This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal’s standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.
Graphical Abstract

We report the use of SAADTC- Ag NPs as colorimetric sensor for the detection of tricyclazole fungicide. The characteristic surface plasmon resonance (SPR) peak of SAADTC- Ag NPs at 400 nm was drastically reduced by the addition of tricyclazole fungicide and yielded a new SPR peak at 550 nm, which can be observed by the discernible color change of the solution from yellow to pink.

Schematic representation for colorimetric detection of tricyclazole by using SAADTC-Ag NPs as colorimetric probe.
5-Sulfo anthranilic acid dithiocarbamate functionalized silver nanoparticles as colorimetric probe for simple and selective detection of tricyclazole fungicide in rice samples

Jigneshkumar V. Rohit and Suresh Kumar Kailasa*

Department of Applied Chemistry, S. V. National Institute of Technology, Surat-395 007, India
*Corresponding author, Phone: +91-261-2201730; Fax: +91-261-2227334
E-mail: sureshkumarchem@gmail.com; skk@ashd.avnit.ac.in

Abstract

A sensitive and selective colorimetric sensor for the detection of tricyclazole fungicide was proposed. The characteristic surface plasmon resonance (SPR) peak of 5-sulfo anthranilic acid dithiocarbamate functionalized silver nanoparticles (SAADTC- Ag NPs) at 400 nm was drastically reduced by the addition of tricyclazole fungicide, and yielding a new SPR peak at 550 nm, which can be observed by the discernible color change of the solution from yellow to pink. This detection mechanism was based on the tricyclazole-induced aggregation of SAADTC-Ag NPs via electron donor-acceptor interactions. The other pesticides such as hexaconazole, propiconazole, tebuconazole and defenoconazole, do not interfere with the sensing of tricyclazole. Under optimal conditions, the linear range of the method was 10 – 100 µM. The limit of detection was $1.8 \times 10^{-7}$ M. The feasibility of this method has been demonstrated by selective and sensitive measurement of tricyclazole in rice samples with good recoveries and relative standard deviation (RSD) $< 2 \%$. This distinct and rapid colorimetric response enables us to readily probe tricyclazole in food samples without more analytical technical demand.

Key words: Tricyclazole, SAADTC-Ag NPs, Colorimetric probe, UV-visible spectrometry.
Introduction

Rice is the most important food grain for a large part of the world’s human population, especially in East and South Asia, Latin America, Middle East and West Indies. Tricyclazole (5-methyl-1,2,4-triazolo[3,4-b]benzothiazole) is a systemic fungicide and used to control the rice blast disease.\(^1\) In recent years, it is widely spraying on the rice foliage to control rice blast.\(^2\) Moreover, it is also used in rice plantation to enhance the quality and quantity of rice production. In this connection, there is a possible ecological risk of tricyclazole in water–soil systems.\(^3\) Furthermore, it has considered as hazardous pesticide by WHO.\(^4\) Therefore, it is important to develop a simple, selective and sensitive analytical method for real-time monitoring of tricyclazole in rice samples.

