pH-Sensing Strips Based on Biologically Synthesized Ly-MgO Nanoparticles

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Supporting Information

ABSTRACT: MgO nanoparticles (NPs) are widely used in diverse areas ranging from catalysis to sensing. Besides this, there is a lack of information regarding their toxicity on fauna and flora. The venture of this work is to evaluate the toxicity behavior and pH-sensing performance of l-lysine-modified MgO (Ly-MgO) NPs synthesized by the green approach using the clove (Syzygium aromaticum) bud extract. The detailed investigations revealed that concentration plays an important role toward in vitro toxicity of Ly-MgO NPs. The Ly-MgO NPs showed 105% biocompatibility toward Vigna radiata (green gram) seeds at 100 ppm concentration. Zero inhibition on microbial growth was observed toward two bacterial strains. Further, pH-sensing strips based on these Ly-MgO nanostructures were developed to test pH-sensing performance at pH values ranging from 2.0 to 13.0. The repeatability as well as recyclability of the prepared pH strips was also analyzed. Nanobased pH paper strips based on Ly-MgO NPs provide a simple, reliable, nontoxic, and affordable method for pH measurements.

1. INTRODUCTION

Metal-oxide nanoparticles (NPs) are of great scientific and technological interest because of their tremendous applications in the fields of electronics, catalysis, sensing, drug delivery, and so forth. Various metal-oxide NPs like CuO, NiO, ZnO, and MgO have attracted a great deal of attention in recent years as they are safe to use and can withstand harsh conditions as well. Among these, MgO NPs are of particular interest due to their remarkable electronic, mechanical, and optical properties. They have been used as a medicine for heart burn, superconducting products, and recently in catalysis also. Because MgO NPs are quite stable and well dispersed, they are well suited for the diverse applications. Widely, various chemical and physical methods have been used for the synthesis of MgO NPs such as sol−gel process, hydrothermal, coprecipitation, and so forth. Most of these methods employ chemical reducing agents and harmful surfactants as stabilizing agents. In general, chemical synthesis of NPs is expensive leading to hazardous effects on human beings and environment. Therefore, the synthesis of NPs through biological integrals is preferred over the conventional methods, owing to its eco-friendly nature and cost-effectiveness. The use of plant extract for the synthesis of NPs has emerged as one of the most favorable biological approach. Plant extracts contain various biomolecules which act as a reducing as well as stabilizing agent. In the present work, the clove bud extract has been used as a reducing and stabilizing agent for the green synthesis of MgO NPs. Further, the synthesized MgO NPs were modified with a water-soluble amino acid, that is, l-lysine. In general, amino acids play an indispensable role in human body because they help to absorb calcium, iron, and zinc, which are useful in bone development and healthy skin and hair. Further, amino acids in form of dose are used to treat cancer because excess of amino acids cause shrinking of tumor cells. L-lysine is an essential amino acid (Figure 1), which cannot be synthesized in our body. It plays an imperative role in the treatment of

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Figure 1. Structures of l-lysine in different pH media.
various diseases like cancer, Alzheimer’s dementia, and cardiovascular diseases. Herein, we report the single-step combination of positively charged L-lysine with negatively charged MgO NPs and their use for fluorimetric pH sensing in aqueous medium.

It is well known that pH plays a key role among various chemical parameters and physiological processes in living organisms and the different environmental niches. pH determination is a strong prerequisite in various chemical, biochemical industries, waste water management processes, and intracellular regulatory processes. Among the different pH measurement methods, use of litmus paper strips is by far one of the most common and convenient method for pH sensing in aqueous solutions in the laboratory. Here, two different litmus paper strips are used to distinguish among acidic and alkaline solutions. Although the use of litmus paper strips is a simple and quick method but they cannot be reused and involve the use of two different paper strips. Here, for the first time, we have developed a single and reusable nanobased pH-sensing strip for the detection of pH in aqueous solutions. A variety of NP-based pH sensors have been developed so far, but their toxicity toward various organisms is still relatively unexplored, and their mechanism of action is still in need of deeper study. The escalated use of NPs in variety of applications related to industrial products ultimately lead their discharge into the environment. Hence, the information regarding the toxicity imparted by NPs on microbiota, fauna, and flora should exist. Therefore, studies of the biocompatibility of NPs are necessary for their rational design and use in sensing applications.

