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Catalytic degradation of β-hematin (malaria biomaker) using some selected metal oxide nanoparticles

Olaoluwa Ruth Obisesan1, Abolanle Saheed Adekunle1,3, John Adekunle O Oyekunle1, Aderemi Okunola Ogungbọnwọkan3, Olarinde Olaniran3, Sabu Thomas4, Thabo TINkambule4 and Bhekie B Mamba1

1 Department of Chemistry, Obafemi Awolowo University, Ilé-Ife, Nigeria
2 Department of Medical Microbiology and Parasitology, Obafemi Awolowo University, Ilé-Ife, Nigeria
3 International and Inter University Center for Nanoscience and Nanotechnology, Mahatma Gandhi University, Kottayam, India
4 Nanotechnology and Water Sustainability Research Unit, College of Science, Engineering and Technology, University of South Africa, Johannesburg, South Africa
5 Author to whom any correspondence should be addressed.

E-mail: sadekpreto@gmail.com

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Abstract

In this study, metal oxide (MO) nanoparticles were synthesized using a microwave and chemical methods and characterized using spectroscopic and microscopic techniques such as scanning electron microscopy (SEM), UV–vis spectroscopy, FTIR spectroscopy and Zeta potential analysis. The sensitivity (growth of inhibition) and catalytic degradation study of the MO nanoparticles with malaria biomarker (β-Hematin) and cultured Salmonella typhi was established. Both the chemical and microwave synthesized metal oxide nanoparticles exhibited effective potential to degrade the malaria biomarker at different degree and to its less active by-product. However, in most cases, microwave synthesized nanoparticles performed better than their chemically synthesized counterparts. The synthesized nanoparticles show high antibacterial activity towards clinical pathogens and S. typhi and therefore can be used for sensor development for monitoring these microorganisms in biological fluids such as blood, and urine samples.

1. Introduction

β-Hematin is a dimer of Hematin in which an iron-oxygen bond links the central iron of one Hematin to the oxygen of one of the carboxylates of the adjacent Hematin. It has a molecular formula C₆₈H₆₀Fe₂N₈O₈ and a molecular weight of 1228.966 g mol⁻¹. β-Hematin is a synthetic compound with the same chemical and structural morphology with malaria pigments known as ‘hemozoin’1. Hemozoin is an insoluble micromicrocrystalline byproduct of hemoglobin digestion by malaria parasites (Plasmodium falciparum) inside host erythrocytes, which can be found freely floating in the bloodstream or inside erythrocytes and leukocytes1,2. Malaria parasites catabolize hemoglobin to produce amino acid and toxic-free heme (ferriprotoporphyrin IX) which is biocrystallize to hemozoin. The presence of hemozoin disturbs normal cellular function and physiology of host, whereas the parasite is less affected by it3. Hemozoin plays a role as a visible marker in identifying malarial parasites and hence, it is popularly termed as malaria pigment4.

Malaria has been a treat to humankind since ancient times and is still a significant threat to half of the world’s population5. Malaria is the fifth most common cause of death from infectious diseases worldwide (after respiratory infections, HIV/AIDS, diarrheal diseases, and tuberculosis) and the second in Africa, after HIV/AIDS6. It is also common in the tropical areas of Asia, Central and South America, where it affects millions of people. Plasmodium falciparum, the most virulent species of the malaria parasite, is responsible for nearly one million deaths each year7. The overwhelming majority of these deaths occur among young children residing in sub-Saharan Africa. Many studies have been carried out to enrich antimalarial drug with new chemotypes so as
to reduce the prevalence of the disease worldwide. Also, researchers are working on good selective and specific diagnostic method for malaria fever using nano-techniques.

Due to their amenability to biological functionalization, nanoparticles are finding important applications in the field of medicine [8]. Nanoparticles (NPs) have high surface areas and unique physicochemical, optical, and biological properties that can be easily tuned, making them ideal candidates for desired applications. Metal oxide nanoparticles have emerged as promising candidates for biological applications (such as drug delivery, biosensing, imaging, antibacterial therapy etc) since they possess greater durability, lower toxicity and higher stability and selectivity [9]. As potential novel antibacterial agents, metal oxide nanoparticles such as copper oxide, aluminium oxide, iron oxide, iron oxide, zinc oxide are being explored by researchers for different medical applications [9]. Considerable studies have been done on using β-Hematin as a biomarker in antimalarial drug synthesis for inhibiting the formation of hemozoin in host system [10, 11], as a biomarker for diagnosis in a magnetic field enriched surface [12], as a detection assay for malaria diagnosis [13]. With so many studies on β-Hematin, however, there is a paucity of data on degradation of β-Hematin with nanomaterials.

