

## ORIGINAL ARTICLE

# NON-NEWTONIAN SUSPENSION FORMULATIONS FOR IMPROVED STABILITY AND DELIVERY OF AUTOINJECTABLE CBRN COUNTERMEASURES

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### Summary

Autoinjectors are commonly employed by the United States and other countries to deliver emergency therapeutics to counteract the effects of various chemical agent threats, including nerve agents. Autoinjector combination products (autoinjector and drug product) used by the military can have drawbacks, including insufficient thermal stability of drug products, limited aqueous solution concentration of active pharmaceutical ingredients (APIs), and complicated and expensive designs for delivering multiple drugs simultaneously. We have developed a novel Enhanced Formulation (EF) technology that solves these problems, using nanoparticle suspensions in biocompatible cottonseed oil (CSO) instead of aqueous solutions of API. The hydrophobic CSO prevents hydrolytic degradation by limiting exposure to water, and the noninteraction of co-suspended particles simplifies multi-drug cocktail therapies. The high API loading (10% or more) gives the formulations non-Newtonian rheological behavior, with high zero-shear viscosity to resist sedimentation, and shear thinning to allow injection with standard autoinjectors and needles.

*Key words: Organophosphate; MMB4; nanoparticle; milling; intramuscular injection; formulation; particle size; suspension; non-Newtonian; shear thinning; enhanced formulation; nerve agent antidote; nerve agent intoxication; medical countermeasure; cottonseed oil*

## INTRODUCTION

The United States Department of Defense currently uses autoinjectors for drug delivery in emergency treatment of nerve agent intoxication [1]. The treatment regimen generally consists of 3 drugs

administered by intramuscular (IM) injection: a muscarinic receptor antagonist (currently atropine), an acetylcholinesterase (AChE) reactivator (currently 2-pralidoxime chloride, 2-PAM), and an anticonvulsant (currently diazepam) [2]. One dose of this treatment can require up to 3 separate autoinjectors, although the more recently developed Antidote Treatment Nerve Agent Autoinjector (ATNAA) combines the atropine and 2-PAM into a single, wet-dual-chamber autoinjector. All currently fielded products suffer from inherent limitations, including limited aqueous solution concentration, drug-drug solution interactions when two or more drugs

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are dissolved in a single chamber as with cocktail drug administration, and implementation of complicated design requirements for wet-dry and wet-wet multi-chamber autoinjectors. Possibly the most critical issue with current key autoinjector products is inadequate stability of active pharmaceutical ingredients (APIs) in aqueous solution, leading to short shelf lives and increased costs to maintain a stockpile. This is particularly critical for bis-pyridinium oximes, which constitute an important class of AChE reactivators that are currently being developed for next-generation broad spectrum nerve agent treatment systems [3,4]. In addition, the pH of aqueous solutions of these salts must be adjusted to maximize the stability of dissolved APIs, leading to local tolerability issues and patient discomfort for products with significantly acidic solutions and high tonicity. Some of the problems caused by aqueous solutions can be mitigated by using multichamber wet-dry autoinjectors, but these are also complicated and have their own set of problems, such as complex filling processes, high risk / performance ratios, controlling solution kinetics to allow for complete drug injection and solubility limitations on concentration and delivery speed [5].

In this article, Southwest Research Institute (SwRI) presents a formulation which solves many of the problems with currently available autoinjector products. Our Enhanced Formulation (EF) [6] consists of drug microparticles and/or nanoparticles suspended in biocompatible cottonseed oil (CSO). EF is a non-Newtonian shear-thinning fluid, making it injectable even at high solid concentrations (400 mg/mL or more), yet it is sufficiently viscous so as to prevent significant sedimentation of suspended particles. As a hydrophobic suspension of solid particles, EF makes many APIs far more stable, because they are not subject to hydrothermal degradation. Shelf life is vastly increased as a result, lowering acquisition costs substantially. Suspension of solid state API particles also enables facile implementation of drug cocktails by combination of multiple APIs in a single formulation without chemical interactions between drugs. We have demonstrated EF using 1,1'-methylenebis-4-[(hydroxyimino)methyl]pyridinium-dimethanesulfonate (MMB4 DMS) as the API, and we have developed a cGMP manufacturing process [7]. A clinical supply batch made by this process is currently under investigation in a Phase I clinical trial [8].

## MATERIALS AND METHODS

### Materials

HPLC-grade acetonitrile, methanol and phosphate buffered saline (PBS) were purchased from Fisher Scientific and used as received. Reagent-grade methanesulfonic acid and 4-pyridine aldoxime (4-PA) were purchased from Sigma-Aldrich and used as received. Cottonseed oil (CSO, USP/NF grade) was purchased from Welch, Holme, & Clark Co., Inc. (Newark, NJ). MMB4 DMS was manufactured by SwRI and Cambrex (Charles City, Iowa). Syringes (1, 3, and 5 mL; Monoject) and needles (16 ga 1½ inch and 21 ga 1 in; Becton-Dickinson) were purchased from Fisher Scientific.

