Supplementary information

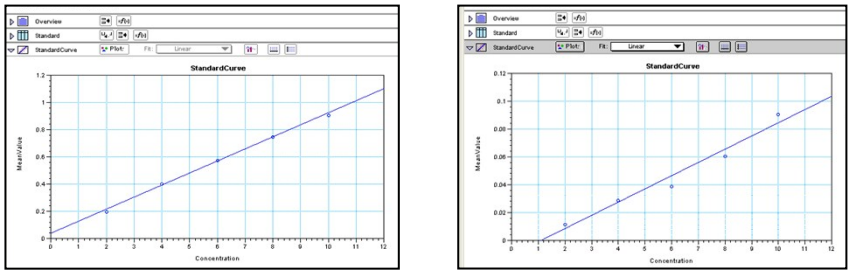
Enzyme free detection of Staphylococcal Enterotoxin B (SEB) using ferrocene carboxylic acid labeled monoclonal antibodies: An electrochemical approach

Arun Sharma, Vepa Kameswara Rao*, Dev Vrat Kamboj, Ritu Gaur, Mahabul Shaik, Anchal Roy Shrivastava

Defence Research and Development Establishment, Gwalior (M.P.), India – 474002

1. Affinity chromatography based purification, estimation and SDS-PAGE analysis of SEB and IgG samples

Unpurified rabbit anti-SEB polyclonal IgG and mice anti-SEB monoclonal IgG was purified by affinity chromatography based method as per details given by the manufacturer. In this method, unpurified serum sample was first mixed properly with the binding buffer. After that the "*Protein A Cartridge"* was washed with regeneration buffer. For this purpose, regeneration buffer was passed through the cartridge at the approx flow rate of 1mL/min. Then the cartridge was equilibrated by binding buffer by passing the binding buffer through the cartridge at the same flow rate. Then, we had loaded the sample-binding buffer mixture by passing it to the "*Protein A Cartridge"* at the approx flow rate of 0.5mL/min. Binding buffer was passed through the cartridge after the sample loading at the flow rate of about 1mL/min. Desalting cartridge was washed with [N-(2-hydroxyethyl)peiperazine-N'-(2-ethanesulfonic acid)] i.e. (HEPES) buffer by passing it through the cartridge at an approximate flow rate of 1mL/min. Then we had attached the one end of the "*Protein A Cartridge"* to another end of the desalting cartridge. Then, we had eluted the cartridges with elution buffer by passing it through the cartridges at an approximate flow rate of 0.5mL/minute. Elute was contained the purified IgG at physiological pH. After it, we had detached both the cartridges and regenerate them. "*Protein A Cartridge"* was regenerated by regeneration buffer by passing it through the cartridge. HEPES buffer was passed through the desalting cartridge and was regenerated. These cartridges are ready and can be used for another affinity chromatographic purification.



(a) **(b)**

Figure S1. (a) Shows the standard graph obtained for the estimation of rabbit anti-SEB IgG (b) shows for estimation of mice anti-SEB IgG using BCA-protein estimation method

Bicinchoninic acid protein assay kit and spectrophotometric method was utilized for the estimation of purified rabbit anti-SEB polyclonal IgG and mice anti-SEB monoclonal IgG concentration. For this purpose, bicinchoninic acid (BCA) working reagent was prepared by mixed with 50 parts of reagent A (containing bicinchonic acid, sodium carbonate, sodium tartrate, and sodium bicarbonate in 0.1N NaOH, pH 11 with 1 part of reagent B (containing 4% (w/v) copper(II) sulfate pentahydrate). In the standard procedure, BCA working reagent was taken in 200µL are mixed with 0, 2, 4, 6, 8, 10µL part of a standard protein (BSA) solution of known concentration. 10µL of water without protein was used as blank. Unknown purified IgG sample was assayed with the known concentration of BSA protein standard. After that, the plate was incubated at 37°C for 30min. In the next step, absorbance was recorded at 562nm and the unknown protein concentration was determined by comparison to the standard curve. It was estimated that 2.4mg/mL IgG is present in rabbit-IgG of SEB and 1.0mg/mL IgG was present in mice-IgG of SEB.

2. SDS-PAGE analysis of purified rSEB, mouse anti-SEB IgG and rabbit anti-SEB IgG

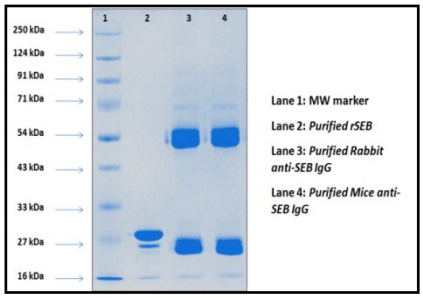


Figure S2. 12% SDS-PAGE gel image of Molecular weight marker (lane-1), purified rSEB (lane 2), purified polyclonal rabbit anti-SEB IgG (lane 3) and purified monoclonal mice anti-SEB IgG (lane 4).

Sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) protocol was followed as per details given by the manufacturer. SDS-PAGE was performed to check the purity of SEB, rabbit anti-SEB IgG and mouse anti-SEB IgG. Two bands are observed that corresponding to heavy and light chain of the purified rabbit and mice IgG raised against SEB (lane 3 & 4 respectively in fig.S2). A sharp band around 28.4 kDa (lane 2 in fig.S2) clearly indicates the presence of SEB. In this experiment, separating gel mixture (12% final concentration in 5mL) was prepared by mixing the stock solution of 30% acryl amide (2mL), 1.5M Tris-buffer, pH 8.8 (1.25mL), 10% SDS (50µL) and distilled water (1.65mL) and then N,N,N′,N′-tetramethylethylenediamine (TEMED) (5µL) and 10% ammonium persulphate (50µL) was added. This mixture was transferred into the gel mould and overlay it with 50µL of butanol and then kept for 40min for polymerization. In the next step, 2.5mL of 5% stacking gel was prepared by mixing 415µL of 30% acrylamide, 315µL of 1M Tris-buffer, pH 6.8, 25µL of 10% SDS and 1.7mL distilled water and then 5µL TEMED and 25µL ammonium persulphate was added. After that, stacking gel was transferred into the gel mould that contains the already polymerized separating gel. At the same time, suitable comb was carefully inserted without any air bubbles to make slots or loading wells for sample application. After complete polymerization, PAGE apparatus was appropriately filled with 1x running buffer (composition of 10x running buffer in 500mL is 93.75gm glycine, 5gm SDS and 15.125gm Tris). Then, the comb was carefully removed and the samples such as purified rSEB (lane 2), purified polyclonal rabbit anti-SEB IgG (lane 3) and purified monoclonal mice anti-SEB IgG (lane 4) were loaded in separate wells. Standard protein molecular weight marker was loaded in separate slot for the accurate molecular weight determination of protein samples. After that 10mA fixed current was applied till the samples crossed the stacking gel and then the electric current was increased to 20mA. It was performed until the molecular weight marker reaches at the bottom of the gel.

3. Validation of antibodies by Western blot

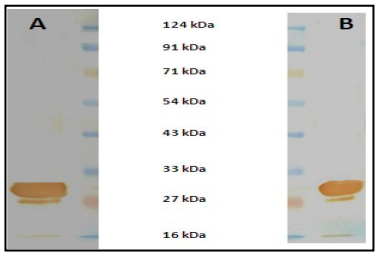


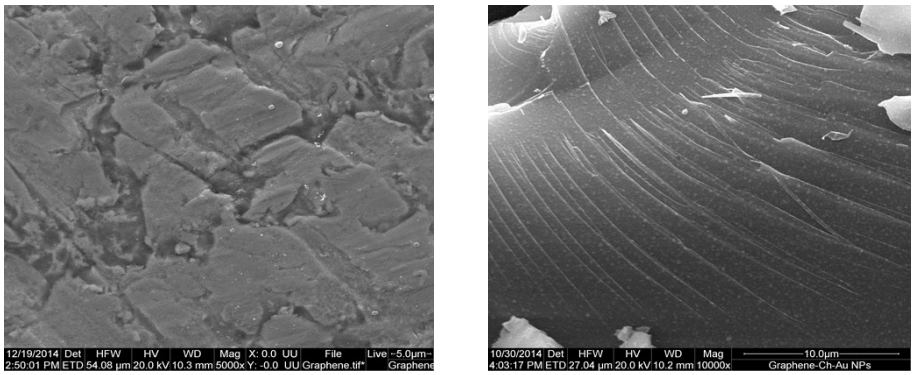
Figure.S3. (A) shows the western blot of SEB with monoclonal mice anti-SEB IgG and (B) is the western blot of SEB with polyclonal rabbit anti-SEB IgG.

In the present study, affinity purified SEB was used to raise antibodies in mice and rabbit models. These antibodies were evaluated by western blot. It is found that the antibodies are pure and bind with SEB [Fig.S3 (A) & (B)]. Furthermore, we can state that the antiserum raised in this study was found to be more specific for SEB detection compare to other commercial antiserum available from M/s Toxin Technology, USA [Kamboj et al., 2006].

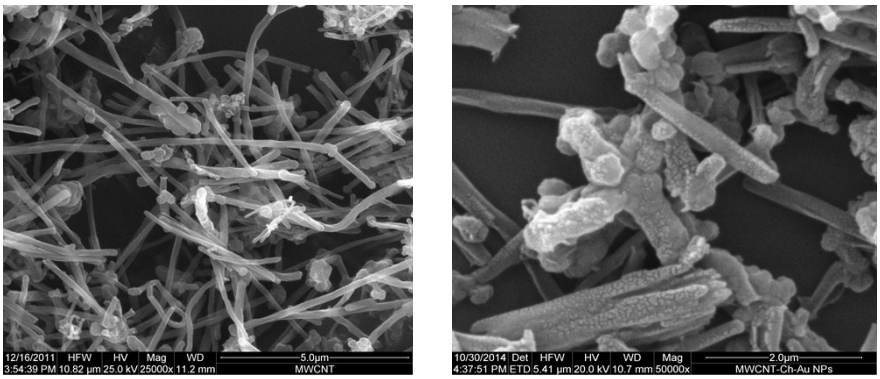
4. Animal care and use

Female BALB/c mice (6-8 weeks old) were obtained from Defence Research and Development Establishment's animal facility and were given water and food *ad libitum*. All mice were housed and used in accordance with the recommendations of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), India. The study had the approval of Institutional Animal Ethics Committee (IAEC).

