



## Evaluation of metal nanoparticles for drug delivery systems

Oluyomi S. Adeyemi<sup>1,✉</sup>, Faoziyat A. Sulaiman<sup>2</sup>

<sup>1</sup>Department of Biological Sciences, Landmark University, Omu-Aran, Kwara State, Nigeria;

<sup>2</sup>Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.

### Abstract

Diminazene aceturate is a trypanocide with unwanted toxicity and limited efficacy. It was reasoned that conjugating diminazene aceturate to functionalized nanoparticle would lower untoward toxicity while improving selectivity and therapeutic efficacy. Silver and gold nanoparticles were evaluated for their capacities to serve as carriers for diminazene aceturate. The silver and gold nanoparticles were synthesized, functionalized and coupled to diminazene aceturate following established protocols. The nanoparticle conjugates were characterized. The free diminazene aceturate and drug conjugated nanoparticles were subsequently evaluated for cytotoxicity *in vitro*. The characterizations by transmission electron microscopy or UV/Vis spectroscopy revealed that conjugation of diminazene aceturate to silver or gold nanoparticles was successful. Evaluation for cytotoxic actions *in vitro* demonstrated no significance difference between free diminazene aceturate and the conjugates. Our data suggest that surface modified metal nanoparticles could be optimized for drug delivery systems.

**Keywords:** drugs, trypanocides, nanoparticles, toxicity, targeting

### Introduction

One major challenge confronting the chemotherapeutic control of trypanosomiasis is the issue of toxicity<sup>[1,2]</sup>. Several trypanocides including melarsoprol and suramin, amongst others, have poor efficacy and limited solubility and major side effects<sup>[3,4]</sup>. Animal trypanosomiasis is also not left off this quagmire, as the major veterinary trypanocides, diminazene aceturate and homidium chloride, have their share of the undaunting toxicity challenges<sup>[5]</sup>. These factors, besides masking the efficacy of the drugs, have also prompted research that could unravel novel treatment strategies for trypanosomiasis. While new drug development for trypanosomiasis are being encouraged, modification of, or investigations on existing trypanocides to increase their therapeutic index is a welcome idea.

Diminazene aceturate is a drug used to treat animal trypanosomiasis. Previously, we have demonstrated the toxicity and limited efficacy of this drug and other trypanocides in experimental infection using rats<sup>[6]</sup>. It is interesting to note that most of the adverse effects have been attributed to the non-specificity of the drug. Furthermore, Asadishad et al.<sup>[7]</sup> proposed the conjugation of drugs to polymers in order to reduce toxicity while sustaining therapeutic efficacy. In order to circumvent drug toxicity challenges, conjugation to polymer-functionalized nanoparticles have been further explored in a parallel study<sup>[8]</sup>. Nanotechnology is an emerging field with potential applications in diagnostic and therapeutic developments. Nanomaterials are defined as having at least one dimension < 100 nm in size, whereas the term nanoparticles applies to substances with all three external dimensions in the nanoscale<sup>[9]</sup>. Worthy of consideration

✉ Corresponding author: Oluyomi S. Adeyemi, Department of Biological Sciences, Landmark University, Omu-Aran, Kwara State, Nigeria. Tel: +234 7034507902, E-mail: [yomibowa@yahoo.com](mailto:yomibowa@yahoo.com).

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is the fact that silver or gold nanoparticles (Ag or Au-NPs) are increasingly being applied for biomedical purposes either because of their respective broad antimicrobial or visible light extinction behavior<sup>[7,10]</sup>. Nanoparticles, because of their remarkable properties, have become attractive for use in drug delivery and/or targeting<sup>[11]</sup>. Nanoparticles have attracted considerable attention worldwide because of the unique functional characters such as small particle size, high stability, tunable hydrophilic-hydrophobic balance and the ability to surface features for target specific localization amongst others<sup>[12,13]</sup>. Hence, nanoparticles offer versatile opportunities for drug delivery system as well as modulation of drug toxicity.

In this study, diminazene aceturate was conjugated to folate-modified silver (Ag) or gold (Au) nanoparticles supported in polyphenolic solution. Folic acid was selected for surface modification targeting because it has high affinity for cells overexpressing folate receptors<sup>[14,15]</sup>. The conjugated complex, folate modified nanoparticle and diminazene aceturate were evaluated for their cytotoxicity and compared.