With the commitment of safer commercialized use, the researchers must be focused on safety concerns owing to potential hazards caused by nanosensors. Toxicity profiling of existing nanosensors has not been investigated so far. With regard to this, we have made an attempt to assess the phytotoxicity and antimicrobial activity of the developed Ly-MgO NP-based fluorescent pH sensor. The phytotoxicity was analyzed by seed germination assay of Vigna radiata seeds, and the antimicrobial activity was tested against two bacterial strains, that is, Staphylococcus aureus (Gram positive) and Pseudomonas aeruginosa (Gram negative). In this scientific report, we present the novel, reusable, inexpensive, biocompatible, and safer Ly-MgO-based pH strips.

2. RESULTS AND DISCUSSION

2.1. Structural Characterization of Ly-MgO NPs.

Figure 2a represents the Fourier transform infrared (FTIR) spectra of l-lysine, MgO, and Ly-MgO NPs. The peak at 652 cm$^{-1}$ (Figure 2a) strongly evidenced the Mg–O stretching.33 The spectrum in Figure 2a showed characteristic $\nu$NH$_2$ stretching frequencies of l-lysine at 2922 and 2865 cm$^{-1}$. Two more important asymmetric and symmetric stretching frequencies of carboxylate ($\nu$COO$^-$) were observed at 1519 and 1402 cm$^{-1}$, respectively. The FTIR spectrum of Ly-MgO showed these symmetric and asymmetric stretching of the carboxyl group at 1574 and 1358 cm$^{-1}$ (Figure 2a). Splitting between asymmetric and symmetric stretching of the carboxyl group, that is, $\Delta\nu$ ($\nu$asym OCO $-$ $\nu$sym OCO) was greater than the splitting in the FTIR spectrum of uncoordinated l-lysine. This observation indicated the unidentate coordination between carboxyl anions and Mg$^{2+}$ ions (Figure 2b). Therefore, l-lysine remains chemisorbed on the surface of MgO NPs. Further, the peak at 3406 cm$^{-1}$ in the FTIR of MgO NPs is prominent in the FTIR spectrum of Ly-MgO, which may be because of the coating of l-lysine on the surface of MgO NPs that were...
covered with phenolic moieties from the plant extract, showing O−H stretching frequency. Other peaks in the FTIR spectrum of Ly-MgO and MgO corresponded to various functional groups present in the biomolecules in the clove extract (CE). FTIR analysis suggested L-lysine coordinated to the MgO surface.

The crystalline nature and size of synthesized MgO NPs and Ly-MgO NPs was determined by X-ray diffraction (XRD) analysis. Synthesized NPs showed a broad peak at 20°−30° (Figure 3a), which clearly indicated the purely amorphous nature of MgO NPs. The absence of peaks at 36.94°, 42.90°, 62.30°, 74.67°, and 78.61° (JCPDS-2-1095) corresponding to planes (111), (200), (220), (311), and (222), respectively, indicated the absence of any crystal plane in MgO NPs. Further, the broadness of peak indicated the biosynthesized MgO NPs to be in the nano range and covered with various biological moieties that provide amorphous nature to MgO NPs. The XRD pattern of Ly-MgO was almost similar to the XRD pattern of MgO NPs.

Various amorphous metal-oxide NPs synthesized using leaf extracts of different plants have also been reported previously.34−36 Moreover, the synthesis of MgO NPs was confirmed by taking the XRD pattern of calcined MgO NPs (Figure S1). For this, Ly-MgO NPs were calcined at 700 °C for an hour to remove the biological moieties from the plant extract and l-lysine. The XRD pattern of calcined Ly-MgO NPs indicated the peaks at 31.23°, 42.91°, and 62.24° (ICSD-01-071-1176), corresponding to the planes (111), (200), and (222), respectively. This XRD pattern was used for crystallite size determination of calcined Ly-MgO from Debye Scherrer equation

\[ D = \frac{k\lambda}{\beta \cos \theta} \]  

where \( D \) is the crystallite size of calcined Ly-MgO NPs, \( \theta \) is the Bragg’s angle, \( \lambda \) is the wavelength of the X-ray source (1.5406 Å) used, \( \beta \) is the breadth of pure diffraction profile in radian on 2θ, and \( k \) is the Scherrer constant with a value from 0.9 to 1. The full-width at half maximum value measured from the most intense peak obtained from the XRD pattern (Figure S1) corresponding to 42.91° was found to be 0.3346. The crystallite size of calcined Ly-MgO NPs determined from the Debye Scherrer equation was found to be 4.5 nm.

