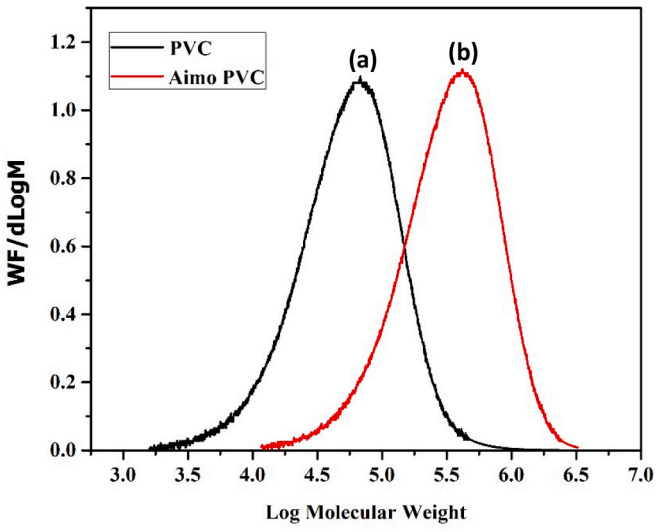

Fig. 4. GPC spectra of (a) PVC and (b) Amino-PVC.

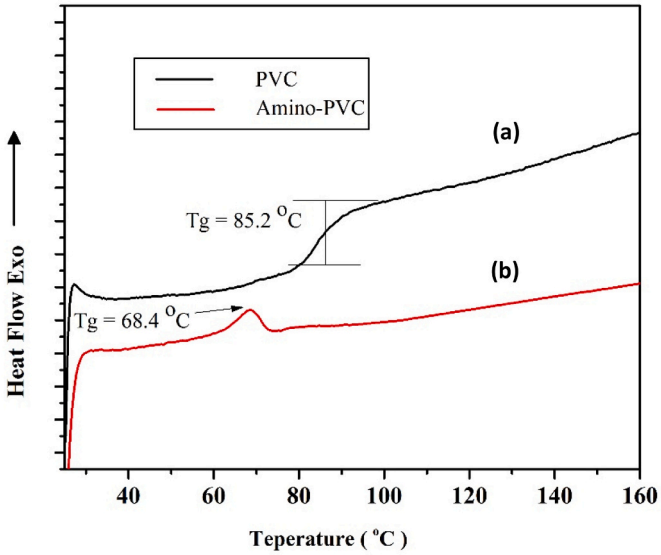

Fig. 5. DSC curves of (a) PVC and (b) Amino-PVC.

working electrode was selected as desired material; Platinum was chosen to be a counter electrode and Silver Chloride was used as a reference electrode. DPV measurements using 100 mM KCl solution contain 1 mM $K_3Fe(CN)_6$ and 1 mM $K_4Fe(CN)_6$ was utilized following these conditions: initial voltage 0.5 V, increate voltage 0.004 V, final voltage 0 V, sample width 0.0167, amplitude 0.05 V, pulse width 0.05 s and pulse period 0.5 s.

3. Results and discussion

3.1. Characterizations

The structures of modified PVC were confirmed by Raman Microscope, NMR and GPC. The Raman Microscope was used to analyze structural fingerprints by vibration modes of molecules. Fig. 2 shows the new Raman shift of Amino-PVC at 1512 cm^{-1}, represents a methylamine group [26]. (See Fig. 1.)

Fig. 3 shows the result of the NMR spectrum of the Amino-PVC indicated different signals of amino group at 3.3 ppm and reduces the signal of C-Cl group at 2.1–2.4 ppm. The molecular weight of Amino-PVC was measured using GPC as shown in Fig. 4, the chromatogram was increased from 30,372 g/mol to 33,488 g/mol. An increment of molecular weight implied an effect of PVC crosslinking with ethylenediamine to form Amino-PVC. All of characterize results indicated that the modification PVC achieved nucleophilic substitution of Chloride atoms on the PVC to produce Amino-PVC.

