Environ. Sci. Technol. **2007**, *41,* 5028−5036

Immunochromatographic Dipstick Assay Format Using Gold Nanoparticles Labeled Protein−Hapten Conjugate for the Detection of Atrazine

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The present study describes a lateral-flow-based dipstick immunoassay format using a novel hapten−protein−gold conjugate for the rapid screening of atrazine in water samples. The immunoassay is based on the competitive inhibition, in which a newly developed hapten−protein−gold conjugate competes with the free antigen present in the sample, for the limited antibody binding sites available at test zone on dipstick membrane, housed in a plastic cartridge. The tracer used as the detection reagent was prepared by first conjugating hapten (a derivative of atrazine) molecules to a carrier protein (bovine serum albumin) via its surface lysine residues and then linking colloidal gold nanoparticles to the hapten−protein conjugate via cysteine residues of the carrier protein. The developed conjugate showed a high level of stability as it did not show any significant loss of activity even after 8 weeks of storage at ambient conditions. The color developed due to conjugate, based on competitive inhibition approach, is correlated with the concentration of atrazine sample. The sensitivity of the developed dipstick was enhanced by gold nanoparticles, as an amplification tag, presenting detection limit of atrazine in standard water samples down to 1.0 ppb level. The kit could serve as a rapid screening methodology for visual screening of atrazine contamination of water samples within 5 min of analysis time, and, when coupled with a portable colorimeter, as an inexpensive semi-quantitative assay. The method reported can be useful for screening a large number of pesticides samples in a very short time in the field.

1. Introduction

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine), because of its selectivity against broadleaf weeds and annual grasses, is one of the most widely used herbicides for combating weeds in corn, sugarcane, and sorghum crops (*1*). Since atrazine readily adsorbs into groundwater and rainwater, the large scale application of this pesticide resulted in the contamination of drinking water and soil, causing serious health risks even at very low (sub ppb) levels (*2*−*3*). Alarmingly, the presence of atrazine has been detected in water and soil at concentrations far exceeding its permissible limit of 3 ppb (*4*). To control and contain such a hazardous pollutant, a rapid method of detection of this organic toxin is urgently required. A number of well established analytical methods such as thin-layer chromatography (*5*), gas chromatography (*6*), high-performance liquid chromatography (*7*), and gas chromatography−mass spectrometry (*8*), have been widely used for the detection and quantification of pesticides at the trace levels. However, these methods of detection of pesticides are complex, time-consuming, and require costly and bulky instrumentation (*9*). Immunoassay-based detection techniques are being developed as an alternative to detect these toxic chemicals in environmental samples (*10*−*16*). These assays, apart from being highly specific, exhibit the desired sensitivity and accuracy for the detection of these low molecular weight contaminants present in our environment.

In recent years, the developments of immunoassay in "dipstick format" for the determination of pesticides are gaining importance because of their low cost and rapid screening capabilities (*17*−*19*). These assay formats are based on the principle of competitive inhibition immunoassay approach, where an anti-pesticide antibody is immobilized on the nitrocellulose membrane. The amount of free antigen (pesticides) present in the sample competes with the conjugate to bind with the available limited antibodies binding sites. The color developed due to the reaction of immuno-conjugate with some suitable substrate is correlated with the concentration of the sample. These dipstick immunoassay formats follow the principles of dot−blot immunoassay procedural schemes that require longer assay time, and therefore are not suitable for field applications. In this paper, we report for the first time a lateral-flow-based dipstick immunoassay using a novel hapten−protein−gold conjugate for the determination of atrazine in water samples. The developed dipstick system is quite simple, rapid, and sensitive enough for pesticide detection. It takes less than 5 min to complete the measurement of atrazine in a sample, and therefore is highly useful for field applications. The use of this newly developed hapten−protein−gold conjugate as a tracer molecule has improved the stability of the dipstick. The tracer trapped on a releasing matrix inside the dipstick module showed no loss of its activity even after 10 weeks of storage at 4 °C. Recently, testing by competitive immunochromatography is gaining importance, and being widely used for the rapid detection of various haptens, such as narcotics, clinical preparations, and pesticides, etc. (*20*, *21*). This technique is based on the distribution of molecules in a biphasic system. In this assay, a specific antibody to test compound is fixed on the surface of a nitrocellulose membrane in the test zone and remains stationary during the analysis. The mobile phase, which contains a test sample and a tracer, moves toward the test zone and competes with the available binding sites of antibodies coated on test zone. The binding of tracer to specific antibodies leads to the formation of a complex which can be evaluated visually and is inversely proportional to antigenic concentration. The kinetics of the process of an antigen binding to its antibody in an immunochromatographic method is higher than in the conventional dot assay (*22*).

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10.1021/es070194j CCC: $37.00

