

2. Mohanta, D., Nath, S. S., Mishra, N. C. and Choudhury, A., Irradiation induced grain growth and surface emission enhancement of chemically tailored ZnS: Mn/PVOH nanoparticles by Cl^{+9} ion impact. *Bull. Mater. Sci.*, 2003, **26**(3), 289–294.
3. Ledentsov, N. N., Ustinov, V. M., Shuchukin, V. A., Koplev, P. S., Alferov, Zh. I. and Bimberg, D., Quantum dot heterostructures: fabrication, properties, lasers (review). *Semiconductors*, 1998, **32**, 343–365.
4. Tuan, A. C. *et al.*, Epitaxial growth and properties of cobalt-doped ZnO on $\alpha\text{-Al}_2\text{O}_3$ single-crystal substrates. *Phys. Rev. Lett. B*, 2004, **70**, 054424–054432.
5. Timp, G., *Nanotechnology*, Springer Verlag, New York, 2005, p. 276.
6. Bandyopadhyay, S. and Nalwa, H. S. (eds), *Quantum Dots and Nanowires*, ASP, New York, 2003.
7. Lockhande, B. J., Patil, P. S. and Uplane, M. D., Studies on cadmium oxide sprayed thin films deposited through non-aqueous medium. *Mater. Chem. Phys.*, 2004, **84**, 238.
8. Clifford, A., Gessner, H. and Hawley, G., *The Encyclopaedia of Chemistry*, Hawley, MN, USA, 1973, 3rd edn, p. 169.
9. Radi, P. A., Brito-Madurro, A. G., Madurro, J. M. and Dantas, N. O., Characterization and properties of CdO nanocrystals incorporated in polyacrylamide. *Braz. J. Phys.*, 2006, **36**, 412–413.
10. Peng, X. S., Wang, X. F., Wang, Y. W., Wang, C. Z., Meng, G. W. and Zhang, L. D., Novel method synthesis of CdO nanowires. *J. Phys. D: Appl. Phys.*, 2002, **35**, L101–L104.
11. Nath, S. S., Chakdar, D., Gope, G. and Avasthi, D. K., Novel effect of swift heavy ion on ZnO quantum dots prepared by quenching method. *Nano Trends*, 2007, **3**, 1–10.
12. Giulian, R., Kluth, P., Araujo, L. L. and Sprouster, D. J., Shape transformation of Pt nanoparticles induced by swift heavy ion irradiation. *Phys. Rev. B*, 2008, **78**, 125413.
13. Virk, H. S., Chandi, P. S. and Srivastava, A. K., Physical and chemical changes induced by 70 MeV carbon ions in PVDF polymer. *Nucl. Instrum. Methods Phys. Res. B*, 2001, **183**, 329–336.
14. Cimino, A. and Mareqio, M., Lattice parameter and defect structure of cadmium oxide containing foreign atoms. *J. Phys. Chem. Solids*, 1960, **17**, 57.
15. Cullity, D. B., *Elements of X-ray Diffraction*, Addison-Wesley, Massachusetts, USA, 1956.
16. Ziegler, J. F. and Biersack, J. P., The stopping and range of ions in matter (SRIM), 2008; <http://www.srim.org>
17. Ridgway, M. *et al.*, Changes in metal nanoparticle shape and size induced by swift heavy ion irradiation. *Nucl. Instrum. Methods Phys. Res. B*, 2009, **267**, 931–935.
18. Kluth, P. *et al.*, Energy dependent saturation width of swift heavy ion shaped embedded Au nanoparticles. *Appl. Phys. Lett.*, 2009, **94**, 113107.
19. Giulian, R. *et al.*, Shape transformation of Sn nanocrystals induced by swift heavy ion irradiation and the necessity of a molten ion track. *Phys. Rev. B*, 2010, **82**, 113410.
20. Giulian, R., Kluth, P., Sprouster, D. J., Araujo, L. L., Byrne, A. and Ridgway, M. C., Swift heavy ion irradiation of Pt nanocrystals embedded in SiO_2 . *Nucl. Instrum. Methods Phys. Res. B*, 2008, **266**, 3158–3161.
21. Toulemonde, M., Dufour, C. and Paumier, E., The ion–matter interaction with swift heavy ions in the light of inelastic thermal spike model. *Acta Phys. Pol. A*, 2006, **109**, 311.

