crystals

MDPI

Article

Fe_2O_3-Ag_2O/TiO_2 Nanocatalyst-Assisted LC-MS/MS-Based Detoxification of Pesticide Residues in Daphnia magna and Algae Mediums

Faheem Ahmed [1,*], Tentu Nageswara Rao [2], Nishat Arshi [3], Y. Prashanthi [4], Shalendra Kumar [1,5] and Adil Alshoaibi [1]

[1] Department of Physics, College of Science, King Faisal University, P.O. Box-400, Al-Ahsa 31982, Saudi Arabia; sjagdish@kfu.edu.sa (S.K.); adshoaibi@kfu.edu.sa (A.A.)
[2] Department of Chemistry, Krishna University, Machilipatnam 521004, India; tnraochemistry@gmail.com
[3] Department of Basic Sciences, Preparatory Year Deanship, King Faisal University, P.O. Box-400, Al-Ahsa 31982, Saudi Arabia; nshastri@kfu.edu.sa
[4] Department of Chemistry, Mahatma Gandhi University, Nalgonda 508001, India; puttaprashanthi@gmail.com
[5] Department of Physics, School of Engineering, University of Petroleum & Energy Studies, Dehradun 248007, India
* Correspondence: fahmed@kfu.edu.sa

1. Experimental Details

1.1. Materials and Methods

1.1.1. Mobile Phase Preparation A

We added 1 ml of formic acid into a 1000 ml volumetric flask containing ¼ of the LC-MS-grade water. The remaining volume was made up to the mark using LC-MS-grade water and sonicated.

1.1.2. Mobile Phase Preparation B

We added 1 ml of formic acid was into a 1000 ml volumetric flask containing ¼ of LC-MS-grade acetonitrile. The remaining volume was made up to the mark using LC-MS-grade acetonitrile and sonicated.

1.1.3. Preparation of Nutrient Stock Solution

Nutrient stock solutions were prepared by dissolving a known quantity of each nutrient into 25 ml of deionized water. The nutrient stock solutions were transferred into amber-colored bottles and stored in a refrigerator for three months.

1.1.4. Method of Preparation of Alga Medium

One day before inoculation, 8.0 liters of OECD TG 201 medium was prepared by adding a known volume of each sterile nutrient stock solution into a sterile beaker. The OECD TG 201 medium was then brought to final volume with deionized water, and the pH was adjusted from 6.88 to 8.13 using 0.1 N NaOH. After measurement of pH, the OECD TG 201 medium was sterilized through 0.20 µm pore-size sterile membrane filters under aseptic conditions. After filtration, OECD TG 201 medium was stored in glass bottles and maintained under aseptic conditions. On inoculation day, the pH of OECD TG 201 medium was checked and adjusted from 7.42 to 8.12 using 0.1 N NaOH and used for the experiments. The details are presented in Table S1

1.1.5. Preparation of *Daphnia magna* (M4 Medium)

Separate stock solutions of individual trace elements were prepared in deionized water. From these different stock solutions (I), a second single stock solution (II) was

prepared (which contained all trace elements). The required volume of M4 medium was prepared by adding a volume of the stock solution II, macronutrient stock solutions, and combined vitamin stock solution to deionized water (Table S2).

1.1.6. Preparation of 500 μg/ml sample stock solution

The stock solution was prepared by dissolving 10.15 mg of pesticide formulation in six different 20 ml volumetric flasks. To the flasks, 5 ml of acetonitrile was added and sonicated the contents for 5 minutes; the flask was allowed to settle at room temperature for 1 hour then filled up to the mark using acetonitrile.

1.1.7. Preparation of 1.0 mg/L working photocatalytic solutions

The working stock solution of pesticide of 1.0 ml was placed into different 500 ml volumetric flasks, and we added 0.1 g of nanocatalyst and finally filled with alga and daphnia media up to the mark and exposed to sunlight.

Table S1. Composition of the alga (OECD TG 201) medium.

