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# Conductimetric immunosensor based on poly(3,4-ethylenedioxythiophene)

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**A conductimetric reagentless immunosensor using the biospecific binding pair of goat antirabbit IgG and rabbit IgG has been designed and fabricated using poly (3,4-ethylenedioxythiophene) as the immobilization matrix-cum-transducer.**

Immunoassays are based on specific binding reactions that occur between antigen and its complementary antibody. In an immunosensor, either an antibody or an antigen forms the immobilized element and the detection and quantification of the complementary biospecific binding pair is carried out. This can be achieved by measuring turbidity, precipitation reaction or agglutination. Electrochemical immunoassays are based on modifications of enzyme immunoassays with the enzyme activities being determined potentiometrically or amperometrically or optically. Sergeyeva *et al.*[1] have used polyaniline label for immunoelectrochemical immunoassay. A very sensitive immunosensor based on polyaniline/nafion/protein composites has been developed for the amperometric analysis with urease labeled immunoreagents.[2] Polyaniline has also been used as a conductivity modulating agent on the colloidal gold surface after immobilizing an antiserum albumin.[3] Lillie *et al.*[4] have used polypyrrole loaded with antibody to lutenising hormone (LH) and have employed electrochemical impedance spectroscopy to detect LH. A capacitive sensor for immunoassay read out has also been developed based on poly(3-hexylthiophene) coated Pt electrode.[5]

From the above discussion it is clear that, to date, there has been no report where change in the conformation of conjugated polymer, as a consequence of antibody : antigen binding, has been used for the design of immunosensors. Swager and coworkers have suggested that this approach may not succeed because there may not be a significant conformational change in the conjugated polymer upon antigen binding to the surface entrapped antibody.[6] However, in the present work, we have developed a conductimetric reagentless immunosensor using the biospecific binding pair of goat antirabbit IgG and rabbit IgG. Another feature of the present work is the easy fabrication of the immunosensor by physical entrapment of the antibody or the antigen during the polymerization. The concept of the present immunosensor is based on the change in the conformation of the polymer due to the formation of antigen–antibody adduct. This is manifested macroscopically in terms of change in the conductivity of the polymer. This is the first time that a direct surface-entrapped antibody–antigen binding has been used for immunosensing applications. Some time back, we reported a potassium ion activated molecular electronic device based on 18 crown 6 (18C6) using polyaniline as the immobilizing matrix-cum-transducer.[7] The ion binding cavities (18C6) in the polymer undergo a conformational change on the occupation of metal ions. This causes a local perturbation in the polymer conformation resulting in change in conductivity of the polymer. In the present communication, we have used conjugated poly(3,4-ethylenedioxythiophene) (PEDOT) as the immobilization matrix-cum-transducer. PEDOT is one of the most promising conducting polymers of the recent times and has been reported to exhibit better stability and conductivity compared to other conducting polymers.[8,9] Unlike polyaniline, it is electroactive and conducting in neutral buffer solutions, and exhibits superior stability compared to polypyrrole. Recently PEDOT and composite films of PEDOT have been used as the amperometric sensor for detection of glucose.[10,11] To the best of our knowledge, this is the first time that PEDOT has been used as a conductimetric sensor in an immunoassay.

50 mM EDOT solution {in pH 7.2 phosphate buffer solution using 6 : 1 water : acetonitrile (vol/vol)} was electrochemically polymerized at a constant potential of 1.1 V *vs.* SCE into the pores (1.2 μm pore diameter) of gold coated polycarbonate membranes. This resulted in formation of polymeric tubules inside the pores of the membrane. PEDOT was found to be conducting and stable in pH 7.2 buffer solution. The immunosensor devices were fabricated by immobilizing different concentrations of goat antirabbit IgG on the polymer matrix. The immobilization was done in two ways: (a) by physical adsorption on to the polymer matrix after polymerization, and (b) by immobilization of the antibody in the polymer matrix during polymerization. These devices were then exposed to solutions containing different amounts of the antigen rabbit IgG. The sensor response is represented by $\Delta g/g_o$ where $\Delta g = g - g_o$; $g_o$ is the conductance of the sensor without any substrate and $g$ is the conductance of the sensor in presence of the substrate. The response of the devices was measured by DC conductivity technique.[12] The measurements were carried out at three gate potentials (−0.8 V, −0.5 V and 0 V *vs.* SCE). The response time of the devices was determined by measuring the conductance as a function of time after exposing it to antigen solution. The $\Delta g/g_o$ was found to increase up to 3 minutes. The gradual increase in conductance as a function of time is attributed to the formation of antigen–antibody adducts. The device response was found to be highest at −0.8 V. Therefore, all the measurements were made at this potential for further studies. Fig. 1 shows the response of the immunosensor devices as a function of the rabbit IgG concentration. In these cases, different concentrations of goat antirabbit IgG was immobilized into the polymer matrix during polymerization. It is clear from Fig. 1 that the sensor response increased significantly when the concentration of antibody loading was changed from $1 \times 10^{-5}$ g ml$^{-1}$ to $3 \times 10^{-5}$ g ml$^{-1}$. Further increase in the antibody concentration to $5 \times 10^{-5}$ g ml$^{-1}$, however, did not increase the sensor response. Furthermore, the linear range for these devices (0.1–30 $\times 10^{-9}$ g ml$^{-1}$ for $1 \times 10^{-5}$ g ml$^{-1}$ and 0.1–100 $\times 10^{-9}$ g ml$^{-1}$ for $3 \times 10^{-5}$ and $5 \times 10^{-5}$ g ml$^{-1}$ of the antibody) was also found to be increasing with increase in the concentration of antibody loading into the polymer matrix. Control experiments were also carried out by fabricating devices without the antibody. These devices showed negligible sensing response when exposed to different concentrations of antigen (Fig. 1) indicating that the sensor response of the devices is due to antibody–antigen interaction. These devices were also contrasted with those fabricated by physical adsorption of antibody after the polymerization (Fig. 1). The sensor response and the linear range for the detection was found to be significantly lower in these cases. Experiments were also carried out by immobilizing different concentrations of the antigen, rabbit IgG, into the polymer matrix during polymerization. These devices were then exposed to the solutions

