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Text should be organized as follows: introduction, methods, results and discussions. The introduction should describe the purpose of the study and its relation to previous work in the area of study. Methods should be concise but sufficiently detailed to permit replication by other researchers. Results should present relevant positive and negative findings of the study, supported where necessary by references to tables and figures. The discussion should interpret the results of the study with emphasis on their relation to the original concept and to previous studies.

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NANOPARTICLES SYNTHESIS PROTOCOLS:
A CASE OF SYNTAX AND SEMANTICS?

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Abstract

Synthesis of metal nanoparticles (MNPs) with DNA template has been approached in two synthetic ways by researchers with some uncertainties about the appropriate conventional method to prepare DNA-templated MNPs. In this exercise effort was made to review these two synthetic approaches and determine which method produced the better approach in relation to results of this study. MNPs were prepared by first attaching metal ion in this case Ag (I) ions from silver nitrate ($AgNO_3$) aqueous solution to Calf thymus DNA (Ct-DNA) and then reducing with sodium borohydride ($NaBH_4$) aqueous solution. And another method requires reducing Ag (I) ions with $NaBH_4$ before adding Ct-DNA. UV-vis results showed that there were no differences in the ABS results of the control (AgNPs) sample from the sample formed by first reducing the metal ion with the reducing agent before adding the DNA template labeled as AgNP/Ct-DNA. Secondly, the DLS results show the same similarity again between the control AgNPs sample and the analyte AgNPs/Ct-DNA. Results were in support of first attaching the metal ions to DNA molecule before the reduction step. It was concluded that the synthesis of MNPs with DNA was step-sensitive and therefore an obvious case of syntax and not semantics.

Keywords: Metal nanoparticles, Calf thymus DNA, Uv-vis, Dynamic light scattering, Fluorescence.

Introduction:

Metal nanoparticles (MNPs) are nanoscale materials that are distinct from their bulk counterparts in possessing interesting and dynamic optical properties . They are characterized by having a high-surface-to-volume ratio which makes them rather susceptible to agglomeration to reduce their thermodynamic instability. The need to control their nanoscale sizes requires ingenious nanoscientists and nanotechnologists to attach suitable capping ligands to them during synthesis.

Several synthetic approaches have been applied by researchers to make NPs including cold synthesis, the use of different reducing agents in a bid to achieve size-control, etching, and the application of several different types of organic molecules as ligands . The length of time, speed, and temperature of the reactions as well as the type of reducing agents have been cited to impact on the size, shape, and optical properties of NPs . The significance of obtaining NPs or indeed nanoclusters (NCs) with definite sizes and shapes is critical in nanoscience and nanotechnology because as small as a unit change in the number of atoms per particle creates a major shift in optical properties including colour changes and magnetic properties (para- or diamagnetism) . This is the famous magic number effect .

Presently, there is no consensus among nano-researchers as to the acceptable reaction protocol in the synthesis of NPs using passivating ligands vis-a-vis the issue of size control. Even though, many researchers adopt the option of attaching template ligands to the metal ion precursors to produce a form of activation complex before the reduction step is effected. Others have simply reduced the metal ions in aqueous/aprotic solutions with appropriate reducing agents before attaching stabilizing ligands; bearing in mind the lability of many surface ligands used as stabilizers.

In this exercise, the aim is to examine these two reaction protocols and study the merits of their synthetic approaches with regards to particle sizes and eventual optical properties as well as future uses.