## Materials and methods

### Synthesis of silver or gold nanoparticles

Silver or gold nanoparticles (Ag or Au-NPs) were synthesized based on established protocols with little modification. Briefly, Ag or Au-NPs were prepared separately by adding 50 mM silver nitrate or 50 mM chloroauric acid to a 1% (w/v) tannic acid solution (pH adjusted to 8 with 150 mM potassium carbonate) of polyvinylpyrrolidone (PVP) with stirring<sup>[16,17]</sup>. The solution turned pale yellow (Ag-NPs) or brown red (Au-NPs). The Ag or Au-NPs were filtered using the 0.22  $\mu$ m filter and characterized by UV/Vis - spectrophotometry (Biotek Epoch, USA), inductively coupled plasma optical emission spectrometry (ICP-OES, Cambridge, UK), and transmission electron microscopy (TEM, Brno, Czech Republic).

### PEG-SH-functionalized and folate modification of nanoparticle

The procedure previously reported by Lee and Low<sup>[18]</sup> was adopted for the preparation of functionalized nanoparticles. Briefly, amino-PEG-SH was prepared by reacting molar equivalents of Traut's reagent (8.2 mg in 200  $\mu$ L of water) and PEG bis-amine (200 mg) in 1 mmol/L EDTA, 100 mmol/L phosphate buffer (1 mL, pH 8.0). Before it was attached to the PEG-SH, functionalized folic acid was first activated. *N*-Hydroxysuccinimide ester of folic acid (NHS-folate)

was prepared by dissolving 5 mg of folic acid in 100 mL dry dimethyl sulfoxide plus 2.5 mL triethylamine and reacted with *N*-hydroxysuccinimide (2.6 g) in the presence of dicyclohexycarbodiimide (4.7 g) overnight at room temperature. The by-product, dicyclohexyurea, was removed by filtration. The dimethylsulfoxide solution was concentrated under reduced pressure and heating. NHS-folate was precipitated in diethylether. The product, NHS-folate, was washed several times with anhydrous ether under vacuum, and stored as a yellow powder.

### Conjugation of diminazene aceturate to functionalized silver or gold nanoparticles

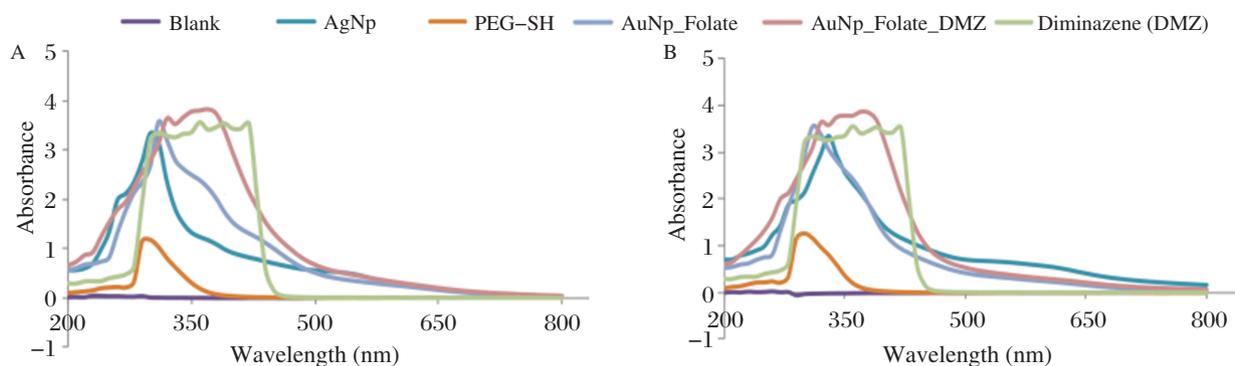
Diminazene aceturate (100 mmol/L) in Tris-HCl, pH 7 was added to folate modified PEG-SH functionalized nanoparticles in aqueous phase and stirred for 24 hours at room temperature. The mixture was characterized and stored at room temperature.

### Characterisation of synthesized conjugates

Synthesized nanoparticles, PEG-SH functionalized, folate-modified and conjugates of the nanoparticles were subjected to characterization using UV/Vis-spectrophotometry (Biotek Epoch), FTIR (Perkin Elmer, USA), and transmission electron microscope.

### In vitro cytotoxicity

MDA-MB-231 cells were used to evaluate the cytotoxicity of free diminazene aceturate, folate-modified nanoparticles and diminazene aceturate-conjugated nanoparticles. MTT assay was performed on MDA-MB-231 cells using a commercial kit (Roche Diagnostics, South Africa). MDA-MB-231 cells were maintained in DMEM containing fetal bovine serum at 37 °C in 5 % CO<sub>2</sub>. A 96 well plate was seeded at  $6 \times 10^4$  cells/mL. After 24 hours of incubation, the cells were treated with various concentrations of free diminazene aceturate, folate-modified nanoparticles, or diminazene aceturate-conjugated nanoparticles. Two times (2x) of the desired concentrations (the concentration ranged from 20, 50 and 100  $\mu$ mol/L – all concentrations were normalized in order to attain unbiased comparison) were prepared in the culture media and 50  $\mu$ L added to the wells appropriately. The control was treated with 50  $\mu$ L culture media. The treated cells were incubated and after 96 hours, 10  $\mu$ L MTT dye was added to each well. The plate was further incubated and after 4 hours, 100  $\mu$ L of the solubilizing reagent was added to each well and incubated overnight. The percentage of cell viability was determined at 595 nm relative to non-treated cells