5. SEM characterization



(A) (B)

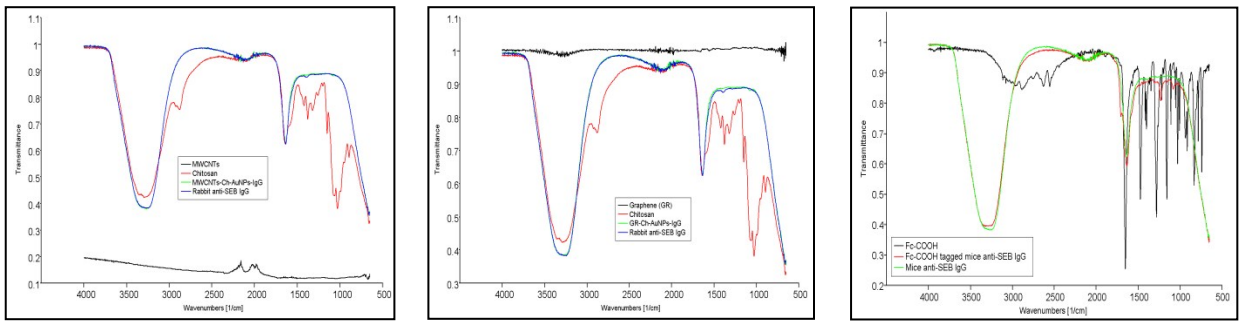


(C) (D)

Figure S4.(A) SEM-image of graphene (GR) sheets, (B) for graphene-Ch-Au NPs, (C) for MWNCTs and (D) for MWCNTs-Ch-Au NPs nanocomposites

GR, GR-Ch-Au NPs, MWCNTs and MWCNTs-Ch-Au NPs were characterized by SEM. Graphene (GR) is giving overlapped multiple layered with fairly smooth-sheet like morphology and due to nanoscale sheet interlocking it is having good film-forming ability [Xu et al., 2008]. The lateral dimensions of the graphene sheets were around 300nm which indicates its atomic thickness composition [Fig.1S(A)]. Due to presence of small cracks on the crumpled island like structure of graphene it can provide a suitable platform for the immobilization of biomolecules for immunosensing applications. After treatment of graphene with chitosan and Au NPs formation on its surface are clearly seen in the SEM-image [Fig.1S(B)]. It can be observed from [Fig.1S(B)] that gold nanoparticles with the average size of 20-50nm were uniformly distributed on the chitosan modified graphene surface. The diameters of carbon nanotubes were ranging from 20 to 60 nm and length range from 1 to 3µ. Moreover, no residuals can be found and the surface of nanotubes was smooth and tidy [Fig.1S(C)]. MWCNTs were successfully functionalized with gold nanoparticles (Au NPs) seen in [Fig.1S(D)]. This confirms that the synthesized materials are falling under the category of nanocompounds.

6. FT-IR characterization of capturing [GR-Ch-Au NPs-CAb], [MWCNTs-Ch-Au NPs-CAb] bionanoconjugates and revealing antibodies [Fc-MAb]



(a) (b) (c)

Figure S5. (a) FT-IR characterization of [MWCNTs-Ch-Au NPs-CAb], (b) for [GR-Ch-Au NPs-CAb] bionanoconjugates and (c) for revealing antibodies [Fc-MAb].

In this work, the formation of the GR-Ch-AuNPs-CAb, MWCNTs-Ch-AuNPs-CAb bionanoconjugates and revealing antibodies [Fc-MAb] was checked by FTIR-characterization method. The FTIR spectra of MWCNTs, chitosan, rabbit anti-SEB polyclonal IgG and [MWCNTs-Ch-Au NPs-CAb] bionanoconjugates are shown in Fig. S5(a). For MWCNTs, the peak at 1653cm^{-1} belongs to the C=O stretching vibration of the -COOH group, which can also be observed in [MWCNTs-Ch-Au NPs-CAb] bionanoconjugates. More importantly, for MWCNTs hybrid, the sharp peak located at 2085 cm^{-1} is attributed to the C≡ stretching vibration [Li et al., 2007]. Besides this in the FTIR- spectrum of graphene solution is shown in Figure S5(b). The absence of graphite and graphene oxide peaks is a strong indication of the obtaining of graphene sheets [Mohammed et al., 2012]. The characteristic absorption of the chitosan is the band at 1559.17 cm^{-1} which is assigned to the stretching vibration of amino group of chitosan and 1333.5cm^{-1} assigned to vibration of C-H. Another band at 3367.1cm^{-1} is due to amine NH symmetric vibration. The peak of 2927.41 cm^{-1} is typical C-H vibration. Chitosan characteristic peaks are coming around 896.73 and 1154.19 cm^{-1} which correspond to saccharide structure of chitosan. The broad peak at 1080.91cm^{-1} indicates C-O stretching vibration [Krishna Rao et al. 2006]. The FT-IR of capturing antibodies bionanoconjugates indicates that it was successfully formed.

