

Sealing Effects on the Storage Stability of the Cyanide Antidotal Candidate, Dimethyl Trisulfide

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Abstract

Background Dimethyl trisulfide (DMTS) is a highly lipid-soluble cyanide (CN) antidote candidate molecule. In prior studies with various US FDA-approved co-solvents, surfactants, and their combinations, aqueous solutions containing 15% polysorbate 80 (Poly80) were found to effectively solubilize DMTS in formulations for intramuscular administration. However, DMTS formulated in 15% aqueous Poly80 solutions showed gradual losses over time when stored in vials with septum-based seals.

Objective The present study tested whether storing DMTS formulations in hermetically sealed glass ampules could mitigate storage losses.

Methods Samples consisted of 1-mL aliquots of a 50 mg/ml stock solution of DMTS in 15% aqueous Poly80. The control samples were stored using a vial-within-a-vial system—the inner and outer vials were sealed respectively, with a snap cap, and with a crimped septum. The hermetically sealed test samples were stored in fire-sealed glass ampules. The DMTS content was measured by HPLC–UV analysis at specific time points over a 100-day period.

Results While the control samples exhibited systematic DMTS losses, no DMTS losses were observed from the test samples stored in hermetically sealed glass ampules over the 100-day testing period.

Conclusion DMTS formulated in 15% aqueous Poly80 solution has excellent stability when stored in fire-sealed glass ampules and thus has the potential to be effectively stored as an intramuscular CN countermeasure for mass casualty scenarios.

Key Points

An improved storage approach is reported for the promising cyanide antidote dimethyl trisulfide (DMTS).

DMTS exhibited no measurable loss over the 100-day study period when stored in hermetically sealed glass ampules.

1 Introduction

Cyanide (CN) causes toxicity by interfering with cellular respiration. In humans, the endogenous sulfurtransferase enzyme rhodanese (Rh) detoxifies CN by converting it to the less toxic thiocyanate [1, 2]; however, this detoxification pathway becomes ineffective when excess CN exposure causes endogenous sulfur donors to be exhausted. For this reason, antidotes have been developed to counter the toxicity of CN. Petrikovics et al. [3] recently reviewed CN detoxification methods, available CN antidotes, and their properties. In the USA, NithiodoteTM [4] and Cyanokit[®] [5] are US FDA-approved remedies for CN intoxication. However, these antidotes require intravenous administration by trained personnel and therefore are not suitable for a mass casualty scenario.

One of the key routes of intervention following CN exposure is the administration of antidotes that serve as sulfur donors [6, 7]. Thiosulfate is the most important endogenous sulfur donor [8] and is a key component of the

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currently available antidote Nithiodote™. Dimethyl trisulfide (DMTS) is a novel sulfur donor that has shown superior antidotal potency when compared with thiosulfate in our previous in vitro and in vivo studies [9]. Acute CN intoxication can be treated with available antidotes [3] such as Nithiodote™ (comprising a combination of sodium thiosulfate and sodium nitrite) [4] and Cyanokit® (hydroxocobalamin) [5].

DMTS is a naturally occurring oily component of garlic that exhibits poor water solubility of approximately 0.13 mg/ml [10]. Studies were performed to find formulations with enhanced solubility. The first attempt to formulate this highly lipophilic molecule for intramuscular administration employed polyethylene glycol-2000 (PEG-2000)-derived lipid surfactants as micelle-forming agents [10]. However, the 2.5 mg/ml encapsulation efficiency of the resulting micellar lipids for DMTS was low. A subsequent investigation of additional co-solvents, surfactants, and combinations identified polysorbate 80 (Poly80), also known as Tween 80, as a promising solubilizing agent for DMTS [11]. Poly80 is a surfactant whose critical micelle concentration (CMC) in water falls in the range of 0.010–0.014 mM [12–14]. Formulation studies showed that 15% (~ 114 mM) Poly80 in water elevated DMTS solubility more than 600 times (from approx. 0.13 to 86 mg/ml) [11].

