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RESEARCH ARTICLE

Optimisation Studies of Probe DNA Concentration on the Surface of Silica Nanobiosensor

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Abstract

Biosensors with immobilized probe that are selective for the identification of toxic compounds at ultra trace levels in industrial products, chemical substances, environmental samples or biological systems (e.g., Bacteria, virus, or tissue components) for biomedical diagnosis. Nanomaterials such as silica nanoparticles works as sensor when probe hybridized with the target sequence. Due to their large surface area to volume ratio, they can achieve rapid and low cost reactions. They can conjugate with a variety of biomolecules (Proteins, enzymes, peptides, and DNA) and used as fluorescence biomarkers to monitor biological events or identify and detect biological targets. DNA biosensors based on nucleic acid hybridization have been extensively developed because of their specificity, speed, portability, and low cost. The immobilization step is essential to ensure high reactivity, orientation, accessibility, and stability of the surface-confined probe and to avoid nonspecific binding. Various studies, i.e., a synthesis of silica nanoparticles, their surface modification, probe immobilization were carried out during optimization of reaction conditions. In this Present work, 3.4% more immobilization of probe DNA onto the silica nano particle was observed in the presence of 10% Butyltrimethoxysilane solution rather than absence of BMS and the concentration of the DNA Probe immobilized on silica nanoparticles was maximum at 1Picomloles/µl was noticed.

Introduction

Bionanotechnology is a subset of nanotechnology where the biological world provides the inspiration and the end goal and is defined as an atom-level engineering and manufacturing using biological precedence for guidance (Nano-Biomimetics) or traditional nanotechnology applied to biological and biomedical needs [1]. It will be employed to describe the use of biological building blocks and the utilization of biological specificity and activity for the development of modern technology at the nano-scale [2]. Nanoparticles have been conjugated with a variety of biomolecules (including proteins, enzymes, peptides, and DNA) and used as fluorescence biomarkers to monitor biological events or identify and detect biological targets. In the last two decades, the field of molecular diagnostics has grown rapidly due to the discovery of new genes involved in different diseases [3]. Silicananoparticle-based solid supports have been effectively used for the immobilization of various biomolecules, because of their large surface area, good biocompatibility and suitability for many surface immobilization mechanisms. Most of these have proven to be excellent substrates in many fields ranging from biosensors to interfacial interaction studies [4-6].The hydroxyl groups on the surface of silica particles can be easily tailored with organic compounds or polymers. Silanol groups can be easily functioned by different chemical procedures. The most convenient technique for silica surface functionalization is the use of the reaction of silanol groups with suitable silane reagents, aminofunctional trialkoxysilanes such as aminopropyltrimethoxysilane (APTMS) and (3-trimethoxysilylpropyl) diethylenetriamine (DETAS) were employed as a surface modification

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molecule for generating monolayer modification on the surface of silica (SiO_2) nanoparticles. Silica nanoparticles were covalently linked with oligonucleotide probes in the preparation of functionalized nanoparticles for DNA/RNA hybridization using the Disuccinimidyl glutarate, which is a homo bifunctional cross linking reagent [7,8]. This covalent immobilization method is an alternative to other methods currently used to modify silica nanoparticles. With these specific linkages, the population of probes is attached in a homogenous manner with good surface coverage and the probes retain full activity, provided that no non-specific attachment occurs through the nucleobases [9].The study was taken up with the objective of the various studies, i.e., synthesis of silica nanoparticles, their surface modification, probe immobilization, effect of 10% Butyltrimethoxysilane (BMS) solution and effect of concentration of probe DNA were carried out during optimization of reaction conditions.

MATERIALS AND EXPERIMENTAL METHODS

Reagents and instruments:

The reagents used in experiment such as ethanol (99.5%), tetraethylorthosilicate (TEOS), ammonium hydoxide(25%), 10% 3-Aminopropyltriethoxysilane solution(1 ml of 3-Aminopropyltriethoxy silane (APS) is added in 9ml of Toluene), 10% Butyltrimethoxysilane solution (1ml of Butyltrimethoxysilane (BMS) is added in 9 ml of toluene), toluene and methanol, Dimethylsulfoxide(DMSO), 1X Phosphate buffered saline (PBS) buffer (1.6g NaCl, 0.04g KCl, 0.288 g Na_2HPO4, 0.048g KH_2PO4, in 200 ml of distilled H_2O. pH is adjusted to 7.4 with HCL. Disuccinimidyl glutarate (DSG) (3.263 mg of Disuccinimidyl glutarate (DSG) is added in 1ml Dimethyl sulfoxide (DMSO) were purchased from Eurofins Genomics India, Bangalore and they were used without any further purification.