The motivation for this study is based on the urgent need to stop the prevalence of the deadly and endemic disease, malaria fever worldwide. Therefore, this study investigated the interaction of copper oxide (CuO), iron oxide (Fe₂O₃), and aluminium oxide (Al₂O₃) nanoparticles with β-Hematin. This will provide insight on the reaction of metal oxide nanoparticles with β-Hematin, and possible application of metal oxide nanoparticles as an anti-malaria drug or in the development of malaria diagnostic devices.

2. Materials and methods

All reagents and solvents used were of analytical grades and obtained from Sigma Aldrich chemical. They include: aluminium nitrate nonahydrate (Al(NO₃)₃·9H₂O), copper chloride (CuCl₂), copper acetate (Cu(CH₃COO)₂), iron nitrate (Fe(NO₃)₂), sodium hydroxide (NaOH), ethylene glycol (C₂H₆O₂), ferrirrprotoporphyrin IX chloride (hemin chloride [Cl-Fe(III)]PPIX), magnetic stirrer, microwave. Cultured and identified Salmonella typhi were obtained from the Department of Medical Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria, while clinical pathogens (Proteus vulgaris, Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli) were obtained from Obafemi Awolowo University Teaching Hospital, Ile-Ife, Osun State, Nigeria.

2.1. Synthesis and characterization of β-hematin and the metal oxide nanoparticles

The synthesis and characterization of β-Hematin, copper oxide (CuO), iron oxide (Fe₂O₃), and aluminium oxide (Al₂O₃) nanoparticles (chemical and microwave synthesized) was recently reported in a study carried out in our group [14].

2.2. Structural and morphological characterization of synthesized nanoparticles

The synthesized nanoparticles were characterized using Ultra-violet visible (UV-visible) spectrophotometer, Fourier transformed infra-red (FTIR) spectrophotometer (Agilent Technology, Cary 600 series FTIR spectrometer, USA), x-ray diffraction (XRD) spectrophotometer, Scanning electron microscope (SEM) Zeiss Ultra Plus 55 HRSEM (Germany) and Transmission electron microscope (TEM).

2.3. Catalytic degradation of β-hematin using synthesized metal oxide nanoparticles

Fifty (50) mg l⁻¹ concentration of β-Hematin solution was prepared in pH 9 phosphate buffer solution. 50 ml⁻¹ of this solution was stirred and reacted with different weight of MO nanoparticles (5–25 mg) for 1 h on magnetic stirrer. After the reactions, the absorbance of the solution was measured using UV-visible spectrophotometer to monitor the catalytic effect of the MO nanoparticles on β—Hematin.

2.4. Antibacterial screening activities of the synthesized MO nanoparticles

Antibacterial activities of the synthesized MO nanoparticles on Salmonella typhi and clinical pathogens was investigated. Cultured and identified Salmonella typhi were obtained from the Department of Medical Microbiology, Obafemi Awolowo University, Ile—Ife, Osun State, Nigeria while clinical pathogens (E. coli, Proteus vulgaris, Klebsiella pneumoniae, Staphylococcus aureus) were obtained from Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife. The microorganism obtained were sub-cultured on Mueller Hilton agar at 37 °C. To test the activities of the synthesized nanoparticles on the sub-cultured microorganisms, Mueller Hilton agar was prepared in Petri dishes and allowed to solidify. After solidification, 10 mm diameter wells were cut on the agar using sterile steel borer. Accurately measured 10 µl of bacteria suspension was inoculated on the solidified plate and 0.1 ml of 1 mg ml⁻¹ synthesized nanoparticle solution was introduced into the wells. The plates were incubated at 37 °C overnight. After incubation, the diameter of the zone of inhibition was measured.
3. Results and discussion

3.1. Microscopic and spectroscopic analysis

The SEM, UV–vis and XRD results of the synthesized MO nanoparticles have recently been reported [14]. The reported SEM images showed aggregated particles for the chemical and microwave synthesized nanoparticles with average size of 11.16–12.72 nm (CuO), 20.92–30.37 nm (Al2O3) and 6.42–6.91 nm (Fe2O3) [14].