Analytical methods were developed for DMTS detection from formulations, blood, and brain [15, 16]. Methods such as these enabled us to follow the storage stability of DMTS in a 15% Poly80 formulation over 29 weeks [17]. By the end of the 29-week study, samples stored at room temperature exhibited 36–58% reductions in DMTS concentration. The DMTS was stored using a “matryoshka doll” approach in which the DMTS formulation was sealed in a small glass vial with a snap-cap, which itself was placed into a larger vial that was sealed with a crimped septum. Samples stored at 2–8 °C exhibited a pattern that was statistically indistinguishable from those stored at room temperature. It was hypothesized that upgrading the sealing mechanism might improve the storage stability of formulated DMTS. The goal of the present study was to test whether DMTS formulations that were hermetically fire-sealed in glass ampules would exhibit better storage stability than formulations stored using the prior snap-cap and septum-sealing approach.

2 Materials and Methods

2.1 Materials

DMTS, and dimethyl disulfide (DMDS) were purchased from Sigma Aldrich (St. Louis, MO, USA). Acetonitrile

(high-performance liquid chromatography [HPLC] grade), water (HPLC), and ethanol were purchased from VWR International (Suwanee, GA, USA). Poly80 was purchased from Alfa Aesar (Haverhill, MA, USA).

2.2 DMTS Solution Preparation and Storage

A DMTS stock solution (50 mg/ml) was prepared by dissolving DMTS in 15% aqueous Poly80. To fully dissolve the DMTS, vigorous hand vortexing was applied for 5 min, followed by 30 min auto-vortexing at room temperature [18].

2.2.1 Control Samples (Snap-Cap and Crimp Sealed)

A set of control samples was prepared by pipetting 1-ml aliquots of DMTS stock solution into 15 snap-cap HPLC vials (VWR International). After capping, each HPLC vial was placed inside a larger 5-ml glass vial (Wheaton, Millville, NJ, USA). Each Wheaton vial was crimp sealed with a rubber septum (Fig. 1). In this manner, 15 samples were prepared and stored at 4 °C for triplicate testing after 7, 14, 21, 60, and 100 days. On each testing day, the appropriate double-sealed vials were opened and the DMTS concentration was measured by HPLC. Each vial was opened and measured only once. Because we were able to observe clear replication of prior results showing a statistically significant decrease of DMTS within 21 days, the 60- and 100-day samples were not measured.

2.2.2 Hermetically Sealed Samples

A hermetically sealed set of samples was prepared by pipetting 1-ml aliquots of DMTS stock solution into 20 ampules (2 ml; Wheaton). The DMTS content of the solutions was determined via HPLC. The ampules were

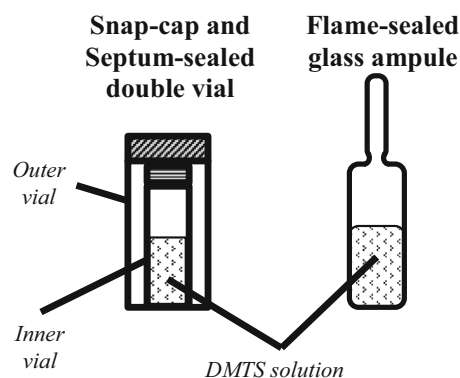


Fig. 1 Two approaches for storing formulated DMTS solution: the snap-cap-sealed vial within a crimp-sealed vial, and the flame-sealed glass ampule

then manually flame sealed, and stored at 4 °C for quadruplicate testing by HPLC after 7, 14, 21, 60, and 100 days.

