ChemComm

RSC Publishing

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2013, **49**, 3802

Received 9th January 2013,
Accepted 17th March 2013

DOI: 10.1039/c3cc00192j

www.rsc.org/chemcomm

Facile degradation of benzenediazonium-grafted thick layers on the electrode surface enabling electrochemical biosensor application†

Al-Monsur Jiaul Haque, Md. Mohibul Islam Khan and Kyuwon Kim*

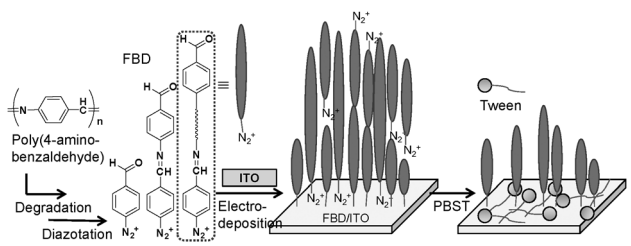
We found that the formylbenzenediazonium (FBD)-grafted organic layers can be degraded by treating with an aqueous solution of salt and Tween. An electrochemical immunoassay was carried out on the degraded surface, which allowed highly sensitive detection of antigen mouse IgG.

The formation of anchoring organic layers on electrode surfaces with desired chemical and electrochemical functions is a critical component in the development of biosensors. Among the various methods used to form such organic layers on the electrode surfaces, the electrochemical reduction of benzenediazonium (BD) cations, introduced by Delamar *et al.*,[1] has become one of the best methods for surface modification because it offers many advantages compared with other methods, for example, self-assembled monolayers, ease of synthesis of diazonium salts with a wide range of functional groups, the rapid formation of stable organic layers on various surfaces such as carbon,[1,2] silicon,[3] metals,[4] and indium-tin-oxide (ITO).[5]

Besides these advantages, one major drawback of this method is that it usually forms thick layers on the surfaces. This is due to the attack of the highly reactive aryl radicals on the already grafted aryl layers, which ultimately leads to formation of poorly defined thick polymeric organic layers on the electrode surfaces.[6] As a result, the BD-modified surfaces often show high barrier properties to interfacial electron transfer which retards their use in the development of high-performance electrochemical biosensors or molecular devices or for carrying out quantitative studies of electron transfer. Therefore, finding the conditions for facile degradation of the polymeric layer to obtain sufficiently thin layers permitting facile electron transfer, and yet still maintain the chemical reactivity toward other molecules for further functionalization is highly desirable. In this connection, Daasbjerg *et al.* used a two-step formation–degradation approach to obtain thin and chemically reactive near-monolayer films of thiophenolates[7] or benzaldehydes[8] through electrochemical or chemical degradation of polymeric layers of rationally designed and synthesized BD salts. On the other hand, formylbenzenediazonium (FBD) salt, among many BDs, is a highly efficient anchor molecule in biochip surface fabrication because it can be easily prepared without complex synthesis and precludes the need for any additional linkers or further activation steps for bio-immobilization. Previously, we reported the direct use of the polymerized film of FBD for multiple bio-immobilization based on electrode-addressing.[9] Nevertheless, the degradation of the FBD-grafted polymerized film providing electrochemically accessible functionalized surfaces has not been achieved to date.[8]

In this study, we report for the first time facile degradation of FBD-grafted layers on ITO electrode surfaces. We demonstrate also that the degraded FBD-modified ITO surfaces can be suitably used in electrochemical biosensor application. We have found that the thick layers can be efficiently degraded by treating with phosphate buffered saline solution containing a surfactant Tween 20 (PBST) and the resulting surface presents a very low barrier property to interfacial electron transfer, which otherwise shows totally blocking behaviour to a redox molecule, potassium ferricyanide. To demonstrate an electrochemical immunosensor, a sandwich-type enzyme-linked immunosorbent assay was carried out on the degraded ITO surfaces.

Scheme 1 shows a process of preparation and electrodeposition of the FBD on the ITO surface, degradation of the thick layers formed during the electrodeposition of FBD. FBD-included solution was prepared by a simple procedure as described in a previous paper.[10] The electrodeposition of FBD on the ITO electrode surfaces was conducted using cyclic voltammetry (CV). The CV results obtained during



Scheme 1 A proposed scheme for the synthesis of FBD from the polymer precursor, electrodeposition of FBD on the ITO surface, and degradation of the electrodeposited layers by PBST.

