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Gold Nanoparticles - Antibody Based Immunosensor for Detection of Arsenic in Ground Water Sample

G. Chaitanya Lakshmi

Department of Chemistry, School of Engineering, Presidency University, Bengaluru - 560064, Karnataka, India

Abstract

The arsenic in groundwater samples is found to harm human health and the environment. Detection of arsenic by gold nanoparticle-antibody based immunosensor plays a vital role in the present day scenario where people need not use sophisticated laboratory detection systems if the efficient immunosensors are developed against metal contaminants. Gold nanoparticles (AuNPs) are chosen because they exhibit unique optical and electronic properties, which in-turn enhances the sensitivity, selectivity and specificity in the field of immunobiosensors. The design of the immunosensor involves conjugation of antibodies that are generated against arsenite (As^3) by functionalizing with gold nanoparticles that helps in specific binding to arsenite (As^3) in real samples. The concentration of arsenite (As^3) under test was ranging from 50ng/l to 500ng/l. The dectection limit of arsenite (As^3) was found to be 38ng/l by the designed gold nanoparticle-antibody based immunosensor.

Keywords: *Antibodies, Gold Nanoparticles, Immunosensor*

1.0 Introduction

Most of the trivial and sophisticated analytical techniques, such as atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectrometry (ICP-MS), anodic stripping voltametry, capillary electrophoresis, X-ray fluorescence spectrometry etc., that are known for metal analysis are associated with lot of disadvantages namely, sophistication in the equipment itself, sample pre-treatments, requirement of skillful operation which makes it very difficult for real sample analysis on site[1]. So, researchers in the field of biosensors are working with an intention of creating the miniaturization of the analytical techniques mentioned above. In this article Immunosensors which operates on the basis of ELISA has been designed with very promising hope in measuring and detecting the metal ions in water samples. On the other hand, we can discuss the noteworthy advantages of Immunosensors like rapid response time, cost effectiveness, ease of use, portability, provides onsite testing and fetches rapid instant result. Immunosensors are a class of biosensors that are used to detect specific target molecules, with the aid of antigen or antibody interaction. Their use in various fields such as medical diagnosis, environmental monitoring, and food safety are gaining importance in recent days[2].

Literature has evidenced that the immunosensors as highly valuable tool for detection of Arsenic metal in ground water sample. The technique involves use of metal nanopartcicles and their surfaces that can directly adsorb arsenic metal ions leading to spectroscopic changes that are monitored in ELISA reader.

However, it is also important to mention that immunosensors also have some limitations. For instance, the immobilization of antibodies or antigens onto the sensor surface can sometimes lead to a loss

**Author for correspondence*

of their reactivity or stability over time. Furthermore, the detection of certain target molecules may require complex or time-consuming sample preparation steps[3].

In conclusion, nanometal enabled immunosensors are found to be accurate and efficient in identification of Arsenic metal contaminant in ground water samples. The a fore mentioned statements motivated to carry out the extensive study on development of nanometal enabled immunosensors for detection of Arsenic metal in water samples.

2.0 Experimental

2.1 Materials

Arsenic trichloride, bovine serum albumin (BSA), gold (III) chloride, and gelatin were procured from M/s Sigma–Aldrich Chemicals, USA. All other reagents were of analytical grade and procured from standard sources.

2.2 Methodology

2.2.1 Immunization

Arsenic metal was conjugated with Bovine serum albumin protein to generate immune response. It was then injected to mouse subcutaneously followed by booster doses every fortnight. Blood was withdrawn every ten days for about three times for antibody isolation. Serum was typically collected 10 days after the administration of the last booster dose. Antibodies was purified using ImmunoPure purification Kit according to manufacturer's recommended protocol[3].

2.2.2 Attachment of Gold Nanoparticles to Antibodies

Conjugation and labelling of Gold Nanoparticles to anti-arsenic antibodies was done by using Gold AB Conjugation Kit. The protocol followed was according to the kit and conjugate was be stored at 2-8°C[4].

2.2.3 Detection of As(III) by Immunoassay Enzyme-Linked Immunosorbent Assay Analysis (ELISA)

Antibodies so obtained from mice was subjected to test for identifying specific binding to antigen by direct ELISA analysis method. The assay plates were first coated with arsenic and then blocked, later antibody preparations were added to the wells and subjected for incubation at 37 °C for 2 h, then the plates were washed with PBS-T and probed with *p*-nitrophenyl phosphatase (*p*NPP)-conjugated secondary antibody. After through washing, the color was developed with *p*NPP, ALP-substrate and the reaction was arrested with 3 M $NaOH_4$.

2.2.4 Checker Board Assay

The concentration of antibody and the antigen required was optimized using checker board assay. The detection limit of the analyte was then determined by competitive ELISA. The lower detection limit was calculated (1µg)[5].

2.2.5 Preparation and Conjugation of Gold Colloidal Nanoparticles (GNPs) to anti-As(III) Antibody

The preparation of the gold nanoparticles was done by the standard citrate-reduction method. To get contaminant free GNPs all apparatus was washed with aquaregia. $HAuCl_4.3H_20$ (1mM) was taken in 250 ml conical flask heated with continuous stirring. The temperature was maintained at 90°C to this 10 ml of 38.8 mM sodium citrate was addedat once. Blue color was developed initially which then turns to deep burgandy of GNPs. The GNPs solution so obtained was cooled to room temperature and was stored at 4°C[6,7].

2.2.6 Analysis of As(III)

ELISA wells were coated with 1µg/100µl Anti As(III) antibodies using bicarbonate buffer of pH 9.4 and was kept for incubation overnight. The wells were blocked using 1% gelatin in PBS (Phosphate Buffer Saline pH 7.4) and then subjected to washing of the wells with PBST (phosphate buffer saline pH 7.4 Tween-20). Later As(III) in the concentration range of 1-1000 $ngmL^{-1}$ was added followed by Anti As(III) antibodies-HRP conjugate (1 g/100 L) and then incubated for 1 hr followed by washing the wells with PBST. Finally, the reaction was arrested by adding 1N H_2SO_4. The absorbance was taken at 450nm[8,9].

3.0 Result and Discussion

3.1 Preparation and Conjugation of Gold Colloidal Nanoparticles (GNPs) to anti-As(III) Antibody

The formation of GNPs by citrate method from gold chloride solution showed an absorption peak at 520 nm. The GNPs were then conjugated with anti As(III) antibodies and the in presence of 0.1 M K_2CO_3 of Ph 9.0. Further, the capping of GNPs with anti As(III) antibodies was confirmed by observing the red shift, the absorption peak now was 535nm. This is because of bioconjugation of antibodies to GNPs[10].

3.2 Optimization of Immunoassay

This protocol can be used for visual detection of As(III) metal ions in water samples. So the color intensity was kept high, so that there is a striking difference between the control and the test samples. The color controlling factor was found to be the GNPs-antibody conjugate around 1.4 mgL^{-1}. The color developed after incubating the samples for 40 min. The concentration of As(III) was inversely related with color intensity (red color)[11].

4.0 Conclusion

The GNP conjugated antibodies used for detecting As(III) in ground water samples through ELISA immunoassay was carried out stepwise. In conclusion, As(III) can be detected quantitatively with notable advantages like the method is selective, sensitive, less time consuming, less sophistication. The technique can be promisingly used for other contaminant metal ions present in ground water samples, food samples, beverages etc in near future.

5.0 References

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