Ever-increasing pesticides use in agricultural sectors leads to increase nonbiodegradable substances in environments, and these contaminants have shown adversely impacts on environmental, public health, and ecosystem. Thus, special attentions have been focused on the development of analytical approaches for determining pesticide residues in agricultural products with greater selectivity and sensitivity. As a result, several analytical techniques such as high performance liquid chromatography (HPLC),\(^5\)–\(^8\) gas chromatography (GC),\(^9\)–\(^11\) gas chromatography-mass spectrometry (GC-MS),\(^12\)–\(^13\) liquid chromatography-mass spectrometry (LC-MS)\(^14\)–\(^15\) and surface enhanced Raman spectroscopic (SERS)\(^16\) techniques have been used for the detection of tricyclazole in rice samples. Even through these methods provide high sensitivity, but they are expensive, and required tedious sample preparations and long analysis time. Importantly, these are not suitable for on-site and real-time monitoring of tricyclazole in agricultural products.
Recent years, the integration of metallic nanoparticles (Au and Ag NPs) with UV-visible spectrometric technique has been attracted much attention to readout trace level target analytes (inorganic, organic and biomolecules) with minimized sample volumes and preparations.\textsuperscript{17} Since, metallic NPs possess intrinsically strong surface-plasmon resonance absorptions and high extinction coefficients, which makes them to act as promising colorimetric probes for sensing of target analytes with the naked eye or with low-cost portable instruments. This sensing mechanism is based on the peak shifts in the SPR-based extinction spectra of metallic NPs, which is due to analyte-induced aggregation of NPs via various interactions (cooperative, covalent, $\pi-\pi$, and charge-transfer).\textsuperscript{18} As a result, NPs-based colorimetric assays have received extensive interest in nanoanalytical sciences because of their outstanding analytical performance such as selectivity, sensitivity, simplicity and on-site analysis with the use of minimal volume of samples.\textsuperscript{19} However, the surface functionality of metallic NPs is deciding factor to act them as probes in colorimetric sensing of target analytes from complex samples.\textsuperscript{20} In this connection, a wide variety of organic derivatives has been assembled on the surfaces of Ag and Au NPs and used as optical probes for inorganic,\textsuperscript{21-22} organic,\textsuperscript{23-26} and biomolecules\textsuperscript{27-28} assays in environmental and biological samples. Among the organic derivatives, dithiocarbamate derivatives have proved as effective supramolecular chemistry on the surfaces of metallic NPs through chemisorptions (small inter-atomic distance between two sulfur atoms) and allowed to act as the potential optical probes for trace analytes assays.\textsuperscript{29} Although metallic NPs-based colorimetric approaches have shown significant advances for sensing of various molecules, unfortunately very few reports have described the use of Au/Ag NPs as colorimetric probes for detection of pesticides/organic pollutants.\textsuperscript{30} For example, Chen \textit{et al.} illustrated the use of cysteamine stabilized Au NPs as colorimetric probe
for detection of glyphosate.\textsuperscript{31} Han and co-workers developed \textit{p}-sulfonatocalix[6]arene modified Au NPs-based colorimetric method for detection of aromatic amine isomers.\textsuperscript{32} Sun’s group designed a simple and rapid Au NPs-based colorimetric method for detection of organophosphates.\textsuperscript{33} Furthermore, various groups have been described the utility of citrate capped Au NPs used as colorimetric sensors for sensing of pesticides (cyromazine, ametryn, mathamidophos and acetamiprid)\textsuperscript{34-37} in environmental samples. Similarly, Xiong et al. developed a colorimetric method for detection of optunal by using calixarene modified Ag NPs as colorimetric probes.\textsuperscript{38} Menon’s team described the use of \textit{p}-sulphonato calix[4]resorcinarene functionalized Ag NPs as optical probe for colorimetric sensing of dimethoate in water samples.\textsuperscript{39} Till date no research has been reported on the use of SAADTC-Ag NPs as colorimetric probes for sensing of tricyclazole in rice samples.

Herein, we describe a simple colorimetric methodology for the analysis of tricyclazole by employing SAADTC-Ag NPs as an optical probe. Referring to the mechanism of Ag NPs induced aggregation with target analytes, we hypothesized that a click-colorimetric-based assay for tricyclazole in rice samples (Scheme 1).

\textbf{Materials and methods}

\textit{Chemicals and reagents}

Tricyclazole (98.5\%), tebuconazole (96\%), difenoconazole (96\%), propiconazole (92\%) were received from Atul Ltd, Gujarat, India. Hexaconazole (98\%) was received from Super Crop Safe Ltd, India. The 5-sulfo anthranilic acid was received from Himalaya Chemicals Ltd, India. Silver nitrate (AgNO$_3$) and sodium borohydride (NaBH$_4$) were purchased from Sigma-Aldrich, USA. Carbon disulfide (CS$_2$), sodium hydroxide and hydrochloric acid were purchased from Merck Ltd, India. Sodium chloride, potassium chloride, disodium phosphate and dipotassium
phosphate were obtained from Finar Chemicals Ltd, India. All chemicals were of analytical grade and used without further purification. Milli-Q-purified water was used in entire practical work.

