

Preparation of colloidal gold immunochromatography strip for detection of methamidophos residue

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Received 15 January 2008; revised 28 February 2008; accepted 5 March 2008

Abstract

Methamidophos (Met) is a broad spectrum organophosphorus insecticide and acaricide. Even a trace of its residue is harmful to humans and many animals. In this study, the synthesis and identification of colloidal gold particles and antibody-colloidal gold conjugates were performed, and the preparation of colloidal gold immunochromatography strip was conducted for detection of Met residue. The size of colloidal gold particles was checked using a transmission electron microscope (TEM). The formation of antibody-colloidal gold conjugates was monitored by UV/Vis spectroscopy. The preparation of colloidal gold immunochromatography strips, analysis of Met standard solutions, and other four pesticides and vegetable samples were operated with common methods and principals. The TEM images showed the average diameter of colloidal gold particles was almost the same size: approximately 40.0 nm in diameter. For the conjugation of colloidal gold and monoclonal antibody (MAb), 0.03 mg/ml of MAb was confirmed to be the minimum amount for stabilization of colloidal gold. With the prepared colloidal gold immunochromatography test strip to determine the standard Met solution, the results demonstrated a detection limit of approximately 1.0 µg/ml. Cross-reaction indicated that the strip had a high specificity to Met. The results of 10 green vegetable sample tests confirmed that one sample was positive by HPLC analysis. There was evidence to suggest that colloidal gold particles and antibody-colloidal gold conjugates were synthesized successfully. The prepared colloidal gold immunochromatography strip was applicable for preliminary screening of Met residue.

Key words: colloidal gold; immunochromatography; methamidophos (Met)

Introduction

Methamidophos (O,S-dimethylphosphor-amidothiolate) (Met), a broad spectrum organophosphorus insecticide and acaricide that has been used all over the world, is the largest tonnage pesticide in the 19th–20th century in China (Lei, 1994). It has been argued by many researchers that even a trace of residual amount of Met is harmful to human and many animals, as it influences the central nervous system acetylcholinesterases, which are responsible for the degradation of acetylcholine at nerve junction (Bouchard *et al.*, 2006; Sidell, 1994; Zhou *et al.*, 2006). Cases have been continuously reported that there were many violations to the national prohibition on the use of Met on fruits, vegetables, and tea plants as well as misuses against the instructions by farmers resulted in Met poisoning (Aguilera *et al.*, 1999; Roy *et al.*, 1995; Chen *et al.*, 2005). From early 2001, Ministry of Agriculture and many provincial governments in China, such as the government of Zhejiang, Shanghai, and Guangdong had published injunctions against the use of Met pesticide, which will eventually

become a national-wide application after 2007. Moreover, the use of pesticide of acephate, a substitute pesticide of Met, has been encouraged by the Chinese government; however, research shows that acephate will degrade into Met in plant and soil, and become more dangerous to soil and human (Annick and Louise, 2004). Therefore, there is a need to detect Met residue in vegetable, food, soil, and drinking water. Although many analysis methods, such as gas chromatography coupled with mass spectrometry, liquid chromatography with electrochemical detection, and liquid chromatography with mass spectrometry, have been developed for the determination of Met (Mol *et al.*, 2003; Ogawa *et al.*, 1997; Tsuchiya *et al.*, 1998), these methods are highly sensitive and specific demanding complicated pre-treatment steps and are, therefore, unsuited for rapid process of multiple samples. What is more, instrumentations for these methods are very expensive and can hardly be equipped for on-site measurement of Met. The method of colloidal gold immunochromatography assay has been used in hospitals and medicine regions for many years (Beggs *et al.*, 1990; Zhou *et al.*, 1998). Recently, the colloidal gold immunochromatography assay method

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has been developed and applied in many no medicine regions, such as for the determination of aflatoxin B1 and Atrazine (Sun *et al.*, 2005; Shim *et al.*, 2006). Nonetheless, the application of the colloidal gold immunochromatography assay method of Met has not been reported yet. Aiming at developing a simple and fast gold-labeled immunochromatography assay method, the researchers have successfully synthesized Met artificial antigen, prepared, and purified monoclonal antibodies of anti-Met (Zhao *et al.*, 2003a, 2003b, 2004). Consequently, the feasibility of building the gold-labeled immunochromatography assay method for on-site monitoring of Met in vegetable and drinking water has been confirmed. Furthermore, the gold-labeled immunochromatography assay method also has been used for rapid analysis in blood serum and emiction to examine Met poisoning in human bodies. This not only benefits human health but also provides a reference to the development of colloidal gold immunochromatography strip for the detection of other pesticide and poison chemical.

