Comparative study of label-free electrochemical immunoassay on various gold nanostructured electrode

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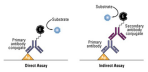
Introduction

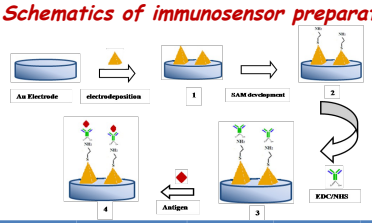
- In labeled immunosensor a 'label' is also incorporated into the assay to provide measurability.
- Most popular labels in immunoassays is Enzyme-linked immunosorbent assay (ELISA).
- ELISAs includes multiple steps of incubation and washing.
- It being an enzymatic reaction, even small quantities of non-specific binding might result in false signal



Advantages of label free detection

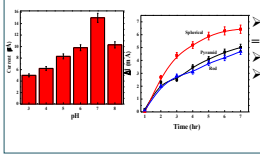
- It eliminates the need for tags, dyes or specialized reagents required for assay development.
- Simplifying assay design, minimizing liabilities created by the use of labels.

Schematics of immunosensor preparation



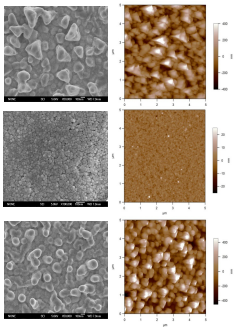
Nanstructures	$HAuClO_4$ (mM)	$HCLO_4$ (M)	Voltage (V)	Time (min)
Pyramid	40	0.1	-0.08	2
Spherical	40	0.1	-0.2	2
Rod	4	0.1	-0.08	2

Optimization of experimental conditions



- The maximum current obtainedat pH = 7.0.
- The 2 hr incubation time was selected.
- To ensure the prevention of saturation and for better performance of immunosensor.

Surface Morphology

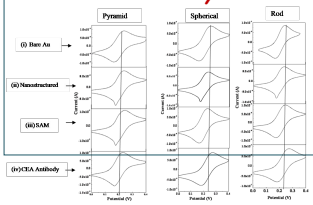


- The average edge length of the pyramid nanostructures is 205 nm.
- The spherical nanostructure had an average diameter of 15 nm.
- Where as nano rods had an average diameter of about 120 nm.

Nanostructures	Surface area (μm^2)	Surface Coverage (%)
Pyramid	45.5	52.9
Spherical	42.3	63.9
Rod	52.1	40.7

FESEM and AFM images of (a) Pyramid (b) spherical (c) Rod like nanostructures.

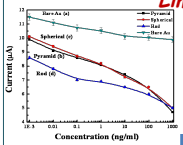
Cyclic Voltammetry



- Peak current of the bare Au was 8.69µA.
- The peak current increased 1.40 times as compared to bare Au electrode.
- With further modification with anti CEA showed 0.83, 0.76 and 0.77 times drop in the peak current for pyramid, spherical and rod like nanostructures, respectively.

CV response of the nanostructured immunosensor.

Limit of Detection (LOD)

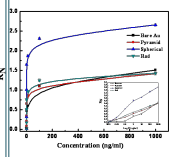


- The current dropped with the increase in the concentration from 1pg/ml to 1000ng/ml.
- The limit of detection (LOD) was calculated using the equation

$$LOD = \frac{3 \times S.D}{Sensitivity}$$

Nanostructures	Bare Au	Pyramid	Spherical	Rod
LOD (ng/ml)	0.07	0.0039	0.0036	0.0045
Sensitivity ($\mu A\ ng^{-1}.ml$)	0.211	0.339	0.457	0.312

Association constant

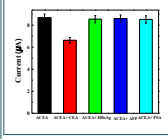


- The association constant was calculated using the equation

$$K_a C = \frac{R_{ct(i)} - R_{ct(o)}}{R_{ct(o)}}$$

- The values came out to be 0.0695, 0.0707, 0.0948 and 0.0696 for bare Au, pyramid, spherical and rod like nanostructures , respectively.

Specificity and stability of immunosensor



- when CEA was used the current decreased to value of 6.62µA.
- Thecurrent retained 83.2% of the original value even after 30 days.

Conclusion

- The spherical nanostructures were smaller in size and has larger value of surface area as compared to the pyramid and rod- like nanostructures.
- Due to the higher surface area the spherical nanostructure showed better electrochemical performance than the other types of nanostructures.
- It was found the limit of detection of the nanostructured electrodes was 4 pg/ml and have stability almost for a month

Reference

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