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Research Article

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Special Issue
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This themed issue pays tribute to Professor Clement Uche Atuanya in recognition of his illustrious career in Metallurgical and Materials Engineering as he retires from Nnamdi Azikiwe University, Awka. We celebrate his enduring legacy of dedication to advancing knowledge and his impact on academia and beyond through this collection of writings.

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DNA-Conjugated Silver Nanoclusters Encapsulated in Microemulsion Droplets

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Abstract
Silver nanoclusters (Ag NCs) coordinated to DNA oligonucleotide molecule in nanowater droplets in emulsion system was studied with the view to determining the possibility of adopting this method in drug delivery systems as well as in medical and allied sciences. DNA oligomer was coordinated to Ag (1) ions in water droplets of microemulsion and then reduced with equimolar portion of sodium borohydride solution in microemulsion. Uv-vis results showed consistency with spectra of scattered water droplets; it also showed the absence of plasmon bands of silver nanoparticles (Ag NPs) which indicates the formation of Ag NCs. Confocal microscopy images displayed evidences of meshed fluorescing samples on silicon wafer in contrast to the control samples. Dynamic light scattering (DLS) spectrum demonstrated that irrespective of the size of the water droplets DNA molecule were encapsulated in the droplets despite their radius of gyration. TEM images showed the formation of dense, globular, spherical micellar mass which underscores the presence of these Ag/DNA globular mesh on holey carbon grid. It was concluded that with the right chemistry and reaction conditions we can direct analyte molecules to target biomolecules in microemulsion systems for diagnostic and therapeutic purposes by adopting similar mechanistic approach presented in this study.

Keywords: Encapsulation, DNA, silver nanoclusters, drug delivery, coordination.

1. Introduction

Nanoparticles (NPs) have evolved as quintessential components of medicine in the crucial area of drug delivery systems (DDS) (Bardhan, 2022; Jadhav et al., 2024; Jebasingh et al., 2023). They are defined as nanoscale materials whose dimensions fall within the size range of 1 – 100 nm (Selmani *et al*., 2022). NPs in DDS come in various types, examples of these are polymer NPs – nanogels, magnetic NPs – magnetite (Fe_2O_3), liposomes – micellar NPs with amphilipid bilayers, and metal NPs – Au, Ag, and other quantum dots like CdSe NPs (Michos et al., 2024). NPs have certain essential properties which make them an indispensable component of medical diagnosis and treatment. Crucial amongst many of their remarkable properties is the areas of precision, sensitivity, and selectivity (Bayda et al., 2020; Zhang et al., 2012). Nanoparticles are unrivaled in their ability to provide accurate data of materials both qualitatively and quantitatively. Nanoscientists and technologists can obtain qualitative and quantitative data of pathogens, pollutants, biomarkers up to the attomolar (10^{-18} M) scale where many of the present conventional methods fail (Liyanage et al., 2018).

Nanoscientists and technologists are able to "engineer" nano-bioconjugates which have been successful in detecting, diagnosing, and treating numerous health conditions. Only recently, scientists evolved a unique and successful protocol for the efficient and indeed accurate diagnosis of COVID – 19 virus (Samuel & Wittstock, 2023). This like in numerous other cases rely heavily on the ability to successfully tag these quantum size probes onto a target material

or indirectly through a 'vehicle". Vehicle here encompasses initially attaching the nanomaterials onto a substrate and transporting it to the site of action especially *in vivo*.

On their own, nanoscale materials already possess quantum size properties which dramatically distinguishes them from their bulk material counterparts (Joudeh and Linke, 2022; Selmani et al., 2022). These size-dependent properties include, local surface plasmon resonance (LSPR), fluorescence (luminescence), catalysis, paramagnetism, and magic number effects (Masterson et al., 2021; Oyem, 2018). Scientists (particularly, nanochemists) can leverage on these distinguishing properties by connecting these nanomaterials with target substances and hope to establish successful bonding (conjugation). The dramatic changes which accompany such reactions are observed and measured using a diversity of important and unique analytical tools which are indispensable and reliable to interpret and quantify these changes. Several approaches have been adopted in these endeavours; including by direct conjugation through covalency in different solvents, tagging to biomolecules which act as capping agents and by simulating these reactions in emulsion droplets (Oyem et al., 2022).

