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Silver nanocluster films for glucose sensing by Surface Enhanced Raman Scattering (SERS)

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ABSTRACT

The detection of glucose by Surface Enhanced Raman Scattering (SERS) is a challenging problem because glucose molecules have a small Raman scattering cross-section and they have a low affinity for adsorption on metal nanoparticle surfaces. In this study we used 2-Thienylboronic acid (2-TBA) as a bridge or linker molecule between the metal surface and the glucose molecule and observed an intense Raman line at 986 cm^{-1} that was used to quantify the glucose concentration in the molar concentration range 1 μM–500 μM. A good correlation was observed between the intensity of this line and molar concentration of glucose. These results would find applications in the development of a non-invasive glucose sensor for diabetic patients using saliva as the body fluid instead of blood serum.

1. Introduction

In recent years, Surface Enhanced Raman Spectroscopy (SERS) has emerged as a very important technique for the ultra-sensitive detection of bio-molecules [1]. This technique combines the advantages of the Raman Effect such as the high specificity (ability to identify a given molecular species in the presence of many other chemicals) with nanoplasmonics for Raman signal enhancement. This makes it a very selective and sensitive method for quantitative detection of molecules down to the single molecule level [2]. We have carried out experiments to establish the efficacy of the SERS technique for quantitative detection of Rhodamine 6G and Crystal Violet [3,4] down to molar concentrations of 10^{-18} M. Recently we have applied our experimental techniques for the quantitative determination of standard amino acids [5].

In this work we apply the SERS technique for the quantitative detection of glucose so that this method can be employed for diagnostic applications in diabetes. Diabetes mellitus is a metabolic disorder and considered as a major problem which affects over 380 million people across the world and about 3.2 million people die annually due to this ailment [6]. It is estimated that people living with diabetes will increase to 55% by 2035. In order to measure the blood glucose levels we need to draw blood from patients and this is a painful procedure. However, studies have also detected presence of glucose in the urine and saliva samples of diabetic patients. The usual molar concentration levels of glucose in urine are in the range 0–0.8 mM, and higher glucose levels in blood lead to higher glucose levels in urine. The glucose levels in saliva are at much lower levels in the range 50 μM–500 μM but given the sensitivity of the SERS technique are measurable. So there is an urgent need to develop non-invasive or minimally invasive methods for frequent glucose monitoring. Accurate quantitative detection and evaluation of glucose has not been possible due to lack of a sensitive and molecule specific method. We show here that it is possible in principle to detect quantitatively glucose levels in aqueous glucose solutions at levels beyond the physiological concentration levels in blood.

SERS detection of glucose is limited by two factors. One factor is the low Raman scattering cross-section of the glucose molecule [7] and the second one is the poor affinity of glucose molecules to be adsorbed on metal surfaces [8,9]. Many approaches have been taken to address the aforementioned challenges. Van Duyne's group [10] came up with the idea to modify the metal surface chemically with alkane thiol so that the alkane molecules sticking out of the metal surface form a partition layer that traps the glucose molecule close to the metal surface. Mixed decanethiol/mercaptohexanol partition layers were also investigated [11]. Other SERS substrates have also been studied for glucose detection [12–14]. In these studies the thiol containing molecule was used to attach the molecule to the metal surface but the trapping of the glucose near the metal surface is not specific to glucose.

Torul et al. reported a paper-membrane based SERS platform [15] and also two component self-assembled monolayer functionalized substrates [16] for glucose detection. In these studies they were able to detect glucose concentration up to 5 mM and 0.5 mM respectively. Kong et al. [17] used tri-osmium carbonyl cluster-boronic acid in SERS based assay. They used CO stretching vibrations (1800 cm^{-1} to 2200 cm^{-1}) of the metal carbonyl for quantification. The limit of detection (LOD)

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was 0.1 mM. Zhong et al. [18] reported D-glucose detection in the physiological concentration range of 0.9 mM to 30 mM. Au nanoparticle coated zinc oxide nanowires and colloidal Ag nanoparticles were used as SERS substrates and 3,3′-boronic benzyl viologen (BBV) as Raman probe molecule. The LOD was 0.25 mM. But for early detection of diabetes it would be desirable to detect glucose levels present in saliva at considerably lower levels and the method proposed in the present work is able to detect glucose to such levels.

In another recent study Kong et al. [19] have used alkyne functionalized boronic acid attached to bi-metallic film over nanosphere (BMFON) as SERS active substrates for detection of glucose. The alkyne Raman signal intensity at 1996 cm^{-1} was shown to change with glucose molar concentration. In this work we propose a more direct method of using 2-Thienylboronic acid as a linker molecule that will attach directly to the silver surface as well as to a glucose molecule. The adsorption of organo-sulphur molecules on Au, Ag, and Cu metal surfaces has been well studied and used extensively for applications [20]. The affinity of boronic acids to bind with glucose and other saccharides is also well known and used for bio-sensing applications [21]. Thienylboronic acid is a thienyl derivative of boronic acid and we have selected this molecule as a linker molecule with a definite purpose since this would attach to both the silver surface and to the glucose molecule. Fig. 1 depicts a schematic diagram of the possible bonding between the 2-TBA and D-glucose structures as well as the bonding between the silver surface and the 2-TBA linker molecule via the Ag—S bond.