Twenty-base synthetic oligonucleotide were purchased from Sigma-Aldrich Chemical Co., St. Louis, USA . Probe sequence:

AmC6F5'GGAGGAGATCTGG C TGGTCA3'

Synthesis of silica nanoparticles:

A sequential addition method has been used to prepare monodisperse and uniform -size silica nanoparticles using ultrasonication the sol-gel process. The monodispersed uniform size silica nanoparticles were prepared by hydrolysis of Tetra Ethyl Ortho Silicate (TEOS) in ethanol medium in the presence of ammonium hydroxide (NH_4OH) which is used as a catalyst.Synthesis of silica nanoparticles were prepared by the following procedure. Firstly 4.67ml of 4M ethanol and 2.68ml of distilled water (H_2O) was taken in 50ml beaker and after 10 minutes 0.200ml of 0.045M Tetra Ethyl Ortho Silicate (TEOS) was added and kept in the sonication bath for 20 minutes, 12.45ml of 25% of 16M ammonium hydroxide (NH_4OH) was added dropwise which acts as a catalyst to promote the condensation reaction. Sonication was continued for further 60 minutes to get a white turbid suspension. Washing is done for 5 times with 2ml acetone and 5times with 2ml water. Silica nanoparticles were prepared at room temperature under air condition, drying was carried by using a microbiological oven at 110^{o}c for 2 hours. The morphology and particle size were studied from AFM product were in the range of 60nm to 400nm, and particles size and distribution was examined under Atomic force microscope(Vicco made ,provide by IIT Hyderabad) by non-contact mode.

Surface modification of silica nanoparticles:

Eight samples were prepared of which two samples were carried out for the estimation of the 10% Butyltrimethoxysilane (BMS) solution effect on the probe DNA immobilization on the silica nano particle and six samples were carried out in the concentration of the probe DNA immobilization on the silica nanoparticles. Firstly 50µg of freshly prepared silicananoparticles was taken and transferred to each 10 ml falcon tube.

In the first Step, (silane treatment): 5ml of 10% 3-Aminopropyltriethoxysilane solution is added to silica N.P of each falcon tube and incubated for 10 hrs, then washed with toluene, toluene and methanol (1:1 v/v) and methanol using a centrifuge, after that heated at 110^{o}c for 1 hour in oven.

In the second Step, (capping treatment): 5 ml of 10% Butyltrimethoxysilane solution is added to first, third, fourth, fifth, sixth, seventh,eighth silica Nanoparticle samples and a second sample of silica Nanoparticle should not undergo any addition of BMS because to estimate the immobilization of probe DNA on the silica nanoparticle without the addition of the 10% Butyltrimethoxysilane (BMS) solution. These samples are incubated for 10 hrs, after that washed with toluene, toluene:methanol (1:1 v/v) and methanol using a centrifuge and heated at 110^{o}c for 1 hour in oven.

Linker treatment:

65.260 mg of DSG (Disuccinimidyl glutarate) which is a homo bifunctional cross linking reagent must be dissolved in 20ml of DMSO which is an organic solvent. The solution is prepared for 8

samples, 2.5ml of Disuccinimidyl glutarate solution is added to each silica N.P sample and wait for 2 hours at room temparature, then washed with 2ml DMSO and 2ml PBS buffer using centrifuge.

Covalent conjugation of DNA Probe on to the silica nanoparticle surface (DNA immobilization):

APS-DSG modified silica nanoparticles transferred into Eppendorf tubes. Probe DNA was prepared by dissolving 40µl of 5' amine modified probe DNA stock concentration of (1 µg/ml) in 39.6ml of 1x PBS buffer. To estimate the effect of immobilization of probe DNA to the silica nanoparticle, 1ml of this solution is added to the sample 1 and sample 2 of the APS-DSG modified silica particles. To examine the concentration of the probe DNA immobilization on to the silica nanoparticle probe DNA is added in this direction, 1ml to sample 3 of the APS-DSG silica nanoparticles , 3ml to sample 4, 5ml to sample 5, 7ml to sample 6, 8ml to sample 7 and 9ml to sample 8. These samples a sealed and allowed to stand at 37°c for overnight. Concentration of probe DNA immobilization is calculated at picomole/µl using UV Visible spectroscopy.

