**An efficient electrochemical biosensor for Vitamin-D3 detection based on**

 **Aspartic acid functionalized gadolinium oxide nanorods**

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**Contact angle measurement**

The contact angle (CA) values of Gd2O3NRs/ITO and Asp-Gd2O3NRs/ITO electrode surface were monitored which analyze the level of wetting on solid-liquid interaction. A small value of CA corresponds to hydrophilicity and vice-versa. For measurement of CA values, a liquid (DI water) droplet was released on the Gd2O3NRs/ITO and Asp-Gd2ONRs/ITO electrode surface and variation in angles was monitored using the Laplace–Young calculation method in static CA mode. The CA values taken were an average of 10 measurements and found to be 38.46◦ and 14.55◦ for Gd2O3 NRs/ITO and Asp-Gd2O3NRs/ITO electrodes, respectively [Fig. S1]. The hydrophilicity properties of electrodes depend on the chemical composition, surface morphology and surface free energy. The decreased CA value for Asp-Gd2O3NRs/ITO electrode as compared to Gd2O3NRs/ITO electrode indicates the hydrophilic nature of Asp-Gd2O3NRs/ITO electrode. This hydrophilic nature helps in better immobilization of Anti-VD on Asp-Gd2O3NRs/ITO surface 1. Hence the Asp (amine) exposes the hydrocarbon chains on the surface of Gd2O3NRs/ITO electrode, and it completely wraps the hydrophilic oxygen-containing groups, which results in increased hydrophilicity of Asp-Gd2O3NRs/ITO electrode 2.



**Fig.S1** Contact angle study of (a) Gd2O3NRs/ITO (b) Asp-Gd2O3NRs/ITO electrodes.

**Effect of pH**

The pH value of an electrolytic solution influences the electrochemical response of immunosensor. So, the effect of pH on electrochemical response of BSA/Anti-VD/Asp-Gd2O3NRs/ITO immunoelectrode was observed for different pH solution ranging from value 6.0 to 8.0. DPV measured the current before each potential change and current difference (ΔI) is plotted as a function of potential 3 and this technique was used to observe effect of pH in potential range of -0.2V to +0.6V with a pulse width of 50 ms and a pulse height of 25 mV in PBS containing [Fe(CN)6]-3/-4. Fig. S2 shows the DPV response of BSA/Anti-VD/Asp-Gd2O3NRs/ITO immunoelectrode obtained for different pH buffer solutions. Initially, the magnitude of ΔI increases from 6.0 to 7.0 pH, after that it decreased from 7.0 to 8.0. However, the maximum value of ΔI observed at pH 7.0 which indicates highest activity of immunoelectrode or biomolecules retained activity at this pH 4. The highest value of ΔI at pH 7.0 can be explained by considering the isoelectric point (pI). The pI value of Asp-Gd2O3NRs complex is 5.74, and individual antibody (Anti-VD; IgG1) is 8.2, obtained from literature (Web link1). Here, the ΔI value obtained was resultant of Asp-Gd2O3NRs and antibody. The resultant pI of these two components (Asp-Gd2O3NRs and antibody) will be 5.74 + 8.2/2=6.97 (~7.0). Therefore the ΔI will be maximum at pH 7.0. Thus, whole electrochemical studies were done at pH 7.0 of electrolyte solution.



**Fig.S2** Effect of pH on BSA/Anti-VD/Asp-Gd2O3NRs/ITO immunoelectrode in PBS containing [Fe(CN)6]-3/-4 in potential range of -0.2V to +0.6V.

**Effect of scan rate**

To investigate the interface kinetics of Asp-Gd2O3NRs/ITO and BSA/Anti-VD/Asp-Gd2O3NRs/ITO immunoelectrode CV was performed with variation of scan rate from 10–100 mV/s [Fig. S3]. Both electrodes show the value of anodic peak current (Ia) and cathodic peak current (Ip) was linearly increased with the increase the scan rate. Fig. S3. inset (a) and (b) show the magnitude of peak current was directly proportional to the value of square root of scan rate. This linear relation reveals that the electrochemical reaction at electrode-electrolyte interface was diffusion- controlled process 5. Moreover, a linear shift in the peaks of redox current toward higher potential was also observed for Asp-Gd2O3NRs/ITO and BSA/Anti-VD/Asp-Gd2O3NRs/ITO immunoelectrode with the increase in square root of scan rate suggesting a quasi-reversible or slow electron transfer kinetics 6.



**Fig.S3** Effect of scan rate on **(**a**)** Asp-Gd2O3NRs/ITO **(**b**)** BSA/Anti-VD/Asp-Gd2O3NRs/ITO immunoelectrode in PBS containing [Fe(CN)6]-3/-4 at different scan rate varies from 10-100 mV s-1.

**Reproducibility and repeatability**

Reproducibility of different (six) BSA/Anti-VD/Asp-Gd2O3NRs/ITO immunoelectrode was observed using DPV technique under similar conditions. Fig. S4 (a) shows the bar graph of values of ΔI obtained for respective electrodes with 2.32% RSD. The repeatability of BSA/Anti-VD/Asp-Gd2O3NRs/ITO immunoelectrode was observed with a specific concentration of Vit-D3 (10 ng mL-1) using DPV technique by taking successive measurements (6 times). Fig. S4 (b) shows the bar graph of the value of ΔI obtained from six successive measurements and found 1.83% RSD value, which was in acceptable range.



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**Fig.S4** (a) Reproducibility and (b) repeatability of BSA/Anti-VD/Asp-Gd2O3NRs/ITO immunoelectrode was monitored using DPV in PBS containing [Fe(CN)6]-3/-4 under similar conditions.

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