ACKNOWLEDGEMENTS. I thank the Principal, DAV Institute of Engineering and Technology, Jalandhar and DAV College Managing Committee, New Delhi for providing research grants. I also thank Dr Navjeet Sharma and Dr Rajiv Sharma, DAV College, Jalandhar for help in irradiation experiments at IUAC, New Delhi.

Received 4 May 2010; revised accepted 22 March 2011

A simple method for estimation of sulphur in nanoformulations by UV spectrophotometry

Rajesh Kumar¹, Kishore Kumar Nair¹,
Md. Imteyaz Alam¹, Robin Gogoi²,
Pradeep Kumar Singh², Chitra Srivastava³,
Saurabh Yadav³, Madhuban Gopal^{1,*},
Samarat Roy Chaudhary⁴, Saheli Pradhan⁴ and
Arunava Goswami⁴

Divisions of ¹Agricultural Chemicals, ²Plant Pathology, and ³Entomology, Indian Agricultural Research Institute, New Delhi 110 012, India

⁴Agricultural and Ecological Research Unit, Biological Sciences Division, Indian Statistical Institute, 203 B. T. Road, Kolkata 700 108, India

We have recently reported the development of nano-sulphur with remarkable fungicidal properties, much better than the conventional sulphur. The nanosulphur could substitute the toxic conventional synthetic fungicides. The lack of estimation protocols for active ingredient in nanoformulations and exploitation of nanosulphur as a new technology for fungal management necessitates the need for estimation protocols to ensure the quality and quantity of sulphur in nanoformulations. Therefore, a method was developed for the estimation of sulphur in its nanoformulations using UV–visible spectrophotometry. Thirty-four samples of nanosulphur were analysed to validate the method. The percentage estimation of the active ingredient in all the samples was above 80.

Keywords: Nanoformulations, polyethylene glycol, synthetic fungicides, UV spectrophotometry.

THE application of synthetic pesticides has caused threat to non-target organisms and the environment due to their overuse¹. Since the release of xenobiotic results in the increase of environmental risk, the goal should be to use such compounds carefully so that they cause least negative impact on the environment into which they are released¹. To remove harmful effects on the non-target organisms, encapsulation of the active ingredient with other materials such as a polymer can allow sensitive ingredients to be physically enveloped into a protective matrix in order to protect core materials from adverse reactions due to factors like air or light².

Sulphur is a widely used element in different applications such as fertilizer, pharmaceutical, anti-microbial agent, insecticide, fungicide, high-density charger in lithium ion battery as well as for rubber and fibre industries^{3,4}. Various methods were reported⁵ for the preparation of nano elemental sulphur (nano-S⁰). Most of these

*For correspondence. (e-mail: madhubangopal@gmail.com)

involved either complex synthetic processes or the use of hazardous chemicals such as H_2S gas⁵.

We have recently reported an improved synthesis of sulphur nanoparticles⁶. These sulphur nanoparticles exhibited fungicidal properties which were significantly superior than the conventional sulphur⁶. Smaller size of nanosulphur and its coating will help resist unwanted environmental processes associated with conventional pesticides, i.e. leaching, evaporation, and photolytic, hydrolytic and microbial degradation.

Though several applications of sulphur have been reported, only a few methods are known to estimate the amount of sulphur^{7,8}. There is no protocol available for the estimation of active ingredient in nanoformulations. The developed nanosulphur could prove a good substitute to the toxic conventional synthetic fungicides and as a new technology for management of pathogens in agriculture. Therefore, a simple method based on UV spectrophotometry was developed for the estimation of sulphur in nanoformulations to ensure its quality and quantity.

Technical sulphur was purchased from Merck. Tetrachloromethane (CCl_4), cyclohexane (sd fine-chem Limited), sodium chloride, sodium bicarbonate and sodium sulphate (Qualigens fine chemicals) and hydrochloric acid (Merck Limited) were procured from the respective companies.