The dynamic light scattering (DLS) method revealed that most of the NPs were centered on 324 nm, as shown in Figure 3b, with the polydispersity index of 0.251, indicating the formation of various sized NPs.

Thermal stability of Ly-MgO was analyzed by thermogravimetric analysis (TGA). TGA curves for MgO and Ly-MgO NPs are represented in Figure 4a,b. The weight-loss scale allows a quantitative comparison of degradation behavior of both samples. MgO NPs showed two staged degradation at 75 and 280 °C. The weight-loss peak at 75 °C was because of physisorbed water molecules, and the peak at 280 °C was because of the loss of CE. In the TGA of Ly-MgO, one additional peak at 393−445 °C was observed, which indicates the weight loss due to degradation of chemisorbed l-lysine molecules on the surface of MgO.

Further, size distribution of Ly-MgO NPs was determined by transmission electron microscopy (TEM) analysis. Figure 5a represents the typical TEM image, and Figure 5b shows the size distribution histogram of Ly-MgO NPs. The TEM image revealed that most of the NPs are spherical in shape.
the NPs are irregular in shape. The TEM image was analyzed by Image J software to obtain the size distribution histogram by measuring at least 356 NPs. The histogram was fitted by using a long normal function. A mean size of 7 nm was obtained for Ly-MgO NPs. Polydispersity of Ly-MgO came to be 23%.

A field-emission scanning electron microscopy (FESEM) micrograph was used to investigate the surface structure and topography of Ly-MgO NPs. The FESEM image of synthesized Ly-MgO is shown in Figure 6a, which clearly demonstrates that Ly-MgO NPs were uniformly distributed with approximately same dimensions. Most of the NPs were irregular in shape, which is the characteristics of biosynthesized NPs. Coating of L-lysine can be confirmed from EDS data (Figure 6b), which demonstrated the elemental composition of Ly-MgO NPs. The peak corresponding to carbon indicates the presence of organic moieties on the surface of MgO NPs. Further, the peaks corresponding to nitrogen, magnesium, and oxygen were because of L-lysine in Ly-MgO NPs. EDS for bare MgO NPs (Figure S2) showed the peaks corresponding to Mg, O, and C. The peak corresponding to N was found absent in MgO because of the absence of L-lysine.

2.2. Toxicological Profiling. 2.2.1. Antibacterial Activity of Ly-MgO NPs. The toxicity of NPs against microorganisms can be evaluated by the antimicrobial activity. Therefore, the antibacterial activity of both MgO and Ly-MgO was assessed for two types of bacteria, that is, P. aeruginosa (Gram negative) and S. aureus (Gram positive) by subjecting them to different concentrations of MgO and Ly-MgO NPs. No zone of inhibition was observed in both Gram negative and Gram positive bacterial strains (Figure 7) in each treatment at each concentration because the diameter of the effective zone of inhibition was found exactly the same as in the control. It can be depicted that the synthesized Ly-MgO NPs have no inhibitory effect on the growth of microorganisms even at concentration as high as 1000 ppm.

Figure 6. (a) FESEM image and (b) EDS spectrum of biosynthesized Ly-MgO NPs.

Figure 7. Toxicity profiling of MgO and Ly-MgO against two bacterial strains.
100% biocompatibility toward both bacterial strains. This can be explained on the basis of the green approach for the synthesis of NPs using CE. Most of chemical and biological properties of nanomaterials depend upon the mode of their synthesis. The biological synthesis provides eco-friendly and biocompatible nature to NPs.

2.2.2. Seed Germination Assay. The green gram seeds were subjected to the seed germination assay (Figure 8a) to ascertain the phytotoxicity imparted by MgO and Ly-MgO NPs. The germination tendency and root lengths of seed are most affected because of the strong tendency of NPs to accumulate on the surface of roots. The toxicity level and biocompatibility of NPs were ascertained by determining the root length difference between the control and NPs at each concentration. The values of different parameters, that is, germination rate (GR), germination index (GI), RL, and percentage of inhibition (PIG) (Table S1 and S2) emerged for green gram seeds were determined for each treatment (Figures 8a–e and 9a–d). The maximum root length of 0.93 cm was observed in 100 ppm treatment, which was even higher than the control experiment (0.88 cm). Root lengths showed only 32% decrease in root length even at concentration as high as 1500 ppm. A negative value of PIG was observed at 100 ppm (Table S1), which depicts the negative inhibition effect of Ly-MgO NPs on the growth of seeds. Even at higher concentrations up to 1500 ppm, PIG was found to be as low as 0.210.

