Micelle bound redox dye marker for nanogram level arsenic detection promoted by nanoparticles†

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The reduction of an SDS micelle bound dye (λ_{max} at 660 nm), methylene blue (MB), by arsine gives a quantitative measure of arsenic concentration in the sub parts per million levels for a test sample. Arsine (AsH_3) is generated *in situ* by $NaBH_4$ from arsenic containing samples while present along with the other reagents. The extent of the micelle bound dye reduction was facilitated in the presence of Ag or Au nanoparticles. The micelle, in turn, helps to increase the collision probability between the dye and arsine and nanoparticles help the electron relay from AsH_3 to the micelle bound dye. This physical effect has been depicted as a micelle catalyzed reaction. The calibration graph is valid for a wide range of concentrations. The linear dynamic range (LDR) is 0–0.11 ppm with the limit of detection (LOD) being 0.03 ppm. This method offers a simple, reproducible and cost effective technique for quantification of arsenic, free from phosphate and silicate interferences and applicable to real sample analysis.

The manifestation of high levels of inorganic arsenic compounds in the human body is well documented. Arsenic has taken its toll (WHO's guidelines, 1993, Geneva) all over the world. The situation is particularly alarming in India and Bangladesh where currently millions of people are suffering from arsenic related diseases by drinking arsenic contaminated underground water. It has been reported that in the Indian subcontinent, underground water reserves are utilized to procure drinking water and geological reasons are usually cited to account for contamination of such water reserves by arsenic. Other major factors reported for causing arsenic related diseases in other countries include excessive mining activity (*e.g.*, copper and gold mining in Chili) and the uncontrolled use of arsenic containing pesticides, paints and pigments, *etc*.

Any treatment of arsenic contaminated water requires methods of detection and decontamination, that is, removal of arsenic from water, which can span a wide range of concentrations. It is important to note that devising such methods is difficult, especially when arsenic is present at relatively low concentrations (below or near the permissible level of 0.05 ppm). An accurate but routine method of detection is of prime importance as this, in turn, ensures the efficiency of removal. While removal is urgent, detection becomes of utmost importance. Although cited references of both processes (detection and removal) summarize well the chemistry at hand, additional efforts are required for real time application of these principles. Innumerable scientists have put forward their efforts to alleviate the problem of arsenic removal from bodies of water. Almost all of the methods are, in one way or another, replicas of Mother Nature's normal and preferential binding tendency of arsenic with iron as arseno pyrites (FeAsS) beneath the earth's crust. Detection procedures/kits usually employed vary from the age-old Marsh test to ion-associate formation,[1,2] atomic absorption spectroscopy[3] (AAS), neutron activation analysis[4] (NAA), *etc*., depending on the gravity and needs of the situation.

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Keeping an eye on this grave situation of arsenic poisoning, we have developed a sensitive one-pot arsenic detection method at the sub parts per million (ppm) level using a micelle catalyzed reaction.[5] The participation of silver or gold nanoparticles as a catalyst[6,7] made the method more efficient. This reaction is simply fascinating and reported for the first time. A simple UV-visible spectrophotometer serves the purpose of arsenic detection well below the WHO's permissible range (0.05 ppm) after concentrating the water sample by boiling. The method is very selective, that is free of effects from common interfering substances.

Scientific compilations of the idea of micelle catalyzed[5] and nanoparticle prompted[8–10] reactions have given birth to this one-pot reaction for a new, simple technique that gives first hand information about the presence of arsenic by spectrophotometry. The method could also be used for a "Yes/No" by eye test for arsenic for which no special instrumention is needed.

Prior to the development of arsenic quantification using the above mentioned procedure, a 'Yes/No' type test has already been reported[1] for arsenic detection. This method is based on the extraction of the arsenic catechol ion-associate of MB, $[As(catechol)_3]^-[MB]^+$, in toluene. This 'Yes/No' type test method has been widely publicized in different news media. The success of this simple technique and the published colour chart[1] help village people to test for arsenic in water samples in remote arsenic affected areas of Bengal.

Here we report a method that is based on the colour bleaching (reduction) of methylene blue (MB) in micellar media by AsH_3 produced *in situ* (one-pot) from arsenite and/or arsenate by sodium tetrahydroborate ($NaBH_4$). The decrease in the colour intensity of MB is a direct measure of the arsenic concentration. The method is free from phosphate and silicate interferences. It is quick and can determine arsenic concentrations in 10 different samples in a 1 h period. The method can determine arsenic on the sub parts per million (ppm) level. The different calibration curves generated for three different ranges of arsenic are reported.