**Fig. 1** UV/Vis spectrum for silver nanoparticle – folate – diminazene aceturate (AgNp\_Folate\_Diminazene) conjugate (A) and gold nanoparticle – folate diminazene aceturate (AuNp\_folate\_diminazene) conjugate (B).

using a microplate reader (Biotek Epoch). The assay was repeated three times in triplicates<sup>[19]</sup>.

### Data analysis

The data on cell viability were analysed using one-way analysis of variance (ANOVA) on GraphPad Prism 3 (GraphPad Prism 3 Software Inc. USA). The results were presented as mean  $\pm$  SEM ( $n = 3$ ). Mean values at  $P < 0.05$  were considered significant.

### Results

The present study attempted to determine the potential of metal nanoparticles for drug delivery systems. **Fig. 1** present the UV/Vis – spectra revealing peaks at the 370 nm range. This corresponds to the presence of diminazene aceturate in the conjugates. The TEM images are shown in **Fig. 2** for silver and gold nanoparticle – diminazene aceturate conjugates, respectively. The nanoparticles had diameter sizes between 6 to 10 nm. The TEM images showed that conjugation of diminazene aceturate to the nanoparticles did not alter nanoparticle morphology. Consequently, the free diminazene aceturate and nanoparticle conjugates were screened for comparative cytotoxicity (**Table 1**). When compared, the cytotoxicity data showed no appreciable ( $P > 0.05$ ) differences in the activities of

free diminazene aceturate or nanoparticles-diminazene aceturate conjugates. Although, the free diminazene aceturate increased in cytotoxicity with increasing concentration, the nanoparticles-diminazene aceturate conjugates showed reduced cellular toxicity at similar concentrations (**Table 1**).

### Discussion

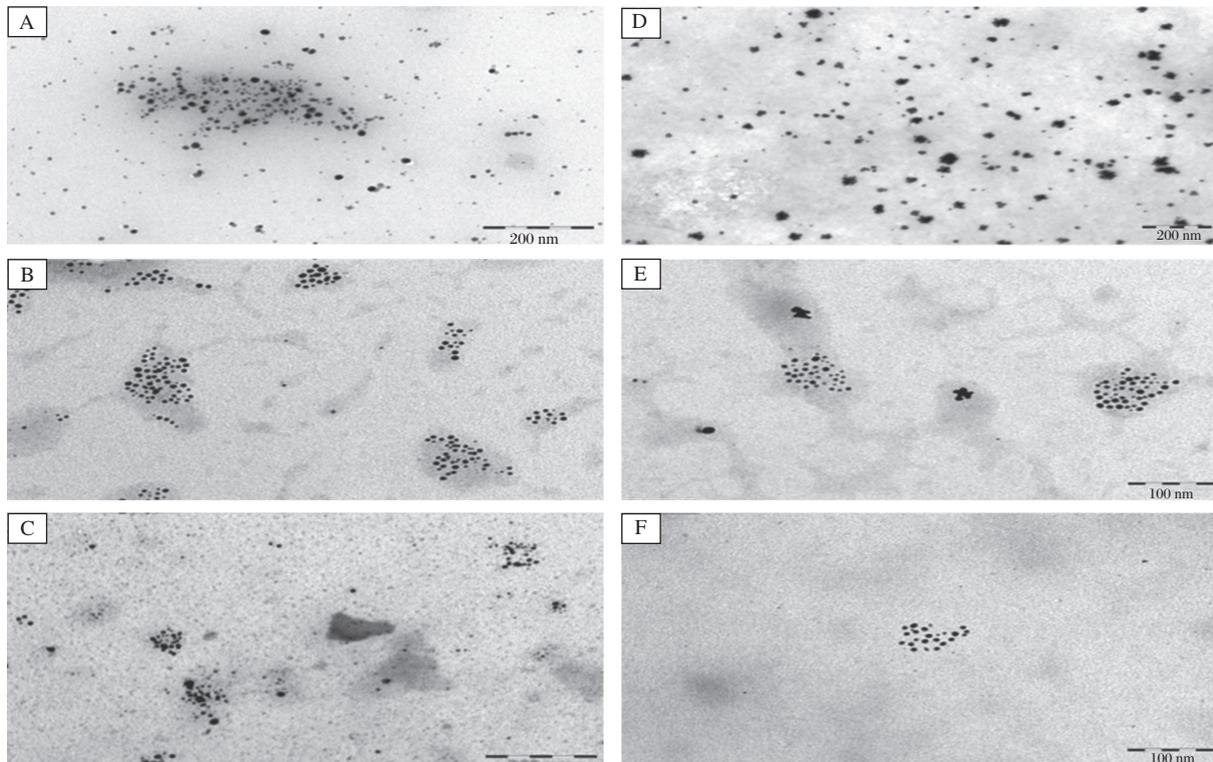
The UV/Vis spectra suggest that the diminazene aceturate conjugation to the modified nanoparticles was successful. In addition, TEM shows nanoparticles of diameter sizes ranging between 6 and 10 nm. The absence of significant changes in the TEM images further reveals the successful attachment of the diminazene aceturate to the nanoparticles and that the surface modification may not have compromised the morphological integrity of the metal nanoparticles.