Published on Web 06/12/2007

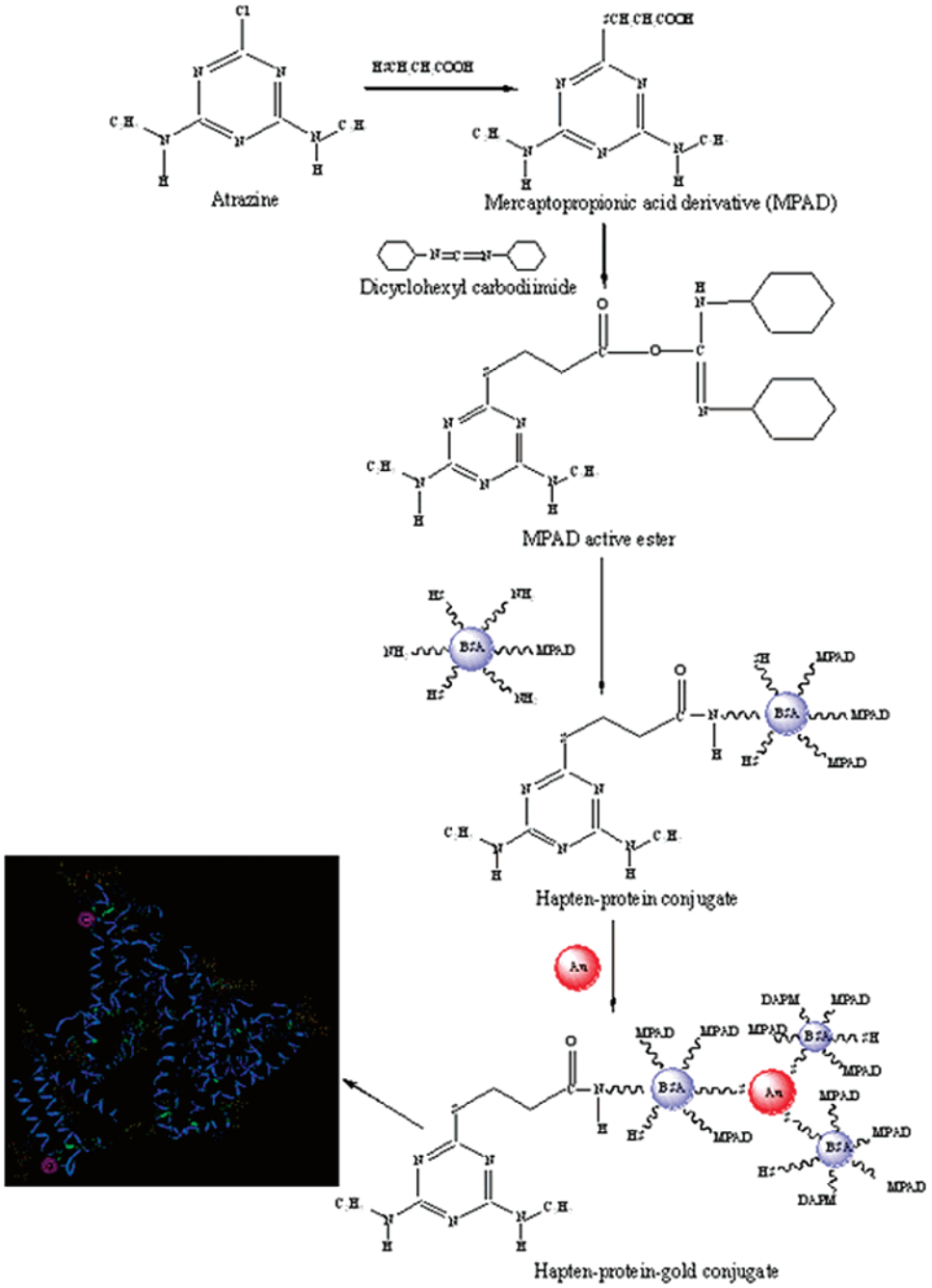

FIGURE 1. Reaction mechanism of hapten−protein−gold conjugate formation. Haptens are shown to link with the carrier protein via its surface lysine residues while colloidal gold particles are linked to the carrier protein through its cysteine residues. Inset shows the computer-generated model of the conjugate. In the model, disulfide bonds (yellow-green) of the protein (represented in sky blue color) are linked to gold nanoparticles (purple) while surface lysine groups (royal blue) of the protein are linked to carboxylated hapten (mustard).

2. Materials and Methods

2.1 Reagents. Hapten-specific antibodies (anti-atrazine antibodies) were generated in our lab by immunizing young New Zealand white rabbits with standard immunization and purification protocols as described earlier (*23*). Bovine serum albumin (BSA), dicyclohexyl carbodiimide (DCC), *N*-hydroxysuccinimide (NHS) ester, hydroxylamine-HCl 3-mercaptopropionic acid, 2-mercaptoethanol, tetrachloroauric acid, and polyethylene glycol (PEG) were purchased from Sigma Chemical Co., USA. Technical grade atrazine was purchased from Supelco, USA. Peroxidase (POD) and 3,3′,5,5′-tetramethylbenzidine (TMB) substrate were obtained from Bangalore-Genei, India. All chemicals, reagents, and solvents used in this study were of high purity analytical grade. Buffers were made in Milli-Q water.

2.2 Preparation of Nanogold Particles. Monodispersed (∼28 nm) colloidal gold was prepared by slight modification of Frens method (*24*). A 200 mL solution of 0.01% tetrachloroauric acid (gold chloride) in Milli-Q water was taken in a 500 mL Erlenmeyer flask. The solution was brought to boiling point on a hot plate. A 4 mL portion of 1% trisodium citrate solution was rapidly added to the boiling gold chloride solution. The solution was allowed to boil for 10 min until it developed a typical bright wine red color of colloidal gold. The average particle size of colloidal gold particles was determined using a Zetasizer particle analyzer (Malvern Instruments) and estimated to be approximately 28 nm.

2.3 Synthesis of Hapten−Protein−Gold Conjugate. For preparing a hapten−protein−gold conjugate, mercaptopropionic acid derivative of atrazine (MPAD) was first mixed