Thermal properties of PVC and Amino-PVC were evaluated by glass transition temperature ($T_{g)}$ using the DSC technique (Fig. 5). The T_g of Amino-PVC and PVC are 68.4C° and 85.2C°, respectively. The T_g of Amino-PVC is lower than that of PVC indicates that PVC reacts with ethylenediamine, increases the molecule of Amino-PVC and decreases molecular mobility of Amino-PVC affects a change in T_g of Amino-PVC [27].

Differential Scanning Calorimeter (DSC) was performed in order to analyze thermal properties of the modified PVC and affect amino group

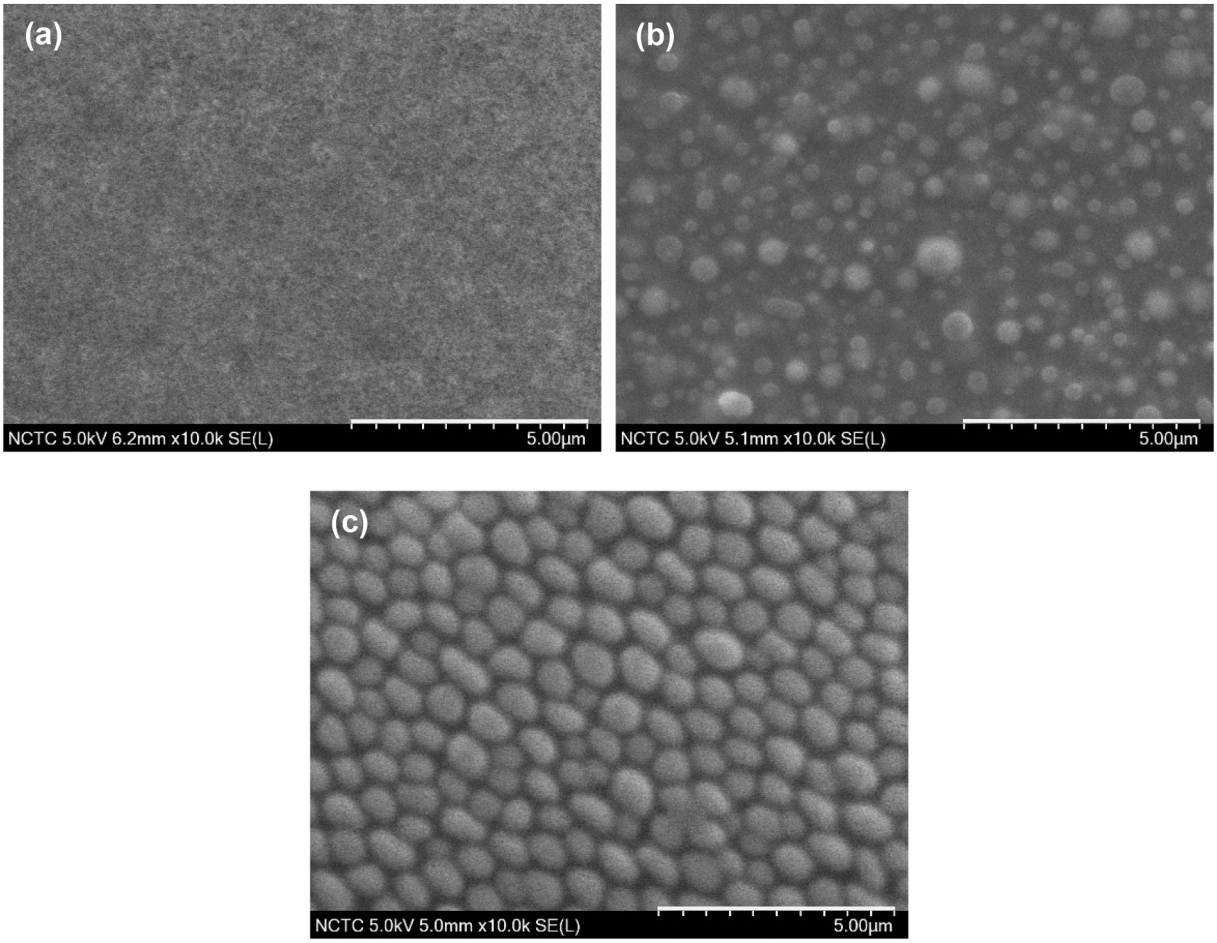

Fig. 6. FE-SEM images of Amino-PVC (a) as-deposited; (b) immobilized antibody and (c) then immobilized ZEN.