Result and Discussion

The size of the nanoparticles was measured using an Atomic force microscope (AFM) and it is confirmed that the size was uniform and monodispersed particle with a size range of 60-200nm determined by AFM were obtained with specific area 32g/m^2 (provided by IITH). Fig 1 shows the AFM images of silica nanoparticles.

The three major steps involved in the covalent immobilization of oligonucleotides onto silica nanoparticles are illustrated in Fig 2. The attachment of modified oligonucleotides to silica nanoparticles was performed via a DSG crosslinking takes place through oligonucleotide reacts with the NH functional group of the DSG layer on the silica nanoparticles. To determine the efficiency of the immobilization method, parameter like, various concentrations of oligonucleotide probes were studied.

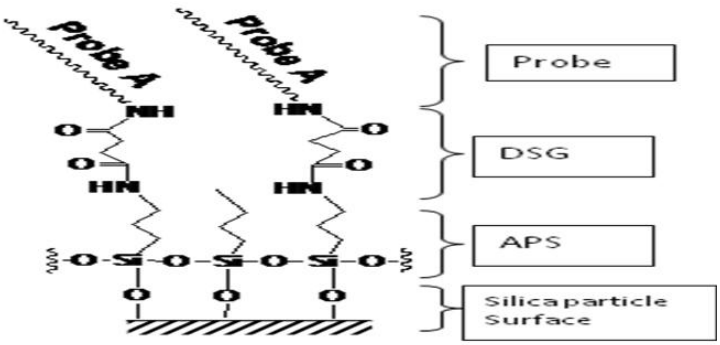


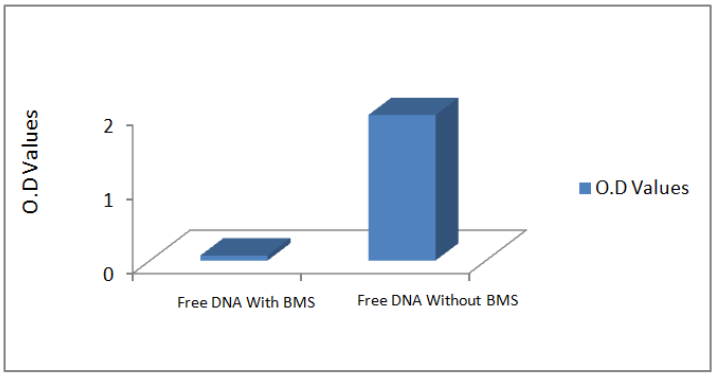
Fig 2 :covalent conjugation of ss oligonucleotides on to the silica nanoparticle surface

It is observed that the effect of 10% Butyltrimethoxysilane (BMS) solution is increasing the immobilization of probe DNA on the silica nanoparticles shown in table 1. Fig 3 shows that the concentration of free DNA is less in the case of presence of BMS i.e more probe DNA is immobilized on the silica nanoparticles [12].

Table 1: Samples with O.D Values

Sample no.	O.D Values
1. Free DNA with BMS	0.0671
2. Free DNA without BMS	1.9584

Fig 3: shows the effect of BMS on the immobilization



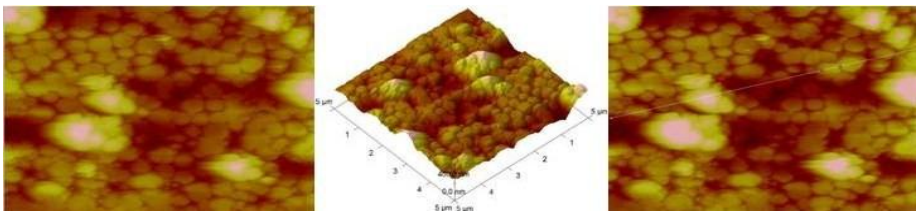
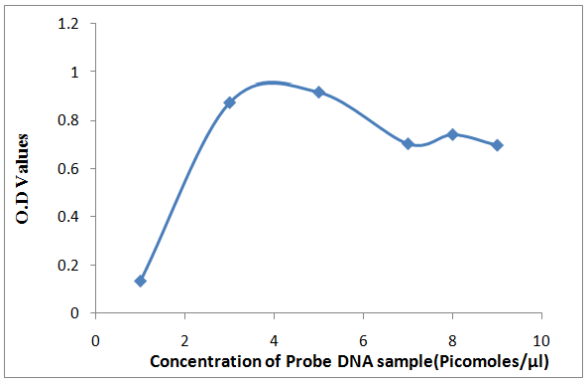


Fig 1: show the AFM images of silica nanoparticles.