Department of Chemistry, University of Incheon, Incheon 406-772, Korea. E-mail: kyuwon_kim@incheon.ac.kr; Fax: +82 32 835 0762; Tel: +82 32 835 8243

† Electronic supplementary information (ESI) available: Experimantal details, AFM, FT-IR, XPS and immunoassay results. See DOI: 10.1039/c3cc00192j

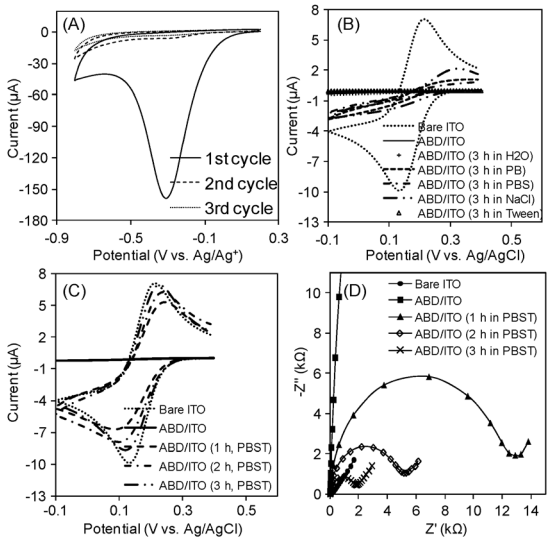


Fig. 1 (A) CVs for the electrodeposition of FBD (2 mM FBD with 0.1 M Bu_4NBF_4 in ACN) on an ITO electrode surface. (B) and (C) CVs for bare and FBD-deposited ITO surfaces recorded in 1 mM $[Fe(CN)_6]^{3-}$–0.1 M KCl solution. Scan rate of 50 mV s^{-1}. (D) Nyquist plots for bare and modified surfaces recorded in 1 mM $[Fe(CN)_6]^{3-/4-}$ (1 : 1)–0.1 M KCl solution with a 20 mV sinusoid superimposed on a dc potential of 0.2 V in the frequency range of 10 kHz–0.01 Hz.

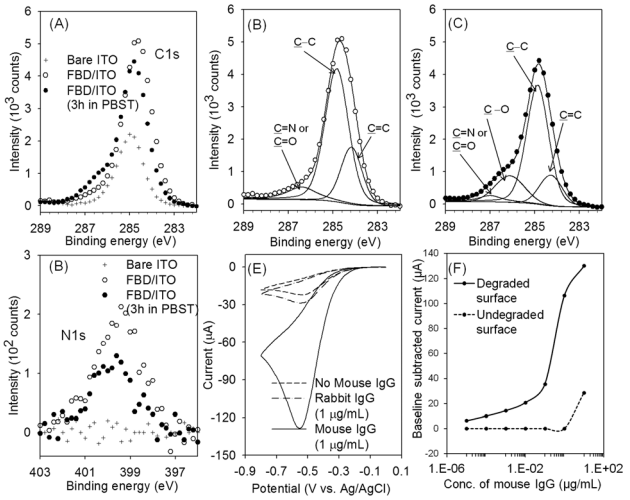


Fig. 2 (A) XPS spectra of C1s for FBD-deposited ITO surfaces before and after PBST treatment. Deconvoluted XPS spectra (B) before and (C) after PBST treatment. XPS spectra of (D) N1s for FBD-deposited ITO surfaces. (E) CV responses of the immunosensor for the detection of mouse IgG and rabbit IgG (1 μg mL^{-1}), scan rate of 50 mV s^{-1}. (F) Calibration curves of the immunosensor responses on the degraded and undegraded surfaces.

the electrodeposition showed a sharp irreversible reduction peak at −0.3 V during the first cycle, and the current was greatly diminished during subsequent cycles (Fig. 1A). The CV response was very similar in nature to that observed previously for other diazonium salt.[1–5]

The degradation of the organic layers by PBST treatment was first investigated from the blocking behavior of FBD/ITO surfaces to a redox probe, $Fe(CN)_6^{3-}$. Fig. 1B and 1C show the CVs from the redox reactions of $Fe(CN)_6^{3-}$ for bare and FBD-modified ITO surfaces before and after treatment with different solutions. While the characteristic redox peaks were observed from the electrochemical reaction of $Fe(CN)_6^{3-}$ on the bare ITO surface, the electrochemical reaction was completely blocked on the FBD-modified ITO surface, indicating formation of very thick organic layers. Complete blocking behaviour of the modified surfaces remained unchanged after treating with only H_2O or an aqueous solution with 0.05% Tween 20. The redox peak currents appeared slightly after treating the modified surfaces with phosphate buffer (PB), phosphate buffer saline (PBS), and 0.15 M NaCl solution suggesting partial degradation of the organic layers with these solutions (Fig. 1B). However, both the anodic and cathodic peak currents were observed clearly after treating with PBST, and increased with increasing treatment time (Fig. 1C). Fig. 1D shows the Nyquist plots of electrochemical impedance spectroscopic (EIS) results for bare and FBD-modified ITO surfaces using $Fe(CN)_6^{3-}$ as a redox probe. As expected, the FBD-modified ITO surface showed very high charge transfer resistance, R_{ct} (diameters of the semicircles), attributed to thick organic layers on the surface. However, the R_{ct} values decreased with increasing PBST treatment time, which was consistent with the CV results indicating considerable degradation of the modified layers.