TEM images of the MO nanoparticles gave a clearer picture (figures 1(a)–(d)). The CuO nanoparticle (chemical and microwave synthesized) appeared rod-like and cylindrical (figures 1(a) and (b)) with an average particle size of 2.14 and 2.15 nm, respectively. The sizes obtained are closer to 6.3 nm reported for CuO nanoparticles by Chen et al [15]. The TEM image of Fe2O3 (C) showed some flaky crystal shaped particles (figure 1(c)) with average size of 4.51 nm while Fe2O3 (M) appeared as aggregated particles (figure 1(d)) with average size of 3.04 nm. The particle sizes obtained for the Fe2O3 nanoparticles compared well with the literature [16]. The differences in morphologies and particle size could be attributed to different methods of synthesis, and this would greatly affect their respective catalysis towards β-Hematin degradation. It was reported that a large effective surface area using nanomaterials could be very beneficial in catalytic applications [17].

The x-ray diffraction analysis confirmed a monoclinic structure of CuO (C) and a cubic face CuO (M) nanoparticles with particles sizes of 21.75 nm and 21.41 nm, respectively [14]. Similarly, a γ-Al2O3 phase and α-Al2O3 phase have been confirmed for both the chemical and microwave synthesized Al2O3 nanoparticles with average particle size of 13.05 and 9.01 nm, respectively, while the XRD pattern for Fe2O3 nanoparticles (chemical and microwave synthesis) showed that the crystallinity phase is γ-Fe2O3 with particles sizes of 14.11 nm and 21.50 nm, respectively [14].

FTIR is an important analysis to know the functional groups of molecules and compounds. It is used to measure vibration frequencies of bonds in the molecule [18]. Figure 2(a)–f presents the FTIR spectra of CuO, Al2O3 and Fe2O3 nanoparticles for both chemical and microwave synthesis. Metal oxides generally have absorption bands below 1000 cm⁻¹ that arise from interatomic vibrations of particles [19]. From figure 2(a), the
CuO (C) peaks at the region 420–987 cm\(^{-1}\) corresponds to Cu–O bond vibration frequencies [20]. Peak at 609 cm\(^{-1}\) indicates Cu–O stretching vibration. The peaks at 3321–3446 cm\(^{-1}\) might be as a result of the vibration of bonded hydroxyl groups, isolated hydroxyl groups and stretching vibration of adsorbed water molecules [21] and the peak at 1631.83 cm\(^{-1}\) can be assigned to stretching and bending modes of adsorbed water [22]. Also, for CuO (M) nanoparticles (figure 2(b)), peaks at region 428–922 cm\(^{-1}\) correspond to Cu–O bond vibration frequencies [20], while peak at 601 cm\(^{-1}\) correspond to Cu–O stretching vibration [20]. FTIR spectra of Al\(_2\)O\(_3\) nanoparticles (figures 2(c) and (d)) showed a broad band at 3444 and 3439 for the chemical and microwave synthesized compounds, ascribed to O–H group of adsorbed water molecules. The peaks at 1645 and 1641 cm\(^{-1}\) from both spectra indicate the bending modes of the hydroxyl group [22].

Figure 2. FTIR Spectra of (a) CuO (C) Nanoparticles, (b) CuO (M) Nanoparticles, (c) Al\(_2\)O\(_3\) (C) Nanoparticles, (d) Al\(_2\)O\(_3\) (M) Nanoparticles, (e) Fe\(_2\)O\(_3\) (C) Nanoparticles and (f) Fe\(_2\)O\(_3\) (M) Nanoparticles.
transmittance of the OH group for Al₂O₃ (M) was 19% compared to 67.5% for Al₂O₃ (C) suggesting three folds' reduction in the water adsorbed during microwave synthesis probably due to conversion of OH group to oxygen. Also, the appearance of a weak peak around 1461 cm⁻¹ assigned to bending modes of hydroxyl group in microwave synthesis further confirms the involvement of fewer water molecules in the process. FT-IR spectra of chemical and microwave synthesized Fe₂O₃ nanoparticles are shown in figures 2(e) and (f). The bands at 661, 570 and 424 cm⁻¹ (figure 2(e)) and 661, 578 and 449 cm⁻¹ (figure 2(f)) indicate the presence of Fe–O bond. Bands at 661 and 570 cm⁻¹ can be assigned to Fe(T)–O–Fe(O) stretching vibration and band at 424 cm⁻¹ can be assigned to Fe(O)–O stretching vibration, where T and O correspond to Fe metal at tetrahedral and octahedral positions [23, 24]. The observed stretching and bending vibration bands for both chemical and microwave synthesis suggest the formation of Fe₂O₃ [25].

