Video Article

Preparation of DMMTA^V and DMDTA^V Using DMA^V for Environmental Applications: Synthesis, Purification, and Confirmation

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Abstract

Dimethylated thioarsenicals such as dimethylmonothioarsinic acid (DMMTA^V) and dimethyldithioarsinic acid (DMDTA^V), which are produced by the metabolic pathway of dimethylarsinic acid (DMA^V) thiolation, have been recently found in the environment as well as human organs. DMMTA^V and DMDTA^V can be quantified to determine the ecological effects of dimethylated thioarsenicals and their stability in environmental media. The synthesis method for these compounds is unstandardized, making replicating previous studies challenging. Furthermore, there is a lack of information about storage techniques, including storage of compounds without species transformation. Moreover, because only limited information about synthesis methods is available, there may be experimental difficulties in synthesizing standard chemicals and performing quantitative analysis. The protocol presented herein provides a practically modified synthesis method for the dimethylated thioarsenicals, DMMTA^V and DMDTA^V, and will help in the quantification of species separation analysis using high performance liquid chromatography in conjunction with inductively coupled plasma mass spectrometry (HPLC-ICP-MS). The experimental steps of this procedure were modified by focusing on the preparation of chemical reagents, filtration methods, and storage.

Video Link

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Introduction

Since dimethylarsinic acid (DMA^V) has been demonstrated to exhibit both acute toxicity and genotoxicity due to undergoing methylation and thiolation upon ingestion^{1,2}, the metabolic pathway of arsenic thiolation has been intensively studied both *in vitro* and *in vivo*^{3,4} as well as in environmental media (e.g., landfill leachate)^{5,6}. Previous studies have found both reduced and thiolated analogs of DMA^V in living cells, for example, dimethylarsinous acid (DMA^{III}), dimethylmonothioarsinic acid (DMMTA^V), and dimethyldithioarsinic acid (DMDTA^V)^{7,8,9}, with dimethylated thioarsenicals such as DMMTA^V exhibiting greater toxicity than other known inorganic or organic arsenicals¹⁰. The abundance of highly toxic thioarsenicals has serious environmental implications, since they may pose a risk to humans and the environment under highly sulfidic conditions¹¹. However, the mechanisms of DMMTA^V and DMDTA^V (trans)formation and their fates in environmental media still require further study. Thus, the quantitative analysis of thioarsenicals is required to improve understanding of the environmental effects of DMMTA^V and DMDTA^V.

Although standard chemicals are the key requirement for quantitative analysis, the standards of DMMTA^V and DMDTA^V are difficult to obtain by replicating previous studies, owing to the high risk of species transformation into other species and unstandardized synthesis procedures¹². Moreover, the methods referenced have limitations that may lead to practical difficulties in synthesizing the standard chemicals and performing quantitative analysis. DMMTA^V and DMDTA^V are commonly prepared by mixing DMA^V, Na₂S, and H₂SO₄ in a certain molar ratio¹ or bubbling H₂S gas through a solution of DMA^V^{13,14}. The bubbling method features substitution of oxygen by sulfur using a direct supply of H₂S gas, which, is highly toxic and difficult to control for an inexperienced user. Conversely, the above mixing method¹, widely used for the qualitative analysis of DMMTA^V and DMDTA^V in environmental studies^{5,6,12}, features the thiolation of DMA^V with H₂S generated by mixing Na₂S and H₂SO₄ and produces DMMTA^V and DMDTA^V, allowing easier stoichiometric control to produce target chemicals, as compared to the direct use of H₂S gas.

The reference mixing method procedures^{1,3,4,8,15} mentioned in this study exhibit limitations in some of their critical experimental steps, which might lead to experimental failure. For example, the details of specific solvent (*i.e.*, deionized water) preparation and the extraction and crystallization of the synthesized arsenicals are over-abbreviated or not described in sufficient detail. Such dispersed and limited information on procedural steps might lead to the inconsistent formation of thioarsenicals and unreliable quantification analysis. Therefore, the modified protocol developed herein describes the synthesis of DMMTA^V and DMDTA^V stock solutions with quantitative species separation analysis.

