A Simple Colorimetric Detection of Malathion Using Peroxidase Like Activity of Fe₃O₄ Magnetic Nanoparticles

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Abstract

This study reports synthesis of Fe₃O₄ magnetic nanoparticles (MNPs) using sodium borohydride (NaBH₄). The leach liquor used for the synthesis of MNPs was obtained by citric acid mediated leaching of scrap iron. X-ray diffraction (XRD) analysis showed the presence of magnetite. The MNPs showed super paramagnetic properties with a saturation magnetization (Ms) of 24.628 emu/g. The transmission electron microscopy (TEM) showed spherical shaped MNPs. These Fe₃O₄ MNPs exhibited intrinsic peroxidase like activity. The optimum concentrations of 3,3',5,5'-tetramethyl benzidine (TMB), H₂O₂ and Fe₃O₄ MNPs for peroxidase like activity were 3 mM, 10 mM and 0.05 mg/ml, respectively. This peroxidase like activity was further used for developing a colorimetric malathion detection method. The increasing concentration of malathion decreased the peroxidase like activity of Fe₃O₄ MNPs. The peroxidase like activity of Fe₃O₄ MNPs was completely inhibited at 3000 ppm malathion concentration.

Keywords: Malathion; Fe₃O₄ MNPs; Peroxidase like activity; Colorimetric detection.

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1. Introduction

The rapid increase in population is putting pressure on agriculture sector for increased supply of food. This can only be achieved by increasing the crop yield. The protection of crop plants from insects, bacteria, weeds, nematodes, rodents and other pests is necessary to achieve the increase in crop yield. Therefore, farmers are using various chemicals for the protection of seeds and crops.^[1] Malathion is one of the important chemicals used for crop plant protection. It is an organophosphate insecticide. It causes nonreversible phosphorylation and inactivation of acetylcholine esterase (AChE) in central nerve system leading to clinical complications and death.^[2] Hence, it is widely used for insect control on agricultural crops, on stored products, in home gardens, and in outdoor sites. It also helps to kill mosquitoes, Mediterranean fruit flies and lice.^[2] This widespread use of malathion is contaminating the food, water and soil with pesticide residues and metabolites. Hence, it is posing a great danger to human health and the ecosystem.^[3] Therefore, it is important to develop pesticide detection approaches considering public safety and environmental protection.

Various methods have been reported for pesticide detection. It includes, liquid or gas chromatography coupled to mass spectrometry, electrochemical analysis, fluorescent bioprobes, enzyme activity inhibition methods and enzyme-linked immunosorbent assays.^[4-14] These methods have several drawbacks. Some of these methods need sophisticated instruments. They need highly trained instrument operators. Sample preparation is also a critical factor.^[15] There are no specific antibodies available for malathion which limit use of immunoassay methods.^[16] Some methods need complex and expensive optical imaging devices and specific software, which increases the cost of the process. It also makes them unsuitable for a rapid on-site application.^[3] The use of pure enzyme is unavoidable for the methods based on enzyme-activity inhibition. This increases the cost of process. The activity of such enzymes gets affected by several factors and also these methods are not sensitive.^[17] Considering the drawbacks of all these methods alternative detection methods is need of time. Recently the colorimetric detection of pollutants based on magnetic nanoparticles (MNPs) has attracted considerable attention. The peroxidase like activity of Fe₃O₄ MNPs has been exploited extensively for various applications. It has been used for biomedical applications,^[18] detection of glucose, hydrogen peroxide, L-lactate, cholesterol, degradation of pollutants, etc.^[19] These types of methods are

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cheaper compared to other reported methods, which don't need sophisticated instruments except a spectrophotometer^[3,20] Considering these benefits of Fe₃O₄ MNPs, a simple colorimetric method was developed in the present study. In a previous study the leaching of iron was carried out from scrap iron using citric acid.^[21] The leached iron solution was used as precursor solution in the present study to produce Fe₃O₄ MNPs. Then the intrinsic peroxidase like activity of the Fe₃O₄ MNPs was used to detect the presence of malathion.

2. Materials and methods

2.1 Materials

Scrap iron was collected from the local market in Pune, Maharashtra, India. Citric acid was purchased from Qualigens, India. Sodium hydroxide (NaOH) and sodium borohydride (NaBH₄, Extra pure) were purchased from LobaChemie, India. The 3,3',5,5'-tetramethyl benzidine (TMB) ($\geq 99\%$) was procured from Sigma Aldrich. H₂O₂ (30%) was purchased from Fisher Scientific. All chemicals are used of analytical grade and used as received without further purification.