Experimental

Reagents and apparatus

All chemicals used were of analytical reagent grade. All aqueous solutions were prepared in distilled water. Arsenic(III) and arsenic(V) solutions were prepared using As_2O_3 (S. D. Fine Chemicals Pvt. Ltd., Bombay, India) and H_3AsO_4 (Merck Ltd., Poole, Dorset, UK), respectively, and standardized using the silver arsenate method.[11] Working solutions of As(III) and As(V) were prepared by appropriate dilution with distilled water. MB was purchased from Qualigens Fine Chemicals (India) and was used after repeated crystallization from alcohol. Sodium dodecyl sulfate (SDS), cetyl trimethyl ammonium bromide (CTAB) and poly(oxyethylene) *iso*-octyl phenyl ether (Triton X-100 or TX-100) were purchased from Aldrich. $NaBH_4$, obtained from BDH Chemical Company, was dissolved in ice cold water to make a 0.1 M solution and was prepared fresh every day. Silver or gold sols were prepared *in situ* from $AgNO_3$ (BDH) or $HAuCl_4$ (Johnson and Matthey), respectively, by $NaBH_4$ reduction.

All UV-visible absorption spectra were recorded in a Shimadzu (Kyoto, Japan) UV-160 digital spectrophotometer equipped with 1 cm quartz cells. A Gilson micropipette with disposable tips was used to add samples.

Procedure

An aliquot of 3 mL of anionic micelle, SDS (10^{-2} mol l^{-1}), was placed in a stoppered cuvette and 50 μL of MB (0.5×10^{-3} mol l^{-1}) was housed in the micellar solution. Then varying amounts (20–100 μL) of sodium arsenate (0.1 mol l^{-1}) was introduced into the cuvette and 40 μL of silver nanoparticles (10^{-4} mol l^{-1}) was added. Finally, 150 μL of $NaBH_4$ (0.1 mol l^{-1}) was introduced into the reaction mixture. After 3 min, the decrease in the absorbance value of the micelle stabilized dye was noted at 660 nm (λ_{max} of the dye) using the stoppered cuvette (Fig. 1). The dilution effect was considered as far as practicable. The colour bleaching of the dye gives a quantitative measure of the amount of sodium arsenate (and hence arsenic) present in the solution. Calibration graphs were set up for three linear dynamic ranges (LDR): 0–8.63, 0–1.11 and 0–0.11 ppm using 1.14×10^{-5}, 8.19×10^{-6} and 6.58×10^{-6} mol l^{-1} MB, respectively. Introducing Ag or Au nanoparticles (10^{-4} mol l^{-1} prepared[12] and added, final concentration 10^{-9} mol l^{-1}) the detection of arsenic in the lower range (0–0.11 ppm of arsenic) was studied in detail.

Results and discussion

The quantitative colour bleaching of methylene blue (MB, 0.5×10^{-3} mol l^{-1}), a well-known non-toxic cationic dye, by arsine (AsH_3) has been studied in aqueous micelles. The arsine is generated *in situ* (*i.e.*, in the reaction medium) from arsenic containing samples with $NaBH_4$ (0.1 mol l^{-1}) while taken along with the reaction mixture containing the dye and micelle. In order to study the quantitative progress of the reaction, an anionic micelle (here it is aqueous SDS, $\sim 10^{-2}$ mol l^{-1}) has been found to be essential. The progress of reduction of the dye was followed spectrophotometrically at the dye λ_{max} at 660 nm. These one-pot and pseudo-first-order reaction ($k = 0.12$ min^{-1}) conditions provide a good measure of the arsenic content of a sample even on the ppm level. The reaction could be followed even when MB is present in trace levels ($\sim 10^{-7}$ mol l^{-1}). This is possible due to the high molar extinction coefficient ($\varepsilon = 1 \times 10^5$ l mol^{-1} cm^{-1}) of the dye at 660 nm. A change in the concentration of MB from 10^{-7} to 10^{-5} mol l^{-1} was required for samples containing a higher amount of arsenic.