**Instrumentation**

UV-visible spectra were measured with a Maya Pro 2000 spectrophotometer (Ocean Optics, USA) at room temperature. Proton nuclear magnetic resonance ($^1$H NMR) spectra were recorded by using Avance-II 400 MHz (Bruker, Switzerland). Fourier transform infrared spectra (FT-IR) were recorded with FT-IR 8400S (Shimadzu, Japan). Transmission electron microscopy (TEM) images were taken by using Tecnai 20 (Philips, Holland). Dynamic light scattering (DLS) were measured by using Zetasizer Nano ZS90 (Malvern, UK).

**Preparation of SAADTC-Ag NPs**

Briefly, 4.5 mg of NaBH$_4$ was added into 50 mL of 0.1 mM AgNO$_3$ solution under constant stirring at 400 rpm for 5 min. The color of the solution was turned into yellow color which confirms the formation of bare Ag NPs. To obtain SAADTC, an ethanolic solution of CS$_2$ (1 mL, 1 mM) was added drop wise into SAA (1 mL, 1 mM) solution and then sonicated for 5 min. The obtained SAADTC derivative was quickly added into bare Ag NPs solution and stirred for 2 h at room temperature. The molecular assembly of SAADTC on Ag NPs surfaces was confirmed by UV-visible, FT-IR and DLS, respectively. Schematic representation for the preparation of SAADTC-Ag NPs is shown in Supporting Information of Figure S1a.

**Extraction of tricyclazole from rice sample**

Tricyclazole was extracted according to the reported method with minor modification.$^{16}$ The pre-treated rice sample was weighted (25 g) and wrapped with filter paper. The wrapped sample was laid in a Soxhlet extractor, soaking it in 50 mL
of methanol for 12 h. Then, 100 mL of methanol was added into the round-bottom flask. The tricyclazole was extracted by heating mantle at 70°C for 6 h. The extracted solution was concentrated to 2 mL. The resulting solution was applied for colorimetric detection of tricyclazole by using SAADTC-Ag NPs as colorimetric probes.

**Determination of tricyclazole by colorimetric assay**

In a typical process, 750 µL of SAADTC-Ag NPs solution was added into a 4 mL sample vials that contained 50 µL of phosphate buffer solution (PBS, pH 8.0). To this, 100 µL of different concentrations of tricyclazole solutions were added into the vials separately and the resulting solutions were vortexed for 1 min. The color changes of the samples were recorded by using digital camera and UV-visible spectrometry.

**Results and discussion**

**Characterization of SAADTC-Ag NPs by spectroscopic techniques**

The prepared SAADTC-Ag NPs were characterized using UV-visible spectroscopy, FT-IR, ¹H NMR, DLS and TEM. As shown in Supporting Information of Figure S1b, a characteristic SPR band of bare Ag NPs is observed at 397 nm, and the color of the solution is yellow (Supporting Information of Figure S1b, inset). However, the SPR band of bare Ag NPs was very slightly shifted to 400 nm, which confirms the SAADTC assembly on Ag NPs surfaces. We also studied the stability of bare Ag NPs and SAADTC-Ag NPs at atmospheric conditions. As shown in Supporting Information of Figure S2a, the SPR peak intensity of bare Ag NPs is decreased with increasing time and the obtained spectra are broadened after 2 hours. However, the SPR peak intensity and shape of SAADTC-Ag NPs did not change even after 48 h, which confirms that the SAADTC-Ag NPs are more stable against oxygen oxidation than the bare Ag NPs (Supporting Information of Figure S2). To confirm
the SAADTC molecules attachment on Ag NPs surfaces, we studied FT-IR spectra of pure SAA, SAADTC and SAADTC-Ag NPs (Supporting Information of Figure S3). As shown in Supporting Information of Figure S3a, the pure SAA molecule exhibited peaks at 1344, 1708, 1222, 3068 and 3400 cm$^{-1}$ corresponding to the stretching and vibrations of S=O, C=O, C–N, O–H and N–H groups in SAA, respectively. However, new peaks are appeared at 1284 and 1029 cm$^{-1}$, which confirmed the –CS–NH and –C–S– stretching modes in SAADTC (Supporting Information of Figure S3b). The peak at 2640 shows the stretching modes of −SH group of SAADTC. These results indicate that SAA molecule was successfully reacted with CS$_2$ to form SAADTC. Importantly, it can be noticed that the chemical environment of SAADTC was completely changed and the peak of −SH group stretching was disappeared in the FT-IR spectrum of SAADTC-Ag NPs, which confirms the formation of covalent bond between SAADTC and the surfaces of Ag NPs via the carbodithioate (-CS$_2$) linkage$^{40}$ (Supporting information of Figure S3c). We also studied $^1$HNMR spectra of pure SAA and SAADTC (Supporting Information of Figure S4). Supporting information of Figure S4a shows the $^1$H NMR spectrum of SAA and the peaks at ~7.0 – 8.2 ppm indicates the aromatic protons (3H) in SAA. It can be noticed that the broad peak was generated at 9.4 ppm, which corresponds to merging of −OH, −NH$_2$, −SO$_3$H protons (4H) in SAA. Evidently, the new peak at 1.1 ppm represents the −SH in SAADTC, which is originated by reacting of CS$_2$ with −NH$_2$ group of SAA (Supporting Information of Figure S4b).