Microemulsion systems were initially conceived to limit the inevitable and spontaneous growth of nanoparticles post synthesis (Oyem et al., 2023), especially in the absence of a veritable capping agent which acts as a stabilizer (Oyem et al., 2022). This produced impressive results and limited the nanomaterials to sub-two nanometer sizes (< 2 nm) (Chen et al., 2024). At these size range, they retain many of the aforementioned properties which in numerous cases are often the focus of nano-researchers. Pileni and coworkers went further to successfully encapsulate deoxyribonucleic acid (DNA) single-stranded oligomers into nanosize droplets using the microemulsion technique (Petit et al., 1993; Pileni et al., 1998; Pileni, 2003).

The successful encapsulation of DNA into nanowater droplets of reverse micelles opened up a new vista in nano-research. These DNA-containing nanodroplets can be likened to biological cells which contain several organelles and biomolecules that constitute the basic units of life. Cells notably contain biomolecules like ribonucleic acids (RNAs) that are produced in the chromatins. Three types of RNAs exist in cells and have been classified into ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), and messenger RNAs (mRNAs); these are renowned for being associated with protein synthesis. Together with DNA, they constitute some of the essential macromolecules known for all forms of life.

But more significantly, the mRNAs have been at the centre of numerous oncological nanoresearch for the detection of cancers where thy serve as target biomarkers (Liyanage et al., 2018; Masterson et al., 2021). It is believed that they can also be involved in efforts aimed at treating this important health challenge. Before now and besides the work by Pileni et al, (2003), the conjugation of metal nanoclusters (MNCs) or any other conjugate *in situ* in microemulsion has not been mentioned in several literature search. Instead, reports on the use of microemulsion technique for the synthesis of nanoclusters (NCs) has significant mention. In this study, focus is centred on demonstrating what the author considers to the best of his knowledge, a pioneering work of synthesizing DNA-capped silver nanoclusters (Ag NCs) encapsulated in nanowater droplets of microemulsion system with implications for medical and pharmaceutical sciences in the area of drug delivery.

2.0 Material and methods

2.1 *Materials*

Silver nitrate ($AgNO_3$, 99.0%), sodium borohydride ($NaBH_4$, 99.0%), sodium bis (2-ethyl hexyl) sulfosuccinate (AOT) ($C_{20}H_{37}NaO_7S$), and 2, 2, 4-trimethyl pentane, isooctane (99.8%) were all bought from Sigma Aldrich, Germany. DNA oligonucleotides (DNA, 29-mer: 5'-AGTCACCCCAACCTGCCCTACCACGGACT-3'), bought from Eurofins Genomics, Ebersberg, Germany. Deionize nanopure water with resistivity of 18.2 ohms.cm was provided by Millipore Diamond Barnstead series, model D11931 by Barnstead International, Iowa, USA.

2.2 *Method*

0.09 *mM* aqueous solution of Ag NO_3 was prepared from its stock solution, and 15 μM aqueous solution of DNA oligomer was added to it. The mixture was thoroughly mixed together for 2 min before it was added to 2.5 mL solution of isooctane containing 0.5 *mM* AOT (surfactant) and allowed to stand in the dark for 15 min. Then an equimolar

aqueous solution of $NaBH_4$ was similarly prepared and added to another solution of 2.5 mL isooctane containing 0.5 *mM* AOT. Afterwards, the freshly prepared 2.5 mL $NaBH_4$/AOT/isooctane solution was slowly added to the second 2.5 mL $AgNO_3$/DNA/AOT/isooctane solution previously prepared while slowly stirring with a magnetic stir bar set at 250 rpm until any colour change observed becomes permanent. Allow the sample to stand overnight before commencing analysis. A control experiment was done in similar manner but without the DNA incorporated; that is, a blank sample. All reactions were done at room temperature.

The idea of this experiment is to produce nanodroplets of water-in-oil emulsion which contain at least one DNA molecule onto which Ag (1) ions have been coordinated onto the nitrogen atoms of the nucleobases by coordinate bonding to form Ag^+/DNA complex in nanodropets. By adding the other 2.5 mL portion containing the $NaBH_4$ nanodroplets into the 2.5 mL Ag^+/DNA complex nanodroplet portion, a reaction occurs as water droplets collide with themselves leading to the reduction of the Ag^+/DNA complex to form Ag/DNA nanodroplet.

