1	Preparation of copper sphere segment void templates for
2	electrochemical SERS and their use to study the interaction of amino
3	acids with copper under potentiostatic control.
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11 Abstract

We describe a simple method to prepare nanostructured copper electrodes based on electrodeposition of copper through a polystyrene nanosphere template to produce a copper sphere segment void (SSV) structure. We show that the SSV copper electrodes give a large SER enhancement under potentiostatic control and we use the SSV copper electrodes to study the adsorption of aromatic and non-aromatic amino acids in basic solution.

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18 **Keywords**: SERS, copper, spectroelectrochemistry, amino acid.

19 Graphical Abstract

20

Graphical abstract

21 Highlights

22 - Simple and reproducible copper substrate preparation for SERS measurements.

23 - Robust surface with good surface enhancement.

- The templates were used under potentiostat conditions to study SERS of amino acids.

- With the SSV Cu surfaces it was possible to obtain SERS of aromatic and non-aromatic amino acids.

26 Introduction.

27 Surface-enhanced Raman spectroscopy (SERS) is recognized as one of the most sensitive 28 spectroscopic analytical techniques for chemical and biochemical analysis. One of the key issues for 29 the application of SERS is the preparation of structurally uniform and highly sensitive SERS substrates 30 [1]. With that in mind, the aim of this paper is the development of sphere segment void (SSV) electrodes for use in electrochemical SERS studies at copper electrodes. The nanostructured copper 31 32 surfaces are produced by electroplating copper through self-assembled templates of uniform sub-33 micron polystyrene spheres [2]. This has the advantage of producing copper electrode surfaces 34 which show large, reproducible and stable SERS enhancements without resorting to roughened surfaces which have high surface areas and where the precise surface area of the electrode is not 35 36 well known.

Copper is an interesting material with unique properties and it has an important role in several diseases, such as in Parkinson's disease where it is associated with protein conjugation [3]. Copper has also attracted great interest as an antimicrobial material [4], due to the reactions of biomolecules with the oxides of copper present on the metal surface. In addition, copper electrodes are widely used to oxidise and detect amino acids [5-7] in analytical applications. The mechanisms that control the oxidation of amino acids on copper electrodes rely on the formation of an
intermediate between copper oxide and the amino acid, but are not very clearly understood.

44 Copper is a SERS active metal and so the technique offers a sensitive method to explore the surface chemistry of the metal in solution [8-10]. However the study of the interaction between 45 46 amino acids and copper surfaces in alkaline media presents a significant challenge due to the high 47 reactivity of the metal. With this fact in mind, it is of interest to develop copper electrodes suitable 48 for use with SERS to investigate the adsorption and oxidation of the amino acids on copper surfaces. 49 To do this requires a copper electrode structure that gives significant surface enhancement, that is 50 easy to prepare, that has a well-defined surface area, and that is reproducible and stable. SSV 51 copper electrodes fulfil all these requirements. As previously reported by Mahajan et al.[11], the use 52 of SSV templates has significant advantages as a way to achieve large surface enhancements at 53 electrodes without high roughness. It is possible to prepare those surfaces for different metals, the 54 most commonly used being gold but also including silver [12] platinum and palladium [13]. Previous 55 SERS studies on copper electrodes have mainly used electrochemically roughened surfaces [14, 15] 56 however these suffer from the problems of high surface area, irreversibility and poor stability particularly at more negative potentials [16]. This development of SSV nanostructured copper 57 electrodes for SERS offers the possibility of using SERS to study the interactions of small molecules 58 59 with copper electrodes with stable, reproducible structures and known surface areas. Thus the main 60 objective of the present work is to describe the preparation and optimization of SSV copper surfaces 61 as working electrodes for SERS.

62

63 Materials and Methods

Evaporated gold electrodes used as substrates were prepared by depositing 10 nm of chromium, followed by 200 nm of gold onto 1 mm thick glass microscope slides. These gold substrates were thoroughly cleaned before use by sonication in deionized water for 30 min,
sonication in isopropanol for 90 min, then rinsed with deionized water and dried under a stream of
argon (BOC Gases).

Templates were made from monodisperse polystyrene latex spheres (Duke Scientific Corporation, 1 wt % solution in water, coefficient of variation in diameter 1.3%). Assembly of the spheres was carried out in a thin layer cell as described elsewhere [17]. Copper was deposited from an electroplating solution containing 0.1 mol L⁻¹ copper sulfate with 5 mL L⁻¹ of PEG 400 MW (Sigma Aldrich – St. Louis) and 1 x 10⁻⁶ mol L⁻¹ KCl at 25° C using a conventional three-electrode cell controlled by an Autolab PGSTAT30 under potentiostatic conditions at - 0.1 V vs. Ag|AgCl (saturated KCl).

