Fabrication of Carboxylated Carbon Nanotube Buckypaper Composite Films for Bovine Serum Albumin Detection

Kuo-Jung Lee 1,*, Ming-Husan Lee 1, Yung-Hui Shih 1,*, Chao-Ping Wang 2,3,*, Hsun-Yu Lin 1 and Sheng-Rui Jian 1

1 Department of Materials Science and Engineering, I-SHOU University, Kaohsiung 84001, Taiwan; e12485772@ym.com (M.-H.L.); carbonfish028@gmail.com (H.-Y.L.); srjian@isu.edu.tw (S.-R.J.)

2 Division of Cardiology, E-Da Hospital, Kaohsiung 84001, Taiwan

3 School of Medicine for International Students, College of Medicine, I-SHOU University, Kaohsiung 84001, Taiwan

* Correspondence: krlee@isu.edu.tw (K.-J.L.); yhshi@isu.edu.tw (Y.-H.S.); ed100232@edah.org.tw (C.-P.W.); Tel.: +886-7-6577-711 (ext. 3126) (K.-J.L.); +886-7-6577-711 (ext. 3115) (Y.-H.S.); +886-7-6151-100 (ext. 5018) (C.-P.W.)

Abstract: The salient point of this study is to fabricate carbon nanotube (CNT) buckypaper composite films prepared through the methods of pumping filtration and spin coating. Firstly, carboxylated CNTs were used to make the original buckypaper specimen and further modify the buckypaper surface by incorporating different surface modifiers. Then, all of original (unmodified) and modified buckypaper composite films had different concentrations of bovine serum albumin (BSA) added, and differential pulse voltammetry (DPV) electrochemical measurement was used to measure the characteristics of the various buckypaper composite films, after adding different concentrations of BSA. The experimental results show that the contact angles for four modified specimens are smaller than that of the original unmodified S–BP specimen (62°). These results indicate that the four modifiers used in this study can improve the hydrophilic properties of the original, unmodified S–BP specimen, and benefit the subsequent bonding of a modified specimen with aqueous BSA. In addition to the improvement of the hydrophilic properties of the modified specimen, which affects the bonding with BSA, the bonding type produced by the modifier also plays an essential role in the bonding between specimen and BSA. Therefore, the S–BP–EDC/NHS and S–BP–TA specimens have better linear dependence between log (BSA concentration) and oxidation current data.

Keywords: buckypaper film; carbon nanotubes (CNTs); bovine serum albumin (BSA); modifiers; electrochemical; hydrophilic

1. Introduction

For biosensors, the efficiency of electronic interactions between the reacting target molecules and the substrate plays a key role in determining the accuracy and efficiency of measurement results. CNTs have the promising advantages of large surface area, high mobilities, and large electrical conductivities to electronic and electrochemical biosensors [1–3]. In addition, many biological molecules, such as proteins or DNA, can be conjugated with CNTs easily. This phenomenon also provides an advantageous solution for the application of CNTs in biosensors. In recent years, there has been a dramatic proliferation of research concerned with the application of CNTs in the biosensors of glucose [4–7], cholesterol [8–12], and DNA [13–18], that achieves excellent properties, including sensitivity, selectivity, and reproducibility.

However, due to the strong inter–tube van der Waals attraction, the aggregation of CNTs acts as an obstacle, in most applications, that deteriorates the mechanical and electrical properties of CNTs. Moreover, the size of CNTs is too small, which makes them difficult to control and disperse in processing, so their application is limited [19,20].
The buckypapers fabricated with CNTs have a macroscopic paper–like structure, which can be easily used and operated [21]. It is an important breakthrough to scale up CNTs to the macroscopic scale by using buckypapers to overcome the difficulties in manipulating CNTs [20–22]. In addition to their excellent CNT–like properties, buckypapers also cover a large area and are easy to control, so the current research uses for buckypapers are fairly extensive and are getting considerable attention. These include biosensors [23–25], electrical and thermal conductivity [26,27], gas filters [28], field emissions [29,30], etc. At present, the research on the application of buckypaper in biosensors has just begun, and there is not much relevant literature. It has been found that buckypaper made up of carboxyl–functionalized CNTs with incorporated gold nanoparticles could be highly sensitive glucose [24,25] and hydrogen peroxide [31] detectors. Buckypapers fabricated using functionalized CNTs are also used as working electrodes to detect dopamine [25].

