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The Effect of Silica Coatings on the Structural, Magnetic and Antimicrobial Properties of Silver Doped Manganite Magnetic Nanoparticles for Biomedical Applications

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Surface modification of magnetic nanoparticles with biocompatible materials is usually required for biomedical applications. $La_{0.80}Ag_{0.15}MnO_3$ (LAMO) magnetic nanoparticles (MNPs) were synthesized by the sol–gel auto combustion synthesis with the nanoparticles subsequently coated with silica. The effect of the coatings on the structural, morphology, magnetic properties, colloidal stability and antimicrobial properties of LAMO nanoparticles were studied using powder X-ray diffraction, thermogravimetric analysis, field emission scanning electron microscopy, zeta potential and vibrating sample magnetometer. The crystalline perovskite structure of LAMO was retained after silica coating but with a reduction in crystallinity. Also, there was a reduction of agglomeration and average crystallite size (48 nm) were the same after silica coating. The zeta potential of the coated sample revealed a considerable high colloidal stability value (−27.70 mV) at physiological pH 7.4. The magnetization values of 26 emu/g and 25 emu/g recorded for bare and coated LAMO samples, respectively show that LAMO MNPs retained its ferromagnetic behaviour after silica coating. Bare and silica coated LAMO gave very high bactericidal effect against *S. aureus* however, there was no change in the antimicrobial properties of LAMO MNPs with silica coating. These results show that while the silica coating influences greatly the morphology and colloidal stability of LAMO nanoparticles, it had virtually no effect on the crystalline structure and size, magnetization and the antimicrobial properties.

Keywords: Silica Coating, Magnetic Nanoparticles, Silver-Doped Maganites, Antimicrobial Properties, Colloidal Stability.

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1. INTRODUCTION

Doped Perovskite manganese oxide with the general formula $Re_{1-x}A_xMnO_3$ (Re is a trivalent rare-earth metal and A is either a monovalent or divalent metal) has attracted huge research interests, over the years, due to their unique properties like colossal magnetoresistance. The magnetic properties seen in these compounds are as a result of the partial chemical substitution of Re^{3+} with A metal ions or the creation of vacancy in the Re^{3+} perovskite site which has been explained traditionally by the Zener double exchange mechanism. Magnetic nanoparticles are being exploited for biomedical applications as their ultra-small size enhances their physiological properties and their inherent magnetic properties allow them to be remotely controlled. They have been used in targeted drug delivery, hyperthermia and magnetic resonance imaging.[1–4] The high incidence of infectious disease and increase in the incidence of antibiotic resistance has led to the application of nanoparticles as novel antimicrobial agents owing to their unique physical and chemical properties. In this regard, different metal and metal oxides nanoparticles have been applied as active antimicrobial agents.[5–7] Also, several spinel ferrites MNPs have been exploited and investigated as potential antimicrobial agents with well documented antimicrobial activity.[8–11] However, the application of doped perovskite manganese oxide as novel antimicrobial agent is scarce in literature except for the use of non-stoichiometric perovskite lanthanum manganese oxide compounds in spontaneous and continuous disinfection of viruses[12] and new layered perovskite compounds with good antibacterial properties.[13]

Prior to biomedical application, MNPs need to be modified to improve their biocompatibility[9] and colloidal stability. In this regard, different biocompatible and biodegradable materials like silica have been employed for the surface modification of MNPs. In biomedicine and

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bioengineering, the interests in silica as a coating material for MNPs stems from its stability against degradation, biocompatibility and ease of surface modification.[14] Also, coating has been used to improve the antimicrobial properties of MNPs. Buteica et al. evaluated the antibacterial activity of magnetite nanoparticles which were previously coated with oleic acid followed by the adsorption coating with cephalosporins antibiotics. They observed that the inhibition zone diameters were greater for cephalosporins coated magnetite nanoparticles than the non-coated ones.[15] Jayandran et al. also carried out the comparative antimicrobial activity studies of iron oxide nanoparticles with their salicylalchitosan functionalized forms and reported the efficiency of the biofunctionalized iron oxide nanoparticles.[16]