S. No.	Chemical Name	mg/L
1.	$NaHCO_3$ (Sodium Hydrogen Carbonate)	50.0
2.	NH_4Cl (Ammonium Chloride)	15.0
3.	$MgCl_2.6H_2O$ (Magnesium Chloride)	12.0
4.	$CaCl_2.2H_2O$ (Calcium Chloride)	18.0
5.	$MgSO_4.7H_2O$ (Magnesium Sulphate)	15.0
6.	KH_2PO_4 (Potassium Dihydrogen Phosphate)	1.60
7.	$FeCl_3.6H_2O$ (Ferric Chloride)	0.064
8.	$Na_2EDTA.2H_2O$ (E.D.T.A. Disodium Salt)	0.100
9.	H_3BO_3 (Boric Acid)	0.185
10.	$MnCl_2.4H_2O$ (Manganese (II) Chloride)	0.415
11.	$ZnCl_2$ (Zinc Chloride)	0.00300
12.	$CoCl_2.6H_2O$ (Cobaltous Chloride)	0.00150
13.	$Na_2MoO_4.2H_2O$ (Sodium Molybdate)	0.00700
14.	$CuCl_2.2H_2O$ (Copper (II) Chloride)	0.00001

Table S2. Daphnia magna (M4 medium) nutrients.

Stock Solution(s) I (Single Substance)	Amount Added to RO/Distilled Water (mg/L)	Concentration (Related to Medium M4)	To Prepare Combined Stock Solution II, Add Following Amount of Stock Solution I to RO/Distilled Water (mL/L)
H_3BO_3	57,190	20,000-fold	1.0
$MnCl_2.4H_2O$	7210	20,000-fold	1.0
LiCl	6120	20,000-fold	1.0
RbCl	1420	20,000-fold	1.0
$SrCl_2$ $6H_2O$	3040	20,000-fold	1.0
NaBr	320	20,000-fold	1.0
Na_2MoO_4 $2H_2O$	1230	20,000-fold	1.0
$CuCl_2.2H_2O$	335	20,000-fold	1.0
$ZnCl_2$	260	20,000-fold	1.0
$CoCl_2.6H_2O$	200	20,000-fold	1.0
KI	65	20,000-fold	1.0
Na_2SeO_3	43.8	20,000-fold	1.0
NH_4VO_3	11.5	20,000-fold	1.0

$Na_2EDTA\ 2H_2O$	5000	2000-fold	-
$FeSO_4 7H_2O$	1991	2000-fold	-
Both Na_2EDTA and $FeSO_4$ solutions were prepared singly, poured together, and autoclaved immediately. This produced:			
21 Fe-EDTA solution	-	1000-fold	20.0

2. Method Validation

2.1. Specificity Analysis

Aliquots of control (alga and daphnia mediums)), mobile phase A, mobile phase B, calibration standard from the linearity solution (25 ppb–CS6), and test item precision samples were injected for specificity analysis.

2.2. Preparation of Calibration solutions for Linearity

From the 50 ppb stock solution, 0.1 mL and 1.0 mL were placed into two different 50 mL volumetric flasks and filled up to the mark with diluent. These concentrations were 0.1 ppb and 1 ppb, respectively. From the 50 ppb stock solution, 0.8, 2, 4 ,and 10 mL were placed into four different 20 mL volumetric flasks and filled up to the mark with diluent. These concentrations were 2, 5, 10, and 25 ppb, respectively. All these calibration solutions were injected onto LCMS-MS and maintained in conditions, and the response obtained was plotted against the respective concentration to assess the linearity in the above concentration range. The correlation coefficient (r), slope (b), and intercept (c) were calculated.

2.3. Limit of Quantification

The lowest validated level with sufficient recovery is defined as the limit of quantification (LOQ). The LOQ was determined using 5 injections of 0.5 ppb fortification recovery samples. Low-level recovery solutions were used for LOQ.