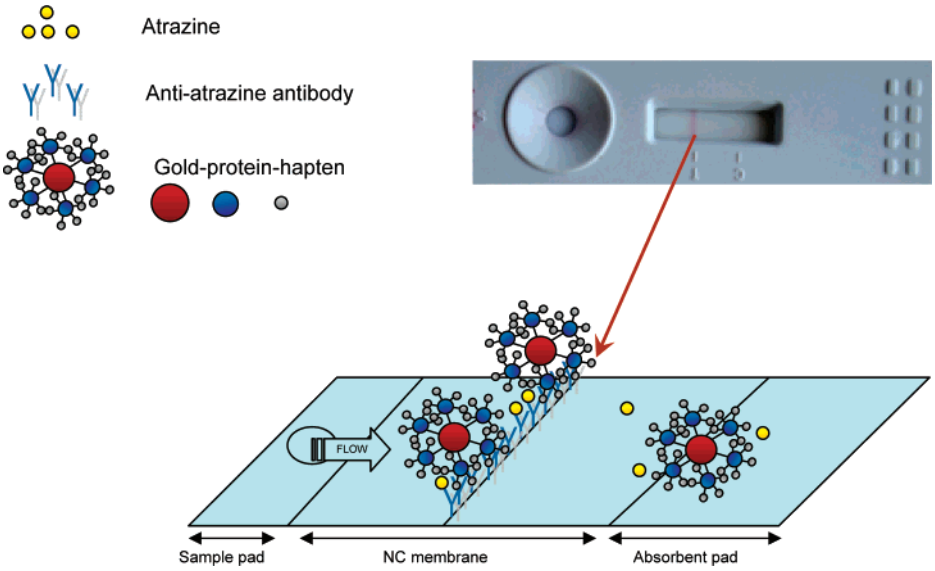

FIGURE 2. Schematic diagram of an immunochromatographic dipstick employed in the described assay showing cross-reaction and assay format.

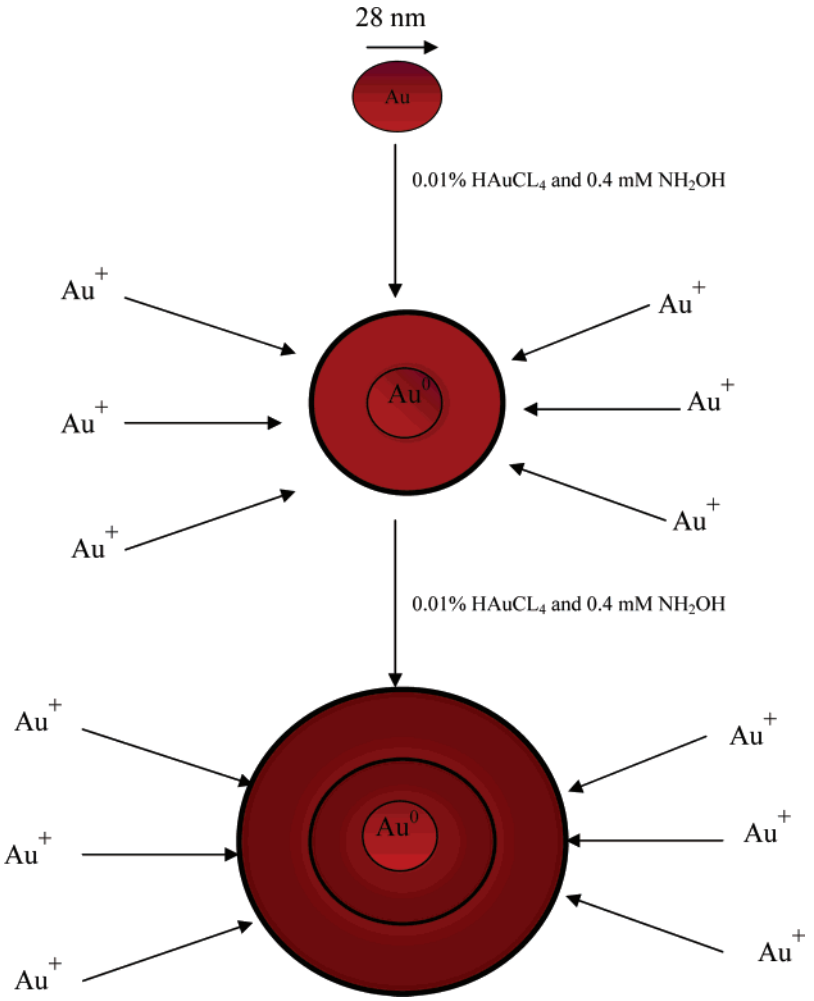

FIGURE 3. Schematic of enlargement of gold nanoparticles by using gold enhancer.

with a carrier protein (BSA) at 1:50 molar ratio of protein and hapten by using a procedure reported earlier (*23*). For this, 50 μmol of atrazine derivative was first mixed in 1 mL of dimethylformamide (DMF) along with 125 μmol of dicyclohexyl carbodiimide (DCC) and 125 μmol of *N*-hydroxysuccinimide (NHS) ester. The mixture was incubated for 4 h and then centrifuged to remove the urea precipitate. A 150 μL aliquot of this supernatant was mixed with the protein solution (10 mg/mL in borate buffer, pH 9.0) to make a final volume of 1 mL. The conjugate was then passed through a P10 gel filtration column. Fractions with maximum protein concentrations were pooled and checked for the final concentration of hapten−protein with a spectrophotometer (Shimadzu 1601A) at 280 nm. Gold particles were labeled

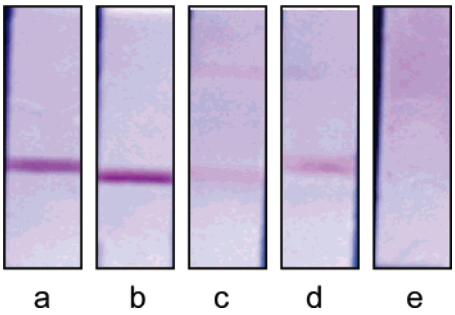

FIGURE 4. Effect of various buffers on antibody coating on dipstick membrane: (a) 50 mmol phosphate, pH 7.2; (b) 50 mmol phosphate + 3% methanol, pH 7.2; (c) phosphate buffer saline, pH 7.2; (d) phosphate buffer saline + 3% methanol, pH 7.2; (e) 50 mmol phosphate + 150 mmol NaCl, pH 7.2. All samples were detected by a 40 nmol gold−hapten conjugated BSA.