XPS was also employed to confirm the degradation by PBST treatment. Fig. 2A shows the C1s XPS spectra of bare and FBD-deposited surfaces. After electrodeposition of FBD, the intensity of the C1s peaks, originating from C═C and C–C bonds of the aromatic ring, increased greatly from that of the bare signal. However, even after treating with PBST for 3 h, the intensity of the peak did not decrease as much as expected. Fig. 2B and C compare the deconvoluted XPS peaks before and after PBST treatment, in which it is observed that the intensities of the peaks from C–C decreased slightly while those of the peaks from C═C and C═N (or C═O) reduced significantly. In addition, a new intensive peak from C–O appeared after the treatment. These changes might be caused by not only the removal of FBD layers but also the adsorption of Tween 20 on the degraded surfaces through hydrophobic interaction between linear alkyl chains of Tween 20 and the residual BD moieties after the degradation, which is reasonable considering that the structure of Tween 20 mainly composed of C–C and C–O bonds. However, the adsorption of Tween 20 may not be so serious that the electron transfer reaction is not blocked. The adsorption of Tween 20 on the hydrophobic surfaces has been well known.[11] For the N1s spectra, as shown in Fig. 2D, the FBD-deposited ITO surfaces exhibited a distinct N1s peak at 399.4 eV corresponding to the nitrogen in the N═C (imine) bonds. After treating with PBST for 3 h, the intensity of the peak decreased considerably.

We carried out atomic force microscopy (AFM) using the scratching method to measure the thickness of the FBD-deposited surfaces.[7] A polished and highly conductive silicon wafer was used as the electrode surface instead of the ITO surface with high surface roughness with the assumption that silicon surfaces have similar electrode properties to ITO surfaces.[5] As shown in Fig. S1A (ESI†), the thickness estimated from 5-measurement average of line scan profiles across the scratch on the FBD-grafted surface was determined to be 4.8 ± 1.2 nm, indicating around 7-layer formation of FBD on the surface with an assumption that the length of the FBD grafting unit is around 0.7 nm. The measurement after the degradation gave a thickness of 2.7 ± 0.7 nm (Fig. S1B, ESI†). Considering the non-negligible adsorption of Tween 20, presumed from the XPS result, and the high accessibility for the electron transfer reaction,

the exclusive thickness of the FBD layer on the degraded surfaces might be much lower than 2.7 nm. The XPS and AFM results lend strong support to our assumption that FBD-grafted organic layers were highly degraded by the PBST treatment.

To understand the degradation phenomenon, we assumed that the degradation originated from the desorption of electrostatically adsorbed BD cations by the action of salts and Tween 20. During the cathodic electrodeposition of FBD, some cationic FBD might be adsorbed on the negatively charged ITO surfaces through electrostatic interaction. Hydrophobic interaction between ITO surface-adsorbed FBD and free FBD can also contribute to the formation of dense insulating layers with the assumption that the FBD-included starting solution contains a considerable amount of diazonium cations with different lengths of aminobenzaldehyde oligomer moieties as well as a FBD monomer due to incomplete hydrolysis of the polymer precursor during the diazonium salt preparation. The steric hindrance around the *ortho*-position of BD of the oligomer might prevent multilayer formation on the ITO surface by the grafting expected for usual BD monomers such as C_6H_5–N_2^+. We have displayed the physical adsorptions in Scheme 1. Upon exposure to PBST solution, the electrostatic bond is broken by excess Cl^- ions, and subsequently the FBD cation is taken by the hydrophobic tails of Tween 20, finally desorbed from the surface, which might make pinholes that enable electron transfer reaction. The hydrophobically trapped FBD cations among the surface-adsorbed FBD are expected to be removed facilely from the surface by Tween 20 alone, without participation of Cl^- ions. However it does not seem to decrease the electrochemical reaction barrier as shown in Fig. 1B. It has also been proposed that electrodeposition of BD on the ITO surface results in strong physical adsorption of BD rather than covalent binding expected at other surfaces such as carbon.[5]

The desorption of FBD from the ITO surface was examined using ATR-FTIR. The absorption band for the N–N stretching mode of the diazonium cation, $N\equiv N^+$, was observed at 2250 cm^{-1} on the FBD-modified surface which disappeared after exposure to PBST (Fig. S2, ESI†).[4] Fig. S3 (ESI†), displaying the deconvolution of the XPS peak for N1s, also supports the adsorption and removal. The peak near 402 eV arising from $\underline{N}\equiv N$ on the FBD-deposited surface decreased significantly with the PBST treatment.[5] Serial treatments on the FBD-deposited surface with PBS solution and 0.05% Tween 20 solution for 3 h each exhibited similar electrochemical accessibility to the surface subjected to the PBST treatment for 3 h (Fig. S4, ESI†). Taken together, all of the results evidence our assumption of the desorption-induced degradation generating some pinholes that allow the electron transfer reaction.