### HPLC

The quantification of MMB4 and its degradation products were analyzed by high performance liquid chromatography (HPLC) (Waters HPLC system). Separations were performed on reversed phase columns with UV or diode array detectors.

For MMB4, HPLC was performed on a Waters 2695 or Agilent 1100 system on a reversed-phase C18 column (Waters Atlantis HILIC, 4.6 x 150mm, 5 micron) at a flow rate of 1.3 mL/min equipped with a UV detector set at 297 nm. The mobile phase consisted of 80/20 MeCN / 0.05 M Ammonium Formate Buffer (pH 4). The major, minor peaks of MMB4 DMS and the impurity have retention times of 18, 21 min and 25 min respectively.

For quantification of the degradation product from MMB4 DMS, HPLC was performed on a reversed-phase C18 column (Discovery C-18, 3.5 x 150mm, Supelco) at a flow rate of 1.5 mL/min with photodiode array detector set at 215nm. The mobile phase consisted of a gradient of 90/10 (v/v) 25mM K<sub>2</sub>HPO<sub>4</sub>, pH 7.0/MeOH (solvent A2) and methanol (solvent B2) with the following conditions: 100% solvent A2 (0 to 20 min), 50% solvent A2/50% solvent B2 (20 to 25 min) and 100% solvent A2 (25.5 to 30 min). 4-PA was eluted after 7 min.

### Particle Size Characterization

The particle size distributions of MMB4 DMS nanoparticles were determined by dynamic light scattering (DLS), also known as photon correlation spectroscopy (PCS), using a Brookhaven ZetaPALS 90Plus BI-MAS. This method is discussed in more

detail in the Results and Discussion section. Samples were prepared by diluting EF with CSO to approximately 50 µg/mL and sonicating in a 100 W bath sonicator for approximately 60 minutes. DLS experiments were conducted at a controlled temperature of 74 °C, and results are reported as unimodal intensity-weighted median particle sizes.

### *Milling*

Four different types of mills were used to reduce the particle size of MMB4 DMS in dry powder form. A benchtop ball mill (Retsch PM100) was used in a batch process; stainless steel and ceramic balls were both used separately to mill the MMB4 DMS. Particle sizes of approximately 72 to 107 µm were achieved using this method. A comminutor impact mill or Fitzpatrick mill (Model LA1 Fitzmill, The Fitzpatrick Company, Elmhurst, IL) was used in a batch process with up to 10 consecutive passes through the mill to produce volume weighted average particle sizes from approximately 6 to 60 µm. A centrifugal impact mill or pin mill (CIM-18-SS, Munsen Machinery, Utica, NY) was used in a batch process, and was also modified to use a closed-loop system for continuous recirculation. Volume weighted average particle sizes of approximately 7 to 8 µm were achieved using this technique. Another type of impact mill, a jet mill (2-inch Micro-Macinazione (M&M) Micronizing Jet Mill, M-3855) produced volume-weighted average particle sizes of approximately 3 to 4 µm. The previously discussed mills all operated with MMB4 DMS as a dry powder. To reduce the particle size to submicron levels, an agitator bead mill (Glen Mills Dyno-Mill Multi-Lab) was used in a wet milling process, combining MMB4 DMS, CSO, and zirconia grinding media. This technique produced intensity-weighted median particle sizes of approximately 250 to 500 nm.

### *Preclinical Study*

This study (*Comparative Pharmacokinetic Study of Original and Enhanced MMB4 DMS Formulations in the Male New Zealand White Rabbit* (Battelle Study Number CG920771-L)) included three dose groups containing six animals each. All doses for this study were prepared from formulated MMB4 DMS EF and MMB4 DMS OF at a target dose of 0.25 mg/kg based on individual body weights. The doses were based on actual animal body weight which was provided on the day of the fill.

### *Physical Properties*

Rheological experiments were carried out using a Rheometrics RFS-1 fluid analyzer. Injectability was determined using a MTS Insight Materials Testing System, which is an electrodynamic frame equipped with a load cell (200 lbf max) to measure the applied force as the frame moved the syringe plunger to eject the samples.

## **RESULTS AND DISCUSSION**

### **Manufacturing of Enhanced Formulation**

MMB4 DMS EF was manufactured by a wet milling process. The API was combined with cottonseed oil (CSO) and zirconia beads in an agitator bead mill. In this type of a mill an agitator shaft in the grinding chamber rotates, imparting kinetic energy to the mixture. A pump and external reservoir were used to continuously circulate the mixture through the mill, allowing production of batches much larger than the grinding chamber. The MMB4 DMS particles were fractured as they impact the beads, chamber walls, and agitator disks, reducing the particle size from hundreds of microns to the nanometer size regime. The grinding chamber was equipped with a cooling jacket which keeps the temperature from rising high enough to cause degradation. Minimum median particle sizes produced by this process were in the range of 200-300 nm (measured by dynamic light scattering, weighted by light intensity).