with protein−hapten conjugate by first cleaving the disulfide bonds of the protein using 2-mercaptoethanol to generate sulfhydryl groups on protein which were used to link with the colloidal gold particles for making a stable hapten−protein−gold conjugate. For this, a solution of protein−hapten conjugate (1 mg/mL) made in phosphate buffer (pH 7.0, 50 mM) was mixed with 2-mercaptoethanol (25 mM) and kept for incubation under mild shaking overnight at 4 °C. After incubation, the conjugate was dialyzed against water for 48 h and stored at 4 °C. 1 mg protein was added dropwise into 2 mL colloidal gold solution under mild stirring condition and the mixture was incubated overnight at 4 C. The conjugate was washed three times with 10 mM Tris containing 3% BSA under centrifugation at 12000 rpm for 30 min. The loose pellet was resuspended in the same buffer and its OD was brought to app. 5.0 at 520 nm. The conjugate was refrigerated at 4 C. The reaction mechanism of hapten−protein−gold conjugate formation is illustrated in Figure 1. A computer-generated model of the hapten−protein−gold conjugate is shown in the inset. The model, generated using "Amber-8" software, highlights the binding of carrier protein (BSA) to carboxylated hapten (MPAD) via its surface lysine residues. All the lysine residues of protein having an accessibility ≥100 were selected for binding. The hapten−protein conjugate is further shown to link with gold nanoparticles through the protein's cysteine residues.

2.4 Immunochromatographic Dipstick and Assay Procedure. A lateral-flow-based dipstick kit was developed using a nitrocellulose (NC) membrane on which an anti-pesticide antibody is immobilized at the test line of the detection zone. Test lines were made with 1 mg/mL anti-atrazine antibody solution dispensed with an Easy Printer (Advance Microdevices, Ambala, India) at 0.7 μL per line on NC membrane supplied with laminated card. After antibody deposition, membranes were dried at RT in a clean air chamber (Laminar hood) for 2 h. The sample pads having glass fiber lining were soaked in hapten−protein−gold conjugate solution with OD ≥ 3.0 and then dried in a laminar hood at RT for 4 h. After assembling sample and adsorbent pads on laminated cards having NC membrane, the cards were cut into strips. A layout of dipstick showing the dipstick schematic and assay format is shown in Figure 2. The strips were housed in a plastic housing and stored in sealed bags with desiccant before their usage. For the sample analysis, 50 μL of sample solution made in PBS containing 1% Tween-20 as detergent was added into the sample well of the dipstick. The sample along with the tracer trapped in sample pad moved through the nitrocellulose membrane where these two different molecules react competitively for the available binding sites of the anti-atrazine antibodies coated on the membrane. The intensity of color developed (reversibly) due to presence of tracer correlates with the amount of analyte present in the sample.

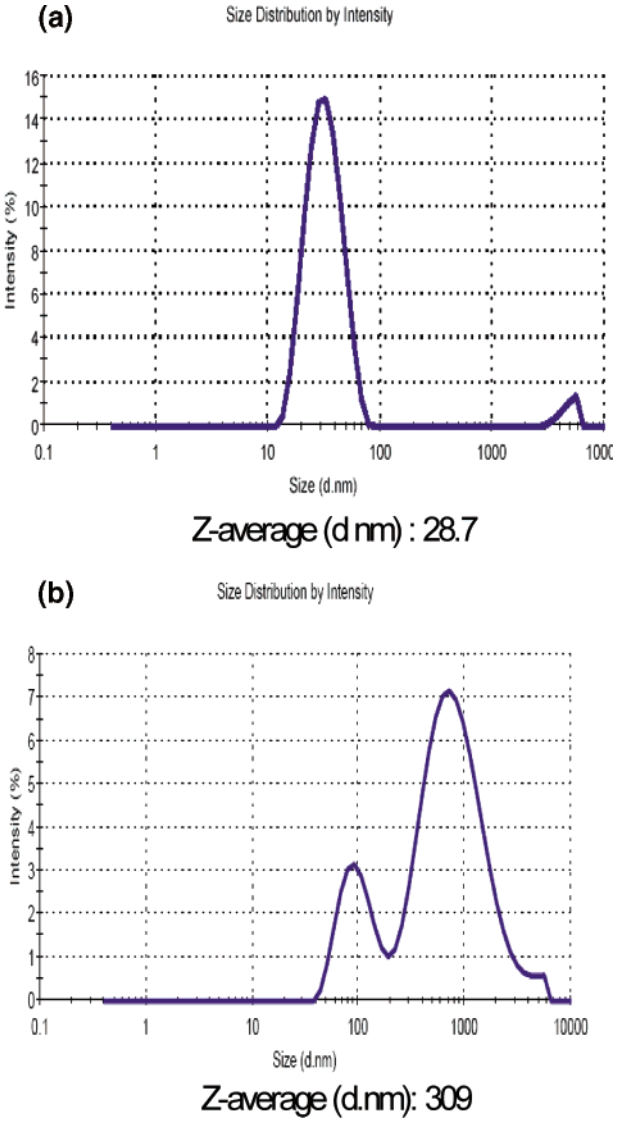

FIGURE 5. Hydrodynamic diameter of colloidal gold particles as measured by dynamic light scattering system (a) before enhancement and (b) after enhancement treatment with a solution of 0.01% gold chloride in 0.4 mM hydroxylamine for 2 min at RT.

The observed color due to binding of colloidal gold reagent at the site of test line is inversely proportional to sample concentration.

The intensity of signal generated at the test line of the dipstick due to competitive inhibition of atrazine samples with tracer was measured by a hand-held scanner (an indigenously developed reflectometer) at 657 nm in the reflection mode. The signal values, relative to the standard atrazine solutions, were fitted to a logistic function using a standard software package (Origin). The signal intensity *"I"* was normalized as $\%B/B_0$, according to the following normalization equation:

$$\%B/B_0 = I - I_{ex}/(I_0 - I_{ex}) \times 100$$

where I is the normal signal intensity, I_0 is signal intensity at zero concentration of the atrazine, and I_{ex} is value at the excess of the atrazine.