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2.0   Materials and Methods

2.1   Materials

Sodium borohydride (NaBH$_4$, 99.0 %) and silver nitrate (AgNO$_3$, 99.0 %) were bought from Sigma Aldrich, USA. Calf thymus DNA (Type 1, highly polymerized) was also bought from Sigma Aldrich, USA. Cysteine (H$_2$SCH$_2$CH(NH$_2$)COOH, 99.0 %) a product of Sigma Aldrich, USA. Deionized (nanopore) water was obtained from Millipore Diamond Barnstead series 1370 manufactured by Barnstead International, Iowa, USA, model D11931 with a resistivity of 18.2 MΩ/cm. A Dynamic Light Scattering (DLS) instrument, a product of Malvern high-performance particle-size analyzer (HPPS) with an invasive backscatter technology was used to determine the particles size. The device has a He-Ne laser source with a wavelength of 633 nm and a photodiode detector. The Uv-vis instrument was the Varian Cary 100 Bio spectrophotometer equipped with a tungsten halogen visible lamp source and an ultraviolet source made of deuterium arc lamp. The fluorescence was obtained using a SPEX FluoroMax spectrofluorimeter instrument, in New Jersey, USA.

2.2   Methods

0.15 mM of aqueous silver nitrate (AgNO$_3$) solution was added to 0.15 mM of aqueous sodium borohydride (NaBH$_4$) solution after which 15 μM aqueous Calf thymus DNA solution was added to the mixture. The resulting sample solution was vortexed and then allowed to stand for 30 – 60 minutes in the dark before analyses.

In the other reverse procedure, 0.15 mM aqueous AgNO$_3$ solution was prepared and treated with 15 μM aqueous Calf thymus DNA solution. The mixture was vortexed for 1 – 2 minutes and allowed to stand in the dark for 15 – 30 minutes before it was reduced with a 0.15 mm portion of aqueous NaBH$_4$ solution. The resulting sample solution was allowed to stand for 30 minutes before analyses began.

A control sample was formed by reacting 0.15 mm aqueous AgNO$_3$ solution with 0.15 mM NaBH$_4$ solution only, without introducing the Calf thymus DNA solution.

Aliquot portions of all the as-synthesized samples were withdrawn into a quart micro-cuvette for uv-vis, DLS, and fluorescence analyses.

3.0   Results

3.1. Absorbance

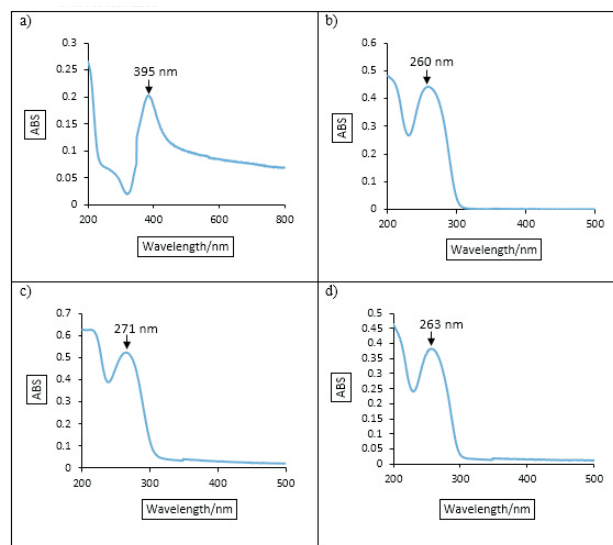**Figure 1**: Absorbance spectra of control samples: a) AgBH$_4$ (AgNPs) without Ct-DNA, b) Ct-DNA only, c) Ag$^+$/Ct-DNA, d) Ct-DNA/BH$_4^-$.

