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FLUORESCENT COPPER NANOCLUSTERS

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ABSTRACT

Clusters of coinage metals like silver and gold have been studied for their opto-physical properties and interesting applications. These nanoscale materials are usually synthesized from their salts by reduction usually in aqueous solution at ambient conditions. Copper nanoclusters were synthesized from copper (II) salts using sodium borohydride as reducing agent in aqueous solution. The resulting sample was observed to be luminescent with emission bands at 346 and 428 nm. Confocal microscopy images of the sample showed small, seemingly spherical "particles" that were visibly fluorescent and photostable. Mass spectrometry data revealed that the clusters had a molecular mass of m/z = 575.8 and a molecular formula of $[Cu_7B_3O_5.H_2O]^-$. These clusters can be applied in the areas of catalysis, optical electronic devices, chemical and biosensors in in vitro systems.

Keywords: copper nanoclusters, fluorescence, emission, wavelength, mass spectroscopy.

INTRODUCTION

Copper is the 25th most abundant element on earth [1,2]. It a member of the transition metal elements and also a coinage metals together with silver and gold. Its clusters are less studied and synthesized as much as with silver and gold which may be attributed to their poor photostability and its tendency to aggregate following synthesis. Nevertheless, their particles are widely used as catalysts [3] and as chemical sensors [4–6].

Copper nanoparticles are nano-sized molecule-like materials which show the characteristic optical properties of nanoscale materials. Among the properties of nanoscale materials include surface plasmon resonance, fluorescence, catalytic and magnetic properties. These clusters usually consist of a few number of atoms of the metal of between 2 – 13 atoms [7] with no more than 2 nm in size.

As materials move from the bulk size towards the nanoscale they reduce in terms of density of state and become molecule-like in properties [8]. They likewise have distinct band gaps as a result of the separation between the valence and conduction bands which allows for electronic transitions when the requisite amount of energy is absorbed. Bulk metals usually have overlapping energy levels as a result of their high density of state resulting in a continuous energy band without bandgaps [9–11].

Fluorescence occurs when materials absorb the requisite amount light energy which causes electronic transition from the ground state to the excited state energy level where it undergoes vibrational relaxation by losing some of it excited energy before returning back to the ground state [12–14]. During luminescence, the wavelength of the emissions falls often in the visible region of light at longer wavelengths with distinct colours. This optical property is the basis of numerous opto-applications of fluorescent nanoclusters scientifically and industrially as well as in medical diagnosis [15].

Until recently majority of the work done on luminescent metal clusters have variously centred on silver and gold, rarely is copper mentioned even though it is relatively more abundant and cheaper. The reason again is attributed to the ease by which the particles readily oxidizes in aqueous medium and the difficulty in making smaller particles are which are fluorescent. In this study, the synthesis of small clusters of copper in aqueous solution which are luminescent is reported.

MATERIALS AND METHODS

Materials

Copper nitrate trihydrate ($Cu(NO_3)_2.3H_2O$, 98 %) product of Fluka Chemika, United Kingdom. Sodium borohydride ($NaBH_4$, 99 %) (213462-25G) also from Sigma-Aldrich, USA. Silicon wafer. Distilled-deionized water.

Method

100 µM aqueous solution $Cu(NO_3)_2$ was prepared from its salt and gently reduce with a stoichiometric portion of aqueous $NaBH_4$ solution in a 250 mL conical flask at room temperature. Insert a clean magnetic stir bar into the mixture and place on a magnetic plate set at 150 rpm for 3-5 minutes to allow the reaction come to completion.

A control sample without the analyte was synthesized also in aqueous solution, this sample was without the copper nitrate salt; however, using the same reaction protocols.

Equation of the reaction

$$Cu(NO_3)_{2.(aq.)} + 2NaBH_{4(aq.)} + 6H_2O \longrightarrow Cu_{(s)} + 2NaNO_{3(aq.)} + 2H_3BO_{3(aq.)} + 7H_{2(g)}$$

Fluorescence determination of copper nanosample

Fluorescence emission measurements of the copper samples were done by taking aliquots of the samples into quarts cuvette and ran at various excitation wavelengths ranging from 290 – 900 nm on a Perkin Elmer FL 6500 model.

Confocal fluorescence microscopy imaging of copper nanosample

Samples were drop casted on previously piranha cleaned silicon wafers and allowed to vacuum dry over night before the images were obtained using a confocal fluorescence microscope instrument manufactured ZEISS LSM 900 model.

Mass spectroscopic determination of as-synthesized copper nanosample

Mass spectrometer data of the copper nanosamples were obtaind by withdrawing 200 **µ**L of the aqueous sample in an electrospray ionisation mass spectrometry (ESI-MS) instrument by Waters LCT Premier electrospray ionization (time of flight (TOF)) mass spectrometer. The instrument having been previously calibrated with *leucine enkephalin* as the reference sample.