2.2 Synthesis and characterization of Fe₃O₄ MNPs

In a previous study the leaching of scrap iron was carried out using citric acid as a leachant.^[21] The iron containing leach liquor obtained from this process was used as precursor solution in present study for synthesis of MNPs. For the synthesis of nanoparticles, a combination of two methods was adopted. Chaki *et al.*^[22] suggested addition of precursor solution to sodium borohydride solution for nanoparticle synthesis. Raza *et al.*^[23] kept the pH of reaction mixture at 12 using sodium hydroxide during synthesis of nanoparticles. These steps were followed in present study with slight modifications. The precursor solution was added drop wise in NaBH₄ (1 M). After complete addition of NaBH₄, 1 M NaOH was added under continuous stirring at room temperature for 1 h to adjust pH 11. The volume ratio of precursor to NaBH₄ was 1:6. After 1 h incubation, black precipitate was formed. Precipitate was separated and further washed thrice with distilled water followed by ethanol (once). The precipitate was collected and dried in oven at 100 °C for 16 h.

The size, morphology, and microstructure of the synthesized MNPs were examined using transmission electron microscopy (TEM) (Philips CM 200, 200 kV), selected-area electron diffraction pattern (SAED) and X-ray diffraction (XRD) (Philips X'pert Diffractometer using Cu KR radiation, $\lambda=1.54178$ Å). The magnetization behavior of the particles was studied using vibrating sample magnetometer (Lakeshore 7307 VSM system). Shimadzu UV-1800 spectrophotometer was used for determination of peroxidase like activity of the Fe₃O₄ MNPs.

2.3 Investigation of the peroxidase-like activity of the Fe₃O₄ MNPs

The peroxidase-like activity of MNPs was evaluated on the basis of catalytic oxidation of the peroxidase substrate TMB

in acetate buffer in the presence of H₂O₂.^[24] In a typical experiment, 100 μ l H₂O₂ (10 mM) and 100 μ l TMB (3 mM) were added in 1700 μ l buffer (0.2 M, pH 4.2). 0.05 mg/ml Fe₃O₄ MNPs were added to above reaction solution and incubated for 10 min. The resulting solution was monitored at 652 nm by using a spectrophotometer.

Effect of various parameters on peroxidase like activity was optimized. The 0.05 mg/ml Fe₃O₄ MNPs was added to various concentrations of TMB such as (1-7 mM) and H₂O₂ (10-40 mM) and incubated for 10 min. The absorbance was taken at 652 nm. The concentrations of TMB and H₂O₂ showing maximum absorbance were chosen as optimum working concentration. Various concentrations of Fe₃O₄ MNPs (0.0025-0.025 mg/ml) were incubated with optimum working concentration of TMB and H₂O₂ for 10 min. Absorbance was taken at 652 nm after 10 minutes using spectrophotometer. The individual experiment approach was used while designing these experiments. The concentration of TMB, H₂O₂ and MNPs was optimized in individual experiments. The optimum concentrations were chosen from individual experiments to perform the next experiment.

2.4 Colorimetric detection assay for malathion

The 0.05 mg/ml Fe₃O₄ MNPs was mixed with increasing concentrations of malathion (200-3000 ppm) and incubated for 30 min. After incubation, optimized amount of 100 μ l TMB and 100 μ l H₂O₂ was added and incubated for 10 minutes. The colorimetric detection of malathion was carried out using peroxidase like activity of MNPs. The negative control contains buffer and substrate only.^[17]

Fig. 1 XRD analysis of as synthesized nanoparticles.

3. Results and discussion

3.1 Synthesis and characterization of MNPs

The present study showed that the leach liquor obtained from scrap iron leaching process was useful for the synthesis of nanoparticles. The NaBH₄ (1 M) was used as reducing agent. The nanoparticle synthesis depends on pH.^[25] They reported

Fig. 2 Characterization of as synthesized nanoparticles, (a) TEM analysis and (b) SAED analysis.

synthesis of Fe_2O_3 (dark green precipitate) at pH value of 6 and 9, while Fe_3O_4 (black precipitate) synthesis at pH 11. Considering these observations, the nanoparticle synthesis process was carried out at pH 11 with the aim to produce Fe_3O_4 MNPs. The pH was adjusted to 11 using NaOH.