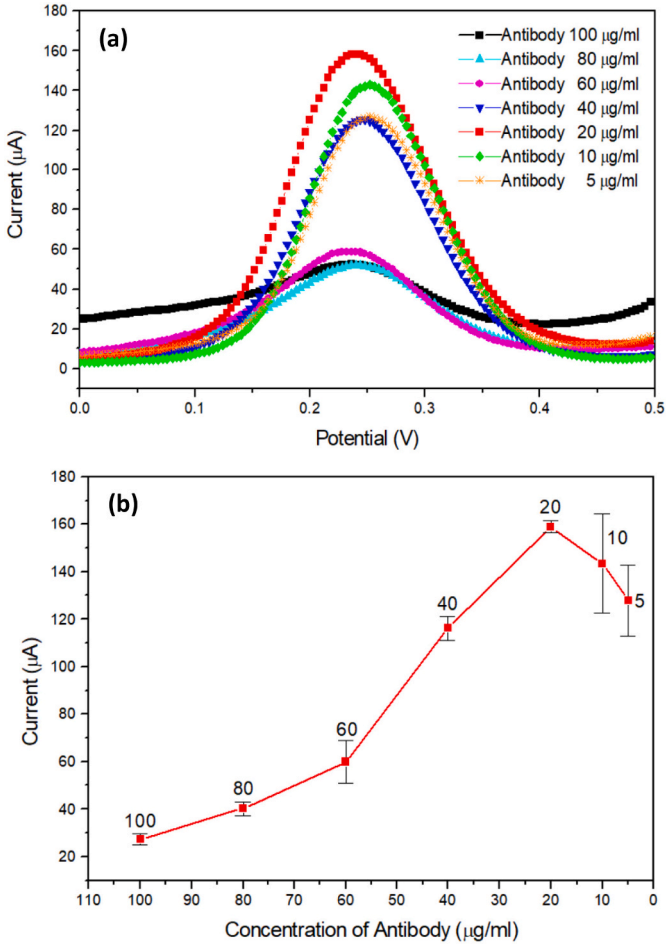

Fig. 7. (a) DPV responses for various antibody concentrations after incubating with a fixed concentration of ZEN at 0.01 µg/ml, (b) Peak current change versus concentration of antibody ($n = 3$) for optimization.