According to early literature [32], the electrical conductivity of buckypapers made of carboxylated (purified) CNTs was 2–4 times higher than that made of as–grown CNTs. By means of carboxylation (functionalization), CNTs can not only be dispersed in solvents, but can attach to molecules physically or chemically without significantly changing their inherent unique properties [24].

This study provides a convenient, rapid, and accurate method to detect BSA, which is expected to be applied to the detection of other biomolecules in the future. In this study, buckypaper composite films were fabricated with carboxylated CNTs through pumping filtration and spin coating. Different modifiers were added by spin coating to modify the surfaces of buckypaper composite films. By combining the excellent conductive characteristics of buckypapers with its modified surface, it can attract and immobilize the BSA. This study will adopt the DPV electrochemical measurement to detect and quantitatively measure various BSA concentrations.

2. Materials and Methods
2.1. Fabrication of Buckypaper Film

First, 80 mg carboxylated multi–wall CNTs (Golden Innovation Business Co., AC tube-100LH, New Taipei City, Taiwan) with the addition of 1 wt.% EDC/NHS were added in 100 mL of 95% alcohol by the process of grinding, stirring, and sonication, respectively, to prepare the CNTs’ suspension solution. Then the suspension solution was poured into the pumping filtration system (Figure 1a) containing Teflon filter paper (Rocker, 201PTFE–47–022–50, Kaohsiung, Taiwan), and the suction filtration process was carried out for about 20 min through the pressure difference formed by the vacuum pump. The obtained buckypaper film, with diameter about 4 cm and thickness of 0.37 ± 0.03 mm (Figure 1b), was dried at 100 °C for 60 min before being cut into square strips for various tests. In this study, the original buckypaper film prepared from CNTs was designated “S–BP” specimen.

![Figure 1. (a) Pumping filtration system; (b) Appearance of the fabricated buckypaper film.](image-url)
2.2. Modification of Buckypaper Film

Four different modifiers of (1–(3 dimethylaminopropyl) ethylcarbodiimide (EDC)+ N–hydroxysuccinimide (NHS), Tannic acid (TA), Poly (Sodium 4–Styrenesulfonate (PSS), and Sodium Dodecyl Sulfate, (SDS)) were used to modify the surface of buckypaper film by spin coating, respectively. The preparation methods of different modifier solutions were mainly based on references [33–36]. During the spin–coating process, 100 µL of the modifier solution was dropped onto the surface of specimen, and rotated at about 1000 rpm for 30 s to make the modifier evenly coated on the surface of specimen. In the following study, the S–BP specimens were modified with four different modifiers. According to the different modifiers added, the modified buckypaper composite films were designated S–BP–EDC/NHS, S–BP–PSS, S–BP–SDS, and S–BP–TA specimens, respectively.

2.3. Addition of Bovine Serum Albumin (BSA)

Due to its cheap price and direct solubility in water, bovine serum albumin (BSA) is frequently used in research as a standard to quantify the concentration of other proteins or biomolecules. Before adding BSA to the buckypaper composite films, the aqueous solutions of 0.02 M NaH$_2$PO$_4$ (Sigma–Aldrich, VE–V900060, Beijing, China) and 0.1 M Na$_2$HPO$_4$ (Sigma–Aldrich, T–3828–01, Bengaluru, India) were mixed to prepare the 0.1 M phosphate buffered saline (PBS). Then, PBS solution and BSA powder (Sigma–Aldrich, A7906, St. Louis, MO, USA) were mixed to prepare BSA solutions with different concentrations (10, 100, 1000, 10,000 and 100,000 ng/mL). Next, in order to render the BSA to bond with the surfaces of specimens evenly, the various specimens and 10 mL of different BSA solutions were put into the serum bottle, respectively, then placed in a horizontal shaker (Dragon LAB, model SK–O180–E, Woodbury, CT, USA), and shaken at 100 rpm for 15 min. These specimens were then removed from the BSA solution and rinsed with PBS solution to wash away excess BSA that remained unbound on the specimens. Finally, these specimens were dried at 30 °C, and the subsequent measurement and microstructure observation were carried out.

2.4. Characterization

2.4.1. Contact Angle Measurement

Since the addition of BSA in this experiment is carried out in the form of an aqueous solution, the hydrophilic/hydrophobic properties of the specimen’s surface may affect the adsorbed amount of BSA. Therefore, it is necessary to further measure the hydrophilic/hydrophobic properties of the specimen’s surface by measuring the contact angle. The contact angles were measured using a contact–angle analyzer (Xinchuangda Technology Co., Ltd., Model 100, Taipei, Taiwan). A drop of deionized water (10 µL) was dropped on the surface of the specimen, and a camera was used to capture the contact angle of water droplet and specimen.