The free diminazene aceturate and the conjugates were screened for comparative cytotoxic actions in order to determine whether conjugating drugs to surface modified nanoparticles could optimize drug delivery to target site while minimizing collateral damage. The free diminazene aceturate and the conjugates exhibited similar toxic potentials in the MDA-MB cell culture. There were non-significant reductions in the toxicity of corresponding dosages of the conjugates

**Table 1** Cytotoxic activities of free diminazene aceturate and nanoparticle – diminazene aceturate conjugates in cell culture mean $\pm$ SEM

Concentration ( $\mu\text{mol/L}$ )	DMZ (%)	AgF (%)	AgD (%)	AuF (%)	AuD (%)
20	64.11 $\pm$ 6.77	59.15 $\pm$ 5.49	64.08 $\pm$ 7.21	59.83 $\pm$ 5.09	61.72 $\pm$ 4.14
50	45.97 $\pm$ 5.85	59.05 $\pm$ 5.99	62.61 $\pm$ 2.85	49.34 $\pm$ 5.47	54.67 $\pm$ 2.55
100	27.61 $\pm$ 2.42	58.04 $\pm$ 3.40	55.56 $\pm$ 3.90	45.35 $\pm$ 5.13	45.06 $\pm$ 2.52

Data are presented as mean  $\pm$  standard error of mean (SEM,  $n = 3$ ); Values are considered significant at  $P < 0.05$ . DMZ – Diminazene aceturate; AgF – silver nanoparticle and folate; AgD – silver nanoparticle, folate and diminazene aceturate; AuF- gold nanoparticle and folate; AuD – gold nanoparticle, folate and diminazene aceturate.



**Fig. 2** TEM images for AgNp\_folate\_diminazene conjugates (A-C) and AuNp\_folate\_diminazene conjugates (D-F). A: Ag nanoparticles (AgNp); B: AgNp\_folate modified; C: AgNp\_folate\_diminazene conjugates; D: Au nanoparticles (AuNp); E: AuNp\_folate modified; F: AuNp\_folate\_diminazene conjugates.

relative to the free diminazene aceturate. In contrast, the nanoparticle conjugates demonstrated consistent toxicity with cell viability higher than 50 %. Dose dependent toxicity was observed for diminazene aceturate. On the other hand, increasing concentration of the nanoparticle conjugates did not result in higher toxicity as opposed to increasing toxicity by free diminazene aceturate. Interestingly, a previous report had demonstrated successful conjugation of diminazene aceturate to lipid nanoparticles<sup>[20]</sup>. However, to our knowledge this is the first attempt that demonstrated a successful conjugation of diminazene aceturate to metal nanoparticles and as well provided preliminary evidence that metal nanoparticles could be explored for drug targeting or delivery. Furthermore, in a parallel study<sup>[21]</sup>, lipid nanoparticle-diminazene aceturate conjugate showed reduced cytotoxicity. This result is consistent with our observation for cytotoxicity of diminazene aceturate-nanoparticle conjugates as obtained in the current study.

In conclusion, we have presented preliminary evidence to show that modification of metal nanoparticles could be exploited for drug delivery and/or used to modulate toxic actions of drug. As the concentration increased, non-significant reduction in the cytotoxic actions for the nanoparticle conjugates were observed

relative to the cytotoxicity of the free diminazene aceturate in MDA-MB-231 cells. Further works on the in vitro kinetic release and cytotoxicity studies in *Trypanosoma brucei* cell culture are ongoing.

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Department of Biochemistry, Rhodes University, South Africa.

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