Nanosulphur formic acid formulation and nanosulphur sodium sulphide formulation were prepared at the Indian Statistical Institute, Kolkata⁶ and authentic commercial nanosulphur was purchased from M. K. Impex Canada, Canada.

The particles were characterized by using standard procedures such as dynamic light scattering (DLS) study, transmission electron microscopy (TEM), scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDAX) to ensure that nanosulphur was indeed taken for study.

The solubility of sulphur was tested in various organic and inorganic solvents. Sulphur was soluble in tetrachloromethane and cyclohexane, but in the latter, the range of detection could be improved from 3–45 to 4–65 ppm. Two stock solutions were prepared using these two solvents, as follows.

For the first stock solution, 15 mg sulphur and 25 ml CCl_4 were taken and sonicated till all the sulphur dissolved and the solution was made up to 50 ml. Further dilutions were made from the above stock solution and absorbance was measured at $\lambda_{\text{max}} = 290$ nm using a spectrophotometer.

For the second stock solution, 15 mg sulphur and 25 ml cyclohexane were sonicated till all the sulphur dissolved and the solution was made up to 50 ml with the same solvent. From the above stock solution, further dilutions were made. The absorbance was taken at 290 nm, as it was the maxima in the UV region.

The extraction procedure used was as follows.

(i) Nanosulphur formic acid formulation: Nanosulphur formulation (0.2 g) prepared using formic acid was dissolved in 15 ml CCl_4 in a 50 ml volumetric flask and the volume was made up to 50 ml. A 2 ml solution of this was extracted in a separatory funnel with 6% aqueous sodium bicarbonate solution (30 ml \times 3). The organic layer was passed through a bed of anhydrous sodium sulphate, which was then washed with 10 ml of the same solvent. The CCl_4 extract was made up to 50 ml. A 1 ml solution of this was diluted to 10 ml with CCl_4 . This solution along with a standard solution of nanosulphur was subjected to UV spectroscopy ($\lambda = 290$ nm).

(ii) Nanosulphur sodium sulphide formulation A: Nanosulphur formulation prepared using sodium sulphide (0.5 g) was mixed with 0.1 M HCl solution. Sodium chloride was added to the resultant solution and it was then extracted with 30 ml CCl_4 four times (30 ml \times 4). The extract was filtered through anhydrous sodium sulphate and made up with 25 ml CCl_4 . This 25 ml solution was subjected to UV spectroscopy at 290 nm.

(iii) Nanosulphur sodium sulphide formulation B: The nanosulphur formulation (0.1 g) prepared using sodium sulphide was mixed with 6% aqueous sodium bicarbonate solution. Sodium chloride was added and it was further extracted with 30 ml cyclohexane four times (30 ml \times 4). The cyclohexane extract was filtered through anhydrous sodium sulphate and finally made up to 50 ml with cyclohexane. The resultant (1 ml) solution was also subjected to UV spectrometry at 290 nm.

In order to ascertain the accuracy of the method, 34 samples of various concentrations were prepared using the solid nanosulphur procured from Canada. The material was processed as mentioned above. After validation, the method was used to estimate sulphur in the nanoformulations of unknown sulphur content.

DLS was used to measure the particle diameter of the sphere (Figure 1). TEM was used to analyse the nanostructured materials with atomic scale resolution. TEM image revealed average particle size around 50 nm (Figure 2). Surface topology of sulphur nanoparticles was determined using SEM at 50,000 magnification under 15.00 kV (Figure 3 a). It showed that majority of the particles were in the 50–70 nm range (Figure 3 b). Purity and chemical composition of the fabricated nanomaterials were confirmed using EDAX (Figure 4). It is evident from the peaks at around 2 keV that the product corresponds to the element sulphur. This value for sulphur was confirmed by SEM studies conducted using standard nanosulphur (purchased from M.K. Impex Canada). The sample was characterized by X-ray diffraction (XRD) for structural studies. XRD spectrum of the synthesized sulphur nanoparticles (Figure 5) showed a number of peaks at 13.57° , 22.98° and 29.3° with planes (1, 1, 0), (0, 0, 2) and (2, 2, 2) respectively [PCPDFWIN card no. 861278]. This indicated the polycrystalline nature of sulphur nanoparticles.