In the FT-IR spectrum of ferrocene carboxylic acid fig.S5(c), C–O stretch appears in the region 1320-1210 cm^{-1}, O-H stretch from 3300-2500cm^{-1}, C=O stretch from 1760-1690cm^{-1} and the O–H bend is in the region 1440-1395 cm^{-1} and 950-910cm^{-1}. The secondary structure of IgG samples were examined by FTIR given in fig.S5(c). The spectrum displays a predominantly

β-sheet structure with a typical minimum at 1636 cm^{-1}. The peptide group, the structural repeat unit of proteins, gives up to 9 characteristic bands named amide A, B, I, II ... VII. Amide A is with more than 95% due to the N-H stretching vibration. This mode of vibration does not depend on the backbone conformation but is very sensitive to the strength of a hydrogen bond. It has vibrations between 3225 and 3280 cm^{-1} for hydrogen bond lengths between 2.69 to 2.85 Å, [Krimm et al., 1986]. The amide A band (about 3500cm^{-1}) and amide B (about 3100cm^{-1}) originate from a Fermi resonance between the first overtone of amide II and the N-H stretching vibration. Amide I and amide II bands are two major bands of the protein infrared spectrum. The amide I band (between 1600 and 1700 cm^{-1}) is mainly associated with the C=O stretching vibration (70-85%) and is directly related to the backbone conformation. Amide II results from the N-H bending vibration (40-60%) and from the C-N stretching vibration (18-40%). This band is conformationally sensitive. Amide III and IV are very complex bands resulting from a mixture of several coordinate displacements. The out-of-plane motions are found in amide V, VI and VII. FTIR analysis of ferrocene conjugated antibodies indicated that the conjugated antibodies maintain the significant native-like secondary structure suggesting that labeling of antibodies led to the minimal structure changes [Brych et al., 2010]. It shows that ferrocene carboxylic acid was successfully tagged with antibodies.

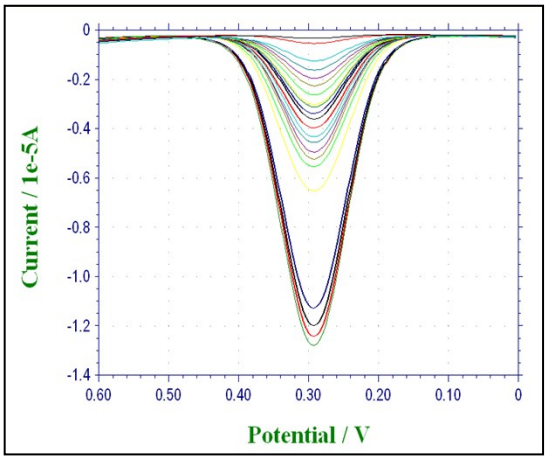
7. Optimization of various parameters

BCA, SDS-PAGE and SWV method was used for the optimization of concentration of polyclonal rabbit anti-SEB IgG (capturing antibodies) attached with GR-Ch-Au NPs and MWCNTs-Ch-Au NPs nanocomposites. The same method is also used for the optimization of monoclonal mice anti-SEB IgG which were conjugated with ferrocene carboxylic acid. In brief, 250µg/mL and 150µg/mL capturing antibodies were optimized and used for the bionanoconjugation with GR-Ch-Au NPs and MWCNTs-Ch-Au NPs nanocomposites respectively. Furthermore, 200µg/mL monoclonal mice anti-SEB IgG were optimized and used for the conjugation with ferrocene carboxylic acid. The details of these optimizations were given in the coming sections.

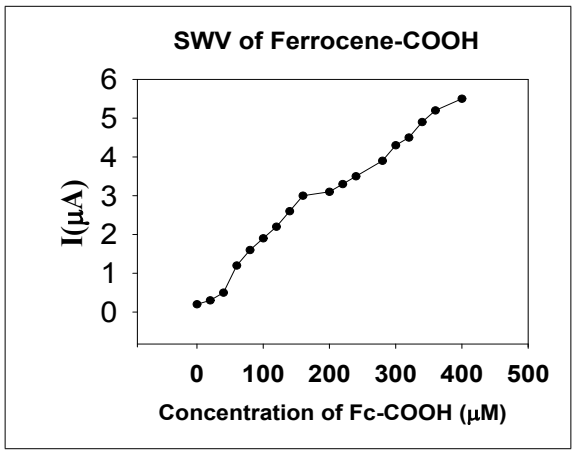
8. Quantitative determination of the [Fc:MAb] ratio present in the 5µL of revealing antibodies conjugates

For the quantitative determination of [Fc:MAb] ratio present in the 5µL of ferrocene carboxylic acid (Fc) tagged mice anti-SEB monoclonal antibodies conjugates that is the final volume of the revealing antibodies which was used for the electrochemical immunoassay of SEB. Simplest two step procedure was utilized for determination. In the first step, amount of Fc present in Fc-antibody conjugates was determined using standard square wave voltammetric method. Amount of IgG present in Fc-antibody conjugates was determined in the second step using standard BCA-protein estimation method. Finally, it was calculated that Fc:MAb ratio in the 5µL of revealing antibodies was 1:7.8 by weight. We can state that about 88.64% antibodies were conjugated with 11.36% Fc by weight. So, there is large amount of specific mice anti-SEB monoclonal antibodies on ferrocene carboxylic acid (Fc) and high sensitivity for the electrochemical detection of SEB was achieved. These steps are described below:

Step 1. *Investigation of amount of ferrocene carboxylic acid present in the revealing antibodies [Fc-MAb]*



(a)

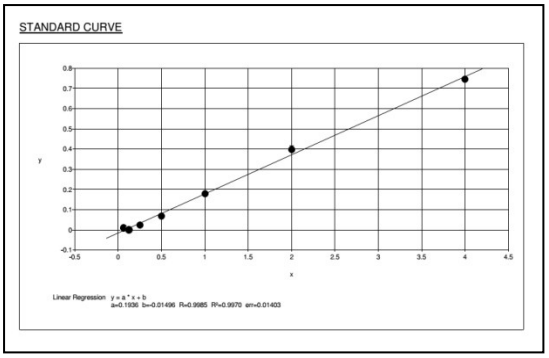


(b)

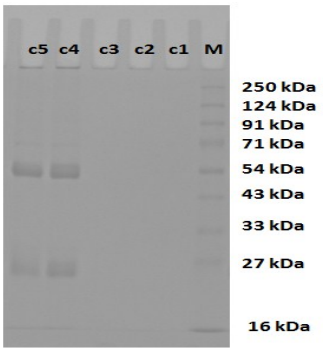
Figure S6. (a). SWV obtained at different concentrations of ferrocene carboxylic acid and (b) shows the standard curve for the determination of ferrocene carboxylic acid present in its antibody conjugates

For the quantitative determination of ferrocene carboxylic acid present in the 5µL of revealing antibodies [Fc-MAb] bioconjugates which was used for the electrochemical immunoassay of SEB. Amount of ferrocene carboxylic acid in revealing antibodies [Fc-MAb] was determined by square wave voltammetric (SWV) analysis. For this purpose, SWV was performed at various concentrations of ferrocene carboxylic acid on bare GCE fig.S6(a) and the standard graph is plotted between the obtained voltammetric peak current and concentration fig. S6(b). Then, 5µL of revealing antibodies [Fc-MAb] is put on GCE surface and kept in the incubator for 15min at 37^0C. After incubation SWV was performed and the corresponding concentration (i.e. 113.5µM) for the observed peak current (i.e. 2.15µA) was found on the bare GCE. It is estimated that 113.5µM of ferrocene carboxylic acid present in the 5µL of revealing antibodies [Fc-MAb] monoclonal antibodies conjugates. Finally, 130.5ng ferrocene carboxylic acid was present in 5µL of revealing antibodies [Fc-MAb]. After modification of GCE the signal is greatly enhanced for the same amount of ferrocene carboxylic acid labeled antibodies and it was 7.13µA and 2.99µA for GR-Ch-Au NPs and MWCNTs-Ch-Au NPs modified GCE. It is verified that the signal amplification strategy was successfully achieved on modified GCE.

Step 2. *Determination of amount of mice anti-SEB IgG present and its confirmatory binding in the revealing antibodies [Fc-MAb]*



(a)



(b)

Figure S7. (a) Standard graph obtained for increasing concentration of BSA using BCA-protein estimation method and (b) shows the SDS-PAGE gel image of different batches of [Fc-MAb] bioconjugates

For the determination of amount of IgG bound with ferrocene carboxylic acid standard solution of BSA (1µg/µL) was prepared and standard curve was plotted using BCA-protein estimation method as shown in fig.S7(a). We had obtained 0.203 µg/µL concentration of protein i.e. mice anti-SEB antibody present in the Fc-antibody conjugates. Since, 1.015µg of mice anti-SEB monoclonal antibody was present in 5µL of the Fc-antibody conjugates which was the final amount used in the electrochemical immunoassay of SEB for the experiments. It was calculated that Fc:MAb ratio in the 5µL of bionanoconjugates was 1:7.8. It means about 88.64% antibodies were conjugated with 11.36% Fc by weight. So, there is high loading of monoclonal antibodies on ferrocene carboxylic acid and high sensitivity for the electrochemical detection of SEB was achieved.

SDS-PAGE is also done for the above prepared batches of [Fc-MAb] bioconjugates. Different concentrations of mice anti-SEB monoclonal IgG [MAb] i.e. 100µg/mL, 150µg/mL and 200µg/mL conjugated with fixed amount of ferrocene carboxylic acid (10mg) not showing any band in their gel image of lane c1, c2 and c3 respectively (fig.S7b). The band of heavy and light chain for unbound or extra mice anti-SEB monoclonal IgG [MAb] is appeared in c4 and c5 lane in the obtained gel image which confirms the excess of unbound antibodies.