3.0 Results and Discussions

3.1 Absorbance (ABS)

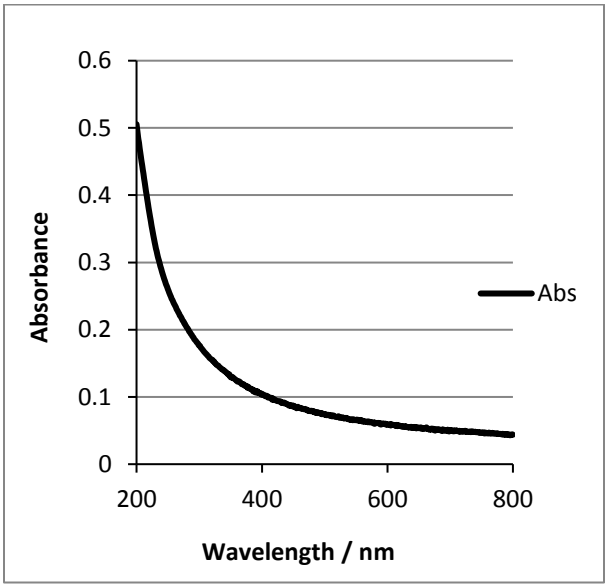


Figure 1: Uv-vis spectrum of 0.09 *m*M Ag/DNA complex in nanowater droplets

Figure 1, is the absorbance spectrum of the Ag/DNA capped NCs in nanowater droplets showing a non-characteristic curve with no obvious absorption bands. Absorption bands of nanoparticles (NPs) are usually obvious and are signature band confirming the presence of surface plasmon resonance frequencies of metallic nanoparticles. For Ag, this band often occurs between 400 – 440 nm and is the tell-tail signals of the presence of particulate silver metal (Aty et al., 2023; Gevorgyan et al., 2022). Therefore, the clear absence of this band in the entire spectrum in Figure 1, is an indubitable inference of the absence of Ag NPs. Invariably, this spectrum signifies the presence of Ag NCs which themselves are particulate Ag but generally contain far fewer atoms (Díez and Ras, 2010). That is, they are obviously below the Fermi wavelength of Ag (Tani et al., 2018; Uesugi et al., 2019) and unable to display the typical SPR of heavier (bigger) Ag particles.

Meanwhile, the absorption curve shown in Figure 1 are also characteristic of Raman scattering by particulate matter in solution. In this respect, it is considered that this curve is rather an obvious demonstration of the presence of dispersed droplets in the medium. This are attributed to the presence of micro- (nano-) droplets dispersed in the oil medium (Oyem et al., 2022). The ABS spectrum confirms the production of nanowater droplet reactors as was originally intended. Furthermore, these nanodroplets are expected to contain the Ag and DNA reactants as well as the

reducing agents. By the sizes of these nanodroplets, we will expect them to be suspended and fully randomly dispersed in the oil continuous phase without being brought down by the effect of gravity (sedimentation). Similarly, the lipophilic end (tail) of the surfactant molecules on the reverse micelles constantly repel these droplets and make it possible for them to be remain suspended in the medium (Indelicato et al., 2017; Khalfallah, 2022).