Further, measured biocompatibility in comparison to control was about 45.4% at 2500 ppm for Ly-MgO, which further enhanced to 59, 63, 77, 96, and 105% for 2000, 1500, 1000, 500, and 100 ppm concentrations. Biocompatibility in case of MgO NPs was 57.9, 62.5, 67, 67, 79, and 83% at 2500, 2000,
1500, 1000, 500, and 100 ppm concentrations. Although bare MgO was also found to be more than 50% biocompatible, still they are comparatively less biocompatible as compared to Ly-MgO NPs. In addition to this, even root lengths of seeds in case of bare MgO NPs were found to be less than those for L-lysine-coated MgO NPs (Table S2). The investigational studies mentioned above demonstrated biocompatibility of both MgO and Ly-MgO NPs toward plants and bacteria. It might be due to the fact that these NPs have been synthesized by the green approach using the plant extract as the reducing and stabilizing agent, which imparted the nontoxic character to the synthesized MgO and Ly-MgO NPs. Further, L-lysine coating on the surface of NPs was responsible for enhanced biocompatibility of Ly-MgO NPs. Therefore, Ly-MgO NPs can be considered as nontoxic and safe candidates for sensing applications in aqueous solutions.

2.2.3. pH-Sensing Applicability. The synthesized MgO and Ly-MgO NPs were found to luminescent, showing emission peaks at 475 and 495 nm. The origin of emission can be explained on the basis of the electron hole recombination process. Luminescence occurs by transition of electrons and holes between electronic states, that is, valence band and conduction band (in crystalline materials), and tail states and gap states, that is, localized states38−40 (in amorphous materials as in the present case).

Interestingly, we noticed that Ly-MgO showed change in fluorescence emission wavelength as well as intensity in acidic and basic pH media while studying the fluorescence emission of Ly-MgO at different pH values. Figure 10a,b shows the emission spectra of Ly-MgO NPs in aqueous solution of pH, ranging from 2.0 to 13.0, and in acidic and alkaline media, respectively. No significant change in fluorescence emission wavelength and intensity was observed in spectra of bare MgO NPs (Figure 10d). Based on this phenomenon, we tried to address the issue that whether Ly-MgO proposed could be an ideal candidate for pH sensing. The observed emission behavior of Ly-MgO NPs in different media can be ascribed to the fact that change in acidic and alkaline media causes protonation and deprotonation of carboxylate and amino groups of L-lysine, which results into increased or decreased electrostatic interactions among L-lysine and MgO NPs. These interactions lead to alterations in n−π* and π−π* transitions in Ly-MgO NPs.

Further, Figure 10b indicated the increase in fluorescent intensity along with emission wavelength. This can be explained on the basis of the fact that the charge on the NP surface depends upon pH of the solution. At some intermediate pH value, at which the charge on the surface of NPs is zero known as the point of zero charge (ZPC). Below this point, there is a net positive charge on the surface of NPs, and at pH above ZPC, negative charge persists. The value of ZPC for synthesized MgO NPs was calculated from the linear plot of charge versus pH (Figure 10e), using relation $y = 349.835 + (-54.471)x$. The ZPC for MgO NPs was observed at a pH value of 6.42. From here, we can conclude that at pH value above 6.42, there is a net negative charge on the surface that could strongly bind to cationic amino acid (L-lysine), resulting into increase in emission wavelength. At pH below 6.42, there is a decrease in emission wavelength with decrease in the pH value because of the reduced electrostatic interaction between cationic L-lysine and positively charged MgO NPs. Hence, the shift in fluorescence emission wavelength and intensity was because of the enhanced binding of L-lysine on the surface of MgO NPs at pH higher than 6.42, which results into red shift in n−π* and π−π* transitions in Ly-MgO NPs.
In addition, careful investigation of emission wavelengths at different pH values revealed that the fluorescence emission wavelength of Ly-MgO varies linearly with pH values from 2.0 to 13.0. The fluorescence peak was shifted from 451 to 496 nm, with the regression constant for linear increase in emission wavelength with the pH value (Figure 10c) found to be 0.994 with equation \( y = 3.97x + 442.03 \).