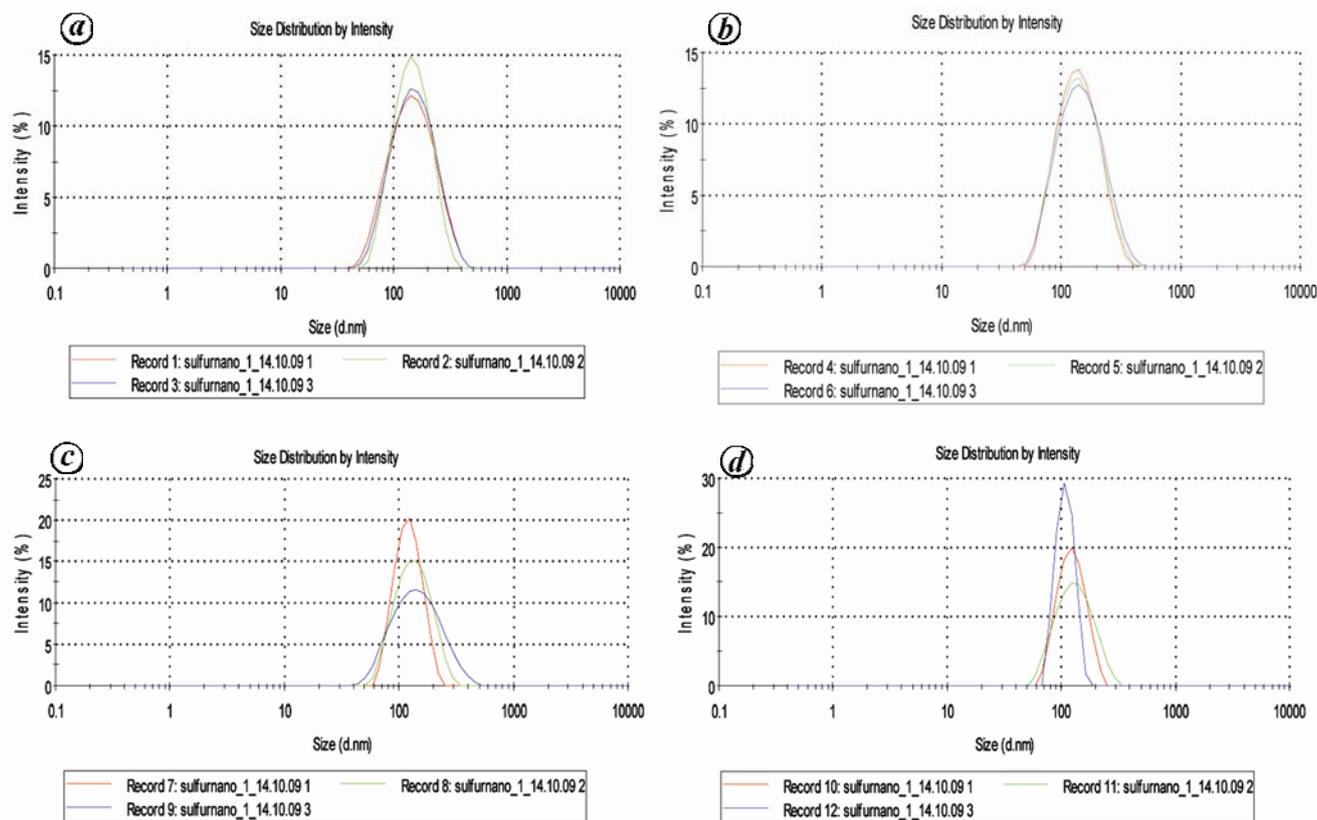


Figure 1. Dynamic light scattering measurements at (a) 25°C, (b) 35°C, (c) 45°C and (d) 55°C.