2.5 Sensitivity Enhancement of Dipstick with Gold Nanoparticles. The sensitivity of colloidal gold particles labeled with hapten−protein conjugates bound to antibodies on the nitrocellulose strips of dipstick was enhanced by using a mixture of gold chloride and hydroxylamine. The nitrocel-

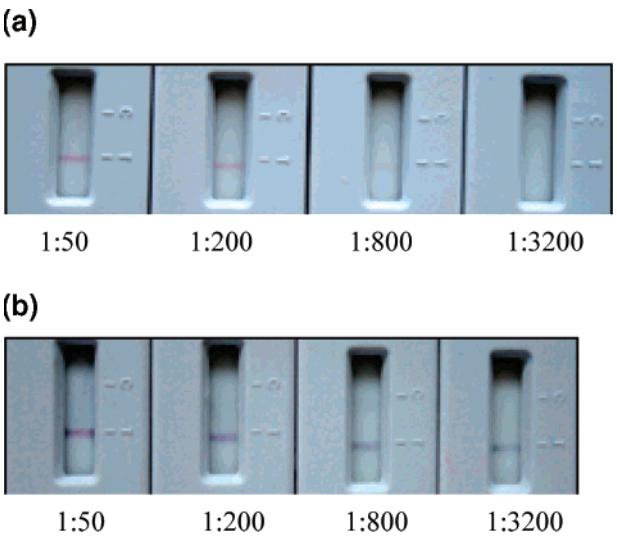

FIGURE 6. Effect of gold-enhancer on improvement of visibility of color of test line on NC membrane (a) before and (b) after enhancement. Tracer dilutions (1:50; 1:200; 1:800, and 1:3200) were prepared in PBS.

lulose strips were treated with the mixture of 0.01% gold chloride in double distilled water and 0.4 mM hydroxylamine hydrochloride ($NH_2OH-HCl$) for 2 min at RT and rinsed with Milli-Q water. A schematic diagram of gold enhancement process increasing the size of gold nanoparticles is shown in Figure 3. Dynamic light scattering measurements of the native and enhanced colloidal gold nanoparticles before and after treatment with enhancer (mixture of gold chloride and hydroxylamine) were performed in solution phase by using a Zetasizer particle analyzer (Malvern Instruments).

2.6 Competitive Inhibition Enzyme Linked Immunosorbent Assay (ELISA). ELISA plates were coated with different concentrations of anti-atrazine antibody made in carbonate buffer (0.05 M, pH 9.6) by incubating the plates overnight at 4 °C. After blocking with 10% skim milk, free atrazine at different concentrations mixed with HRP-labeled MPAD (*25*) were added into the plates, and incubated for 2 h at RT. The competition was also checked with MPAD, used as free antigen. After washing the plates thoroughly, the color was developed by the addition of TMB substrate (100 μL/well) and absorbance was recorded at 450 nm using ELISA reader (Microdevices, USA).

3. Results and Discussion

3.1 Characterization of the Hapten−Protein−Gold Conjugate. The present immunochromatographic detection of atrazine in water samples is based on the competitive inhibition, in which a newly developed hapten−protein−gold conjugate (tracer) competes with the free antigen present in the sample, for the limited antibody binding sites available at test zone on dipstick membrane. In most cases, the immunochromatography is carried out using a complex of protein with gold nanoparticles as a detecting agent. The advantages of gold nanoparticles as a marker in immunochromatographic detection are their ease of preparation of required size, monodispersity in aqueous phase, and also making conjugates with biomolecules to be used as detector reagents. However, a considerable drawback of complexes with colloidal gold particles with biomolecules is their insufficient stability (*21*).

For making a stable hapten−protein−gold conjugate, we first prepared gold nanoparticles using a standard method (*24*) with slight modification. Gold nanoparticles prepared by this method resulted monodispersed colloidal particles of 28 ± 5 nm size. This was confirmed by a Zetasizer particle analyzer (Malvern Instruments) as well as by absorption spectrum that showed maxima at 525 nm.

Gold−protein complexes, in general, are formed by electrostatic attraction between the negatively charged gold particle and the positively charged protein molecules (amine groups present at the surface of the proteins are primarily responsible for the positive charge on the protein molecule). These types of protein−gold complexes are usually not stable for a long time due to weak interaction forces applicable between them. Also, for making hapten−protein conjugates, the amine groups of the carrier protein (BSA) are used for binding with carboxylated haptens (MPAD). By utilizing some of the surface amine groups, the protein has become less positive, and hence unsuitable for binding with negatively charged colloidal gold particles. In order to overcome the loss of amine groups in the conjugate, we have attempted to use the cysteine residues of the protein to link with the gold nanoparticles via thiol−gold dative bonds which are very stable, in general. In the present study, we demonstrate for the first time the synthesis of a highly stable hapten−protein−gold conjugate used as the detection reagent for the immunochromatographic dipstick application. The conjugate was made by first conjugating hapten (MPAD, a derivative of atrazine) to a carrier protein (BSA) via its surface lysine residues and then linking colloidal gold nanoparticles to hapten−protein conjugate via cysteine residues of the carrier protein (Figure 1). The reduced protein forms a stable complex with gold particles through its thiol linkage.

3.2. Effect of Buffers on Antibody Coating on the Dipstick Membrane. The use of various agents to make a sharp and dense capture line of anti-atrazine antibodies on NC membrane was studied by dispensing 1 μL/line of antibody solution using a microdispenser (Easy Printer, Advance Microdevices, Ambala, India). The effect of various buffers ((a) 50 mmol phosphate, pH 7.2 (b) 50 mmol phosphate + 3% methanol, pH 7.2 (c) phosphate buffer saline, pH 7.2 (d) phosphate buffer saline + 3% methanol, pH 7.2, and (e) 50 mmol phosphate + 150 mmol NaCl, pH 7.2) on the coating of antibody on dipstick membrane is depicted in Figure 4. It was observed that the saline buffers with 150 mmol NaCl (lines c, d, and e) could not produce a sharp line of protein on NC membrane since the ionic strength of the buffer interferes with electrostatic interactions essential for protein binding. It is therefore important to determine the optimum buffer conditions that will result in a sufficient concentration of capture protein in the solution. It was observed that by adding a small percentage of methanol (3%) in the buffer gave considerable improvement in antibodies coating on NC membrane, giving a sharp line to improve the performance of the immunoassay (line b). The use of a coprecipitating agent, such as alcohol, helps to rewet the membrane and reduces any static charge on it. Partial destabilization of the protein with such a coprecipitating agent may also lead to the exposure of more-hydrophobic groups that are normally hidden within the protein structure. The increase in protein hydrophobicity resulting from the use of such coprecipitating agents improved the protein binding on NC membrane, which produced a sharp band of protein on NC membrane.