XRD analysis was carried out to check the diffraction patterns of the MNPs. The peaks correspond to Fe_3O_4 MNPs (Fig. 1). These peaks match well with the peaks reported in the literature for magnetite.^[26] Also, the interplanar spacing and diffraction indexes match with the standard JCPDS card No.19-0629 for Fe_3O_4 .^[27] TEM images showed spherical shaped MNPs. The agglomeration of MNPs was observed (Fig. 2a). The SAED analysis confirmed the crystalline nature of particles (Fig. 2b). The results of XRD analysis are in agreement with the SAED results.

Fig. 3 Optimization of the peroxidase like activity of Fe_3O_4 MNPs using various TMB concentration.

The hysteresis loop at 300 K shows a M_s of 24.628 emu/g with the coercivity 7.31 G (Fig. S1). This indicates the presence of super paramagnetic properties in the Fe_3O_4 MNPs. The Fe_3O_4 MNPs retain residual magnetism in presence of an external magnetic field. The superparamagnetic properties of

Fe_3O_4 nanoparticles are beneficial for interference-free measurement during detection process.^[28] Hence, the Fe_3O_4 MNPs can be separated from pesticide containing reaction mixture by a simple magnet.

3.2 Study of the peroxidase-like activity of the Fe_3O_4 MNPs

In the present study, the synthesis and characterization of Fe_3O_4 MNPs was carried out. These Fe_3O_4 MNPs exhibited intrinsic peroxidase like activity. Effect of various concentrations of TMB and H_2O_2 on peroxidase like activity of Fe_3O_4 was studied. The peroxidase like activity increased from 1 to 3 mM TMB. It decreased beyond 3 mM TMB concentration. It showed 4091.66% (± 158.64) relative activity at 3 mM TMB concentration (Fig. 3). Therefore, 3 mM TMB concentration was considered as an optimum and used in further experiments. The peroxidase like activity decreased with an increase in H_2O_2 concentration. The maximum peroxidase like activity was observed at 10 mM H_2O_2 concentration. It showed 3944.44% (± 160.71) relative activity at 10 mM H_2O_2 concentration (Fig. 4). Another experiment was carried out to study the effect of various concentrations of Fe_3O_4 MNPs on peroxidase like activity. It showed maximum relative activity (5600% ± 90.5) at 0.05 mg/ml MNPs concentration (data not shown).

3.3. Colorimetric detection of malathion

The peroxidase like activity of Fe_3O_4 MNPs was further used to develop a colorimetric assay method for the detection of malathion. The optimum concentrations of MNPs, TMB and H_2O_2 (as reported in previous section) were taken in buffer. To this reaction mixture increasing concentration of malathion (200 to 3000 ppm) was added to study the effect of malathion on peroxidase like activity. Previous studies reported that presence of malathion inhibited peroxidase like activity of gold nanoparticles.^[17,29,30] Therefore, the present study was started with an expectation that the presence of malathion might inhibit the peroxidase like activity of Fe_3O_4 MNPs as

Fig. 4 Effect of various H₂O₂ concentrations on the peroxidase like activity of Fe₃O₄ MNPs.

well. In absence of malathion the color of the reaction mixture

changed from colorless to blue showing maximum absorbance at 652 nm (Fig. 5a & b). The color change occurred due to oxidation of TMB by peroxidase like activity of Fe₃O₄ MNPs. When the similar reaction was carried out in presence of increasing malathion concentration (200 ppm to 1400 ppm) then the absorbance also increased (Fig. 5a). The wavelength scan analysis of these reaction mixtures showed that the λ_{max} for each of the reaction mixture changed slightly (Fig. 5b). The presence of malathion may have affected the particles stability. Therefore, the scattering contribution might have increased with malathion concentration and it made the whole spectrum shift to large absorbance values. Another interesting phenomenon was observed in the present study. The color intensity of the reaction mixture decreased with an increase in malathion concentration from 1600 to 3000 ppm (data not shown). Hence, for better understanding, the results were analyzed by plotting a semilog graph between malathion concentration on log scale and % signal ($A_{\text{max}}/A_{800} \times 100$) on linear Y axis, where, A_{max} is absorbance at 652 nm for various malathion concentrations and A_{800} is absorbance at 800 nm.