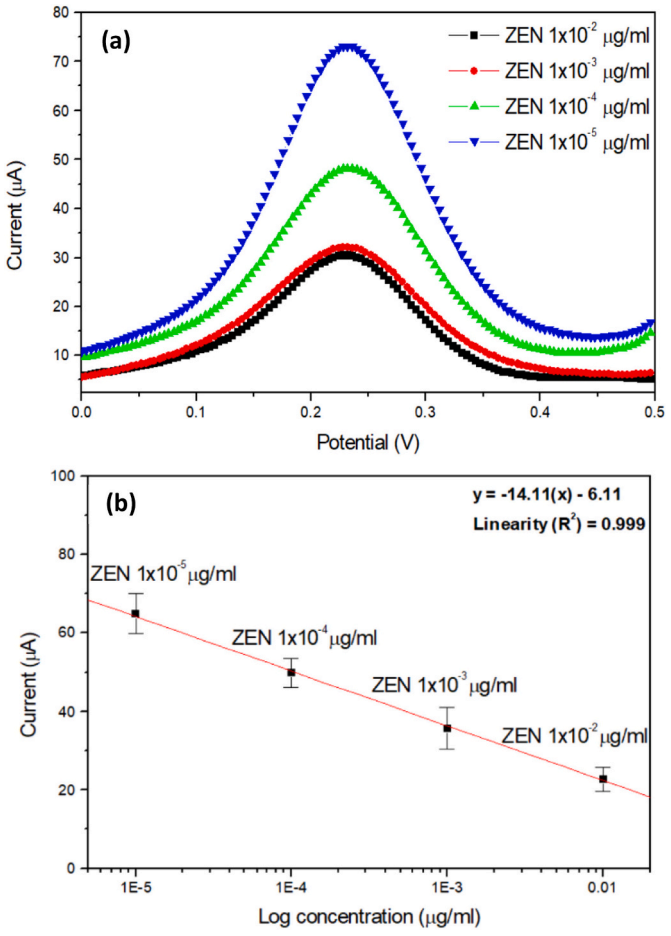

Fig. 8. (a) DPV responses for various ZEN concentrations with a fixed concentration of antibody at 20 µg/ml, (b) Calibration curve for the detection of ZEN.

on Tg. The value of Tg depends on chain flexibility and side group of the polymer. Tg is independent of molecular weight of the polymer and polymers with more chain flexibility have lower Tg values.

Increasing in molecular weight was a result of PVC crosslinking with ethylenediamine to form the Amino-PVC shown in Fig. 4. The Amino-PVC chain is more flexible than that of PVC leading to decrement of Tg.

The FE-SEM photographs of surface morphology of the Amino-PVC, immobilized antibody and immobilized ZEN are presented in Fig. 6 (a) - 6 (c). The image of the Amino-PVC displays a uniform surface with microstructures in Fig. 6 (a). After antibody immobilization, the surface was induced to form small spheres Fig. 6 (b). Finally, a uniform sphere morphology that confirms the immunosensor to assay ZEN based on indirect competitive ELISA technique has been successful shown in Fig. 6 (c).

3.2. *Optimal antibody and detection of ZEN using DPV*

Differential Pulse Voltammetry (DPV) was used to make the transduction mechanism for this immunosensor by which performed linear sweep voltammetry at series of regular voltage pulses modulated on the linear potential sweep itself. The current was simultaneously measured at each potential to minimize the effect of the charging current. This method then gains high sensitivity of measurement since the concentrations of a specific antibody were an important parameter leading to high sensitivity of the immunosensor such as low antibody concentration can render a high sensitivity detection [28].

In this study, the optimization of antibody loading concentrations on this immunosensor for effective detection of ZEN. The DPV responses vary the number of antibody concentrations of 5, 10, 20, 40, 60, 80 and 100 µg/ml loading on the Amino-PVC membranes and then incubating with a fixed 0.01 µg/ml of ZEN (as the maximum concentration of antigen in this study). The DPV results were performed using 100 mM KCl solution consisting of 1 mM $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ in Fig. 7 (a). The peak currents decreased with increasing concentrations of antibody that the maximum peak current occurs at 20 µg/ml of antibody, indicating the optimum formation of antibody-antigen complex and insulating effect. Hence, 20 µg/ml of antibody was selected as the optimal for ZEN detection in Fig. 7 (b).

The approach to direct detection of ZEN using voltammetry involves incorporating redox-active moieties directly onto the electroactive surface and relied on inhibition of efficient electrons transfer caused by the formation on insulating layer through accumulation of the target on the surface. Fig. 8 (a) shows the current response of various ZEN concentrations based on the activity of antibody (using the optimized antibody fixed concentration at 20 µg/ml) was immobilized onto the electrode surface and changed in the electrochemical response of the redox core upon binding of mycotoxin which could be measured using DPV. Current outputs showed reverse proportional to the concentration gradients because the higher concentration of antigen (ZEN) is, the lower conductivity on the surface will be due to increment of surface resistivity. Since a higher concentration of ZEN, more binding sites of available antibody were fully been occupied and then being washed out from the system during the cleaning step [29]. The calibration curve was performed by using obtained results in a logarithm of ZEN concentration