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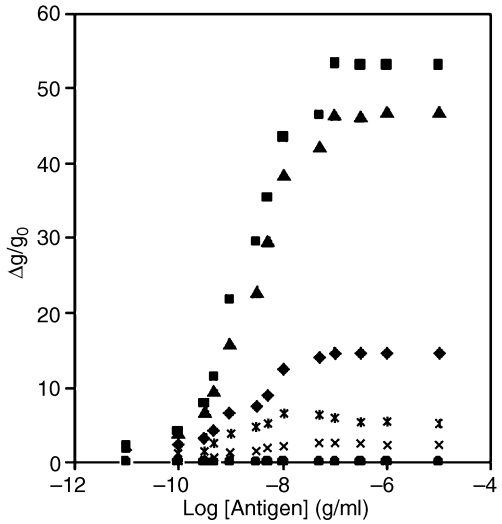


**Fig. 1** Sensor response as a function of antigen concentration. (■, ▲and ◆are for 3, 5, and $1 \times 10^{-5}$ g $ml^{-1}$ of the antibody, respectively, immobilized during polymerization. ★and × are for 3 and $1 \times 10^{-5}$ g $ml^{-1}$ of the antibody, respectively, immobilized by physical adsorption after the polymerization. ●is for the control experiment where no antibody was immobilized.)

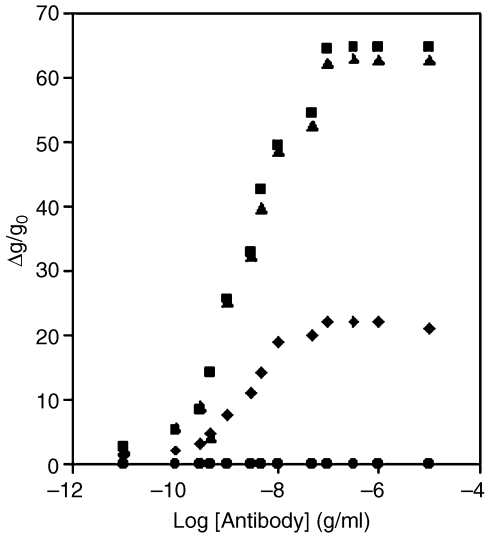


**Fig. 2** Sensor response as a function of antibody concentration. (■, ▲and ◆ are for 3, 5, and $1 \times 10^{-5}$ g $ml^{-1}$ of the antigen, respectively, immobilized during polymerization. ● is for the control experiment where no antigen was immobilized.)

containing different concentrations of antibody and the sensor response was measured at −0.8 V (Fig. 2). In these devices also, the sensor response and the linear range ($0.1–100 \times 10^{-9}$ g $ml^{-1}$) was found to increase with increase in concentration of rabbit IgG on the polymer matrix. This indicates that these devices can be used for the detection of either the antibody or the antigen.

In conclusion, we have shown for the first time that a direct surface-entrapped antibody : antigen binding can be used for the design of immunosensors. A direct reagentless conductimetric immunosensor based on conducting PEDOT has been developed for the detection of either the antigen or the antibody. These devices were capable of detecting as low as $1 \times 10^{-10}$ g $ml^{-1}$ of the antigen with a response time of 3 minutes. It should, however, be noted that the present concept will only work, at least with realistic sensitivities, when the antigen is a macromolecule, *i.e.* small ligands will have little or no effect.

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