Simulated spectrum

The University of Manchester, United Kingdom, mass spectrometer software tool: http://fluorine.ch.man.ac.uk/research/mstool2.php was used to confirm mass spectrometry data obtained for the copper nanosamples by simulating the copper spectra in line with molecular mass obtained from the ESI-MS.

RESULTS AND DISCUSSION

Fluorescence

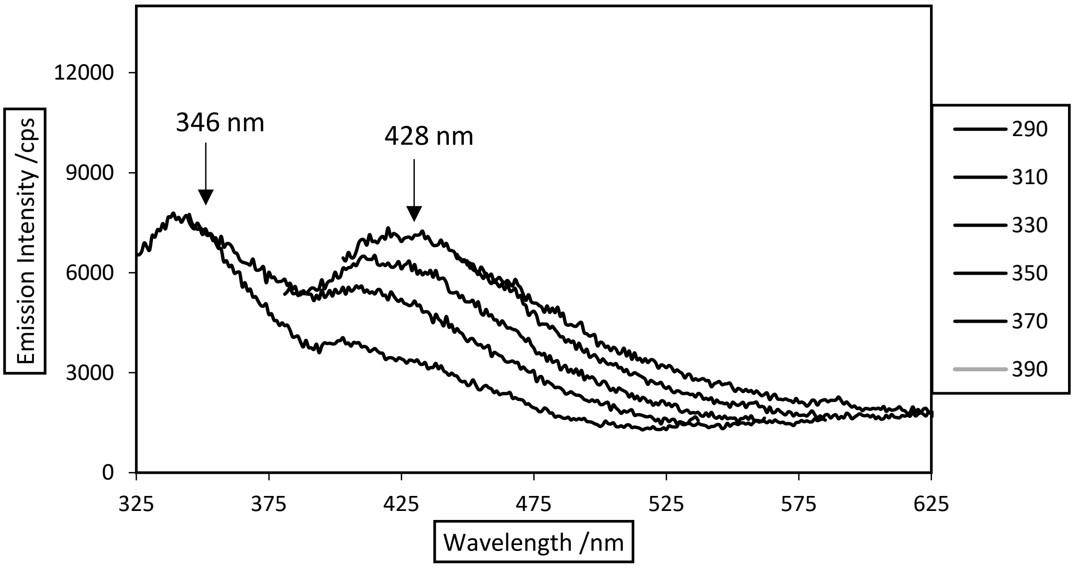

Figure 1: *Fluorescence emission spectra of (a) 100 µM CuNCs in aqueous solution. Excitation wavelengths are shown in the legends.*

Figure 1 is the emission spectrum of the copper nanosample showing that the sample is fluorescent. The display of luminescence by metal nanosamples is a confirmation that indeed nanoclusters have been formed. In this case, confirming that copper nanoclusters (CuNCs) are present in the sample under analysis; especially as the control samples comprising only of sodium borohydride solution and *vice versa* showed no luminescence. This is because as was explained in the previous section, only molecule-like clusters of metal atoms with low density of state and having bandgaps which allows for electronic transition can therefore exhibit luminescence.

In the emission spectrum above, two prominent bands are observed, one at 346 nm and the other at 426 nm. This infers also that the samples are not monodispersed but may contain different morphologies. Both bands are weak considering that the values of their intensity signals averages at about 8000 counts per second (cps). By further inference therefore, it implies that the population (concentration) of the fluorescent species in the sample medium is low. Low fluorescence intensities with copper has also been reported in the literature [16]. Fluorescence intensity has been variously linked with the concentration of the fluorophores in any given sample [12,17,18]. The low intensities observed in this sample is not strange considering that the aqueous synthesis of CuNCs is froth with the problem of oxidation which leads to fluorescence quenching [19]. Nonetheless, since biological systems are basically aqueous media, it is inevitable to simulate the synthesis of CuNCs in aqueous conditions.

Meanwhile, the positions of the two emission bands in figure 1 indicate two morphological cluster sizes. The band at longer wavelength is broader with a comparatively larger full-width at-half-maximum (FWHM), even though both of them are about the height (intensity). Nevertheless, both set of CuNCs are both blue emitters emitting in the near visible region of the electromagnetic spectrum [20,21]. This implies that their wavelengths of absorption (290 nm) falls well inside the ultraviolet region. Furthermore, the implication is that the samples have large bandgaps [22].