9. Optimization of amount of mice anti-SEB IgG in the revealing antibodies [Fc-MAb] for the immunosensing of SEB

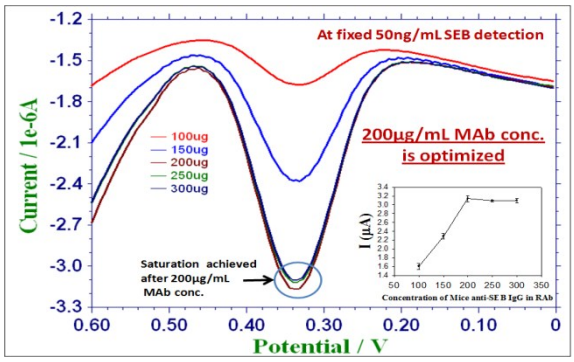


Figure S8. SWV obtained for immunosensing of 50ng/mL SEB at different concentrations (100, 150, 200, 250 and 300µg/mL) of mice anti-SEB monoclonal IgG [MAb] in their [Fc-MAb] bioconjugates

A series of revealing antibodies [Fc-MAb] bioconjugates were prepared in the batches at different concentrations (100, 150, 200, 250 and 300µg/mL) of mice anti-SEB monoclonal IgG [MAb] under a fixed amount of ferrocene carboxylic acid (10mg). The amount of MAb attached to ferrocene carboxylic acid was determined by SWV and BCA method. The SWV experiment is performed for the immunosensing of 50ng/mL SEB using GR-Ch-Au NPs-CAb immobilized GCE with each batch independently for three times (Fig.S8). The amount of CAb is arbitrarily fixed (250µg/mL) in GR-Ch-Au NPs-CAb bionanocomposite. The obtained peak current is increased with increasing the concentration of MAb and saturation is achieved after 200µg/mL MAb concentration (inset of fig.S8). Control experiment is also performed in absence of SEB and revealing antibody and baseline current is recorded. Finally, 200µg/mL MAb concentration is optimized and used as such in the subsequent experiments.

10. Determination of the amount of rabbit anti-SEB IgG (capturing antibodies) present and its confirmatory binding in the [GR-Ch-Au NPs-CAb] bionanoconjugates

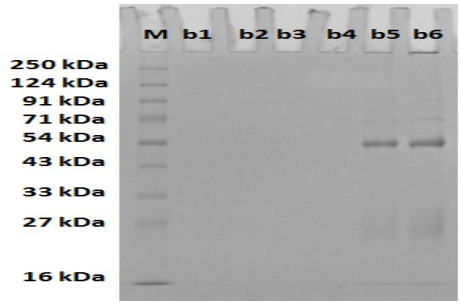


Figure S9. Shows the SDS-PAGE gel image of different batches of [GR-Ch-Au NPs-CAb] bionanoconjugates

For the determination of amount of rabbit anti-SEB IgG bound with GR-Ch-Au NPs nanocomposite, standard solution of BSA (1µg/µL) was prepared and standard curve was plotted using BCA-protein estimation method as shown in fig.S7(a). We had obtained 0.275µg/µL

concentration of protein i.e. rabbit anti-SEB antibody present in the GR-Ch-AuNPs-capturing antibody conjugates. Since, 1.375µg of rabiit anti-SEB antibody was present in 5µL of the GR-Ch-AuNPs-CAb bionanoconjugates which was the final amount used in the experiments.

SDS-PAGE is also done for the above prepared batches of [GR-Ch-Au NPs-CAb] bionanoconjugates. Different concentrations of rabbit anti-SEB monoclonal IgG [CAb] i.e. 100µg/mL, 150µg/mL, 200µg/mL and 250µg/mL conjugated with fixed amount of GR-Ch-Au NPs nanocomposite (1mL) not showing any band in their gel image of lane b1, b2, b3 and b4 respectively (fig.S9). The band of heavy and light chain for unbound or extra capturing antibodies is appeared in b5 and b6 lane in the obtained gel image which confirms that these unbound antibodies were detached during washing step and does not play any significant role in the immunosensing of SEB.

11. Optimization of the amount of rabbit anti-SEB IgG (capturing antibodies) in the [GR-Ch-Au NPs-CAb] bionanoconjugates for immunosensing of SEB

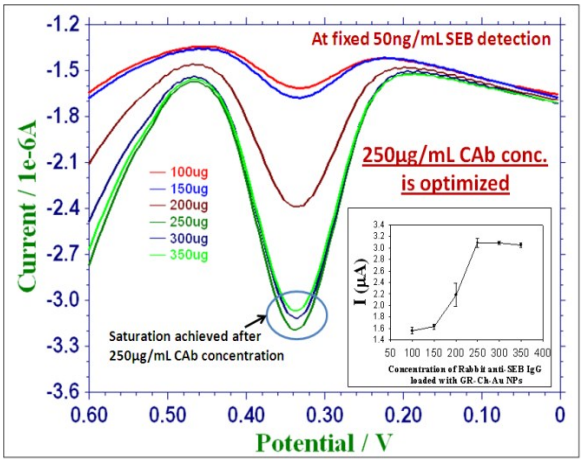


Figure S10. SWV obtained for immunosensing of 50ng/mL SEB at different concentrations (100, 150, 200, 250, 300 and 350µg/mL) of rabbit anti-SEB polyclonal IgG [CAb] in their [GR-Ch-Au NPs-CAb] bionanoconjugates