2.4.2. Electrochemical Measurement

In this experiment, an electrochemical workstation (CHI Instruments, 6114E, Austin, Texas, TX, USA) was used to carry out the measurement via Differential Pulse Voltammetry (DPV). The electrochemical system that has three electrodes, including the counter electrode of platinum, the reference electrode of Ag/AgCl (CHI Instruments, RE–1BP, Tokyo, Japan), and the working electrode of the specimen, was used in the experiment. During the measurement, a square specimen of 2 × 2 cm$^2$ with thickness of 0.37 ± 0.03 mm was used, and the electrolyte was the 5 mM K$_3$Fe(CN)$_6$ PBS solution.

2.4.3. Functional Group Detection

In this experiment, Fourier transform infrared spectroscopy (FTIR) (PerkinElmer, model Nicolet 460, Akron, OH, USA) was applied to the functional group detection (scanning range: 400–4000 cm$^{-1}$), which could detect the changes of functional groups on
surfaces of specimens before and after modification. Because the specimens were black and opaque, the mode of Attenuated Total Reflection (ATR) was adopted for measurement.

2.4.4. Observation of Microstructure

Before observation, a gold plating machine (Hitachi, E–1010, Lbaraki, Japan) was used to coat the surface of specimens with a platinum film to increase the electrical conductivity of the specimen. The scanning electron microscope (Hitachi, FE–SEM4700, Lbaraki, Japan) with a cold–field emission emitter was used to observe microstructures of various specimens before and after the addition of different concentrations of BSA, respectively. Meanwhile, energy–dispersive X–ray spectroscopy (EDX, Horiba, Osaka, Japan) was also used to analyze the element composition of the modified specimen surface.

3. Results and Discussion

3.1. Contact Angle Measurement

The hydrophilic/hydrophobic properties of the specimen can be carefully researched by the contact angle measurement. The smaller the contact angle, the better the hydrophilicity of the specimen. In this study, if the specimen has fine hydrophilic properties, it will be beneficial to combine it with aqueous BSA. As shown in Figure 2, it can be found that the contact angle of the original, unmodified S–BP specimen (62°) is larger than that of all modified specimens. Among the modified specimens, the contact angle of the S–BP–EDC/NHS specimen (53°) is the largest, followed by the S–BP–PSS specimen (38°), the S–BP–TA specimen (22°), and lastly, the S–BP–SDS specimen (17°). It is important to note that all of the contact angles for these four modified specimens are smaller than that of the S–BP specimen (62°). Namely, it indicates that the four modifiers used in this study can improve the hydrophilic properties of the original S–BP specimen and benefit the subsequent bonding of the modified specimen with aqueous BSA.

**Figure 2.** Contact angle measurement of various specimens. (a) S–BP; (b) S–BP–EDC/NHS; (c) S–BP–PSS; (d) S–BP–SDS; (e) S–BP–TA.
3.2. Differential Pulse Voltammetry (DPV) Measurement of Buckypaper Composite Films

This part mainly uses the DPV electrochemical method to measure the reaction feedback (oxidation current) of various specimens, adding different concentrations of BSA (0 ng/mL, 10 ng/mL, 100 ng/mL, 1000 ng/mL, 10,000 ng/mL, 100,000 ng/mL). The change rate of the oxidation current of the specimen before and after adding different concentrations of BSA was further calculated. The correlation between the logarithm BSA concentration (log (BSA) ng/mL) and the change of oxidation current was analyzed by linear regression analysis.

Figure 3a shows the DPV measurement results of the original, unmodified S–BP specimen after adding different concentrations of BSA. It can be found that there is no obvious difference in the oxidation current of the S–BP specimen when adding lower concentrations of BSA (10 ng/mL, 100 ng/mL). However, after adding a high concentration of BSA (1000 ng/mL, 10,000 ng/mL, 100,000 ng/mL), the oxidation current of the S–BP specimen begins to decrease gradually. When adding BSA with a low concentration, it has difficulty bonding stably to the surface of the S–BP specimen, leading to an inconspicuous change in the oxidation current. The main cause is conceivably due to the fact that the S–BP specimen only exhibits weak hydrophilic properties (contact angle: 62°). When the concentration of BSA is higher, the bonding chance of BSA to the specimen increases, so the oxidation current reflected by the electrochemical system also changes significantly.