Protocol

1. Synthesis of DMMTA^V

1. Chemical preparation and molar ratio mixing of DMA^V, Na₂S, and H₂SO₄

NOTE: DMA^V:Na₂S:H₂SO₄ = 1:1.6:1.6

1. Dissolve 5.24 g of DMA^V in 40 mL deionized and N₂-purged (purged for at least 30 min) water in a 50 mL centrifuge tube.
2. Prepare Na₂S reagent by dissolving 14.41 g of Na₂S·9H₂O in 50 mL deionized and N₂-purged water in a 250 mL flask.
3. Prepare H₂SO₄ reagent by adding 3.3 mL of concentrated sulfuric acid (96%) to 40 mL of deionized and N₂-purged water in a 50 mL centrifuge tube.

NOTE: Final molar ratio of DMA^V:Na₂S:H₂SO₄ = 1:1.6:1.6^{1,3,4,8,15}.

4. Add the prepared 40 mL of DMA^V solution (step 1.1.1) to the 50 mL of Na₂S solution contained in the 250 mL flask (step 1.1.2). Rinse the tube containing DMA^V with 10 mL of purged water and add this to the 250 mL flask as well.
5. Close the flask with a three-hole rubber stopper fitted with glass tubes. Use the glass tubes for N₂ gas inflow, outflow, and H₂SO₄ solution inlet, respectively. Immediately after closing the flask, allow N₂ gas flow into the flask.
NOTE: Gas pressure should be maintained to flow over the surface of the reaction solution without splashing.
6. Connect acid resistant tubing to the glass tube of the H₂SO₄ solution inlet with a 50 mL syringe to add the prepared 40 mL of H₂SO₄ solution (step 1.1.3). Add 40 mL of H₂SO₄ solution, slowly and in a stepwise manner.
Caution: Upon adding sulfuric acid, white fumes will be generated; use a well-ventilated fume hood.
7. Monitor color change of the reaction mixture in the flask while adding sulfuric acid at regular intervals. Maintain an interval of 4 - 5 mL drops of H₂SO₄; the mixture should be a white cloudy solution.
NOTE: Instantaneous yellow precipitation might appear due to rapid addition of concentrated sulfuric acid.
8. Ensure the reaction solution has been standing for 1 h since the start of step 1.1.4.

2. DMMTA^V extraction using liquid-liquid extraction method

1. After 1 h, pour the reaction solution into a separatory funnel containing around 200 mL of diethyl ether.
2. Shake the funnel for 5 - 10 min, releasing gas several times by switching the stopcock.
NOTE: Synthesized DMMTA^V will be transferred to the upper layer of diethyl ether (0.713 g·mL⁻¹).
Caution: Diethyl ether gas might be harmful; use a well-ventilated fume hood.
3. Collect the reaction solution in a beaker, and separately collect the diethyl ether containing DMMTA^V in a bottle. Place the reaction solution back in the same separatory funnel and add about 200 mL of fresh diethyl ether for reshaking. Repeat steps 1.2.2 - 1.2.3 three times.
4. Pour the collected diethyl ether from step 1.2.3 into the same separatory funnel again, and add about 100 mL N₂-purged deionized water. Shake for 5 - 10 min, and discard the N₂-purged deionized water and a few mL of diethyl ether for purity purposes. Collect the remaining diethyl ether in a glass petri dish (minimum inner diameter of 160 mm and minimum height of 50 mm).
5. Transfer the glass petri dish into a N₂ atmosphere glove box to prevent species transformation.
Caution: Ensure the solvent is not pulled into the vacuum pump through the outlet of the pass box.
6. Dry until a white precipitate of dimethylmonothioarsinate (crystallized DMMTA^V) is formed on the glass petri dish.
NOTE: The protocol can be paused here.