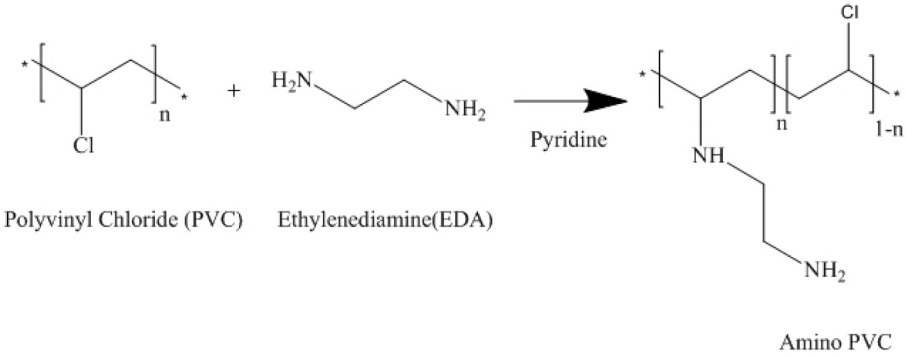

Fig. 1. The preparation of modified PVC.

range 10^{-5} - 10^{-2} μg/ml. The correlation coefficient for linear fitting was 0.999 and sensitivity (slope) was 14.11 μA/decade as Fig. 8 (b).

4. Conclusions

Modification of Amino-Polyvinyl Chloride membrane as a detector of zearalenone (ZEN) based on indirect competitive enzyme-linked immunosorbent assay (ELISA) technique was demonstrated. The chemical compositions and morphology characterizations of the samples were characterized using FTIR, NMR, GPC and FE-SEM assuring the correct results. Using the optimized antibody concentration at 20 μg/ml for immobilization that was found that the ZEN detections can be accomplished these performances: sensitivity = 14.11 μA/μg, linear range 10^{-2} - 10^{-5} μg/ml and linearity 0.999. The electrochemical immunosensor provides high sensitivity and reproducibility which may conveniently be utilized in many applications in foods and agriculture products.

Author statement

A. Pankiew, W. Chaisriratanakul, W. Bunjongpru and W. Jeamsaksiri designed these experiments. A. Pankiew, W. Chaisriratanakul and P. Pengpad prepared the manuscript. A. Pankiew and W. Chaisriratanakul, N. Thornyanadacha, A. Srisuwan, M. Horprathum, and K. Chauyrod have analyzed the results and discussed the manuscript during preparation. All authors discussed the results and implications and commented on the manuscript at all stages.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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References