From the diagram of linear regression analysis (Figure 4), it is evident that the oxidation current of all specimens roughly decreases with the increase of logarithm concentration of BSA (log (BSA) ng/mL). It can be found that there is no obvious difference in the oxidation current of various specimens when adding lower concentrations of BSA (10 ng/mL, 100 ng/mL). However, after adding a high concentration of BSA (1000 ng/mL, 10,000 ng/mL, 100,000 ng/mL), the oxidation currents of the various specimens apparently start to become different. It also can be found that the regression line slope of the unmodified S–BP specimen (−0.022) is “less tilted” than that of the modified specimens (S–BP–EDC/NHS specimen: −0.0318, S–BP–PSS: −0.0438, S–BP–SDS specimen: −0.034, and S–BP–TA specimen: −0.0388). This phenomenon implies that, with the increase of BSA concentration, the change in oxidation current of the unmodified S–BP specimen was less obvious than that of the modified specimens.

Figure 3b shows the DPV measurement results of the S–BP–EDC/NHS specimens after adding different concentrations of BSA. It can be found that the oxidation current of the S–BP–EDC/NHS specimen, compared with that of the S–BP specimen, trends prominently downwards with the log (BSA concentration). It is conceivable that an amine–reactive O–acylisourea intermediate develops [37] when the carboxyl groups on the surface of the S–BP specimens are modified by EDC/NHS. This O–acylisourea intermediate will form covalent amide bonds with the amine group on BSA. The covalent bond has strong bonding strength to conjugate with BSA more stably. Therefore, the measurement results also demonstrate that the S–BP–EDC/NHS specimens have a more obvious oxidation current feedback when adding different concentrations of BSA.

According to the linear regression analysis of the S–BP–EDC/NHS specimens (Figure 4b), it can be found that the added log (BSA concentration) presents a good inverse linear relationship with the feedback oxidation current, which represents that the S–BP–EDC/NHS specimens have a good linear dependence between log (BSA concentration) and oxidation current data.

The DPV measurements of the S–BP–PSS and S–BP–SDS specimens were quite similar (Figure 3c–d). After the concentration of BSA was higher than 100 ng/mL, the oxidation current of these specimens began to change significantly, and the change extent was also more obvious than that of the unmodified S–BP specimen. It is evident that these modified specimens, owning better hydrophilicity, improve the bonding effect between these specimens and aqueous BSA. Moreover, the oxidation currents measured by these specimens also differed with changes in BSA concentration. In the linear regression analysis
of these specimens (Figure 4c–d), it is clear that the oxidation currents of these specimens also roughly decrease with the increase of the logarithm concentration of BSA.

**Figure 3.** Differential Pulse Voltammetry (DPV) measurement of various specimens. (a) S–BP; (b) S–BP–EDC/NHS; (c) S–BP–PSS; (d) S–BP–SDS; (e) S–BP–TA.
Figure 4. Linear regression analysis diagram of various specimens. (a) S–BP; (b) S–BP–EDC/NHS; (c) S–BP–PSS; (d) S–BP–SDS; (e) S–BP–TA.
When comparing these four modified specimens, it can be observed that the S–BP–PSS and S–BP–SD specimens have similar measurement data, while the S–BP–EDC/NHS and S–BP–TA specimens are still slightly different from the other specimens. The possible reason is that the EDC/NHS and TA modifier can not only improve the hydrophilic properties of the specimens, but also has more O–acylisourea intermediate and conjugated structure (benzene), which is also beneficial to further bonding with BSA with covalent amide bond or conjugated structure. Therefore, the measured data of the S–BP–EDC/NHS and S–BP–TA specimens in the linear regression analysis showed a better linear dependence.

By comprehensively analyzing the measurement results of each modified specimen, it can be found that the hydrophilic/hydrophobic properties of the modifier affect the bonding effect between the specimen and BSA. However, if the modifier can produce an amide bond or conjugate structure on the specimen, it will have a more significant and positive influence on the bonding effect between the specimen and BSA.

3.3. Functional Group Detection

In order to explore whether the various modifiers were successfully attached on the surface of S–BP specimens to achieve the effect of modification, the functional groups of various S–BP specimens before and after modification were analyzed by FTIR.

Figure 5 shows the FTIR analyses of various specimens. The C=C peak (1600~1650 cm$^{-1}$) and hydroxyl functional groups (–OH) peak (2900~3100 cm$^{-1}$) denote the CNTs and carboxylated CNTs respectively, which can be found in the unmodified S–BP specimens. The S–BP–EDC/NHS specimen has the C=O peak (1650~1700 cm$^{-1}$) and the N–H peak (3200~3400 cm$^{-1}$), which is consistent with the chemical formula of EDC/NHS ($C_8H_{17}N_3/C_4H_5NO_3$). It indicates that EDC/NHS is successfully attached to the surface of the specimen.