3.2. Zeta potential of synthesized metal oxide nanoparticles
The Zeta potential graphs for the synthesized MO nanoparticles are presented in figures 3(a)–(f). Zeta potential analysis determines the surface charges on the synthesized metal oxide nanoparticles in solution [26]. This also can be used to have knowledge about the stability of the nanoparticles synthesized in fluid [27]. Particles with zeta potential values within ±30 to 40 mV are considered to be moderately stable and zeta values within ±40 to 60 mV are very stable [28, 29].

For this study, chemical and microwave synthesized CuO have zeta values of -31 and -38 mV, respectively (figures 3(a) and (b)) which implies that they are moderately stable and will not flocculate or aggregate in fluid. On the other hand, Al₂O₃ (C) and Al₂O₃ (M) have zeta values of -14 and -20 mV (figures 3(c) and (d)). According
to stability assessment standard, Al₂O₃ for chemical synthesis will agglomerate while that of microwave synthesis will disperse in solution (incipiently stable) [30, 31]. For Fe₂O₃ nanoparticles, the zeta values for chemical and microwave synthesis are -27 and -33 mV, respectively (figures 3(e) and (f)). This suggests that the microwave synthesized nanoparticles of Fe₂O₃ are moderately stable while the chemically synthesized are incipiently stable. From zeta potential analysis result, it can be concluded that microwave synthesized metal oxide nanoparticles are more stable in solution than their chemically synthesized counterparts suggesting that the microwave metal oxide nanoparticles will not coagulate or flocculate in solution.

### 3.3. Catalytic degradation of β-hematin using synthesized metal oxide nanoparticles

In this study, synthetic hemozoin (β-Hematin) was used as biomarker instead of the organism (P. falciparum) due to the challenges of culturing P. falciparum outside the biological system. Hemozoin (β-Hematin) is a product of hemoglobin degradation in the bloodstream during infection by the malaria parasite. The UV—visible spectroscopy results of hemin chloride (the starting material for β-Hematin) and the synthesized β-Hematin (not shown) showed two different maximum absorbance (λmax) for the two molecules at 370 nm and 650 nm respectively confirming successful conversion of hemin chloride to β-Hematin and the 650 nm
absorption band of β-Hematin obtained in this study agreed with that reported by Coy and Phitsamai [32] and Johann et al [33] for hemozoin or β-Hematin.

Figure 4, therefore, presents the UV—visible spectra of β-Hematin after interaction with different weights (5–25 mg) of CuO nanoparticles. Similar results were obtained for Al₂O₃ and Fe₂O₃ nanoparticles and presented in figures 6 and 7. It is interesting to note that the β-Hematin absorption intensity of 0.66 around 650 nm reduced to 0.41 with a blue shift in the absorption peak to 585 nm after interaction with 5 mg of chemical and microwave synthesized CuO nanoparticles (figure 4(a)). Also, the two broad soret bands at 383 and 400 nm shifted to 359 and 375 nm and became narrow, but with an increase in absorbance after interaction with CuO nanoparticles suggesting the formation of a new compound or complex (figure 4(a)). Similar results were observed for 10 and 15 mg of CuO nanoparticles (figures 4(b) and (c)). However, as the weight of the CuO catalyst increased further (20–25 mg), although an interaction occurred with β-Hematin, absorption peak shifted to around 585 nm but there was no observable drop suggesting catalyst saturation therefore reducing the available active sites for β-Hematin degradation (figures 4(d) and (e)). Thus, the result clearly confirmed the effect or impact of the method of CuO nanoparticles synthesis on the degradation of β-Hematin. Similar results were obtained for Al₂O₃ nanoparticles interaction with β-Hematin (figures 5(a)–(e)) where β-Hematin
absorption peak at around 650 nm shifted to 585 nm with reduced in absorption peak intensity confirming degradation of β-Hematin.