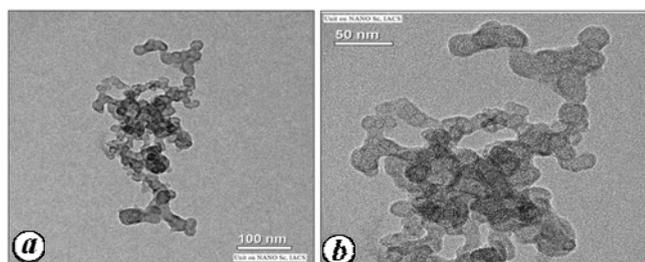


Figure 2. *a*, Transmission electron microscopic image of sulphur nanoparticles. *b*, High magnification of (*a*).

Unlike pesticides, which are mostly stable organic molecules, sulphur is an inorganic material and its quantification is not facile as those reported for organic compounds. Few methods have been reported using GLC, HPLC, etc. due to its limited solubility in common solvents, non-volatile character and lack of any chromophoric group present in it^{7,8}. The problem is compounded as methods for estimating nanoformulations are not available. While ordinary sulphur could be estimated, nano-sulphur is made by adding chemicals to keep these particles apart, to avoid their agglomeration. For example, pegylated sulphur may have an envelope of polyethylene glycol (PEG) and there is a need to know the amount of active ingredient before bioefficacy can be studied.

Since nanoparticles tend to coalesce or agglomerate and return back to more stable micro–macro forms, additional reagents are essential to stabilize them. Nanoparticles need special processing before they can be analysed by a routine UV method. In this method, solutions of sulphur were made at different concentrations in the two solvents, viz. tetrachloromethane and cyclohexane. Satisfactory linearity was found by plotting a standard curve of concentration versus absorbance for the two solvents (Figure 6) separately. The range of estimation with tetrachloromethane was 3–50 ppm. It can be seen from Figure 6*a* that below 3 and above 57 ppm, the linearity gets disturbed. The portion of the graph below 3 ppm on magnification clearly indicated the deformity from 0.1438 to 2.5884 ppm. The equation between observed absorbance recorded at different concentrations was $y = 0.0143x$, with a $R^2 = 0.9786$. The usage of cyclohexane as a solvent improved the range of estimation from 4 to 65 ppm, which was thus found to be better than that of tetrachloromethane (Figure 6*b*). Being a nonchlorinated solvent, cyclohexane was chosen for further analytical studies.

The method was validated by analysis of 34 different samples of known concentration which were prepared using the nanosulphur procured from M. K. Impex Canada. Data for percentage estimation for all the