2.3 HPLC Measurements

Samples were diluted before HPLC analysis: 990 μ l of ethanol with 0.1 mg/ml DMDS (internal standard) was transferred into an HPLC vial; 10 μ l of DMTS sample solution was then added and the mixture was vortexed well. For the HPLC measurement, the system was a Dionex Ultimate 3000 UHPLC with ultraviolet–visible (UV–VIS) detector (Thermo Scientific). Ten microliters of the diluted sample was injected into a Phenomenex Luna 5 μ m C8 (2) 100A, 250 \times 4.6 mm column. The mobile phase consisted of 60% acetonitrile and 40% water with a flow rate of 1 ml/min. The detector wavelength was 215 nm. Peak integration was performed by using Chromeleon 7 software. The calibration curve for this assay gave an equation of $y = 4.2038 \times - 2.0367$ with an $R^2 = 0.9972$.

2.4 Statistical Analysis

All data presented are mean \pm standard deviation. Values were compared using analysis of variance (ANOVA) followed by Dunnett's test (GraphPad Prism 5.0, GraphPad

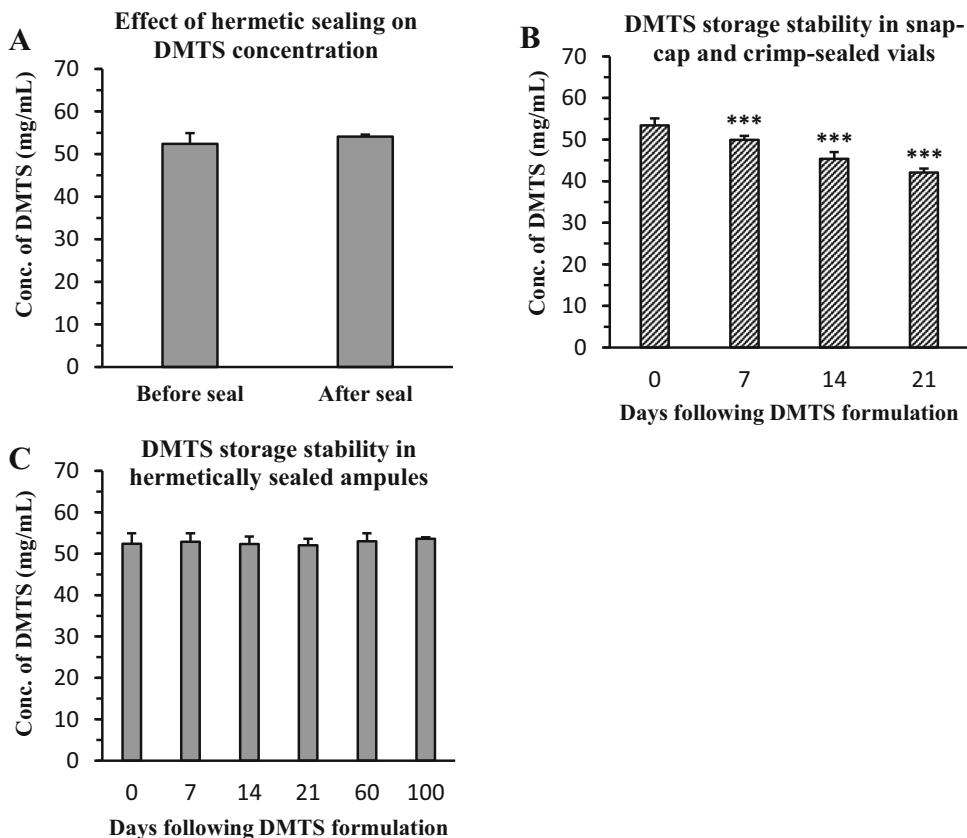
Software Inc., San Diego, CA, USA). An unpaired t test was applied to assess the ampule sealing effect (Fig. 2a). Changes were considered statistically significant at $p < 0.05$.

3 Results

3.1 DMTS Stability Studies in Double-Sealed Vials and Ampules

To allay initial concern that the flame used to hermetically seal the glass ampule samples might itself influence concentrations, we measured the DMTS in six ampules immediately before and after fire sealing the ampules. As seen in Fig. 2a, no significant changes in DMTS concentration were induced by the flame sealing of the ampules. This gave us confidence to proceed with the storage-stability study in the hermetically sealed vials. The DMTS concentrations measured in control samples decreased systematically by 20% over 21 days (Fig. 2b). In sharp contrast, no statistically significant changes in DMTS concentration (Fig. 2c) were observed over a 100-day period when the DMTS formulation was hermetically stored in fire-sealed ampules.