3. Verification of synthesized DMMTA^V and storage

1. Take the white precipitate of crystallized DMMTA^V, and measure and record its total weight.
Caution: Use a well ventilated fume hood or glove box to prevent inhalation of hydrogen sulfide gas.
2. Dissolve crystallized DMMTA^V in 50 mL of N₂-purged deionized water, and filter the yellow precipitate through a 0.2-μm syringe filter.
3. Assume the total As of used DMA^V is converted into DMMTA^V, i.e. ≈9,649 mg As·L⁻¹. Dilute DMMTA^V stock solution to ≈1 mg·L⁻¹ and ≈40 μg·L⁻¹ for verification analysis using Electrospray ionization Mass Spectrometry (ESI-MS) and HPLC-ICP-MS, respectively.
4. Analyze *m/z* of DMMTA^V using ESI-MS^{11,16} and fragments at *m/z* 155 in the positive-ion mode or at *m/z* 153 in the negative-ion mode (Table 1).
NOTE: See reference values of *m/z* (Table 1).
5. Analyze chromatogram of DMMTA^V in the stock solution using HPLC-ICP-MS^{11,16,17} with appropriate eluent conditions and confirm a major peak is found at the retention time described in the literature.
NOTE: Purity of synthesized DMMTA^V should be calculated using the analysis results from step 1.3.5.
6. Analyze the total arsenic concentration using ICP-MS after acid digestion¹¹ and calculate the true DMMTA^V concentration in synthesized DMMTA^V stock solution using dilution factors and purity, as in the following equation:
Analyzed total As concentration (μg·L⁻¹) · Dilution factor · Purity (%) = True DMMTA^V concentration in DMMTA^V stock solution (μg·L⁻¹)
7. Store DMMTA^V stock solution at 4 °C in the dark for further quantitative speciation analysis¹⁸.

2. Synthesis of DMDTA^V

1. Chemical preparation and molar ratio mixing of DMA^V, Na₂S, and H₂SO₄

NOTE: DMA^V:Na₂S:H₂SO₄ = 1:7.5:7.5

1. Dissolve 1.38 g of DMA^V in 40 mL deionized water in a 50 mL centrifuge tube.
2. Prepare Na₂S reagent by dissolving 18.01 g of Na₂S·9H₂O in a 50 mL deionized water in a 250 mL flask.
3. Prepare H₂SO₄ reagent by adding 4 mL of concentrated sulfuric acid (96%) to 40 mL of deionized water contained in a 50 mL centrifuge tube.

NOTE: Final molar ratio of DMA^V:Na₂S:H₂SO₄ = 1:7.5:7.5^{1,3,4,8,15}.

4. Add the prepared 40 mL of DMA^V solution (step 2.1.1) to the 50 mL of Na₂S solution contained in the 250 mL flask (step 2.1.2). Rinse the tube containing DMA^V with 10 mL of deionized water and add this to the 250 mL flask as well.
5. Add the prepared 40 mL of H₂SO₄ solution (step 2.1.3), slowly and in a stepwise manner, into the flask.
6. Monitor the color change of the reaction mixture in the flask while adding sulfuric acid at regular intervals. Maintain an interval of 4 - 5 mL drops of H₂SO₄; the mixture should be a white/yellow cloudy solution.
NOTE: Instantaneous yellow precipitation might appear due to a rapid addition of concentrated sulfuric acid.
Caution: Upon adding sulfuric acid, white fumes will be generated; use a well ventilated fume hood.
7. Maintain the reaction solution in the flask overnight without covering.
NOTE: The protocol can be paused here.

2. DMDTA^V extraction using solid-phase extraction (SPE) method

1. After the overnight standing reaction, filter the reaction solution using a C18 syringe type silica-based SPE in order to trap synthesized DMDTA^V on the resin.
Caution: Use a well-ventilated fume hood.
2. Prepare 10 mM ammonium acetate (pH 6.3) by dissolving 0.77 g ammonium acetate in 1 L of deionized water. Elute a sufficient volume of 10 mM ammonium acetate through the C18 syringe (step 2.2.1) to extract the adsorbed DMDTA^V. Collect the filtered ammonium acetate in a glass Petri dish (minimum inner diameter of 160 mm and minimum height of 50 mm).
3. Transfer the glass petri dish into a N₂ atmosphere glove box to prevent species transformation.
Caution: Ensure solvent is not pulled into the vacuum pump through the outlet of the pass box.
4. Dry until a white precipitate of dimethyldithioarsinate is formed (crystallized DMDTA^V) on the glass petri dish.
NOTE: The protocol can be paused here.