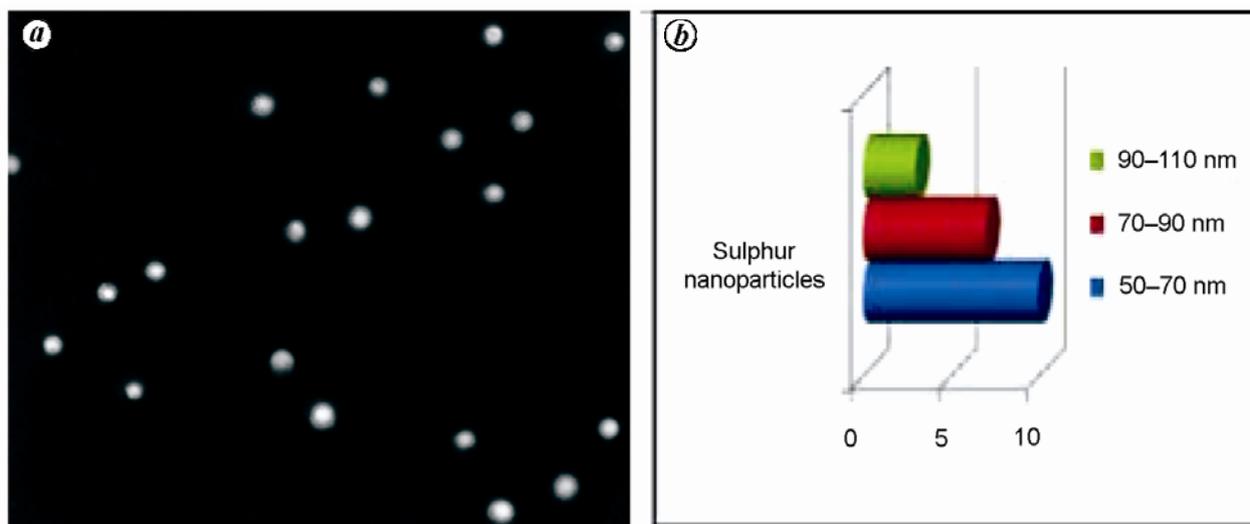


Figure 3. *a*, Scanning electron microscopic (SEM) image of sulphur nanoparticles. *b*, SEM particle distribution of sulphur nanoparticles.

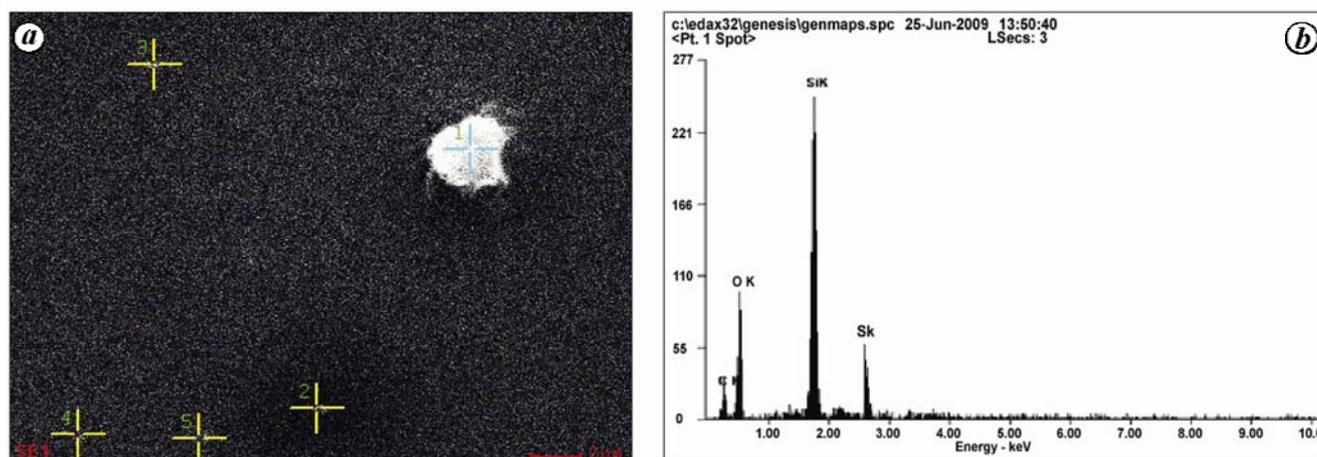


Figure 4. *a*, Particle selection for energy-dispersive X-ray spectroscopy (EDAX). *b*, EDAX spectra for sulphur.

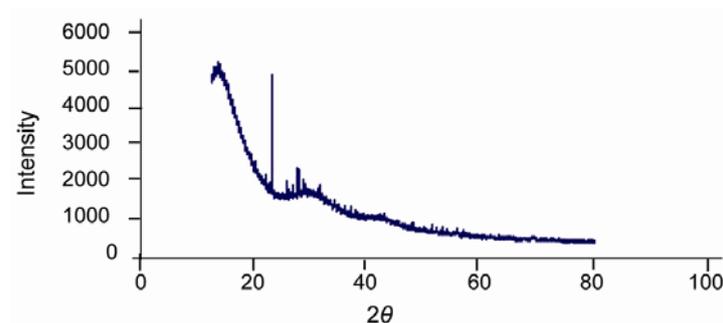


Figure 5. X-ray diffraction spectrum of nanosulphur.

samples are given in Figure 7. The percentage estimation for samples having concentration in the 10–35 ppm range was above 95, whereas the same for samples at the lower and higher extremes of the estimation range of the method was 80–90 (Figure 7).