3.2 Confocal Fluorescence Microscopy

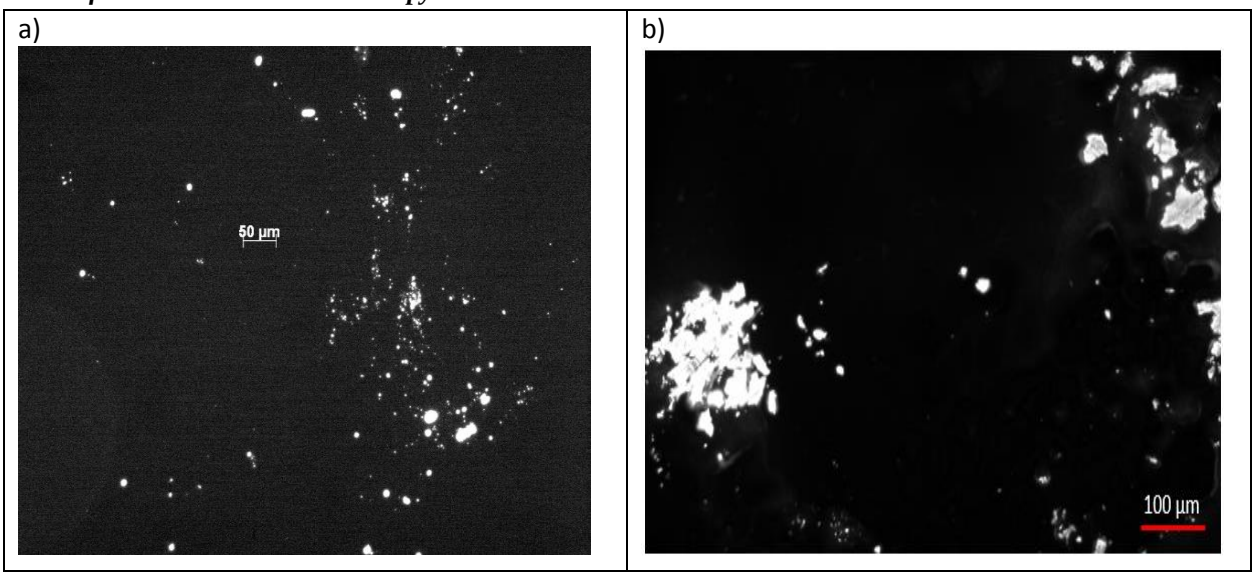


Figure 2: Fluorescence confocal microscope images of a) 0.09 *m*M Ag NCs (without DNA) in nanowater droplets, and b) 0.09 *m*M Ag/DNA in nanowater droplets.

Confocal microscopy is an instrument which provides evidence of luminosity of a substance, it also provides evidence of the morphology of materials being studied. It is the go-to instrument for visible confirmation of a samples luminescence besides fluorescence spectrophotometry which is obviously limited in showing the fluorescing sample. Figure 2 above displays the luminescence of the Ag NCs samples and their morphology, which is also critical. In Figure 2a, we can see the clusters fluorescing to be small generally dispersed spherical clusters of Ag. We describe these as monodispersed Ag NCs and the confocal instrument focusses on the particular regions of light illumination and cuts out other light interference. The sample in Figure 2a contain no DNA adduct, and so, serve as the control sample in this study.

However, in Figure 2b, we have the confocal fluorescence images of the DNA-containing Ag NCs, this is the analyte sample. The image displayed in Figure 2b speaks for itself. It shows contrary to that in Figure 2a cluttered mesh of obviously fluorescing material – the Ag/DNA sample that are in obviously distinct, essentially non-spherical morphology with the control sample. In this image (Figure 2a), we the tell-tail signs of the presence of DNA moiety in the wide-spread of luminescence entity which are clearly not small spherical Ag NCs as can be seen in Figure 2a. The obvious luminescence of these DNA moiety is adduced to the presence of embedded Ag NCs which have been coordinated to DNA nucleobases at the nitrogen centres of the adenine, cytosine, guanine, and thymine nucleobases (Oyem, 2022).

These observations mentioned above are clear pointers of successfully encapsulating DNA into nanowater-droplets and coordinating Ag to DNA molecule in the droplets which is the objective of the exercise. This practically demonstrates that we can go further than merely encapsulating DNA in water droplets, to targeting special biomolecules with the desired drug components in drug delivery, immunology and immunochemistry with careful consideration.