3. Verification of synthesized DMDTA^V and storage

1. Take the white precipitate of crystallized DMDTA^V, and measure its total weight, recording the measurement.
Caution: Use a well-ventilated fume hood or glove box to prevent inhalation of hydrogen sulfide gas.
2. Dissolve crystallized DMDTA^V in 50 mL of N₂-purged deionized water in a 50 mL centrifuge tube, and filter any precipitate through a 0.2-μm syringe filter.
3. Assume that the total As of used DMA^V is converted into DMDTA^V, i.e. ≈2,539 mg As·L⁻¹. Dilute DMDTA^V stock solution to ≈1 mg·L⁻¹ and ≈40 μg·L⁻¹ for verification analysis using ESI-MS and HPLC-ICP-MS, respectively.
4. Analyze *m/z* of DMDTA^V using ESI-MS^{11,16} and fragments at *m/z* 171 in the positive-ion mode or at *m/z* 169 in the negative-ion mode (Table 1).
NOTE: See reference values of *m/z* (Table 1).
5. Analyze the chromatogram of DMDTA^V in the stock solution using HPLC-ICP-MS^{11,16,17} with appropriate eluent conditions and confirm that a peak is found at the retention time described in the literature.
NOTE: Purity of synthesized DMDTA^V should be calculated using the analysis results from step 2.3.5.
6. Analyze the total arsenic concentration using ICP-MS after acid digestion¹¹ and calculate the true DMDTA^V concentration in synthesized DMDTA^V stock solution using dilution factors and purity as the following equation:
Analyzed total As concentration (μg·L⁻¹) · Dilution factor · Purity (%) = True DMDTA^V concentration of DMDTA^V in stock solution (μg·L⁻¹)
7. Store DMDTA^V stock solution at 4 °C in the dark for further quantitative speciation analysis¹⁸.

Representative Results

Since DMMTA^V has been mistakenly prepared by the DMA^{III} synthesis method¹⁹, verification of synthesized DMMTA^V and DMDTA^V is a critical step for synthesis and extraction and determining the ideal standard chemical materials. Synthesized chemicals can be verified by the peak of DMMTA^V (MW 154 g·mol⁻¹) and DMDTA^V (MW 170 g·mol⁻¹) mass-to-charge ratio (*m/z*) using either the positive or negative ion mode of electrospray ionization-mass spectrometer (ESI-MS) through real-time injection. The reference values of *m/z* are listed in Table 1¹⁹. Additional verification of the successful synthesis of DMMTA^V and DMDTA^V was conducted by comparing the species separation analysis results of the retention time (RT) of major peaks to reference data using HPLC-ICP-MS. Figure 1 shows similar RT of major and minor peaks, DMA^V and DMMTA^V (Figure 1a) or DMDTA^V (Figure 1b), using 1.0 mL·min⁻¹ of 5 mM formic acid as an eluent and a C18 Liquid Chromatography (LC) column as described in¹⁷. Note that the RT of the major peaks may vary depending on the instrumental and eluent conditions, and which LC column is used. Species included in the stock solutions of DMMTA^V and DMDTA^V should be examined prior to every analysis, although this protocol suggests storage conditions of 4 °C in the dark, which maintains synthesized DMMTA^V and DMDTA^V with, respectively, 2.2% and 5.8% transformation during the 13 weeks of analysis (Figure 2).

Figure 1: HPLC-ICP-MS chromatogram of the synthesized DMMA^V and DMDTA^V. 1: DMA^V, 2: DMDTA^V, and 3: DMMA^V were measured as major peaks at 3.8 min, 5.9 min, and 8.0 min in each stock solutions of a: DMDTA^V and b: DMDTA^V, which were corresponded to those reported by Li *et al.* in 2010¹⁷. Instrumental conditions of ICP-MS were 1550 V RF power and 50 μ L injection volume. A C18 column was used with 5 mM formic acid as an eluent¹⁷. [Please click here to view a larger version of this figure.](#)

Figure 2: Stability of DMMA^V and DMDTA^V at 4 °C in the dark. Percentage changes of As species distribution in each of the stock solutions DMMA^V (a) and DMDTA^V (b) for 13 weeks. [Please click here to view a larger version of this figure.](#)

Synthesized stock solution	ion mode	Fragments	m/z	References
DMMTA ^V	Positive	[Me2As(SH)OH] ⁺	155	13,14,19,17,21,24
		[Me2As(OH)2] ⁺	139	19,17
		[Me2AsS] ⁺	137	13,19,17,24
		[Me2As(S)SAsMe2] ⁺	275	13
	Negative	[Me2AsOS] ⁻	153	17,5,23
		[MeAsSO] ⁻	138	17,5
		[AsSO] ⁻	123	17,5
DMDTA ^V	Positive	[Me2As(SH)2] ⁺	171	19,17
		[Me2As(SH)OH] ⁺	155	19
		[Me2AsS] ⁺	137	19,17
	Negative	[Me2AsS2] ⁻	169	20,17,5,22
		[MeAsS2] ⁻	154	20,17,5
		[AsS2] ⁻	139	17,5,22

Table 1: Suggested structure of synthesized DMMTA^V and DMDTA^V, and minor fragment ions by positive and/or negative ion mode of ESI-MS. The list of DMMTA^V, DMDTA^V, and minor fragments *m/z* measured by ESI-MS was reproduced from literature^{5,13,14,17,19,20,21,22,23,24}. Note that *m/z* peaks may vary with instrumental conditions and/or matrices of the stock solutions.