2.4. Limit of Detection

The limit of detection (LOD) is defined as the lowest detectable concentration of an analytic in a sample. It is expressed as the lowest calibration standard in the linearity test. The limit of detection was established by dividing the concentration of the LOQ by 3.3 values. Spirodiclofen was detected, and the LOD was found to be 0.15 ppb

2.5. Assay Accuracy and Precision in Alga and *Daphnia magna*

Accuracy was performed at two fortification levels (0. 5 ppb and 5 ppb).

2.6. Low-Range preparation (LOQ level)

Six replications were prepared by using the high range of the stock to determine assay accuracy and precision in the low range. For each replication, an aliquot of 100 µL of reference item stock (50 ppb) was pipetted and transferred into a 50 ml volumetric flask. The content in all volumetric flasks was diluted up to the mark using alga and daphnia media. The solutions were marked and injected into LCMS-MS.

2.7. High-Range preparation (10 × LOQ):

Six replications were prepared by using the high range of the stock to determine assay accuracy and precision in the low range. For each replication, an aliquot of 500 µL of reference item stock (50 ppb) was pipetted and transferred into a 10 ml volumetric flask. The content in all volumetric flasks was diluted up to the mark using alga and daphnia media. The solutions were marked and injected into LCMS-MS. The calculation of recovery is given below:

$$\text{Residue content (mg/L)} = \frac{\text{P1} \times \text{V} \times \text{C}}{\text{P2}}$$

where
P1: active content's peak area in the sample;
V: sample's volume (mL);
C: standard solution concentration (mg/L);
P2: active content's peak area in the reference solution.

$$\text{Recovery \%} = \frac{\text{Recovered residue} \times 100}{\text{Fortified concentration}}$$

$$\%\text{RSD} = \frac{\text{SD} \times 100}{\text{Mean}}$$

where SD is standard deviation; RSD is relative standard deviation.

3. Results and Discussion

Table S3. Recovery and repeatability in alga medium.

Sample Code	Area	Average Std Area	Std Conc. (ppb)	Recovered Con. (ppb)	Nominal Conc. (ppb)	Recovery (%)
WSTD	225,477			-	-	-
A0R1	BDL			ND	NF	-
A0R2	BDL			ND	NF	-
A1R1	21,311			0.473	0.5	94.50
A1R2	21,614			0.479	0.5	95.84
A1R3	21,965			0.487	0.5	97.40
A1R4	21,415	225,515	5.00	0.475	0.5	94.96
A1R5	21,369			0.474	0.5	94.76
A2R1	223,141			4.947	5.0	98.95
A2R2	221,985			4.922	5.0	98.43
A2R3	222,158			4.926	5.0	98.51
A2R4	220,415			4.887	5.0	97.74
A2R5	221,174			4.904	5.0	98.08
WSTD	225,552			-	-	-
Statistical evaluation of recovery %						
Fortified Concentration (mg/L)		0.50	5.0			–
Average Recovery %		95.49	98.34			
Standard deviation		1.18	0.46			
% Relative standard deviation		1.24	0.47			

Table S4. Recovery and repeatability in Daphnia magna medium.

Sample Code	Area	Average Std Area	Std Conc. (ppb)	Recovered Con. (ppb)	Nominal Conc. (ppb)	Recovery (%)
WSTD	225,105			-	-	-
D0R1	BDL			ND	NF	-
D0R2	BDL			ND	NF	-
D1R1	21,047			0.469	0.5	93.72
D1R2	21,125	224,579	5.00	0.470	0.5	94.06
D1R3	21,323			0.475	0.5	94.94
D1R4	21,327			0.475	0.5	94.96
D1R5	21,236			0.473	0.5	94.56
D2R1	223,325			4.972	5.0	99.44

D2R2	221,741			4.937	5.0	98.74
D2R3	222,235			4.948	5.0	98.96
D2R4	221,257			4.926	5.0	98.52
D2R5	219,853			4.895	5.0	97.90
WSTD	224,053			-	-	-
Statistical evaluation of recovery %						
Fortified Concentration (mg/L)		0.50	5.0			–
Average Recovery %		94.45	98.71			
Standard deviation		0.55	0.57			
% Relative standard deviation		0.58	0.58			

ND—not detected; NF—not fortified.