The effect of 10% Butyltrimethoxysilane(BMS) solution in the process of conjugation of the probe DNA on to silica nanoparticle is estimated. It is illustrated that probe DNA immobilization on the silica nanoparticle is increased in the presence of Butyltrimethoxysilane(BMS) solution (capping treatment) compared to the absence of BMS (Butyltrimethoxysilane). There is approximately 3.4% more immobilization of probe DNA onto the silica nano particle in the presence of BMS (Butyltrimethoxysilane) rather than absence of BMS.

The probe DNA concentration is another important parameter, which affect the hybridization and amenability. From Fig 4, it is observed that an increase in O.D value i.e probe concentration on modified silica nanoparticles was noticed with increasing concentration of the sample up to 7 **(**Picomoles/µl) and after 7 **(**Picomoles/µl) there is decrease in O.D value was observed, as we are measuring the free oligonucleotide concentration in the sample (free DNA is inversely proportional to immobilized DNA), from the graph it is shown at 1picomoles/µl, less free DNA and highest immobilization is achieved. The further experiments were carried by using the above optimized reaction conditions [13].

Fig 4: O.D values Vs. Concentration of Probe



CONCLUSION

We have shown the preparation and surface modification for silica nanoparticles and demonstrated the immobilization of oligonucleotides probes on APS-DSG-modified silicananoparticles. We have optimized the concentration of probe DNA on APS-DSG-modified silicananoparticles. It is examined that 3.4% more immobilization of probe DNA onto the silica nano particle in the presence of 10% Butyltrimethoxysilane solution rather than absence of BMS and at 1picomole/µl, the immobilization of probe DNA is increased on APS-DSG-modified silicananoparticles (concentration of probe sample is inversely proportional to the immobilized DNA in the sample). These oligonucleotide-modified silica nanoparticles can be used for efficient nucleic acid hybridization. These DNA conjugated nanoparticles can be used to detect nanomolar range target DNA probes for biomedical diagnosis.

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REFERENCES

[1] D. S. Goodsell (2004). Bionanotechnology, Lessons from Nature,Willey-Less, New Jersey, USA,pp. 1–8.

[2] Ehud Gazit (2007). Plenty of room for biology at the bottom: An introduction to bionanotechnology. Imperial College Press, ISBN 978-1-86094-677-6.

[3] H.S. Nalwa (1999).Handbook of Nanostructured Materials and Nanotechnology, Vol. IV. Academic Press, San Diego, CA, pp. ix–x.

[4]. C. Kneuer, M. Sameti, E.G. Haltner, T. Schiestel, H. Schirra, H. Schimdt,C.M. Lehr, (2000).Int. J. Pharm. 196 ,257.

[5] J.E.G.J. Wijnhoven, S.J.M. Zevenhuizen, M.A. Hendriks, D. Vanmaekel-bergh, J.J. Kelly, W.L. Vos v (2000). Adv. Mater. 12 , 888.

[6] L.K. Van Vugt, A.F. Van Driel, R.W. Tjerkstra, L. Bechger, W.L. Vos,D. Vanmaekelbergh, J.J. Kelly v. (2002). Chem. Commun. 18 ,2054.

[7]L.M. Demers, S.J. Park, T.A. Taton, Z. Li, C.A. Mirkin, Angew (2001). Chem.Int. Ed. 40 ,3071.

[8] D. Guschin, G. Yershov, A. Zaslavsky, A. Gemmell, V. Shick, D. Proud-nikov, P. Arenkov, A. Mirzabekov (2001). Anal. Biochem. 250 ,203.

[9]. G. Nonglaton, I.O. Benitez, I. Guisle, M. Pipelier, J. Leger, D. Dubreuil,C. Tellier, D.R.

Talham, B. Bujoli (2004). J. Am. Chem. Soc. 126 1497.
[10] Nagata, L.T. Kubota, I.M.S.B. Maria, P.G. Peralta-Zamora (2004). J. Colloid Interf. Sci. 200,121.

[11] L.T. Kubota, A. Gambero, A.S. Santos, J.M. Granjeiro (1996). J. Colloid Interf.Sci. 183 , 453.
[12] G.Y. Lee, C.H. Chen, T.H. Wang, W.C. Lee (2003). Anal. Biochem. 312 ,235.

[13] N. Zhu, H. Cai, P. He, Y. Fang (2003). Anal. Chim. Acta 481 ,181.