Further, a prominent visible change in the color of Ly-MgO NPs-based pH-sensing strips was observed in acidic and basic media from pale yellow to orange yellow, respectively, as shown in Figure 11. Reversibly, the color of Ly-MgO strip again came back to pale yellow when adjusted to the acidic range, proposing that the variation of pH may affect electrostatic interactions between coated L-lysine and MgO, which leads to variation in \( n-\pi^* \) and \( \pi-\pi^* \) transitions.

In addition to this, the pH-sensing activity of Ly-MgO-based strips was tested for all strong (H\(_2\)SO\(_4\), HNO\(_3\)) and weak acids (CH\(_3\)COOH) and bases (KOH, NH\(_4\)OH) available in the laboratory. The Ly-MgO-based pH-sensing strips showed excellent and consistent response to each acidic and basic solution.

Further, repeatability and reproducibility of the Ly-MgO sensor was explored. Sensor’s repeatability or reusability refers to the consecutive runs made by using a single sensor to check consistency in its response. The reusability of Ly-MgO sensor was studied by using a single-sensor solution (Ly-MgO)
The current scientific study accentuated on the toxicological profiling of synthesized Ly-MgO NPs for pH-sensing applications. The Ly-MgO NPs were successfully synthesized using the green approach and characterized by various techniques like FTIR, DLS, XRD, TGA, TEM, and FESEM. Toxicity evaluation using the multiassay approach demonstrated the appreciable biocompatibility and nontoxicity toward bacteria and green gram seeds. Here, for the first time, we have developed pH-sensing strips based on green-synthesized l-lysine-coated MgO NPs. These pH-sensing strips and pH-sensing strip for six times at two different pH values of 2.0 and 13.0 (Figure 12), illustrating the excellent repeatability of Ly-MgO. It can be explained on the basis of alternative protonation and deprotonation of Ly-MgO NPs in acidic and basic media. It was observed that there was slight fatigue for the pH-sensing response after six runs. On the other hand, sensor reproducibility refers to the sensor consistency in the response of a batch of similarly synthesized sensor samples. The reproducibility of three similarly synthesized Ly-MgO sensor samples was checked to measure the pH-sensing response at pH 2.0 and 13.0.

2.3. Effect of Ionic Strength. The effect of ionic strength of solution on the pH sensor was examined by exposing the pH sensor to 100 μM NaCl solution. A 3 mL aliquot of Ly-MgO NP solution was diluted with 100 μM NaCl solution instead of double-distilled water (DDW). The pH of each solution was varied from 2.0 to 13.0. Each solution of different pH values was analyzed by a fluorescence spectrophotometer. Figure 13a represents the emission spectra of Ly-MgO NPs at different pH values in the presence of 100 μM NaCl solution. Similarly, Figure 13b represents the comparison of emission wavelength at different pH values in the presence of 100 μM NaCl with emission spectra in the presence of DDW (Table 1).

2.4. Comparison with Literature. The advantage of the present work is the green and environment friendly synthesis of the pH-sensing system. Further, ease of fabrication in terms of cost and time makes it a better candidate for simple and quick detection of pH.

3. CONCLUSIONS

The current scientific study accentuated on the toxicological profiling of synthesized Ly-MgO NPs for pH-sensing applications. The Ly-MgO NPs were successfully synthesized using the green approach and characterized by various techniques like FTIR, DLS, XRD, TGA, TEM, and FESEM. Toxicity evaluation using the multiassay approach demonstrated the appreciable biocompatibility and nontoxicity toward bacteria and green gram seeds. Here, for the first time, we have developed pH-sensing strips based on green-synthesized l-lysine-coated MgO NPs. These pH-sensing strips...
were tested over a range of pH, that is, from 2.0 to 13.0. Ly-MgO NP-based pH-sensing strips provided the advantages of excellent repeatability, reproducibility, cost-effectiveness, safe, and quick detection of pH. Effect of ionic strength on the pH sensing ability was also illustrated. The endeavor of the present work signifies the potential applicability of the developed environmentally benign pH-sensing strips for the detection of pH in aqueous solutions for commercial purposes in the near future.