2. Experimental methods

Silver nanocluster films were deposited on 1 × 1 cm^2 glass slides using inert gas phase condensation technique. A nanocluster deposition system (Nanodep 60 from Oxford Applied Research, UK) was used for Ag nanocluster deposition. A detailed description of the nanocluster deposition system is found elsewhere [22]. The substrate glass sides were sonicated and cleaned using isopropan-2-ol, acetone and Milli-Q water before deposition of clusters. Surface morphological studies of silver nanocluster films were carried out using Field Emission Scanning Electron Microscope (FESEM model Ultra 55 from Carl Zeiss). Fig. S1 (A) shows the FESEM image of the Ag nanocluster substrate and Fig. S1 (B) shows the cluster cross-sectional area distribution (supplementary information). The distributions could be fitted to two lognormal peaks. The average diameters of the clusters deduced from the areas of the peaks were 60 nm and 150 nm with standard deviations of the areal log-normal distributions being 0.69 nm and 0.29 nm respectively.

SERS measurements were carried out using a micro-Raman Spectrometer (Lab Ram HR800) with a laser excitation wavelength of 632.8 nm. First, a 0.1 M stock solution of 2-Thienylboronic acid (2-TBA) was prepared and stored. Different molar concentrations (500 μM to 1 μM) of D-glucose were prepared by sequential dilution method. Analyte solutions were prepared by mixing 500 μL of the 0.1 M 2-TBA solution and 250 μL solutions of D-glucose of different molar concentrations. 50 μL of the analyte solution was dropped on the Ag nanocluster substrate and allowed to dry naturally overnight under ambient conditions. For practical applications the drying process can be carried out under dry nitrogen to accelerate the drying process.

We first carried out SERS measurements on mixtures of different molar concentrations of 2-TBA (0.1 M–1 μM) and D-glucose (1 mM–1 μM). From these measurements we observed that 0.1 M concentration of 2-TBA with different molar concentrations of D-glucose showed all the characteristic peaks of D-glucose. The possible reason for this is that the surface coverage of 2-TBA on Ag nanoclusters may be optimum at this molar concentration. All subsequent measurements were carried out by using 0.1 M concentration of 2-TBA as constant and varying the D-glucose molar concentration from 500 μM to 1 μM.

3. Results and discussion

The SERS spectra of the 0.1 M base solution of 2-TBA were recorded first to see if the bridge molecule attaches to the silver nanocluster substrate. Fig. 2(A) shows the SERS spectrum of 2-TBA. The spectra shown in the figure are baseline corrected using line-segmented baseline correction. Table T1 (supplementary information) shows a comparison of the Raman peak assignments with reported FT Raman measurements [23]. The intense Raman line at 880 cm^{-1} is assigned to C—H out of-

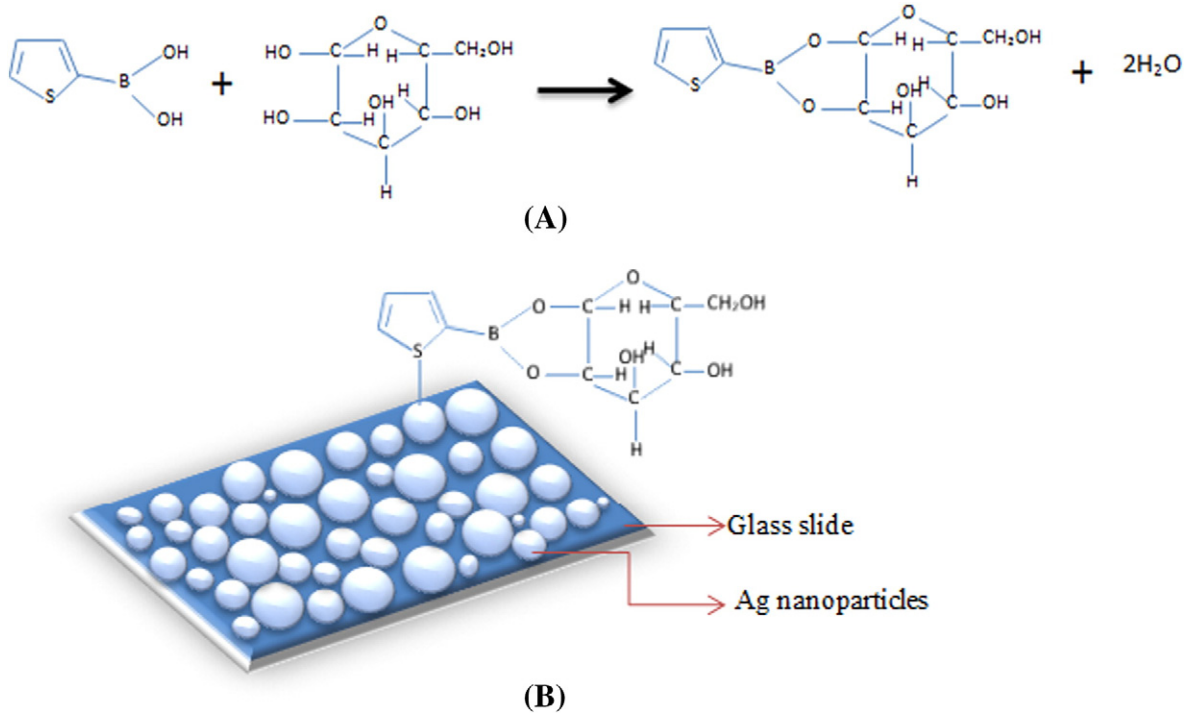

Fig. 1. Schematic diagrams depicting (A) the bonding between the 2-Thienylboronic acid and D-glucose and (B) the attachment of the 2-Thienylboronic acid molecule to the silver surface via Ag—S bond.