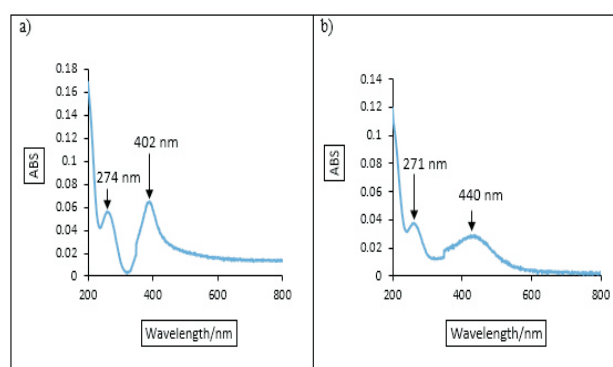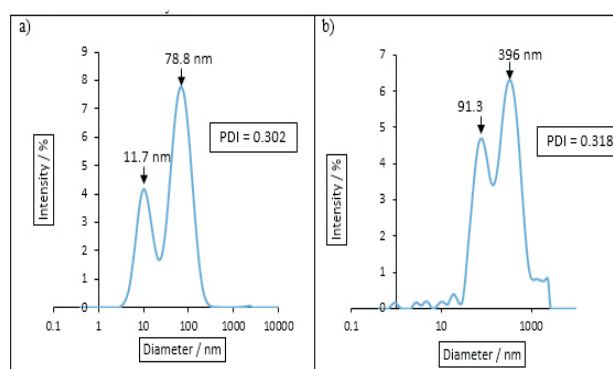**Figure 2**: Absorbance spectra of, a) AgBH$_4$/Ct-DNA, b) AgCt-DNA/BH$_4$ representing the reduction of Ag (I) ions before and after adding Ct-DNA respectively.

3.2   Particle size Analysis
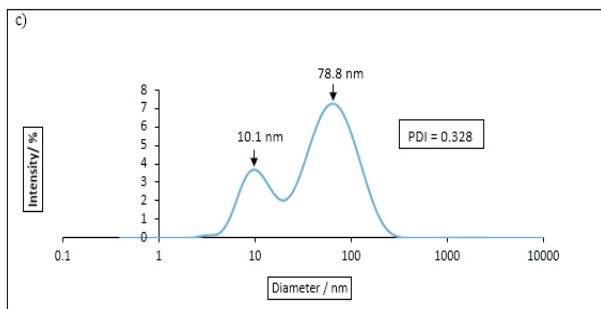
Figure 3: DLS spectra of: a) AgNPs/Ct-DNA, b) AgCt-DNA/BH$_4$ representing the addition of Ct-DNA after and before reduction, c) AgBH$_4$ (AgNPs) as control sample respectively showing polydispersity index (inset).

3.2   Fluorescence
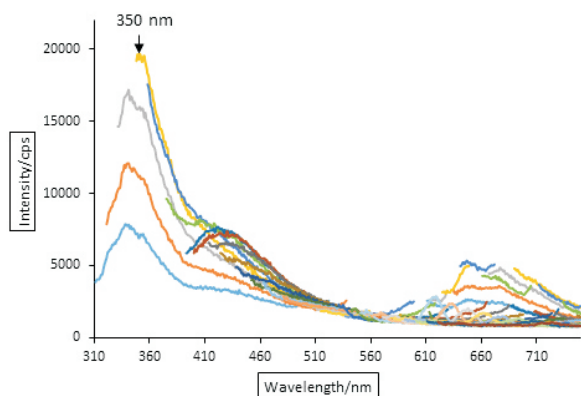
Figure 4: Emission spectrum of AgCt-DNA excited at 280 nm.
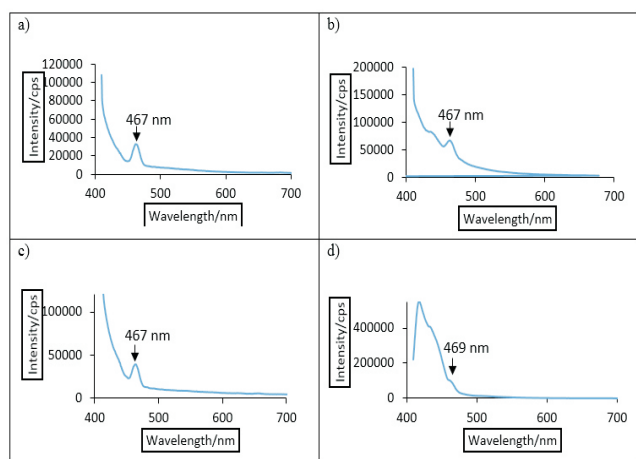
Figure 5: Fluorescence spectra of a) AgNPs/Ct-DNA and b) AgCt-DNA/BH$_4$ are analyte samples, while c) AgNPs only, and d) Ct-DNA/BH$_4^-$ are positive and negative control samples respectively, and excited at 400 nm.