In the S–BP–PSS specimen, there are the S=O peaks (1380~1435 cm$^{-1}$) of sulfonates and the C–H peaks (2840~3000 cm$^{-1}$) of alkenes. Both peaks are consistent with the chemical formula of PSS ($C_8H_7NaO_3S_n$). It is believed that the PSS is also successfully attached to the specimen.

![Figure 5. Fourier transform infrared spectroscopy (FTIR) analyses of various specimens.](image-url)
The S–BP–SDS specimen has the S=O peak (1250~1315 cm\(^{-1}\)) and the C–H peak (2900~3000 cm\(^{-1}\)) that denote the elements of sulfate and alkane respectively. Both peaks are consistent with the SDS chemical formula of NaC\(_{12}\)H\(_{25}\)SO\(_4\), which indicates that SDS can be successfully attached to the specimen.

In the S–BP–TA specimen, in addition to the C=O peak (1650~1700 cm\(^{-1}\)) representing the carboxyl group (–COOH), the intensity of the hydroxyl (–OH) peak (2900~3000 cm\(^{-1}\)) is also higher than that of the unmodified S–BP specimen. This means that the S–BP–TA specimen not only contains the hydroxyl group of the S–BP specimen itself, but also adsorbs the hydroxyl group in the modifier TA, and these two peaks are also consistent with the chemical formula of TA (C\(_{76}\)H\(_{52}\)O\(_{46}\)). It is speculated that TA is also successfully attached to the specimen.

3.4. Observation of Microstructure

Figure 6 shows the microstructure of various specimens before adding BSA. It can be found that in the original unmodified S–BP specimen (Figure 6a) prepared from carboxylated CNTs, the CNTs have some cutting surfaces due to the carboxylation treatment (as indicated by the arrow). The CNTs of the specimen, without agglomeration, intertwined and stacked with each other, as well as distributed evenly. This implies that using pumping filtration to make buckypaper films has a good effect. By observing the microstructure of various specimens modified by different modifier (Figure 6b–e), it is found that all modified specimens showed similar surface morphologies, and the differences cannot be distinguished clearly by SEM.

In order to investigate whether the modifier is successfully attached to the specimen, this study not only uses FTIR to analyze the functional groups of each modified specimen, but also employs EDX to analyze the element compositions of the modified specimen surfaces. As shown in Figure 7, it can be found that in the unmodified S–BP specimen (Figure 7a), the EDX signal is mostly dominated by the element of carbon (C), and contains a weak signal of the element of oxygen (O). This weak signal is caused by the oxygen-containing functional groups on carboxylated CNTs.
Figure 7. EDX analyses of various specimens. (a) S–BP; (b) S–BP–EDC/NHS; (c) S–BP–PSS; (d) S–BP–SDS; (e) S–BP–TA.
In the S–BP–EDC/NHS specimen (Figure 7b), it can be found that the signal of the element of oxygen (O) is slightly stronger than that of the unmodified S–BP specimen, but the element of nitrogen (N) in the chemical formula of EDC/NHS (C₈H₁₇N₃/C₄H₅NO₃) cannot be detected. It is believed to be due to the fact that the content of the nitrogen element (N) is too scant to be detected. Nevertheless, from the EDX signal of the oxygen element (O) and the analytic results of the functional groups in Figure 5, it can be inferred that EDC/NHS is successfully attached to the surface of the specimen. In the S–BP–PSS (Figure 7c) and S–BP–SDS specimens (Figure 7d), the detectable EDX signals of the sulfur element (S) and the sodium element (Na), corresponding to the chemical formula (C₈H₇NaO₃S)_ₙ of PSS and the chemical formula of SDS (NaC₁₂H₂₅SO₄), indicate that both PSS and SDS are attached to the specimen surface. In the EDX element analysis of the S–BP–TA specimen (Figure 7e), it can be found that the signal of the oxygen element (O) is stronger than that of the unmodified S–BP specimen, which also accords with the chemical formula of TA (C₇₆H₅₂O₄₆). Therefore, it is reasonable to infer that TA is attached to the surface of the specimen. Based on the analytic results of FTIR and EDX, it can be proven that the various modifiers can successfully attach to the surfaces of the specimens by spin coating.