Finally, the broadness of the band at 428 nm indicates ineffective metal-ligand orbitas mixing leading to longer deep trapped-states (dark phase- nonradiative) energy loss before returning to the ground state by losing the residual energy in the form of fluorescence. This accounts for the longer emission wavelength since also, it have been established that wavelength is inversely proportional to energy (Einstein’s equation). Secondly, the species responsible for the 428 nm band are obviously aggregating CuNCs which are spontaneously obeying the thermodynamic requirement for stability brought about by their high surface-to-volume ratio conferred on these clusters by their small sizes [23] hence they aggregate and form larger clusters/particles.

Fluorescence microscopy

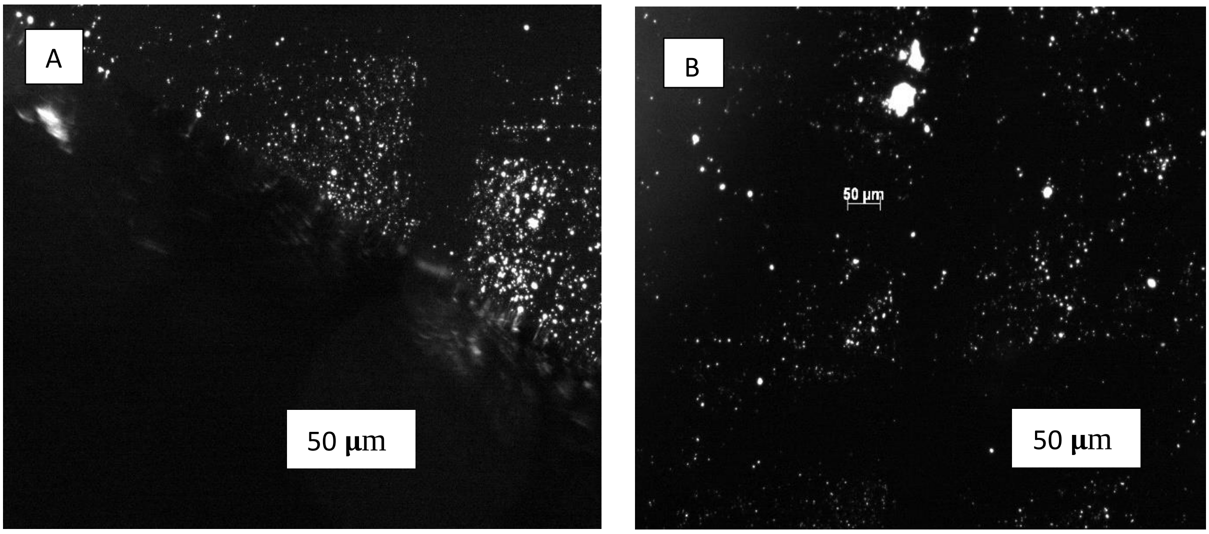

Figure 2: *Fluorescence microscopy images of (*A and B*) 100 µM CuNCs exposure time of 1450 ms.*

Figure 2 are epifocal fluorescence images of the CuNCs samples obtained by drop casting aliquots of the sample on cleaned silicon wafer and allowed to dry before imaging. These images not only serve as further confirmation of the fact that the sample is indeed fluorescent, they also show to the human eyes that there are factually small, seemingly spherical and diverse clusters of copper which are spread on the silicon wafer. Besides, the presence of obviously clear and dark backgrounds especially on the bottom left hand side of the image A above where the sample did not apparently spread up to is a confirmation that the as-synthesized sample contained fluorescent CuNCs.

Meanwhile, we can also observe some larger "particles" in these images (especially in image B) which confirms the initial assertion that the sample was not monodispersed but already contained aggregating species which are still luminescent. The bigger clusters gives an indication of growth to a more stable particle and a shift to longer wavelengths as the energy of emission has an inverse correlation with the "particle" size [24].

Finally, both images were obtained under an exposure time of 1450 milliseconds showing that the CuNCs were photostable.