4.0   Discussion

Figure 1 above contains the absorbance (ABS) spectra of control samples used to monitor the formation of AgNPs by templating with Calf thymus-DNA molecules before and after reduction with sodium borohydride. The control reactions helps us to understand the reaction pathway and so arrive at an informed conclusion of the reaction mechanisms.

In Figure 1a, the ABS spectrum of AgNPs only (without the Ct-DNA) show the expected surface plasmon resonance (SPR) typical of AgNPs. This plasmon peak of AgNPs typically occurs at approximately 400 to 420 nm and are characterized by a sharp and intense band . The spectrum in Figure 1b, is the ABS spectrum of Ct-DNA. DNA absorbance spectrum often occurs at 260 nm for samples containing DNA moiety. The ABS spectrum in Figure 1c is that of Ag (I) ions attached to the Ct-DNA structure prior to reduction. Aliquot portion of the sample was withdrawn and quickly ran in a uv-vis spectrophotometer. The spectrum shows a bathochromic shift from the original DNA absorbance wavelength from 260 to 271 nm. This notable shift in wavelength is taken as an indication of the successful attachment of the Ag (I) ions on the Ct-DNA molecule. The last spectrum in Figure 1d, is that of the Ct-DNA to which sodium borohydride reducing agent was added. This spectrum shows a marginal shift in the Ct-DNA ABS peak from 260 to 263 nm. Chemically speaking, this negligible shift in ABS wavelength is not considered significant nor indicative of successful bonding between these two species. DNA molecule is a polyanionic molecule and it is not expected to observe bonding between it and borohydride ion due to columbic repulsion. Rather, the marginal shift in ABS wavelength is attributed to a change in the optical density of the medium occasioned by the change in the dielectric environment of the samples upon addition of the borohydride solution.

Figure 2 contains the ABS spectra of the analyte samples being investigated. Figure 2a is the ABS spectrum of the AgBH$_4$/Ct-DNA sample which at this point is renamed AgNPs/Ct-DNA. The spectrum of this sample reveal two ABS bands at 274 and 402 nm corresponding to Ct-DNA and AgNPs respectively. Recall, that this sample was formed by first reducing the Ag (I) ions before adding Ct-DNA. Indications from this spectrum suggest that the Ag (I) ions may not have bonded to

the Ct-DNA nucleobases even though, there was an apparent shift in the Ct-DNA absorption peak position. Interestingly, the 402 nm band is attributed to AgNPs, more so, as the peak is almost a precise replica of the AgNPs peak previously obtained in Figure 1a above. Both bands are mirror images of each other – they are sharp and intense and are equivalent in terms of their full-width-at-half-maximum (FWHM). Also noteworthy is the intensity of the AgNPs band in Figure 2a in contrast to the Ct-DNA band. Apparently they are the same SPR bands of AgNPs which occurs usually at approximately 400 nm.

In Figure 2b, there are similarly two bands here which are again ascribed to the Ct-DNA and this time, to AgNCs and not AgNPs as was the case in Figure 2a. These bands occur approximately at a peak maximum of 271 and 440 nm, the former being red-shifted from the 260 nm and ascribed to Ct-DNA. But unlike the bands in Figure 2a, these peak positions (Figure 2b) as well as their intensities are different. The striking dissimilarities between the bands in Figure 2a and 2b is the second band occurring at 440 nm. This band is broad, weak, and occur at a longer wavelength with a larger FWHM. The nature of the 440 nm bands is *akin* to numerous Ag-template DNA bands reported by several researchers who have previously worked with Ag and DNA ligands and observed these similar optical phenomena .