1 Materials and methods

1.1 Materials

Methamidophos ($\geq 99\%$) was purchased from Institute of Chemistry and Industry (Shenyang, China). Chloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) and sodium citrate were purchased from the company of Australia Chemical Reagents (19 Kensal St Moorooka, Australia). Bovine serum albumin (BSA) and goat anti-mouse IgG were purchased from Sigma (USA). High-flow nitrocellulose membrane, glass fiber, and absorption pad were purchased from Millipore (Bedford, MA). Artificial antigen of Met-BSA was synthesized by the researchers themselves (Zhao *et al.*, 2004). Purifying monoclonal antibody to Met was generated in mice by immunizing the animals with artificial antigen of Met-BSA (Zhao *et al.*, 2003a, 2003b). Phosphate-buffered saline (PBS, pH 7.4, 0.01 mol/L in 0.85% NaCl) and 0.01 mol/L Na_2CO_3 solution was prepared. Distilled water was used in this experiment. All other chemicals used in the present study were analytically pure.

1.2 Synthesis and characterization of colloidal gold

Colloidal gold particles with a mean diameter of 40 nm were prepared according to the method by Xu *et al.* (2006). Briefly, an aqueous solution of chloroauric acid (30 ml of 0.01% (W/V) $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) was heated to boiling point, followed by 300 μl of 1.0% (W/V) sodium citrate solution added into it. The reaction solution was stirred simultaneously and gently boiling it for 15 min until the color of the solution turned from straw yellow into black and eventually red. The obtained colloidal gold solution can be stored at 4°C for several months and they will be used for conjugation with monoclonal antibody (MAb) to Met. Colloidal gold particles were checked by a transmission electron microscope (TEM) performed on a CM 100 (Philips Co., The Netherlands) and operated at an acceleration voltage of 80 kV with a magnification of

66,000. The sample was prepared by placing a drop of colloidal gold on a carbon-coated TEM copper grid. The film was allowed to dry for 10 min, and the excess solution was removed using a tissue paper. The size distribution of the colloidal gold particles from photographs enlarged three times was measured, and the TEM images were selected at least 50 counts.

1.3 Minimum amount of antibody study for stabilizing colloidal gold particles

Colloid gold suspension ($C_{\text{Au}} = 2.5 \times 10^{-4}$ mol/L) was adjusted to the same pH (ranges about 7–8) with 0.01 mol/L Na_2CO_3 solution firstly and pipetted into a series of tubes (tube/0.95 ml). Then, monoclonal antibody solution (0.01–0.07 mg/ml, 0.95 ml) was added to the above each colloidal gold solution. After that, each tube received 0.1 ml of 10% NaCl and was shaken for observation of color change after 2 h.

1.4 Formation of antibody-colloidal gold conjugates and characterization

The pH of the colloidal gold solution was adjusted to pH around 7.4 by additional 0.01 mol/L Na_2CO_3 before the monoclonal antibody was added. This was used in as much as the laboratory experience supports the optimum pH at 7–8 for labeling monoclonal antibody with colloidal gold. The least concentration of monoclonal antibody for stabilizing colloidal gold particles is on a basis of 0.03 mg/ml for the above experimentation. Therefore, 2.5 ml MAb (0.04 mg/ml prepared in pH 7.4, PBS 0.01mol/L) was added drop-wise to 2.5 ml of colloidal gold solution. The solution was stored for 2 h at 4°C and then, was centrifuged to remove unconjugated MAb. The centrifugation was undertaken at 14,000 r/min at 4°C followed by the pellet redispersed in total 5 ml of pH 7.4 PBS and stored at 4°C for further experiments. The formation of antibody-colloidal gold conjugation was monitored with UV/Vis spectroscopy using a double-beam spectrophotometer (Lambda 25, Perkin Elmer Instruments, USA). The colloidal gold solutions were monitored immediately after the antibody being added and after the centrifugation and resuspension of the conjugation in 5 ml of pH 7.4 PBS.