3.1. Limit of Quantitation (LOQ)

The limit of quantitation (LOQ) was established to be 0.5 ppb from the lower level recovery test in alga medium and Daphnia magna medium.

3.2. Limit of Detection (LOD)

The limit of detection (LOD) was established to be 0.15 ppb, which was the lowest limit of the calibration curve.

3.3. Morphological Studies by SEM

Scanning electron microscopy was used to examine the morphology and structure of the as-prepared samples. The FE-SEM image revealed that the morphology of the Fe_2O_3-Ag_2O/TiO_2 nanocomposite was approximately spherical, with the Fe_2O_3 and Ag_2O deposited on the surface of TiO_2 nanoparticles that were found to be aggregated. It could also be seen that the size of Fe_2O_3 and nanoparticles is smaller than that of TiO_2, and the size of nanoparticles of Ag_2O is very small. This demonstrates that the powder particles are slightly agglomerated, as evidenced by the closed view of spherical nanoparticles (Figure 5).

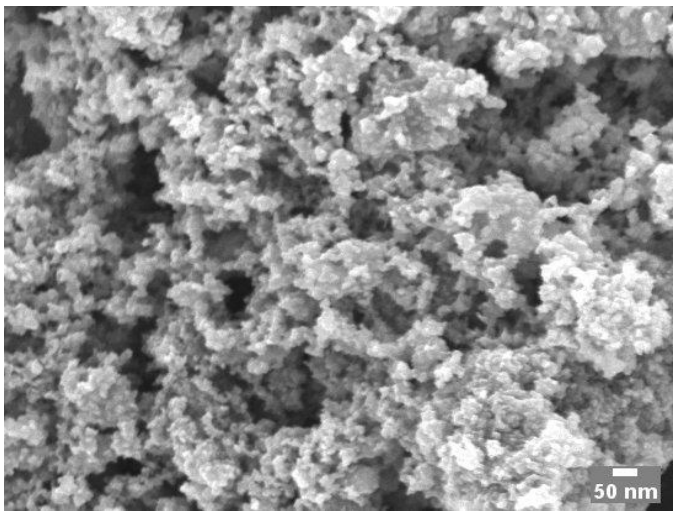

Figure S1. SEM images of Fe_2O_3-Ag_2O/TiO_2 nanocatalyst.

Table S5. Kinetic parameters for photocatalytic decontamination of Spiromesifen in Alga and Daphnia under direct sunlight using Fe2O3-Ag2O-TiO_2 nanocomposites.

Alga Medium		
Occasion (Hours)	**Log**	**Kinetic parameters**

Residue Level (ppb)				
0	0.992	−0.003		
3	0.574	−0.241	**Slope**	−0.037
8	0.412	−0.385	**Half-life (Days)**	**8.15**
16	0.268	−0.572	**Intercept**	−0.060
24	0.105	−0.979	**CC**	−0.985
48	BDL	BDL		
Daphnia Medium				
0	0.995	−0.002		
3	0.487	−0.312	**Slope**	−0.041
8	0.341	−0.467	**Half-life (Days)**	**7.41**
16	0.181	−0.742	**Intercept**	−0.102
24	0.087	−1.060	**CC**	−0.985
48	BDL	BDL		

CC, correlation coefficient.