**Size and morphology confirmation by DLS and TEM**

The size and morphology of Ag NPs were very important to verify its aggregation with target analytes. Figure 1a-b shows the DLS data of bare Ag NPs and SAADTC-Ag NPs and possesses the hydrodynamic diameters 4 and 6 nm for bare Ag
NPs and SAADTC-Ag NPs, respectively. Figure 1d shows the TEM image of SAADTC-Ag NPs. It can be observed that the SAADTC-Ag NPs are well dispersed with diameter of 5 nm. However, the size and morphology of Ag NPs were slightly changed by SAADTC molecular assembly onto the surfaces of Ag NPs. The size of Ag NPs was slightly increased, which is due to the attachment of SAADTC molecules onto the surfaces of Ag NPs. Finally, it can be observed that Ag NPs are in spherical shape and well dispersed in water.

**SAADTC-Ag NPs-based colorimetric detection of tricyclazole**

To examine the colorimetric sensing ability of SAADTC-Ag NPs towards tricyclazole, we applied SAADTC-Ag NPs as optical probe for sensing of triazole-group pesticides (hexaconazole, propiconazole, tebuconazole, defenoconazole and tricyclazole). Tricyclazole is an unclassified pesticide, however it can be considered as triazole-group pesticides due to its structural similarities with triazole-group pesticides (Supporting Information of Figure S5). On the basis of this strategy, we have applied SAADTC-Ag NPs as colorimetric probes for colorimetric sensing of triazole-group pesticides (hexaconazole, propiconazole, tebuconazole, defenoconazole and tricyclazole, 100 µM) in the presence of PBS buffer at pH 8.0. Figure 2 displays the UV-visible spectra and photographic image of SAADTC-Ag NPs solutions upon the addition of triazole-group pesticides including tricyclazole (100 µM). Significantly, it can be observed that the color of SAADTC-Ag NPs was changed from yellow to pink (Figure 2, inset), only after the addition of tricyclazole. This result proved that tricyclazole can induce the aggregation of SAADTC-Ag NPs. As a result, the characteristic SPR peak of SAADTC-Ag NPs at 400 nm was shifted to longer wavelength 550 nm. To confirm this, we studied the DLS and TEM. Figures 1c and 1e show the DLS and TEM image of SAADTC-Ag NPs with tricyclazole. It can
be noticed that the hydrodynamic diameter of SAADTC-Ag NPs was increased from 5 nm to 40 nm, which is due their induced aggregation with tricyclazole. To evaluate the selectivity of SAADTC-Ag NPs system toward tricyclazole, we studied the UV-visible spectra of SAADTC-Ag NPs in the presence of various amino compounds (primary amines - aniline, p-nitroaniline, L-phenylalanine, glycine; secondary amines - N-methyl aniline, L-proline, uracil; tertiary amines - N,N-dimethyl aniline, 2-2'bipyridine, N-hydroxy succinimide, imipramine) and metal ions (Na⁺, K⁺, Cu²⁺, Zn²⁺, Cd²⁺, Fe²⁺, Mn²⁺, Mg²⁺, Co²⁺, Pb²⁺, Hg²⁺, Ni²⁺, Ca²⁺, Ba²⁺, Cr³⁺, Fe³⁺, and Al³⁺) (Supporting Information of Figure S6-S7). It can be observed that the color of SAADTC-Ag NPs solution was remained yellow in color, however, the color of SAADTC-Ag NPs is changed into pink in the presence of tricyclazole only, indicating that our colorimetric probe exhibited good selectivity towards tricyclazole.

