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Surface Enhanced Raman Scattering studies of L-amino acids adsorbed on silver nanoclusters

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ABSTRACT

Silver nanocluster films were prepared using plasma inert gas phase condensation technique. These were used as Raman active substrates for Surface Enhanced Raman Scattering (SERS) studies of 19 standard L-amino acids adsorbed on the surface of Ag nanoclusters via Ag—N bonds. A detailed study of two essential aromatic amino acids viz. L-Phenylalanine and L-Tryptophan showed a correlation between the Raman intensity of the characteristic lines of phenol and indole side chains and their molar concentrations in the range 1 μM–1 mM. This indicates that Raman studies can be used for quantitative determination of the amino acids in proteins.

1. Introduction

The intensity of Raman scattered lines from molecules can greatly be enhanced by adsorption of the molecules on the surface of nanosize metal structures. This was shown first by Fleischmann in 1974 by adsorbing molecules on roughened silver electrodes [1]. This technique is called Surface Enhanced Raman Scattering (SERS) and has been found to be very effective in ultrasensitive detection of molecules down to almost a single molecule level [2]. Because of its sensitivity and selectivity it has many applications in the field of biological sciences [3], analytical chemistry [4], forensic sciences [5], art and archeology [6], clinical diagnostics [7], and food safety [8]. The basic mechanism of the large signal enhancement is the excitation of a localized surface plasmons and the consequent increase in the electric field near the metal nanoparticle surface where the molecule to be studied is adsorbed.

Various methods to prepare SERS active substrates presently exist, such as colloidal solution method [9,10], core shell metal nanoparticle [11], thermal inkjet technology [12], vacuum synthesized nanoparticles [13], electron beam lithography and nano-transfer printing [14], laser ablation [15] and sputtering techniques [16]. In the present report we prepared Ag nanocluster films using inert gas phase condensation technique. In this method there is a production of pure metal nanoparticles and there is no surface contamination with other chemicals.

In this work we report SERS measurements on 19 out of a total of 20 standard L-amino acids. These protein-building amino acids are characterized by a carboxylic acid group (COOH) and an amine group (NH_2) attached to the same alpha carbon atom and a side chain specific to each acid. Their general formula is: NH_2—CH(R)—COOH and they are classified according to the different side chains in them: aliphatic amino acids [L-Alanine (L-Ala), L-Glycine (L-Gly), L-Isoleucine (L-ILe), L-Leucine (L-Leu), L-Proline (L-Pro) and L-Valine (L-Val)], Aromatic amino acids [L-Phenylalanine (L-Phe), L-Tryptophan (L-Trp) and L-Tyrosine (L-Tyr)], Acidic amino acids [L-Aspartic acid (L-Asp), L-Glutamic acid (L-Glu)], Basic amino acids [L-Arginine (L-Arg), L-Histidine (L-His), and L-Lysine (Lys)], Hydroxylic [L-Serine (L-Ser) and L-Threonine (L-Thr)], Sulphur containing amino acids [L-Cysteine (L-Cys) and L-Methionine (L-Met)] and Amidic amino acids [L-Asparagine (L-Asn) and L-Glutamine (L-Gln)]. Amongst these, we have further chosen two aromatic essential amino acids: L-Tryptophan (indole side chain), and L-Phenylalanine (Phenyl side chain) for a detailed study. Although some preliminary qualitative SERS studies of these amino acids are documented in literature [17–20], we present here for the first time a quantitative analysis of the SERS data of these two amino acids in the concentration range of 1 mM to 1 μM.

2. Experimental methods

The Ag clusters were deposited on 1 cm^2 glass plates using a NANODEP60 cluster deposition system from Oxford Applied Research, UK. This deposition system is based on the principle of inert gas phase condensation [21]. Atoms are sputtered using a DC magnetron source and are made to move in an agglomeration zone

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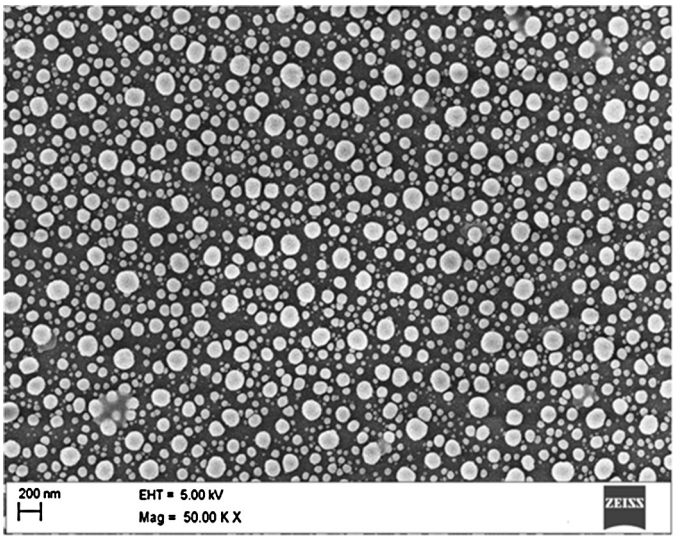

Figure 1. FESEM image of Ag nanoclusters deposited using Nanodep60 cluster deposition system on glass substrate followed by annealing for 2 h at 300 °C.