We carried out an electrochemical sandwich immunoassay on the PBST-treated ITO surface for detecting an antigen, mouse IgG, using an HRP-labelled secondary antibody, as described in the experimental section. Fig. 2E shows the CVs obtained for the PBS buffer solution containing HQ and H_2O_2. A high reduction peak current was observed for mouse IgG (1 μg mL^{-1}), indicating the catalytic activity of the HRP molecules immobilized through the immunoreactions on the surface. A very small reduction peak current was observed in the CV for the control experiment (without any antigen) which is attributed to the nonspecific binding of proteins and electroreduction of H_2O_2. The CV for rabbit IgG was almost the same as that obtained without using any antigen. This result indicates that the immunosensor is highly specific. We have examined CV responses with different concentrations of mouse IgG from 10 pg mL^{-1} to 10 μg mL^{-1} (Fig. S5, ESI†). The reduction peak current of BQ increased with increasing concentration of mouse IgG, and exhibited a good linear relationship up to 10 ng mL^{-1} (Fig. 2F).

To confirm the usefulness of the degraded surface as a platform for electrochemical immunosensors, the immunoassay has been conducted on the undegraded surface that was prepared only from PBS treatment. As shown in Fig. 2F, considerable signal current was observed only for 10 μg mL^{-1} of mouse IgG but this was much lower than that observed from the degraded surface (Fig. S6, ESI†), which clearly indicates that the use of the degraded surface with low charge transfer resistance is essential to develop a highly sensitive electrochemical immunosensor. The high sensitivity might be attributed to the efficient immobilization of high density primary antibodies on the aldehyde-terminated surface which precludes the need for any additional linkers or further activation steps, and the low charge transfer resistance property of the degraded FBD-modified ITO surfaces. Moreover, the degradation process by PBST does not need any harsh chemical or electrochemical treatment which may damage the integrity of the electrode surface and affect further applications.

To examine the stability of the degraded surface it was stored in a dry nitrogen atmosphere at 25 °C. Fig. S7 (ESI†) shows storage time-dependent CV responses of the immunosensor. The current decreased with increasing time after 24 h, which might be attributed to the chemical damage of aldehyde groups and the natural degradation of FBD layers.

In conclusion, we have demonstrated unexpected desorption based-degradation of FBD-deposited organic layers on ITO surfaces by PBST treatment. However, more detailed study of the degradation mechanism is still needed. Nevertheless, our strategy can be a simple and promising tool to overcome the drawback in the use of the BD reduction method for the development of high-performance electrochemical biosensors.

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. NRF-2011-0017219).

Notes and references

1 M. Delamar, R. Hitmi, J. Pinson and J. M. Savéant, *J. Am. Chem. Soc.*, 1992, **114**, 5883.
2 P. Allongue, M. Delamar, B. Desbat, O. Fagebaume, R. Hitmi, J. Pinson and J. M. Savéant, *J. Am. Chem. Soc.*, 1997, **119**, 201.
3 P. Allongue, C. Henry de Villeneuve, G. Cherouvrier, R. Cortès and M. C. Bernard, *J. Electroanal. Chem.*, 2003, **161**, 550.
4 A. Adenier, C. Combellas, F. Kanoufi, J. Pinson and F. Podvorica, *Chem. Mater.*, 2006, **18**, 2021.
5 S. Maldonado, T. J. Smith, R. D. Williams, S. Morin, E. Barton and K. J. Stevenson, *Langmuir*, 2006, **22**, 2884.
6 C. Combellas, F. Kanoufi, J. Pinson and F. Podvorica, *Langmuir*, 2005, **21**, 280.
7 L. T. Nielsen, K. H. Vase, M. Dong, F. Besenbacher, S. U. Pedersen and K. Daasbjerg, *J. Am. Chem. Soc.*, 2007, **129**, 1888.
8 K. Malmos, M. Dong, S. Pillai, P. Kingshott, F. Besenbacher, S. U. Pedersen and K. Daasbjerg, *J. Am. Chem. Soc.*, 2009, **131**, 4928.
9 A. M. J. Haque and K. Kim, *Langmuir*, 2011, **27**, 882.
10 L. L. Lai, C. H. Ho, Y. J. Lin, E. Wang, Y. H. Liu, Y. Wang, Y. C. Lin and K. L. Cheng, *Helv. Chim. Acta*, 2002, **85**, 108.
11 M. Zhang and M. Ferrari, *Biotechnol. Bioeng.*, 1997, **56**, 618.