1.5 Preparation of immunochromatography strip

Immunochromatography strip was constructed as a method by Xu *et al.* (2006) and Verheijen *et al.* (1998). Colloidal gold-labeled antibody conjugate was jetted onto glass fiber and dried at 37°C. Goat anti-mouse antibody (1.0 mg/ml) was dispensed onto a nitrocellulose membrane on the upper line for control with a volume of 1 μl per 1 mm line, and Met-BSA antigen (1.0 mg/ml) in PBS was jetted into the lower part for test line; the dispensed volume was also of 1 μl per 1 mm line. The remaining active sites on the membrane were blocked by incubation with 2% BSA in PBS (1 ml/cm membrane) for 30 min at room temperature. The membrane was washed once with PBS and again with distilled water and then, dried at 37°C. Subsequently, the absorption pad, the nitrocellulose membrane, the released antibody-gold conjugation pad,

and the sample application pad were assembled into a sheet of plastic backing orderly (Fig.4) and cut into individual strips (2.0 mm/strip) with a pair of small medicine scissors.

1.6 Procedure for analysis of Met standard solutions

The standard solutions of sample Met pesticides were prepared in PBS (pH 7.4) by serial dilutions in 0.5 ml graduated microtubes. The sample of distilled water was used for the control test. During the detection process, four drops (160 μ l) of different concentration of the above liquid samples were pipetted onto the sample pad, allowing the sample to migrate upward. After 10 min, image containing the color signal was produced in the strip and the results were judged by the color in the test and the control lines.

1.7 Cross reactivity to other pesticides and sample analysis

The four pesticides which are structurally related to Met: acephate, dichlorvos, dimethoate, and monocrotophos were obtained from Plant Protection Research Institute of Guangdong Academy of Agricultural Sciences, China. The cross-reactivity for the four pesticides with different concentrations was tested using the procedure described above. Ten green vegetable samples were bought from Changban Market in Guangzhou City. The vegetable leaves were squeezed and the juices were prepared and tested using the procedure described above, the test of every sample repeated twice. The Met positive samples were determined by HPLC (Agilent 1100, USA) for corroboration.

2 Results

2.1 Characterization of the colloidal gold particle

Colloidal gold particles were synthesized by chemical method using the reduction of chloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$). Chloroauric acid was reduced to gold atoms by sodium citrate and many of the gold atoms accumulated into colloidal gold solution. The TEM image shows well-dispersed colloidal gold particles (Fig.1). The average diameter of the colloidal gold particles was about 40.0 nm. TEM image indicates that the colloid gold particles were almost of the same diameter, which provided the best basis for preparation of immunochromatography test strip.

2.2 Minimum amount of MAb for stabilizing colloidal gold particles

For the conjugation of colloidal gold, a minimum amount of MAb concentration was needed for stabilizing colloidal gold particles. The minimum amount of MAb was determined by adding 10% NaCl to colloidal gold particles containing MAb of different amount. After the

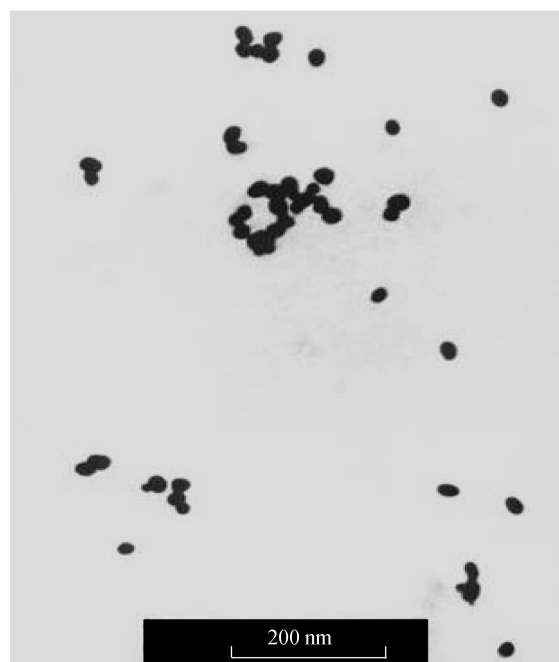


Fig. 1 Colloidal gold observed by transmission electron microscope (66000 \times).