Electrospray ionisation mass spectroscopy

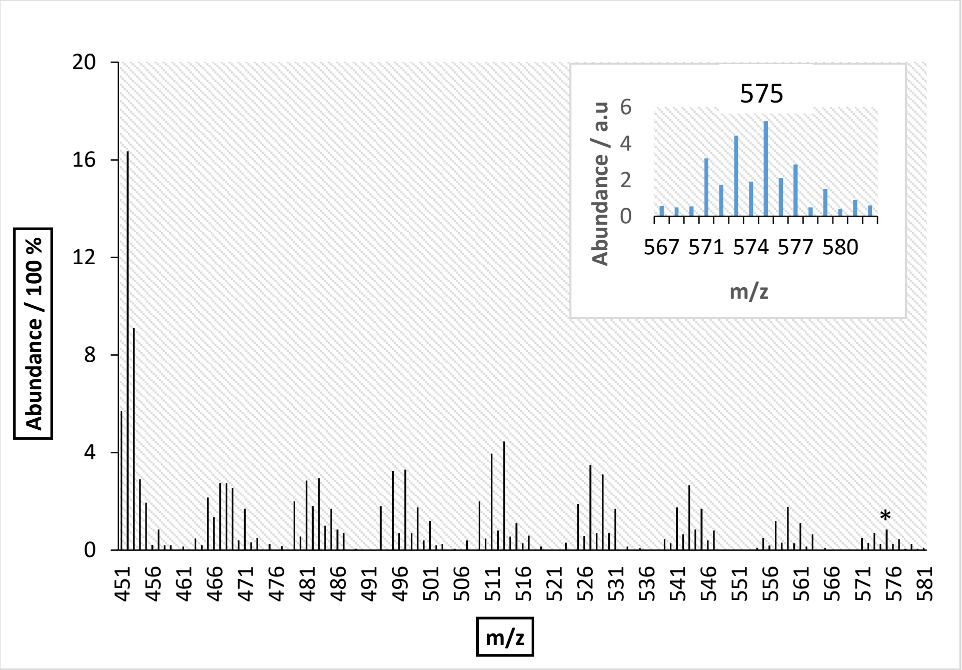

Figure 4 is the *ESI-MS spectrum of 100 µM CuNCs in aqueous solution showing clusters of peaks ascribed to Cu species. The asterisk are peaks attributed to* Cu_7 *nanoclusters* **$[Cu_7B_3O_5.H_2O]^-$**. *Inset is the simulated version of the set of asterisked peaks at 575 m/z.*

It makes scientific sense to be able to determine the molecular mass and indeed composition of the clusters. This can be done with the help of a mass spectrometer instrument which can give the molecular mass without decomposing the sample. Figure 4 is the spectrum of the CuNCs sample obtained at the negative mode of the instrument (the positive mode did not produce explicit patterns of CuNCs).

Copper has two isotopes ^{63}Cu and ^{65}Cu with percentages abundance of 68.94 and 31.06 % respectively [25]. This would be expected to have a split pattern of 2:1 in a mass spectrum. The group of peaks between m/z 461 – 581 are recognizable by their distinct split patterns for Cu_7 clusters, that is, a group of clusters containing seven atoms of copper mixed with patterns of boron. Boron hydride, borate and water molecules comprises the ligands coordinated to the metal clusters. The borates are products of the oxidation of borohydride anions in the reaction medium.

The last set of peaks at m/z 575.8 are of particular interest because they signify the species with the highest molecular mass in the spectrum. Further analysis of the signals in this spectrum reveals a difference of $\Delta m/z = 1$ between the isotopic signals of copper. For example, in the last set of peaks with a maximum at m/z = 575 on the spectrum, the difference between the 575 isotope signal and the next copper signal at m/z = 577 is m/z = 2. By interpretation therefore, this m/z of 2 gives a charge of one (1); meaning that the molecular mass of the substance at m/z = 575. Meanwhile as the sample data was obtained in the negative mode meaning ionization was done by the loss of a proton from the sample, the resulting analyte will carry a net negative charge.

From the foregoing, this group of peaks which is likened to the parent peaks have been resolved to as $\mathbf{[Cu_7B_3O_5.H_2O]^-}$. In order to confirm this, a simulation of the mass spectrum signals for $[Cu_7B_3O_5.H_2O]^-$ was undertaken using the software provided by the University of Manchester. The resulting peaks (inset in figure 4) matched perfectly with the experimental peaks obtained from the mass spectrometer data in figure 4. The borates and water ligands were determined from the difference between each successive sets of peaks in the region under focus. Put together therefore, evidence from this study points at the formation of clusters of copper comprising of seven atoms coordinated by borates and water molecules.

CONCLUSION

From the foregoing, copper nanoclusters were synthesized from their metal precursors in aqueous medium using sodium borohydride as the reducing agent. No passivating ligand was added to the reaction medium as stabilizers. The sample was observed to be fluorescent *albeit* weakly. Two emission signal bands were recorded at 326 and 428 nm, showing that the CuNCs produced were blue emitters whose absorption wavelengths were in the ultraviolet region; meaning they have high bandgaps of approximately 3.58 and 2.90 eVs respectively.

The fact that these samples are luminescent was confirmed by their fluorescent and epifocal data. The epifocal data further demonstrate the existence of small spherical species which were obviously fluorescing.

Mass spectrograph data also confirmed the molecular mass of the as-synthesized copper nanoclusters. The molecular mass was resolved as m/z = 575.8 with the formula given as $[Cu_7B_3O_5.H_2O]^-$.

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