3.3 Effect of Blocking Agents to Reduce the Nonspecific Binding. Nitrocellulose membranes are naturally hydrophobic. Because of the hydrophobicity of the membrane, nonspecific interaction of a hydrophobic analyte with the membrane can generate significant nonspecific signal. This is more relevant with the detection of pesticide molecules, which usually are nonpolar in nature. Also, the size of pesticide molecules present in the sample is too low to flow across the membrane along with tracer in immunochromatography dipstick assay. The selection of a suitable blocking agent for the dipstick application for pesticide

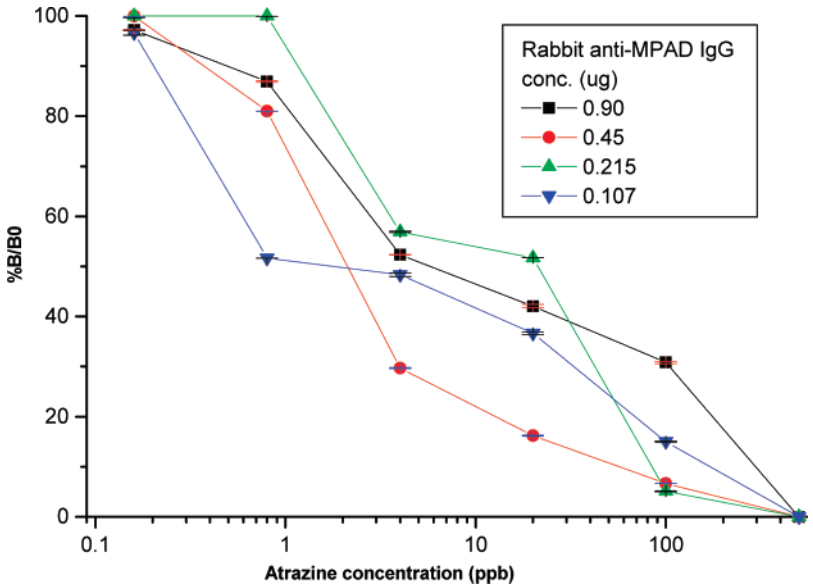

FIGURE 7. Competitive inhibition between hapten−protein−gold conjugate and free antigen with antibody-coated test line of dipstick. Antibody lining was made on the NC membrane by using different concentrations of antibody in PBS solution. SD values are indicated as error bar ($n = 3$).

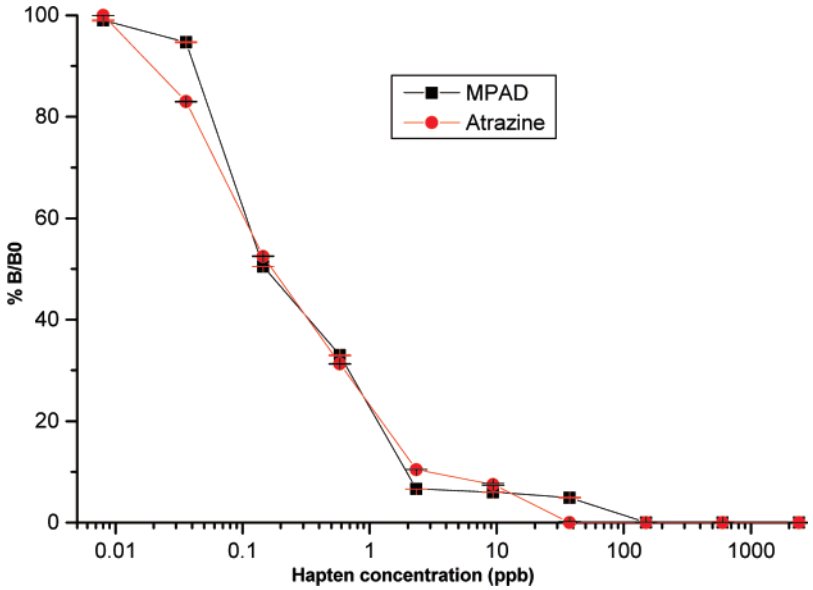

FIGURE 8. Reactivity of anti-MPAD antibody with atrazine and hapten (MPAD) in direct ELISA format. The competition was done between free antigens at different concentrations between 0.01 and 1200 μg/L and labeled MPAD−HRP conjugate at 1:8000 dilution against binding on 0.5 μg/mL coated anti-MPAD antibody. SD values are indicated as error bar ($n = 3$).

molecules is the critical part of its development. Ideally, the blocking materials should be free from interference with the interaction of antibodies and antigens at the capture line, where such interference can substantially reduce the signal intensity of the test. A range of blocking reagents such as polymers (PVP, PVA, and PEG), surfactant (Tween 20, Triton-X), and protein (BSA) were tested to demonstrate the minimum nonspecific binding in dipstick applications. A solution of 2% PVA mixed with 5% skim milk showed the best results in terms of nonspecific binding in dipstick applications (comparative data not shown). Protein molecules (2−5% BSA), in general, help to reduce the levels of nonspecific background signal in various immunoassay formats such as dot assay, ELISA, etc. However, they also reduce the lateral wicking rate of the test system in dipstick (*26*), and hence are not recommended as suitable blocking materials for dipstick applications.