Micellar catalysis

The reaction between MB and AsH_3 is thermodynamically favourable but does not occur appreciably in water. This may be due either to a high activation energy or to the low encounter probability because of the solvated reactants. The rates of MB reduction by AsH_3 carried out in different micelles are in the order

$$Rate_{SDS} > Rate_{TX-100} > Rate_{CTAB} > Rate_{water}$$

The bleaching of MB by arsine could be explained with the concept of encounter probability[5,13] and the fractal nature of the micelle surface.[14] Anionic micelles bind the dye and thus help to increase the collision probability between the dye and arsine through a physical factor, their incorporation in the micellar Stern layer. The reduction occurs in the diffusion space of the micelle of dimension $d < 3$. Gradual increase of the arsine concentration (a direct measure of the amount of any arsenic compound present in the solution even on the sub ppm level) increases the extent of reduction of MB in the presence of anionic micelles. Dynamic light scattering (DLS) experiments revealed a 13-fold increase in the size of the SDS micelle (schematically represented in Scheme 1) while AsH_3

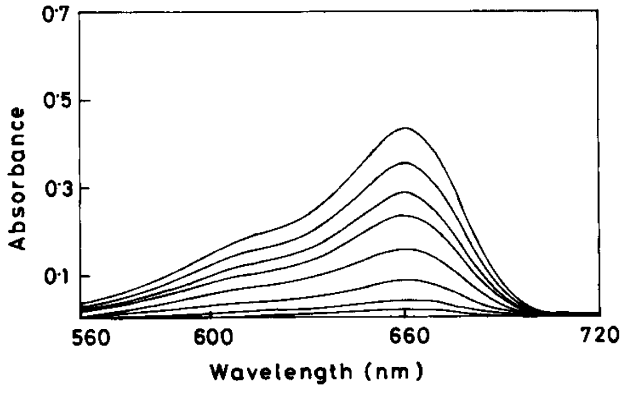
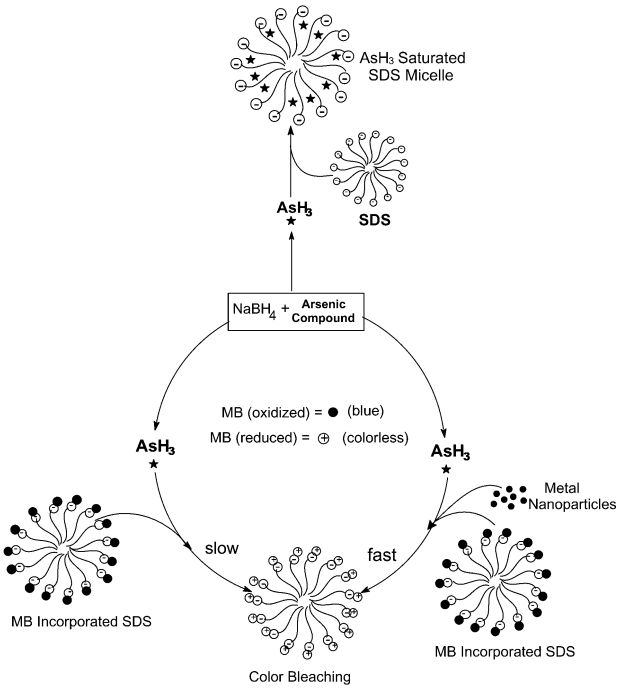


Fig. 1 UV-visible spectra of MB reduction with variable concentration of arsenic.



Scheme 1 Schematic diagram of incorporation of MB and arsine in an SDS micellar environment.

saturates SDS at 25 °C. The average diameter (hydrodynamic diameter) of the SDS micelle and arsine incorporated into the SDS micelle were measured at different angles. The average diameter was 6.3 nm and that for arsine incorporated into the SDS micelle was 79 nm for a 90° measurement. Thus, from the above measurements it is clear that arsine is definitely incorporated into the micelle. Thus, there are no restrictions on the movement of AsH_3 in the micelle and hence it can easily reduce micelle bound MB.

Thermodynamic parameters and nanoparticle catalysis

From kinetic data the activation energy of this reaction was found to be 13.36 kJ mol^{-1}, which indicates that the reaction is nearly diffusion controlled. In the absence of SDS micelles the reaction does not occur at all. Even in SDS solution the colour bleaching is relatively slow. Hence, it has been thought that there is a kinetic barrier that prevents dye reduction. In order to remove the kinetic barrier between the donor (AsH_3) and the acceptor (micelle bound methylene blue) the addition of metal nanoparticles was thought of and tested successfully. Metal nanoparticles help the electron relay (promoting the extent of reaction) from the donor to the acceptor. Interestingly, it has been observed that silver or gold nanoparticles served this purpose; they could easily eliminate the kinetic barrier between the dye-micelle aggregate and arsine. The size of the silver nanoparticles (prepared by $NaBH_4$ reduction[12] method) lies in the range of ~7 nm. The S donor of the dye readily poisons gold nanoparticles, however, silver nanoparticles are not. Hence silver works better than gold.[8]