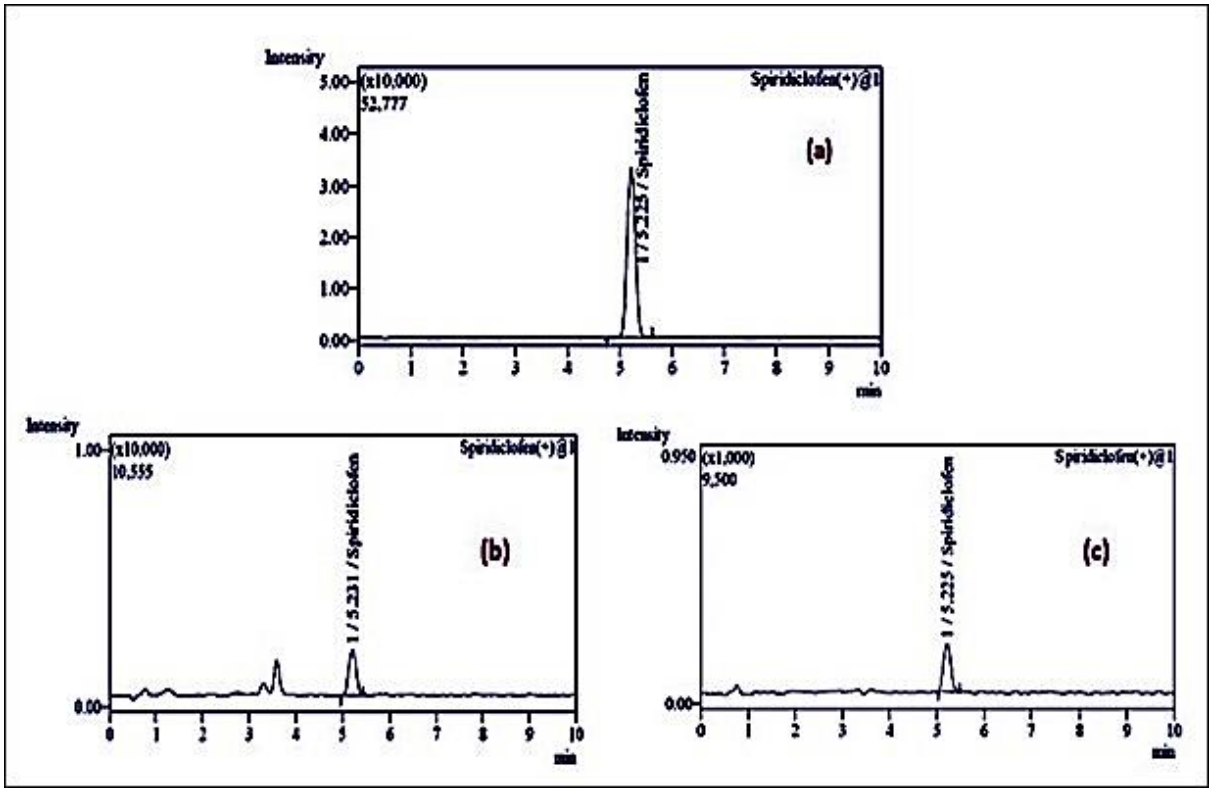

Figure S2. Representative photocatalytic 0th-hour Spiromesifen standard (**a**), alga (**b**) and daphnia (**c**) samples' LC-MS/MS MRM chromatograms.

Table S6. Kinetic parameters for photocatalytic decontamination of Spiromesifen in alga and daphnia under direct sunlight using TiO_2, Fe_2O_3-TiO_2, and Ag_2O-TiO_2 nanocomposites.

Alga Medium			
Occasions in Hours	**TiO_2 NPs Residue Level (ppb)**	**Fe-TiO_2 NPs Residue Level (ppb)**	**Ag-TiO_2 NPs Residue Level (ppb)**
0	0.841	0.723	0.761
4	0.420	0.692	0.492

12	0.362	0.480	0.454
24	0.241	0.281	0.373
72	0.151	0.101	0.131
96	0.062	0.060	0.031
120	0	0	0
Half-life (DT50) in Hours	32.25	26.73	23.77
Daphnia Medium			
Occasions in Hours	**TiO_2 NPs Residue Level (ppb)**	**Fe-TiO_2 NPs Residue Level (ppb)**	**Ag-TiO_2 NPs Residue Level (ppb)**
0	0.931	0.953	0.922
4	0.823	0.883	0.864
12	0.615	0.651	0.533
24	0.452	0.452	0.334
72	0.271	0.203	0.162
96	0.104	0.071	0.052
120	0	0	0
Half-life (DT50) in Hours	33.96	27.4	25.18