3.4 Sensitivity Enhancement of Dipstick with Gold Nanoparticles. The quantification of immunoassays is generally achieved by measuring the specific activity of a label. The observation of larger Au nanoparticles is beneficial for the visualization or quantification of the target molecules on a nitrocellulose membrane used in dot−blot or dipstick immunoassays. However, to increase the labeling efficiency, smaller Au nanoparticles are usually used to prepare the conjugate. Silver enhancement, also called autometallography, is a commonly used enhancement approach for improving the sensitivity of any assay using colloidal gold nanoparticles (*27*). In this process, silver is deposited on the gold surface. Because the metal particle acts as a catalyst, the plating is highly specific. Various silver salts have been used to increase the size of gold particles for giving better visual effect or usefulness in electron microscopy. However, the distribution of particle sizes after the silver enhancement

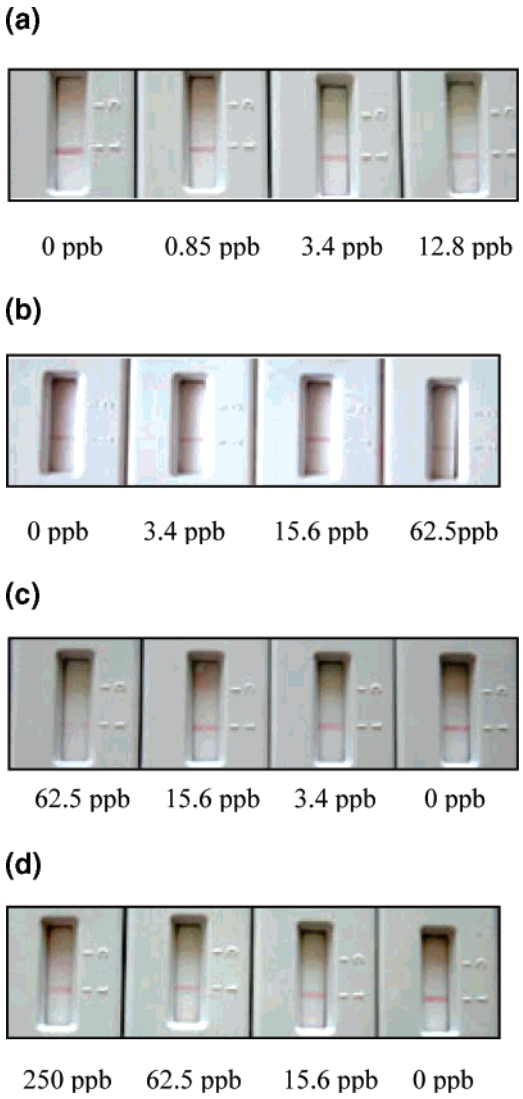

FIGURE 9. Cross-reactivity of immunochromatographic dipstick coated with anti-atrazine antibody with atrazine and its analogues: (a) atrazin, (b) simazin, (c) prometon, and (d) terbutryn. Stock solutions of samples were prepared in methanol (1 mg/mL) and further diluted in PBS buffer for assay.

is not uniform because post staining with silver can get oxidized which may reverse the silver staining.

In the present study, we have studied the signal enhancement of colloidal gold particles by using a solution of gold chloride enhancer. Figure 3 shows the schematic diagram of the gold nanoparticles' enlargement with gold chloride and hydroxylamine. The hydrodynamic diameter of gold nanoparticles increased to around 310 nm, as confirmed by a Zetasizer particle analyzer (Figure 5a and b). Hydroxylamine used in the enhancer solution is thermodynamically capable of reducing Au^{3+} ions to bulk metal (*28*), where all the added Au^{3+} ions go into the production of larger gold particles by making contact with gold on NC membrane, causing enlargement of the gold nanoparticles.

Treating colloidal gold-labeled hapten−protein conjugate linked to antibody coated NC membrane with 0.01% gold chloride and 0.4 mM hydroxylamine for 2 min at RT resulted in increased visibility of the test line color due to enlargement of the gold particles. The result suggests that by dipping nitrocellulose membrane in a solution of 0.01% gold chloride in 0.4 mM hydroxylamine for 2 min at RT effectively improved the visual effect of test line of the dipstick (Figure 6a and b). Without employing an enhancer tag, the test lines were not visible even at 1:800 dilution of tracer (Figure 6a). However, test line visibility was drastically improved by using gold enhancer as an amplification tag. The test line band became quite visible even at 1:3200 tracer dilution (Figure 6b).

Au nanoparticle, as an amplification tag, is therefore an important aspect to enhance the sensitivity of immunoassay in dipstick format where gold−protein complex is employed as a detector reagent.

3.5 Assay Development. The immunochromatographic detection of atrazine in water samples is based on the competitive inhibition approach where the pesticide in water samples and hapten−protein−gold conjugate (tracer) compete with the limited binding sites of available antibody on the test line of the dipstick. The hapten−protein conjugate is labeled with colloidal gold particles for one-step qualitative assay development. No additional steps are needed for the detection, as required in the case of enzymes where substrate is added for the color development (*18*). Figure 7 shows the competitive inhibition between hapten−protein−gold conjugate and free antigen present in standard samples with antibody coated on test line of dipstick. Different concentrations of antibody were selected to make the test line. Concentrations of antibody as low as 0.45 μg/line showed a good inhibition with standard atrazine samples, presenting IC_{50} values ~1.4 ppb. A hand-held scanner in the reflection mode measured the intensity of the signal generated at the test line. Data were obtained on duplicates of each standard solution in three different experiments ($n = 3$). The signal values, relative to the standard atrazine solutions, were fitted to a logistic function using a standard software package (Origin).