Sequence of addition

The sequence of addition of substrates has been observed to be extremely important for the micellar catalysis. If AsO_3^{3-}/AsO_4^{3-} is added prior to the addition of MB in SDS solution no effective catalysis is observed. One possible reason for this is that if AsO_3^{3-}/AsO_4^{3-} is added before MB then MB forms a complex with it. This has been evidenced from the slight (~3 nm) blue shift of the absorption maximum of MB while present in the SDS micellar medium in the presence of AsO_3^{3-}/AsO_4^{3-}. Thus, the probability for MB to attach to the micelle and to be reduced by AsH_3 is decreased. Similarly, when $NaBH_4$ is added prior to the addition of AsO_3^{3-}/AsO_4^{3-} but after the addition of MB, the reaction is not that significant. Thus, the best addition sequence is found to be as follows:

$$SDS \rightarrow MB \rightarrow As(III)/As(V) \rightarrow NaBH_4$$

The thermodynamically favourable reduction of the dye (E^0 for MB = −0.18 V, BH_4^- = −1.33 V and AsH_3 = +0.60 V *vs.* NHE) was not observed in water, cationic and non-ionic micellar solutions under the experimental reaction conditions and timescale.

Calibration graph and other statistical parameters

Three different calibration graphs were obtained in three different ranges of arsenic concentration. Arsenic quantification can be done in the range of 0–150 ppm using any one of the calibration graphs. The concentration of MB was varied for the three different ranges of arsenic concentration. The method can attain lower sensitivity if an initial pre-concentration step (boiling) is used before the AsH_3 generation and MB bleaching. Statistical parameters such as the linear dynamic range (LDR), limit of detection (LOD), correlation coefficient, slope and intercept are shown in Table 1. The RSD for all 3 curves are within ±5%.

Table 1 Statistical parameters for arsenic determination

LDR/ppm	Slope	Intercept	Corr. co-eff.	LOD[a]/ppm
0–8.63	-0.0158×10^3	0.5249	0.9892	1.3
0–1.11	-0.0393×10^3	0.3405	0.9937	0.53
0–0.11	-0.0071×10^5	0.4682	0.9892	0.03

[a] All LODs were calculated as $LOD = 3S_B/m$ (S_B = standard deviation of the blank, m = slope of the calibration graph).

Table 2 Quantification of total arsenic in a real samples using spectrophotometric,[2] spectrofluorimetric[15] and the present methods

	Concentration[a]/ppm		
Sample	Spectrophotometric[2]	Fluorimetric[15]	Present method
1.	0.269	0.261	0.264
2.	0.158	0.152	0.159

[a] Average of three determinations.

Applicability of the method for real sample analysis

This method is applicable for the analysis of both surface and ground water and can be applied for real sample analysis. The standard deviations for all three curves lie within 0.0021 to 0.0083. The results were obtained by following the described procedure and compared with the results obtained using the spectrophotometric[2] and fluorimetric[15] methods (Table 2). The results from all 3 method are comparable.

Interference

The method (one-pot) is free from phosphate and silicate interferences. Most metal ions such as Fe^{2+}/Fe^{3+}, Ca^{2+}, Mg^{2+}, Na^+, K^+ and common halides such as Cl^-, Br^-, I^- do not interfere in the determination. Using the two-pot method (arsine generated separately, reduction of micelle bound methylene blue carried out in another container) the selectivity of the proposed method can be improved further.

Conclusion

This paper describes a method for total arsenic determination using the reduction and thereby colour bleaching of MB, a cationic dye, by AsH_3, generated by the $NaBH_4$ reduction of AsO_3^{3-}/AsO_4^{3-}, in an anionic micelle. This one-pot determination of arsenic is simple and easy to carry out. The study reports the catalytic effect offered by the micellar environment through electrostatic attractions. The colour intensity decrease due to the reduction of MB is inversely proportional to the arsenic concentration. Hence, it is used for arsenic quantification in the ppm range. The method is simple, rapid, reproducible, cost effective and environmentally friendly. It does not require any toxic chemicals and the method is also not time-consuming (an hour for the analysis of 10 samples). The method is free from common interferences and could be recommended for routine analysis.

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