[1] E.E. Creppy, Update of survey, regulation and toxic effects of mycotoxins in Europe, Toxicol. Lett. 127 (1–3) (2002 Feb 28) 19–28, https://doi.org/10.1016/s0378-4274(01)00479-9.
[2] J.W. Park, E.K. Kim, D.H. Shon, Y.B. Kim, Natural co-occurrence of aflatoxin B1, fumonisin B1 and ochratoxin a in barley and corn foods from Korea, Food Addit. Contam. 19 (11) (2002 Nov) 1073–1080, https://doi.org/10.1080/02652030210151840.
[3] B. Fazekas, A. Tar, M. Kovács, Aflatoxin and ochratoxin a content of spices in Hungary, Food Addit. Contam. 22 (9) (2005 Sep) 856–863, https://doi.org/10.1080/02652030500198027.
[4] Y.S. El-Sayed, R.H. Khalil, T.T. Saad, Acute toxicity of ochratoxin-a in marine water-reared sea bass (*Dicentrarchus labrax* L.), Chemosphere. 75 (7) (2009 May) 878–882, https://doi.org/10.1016/j.chemosphere.2009.01.049.
[5] J. Meneely, F. Ricci, H.P. Van Egmond, C. Elliott, Current methods of analysis for the determination of trichothecene mycotoxins in food, TRAC-Trends Analyt. Chem. 30 (2) (2011 Feb) 192–203, https://doi.org/10.1016/j.trac.2010.06.012.
[6] Han Zheng, Yiping Ren, Junfeng Zhu, Zengxuan Cai, Yong Chen, Lianjun Luan, Yongjiang Wu, J. Agric. Food Chem. 60 (33) (2012) 8233–8247, https://doi.org/10.1021/jf301928r.
[7] T.C.-H. Lee, F.L.-Y. Fong, K.-C. Ho, F.W.-F. Lee, The mechanism of diarrhetic shellfish poisoning toxin production in *Prorocentrum* spp.: physiological and molecular perspectives, Toxins 8 (10) (2016) 272, https://doi.org/10.3390/toxins8100272.
[8] B.S. Shin, S.H. Hong, S.W. Hwang, H.J. Kim, J.B. Lee, H.S. Yoon, D.J. Kim, S. D. Yoo, Determination of zearalenone by liquid chromatography/tandem mass spectrometry and application to a pharmacokinetic study, Biomed. Chromatogr. 23 (9) (2009 Sep) 1014–1021, https://doi.org/10.1002/bmc.1217.
[9] V. Ostry, J. Skarkova, A HPTLC method for the determination of the mycotoxin zearalenone in cereal products, Mycotoxin Res. 19 (1) (2003 Mar) 64–68, https://doi.org/10.1007/BF02940096.
[10] Ran Ran, Canhua Wang, Han Zheng, Aibo Wu, Dabing Zhang, Jianxin Shi, Corrigendum to "Determination of deoxynivalenol (DON) and its derivatives: Current status of analytical methods" [Food Control 34 (1) (2013) 138–148], Food Control 51 (2015) 453. ISSN 0956-7135, https://doi.org/10.1016/j.foodcont.2014.12.025.
[11] M.M.P.S. Neves, M.B. Gonzalez-Garcia, A non-enzymatic ethanol sensor based on a nanostructured catalytic disposable electrode, Anal. Methods 9 (2017) 5108–5114, https://doi.org/10.1039/C7AY01078H.
[12] L.C. Rosales-Rivera, J.L. Acero-Sánchez, P. Lozano-Sánchez, I. Katakis, C. K. O'Sullivan, Electrochemical immunosensor detection of antigliadin antibodies from real human serum, Biosens. Bioelectron. 26 (11) (2011 Jul 15) 4471–4476, https://doi.org/10.1016/j.bios.2011.05. 004.
[13] W.I. Riberi, L.V. Tarditto, M.A. Zon, F.J. Arévalo, Development of an electrochemical immunosensor to determine zearalenone in maize using carbon screen printed electrodes modified with multi-walled carbon nanotubes/polyethyleneimine dispersions, Sensors Actuators B Chem. 254 (2018) 1271–1277, https://doi.org/10.1016/j.snb.2017.07.113.
[14] S. Piermarini, L. Micheli, N.H. Ammida, G. Palleschi, D. Moscone, Electrochemical immunosensor array using a 96-well screen-printed microplate for aflatoxin B1 detection, Biosens. Bioelectron. 22 (7) (2007 Feb 15) 1434–1440, https://doi.org/10.1016/j.bios.2006.06.029.
[15] N.V. Chuc, N.H. Binh, C.T. Thanh, N.V. Tu, J. Mater, Electrochemical Immunosensor for detection of atrazine based on polyaniline/graphene, J. Mater. Sci. Technol. 32 (6) (2016) 539–544, https://doi.org/10.1016/j.jmst.2016.04.004.
[16] G. Evtugyn, T. Hianik, Electrochemical Immuno- and Aptasensors for mycotoxin determination, Chemosensors. 7 (1) (2019) 10, https://doi.org/10.3390/chemosensors7010010.
[17] F. Zhang, B. Liu, W. Sheng, Y. Zhang, Q. Liu, S. Li, S. Wang, Fluoroimmunoassays for the detection of zearalenone in maize using CdTe/CdS/ZnS quantum dots, Food Chem. 255 (2018) 421–428, https://doi.org/10.1016/j.foodchem. 2018.02.060.
[18] M. Khairy, H.A. Ayoub, C.E. Banks, Non-enzymatic electrochemical platform for parathion pesticide sensing based on nanometer-sized nickel oxide modified screen-printed electrodes, Food Chem. 255 (2018 Jul 30) 104–111, https://doi.org/10.1016/j.foodchem.2018.02.004.
[19] J.J. Park, Y. Kim, C. Lee, J.W. Kook, D. Kim, J.H. Kim, K.S. Hwang, J.Y. Lee, Colorimetric visualization using polymeric Core-Shell nanoparticles: enhanced sensitivity for formaldehyde gas sensors, Polymers (Basel). 12 (5) (2020 Apr 25) 998, https://doi.org/10.3390/polym12050998.
[20] W. Chaisriratanakul, et al., Modification of polyvinyl chloride ion-selective membrane for nitrate ISFET sensors, Appl. Surf. Sci. 512 (2020) 145664, https://doi.org/10.1016/j.apsusc.2020.145664.
[21] W. Chairatanakul, W. Bungru, J. Wutthinan, Jpn. J. Appl. Phys. 59 (2020) SIIJ10, https://doi.org/10.35848/1347-4065/ab7411.