4. EXPERIMENTAL SECTION

4.1. Materials. Magnesium acetate tetrahydrate [Mg-(COOCH₃)₂·4H₂O] (99%), HCl, NaOH, HgCl₂, and NaCl were purchased from Sigma-Aldrich. l-lysine was obtained from Fluka. For the strip preparation, Whatman filter paper (Grade-1) was purchased from Sigma-Aldrich. All chemicals were of analytical grade unless specified otherwise and were used as such. Clove buds and V. radiata seeds were purchased from local market (Sector 32, Chandigarh). DDW was used in all the experiments.

4.2. Fabrication of Ly-MgO NPs. Initially, CE was prepared according to our published procedure. For this, 1 M solution of clove bud powder was put into 50 mL of DDW and boiled for 2 min. This solution was cooled and centrifuged twice at 7000 rpm for 10 min to get a clear solution. 25 mL of CE was injected into the cuvette using a 5 mL disposable syringe such that a 5 mL disposable syringe such that no air bubble was entrapped. Each sample was analyzed using Zeta sizer software on automatic mode with 3 min equilibration time.

4.3. Characterization. 4.3.1. Fluorescence Measurements. All experiments were performed using a fluorescence spectrophotometer (Hitachi-7000) under fluorescence mode with a Xenon lamp. The emission slits were set at 10 nm slit width. A 3.5 mL quartz cuvette with 10 mm path length containing 3 mL of solution was used for spectral measurement. The excitation and emission wavelengths were 390 and 415 nm with a scan rate of 500 nm min⁻¹, respectively.

4.3.2. FTIR Measurements. To identify the functional groups, Ly-MgO NPs were subjected to FTIR spectroscopy (Shimadzu, Japan) in the working range of 4000–400 cm⁻¹. For this, 2 mg of powdered sample was transferred to a sample cabinet. A good signal to noise ratio was obtained by taking 256 scans per sample.

4.3.3. Particle Size Analysis. Initially, an approximate size of NPs was determined by DLS using a particle size analyser (Malvern, ZEN 1690). For this, an aliquot of the 100 μL Ly-MgO NPs was diluted with 2 mL of DDW and was dispersed using a probe sonicator for 5 min. The samples were then injected into the cuvette using a 5 mL disposable syringe such that no air bubble was entrapped. Each sample was analyzed using Zeta sizer software on automatic mode with 3 min equilibration time.

4.3.4. X-ray Diffraction. Crystallographic analysis of NPs was carried out using a Panalytical D/Max-2500 powder diffractometer with monochromatic Cu Kα radiation (λ = 1.5406 Å) over 2θ range of 5°–90° at the scan rate of 2° min⁻¹. The operational voltage and current were 40 kV and 30 mA, respectively. The size of NPs was calculated using the Debye Scherrer equation.

4.3.5. Thermogravimetric Analysis. The thermal stability of Ly-MgO NPs was determined using thermal gravimetric analysis (TGA-SDTQ600). TGA thermograms were recorded for 5 mg of the powdered sample at the heating rate of 10 °C in the temperature range of 20–1000 °C under a nitrogen atmosphere.

4.3.6. Transmission Electron Microscopy. Transmission electron microscopic analysis was performed to determine the size and morphology of Ly-MgO NPs using a Hitachi H-7500 microscope. For TEM studies, the carbon-coated 200 mesh copper grid was dipped into a solution of MgO NPs dispersed in DDW.

4.3.7. Field Emission Scanning Electron Microscopy. The morphology and elemental composition of Ly-MgO were determined by FESEM micrograph and EDS on the Hitachi SU8010 field-emission scanning electron microscope and Oxford energy-dispersive X-ray spectrometer, respectively.

4.4. Toxicological Profiling of Ly-MgO NPs. Applications of MgO NPs in various potential fields, that is, catalysis,2,3 sensing,4,5 and drug delivery,6,7 require toxicological profiling of these NPs to determine their impact on the ecosystem. In this study, we have used a multiassay approach to evaluate the toxicity imparted by MgO and Ly-MgO NPs on commonly found microorganisms and flora in the environment prior to their use as the pH sensor.