After deposition, the samples were soaked in 50 mL of DMF for two hours to remove the polystyrene template. A Philips XL30 ESEM was used to image the macroporous metal films. All Raman spectra were recorded on a Renishaw Raman 2000 system using a 633 nm HeNe laser with 5 µm diameter spot and 3 mW power using a single 10 s accumulation unless otherwise stated. Benzenethiol was adsorbed onto the gold surface by soaking in a 1.4 x 10⁻³ mol L⁻¹ ethanolic solution for 30 min. The samples were left to dry in air for 15 min before measurement.

82 Amino acid spectra were recorded on a Renishaw Raman 2000 system using a 633 nm HeNe laser 83 with 5 μ m diameter spot and 3 mW power using a single 10 s accumulation under potentiostatic 84 control using a 3 electrode cell unless otherwise stated. The spectroelectochemical cell has the SSV 85 copper as the working electrode, a stainless steel counter electrode and Ag|AgCl (saturated KCl) 86 reference electrode. In the cell the SSV template working electrode is in the center surround by the 87 counter electrode with the laser beam of the spectrometer normal to the SSV surface (see Figure S1 in Supplementary Information). All solutions were prepared in 0.1 mol L⁻¹ sodium hydroxide (Fischer 88 Scientific) with the amino acid concentration around 10⁻⁴ mol L⁻¹. Details of the experimental setup 89 90 are presented in the Supplementary Information.

92 Results.

93 Electroplating of copper SSV structures.

The conditions for the electrodeposition of copper through the template are discussed in some detail in this work since the optimization of the deposition step is important in order to produce well-structured electrodes with a uniform surface. Figure 1A shows a graphic of the desired SSV structure. To achieve this, the parameters such as applied potential, solution concentration and additives in the electroplating bath need to be optimized to ensure uniform electrodeposition of copper on the 10 nm scale.

100

FIGURE 1

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102 Copper is one of the easiest, and most widely, electroplated metals, with several books 103 dedicated to the topic [18-21]. The applied potential plays an important role in the electroplating of 104 copper and in order to prepare SSV surfaces in a simple and reproducible way, the applied potential 105 was chosen to meet the following criteria: nucleation should be fast in order to generate a large 106 number of nuclei to obtain a small grain size; the deposition of the template film should not take too 107 long in order to avoid an excessive formation of copper oxide; the surface of the deposit should be 108 smooth on the 10 nm scale and the formation of dendrites avoided.

For this particular system, using a nanosphere template covered evaporated gold electrode, the open circuit potential in the 0.1 mol L⁻¹ copper sulfate plating solution was around 0.0 V vs Ag|AgCl (saturated KCl). A range of deposition potentials were investigated between -0.15 to 0 V vs Ag|AgCl (saturated KCl). At -0.05 V no deposition was observed after 15 min, at -0.10 V after 3 to 5 min a visible copper layer was deposited on the surface. As shown in Figure 1B the resultant film has the expected gross structure, however there are undesired micron scale copper grains on the surface. This is due to the fact that, even at low overpotentials deposition still occurs in preferential areas, leading to rough over growths on the electrode surface. To overcome this we turned to the use of additives in the electroplating bath.

118 Polyethylene glycol (PEG) and KCl are commonly used additives in copper plating baths. PEG 119 has the property of inhibiting the deposition of copper, suppressing dendritic growth. Chloride was 120 added as it is known to work together with PEG to promote a better quality copper deposition [22-121 24]. This can be clearly seen in Figures 1C and 1D were the template surface is now much smoother 122 than that obtained using copper sulfate alone in the electroplating bath and is free from the micron 123 sized copper grains seen previously. The optimized conditions for copper deposition were 0.1 mol L⁻¹ CuSO₄ with 1 mL L⁻¹ PEG and 1 10⁻⁶ mol L⁻¹ KCl as electroplating solution. Deposition was carried out 124 125 for 300 s at -0.1 V vs Ag | AgCl (saturated KCl).