Effect of pH

To investigate effect of buffer pH on the UV-visible absorption spectra of SAADTC-Ag NPs and tricyclazole-induced SAADTC-Ag NPs, we measured UV-visible absorption spectra of SAADTC-Ag NPs upon the addition of tricyclazole at PBS (10 mM) buffer pH ranging from 2.0 to 12.0 (Figure 3). It can be observed that at low pH (pH 2–3), the characteristic SPR peak of SAADTC-Ag NPs was drastically reduced without generation of new SPR peak at longer wavelength (550 nm). This is due to the surface charge neutralization of Ag NPs. At pH 4 – 7, the absorbance spectra are quite similar to the original spectra of SAADTC-Ag NPs, which confirms that there is no induced aggregation with tricyclazole. As shown in Figure 3, the characteristic SPR peak of SAADTC-Ag NPs at 400 nm was greatly reduced with the generation of new SPR peak at 550 nm, which confirms the tricyclazole-induced aggregation of SAADTC-Ag NPs. Therefore, PBS buffer (pH 8.0) was chosen as the optimum buffer condition for colorimetric sensing of tricyclazole.
Mechanism of SAADTC-Ag NPs-based colorimetric assay for tricyclazole

The SAADTC molecules on the surface of Ag NPs play a key role to act as the receptor for sensing of tricyclazole. As per the literature,\textsuperscript{26} we assume that there is a strong molecular interaction between the electron-deficient aromatic ring of SAADTC and the electron-rich triazole group of tricyclazole. Since, tricyclazole (nitrogen and sulphur groups) acted as electron donor to the lowest vacant molecular orbital of SAADTC.\textsuperscript{26} Therefore, we can draw the conclusion that tricyclazole-induced aggregation of SAADTC-Ag NPs is mainly due to electron donor–acceptor interaction between tricyclazole and SAADTC. As a result, the characteristic SPR peak (400 nm) intensity was drastically decreased after addition of tricyclazole and a new and strong absorbance peak was appeared at 550 nm, which was verified by the naked eye and UV-visible spectrometry (Figure 4a, inset).

Analytical data

Under the optimized conditions, various concentrations of tricyclazole are added into the SAADTC-Ag NPs solution at PBS buffer pH 8.0, and the measured UV-visible spectra and photographs are shown in Figure 4a. As shown in Figure 4a, the color of the solutions was gradually changed from yellow to pink upon increasing concentration of tricyclazole. Furthermore, the SPR peak of SAADTC-Ag NPs was gradually decreased at 400 nm and new peak was generated at 550 nm by increasing concentration of tricyclazole. These results revealed that the higher concentration of tricyclazole can be induced a higher degree of aggregation of SAADTC-Ag NPs, which yields an intense pink color. As shown in Figure 4b, the intensity of absorbance ratio (A\textsubscript{550}/A\textsubscript{400}) is gradually increasing with increasing the concentration of tricyclazole. The intensities of absorbance ratios at A\textsubscript{550}/A\textsubscript{400} were found to be linear with the logarithm concentration of tricyclazole ranging from 10 to 100 µM, with the
regression equation $y = 0.097x + 0.621$ ($R^2 = 0.997$). The limit of detection (LOD) was found to be $1.8 \times 10^{-7}$ M, which describes the use of SAADTC-Ag NPs for colorimetric analysis of tricyclazole in rice samples. Table 1 shows the comparison of present method with the reported chromatographic and mass spectrometric methods in the literature. The results obtained by the proposed method were in good agreement with those obtained by the other analytical methods (GC, HPLC, GC-MS, LC-MS and SERC) in the literature. Based on this result, it is obvious that the proposed method has great potentiality for the analysis of tricyclazole in rice samples.