Based on appropriate formulation, the extraction procedure needs subtle modifications, e.g. sodium hydrogen carbonate was added to formic acid formulation. Nanosulphur prepared in our laboratory using PEG and in two other formulations of nanosulphur was estimated and

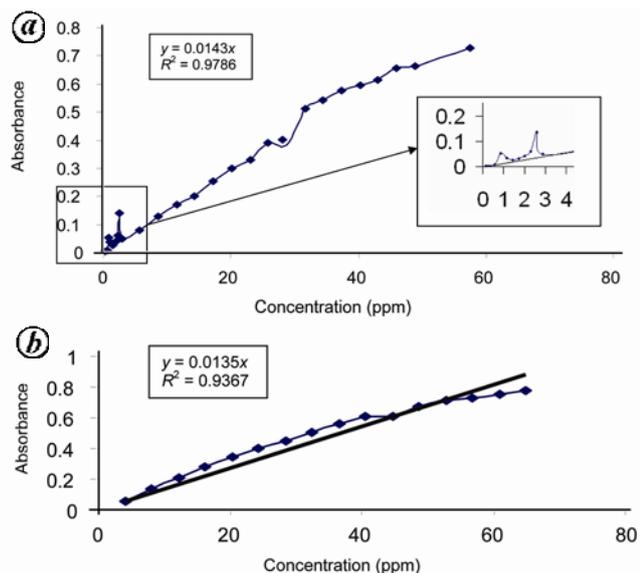


Figure 6. Standard curve of sulphur in tetrachloromethane (a) and cyclohexane (b).

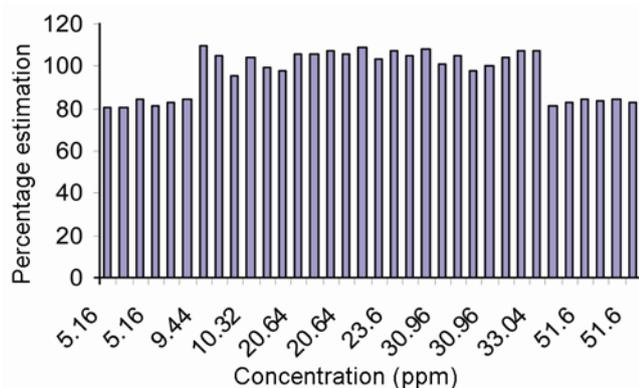


Figure 7. Percentage estimation of sulphur nanoparticles at various concentrations.

calculated on the basis of the above-mentioned standard equation. As expected, the additional chemical had masking effect in estimating sulphur in formic acid nanoformulation. It was therefore neutralized using sodium bicarbonate. The method was then employed for estimation of sulphur in nanoformulations. The concentration of nanosulphur in formic acid formulation, sodium sulphide and nanosulphur in pegylated formulation was estimated to be 1176, 125.1 and 5194.3 ppm respectively. The method was found useful for estimating varied amounts of sulphur in three formulations before utilizing them for studying their bioefficacy against fungal pathogens⁶.

1. Gupta, P. K., Pesticide exposure – Indian scene. *Toxicology*, 2004, **198**, 83–90; Abhilasha, P. C. and Singh, N., Pesticide use and application: An Indian scenario. *J. Hazard. Mater.*, 2009, **165**, 1–12; Ahtiainen, J. H., Vanhala, P. and Myllymäki, A., Effects of different plant protection programs on soil microbes. *Ecotoxicol. Environ.*

Saf., 2003, **54**, 56–64; Gliotti, C. and Allievi, L., Differential effects of the herbicides bensulfuron and cinosulfuron on soil microorganisms. *J. Environ. Sci. Health, Part B*, 2001, **36**, 775–782.

2. Jacob, S. R., Arunkumar, M. B., Gopal, M., Srivastava, C. and Sinha, S. N., An analysis of the persistence and potency of film-coated seed protectant as influenced by various storage parameters. *Pest Manage. Sci.*, 2009, **65**, 817–822.