reaction with NaCl, a colloidal gold solution containing minimum MAb could be kept in red color. In the experiment, 0.03 mg/ml of MAb was confirmed to be the minimum amount for stabilizing colloidal gold solution. However, the 0.04 mg/ml of MAb for the conjugation was considered as the selected MAb concentration must be greater than minimum amount MAb concentration, which will be a better stabilization of colloidal gold (Table 1).

2.3 Characterization of antibody-gold conjugates with UV/Vis spectra

Figure 2 shows the UV/Vis spectra of the colloidal gold and antibody-colloidal gold conjugate. A peak at about 527 nm in curve a was a result of the surface resonance of colloidal gold particles. Added with the antibody, the surface resonance band shifted a little into the red direction. This was caused by the interaction of the antibody and colloidal gold particles. A peak at approximately 280 nm in the conjugation system arose because of the absorbance from the tyrosine and tryptophan residues in the protein of monoclonal antibody. After centrifugation and resuspension of the antibody-colloidal gold conjugation, the intensity of the surface resonance band and protein absorbance band at 280 nm decreased because of the reduced colloidal gold concentration, and removal of the unconjugated MAb molecules in solution.

2.4 Preparation immune test strips and analysis of Met standard solutions

The construction of the method of colloidal gold

Table 1 Color of colloidal gold and different concentration antibody solutions after adding 10% NaCl

| Antibody concentration (mg/ml) | 0 | 0.01 | 0.02 | 0.03 | 0.04 | 0.05 | 0.06 | 0.07 |
|--------------------------------|------|------|------------|------|------|------|------|------|
| Color | Blue | Blue | Faint-blue | Red | Red | Red | Red | Red |

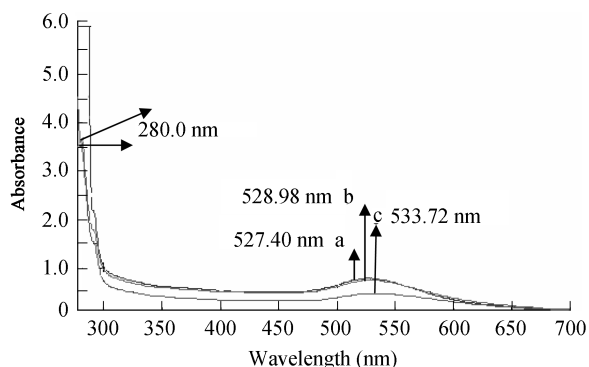


Fig. 2 UV/Vis spectra of colloidal gold and antibody-gold conjugate. Curve a: colloidal gold solution; curve b: the antibody-colloidal gold conjugate immediately after addition of the antibody at pH 7.4 PBS; curve c: the antibody-colloidal gold conjugate after centrifugation and resuspension at pH 7.4 PBS.

immunochromatography strip and the principle of the analysis of immunochromatography test are shown in Fig.3. If the test result could be judged with the appearance of two lines in the control and test line on the membrane, it indicates that the Met concentration is below the detection limit. Only one red line in the control line indicates the Met concentration is above the detection limit. The test results (Fig.4) shows 2 obvious red bands on the test line and the control line for the distilled water sample (line 1). The concentration of 0.10 $\mu\text{g/ml}$ of Met (line 2) and the concentration of 0.50 $\mu\text{g/ml}$ of Met (line 3) displayed 2 obvious red bands on both the test line and the control line. The concentration of 1.00 $\mu\text{g/ml}$ of Met was found to cause a considerable decrease with a very faint red color band at the test line (line 4). The concentration of 2.00 $\mu\text{g/ml}$ of Met displayed only 1 red band on the control line (line 5). Therefore, the detection limit was about 1.0 $\mu\text{g/ml}$.