The broad nature of the 440 nm band in Figure 2b is attributed to several vibrational states and ineffective orbital mixing (Coulson-Fischer theory) . The observation of these ABS bands at longer wavelength positions is a pointer to the presence of NCs with deep-trapped states for transfer of energy . Without a doubt, the ABS spectrum in Figure 2b clearly demonstrates that the attachment of Ag (I) ions directly to the Ct-DNA before reduction produced larger NPs owing to the wavelength of ABS than the one in Figure 2a. The 440 nm band is apparently different from the approximately 400 nm band obtained for the AgNPs control sample in Figure 1a above. The obvious deviation from the control sample by this 440 nm band is a credible pointer of a significant change from the known (control AgNPs sample) to an unknown (Ct-DNA templated AgNPs).

One way of ascertaining the sizes of the as-synthesized AgNPs/Ct-DNA is to analyze their

particle sizes using a particle-size DLS analyzer. Results are presented in in Figure 3 above. These show DLS spectra of the two analyte samples (Figure 3a and 3b) and the control AgNPs (Figure 3c). Figure 3a and 3c are almost replica of each other and are the closest indication of a similarity in species. These indicate that both samples followed similar methods of formation and are apparently the same specie-wise. It follows the earlier interpretation that the Ag (I) ion did not bind to the Ct-DNA molecule in the first place.

Whereas, in the case of the AgCt-DNA/BH$_4$ sample in Figure 3b, there is an obvious difference between this and the control sample (Figure 3c). This obvious difference is a veritable marker demonstrating that a new specie different from the former (the control, AgNPs) has been formed. This implies that the reaction protocol involved in this case was different. All three DLS spectra reveal two basic particle types even though the spectrum in Figure 3b shows several other smaller entities below 30 nm particle size. The particle sizes indicated in these spectra clearly demonstrates that the Ag (I)Ct-DNA sample reduced after adding the Ct-DNA produced species of larger particle size of approximately 91 and 400 nm. This should be expected if Ag has successfully bonded to Ct-DNA. All samples possess good polydispersity indexes (PDI) in the region of 0.30. PDI are a measure of the diversity of particle sizes in a sample. It ranges from between 0 to 1 with values closer to zero being significant and an indication of monodispersity which is the ideal.

Fluorescence is a chemo-physical property of matter which it exhibits in response to the acquisition of photons of energy from light. This phenomenon occurs when matter absorbs requisite photons of light and electrons then transit from lower energy state (valence band) or highest occupied molecular orbital (HOMO) to the higher energy state (conduction band) or lowest unoccupied molecular orbital (LUMO) energy level provided a bandgap exist between these two energy levels . The excited electrons (excitons) while in the higher energy states (lifetime) undergo vibrational relaxation which is synonymous with dark state (non-radiative energy decay) before returning back to the ground state by loss of residual excited energy in the form of emission . Hence, the observation is also termed as luminescence.

The reaction of Ag (I) ions with Ct-DNA followed by reduction of the metal complex obviously produced fluorescent species as can be seen in

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Figure 4 above. These are luminescent AgNCs templated on the DNA molecule showing quantized LUMO – HOMO transitions attributed to a mixture of both intraband and interband sp–sp and d–sp electronic transitions . The spectrum in Figure 4 shows three fluorescent bands at 350, 435, and 678 nm respectively. These are indications of multiple types of AgNCs of different sizes . The size of the NCs determine the bandgap and thus the eventual wavelength of emission. However, the band at 350 nm is attributed to Ag_4^{2+} species which are formed during the nucleation stages of AgNPs formation and obviously abundant in the sample . The band at 435 nm are the main NCs in the sample followed by the clusters emitting at 678 nm which by all indications is the larger AgNCs in the sample . These clusters apparently have bandgaps estimated at 2.85 and 1.83 eV respectively.

Furthermore, The intensities of these AgNCs in the emission spectrum in Figure 4 signifies that the AgNCs that are emitting at 435 nm is the slightly more abundant specie in the sample besides the Ag_4^{2+} clusters at 350 nm, since fluorescence intensity is directly related to the concentration of the sample (fluorophores). Again, this means that the specie emitting at 678 nm are the least abundant concentration-wise according to the intensity data. Meanwhile, these emissions were not observed in the AgNPsCt-DNA sample (Figure 5). The observation of luminescence in the AgCt-DNA sample is a confirmation of this samples DLS results in Figure 3b above, which showed weak intensities for "particles" in the 1.00 nm or less region.