Recently, silver doped perovskite manganites have been exploited as new materials with tunable T_c in temperature-controlled magnetic hyperthermia.[17–20] Particularly, the $La_{1-x}Ag_yMnO_3$ ($y \leq x$) compositions with nominal y values of 0.1, 0.15, and 0.2 have been shown to have a T_c of between 42–47 °C which falls in the therapeutic temperature range needed for hyperthermia. However, none of these reports have investigated the surface modification of silver doped perovskite manganites even though it is a requirement for biological applications. Also, it is well known that surface modification of MNPs can influence a number of their properties such as the composition, shape, coating, magnetic properties, size and size distribution which in turn can further influence their properties and applications. Therefore, the current study concerns the sol–gel auto combustion synthesis of LAMO MNPs which was subsequently coated with silica. The effects of the surface modification on the structural, morphological, magnetic properties, colloidal stability and antimicrobial activity of the synthesized LAMO MNPs have been studied.

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2. EXPERIMENTAL DETAILS

2.1. Synthesis of LAMO MNPs

The perovskite type LAMO MNPs were prepared by the sol–gel auto combustion method. The detailed synthesis of $La_{1-x}Ag_yMnO_3$ ($y \leq x$) compositions using glycine mediated sol–gel auto combustion synthesis has been reported in our recent publication.[21]

2.2. Formulations of Silica Coated LAMO MNPs

The LAMO MNPs were coated with silica using the modified Stober method.[22] Briefly, 100 mg MNPs were added to a solution of 150 ml of ethanol in which 10 ml distilled water and 2 ml ammonium hydroxide have been added. 2 ml tetraethoxy silane (TEOS) was added to the solution and sonicated for 15 min while the solution was maintained in an ultrasonic bath for 1 hr. This process was repeated twice and the mixture was allowed to stand for 24 hrs. The solution was filtered and the MNPs were washed with ethanol five times by centrifugation. The MNPs were dried at 60 °C for about 12 hrs and the silica coated MNPs were obtained.

2.3. Physico-Chemical Characterisation

The structure and phase of the bare and silica coated MNPs were identified by X-ray diffraction using a D8 Advance Bruker diffractometer with Cu-Kα radiation source at $\lambda = 0.15406$ nm in the 2θ scan range between 10° and 80° at 40 kV, 40 mA and at room temperature. The mean crystallite sizes (D) of the bare and silica coated MNPs were estimated using the Debye Scherrer formula.

$$D = \frac{0.9\lambda}{\beta \mathrm{Cos}\theta}$$

where β is the full width at half maximum of the strongest intensity diffraction peak (110), θ is the Bragg angle and λ is the radiation wavelength of X-ray used. The surface morphological images of the bare and silica coated MNPs were obtained using field emission scanning Electron Microscopes (Nova Nano SEM 600, FEI Co., Netherlands). Thermal decomposition behavior of silica coated MNPs was done using STA 409 PC Luxx (NETZSCH-Geratebau, Germany) under the temperature range of 30–1000 °C in argon atmosphere with a heating rate of 10 °C/min. Magnetic measurements of the bare and silica coated MNPs were done with a Vibrating Scanning Magnetometer (Lake Shore cryotronics-7400 series) with a magnetic field up to ±20,000 G at room temperature. Colloidal stability studies of the bare and silica coated MNPs in water and in PBS were done using a zetasizer Nano Zs (Malvern instruments). Zeta potential measurements were done thrice for each sample at 30 electrode cycles.

2.4. Antimicrobial Study

The bacterial cultures of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and fungal strains of *Candida albicans* were obtained from the Department of Biological Sciences, Applied Biology and Biotechnology Unit, Covenant University, Nigeria. The modified antimicrobial test procedure[23] was followed. The bacteria were cultured in nutrient broth and were allowed to grow in an incubator at 37 °C for 24 hrs and used for further experiments. The fungi were maintained in slants of SDA. Microbial suspensions of 0.5% McFarland standard obtained from bacterial cultures developed on solid media were used. The nanoparticles were suspended in dimethyl sulfoxide (DMSO) to prepare a stock solution of 20 mg/mL concentration. Gingasu et al. have shown that DMSO does not show any activity against the test organisms except for *Escherichia coli* where partial inhibition of its growth was recorded.[11] Agar well diffusion method was used for the antibacterial activity. The agar plates were inoculated in an overnight culture of each bacteria isolate in sterile petri-dishes. Holes were

drilled in the agar layer of each plate using a 9 mm diameter standard sterile cork-borer. Equal volumes of the ferrite compounds were introduced in the holes using a micropipette and allowed to diffuse for one hour at room temperature. Gentamicin was used as reference drugs for the microbes at the concentration of 10 μg/ml. The plates were incubated at 37 °C for 24 h for the bacteria, the plates for fungi were incubated at 25 °C for 3 days.