3. Scherer, H. W., Sulphur in crop production. *Eur. J. Agron.*, 2001, **14**, 81–111; Lee, J. *et al.*, Anti-cancer activity of highly purified sulfur in immortalized and malignant human oral keratinocytes. *Toxicol. In vitro*, 2008, **22**, 87–95; Williams, J. S. and Cooper, R. M., The oldest fungicide and newest phytoalexin: A reappraisal of the fungitoxicity of elemental sulphur. *Plant Pathol.*, 2004, **53**, 263–279; Bostanian, N. J., Thistlewood, H. M. A., Hardman, J. M. and Racette, G., Toxicity of six novel fungicides and sulphur to *Galen-dromus occidentalis* (Acari: Phytoseiidae). *Exp. Appl. Acarol.*, 2009, **47**, 63–69.

4. Yong, Z., Wei, Z., Ping, Z., Lizhen, W., Tongchi, X., Xinguo, H. and Zhenxing, Y., Novel nanosized adsorbing composite cathode materials for the next generational lithium battery. *J. Wuhan Univ. Technol. – Mater. Sci. Edn.*, 2007, **22**, 234–239; Johnson, N. C., Manchester, S., Sarin, L., Gao, Y., Kulaots, I. and Hurt, R., Mercury vapor release from broken compact fluorescent lamps and *in situ* capture by new nanomaterial sorbents. *Environ. Sci. Technol.*, 2008, **42**, 5772–5778; Fuhrmann, M., Melamed, D., Kalb, P. D., Adams, J. W. and Milian, L. W., Sulfur polymer solidification/stabilization of elemental mercury waste. *Waste Manage.*, 2002, **22**, 327–333; Oji, L. N., Mercury disposal via sulfur reactions. *J. Environ. Eng.*, 1998, **124**, 945–952.

5. Xie, X., Zheng, W., Bai, Y. and Liu, J., Cystine modified nanosulfur and its spectral properties. *Mater. Lett.*, 2009, **63**, 1374–1376; Guo, Y., Zhao, J., Yang, S., Yu, K., Wang, Z. and Zhang, H., Preparation and characterization of monoclinic sulfur nanoparticles by water-in-oil microemulsions technique. *Powder Technol.*, 2006, **162**, 83–86; Aniruddha, S. D., Khomane, R. B., Vaidya, B. K., Joshi, R. M., Harle, A. S. and Kulkarni, B. D., Sulfur nanoparticles synthesis and characterization from H₂S gas, using novel biodegradable iron chelates in w/o microemulsion. *Nanoscale Res. Lett.*, 2008, **3**, 221–229; Chaudhuri, R. G. and Paria, S., Synthesis of sulfur nanoparticles in aqueous surfactant solutions. *J. Colloid Interface Sci.*, 2010, **343**, 439–446; Messalimov, I. A., Abdurakipova, L. F., Khusainov, A. N. and Mustafin, A. G., Preparation of nanosized sulfur particles from aqueous solutions of calcium and sodium polysulfides. *Russ. J. Appl. Chem.*, 2009, **82**, 2087–2092.

6. Choudhury, S. R. *et al.*, Nanosulfur: potent fungicide against food pathogen, *Aspergillus niger*. *AIP Proc.*, 2010, **1276**, 154–157; Gopal, M. *et al.*, Samfungin: A novel fungicide and the process for making the same. Patent submitted to ITMU, IARI, New Delhi vide letter No. AC/2010/615 dated 16/09/2010.

7. McGuire, M. M., Banfield, J. F. and Hamers, R. J., Quantitative determination of elemental sulfur at the arsenopyrite surface after oxidation by ferric iron: Mechanistic implications. *Geochem. Trans.*, 2001, **4**.

8. Richard, J. J., Vick, R. D. and Junk, G. A., Determination of elemental sulfur by gas chromatography. *Environ. Sci. Technol.*, 1977, **11**, 1084–1086.

ACKNOWLEDGEMENTS. We thank National Agricultural Innovation Project–Indian Council of Agricultural Research (NAIP–ICAR) for providing financial assistance and sanctioning the project on nanopesticides.

Received 3 November 2010; revised accepted 5 April 2011