[22] W. Chaisriratanakul, et al., Durable nitrate sensor by surface modification, Surf. Coat. Technol. 306 (2016) 58–62, https://doi.org/10.1016/j.surfcoat.2016.04.052.
[23] M.S. Mohy Eldin, M.R. Elaassar, A.A. El-Zatahry, M.B. El-Sabbah, Covalent Immobilization of β-galactosidase onto amino functionalized polyvinyl Chloride (PVC) Microspheres: Enzyme immobilization and characterization, Adv. Polym. Technol. (2013) 21379, https://doi.org/10.1002/adv.21379.
[24] C. McCoy, N. Irwin, J.G. Hardy, S. Kennedy, L. Donnelly, J. Cowley, G. Andrews, S. Pentlavalli, Systematic optimization of poly(vinyl chloride) surface modification with an aromatic thiol, Eur. Polym. J. 97 (2017) 40–48, https://doi.org/10.1016/j.eurpolymj.2017.09.030.
[25] Jin Sun Koo, Noh-Seok Kwak, Taek Sung Hwang, Synthesis and properties of an anion-exchange membrane based on vinylbenzyl chloride–styrene–ethyl methacrylate copolymers, J. Membr. Sci. 423–424 (2012) 293–301, https://doi.org/10.1016/j.memsci.2012.08.024.
[26] M.G. Giorgini, M.R. Pelletti, G. Paliani, R.S. Cataliotti, Vibrational spectra and assignments of ethylene-diamine and its deuterated derivatives, J. Raman Spectrosc. 14 (1983) 16–21, https://doi.org/10.1002/jrs.1250140106.
[27] B. Erwin, R. Katja, W. William, Chemical Resistance of Commodity Thermoplastics, William Andrew, 2016.
[28] A. Molazemhosseini, L. Magagnin, P. Vena, C.-C. Liu, Single-use disposable electrochemical label-free Immunosensor for detection of glycated hemoglobin (HbA1c) using differential pulse voltammetry (DPV), Sensors. 16 (7) (2016) 1024, https://doi.org/10.3390/s16071024.
[29] X. Zhang, C.R. Li, W.C. Wang, J. Xue, Y.L. Huang, X.X. Yang, B. Tan, X.P. Zhou, C. Shao, S.J. Ding, J.F. Qiu, A novel electrochemical immunosensor for highly sensitive detection of aflatoxin B1 in corn using single-walled carbon nanotubes/chitosan, Food Chem. 192 (2016 Feb 1) 197–202, https://doi.org/10.1016/j.foodchem.2015.06.044.