At 5 mg weight, the catalyst, Fe₂O₃ (C) degraded β-Hematin better than Fe₂O₃ (M), with reduction in β-Hematin absorption intensity from 0.66 to 0.35 (Figure 6(a)). The poor activities of Fe₂O₃ (M) might be due to the method of synthesis or availability of few catalytic sites for β-Hematin degradation. However, with an increase in weight of Fe₂O₃ (10–15 mg), the activities of Fe₂O₃ (C) towards the biomarker degradation increases with almost zero absorbance observed at 15 mg of Fe₂O₃ (C) (Figures 6(b) and (c)). Further increase in Fe₂O₃ (C) weight (20–25 mg) led to a decrease in catalytic activities towards the biomarker. This can be attributed to blockage of an active site (saturation) as the weight of the nanoparticle is increased (Figures 6(d) and (e)). Interestingly, while Fe₂O₃ (C) catalytic activities decreased with increasing catalyst weight, Fe₂O₃ (M) activities increased towards β-Hematin degradation with the malaria biomarker peak around 650 nm reduced significantly, while its soret peak around 400 nm was reduced completely to 0.0 absorbance (Figures 6(d) and (e)).

Figure 5. UV–vis Spectra (wavelength nm) (a) β-Hematin with 5 mg Al₂O₃ (C) and Al₂O₃ (M) (b) β-Hematin with 10 mg Al₂O₃ (C) and Al₂O₃ (M) (c) β-Hematin with 15 mg Al₂O₃ (C) and Al₂O₃ (M) (d) β-Hematin with 20 mg Al₂O₃ (C) and Al₂O₃ (M) (e) β-Hematin with 5 mg Al₂O₃ (C) and Al₂O₃ (M).
3.4. Antibacterial activities of synthesized metal oxide nanoparticles

The results of the antibacterial activities of the synthesized metal oxide nanoparticles carried out against clinical pathogens and *S. typhi* are presented in Figure 7. The microorganism growth inhibition results in the presence of the nanoparticles showed that CuO and Fe$_2$O$_3$ (microwave and chemical synthesized) have better antibacterial activities towards most of the microorganisms than Al$_2$O$_3$ nanoparticles. Growth inhibitory activities of Al$_2$O$_3$ have been reported to occur at high concentration [34], and this might be the reason for the mild activity exhibited against the microorganisms in this study. Microwave synthesized Fe$_2$O$_3$ and CuO nanoparticles showed greater inhibitory growth than their chemically synthesized counterparts towards clinical pathogens and the *S. typhi* (figure 7). For gram-negative clinical pathogens (*Proteus vulgaris, Klebsiella pneumoniae, Escherichia coli*), CuO (M) showed maximum zone of inhibition at 10 mm against *P. vulgaris* and 6 mm zone of inhibition towards *E. coli* but mild inhibition of 1 mm against *K. pneumoniae*. On the other hand, Fe$_2$O$_3$ (C) showed maximum inhibition of 5 mm for *K. pneumoniae*. For gram-positive pathogen (*Staphylococcus aureus*), Fe$_2$O$_3$ (M) showed the highest inhibition of 6 mm. The reason for the low activities towards gram-positive might be due to the presence of thicker peptidoglycan layer in their cell walls which provide resistance to the passage of nanoparticles and it has been concluded that due to this reason, high concentration of nanoparticles will be needed to inhibit their growth [35]. For *S. typhi* (gram-negative), Fe$_2$O$_3$ (M) nanoparticle showed maximum growth of inhibition except for *S. typhi* 1 where Fe$_2$O$_3$ (C) has the highest inhibition. Mild growth of inhibition...
was observed for CuO and Al₂O₃ nanoparticles towards S. typhi, except for S. typhi 161 where zone of inhibition of 5 mm was observed for CuO (M) nanoparticles.

4. Conclusion

From this study it was observed that both the chemical and microwave synthesized metal oxide nanoparticles have demonstrated a good potential to degrade malaria biomarker (β-Hematin) at different degree to its less active byproduct. The MO nanoparticles also demonstrated high antibacterial activity towards clinical pathogens and S. typhi. However, the study also showed that in most cases, microwave synthesized nanoparticles demonstrated better antimicrobial activities than their chemically synthesized counterparts. It can be concluded that the MO nanoparticles have good potential to be used as materials for fabrication of chemical and biological sensors that will produce desirable signals for the detection and quantification of biological microorganisms (e.g. bacteria, virus, etc).

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Author contribution

ASA and JAO, AOO conceptualized, designed the work and were part of the manuscript write-up. ST, TTIN and BBM provide the research platform for nanoparticles characterization and spectroscopic study and involved in manuscript preparation, ORO carried out the experiment, interpret the results and prepare the manuscripts. All authors reviewed the manuscript and agree to its publication.
Conflicts of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest or competing financial interest.

ORCID IDs

Olaoluwa Ruth Obisesan https://orcid.org/0000-0002-7265-9010
Abolanle Saheed Adekunle https://orcid.org/0000-0002-2174-7600

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