126 Electrochemical measurements.

127 In order to assess the suitability of the SSV copper surface as an electrode the voltammetry 128 of the surface in 0.1 mol L⁻¹ NaOH was compared to that for bulk copper, Figure 2. The voltammetry 129 for the two electrodes shows identical surface oxidation and reduction features associated with the 130 formation and stripping of surface copper (I) and copper (II) oxides [25]. Comparing the current 131 densities in the two cases we see that for the SSV copper surface the current density, based on the 132 projected geometric area, is about three times that of the bulk copper electrode. This is consistent 133 with the increase in surface area expected for the template structure in which there is a smooth 134 copper metal surface electrodeposited around the template spheres. These results show that the 135 SSV copper surface behaves well as an electrode and has low surface roughness.

136

FIGURE 2

138 SERS studies.

139 As described in the text a three-electrode cell was used in these measurements, pictures of 140 the system can be seen in Figure S1 in the Supplementary Information. The cell consisted in a PEEK 141 (polyether ether ketone) circular body with a stainless steel ring attached to act as the counter 142 electrode. The SSV template is then attached to the centre of the cell and is used as the working 143 electrode. See FigureS1 A which shows the cell without its lid and with the working (WE) and counter electrodes (CE) highlighted. Figure S1B shows the assembled cell with reference electrode (RE) 144 145 entering the cell from the top and with the lid covering the cell. The lid is a circular piece of PEEK 146 with a quartz window. The space between the lid and the electrodes is filled with the electrolyte that 147 contains the amino acid solution that is to be studied. After sealing the cell and positioning the electrodes, the electrodes are connected to the potentiostat and the laser is focused on the SSV 148 149 surface. Figure S1C shows a picture of the system in use; it is possible to see that the laser that 150 comes through the microscope lenses reaches the template at an angle of 90 degrees.

151 The diameter of the voids and the thickness of the film both affect the plasmonic behavior of 152 the SSV structure [26] and hence affect the magnitude of the surface enhancement as discussed in 153 the literature [1, 11, 27]. This is because, for efficient surface enhancement it is necessary to ensure 154 coupling of both the incoming laser light and the outgoing Raman scattered light to plasmon modes 155 of the surface [28]. In this work templates varying from 400 to 900 nm were tested in order to seek 156 the best sphere diameter to obtain the correct coupling. In order to quantify the surface 157 enhancement on the SSV copper surfaces benzenethiol [29] was used as a model system to compare 158 the quality of the spectra and the enhancement to that seen on other metals, and in particular gold. 159 Benzenethiol is a good choice because it forms well defined self-assembled monolayers on gold, 160 silver and copper through the formation of metal-sulphur bonds [29, 30]. To assemble the 161 benzenethiol on the copper surface it was incubated in an ethanolic solution of benzenthiol for 3 162 hours, and then dried with the stream of nitrogen. All spectra were recorded in air immediately

after preparation. It is possible to observe the difference in quality and intensity for the spectra foreach template sphere diameter.

Several parameters had to be optimized in order to obtain the best coupling between the incoming laser light and the outgoing Raman scattered light. In order to find this optimum condition template spheres from 400 to 900 nm diameter were tested. As discussed in the text the sphere size and film thickness plays a crucial role in determining the size of the enhancement. For copper these needed to be optimised from scratch as there is no previous report on the use of SSV copper surfaces.

Figure 3 shows spectra for different copper SSV surfaces recorded using benzenethiol as a model compound. It can be seen that, even though the 700 nm sphere structures do not give the highest global intensity, the difference between the background and the peak height, and the signal to noise ratio, is highest for this sphere diameter. In addition the 400 nm spheres are more difficult to pack uniformly and so give less reproducible SSV substrates. For the 900 nm spheres the SSV structures show a large background.

177 The film thickness was a consequence of the deposition period. The deposition was 178 performed for 300 seconds; this is because longer deposition times led to uneven surfaces, with the 179 presence of copper blocks, which would interfere in the measurements. The obtained film thickness 180 was calculated according to the average size of voids, which had a correlation with the total sphere 181 size. As the voids have an open sphere shape, the film thickness is smaller than the total diameter of 182 the sphere. Therefore, it was possible to calculate the film thickness, which led to the following 183 results. For the polystyrene particle used as template for the deposition with a diameter of 400 nm led to a film with a thickness of 140 ± 5 nm, with 600 nm the film thickness was 220 ± 5 nm, for 700 184 nm it was 300 ± 5 nm and for 900 nm it was 350 ± 5 nm. 185

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FIGURE 3

For the conditions of this study, 700 nm diameter template spheres were found to give the best signal to background ratio when using the 633 nm laser and a film thickness of 300 nm. These SSV copper substrates are robust and show good stability. We were able to store samples for at least 5 days without loss of SERS activity provided care was taken to slow down surface oxidation (storage in a sealed container filled with nitrogen) and any surface oxide was electrochemically reduced before use.