3.3 Dynamic Light Scattering (DLS)

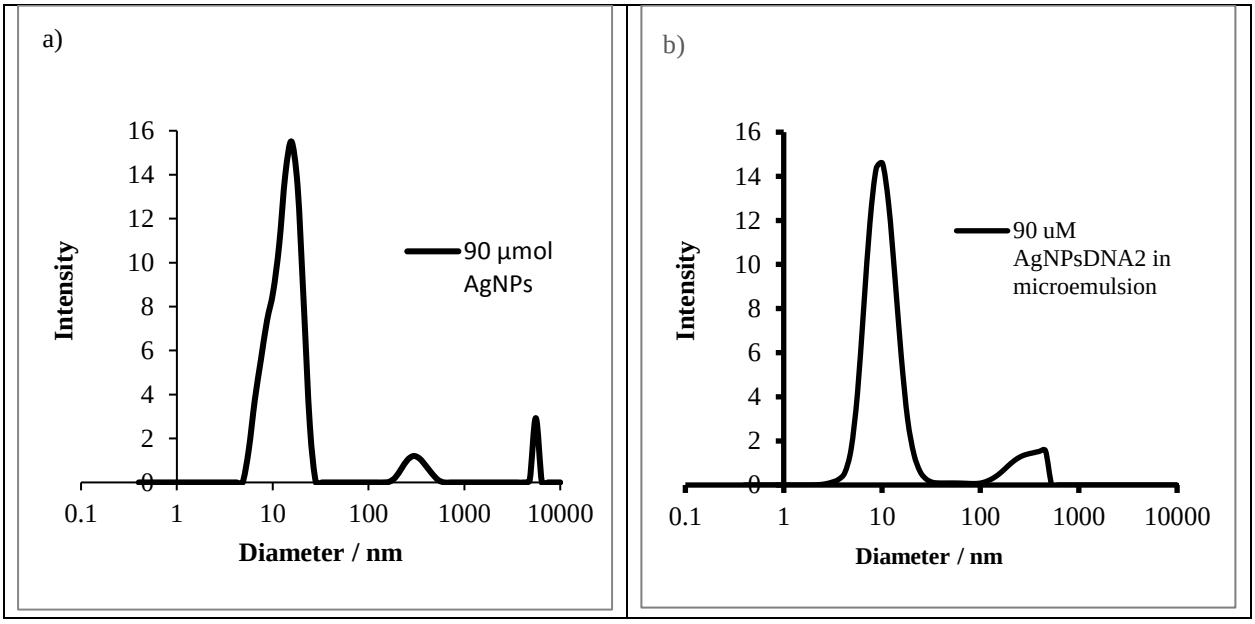


Figure 3: DLS spectra of a) 0.09 *m*M Ag NCs (without DNA) in nanowater droplets, b) 0.09 *m*M Ag/DNA in nanowater droplets.

Dynamic light scattering spectrum of the control and as-synthesized samples are presented in Figure 3a&b respectively. This technique presents the particle size information in the samples. It works by observing the attenuation of laser light incident on a sample and measuring the extent (degree) of light scattered by the particles in a sample, and correlates them by computation into different sample sizes.

Figure 3a contains DLS data of the control sample, that is, Ag NCs prepared by the same reaction protocol but does not contain DNA adducts in the water droplets. This spectrum shows three signals at different diameter positions which portent the presence of three particle size disparity. There is a particular class of sample with a diameter of approximately 10, 400, and 10000 nm respectively. The 10 nm particle is ascribed to reverse micelles in the sample solution. The second one at 400 nm is believed to be the nanowater droplets which contains the sample analytes. However, the third particle size is ascribed to critical micelles which have grown into large shapes (cylindrical) as the sample aged over time. This position is supported by the clear absence of this particle size in the spectrum of the as-synthesized sample in Figure 3b. Sequel to previous studies, it has been observed that this third particle signal at 10000 nm are noticeable absent when DLS samples of an analyte is freshly taken after synthesis, but often appear when the sample has aged awhile (Oyem, 2018).

The spectrum in Figure 3b present similar scenario as that of Figure 3a, apart from the clear absence of the 10000 nm signal, it would almost pass for the same spectrum. The absence of the 10000 nm signal has just been explained, and confirms the stated observation that this spectrum was obtained not long after synthesis, which was the case; so, obviously the surfactant molecules have not grown into large micelles yet. Meanwhile, if we focus on the 400 nm particle size, we can discern that this is almost the same with the control sample in Figure 3a. This tells us that although we might have anticipated an increase in size of this particle after incorporating DNA, but studies have shown that DNA molecules have successfully been encased into water droplets quite below their radius of gyration (Lisiecki and Pileni, 1993; Lisiecki *et al*., 1995; Pileni, 2003). The radius of gyration is the distance between the axis of rotation and the centre of mass. Hence, there is ostensibly no obvious change in the droplet hydrodynamic diameter post encapsulation of DNA, thus the molecule is able to coil into a mesh in the water entrapment.