(a)

(b)

Fig. 5 (a) Colorimetric malathion detection in the reaction mixtures containing various malathion concentrations (200-1400ppm) (b) wavelength scan of reaction mixtures containing various malathion concentrations (200-1400ppm).

Fig. 6 Semilog calibration plot of peroxidase like activity of Fe_3O_4 MNPs in reaction mixtures with increasing malathion concentration.

This semilog graph showed that % signal decreased with an increase in malathion concentration (Fig. 6). From the semilog graph results it can be interpreted that the presence of malathion affected the peroxidase like activity of MNPs. Our results are in accordance to Biswas *et al.*,^[30] Singh *et al.*,^[29] and Wu *et al.*,^[17] Biswas *et al.*,^[30] and Singh *et al.*^[29] proposed that malathion interact with gold nanorods (GNRs) and this interaction inhibit the peroxidase like activity of GNRs. According to Biswas *et al.*^[30] sulfanyl present in malathion disturbed the overall positive charge on the surface of GNRs. The positive charge on GNRs is necessary for the generation of free radical ($\text{HO}^\cdot$), which take part in partial electron transfer and play an important role for catalytic activity of GNRs. Hence, the presence of malathion inhibited the peroxidase like activity of GNRs.^[30] Similarly, the presence of malathion might be interfering with the properties of Fe_3O_4 MNPs, which resulted in decrease in % signal. The method described by Wu *et al.*^[17] is based on the hydrolysis reaction of AChE. In present study only Fe_3O_4 MNPs were involved. The system is not based on AChE enzyme and dissolution of nanoparticles. Guan *et al.*^[28] reported detection of various pesticides using intrinsically selective chemiluminescence (CL) switching at the surface of Fe_3O_4 nanoparticles. They suggested that, the peroxidase-like catalytic activity of Fe_3O_4 nanoparticles produced superoxide anions in acidic conditions. The resultant superoxide anions further oxidize luminol in basic media to produce the strong CL emission.^[28] They used this CL emission phenomenon for pesticide detection. The present method is comparatively simple than the method described by Guan *et al.*^[28] Present method just relies on peroxidase like activity of MNPs and don't require the acidic and basic conditions for pesticide detection as mentioned by Guan *et al.*^[28] Another study, reported Fe_3O_4 nanoparticle based colorimetric assay to detect organophosphate and nerve gas. In that method the enzymes AChE and choline oxidase (CHO) were used to produce H_2O_2 . This H_2O_2 activated Fe_3O_4

nanoparticle to catalyze the oxidation of substrates to produce a color reaction. This process was inhibited in the presence of pesticide resulting drop in color intensity.^[3] In present method no such activation of Fe_3O_4 nanoparticle is required for oxidation of substrate. Also AChE and CHO are natural enzymes with inherent limitations. They will be affected by environmental factors. Their use will increase the cost of process.^[1] Therefore, the present method describing pesticide detection independent of AChE and CHO using peroxidase like activity of Fe_3O_4 nanoparticle is more advantageous. However, the present study has a limitation as well. The lowest malathion concentration detected using this method was 200 ppm. Below 200 ppm malathion concentration, it was difficult to differentiate the color of reaction mixture with and without malathion. Further research is required to improve the method for detection of malathion below 200 ppm.

4. Conclusions

This study showed that the leach liquor obtained from scrap iron leaching process was useful for the synthesis of MNPs. The XRD analysis confirmed the presence of Fe_3O_4 MNPs. The VSM analysis showed that the MNPs have magnetic property. These MNPs can be removed from reaction mixture using a simple external magnet. The obtained MNPs also showed peroxidase like activity. They exhibited TMB oxidation ability. The MNPs showed optimum peroxidase like activity at 3 mM, 10 mM and 0.05 mg/ml of TMB, H_2O_2 and Fe_3O_4 concentrations respectively. This study also reported the use of peroxidase like activity of Fe_3O_4 MNPs for the detection of malathion. It provided a proof of concept for development of colorimetric assay of malathion detection. The results showed the decrease in % signal with an increase in malathion concentration. This method used only Fe_3O_4 MNPs and it was independent of specific recognition element like enzyme/antibody/aptamer. The reported method has a limitation of detecting lowest malathion concentration up to 200 ppm only.

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Conflict of interest

There are no conflicts to declare.

Supporting information

Not applicable

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