194 Figure 4 shows an SER spectrum obtained for benzenethiol on the optimised SSV copper 195 surface. The spectrum corresponds to that of benzenethiol as expected [29]: the peak at 1579 cm⁻¹ is 196 assigned to $a_1 v$ (C-C-C) mode; the peak at 1079 cm⁻¹ to the $a_1 v$ (C-C-C) and v (C-S) modes; 1022 cm⁻¹ 197 to the $a_1 v$ (C-H) mode; 998 cm⁻¹ to the $a_1 v$ (C-C-C) mode. The broad peaks between 400 cm⁻¹ and 700 198 cm⁻¹ are not due to the benzenethiol and are believed to be due to several overlapping Cu₂O-related 199 bands such as those reported at 616 and 531 cm⁻¹ [31]. The intensity of the benzenethiol spectra 200 obtained on the SSV copper surfaces is around one tenth of that previously reported for an SSV gold 201 surface [29], but this is as expected when one takes account of the optical properties of the two 202 metals [32] and is consistent with studies of gold and copper metal colloids [33].

203

Figure 4

204 Spectroelectrochemistry measurements.

The SSV copper surfaces were used in a preliminary study of the adsorption of amino acids at copper electrodes in basic solution under potentiostatic control. Six amino acids were selected, 3 aromatic amino acids (tryptophan, histidine and phenylalanine) and 3 non-aromatic ones (serine, alanine and glycine). Non-aromatic amino acids have much lower Raman cross sections than those that have an aromatic ring in the structure [34] and are therefore more challenging to observe by SERS. The electrode potential is a key parameter in these experiments since copper readily undergoes oxidation, especially in the alkaline media (0.1 mol L⁻¹ sodium hydroxide) used for electrochemical oxidation of amino acids. The presence of an oxide layer at the copper surface is
believed to be important in the process; however, copper oxide may absorb light and could quench
the SERS process if the layer becomes too thick.

215 Figure 5 shows a set of spectra recorded for 7.5 10⁻⁴ mol L⁻¹ tryptophan in 0.1 mol L⁻¹ NaOH 216 at different applied potentials. Starting at the open circuit potential (around -0.2 V vs Ag|AgCl 217 (saturated KCI)) and then at +0.25 V there is a broad SERS band or set of bands between 400 and 700 218 cm⁻¹ corresponding to copper oxide but no features that could be attributed to the tryptophan. At 219 open circuit there is no significant different between the spectra with and without tryptophan (see 220 Figure S3). However on stepping the potential to -0.65 V, where the surface oxide is reduced to the 221 copper (I) oxide (see Figure 2), the spectra change significantly with new bands attributable to 222 tryptophan appearing at higher wave number and rapidly stabilising (Figure 5).

223

Figure 5

224 Figure 6 shows spectra for serine and tryptophan obtained under potentiostatic control at -225 0.65V vs Ag AgCl (saturated KCl). Under these conditions, in contrast with the results at open circuit 226 (Figure S3 and Figure 5), there are clear SERS bands associated with the adsorbed amino acids and 227 the broad bands associated with the copper oxide are significantly diminished. The spectra obtained 228 for serine and tryptophan were assigned by comparison with the literature, Tables 1 and 2. It is 229 notable that although the spectra for serine are less intense than those for tryptophan the bands are 230 still readily visible. Corresponding spectra for phenylalanine, alanine, histidine and glycine are given 231 in the supplementary information (Figures S4 to S6)

232

Figure 6

- 233
- 234

Table 1

Table 2

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239 There are three points to note. First, the copper SSV substrates show good stability during 240 use. Our experiments lasted up to 4 hours and over this time there was no problem with loss of 241 activity and the templates were stable upon potential cycling from +0.5 to - 0.7 vs Ag|AgCl (saturated KCl) with spectra being unchanged when the potential was returned to its initial value. 242 243 Second, this is the first work to report amino acid SERS on SSV copper surfaces and further studies 244 will be needed to fully understand all the vibration modes involved in the interaction of the molecule 245 and the copper electrode. Third, it is well established that the mechanism of interaction between 246 amino acids and the copper electrode involves the formation of some form of complex [35], it is 247 therefore reasonable to infer that the molecules are bound in some way to the surface of the electrode [36]. Considering these facts it is interesting to point out that a clear difference between 248 249 the spectra obtained on copper and those reported on silver in the work of Stewart et al. [37] is the 250 absence of vibrations associated with the carboxylate. This suggests that interaction between the 251 carboxylate group and the copper oxide may play an important role.