Figure 8 shows the microstructure of various specimens after adding two different concentrations (100 ng/mL and 100,000 ng/mL) of BSA. It is clear that there is no obvious BSA particle adhesion on the surface of the S–BP specimen with 100 ng/mL added BSA (Figure 8a). The main cause is due to the low concentration of added BSA and the weak bonding strength between BSA and the surface of the S–BP specimen. When the added BSA concentration was 100,000 ng/mL, more BSA particles could be obviously observed on the surface of the S–BP specimen (as indicated by the arrow) (Figure 8b), so the corresponding electrochemical measurement results also showed more obvious changes.

However, the microstructures of the modified specimens after adding BSA are different from those of the S–BP specimens (Figure 8c–j). It can be found that even after adding a low concentration (100 ng/mL) of BSA to the S–BP–EDC/NHS specimen, some BSA particles can still be observed on the surface of the S–BP–EDC/NHS specimen (Figure 8c). This shows that the bonding effect between the S–BP–EDC/NHS specimen and BSA is better than that of the S–BP specimen. When the added BSA concentration was 100,000 ng/mL (Figure 8d), it reveals that the surface of the S–BP–EDC/NHS specimen is covered with a large range of BSA particles, which also explains the reason for the most obvious change in electrochemical properties of the S–BP–EDC/NHS specimen after adding BSA.

In the microstructure of the S–BP–PSS, S–BP–SDS and S–BP–TA specimens with 100 ng/mL added BSA, it can be observed that BSA with a membrane–like structure is bonded to the surface of the specimens (Figure 8e,g,i). According to the contact angle measurements (Figure 2c–e), the modifiers of PSS, SDS, and TA can reduce the surface tension, improve the hydrophilic property of these three specimens, and avail the combination of the specimen and aqueous BSA to achieve a membrane–like structure [38]. When 100,000 ng/mL BSA is added (Figure 8f,h,j), a large area of membrane–like BSA can also be observed on the surface of these three specimens. These phenomena also provide the main reason why the electrochemical measurement performance of these three modified specimens is different from that of the unmodified S–BP specimen.

From the microstructure observation of all modified specimens with 100,000 ng/mL added BSA, it can be found that the BSA attached on the surfaces of the S–BP–EDC/NHS and S–BP–TA specimens seems to show a larger area and denser morphologies. Furthermore, the corresponding data of these two specimens in the linear regression analysis also exhibit a better linear dependence. For the modified specimens, this phenomenon once again shows that the hydrophilic property affects the bonding effect with BSA, and the bonding type (such as amide bond or conjugate structure, etc.) produced by the modifier on the specimens also performs an important role in the bonding effect between the specimen and BSA.
Figure 8. Microstructure of various specimens after adding two different concentrations (100 ng/mL and 100,000 ng/mL) of BSA. (a) S–BP, 100 ng/mL; (b) S–BP, 100,000 ng/mL; (c) S–BP–EDC/NHS, 100 ng/mL; (d) S–BP–EDC/NHS, 100,000 ng/mL; (e) S–BP–PSS, 100 ng/mL; (f) S–BP–PSS, 100,000 ng/mL; (g) S–BP–SDS, 100 ng/mL; (h) S–BP–SDS, 100,000 ng/mL; (i) S–BP–TA, 100 ng/mL; (j) S–BP–TA, 100,000 ng/mL. “Arrows” indicate the positions of part of the BSA covering on the specimen surface.
4. Conclusions

In this study, carboxylated CNTs were used to make buckypaper composite films and further modify them by incorporating different surface modifiers. After adding different concentrations of BSA, DPV electrochemical measurement was applied to measure the characteristics of the specimens. It is observed that the hydrophilic properties of all modified specimens are better than that of the original unmodified S–BP specimen. Succinctly, the changes brought about by using modifiers can improve the bonding effects between modified specimens and aqueous BSA. Therefore, the variation of electrochemical properties for the modified specimens after adding BSA is more apparent than that of the original S–BP specimen. In the linear regression analyses of all specimens, the oxidation current of a specimen roughly decreases with the increase of logarithm concentration of BSA (log(BSA) ng/mL). The regression line slope of the unmodified S–BP specimen is less tilted than that of the modified specimens. This phenomenon implies that, with the increase of BSA concentration, the change in oxidation current of the unmodified S–BP specimen was less obvious than that of the modified specimens. It is noteworthy that the bonding type produced by the modifier has a more prominent influence than the hydrophilic property on the bonding effect between the specimen and BSA.

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