2.5 Test results of cross reactivity to other pesticides and sample analysis

The specificity of the strip was evaluated in comparison to other four pesticides. As shown in Table 2, two clear red lines were observed in the test and the control lines

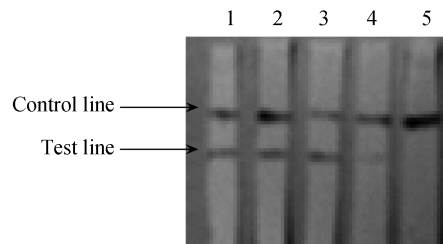


Fig. 4 Immunochromatographic detection results of methamidophos (Met). Line 1: only distilled water; line 2: 0.10 $\mu\text{g/ml}$ Met; line 3: 0.50 $\mu\text{g/ml}$ Met; line 4: 1.00 $\mu\text{g/ml}$ Met; line 5: 2.00 $\mu\text{g/ml}$ Met.

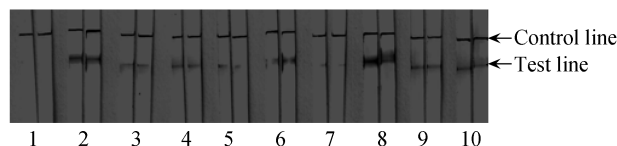


Fig. 5 Immunochromatographic detection results of 10 green vegetable samples. The test of every sample repeat two times.

on various concentrations of the four pesticides with few exceptions to acephate pesticide. This indicated that the developed strip had a high specificity to Met without cross-reaction for other pesticides. The results of the 10 green vegetable sample tests showed that one vegetable sample (sample 1) was positive (Fig.5) and in agreement with the HPLC analysis (Fig.6).

In a conclusion, the strip is easy to perform, and the results can be shown within 10–15 min without a need

Table 2 Cross-reactivity of the strip to other pesticides

| Other pesticide | | Concentration ($\mu\text{g/ml}$) | | | | | | |
|-----------------|--------------|------------------------------------|------|-----|---|---|----|----|
| | | 0 | 0.01 | 0.1 | 1 | 5 | 10 | 50 |
| Acephate | Test line | + | + | + | + | + | ± | – |
| | Control line | + | + | + | + | + | + | + |
| Dichlorvos | Test line | + | + | + | + | + | + | + |
| | Control line | + | + | + | + | + | + | + |
| Dimethoate | Test line | + | + | + | + | + | + | + |
| | Control line | + | + | + | + | + | + | + |
| Monocrotophos | Test line | + | + | + | + | + | + | + |
| | Control line | + | + | + | + | + | + | + |

+: obvious red band was observed; ±: faint red band was observed; –: no band was observed.

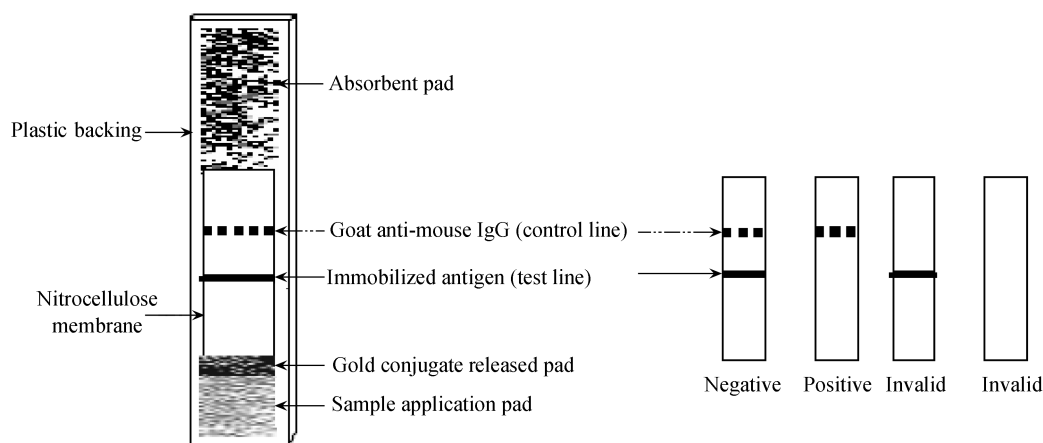


Fig. 3 Construction of colloidal gold immunochromatography strip and illustration of immunochromatography result. The positive result is judged by the appearance of only a single color line in the control line. The negative result is judged by the appearance of two lines in the control and test line. The test is invalid if no line is present in the control line.