252 Conclusion.

In this paper we describe for the first time the fabrication of SSV copper surfaces for electrochemical SERS. Using benzenethiol as a model system, we have shown that these surfaces give strong SERS enhancements with the intensity of the spectra about one tenth of that found on gold. We have also shown that these SSV copper electrode surfaces can be used to obtain SER spectra for the aromatic amino acids (tryptophan, histidine and phenylalanine) and for non-aromatic amino acids (serine, alanine and glycine) under potentiostatic control in 0.1 mol L⁻¹ NaOH. This preliminary work on electrochemical SERS on SSV copper electrodes opens up a great number of

- 260 possibilities as it allows the study the interaction of molecules with copper electrodes at different
- applied potentials.

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Figures

Graphical Abstract





Figure 2



Figure 3











Subtitles

398 Graphical abstract presenting a sketch of the template developed in this work. Presenting a nanostructured 399 copper sphere void template that provides the surface enhancement.

Figure 1. SSV copper template. A) graphic of the desired template structure. B) scanning electron micrograph of a templated film produced without the use of additives, the electroplating solution was 0.1 mol L⁻¹ of CuSO₄, deposition was carried out for 150 s at -0.1 V vs Ag|AgCl (saturated KCl). C and D) scanning electron micrographs of templated films prepared from solution containing additives (5000 and 15000 magnification) of 0.1 mol L⁻¹ of CuSO₄ with 1 mL L⁻¹ of PEG and 1 10⁻⁶ mol L⁻¹ KCl, deposition was carried out for 300 s at -0.1 V vs Ag|AgCl (saturated KCl).

Figure 2 Cyclic voltammetry of an SSV copper electrode and a bulk copper electrode in 0.1 mol L⁻¹ sodium hydroxide, scan rate 10 mV s⁻¹. The bulk copper electrode was 3 mm copper rod insulated in PTFE, the SSV copper electrode had an exposed area of 1.5 mm by 2 mm.

Figure 3: Raman spectra for copper SSV substrates made using different template sphere diameters. Film
 thickness are for 400 nm, 600 nm, 700 nm and 900 nm spheres 140 ±5 nm, 220 ±5 nm, 300 ±5 nm and 350 ±5
 nm respectively, recorded for benzenethiol in air, 633 nm laser,10 s single acquisition.

Figure 4. Baseline corrected SER spectrum for benzenethiol recorded in air on an SSV (700 nm diameter, 300 nm thick) copper surface. The benzenethiol was deposited from a 14 mM ethanol solution, 633 nm laser of 3 mW, single 10 s acquisition.

Figure 5. A series of spectra obtained at different potentials for an SSV copper electrode in solution of 7.5 x
10⁻⁴ mol L⁻¹ tryptophan in 0.1 mol L⁻¹ NaOH, 633 nm laser, of 3 mW, single 10 s acquisition. The potential was
stepped from the open circuit potential (-0.2 V) to +0.25 V and then to -0.65 V vs Ag|AgCl (saturated KCl).
Spectra were recorded every 154 s.

Figure 6. Baseline subtracted spectra for (Black line) 7.7 x 10^{-4} mol L⁻¹ serine and (Red line) 7.5 x 10^{-4} mol L⁻¹

420 tryptophan at an SSV copper electrode (700 nm diameter, 300 nm thick) recorded in 0.1 mol L⁻¹ NaOH at -0.65
421 V vs Ag | AgCl (saturated KCl).

422

Tables

Table 1

Table 1. Serine peak assignment

Wavenumber / cm ⁻¹ This work	Wavenumber / cm ⁻¹ Ref [37–38]	Assignment
	1120	- NUL +
1145	1129	ρΝH ₃
1324	1325	
1348	1354	δ CH + δChain framework
1388	1422	ωCH ₂ + $δ$ COH + + $δ$ Chain framework
1478	1464	δCH₂
1532		

Table 2

Table 2. Tryptophan peak assignment.

Wavenumber / cm ⁻¹ This work	Wavenumber / cm ⁻¹ Ref [39]	Assignment
1071	1066	γNH₃⁺, βH(C)
1324	1321	βH(C), ωCH₂
1378	1367	ωCH₂, βCH
1426	1418	v(r), v(R)
1508	1486	v(R), v(r)
1574	1564	v(R), v(r)