3. RESULTS AND DISCUSSION

3.1. Surface Modification of LAMO Sample with Silica

The coating of $LAMO_3$ core with silica was used to passivate the MNP surface. The nanoparticles are coated in order to prevent agglomeration, improve colloidal stability and provide a positive effect on biodistribution.[24] Silica coatings on nanoparticles provide a rich surface chemistry, high biocompatibility and an anomalously high stability, especially in aqueous media. It is assumed that silica adsorbed on the surface of magnetic core of LAMO MNPs and forms a shell as shown graphically in Figure 1.

3.2. XRD Analysis

XRD measurement was performed on the bare and silica coated crystalline LAMO powder and it is shown in Figure 2. The diffraction peaks of bare and silica coated MNPs gave similar relatively strong reflection peaks which were indexed to the rhombohedral perovskite structure [R-3c (167) space group]. This clearly showed that there was no change in phase in the case of the silica coated MNPs but there was a slight suppression of diffraction peaks compared with the bare MNPs. Therefore, the XRD data suggests that the silica shell consists mainly of amorphous phase rather than polycrystalline one[25] since there is the absence of silica-derived diffraction peaks. The average crystallite size obtained for the silica coated sample (48 nm) was the same with the uncoated sample (48 nm).

3.3. Morphological Analysis

The FE-SEM images of bare and silica coated LAMO MNPs are given in Figure 3. A reduction in the agglomeration of the silica coated MNPs is observed compared with the bare sample (Fig. 3(a)). The reduced agglomeration confirms the presence of silica coating on the MNPs

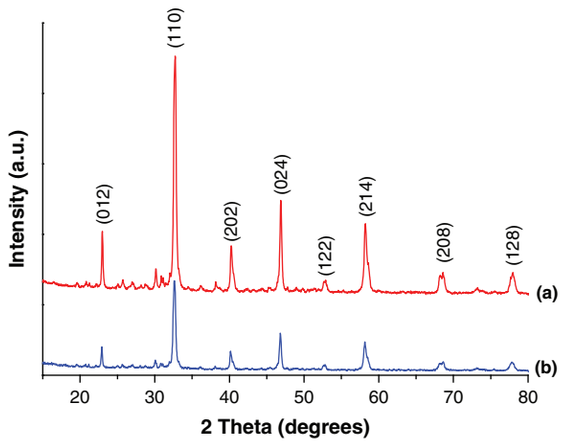

Fig. 2. XRD patterns of (a) bare and (b) silica coated nanocrystalline LAMO powders.

which helps to reduce the magnetic interactions between MNPs and gives a fair homogeneous particle size distribution which improves their biomedical applications. The MNPs after silica coatings gives a core–shell like structure with LAMO MNPs as the core and the silica (indicated as lighter contours enveloping the MNPs) as the shell. It can be seen that the LAMO particles were coated with silica, which is a favourable precondition for the biomedical application of MNPs to prevent their recognition by macrophages that clears the particles from the system, thereby preventing the MNPs from reaching the tumour sites.[26]

3.4. Thermal Analysis of Silica Coated LAMO Sample

The results of simultaneous thermal analysis (TGA and DTA) on the silica coated LAMO powders are presented in Figure 4. The TGA showed a sudden weight loss of ~98%, occurring in the 35–140 °C temperature range, is observed which is due to the decomposition of the coated silica layer from the MNPs' surface (it is also possible that the vapourisation of residual moisture was part of this decomposition process). This sudden weight loss corresponds to a sharp endothermic peak at ~150 °C on the DTA curve. It is well known that silica synthesized by the Stöber process possess high amounts of water and ethanol adsorbed on the surface, and both are removed by heating up to 150–200 °C.[27, 28] This might have accounted for