**Interference study**

To evaluate the selectivity of the proposed method, the proposed method was challenged with other similar pesticides including hexaconazole, propiconazole, tebuconazole and defenoconazole. In practice, SAADTC-Ag NPs solution was added into tricyclazole ($100 \mu M$) that contained hexaconazole, propiconazole, tebuconazole and defenoconazole ($100 \mu M$ to $1.2 \text{ mM}$). As shown in Supporting Information of Figure S8, SAADTC-Ag NPs shows color as well as spectral changes with tricyclazole ($100 \mu M$) in presence of other interfering pesticides ($100 \mu M$ to $1.2 \text{ mM}$). This result indicates that the proposed method exhibited good selectivity towards tricyclazole over other similar pesticides ratio up to 1:6 ($100 \mu M$:$600 \mu M$).

**Determination of tricyclazole from rice samples**

In order to validate the practical application of the method, we applied this method for analysis of tricyclazole in rice samples. To this, tricyclazole was extracted from rice samples as per the described procedure in the experimental section. Figure 5 shows the UV-visible spectra of extracted tricyclazole from rice samples. Additionally, we also studied the recoveries of tricyclazole from rice by spiking different concentration of tricyclazole. This method showed good recoveries in the
range of 82 – 85 %, with RSD < 2% (Table 2). These results illustrated that the SAADTC-Ag NPs acted as an effective colorimetric probe for analysis of tricyclazole in rice samples.

Conclusions

In this work, we developed SAADTC-Ag NPs as colorimetric sensor for visual detection of tricyclazole in rice samples. The detection of tricyclazole was based on the aggregation of SAADTC-Ag NPs via electron donor-acceptor interactions. With the addition of tricyclazole into the SAADTC-Ag NPs, a visible color change was observed by the naked eye, which was originated by the aggregation of SAADTC-Ag NPs. The SAADTC-Ag NPs probe provides a reliable option to determine tricyclazole with good sensitivity and selectivity. Moreover, the SAADTC-Ag NPs colorimetric probe has been successfully applied to determine tricyclazole in rice samples, which validate the efficiency of visual on-site analysis of tricyclazole in complex food samples.

Acknowledgements

We gratefully acknowledge grant from the Department of Science and Technology, India (No.SR/FT/CS-54/2010). We thank Atul Ltd., and Super Crop Safe Ltd., for providing pesticides samples to this work. We also grateful to Himalaya Chemicals Ltd., for providing SAA. We thank Prof. Z.V.P. Murthy and Mr. Chetan Patel, Chemical Engineering Department, SVNIT for providing DLS measurements.
References


Figure captions

Scheme 1. Schematic representation for colorimetric detection of tricyclazole by using SAADTC-Ag NPs as colorimetric probe.

Figure 1. DLS of (a) bare Ag NPs, (b) SAADTC-Ag NPs and (c) SAADTC-Ag NPs with tricyclazole. TEM images of (d) SAADTC-Ag NPs and (e) SAADTC-Ag NPs with tricyclazole.

Figure 2. UV-visible absorbance spectra of SAADTC-Ag NPs with various pesticides (hexaconazole, propiconazole, tebuconazole, defenoconazole, and tricyclazole, 100 µM) at PBS buffer pH 8.0. Inset: photograph of SAADTC-Ag NPs with pesticides (i-SAADTC-Ag NPs, ii-hexaconazole, iii-propiconazole, iv-tebuconazole, v-defenoconazole, vi-tricyclazole).

Figure 3. UV-visible absorption spectra of SAADTC-Ag NPs with tricyclazole (100 µM) at PBS buffer pH ranging from 2.0 to 12.0.

Figure 4. (a) UV-visible absorption spectra of SAADTC-Ag NPs with the increasing concentration of tricyclazole from 1 to 100 µM by using PBS buffer pH 8.0. Inset: Photographs showing solutions (i) SAADTC-Ag NPs, SAADTC-Ag NPs with different concentration of tricyclazole (ii) 1 µM, (iii) 2.5 µM, (iv) 5 µM, (v) 7.5 µM, (vi) 10 µM, (vii) 25 µM, (viii) 50 µM, (ix) 75 µM and (x) 100 µM and (b) calibration graph between the absorbance ratio at A550/A400 and different concentration of tricyclazole from 1-10 µM.

Figure 5. UV-visible absorbance spectra of extracted tricyclazole from rice samples.
Table 1. Comparison of present method with the other chromatographic and mass spectrometric methods in the literature for the determination of tricyclazole.