Different batches of capturing antibodies [GR-Ch-Au NPs-CAb] bionanoconjugates were prepared at different concentrations (100, 150, 200, 250, 300 and 350µg/mL) of rabbit anti-SEB polyclonal IgG under a fixed amount of GR-Ch-Au NPs nanocomposite (1mL). The amount of capturing antibodies attached to GR-Ch-Au NPs nanocomposite was optimized by SWV and

BCA-method. The SWV experiment is performed for the immunosensing of 50ng/mL SEB using different batches of [GR-Ch-Au NPs-CAb] immobilized GCE independently for three times (fig.S10). The obtained peak current is increased with increasing the concentration of capturing antibodies and saturation is achieved after 250µg/mL capturing antibodies concentration (inset fig.S10). Control experiment is also performed in absence of SEB and revealing antibody and baseline current is recorded. Finally, 250µg/mL capturing antibodies concentration is optimized and used as such throughout the experiments.

12. Determination of the amount of rabbit anti-SEB IgG (capturing antibodies) present and its confirmatory binding in the [MWCNTs-Ch-Au NPs-CAb] bionanoconjugates

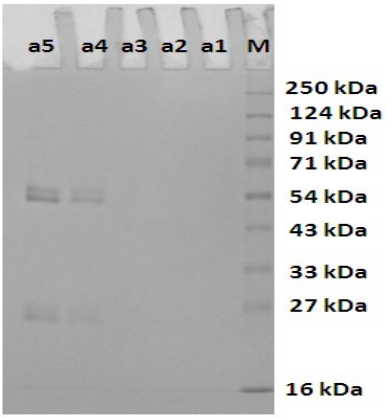


Figure S11. Shows the SDS-PAGE gel image of different batches of [MWCNTs-Ch-Au NPs-CAb] bionanoconjugates

For the determination of amount of rabbit anti-SEB IgG bound with MWCNTs-Ch-Au NPs nanocomposite, standard solution of BSA (1µg/µL) was prepared and standard curve was plotted using BCA-protein estimation method as shown in fig.S7(a). We had obtained 0.145µg/µL concentration of protein i.e. rabbit anti-SEB antibody present in the GR-Ch-AuNPs-capturing antibody conjugates. Since, 0.725µg of rabbit anti-SEB antibody was present in 5µL of the MWCNTs-Ch-AuNPs-CAb bionanoconjugates which was the final amount used in the electrochemical immunoassay of SEB for the experiments.

SDS-PAGE is also done for the above prepared batches of [MWCNTs-Ch-Au NPs-CAb] bionanoconjugates. The band of heavy and light chain for unbound or extra capturing

antibodies is appeared in a4 and a5 lane in the obtained gel image (fig.S11) which confirms that these unbound antibodies were detached during washing step and does not play any significant role in the immunosensing of SEB.

13. Optimization of the amount of rabbit anti-SEB IgG (capturing antibodies) in the [MWCNTs-Ch-Au NPs-CAb] bionanoconjugates for immunosensing of SEB

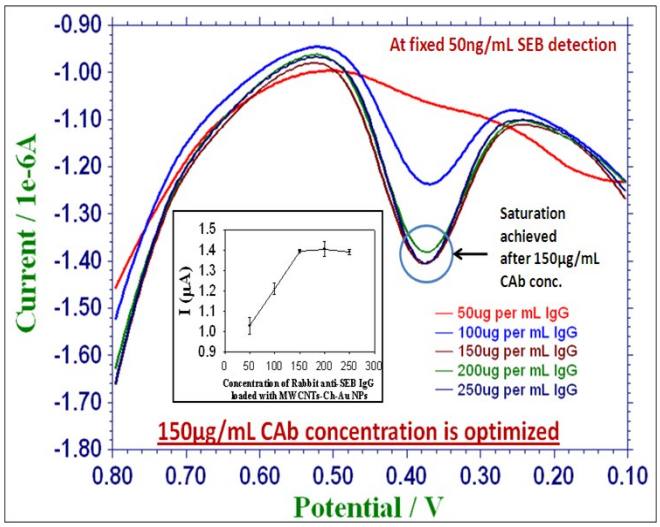
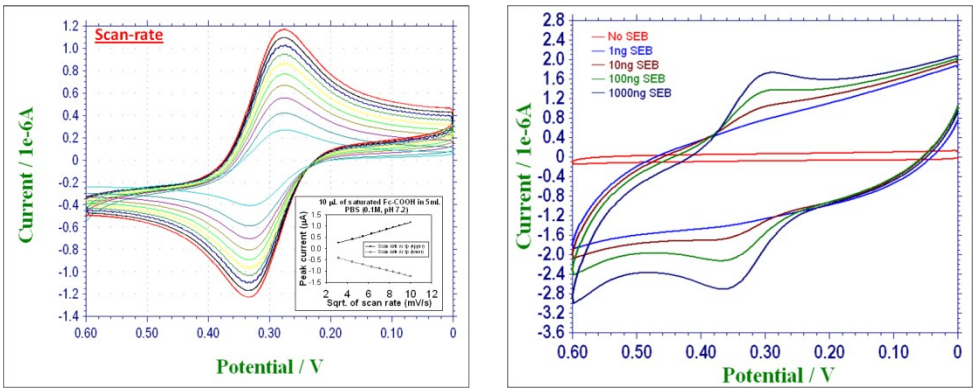