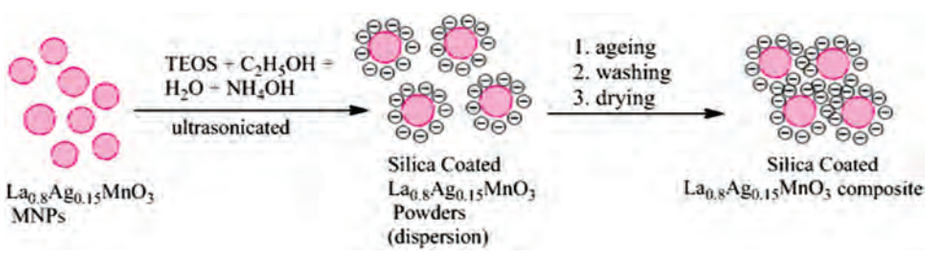

Fig. 1. Schematic of silica coating on $La_{0.80}Ag_{0.15}MnO_3$ MNPs.

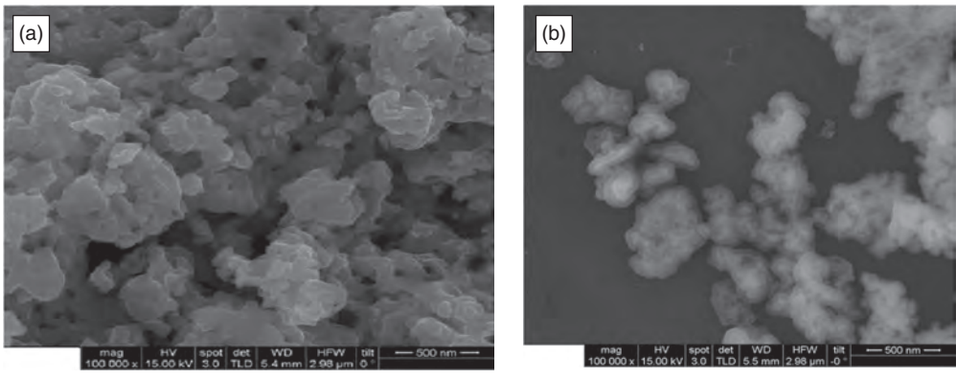

Fig. 3. FE-SEM images of LAMO powders (a) bare (b) silica coated.

the high weight loss associated with the detachment of silica coatings. It can be seen that in the 140–1000 °C temperature range, no weight loss is observed confirming the presence of pure LAMO phase. After the decomposition, ~2% LAMO yield is obtained.

3.5. Magnetic Studies of Silica Coated LAMO Sample

Figure 5 shows the M–H hysteresis loops for the bare and silica coated LAMO samples measured at room temperature. Table I gives the summary of the saturation magnetization (M_s), coercivity (H_c), remanent magnetization (M_r) and squareness (M_r/M_s) of the bare and silica coated samples. Like the bare sample, the applied magnetic field (20,000 G) was obviously not enough to saturate all the magnetic moments in the coated sample in its direction. Also, it is observed that the M_s of the silica coated sample (25 emu/g) is slightly smaller compared to the bare sample (26 emu/g) at the applied magnetic field even though TGA results showed high amounts of silica coatings (~98%). Surface modification of MNPs by non-magnetic coatings like polyvinyl alcohol has been implicated in the reduction in magnetization of the coated MNPs. This reduction in magnetization of the coated samples could be due to the reduction of particle–particle interaction and lowering of the exchange coupling energy.[29] The reduction in magnetization might also be due to the lesser amount of magnetic substance per gram in the silica coated sample compared with the bare sample.[2] The coated sample had lesser M_r, M_r/M_s and H_c values than the bare sample which also demonstrates the effects of the silica coating on the magnetic properties of LAMO samples. The relatively comparable M_s of the coated sample with the bare sample shows that the silica coating did not significantly reduce the M_s which is critical for its potentials as a heating agent in magnetic fluid hyperthermia.