3.4 Transmission Electron Microscopy (TEM)

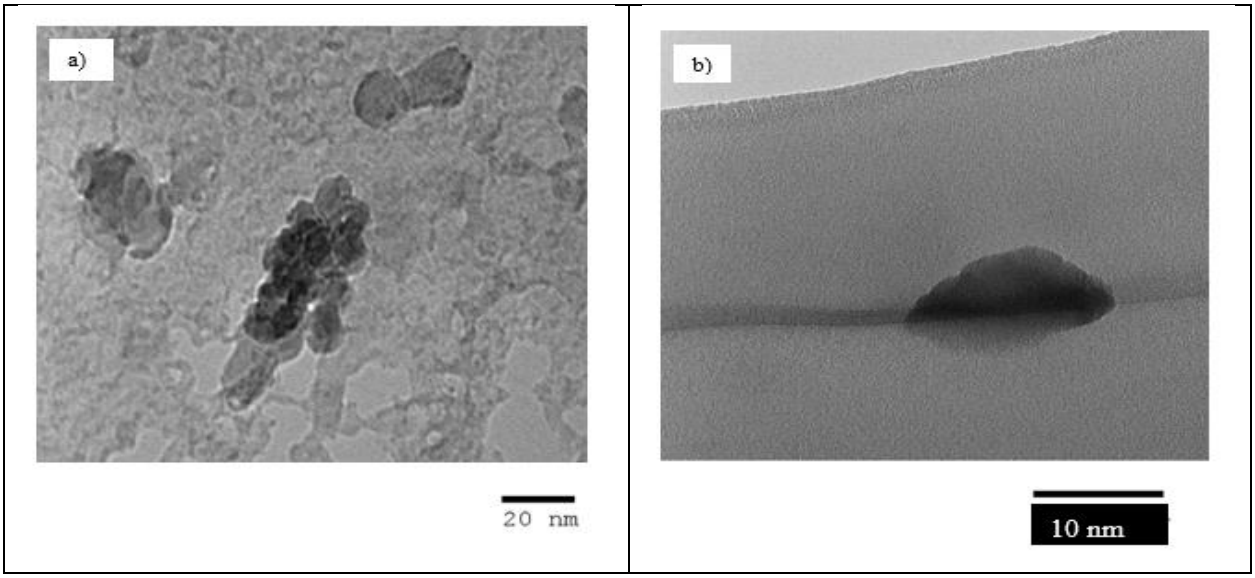


Figure 4: TEM images of **a)** 0.09 *m*M Ag NCs (without DNA) in nanowater droplets, and **b)** 0.09 *m*M Ag/DNA in nanowater droplets

In other to further confirm the encapsulation of DNA in the nanodroplets, TEM images of the samples were obtained and shown in Figure 4a&b above. Again, the first figure (Figure 4a) is the control sample (with no DNA). It shows dots of black tiny particles of Ag within the matrix of surfactant moieties. The sample is different from that of Figure 4b on the right, in the sense of the obvious dissimilarity in conformation. There are no cluttered mass in the image of Figure 4a, but we can see the dense, spherical, globular mass in Figure 4b which is similar to what we saw previously in the confocal image of the sample in Figure 2b above. This is further testament of a successful DNA conjugation to Ag NCs in microemulsion system. The random coil of the DNA molecule accounts for why the Ag NCs formed are not easily discernable as in the control sample in Figure 4b above because the Ag NCs is believed to have been enclosed in these dense micellar globules. Long monomeric polymer molecules characteristically exhibit random walk which manifest in random coils into micellar spherical globules. The fact that these samples contain Ag NCs is already established in Figure 2b by their luminescence. TEM technique is a remarkable technology which is used for detailed probe of cellular structure at the electron microscope level in medical sciences.

Effort was made to obtain the molecular mass of the analyte using the time-of-flight electrospray ionization mass spectrometer (TOF ESI-MS), but data (not provided) showed that the sample was not detected by the instrument; suggesting that the sample may have been too heavy to "fly" to the instrument's detector as would ordinarily be expected. Further studies will seek the use of matrix assisted laser desorption ionization mass spectrophotometer (MALDI) to attempt to obtain the molecular mass of the sample.

4.0. Conclusion

The conjugation of Ag NCs onto DNA molecules entrapped in water droplets of microemulsion was the focus of this study. The essence of this was to demonstrate the possibility of adopting this strategy in the areas of drug delivery and immunology. The ABS result showed that indeed nanodroplets were formed as the spectra was indicative of Raman scattering. In addition, it also demonstrated that plasmonic Ag NPs were not formed by the absence of distinct plasmon peak. Confocal fluorescence as well as TEM images were unanimous in confirming the presence of dense, micellar spherical globular mesh of obvious DNA-containing luminescent Ag NCs which were ostensibly in contrast with the control samples. It was concluded that we could attach an analyte by the appropriate chemical means onto a target molecule in nanodroplets in emulsion systems. This should open a new vista into future research in the area of drug delivery by extracellular and intracellular mechanisms.

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