The absence of luminescence is confirmed by the absence of a broad band in the absorption spectrum of the AgNPsCt-DNA sample in Figure 2a above but instead replaced by a sharp narrow SPR band confirming the formation of metallic AgNPs whose size is already close to the de Broglie wavelength of electrons at the Fermi energy level of 5.49 eV (226 nm wavelength) for metallic silver, thus hindering electronic transitions between the valence to the conduction bands.

In Figure 5a and 5b, we have the spectra of the two analyte samples being studied. Figure 5c and 5d are control samples. These four samples show "luminescent" – the AgNPs/Ct-DNA, AgCt-DNA/BH$_4$, AgNPs, and Ct-DNA/BH$_4^-$ (appearing as a shoulder in the spectrum). The observation of these bands is not associated with neither NPs nor nanoclusters (NCs) because the latter is already comprised of numerous atoms and so possess a high density of state . This means that they do not have bandgaps and therefore should not display fluorescence since their size is already close to the de Broglie wavelength of electron . The question therefore is, what species are responsible for the observed weak fluorescence in the samples showing luminescence in Figure 5a, b, and c above?

The answer is that all four samples show "fluorescence" at 465 nm but the species fluorescing are not clusters of Ag (AgNCs) but Raman scattering of water in these samples. This accounts for why what is now better described as Raman peaks appear in all of these spectra in Figure 5. The signal observed in these spectra are ostensibly not associated with the Ct-DNA as can be attested to by the fact of this signal also appearing in the AgNPs (control) spectra which contained no DNA.

Lastly, although we can clearly discern small particles in the DLS spectrum in Figure 2b which confirms their presence, however, these DLS spectra for the AgNPs/Ct-DNA and AgNPs samples were not well resolved and are rather weak. The DLS data is often reported in terms of intensity (%) (and sometimes in number), with larger particles being able to scatter light more distinctly. It is clear that the intensities of the AgNCs associated with the observed fluorescence were as earlier stated not well resolved in these two instances.

Conclusion

Synthesis of MNPs from the metal ion precursor often requires the use of DNA molecule to stabilize them against their spontaneous growth and aggregation. However, the appropriate steps to follow in making these particles nanoparticle with DNA is a subject of some controversy. Two synthetic approaches were studied and analyzed for their merit according to the results obtained.

Uv-vis results point towards the synthetic approach which entails first attaching metal ion to the DNA structure before the subsequent reduction with an appropriate reducing agent. The uv-vis results of the control sample were in proximate agreement with the results of samples which were formed from the approach of first reducing the metal ion with the reducing agent before adding the templating DNA.

The DLS results follow suit those of the uv-vis in agreeing with the attachment of the DNA template first before reducing the metal-DNA complex. This showed in the DLS results obtained from this synthetic approach.

Fluorescence results show luminescence in the sample produced from attaching Ag (I) ions to Ct-DNA before reduction, whereas, the other sample from the second approach did not produce luminescence, even though a band was observed at 465 nm which is also present in all the samples including the controls that has been ascribed to Raman scattering for water.

In conclusion, it is apparent from this study that the first protocol which requires the reduction of the metal ions before adding the templating DNA ligand is certain to produce NPs, whereas, the second protocol that entails the addition of the ligand before reduction tends towards the formation of NCs. Invariably, there would be no need to add the ligands in the first place for the first synthetic approach since either ways NPs would be the result. But if NCs are desired, particularly with DNA ligand, then the more appropriate synthetic approach would be the attachment of metal ions to the ligand before the reduction step.

In the final analysis, the reaction protocols for NPs (including NCs) is sequence-specific rather than the case of having two different methods which should ultimately produce the same end-product. Therefore, the synthesis of MNPs/MNCs using passivating ligands like DNA follows a definite stepwise approach and therefore it is clearly a case of syntax rather than semantics.

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