3.6. Colloidal Stability Studies of Silica Coated LAMO Sample

Generally, in biomedical applications like magnetic fluid hyperthermia, the successful application of MNPs is highly dependent on their colloidal stability in aqueous and in biological media.[3] The average zeta potential values in distilled water recorded for the bare and coated samples were −4.54 mV and −5.96 mV, respectively. The pH dependent zeta potentials of the silica coated sample is −27.70 mV at physiological pH 7.4. The results show that there is increased colloidal stability with the silica coatings mostly at physiological pH.

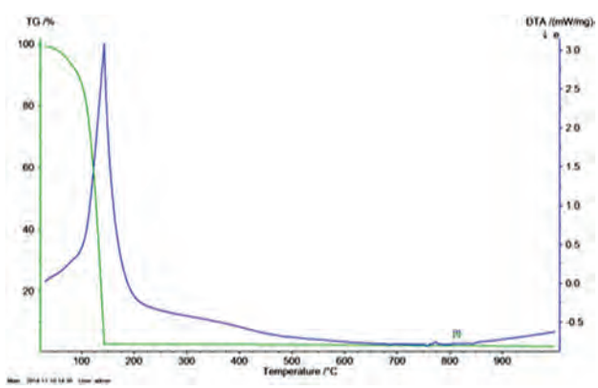

Fig. 4. TG-DTA curves of silica coated LAMO powders.

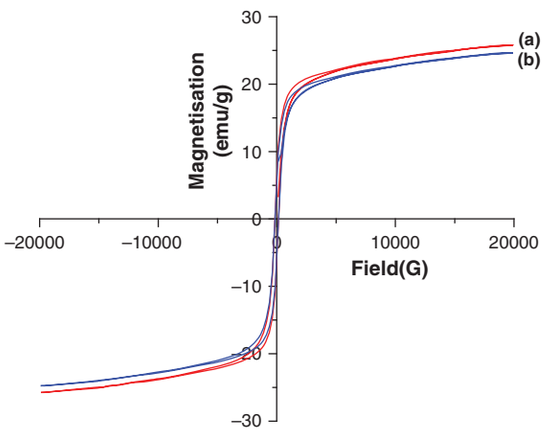

Fig. 5. Magnetic hysteresis curves of LAMO for (a) the bare sample (b) silica coated sample.

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Table I. Magnetic properties of the bare and the silica coated LAMO MNPs.

Sample	M_s (emu/g)	M_r (emu/g)	H_c (Gauss)	M_r/M_s
Bare sample	26	8.0	150	0.31
Silica coated sample	25	7.5	140	0.30

3.7. Antibacterial Studies

In this work, bare and silica coated LAMO MNPs were tested for their antimicrobial activity against gram-positive and gram-negative bacteria and also against fungi strain. The antimicrobial effects of the nanoparticles were qualitatively measured by performing agar diffusion test against all the test microorganisms. The results of zones of inhibition are given in Figure 6 and the zone of inhibition diameters are shown in Table II. The absence of microbial growth around the nanoparticles is an indirect measure of the ability of the nanoparticle to inhibit the growth.[10] Bare and silica coated LAMO MNPs gave bactericidal effect against *S. aureus*, *E. coli* and *P. aeruginosa*. Bare and silica coated LAMO gave very high bactericidal effect against *S. aureus* which was also found to be higher (over two orders of magnitude) than the conventional antibiotics (gentamycin) used and also the zone of inhibition found in literature for these organisms by other nanoparticle based antibiotics.[10, 11, 30] However, they gave lesser bactericidal effect against *E. coli* and *P. aeruginosa* compared to gentamycin. The results reveal that there was no difference in the antibacterial activity of the bare and silica coated LAMO MNPs. It is well known that the antibacterial activities of nanoparticles depend on their physicochemical properties and type of bacteria.[31] However, the mechanism of nanoparticles in their bactericidal activity is still speculative and not fully understood.[32] Reports[33, 34] have shown that nanoparticles, and in fact antibiotics generally,[35] give better bactericidal effect against the gram positive bacteria (e.g., *S. aureus*) than the gram negative bacteria (e.g., *E. coli* and *P. aeruginosa*). The presences of the cytoplasmic and outer cell membranes in gram-negative bacteria screen antibiotics and hence reduce the bactericidal effect as compared to the gram-positive bacteria which are bound by a single cell membrane. Also, the gram-positive bacteria contain a thick layer (20–80 nm) of peptidoglycan which might be responsible for retaining the colloidal form of the antibiotics hence improving its bactericidal effect.[36] Only bare LAMO MNPs recorded bactericidal effect against *C. albicans* but was lower than the standard antibiotics (tioconazole) used.