Figure S12. SWV obtained for immunosensing of 50ng/mL SEB at different concentrations (50, 100, 150, 200 and 250µg/mL) of rabbit anti-SEB polyclonal IgG [CAb] in their [MWCNTs-Ch-Au NPs-CAb] bionanoconjugates

Different batches of capturing antibodies [MWCNTs-Ch-AuNPs-CAb] bionanoconjugates were prepared at different concentrations (50, 100, 150, 200 and 250µg/mL) of rabbit anti-SEB polyclonal IgG under a fixed amount of MWCNTs-Ch-Au NPs nanocomposite (1mL). The amount of capturing antibodies attached to MWCNTs-Ch-Au NPs nanocomposite was optimized by SWV and BCA-method. The SWV experiment is performed for the immunosensing of 50ng/mL SEB using different batches of [MWCNTs-Ch-Au NPs-CAb] immobilized GCE independently for three times (fig.S12). The obtained peak current is increased with increasing the concentration of capturing antibodies and saturation is achieved after 150µg/mL capturing antibodies concentration (inset fig.S12). Control experiment is also performed in absence of SEB and revealing antibody and baseline current is recorded. Finally,

150µg/mL capturing antibodies concentration is optimized and used in the subsequent experiments.

14. Cyclic voltammetric characteristics of ferrocene-based immunosensor



(a) (b)

Figure S13. (a) Cyclic voltammograms at different scan rates for ferrocene carboxylic acid and (b) is the cyclic voltammograms at different concentrations of SEB at [GR-Ch-Au NPs-CAb-SEB-Fc-MAb] modified electrode at 50mV/s scan rate.

The key components in the immunosensor included the GR-Ch-Au NPs-CAb and MWCNTs-Ch-Au NPs-CAb biofilm that fix the SEB toxin on GCE surface and the Ferrocene-labeled antibody for signal generation. The electrochemical properties of Fc-MAb for SEB detection in PBS were investigated with cyclic voltammetric measurements. When the sandwich format reaction was completed, a pair of stable and well-defined redox peaks (Figure S13b) could be observed at the [GR-Ch-Au NPs-CAb-SEB-Fc-MAb] modified electrode which may recommend the efficient electrochemical activity of the ferrocene–mice anti-SEB IgG bioconjugate labels. Since, there is fine electron communication between the ferrocene moieties and the modified GC-electrode. However, no peaks could be found in the control experiments (i.e. without SEB). By exploiting the unique structure and electronic properties of ferrocene, a specific target antigen can be sensitively detected. Additionally, the cyclic voltammograms is varied at different scan rates (Figure S13a), which corresponded to the oxidation of the ferrocene moiety into the ferricinium cation [Dou et al., 2012]. In addition, both the anodic and cathodic

peak currents increased linearly with the square root of the scan rate within the range from 1 to 100mV/s (inset of Figure S13a), which confirmed the diffusion-controlled nature of the redox species in this process. We observed the charge transfer mechanism in our present system which may be due to the flexibility of the ferrocene moieties on the [GR-Ch-Au NPs-CAb-SEB-Fc-MAb] immunocomplex. This immunocomplex may physically impinge the ferrocene moieties on the electrode surface and provide a sufficiently high current signal [Lu et al., 2012].

15. References:

[1] J. Li, J.D. Qiu, J.J. Xu, H.Y. Chen, X.H. Xia, The synergistic effect of prussian-blue-grafted carbon nanotube/poly(4-vinylpyridine) composites for amperometric sensing, Adv. Funct. Mater. 17 (2007) 1574–1580.

[2] Mohammed Khenfouch, Mimouna Baïtoul, Hassane Aarab, Malek Maaza, Morphological, Vibrational and Thermal Properties of Confined Graphene Nanosheets in an Individual Polymeric Nanochannel by Electrospinning. Graphene, 2012, 1, 15-20.

[3] Krishna Rao K.S.V., Vijaya Kumar Naidu B., Subha M.C.S., Sairam M., Aminabhavi T.M., 2006, Novel chitosan-based pH-sensitive interpenetrating network microgels for the controlled release of cefadroxil, Carbohydrate Polymers, 66, 333–344.

[4] Brych SR1, Gokarn YR, Hultgen H, Stevenson RJ, Rajan R, Matsumura M., J Pharm Sci. 2010 Feb;99(2):764-81. doi: 10.1002/jps.21868.

[5] Krimm & Bandekar Adv Protein Chem 1986;38:181-364.

[6] Dou, Y. H., Haswell, S. J., Greenman, J. & Wadhawan, J. Voltammetric immunoassay for the detection of protein biomarkers. Electroanalysis 24, 264–272 (2012).

[7] Lu, N., Pei, H., Ge, Z. L., Simmons, C. R., Yan, H. & Fan, C. H. Charge transport within a three-dimensional DNA nanostructure framework. J. Am. Chem. Soc. 134, 13148–13151 (2012).