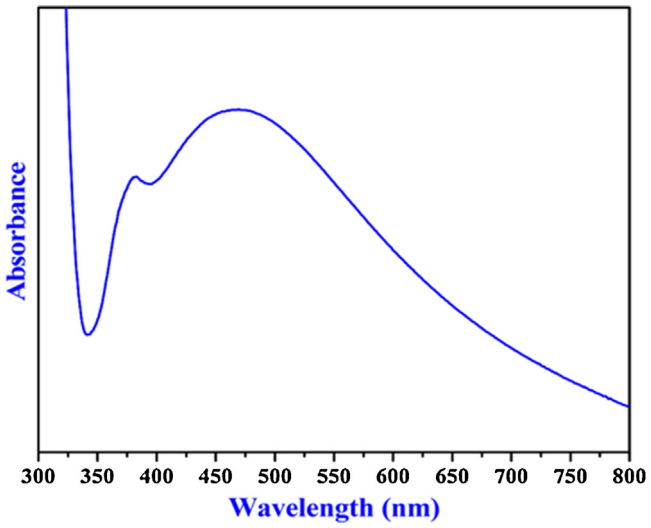

Figure 2. Optical absorption spectrum of the SERS substrate showing surface plasmon peaks at 383 nm and 470 nm due to quadrupole and dipole oscillations.

under an inert gas flow. Here they coalesce to form clusters whose size depends on the length of the agglomeration chamber, the gas flow rate, the temperature of the chamber, and the magnetron power. The clusters then move into the deposition chamber through an aperture and get deposited onto the substrate. The deposition chamber is maintained at a lower pressure as compared to the agglomeration chamber using a turbo pump of higher pumping rate as compared to the turbo pump of the agglomeration chamber, thus providing a differential pressure for the flow gas together with the agglomerated clusters to flow toward the deposition chamber. Our method of making nanoclusters is by direct deposition of Ag nanoclusters in an Ultra High Vacuum (UHV) cluster deposition system and differs from chemical methods that leave residues on the Ag nanoparticle surface. The substrate glass sides were sonicated and cleaned using iso-propan-2-ol, acetone and Milli-Q water before deposition of clusters. A deposition time of 8 min followed by a heat treatment of 300 °C for 2 h gave the best size of the nanoclusters for optimum surface plasmon absorption at the excitation wavelength (514.5 nm) of the laser used for Raman studies [16].

Surface morphological studies of silver nanocluster films were carried out using Field Emission Scanning Electron Microscope (FESEM model Ultra 55 from Carl Zeiss). Optical measurements were carried out using a UV–Vis Spectrophotometer (Jasco V-670). The SERS spectra of were recorded with a micro-Raman spectrometer (HORIBA, LabRam HR). An FESEM image of the annealed silver nanocluster (AgNC) film is shown in Figure 1. The optical absorption behavior of the AgNC film is shown in Figure 2. Optical absorption spectrum has two maxima at ~383 nm and ~470 nm and these are due to the excitations of quadrupolar and dipolar oscillations respectively. Dipolar oscillations correspond to smaller size nanoclusters and quadrupolar oscillations correspond to bigger size nanoclusters [22].

Initially 1 dM solutions of the amino acids were prepared by dissolving in Milli-Q water. Sequential dilution of this solution was carried out to get solutions in the range of 1 mM to 1 μM. Samples for SERS measurements were prepared by dropping a 40 μL droplet of the amino acid solution on the Ag nanocluster substrate and were allowed to dry naturally overnight under ambient conditions. Raman spectra were recorded with a laser excitation wavelength of 514.5 nm. Typical laser power and spot size used was 0.7 mW and 2 μm diameter respectively. The estimated number of molecules present in the laser beam probe area was about 10^4.

3. Results and discussion

SERS spectra of the 19 L-amino acids at 1 μM concentration level (which is the lowest concentration of the amino acids studied here) are shown in Figures 3 and 4. The Raman spectra shown in these figures have been grouped into different categories according to the type of side chains present in them viz. aromatic, aliphatic, acidic, basic, amidic, hydroxylic, and sulphur containing. The peak assignments of the Raman lines of these amino acids are given in Supplementary information of this manuscript. A comparison of the Raman spectra of the as-received amino acids with the SERS spectra of the acids adsorbed on the Ag nanocluster substrates shows an additional medium intensity line between 230 cm^{-1} and 242 cm^{-1}. This additional line has been previously attributed to the formation of a Ag—N bond through which the nitrogen containing molecules attach to the metal surface [16,23]. Accordingly we can assign these additional medium intensity lines in the SERS spectra of L-amino acids to the formation of Ag—N bond through which these biomolecules attach to the metal surface. There is a weak Raman line in the region of the Ag—N bond line observed in the normal Raman spectrum of L-Trp. This has been attributed to the bending of the benzene ring or pyrrole ring [20]. This line is however weak and the intense line observed in the SERS spectrum of L-Trp is also present in the SERS spectra of all the other amino acids which supports our assignment of this line to a Ag—N bond.

To be able to use the intensity of the Raman lines for quantitative measurements it is essential to identify the characteristic lines specific to the amino acid. The intensity of the Raman line from the carboxylic group shows a variation with concentration for all the amino acids. However we discuss here in detail quantitative measurements on two aromatic acids: L-Phe and L-Trp that have the phenol and indole side chains respectively. The characteristic Raman lines from these groups could be identified in the SERS spectra. Their Raman spectra of these two amino acids were first recorded in the as-obtained powder state to identify the positions of various Raman lines. The spectra are shown in Figure 5.

The peaks observed for L-Phenylalanine (L-Phe) powder were in good agreement with literature values [24,25]. The weak Raman line at 619 cm^{-1} is due to ring deformation mode of mono substituted benzene ring, the very intense peak at 1000 cm^{-1} and slightly weaker one at 1031 cm^{-1} are attributed to mono substituted benzene ring vibration or ring breathing mode. The SERS peak positions (see Figure 4B) are close to but do not exactly match with the