Table II. Susceptibility test of bare LAMO and silica coated LAMO MNPs.

	Zone of inhibition (mm)			
Test organism	LAMO	LAMO-Si	GENT	TIOC
Staphylococcus aureus	30	30	12	–
Escherichia coli	11	10	13	–
Pseudomonas aeuruginosa	10	10	15	–
Candida albicans	12	–	–	15

Notes: – Nil; GENT—Gentamycin; TIOC—Tioconazole; 0—absence of growth inhibition. Assays run @ 20 mg/ml; controls @ 10 μg/ml.

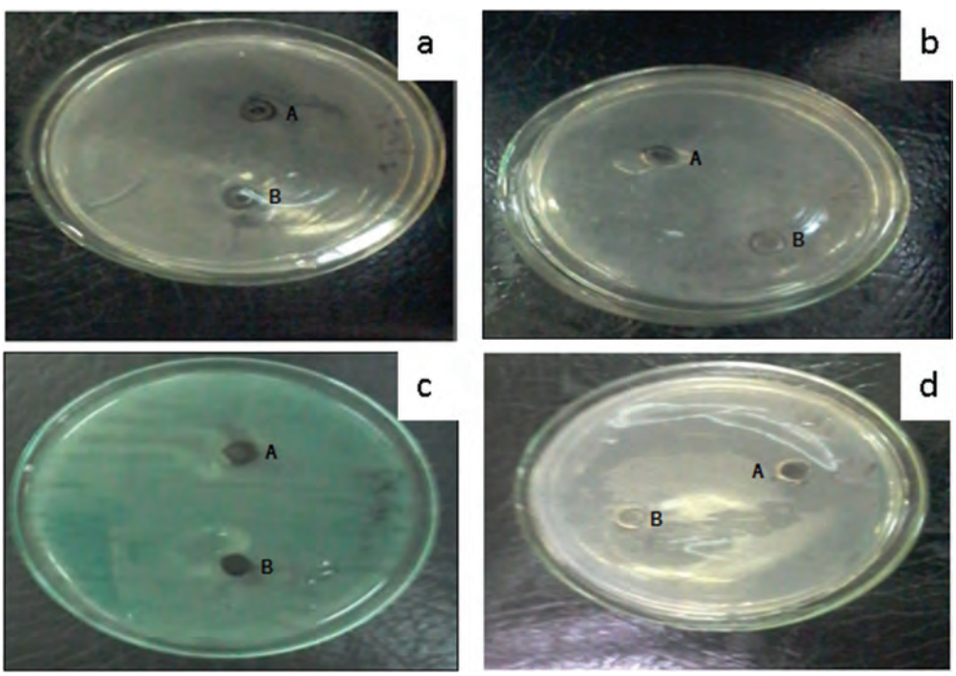

Fig. 6. Petri-dishes showing the antimicrobial activity of sample A (LAMO) and sample B (LAMO-Si) against microorganisms. (a) *Staphylococcus aureus* (b) *Escherichia coli* (c) *Pseudomonas aeruginos* (d) *Candida albicans.*

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4. CONCLUSION

The perovskite type LAMO MNPs were prepared by the low-temperature combustion method which was subsequently coated with silica to be evaluated for their biomedical applications. The results show that the crystalline perovskite phase and crystallite sizes were retained after silica coatings. There was a reduction in agglomeration of MNPs after silica coatings. Diamagnetic silica coated on the MNPs only marginally reduced the magnetization of the coated samples. The zeta potential measurements of the silica coated sample revealed a considerable high colloidal stability value at physiological pH 7.4. Antimicrobial results reveal that there was no difference in the antibacterial activity of the bare and silica coated LAMO MNPs. These results show that while the silica coating influences greatly the morphology and colloidal stability of LAMO MNPs, it had virtually no effect on the crystalline structure and size, magnetization and the antimicrobial properties. The results have implication for the exploitation of LAMO in many biomedical applications like hyperthermia, drug delivery and as antibiotics.

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