Standard ELISA was employed using the same anti-atrazine antibody (0.5 μg/well) coated on a microtiter plate (Nunc Maxisorp) to compare the dipstick results for atrazine detection. ELISA results showed a good inhibition with standard atrazine samples, presenting IC_{50} values around 0.15 ppb (Figure 8). Data show a good agreement with dipstick data. The differences in sensitivity limits between results from ELISA and dipstick could be justified because of several factors which may combine to determine the difference in performance between dipstick and ELISA results. The higher binding capacity of the antibodies on polystyrene microtiter plate in comparison to that on NC membrane, and also making use of a sophisticated ELISA reader for signal detection contribute to obtain the higher sensitivity of ELISA.

The test line coated with the above antibody concentration was used in a single-step lateral-flow colloidal gold-based assay system. The inhibition of binding is given by a lack of color formation at the test line where the capture antibody is coated; images of the sticks were taken using a digital camera (Nikon S3). The cross-reactivity of atrazine and some of its analogues (a, atrazine; b, Simazin; c, Prometon: and d, Terbutryn), as tested with anti-atrazine antibody coated immunochromatographic dipstick, is shown in Figure 9. Samples were added to the sample well and the response was recorded using a digital color camera after 3 min of sample addition. Anti-atrazine antibody showed very high reactivity with atrazine in comparison to other atrazine analogues. Similar results were obtained by ELISA assay for confirming the cross-reactivity of atrazine analogues.

Figure 10 shows the lateral-flow dipstick assay performed for detection of atrazine in aqueous samples using hapten−protein−gold conjugate. Anti-atrazine antibody was used as capture antibody immobilized on the nitrocellulose membrane (1 μL/line) at a concentration of 1 mg/mL (test line). Calibration curve was obtained using standard atrazine solutions in the concentration range of 0 to 500 ppb. The inhibition of tracer binding to the immobilized antibody is quite evident even at <1.0 ppb and no binding of conjugate is observed above 100 ppb. A relatively low coefficient of

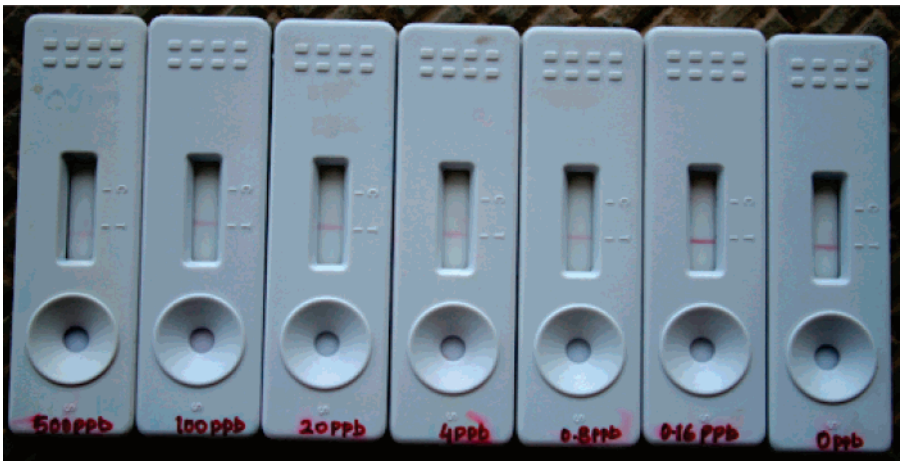

FIGURE 10. Sensitivity of immunochromatographic dipstick for atrazine samples. Samples spiked with different concentrations of atrazine were added to the sample well and the response was recorded using a digital color camera after 3 min of sample addition.

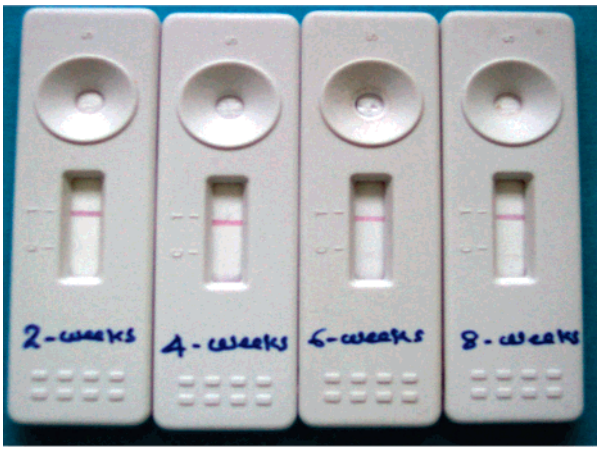

FIGURE 11. Stability test of developed gold−protein−hapten conjugate. The dipsticks were subjected to tracer solution (hapten−gold−protein conjugate) after 2, 4, 6, and 8 weeks of their storage in ambient conditions (room temperature in a desiccator).

variation (CV) among three inhibition assays with dipstick was observed with not much difference in color intensity, as observed by hand-held scanner. The kit could therefore serve as a rapid and reproducible screening test for visual screening of atrazine contamination of water samples, and, when coupled with a portable colorimeter, as an inexpensive semiquantitative assay.

The newly synthesized colloidal gold-labeled hapten−protein conjugate showed high stability and longer shelf life while trapped in the sample pad of the dipstick at ambient storage condition (Figure 11). The conjugate did not show any significant loss of its activity and color intensity even after 8 weeks of its use in the dipstick assay format. This was attributed to strong dative bonds between the gold and the protein molecules.

The proposed dipstick assay seems to be a fast and reliable field test for a preliminary qualitative/semiquantitative screening of atrazine samples. The real samples can be analyzed without any pre-enrichment or cleanup treatment. The developed dipstick shows good accuracy, acceptable precision, higher sensitivity, and longer shelf life.

Acknowledgments

We thank Mr. Nalini Kant of M/s Microdevice, Ambala (India) for the technical support. The Swiss Development Corporation (SDC) Switzerland and Department of Biotechnology (DBT), India are gratefully acknowledged for the financial support under the ISCB programme. R.B. is a pre-doctoral student supported by a fellowship from CSIR, India. Authors J.K. and K.V.S. had equal contributions to this work.

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Received for review January 25, 2007. Revised manuscript received April 24, 2007. Accepted May 1, 2007.

ES070194J