Discussion

The developed protocol has clarified critical steps that previous studies^{1,3,4,8,15} omitted or abbreviated, which may have led to difficulties with or failure during DMMTA^V and DMDTA^V synthesis. As DMMTA^V is oxidation-sensitive^{1,5}, chemical reagents for its synthesis were prepared using N₂-purged deionized water (steps 1.1.1 - 1.1.3) to prevent the possible retardation of DMA^V thiolation and the oxidation of DMMTA^V. DMMTA^V prepared using this protocol had a purity of 92%. The reference DMDTA^V extraction method featured silica-based C18 column extraction with ammonium acetate or phosphate buffer solution as an eluent,^{3,4,7,8} with the purity of the thus prepared DMDTA^V varying depending on the column compartments used, which is not described in references studies^{3,4,8}. In contrast, the use of disposable SPE in the developed protocol (step 2.2.1) allowed the extraction of 88% pure DMDTA^V. In addition, previous methods of crystallizing DMDTA^V referenced only a freeze-drying procedure²⁰, whereas in this protocol, simple drying of the extracted DMDTA^V solution in an atmosphere of N₂ inside the glove box (step 2.2.3) produced a white residue of crystallized dimethyldithioarsinate.

Identity verification of the synthesized DMMTA^V and DMDTA^V is a critical step for determining ideal standard chemical. In our previous work¹⁶ based on this protocol, major fragments at *m/z* 155 in the positive-ion mode and *m/z* 169 in the negative-ion mode were detected by ESI-MS analysis of as-synthesized DMMTA^V and DMDTA^V, respectively. The latter fragment was ascribed to [CH₃]₂As(=S)S⁻ (*i.e.*, [M-H]⁻), in good agreement with previous results^{5,17,20,22}, whereas the fragment at *m/z* 155 was assigned to [CH₃]₂As(=S)(OH) + H⁺ (*i.e.*, [M+H]⁺), again, in agreement with references^{13,14,17,19,21,24}. Since ESI-MS analysis cannot be used as the only verification method, major peaks in the chromatograms of DMMTA^V and DMDTA^V stock solutions obtained by HPLC-ICP-MS were compared to those reported by Li *et al.*¹⁷ (Figure 1). Although DMA^{III} is known as an intermediate produced in the initial stage of DMMTA^V formation^{1,7,8,25}, it exhibits low stability, disappearing within 70 min¹⁹ and therefore is not detectable by this procedure (Figure 1).

Another goal of this study was to suggest storage conditions for DMMTA^V and DMDTA^V stock solutions with fewer impurities to prevent sulfide-to-oxide conversion and thus achieve greater stability¹⁸. The storage conditions used here (*i.e.*, 4 °C in the dark) allowed the preservation of the synthesized DMMTA^V and DMDTA^V as major species in stock solutions (Figure 2) for four weeks, with no drastic decomposition observed even after 13 weeks. Although the solvent pH (*i.e.*, that of deionized water) may affect the transformation of species during storage due to the presence of native¹⁰ and excess sulfide species originating from chemical reagents such as HS⁻ or H₂S, the stock solutions maintained neutral pH without significant transformation of the species contained therein (Figure 2). Therefore, stock solutions could be stored at 4 °C in the dark for 13 weeks prior to quantitative speciation analysis.

In this study, the reference molar ratio mixing method of synthesizing DMMTA^V and DMDTA^V^{1,3,4,8,15} was modified to produce stable DMMTA^V and DMDTA^V stock solutions for HPLC-ICP-MS quantitative analysis. Due to a lack of critical step details, including not only chemical reagent preparation, extraction, and crystallization steps, but also the storage conditions of DMMTA^V and DMDTA^V, stock solutions had to be modified and optimized. Therefore, stock solutions prepared using this protocol are sufficiently applicable for quantitative analysis of DMMTA^V and DMDTA^V for environmental monitoring purposes.

Disclosures

The authors have nothing to disclose.

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