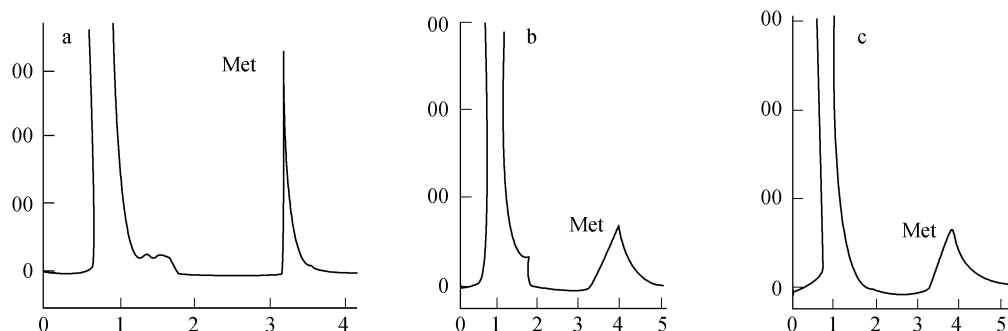


Fig. 6 HPLC analysis in positive green vegetable sample. a: standard of Met; b and c: positive vegetable sample (repeated two times).

of expensive equipment or washing steps. Moreover, with respect to its simplicity and rapid speed, the colloidal gold immunochromatography is superior to enzyme linked immunosorbent assay (ELISA). The method has a potential as a rapid, cost-efficient, and on-site screening tool to detect Met contamination in agricultural and environmental samples. The method can be used, for example, by vegetable companies and water inspection centers and hospital for the preliminary screening of Met residues.

3 Discussion

Since monoclonal antibodies are labeled with colloidal gold particles instead of enzyme, the substrate is no longer needed in the reacting system for colloidal gold immunochromatography. This is the reason why colloidal gold particles have been gradually applied in immunoanalysis and biosensor (Deng *et al.*, 2000; Sithigorngul *et al.*, 2007). Usually the strength of color demonstration is closely related to result judging, whereas the color of colloidal gold particle is related to the size of the colloidal gold particle, which is directly dependent on the amount of trisodium citrate used in its preparation process. When all other prepared conditions including procedures and reagents are kept stable, changes to the amount of trisodium citrate will result in different colloidal gold particles in diameter and in color. In this study, 1.0 ml of trisodium citrate (1.0%) was added into 100 ml (0.01%) of chloroauric acid solution. The formed colloidal gold particles were red with a diameter of around 40 nm. After the particles keeping it in steady state for two more months, the colloidal gold particles combined with monoclonal antibody were found to be stable. The indicating color was obvious and easy to distinguish. The highest UV/Vis absorbance of 530 nm was wholly coincident with a diameter of 40 nm colloidal gold. Therefore, the prepared colloidal gold particle and monoclonal antibody-gold conjugation in this study is suitable for immunochromatography detection of Met. The strip has few cross-reactivity to acephate pesticide. This is because Met and acephate pesticide have the same basic chemical structure. The foregoing study also confirmed the same cross-reactivity with the ELISA test (Zhao *et al.*, 2003a). Compared with other analysis methods, the immunochromatography strip test has many advantages. There is no requirement for any other instruments. Unlike

ELISA, the method does not require enzyme. There is no need for washing repeatedly and adding different chemical reagents so as to largely shorten the whole detection time. Moreover, compared with ELISA method, temperature becomes less influential on this testing method (Grif *et al.*, 2007). The immunochromatography detection method is, therefore, a ideal and rapid determination method for monitoring of pesticide residue.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 39970519), the Guangdong Province Natural Science Foundation (No. 05001814, 06012298), the Scientific Research Foundation for the Returned Overseas Chinese Scholars of State Education Ministry and Personnel Ministry, the Tackle Key Research Project of Guangxi Autonomous Region and Nanning City (No. 0537007-7-2, 200501065B). The authors would like to thank Dr. ZHANG Zhenyu for proofreading of the article.

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