<table>
<thead>
<tr>
<th>Analytical method</th>
<th>Analytes</th>
<th>LOD (M)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>Penoxsulam, propanil and tricyclazole, etc..</td>
<td>$3.2 \times 10^{-8}$</td>
<td>[6]</td>
</tr>
<tr>
<td></td>
<td>Tricyclazole</td>
<td>$4.2 \times 10^{-11}$</td>
<td>[7]</td>
</tr>
<tr>
<td></td>
<td>Tricyclazole</td>
<td>$2.6 \times 10^{-7}$</td>
<td>[8]</td>
</tr>
<tr>
<td>GC</td>
<td>Tricyclazole</td>
<td>$4.2 \times 10^{-9}$</td>
<td>[10]</td>
</tr>
<tr>
<td></td>
<td>Tricyclazole and alcohol metabolite</td>
<td>$1.1 \times 10^{-7}$*</td>
<td>[11]</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Dichlorvos, acephate and tricyclazole, etc..</td>
<td>$7.9 \times 10^{-8}$*</td>
<td>[13]</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Thiamethoxam, acetamiprid and tricyclazole, etc..</td>
<td>$6.8 \times 10^{-9}$*</td>
<td>[14]</td>
</tr>
<tr>
<td></td>
<td>Azoxyosrobin, carbendazim and tricyclazole, etc..</td>
<td>$2.6 \times 10^{-8}$*</td>
<td>[15]</td>
</tr>
<tr>
<td>SERS</td>
<td>Tricyclazole</td>
<td>$1.1 \times 10^{-8}$</td>
<td>[16]</td>
</tr>
<tr>
<td>SAADTC-Ag NPs based UV-visible spectrometry</td>
<td>Tricyclazole</td>
<td>$1.8 \times 10^{-7}$</td>
<td>present study</td>
</tr>
</tbody>
</table>

*The LOD only for tricyclazole.

Table 2. Analysis of tricyclazole in rice samples by using SAADTC-Ag NPs as colorimetric probe.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added (µM)</th>
<th>Found (µM)</th>
<th>Recovery (%)(^a)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>50</td>
<td>42.41</td>
<td>84.82</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>61.28</td>
<td>81.70</td>
<td>1.79</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>83.57</td>
<td>83.57</td>
<td>1.15</td>
</tr>
</tbody>
</table>

\(^a\)Mean±standard deviation (n=3)
Scheme 1.
Figure 1.
Figure 2.

![Absorption spectra of SAADTC-Ag NPs and their combinations with various fungicides.](image)

- **SAADTC-Ag NPs**
- **SAADTC-Ag NPs + hexaconazole**
- **SAADTC-Ag NPs + propiconazole**
- **SAADTC-Ag NPs + tebuconazole**
- **SAADTC-Ag NPs + defenoconazole**
- **SAADTC-Ag NPs + tricyclazole**
Figure 3.
Figure 4.

Graph (a) shows the absorbance spectra of various solutions of SAADTC-Ag NPs with different concentrations of tricyclazole. Each line represents a different concentration level:
- Black line: SAADTC-Ag NPs
- Red line: SAADTC-Ag NPs + 1 µM tricyclazole
- Green line: SAADTC-Ag NPs + 2.5 µM tricyclazole
- Blue line: SAADTC-Ag NPs + 5 µM tricyclazole
- Cyan line: SAADTC-Ag NPs + 7.5 µM tricyclazole
- Pink line: SAADTC-Ag NPs + 10 µM tricyclazole
- Yellow line: SAADTC-Ag NPs + 25 µM tricyclazole
- Brown line: SAADTC-Ag NPs + 50 µM tricyclazole
- Purple line: SAADTC-Ag NPs + 75 µM tricyclazole
- Maroon line: SAADTC-Ag NPs + 100 µM tricyclazole

Graph (b) shows a linear relationship between the absorbance ratio $A_{550}/A_{400}$ and the logarithm of the concentration $C$. The equation of the line is $y = 0.097x + 0.621$ with a coefficient of determination $R^2 = 0.998$. The data points on the graph are plotted according to $\log C$ and the absorbance ratio.
Figure 5.