Fig. 2 Comparing the storage stability of formulated DMTS (50 mg/ml in 15% w/w aqueous Poly80 solution): **a** The effect of ampule flame sealing on DMTS concentration. Statistical analysis for A: unpaired t -test, $n = 6$. **b** The DMTS concentration when the formulation was stored in double-sealed vials (snap-cap-sealed vial within a crimp-sealed vial), $n = 3$. **c** The DMTS concentration when the formulation was stored in flame-sealed glass ampules, $n = 4$. Data are presented as mean \pm standard deviation. Statistical analysis for (b) and (c): analysis of variance (ANOVA) followed by Dunnett's test; $***p < 0.001$, all groups were compared with control



4 Discussion

The Poly80 formulation provided a solvent system that enhanced the solubility of the lipophilic DMTS. When the Poly80 concentration exceeds the CMC (in this case by about 600 times), Poly80 forms micelles. Kovacs and colleagues determined the solubility of DMTS to be 0.13, and 86 mg/ml, respectively, in water [10] and in the 15% aqueous Poly80 formulation [11]. No changes in DMTS concentration, formulation clarity, or phase were observed upon storage. Based on this assessment, and the encapsulation efficiency (EE%) calculation shown below, we estimate that 0.2% of the DMTS in this formulation was present as free DMTS in the water and 99.8% was associated with the micelles both before and after storage.

$$\begin{aligned} \text{EE\%} &= \frac{\text{amount of encapsulated DMTS}}{\text{total amount of DMTS}} \times 100 \\ &= \frac{(\text{total amount of DMTS} - \text{amount of DMTS in water})}{\text{total amount of DMTS}} \\ &\quad \times 100. \end{aligned}$$

The enhanced solubility enables DMTS to be formulated for intramuscular injections that can be rapidly administered in a scenario of mass intoxication. In a prior study of the storage stability of DMTS in double-sealed containers, Bartling et al. [17] observed DMTS concentration decreased in the range of 7–45% over 10 weeks of storage at 2–8 °C. They carried out careful experiments attempting to isolate the loss mechanisms. Although these experiments did not lead to a conclusive explanation, the authors suggested that two potential causes of DMTS losses from non-hermetically sealed vials might be reactions with airborne molecules and evaporation. Based on these suggestions, it was hypothesized that higher-quality seals might improve the storage stability of DMTS formulations. The present study tested this hypothesis by replacing snap-cap and crimped-septum seals with hermetic glass seals prepared in a flame-sealing process. The hermetic glass seals did not perturb DMTS concentrations. More importantly, when formulated DMTS was stored at 4 °C in these hermetically sealed ampules, the storage instability problem was overcome. No losses in DMTS concentration were observed over the 100-day period of this study. Because this hermetic sealing approach shows great promise, studies of DMTS formulation stability over longer storage periods and at different temperatures are ongoing.

Compliance with Ethical Standards

Conflict of interest This research was supported by the CounterACT Program, National Institutes of Health Office of the Director, and the National Institute of Allergy and Infectious Diseases, NIH/Department of Defense Interagency Agreement (AOD14020-001-00000/

A120-B.P2014-01), and the Robert A. Welch Foundation (X-0011) at Sam Houston State University, Huntsville, TX, USA. L. Kiss, A. Duke, T. Barcza, M. Kiss, and D.E. Thompson have no known conflicts of interest. Dr. Petrikovics [18, 19] is a coauthor of two, and Dr. Kovacs [18] is a coauthor of one, US patents relating to the use of DMTS as a novel cyanide antidote. The authors alone are responsible for the content and writing of the paper.

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