4.4.1. Toxicity Evaluation on the Flora—V. radiata Seed Germination Assay. For the evaluation of phytotoxicity, V. radiata seeds were washed using 0.1% mercuric chloride solution for sterilization, followed by 3–4 washings with DDW to completely remove the mercuric chloride residues.52 The sterilized seeds were soaked overnight in DDW. Next day, 10 seeds were transferred to Petri dishes. Further, the seeds in the Petri dishes were soaked in 8 mL aqueous solutions of MgO and Ly-MgO NPs at different concentrations, that is, 2500, 2000, 1500, 1000, 500 and 100 ppm. The Petri dishes containing soaked green gram seeds were kept under the dark and warm conditions for 72 h. Number of seeds germinated and root lengths in each case was measured. All the experiments were performed in triplicates to avoid the chances of error.

\[ \text{GR} = \frac{\text{no. of germinated seeds}}{\text{total no. of seeds}} \times 100 \]

\[ \text{GI} = \frac{N_c \times L_N}{N_c \times L_c} \times 100 \]

\[ \text{PIG} = \frac{L_c - L_N}{L_c} \]

\[ \text{VI} = \text{GR} \times L_N \]
$N_1$ = no. of germinated seeds for each treatment, $N_2$ = no. of germinated seeds in control $L_0$, and $L_n$ = mean root length of germinated seeds in control and each treatment, respectively.

4.4.2. Toxicity Evaluation on Microorganisms—Antibacterial Activity Testing. The antibacterial activity of synthesized MgO and Ly-MgO NPs was tested against two different strains of bacteria S. aureus (Gram positive) and P. aeruginosa (Gram negative). The antibacterial activity is useful to assess the toxicity of NPs to ensure their safe use in various biological applications. For this, the well diffusion method was employed. In this method, sterilized Petridishes were filled with 25 mL of autoclave-sterilized Muller Hinton (MH) agar and allowed to solidify. Further, 200 μL of activated bacterial cultures were spread over the surface of MH agar, and the wells of 5 mm diameters were punctured in each Petri dish. Aliquots (100 μL) of NP solutions of different concentrations (1000, 500, 200, and 100 ppm) were poured into the wells. The plates were incubated at 37 °C for 24 h to develop the zone of inhibition. The sterilized water was used as the control, and each experiment was carried out in triplicates. The effective zone of inhibition was measured with ruler and determined by subtracting the zone of inhibition observed in control experiment.

4.5. pH Sensing by Ly-MgO. To check the pH-sensing performance of Ly-MgO NPs, 10 mg of Ly-MgO NPs was dispersed in 20 mL of DDW by using a probe sonicator for 15 min. This solution was further diluted with DDW to 60 mL. The pH of this solution was adjusted to 3.0. Then, 3 mL portions of this solution were diluted two times with DDW. The pH of each solution was set from 2.0 to 13.0 using 0.1 N HCl and 0.1 N NaOH. These solutions were analyzed as such using a Hitachi F-7000 Fluorescence Spectrophotometer at an excitation wavelength of 390 nm.

4.6. Effect of Ionic Strength. To check the effect of ionic strength on the pH sensing ability of Ly-MgO NPs, 3 mL portions of Ly-MgO NP solution of different pH values were taken. To the 3 mL of each solution, 3 mL of 100 μM NaCl solution was added. Each solution was again analyzed by a fluorescence spectrophotometer.

4.7. Preparation of Ly-MgO-Based pH-Sensing Paper Strips. For the preparation of pH-sensing paper strips, 10 mg of Ly-MgO NPs was dispersed in 5 mL of water by sonication for 30 min. A strip with dimensions 3.5 cm × 0.5 cm was cut from the Whatman filter paper. This strip was impregnated with Ly-MgO solution. Then, Ly-MgO-based pH-sensing paper strips were dried in an oven at 40 °C. The process of impregnation followed by drying was repeated five times to saturate the paper strip with Ly-MgO NPs. These Ly-MgO pH-sensing paper strips were used as such for the detection of pH.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b01306.

XRD pattern of Ly-MgO NPs calcined at temperature 700°C, EDS spectra of MgO nanoparticles synthesized using the clove extract, the value of various seed growth parameters from the seed germination assay for Ly-MgO, and the value of various seed growth parameters from the seed germination assay for MgO (PDF).

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