### **Stability**

Several stability studies have been conducted with MMB4 DMS EF to assess the stability advantages of EF over the traditional aqueous formulation. MMB4 degrades primarily to 4 pyridinealdoxime (4-PA), which is tracked as a precise marker for stability for small amounts of degradation. In cases of more significant degradation, MMB4 can be assayed directly.

Preliminary stability study results are aggregated in Table 1. Aqueous solutions contained the preservative benzyl alcohol, which was also investigated as a preservative for EF. Initial stability results indicated that benzyl alcohol did not enhance EF stability, and later it was determined that MMB4 DMS EF itself has antimicrobial properties.

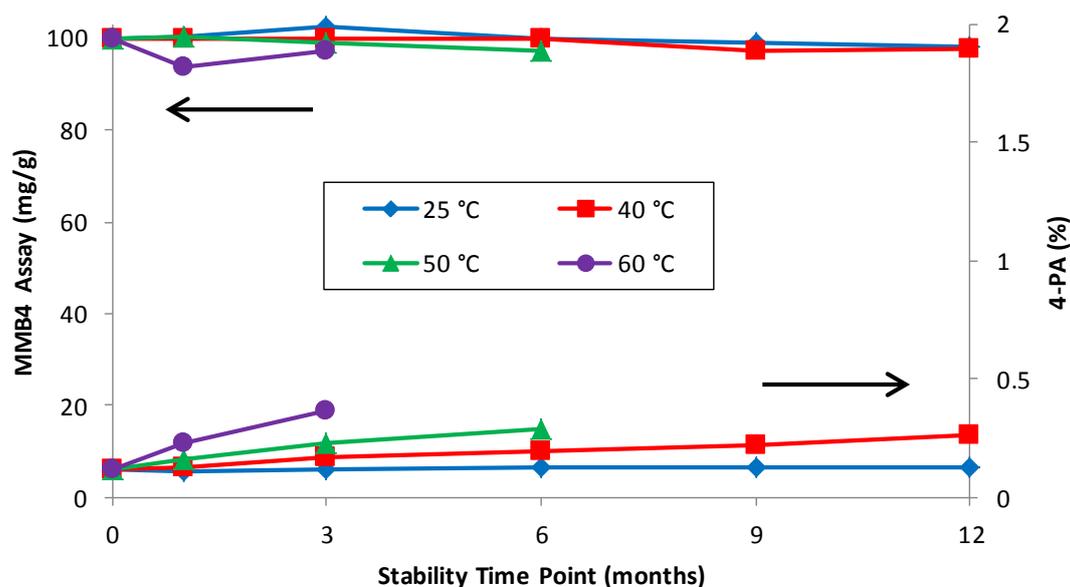
**Table 1.** MMB4 DMS 2-Year Preliminary Stability Results Summary.

Description	MMB4 DMS Stability
120 mg/g MMB4 DMS in CSO	< 0.25% degradation after 2 years at 80 °C
120 mg/g MMB4 DMS with 9.45 mg/g benzyl alcohol in CSO	< 0.30% degradation after 2 years at 80 °C
120 mg/g MMB4 DMS with 1.0 mg/g atropine sulfate in CSO	< 0.25% degradation after 2 years at 80 °C
120 mg/g MMB4 DMS with 9.45 mg/g benzyl alcohol and 1.0 mg/g atropine sulfate in CSO	< 0.50% degradation after 2 years at 80 °C
Aqueous 120 mg/g MMB4 DMS with 5mg/g benzyl alcohol	100% degradation within 2 years at 60 °C ~ 80% degradation after 9 months at 60 °C ~ 10% degradation after 2 years at 40 °C
Aqueous 120 mg/g MMB4 DMS with 5mg/g benzyl alcohol and 1.0 mg/g atropine sulfate	100% degradation within 2 years at 60 °C ~ 80% degradation after 9 months at 60 °C ~ 10% degradation after 2 years at 40 °C

The choice of MMB4 DMS concentration in these stability studies (120 mg/mL) was based on preclinical safety and efficacy studies. It is evident that aqueous MMB4 DMS cannot be stored above 40 °C for more than a few months. After 2 years at 40 °C, approximately 10% of the MMB4 had degraded, which is a common limit for drug product shelf life. Approximately 80% of MMB4 degraded after 9 months at 60 °C. Temperatures significantly in excess of 40 °C are commonly encountered in military theaters of operation. Therefore, a formulation with enhanced thermal stability

is required. In EF, negligible degradation was observed after 2 years at temperatures up to 80 °C.

In addition to the preliminary stability studies with results in Table 1, ICH stability studies were initiated using the clinical trial batch of MMB4 DMS EF. Initial results from samples stored under accelerated conditions indicate that the drug product is stable at 50 °C and 60 °C. Data has been obtained up to the 12 month time point (60 °C samples were held for a maximum of 3 months, and 50 °C for 6 months). Stability indicating parameters, which include



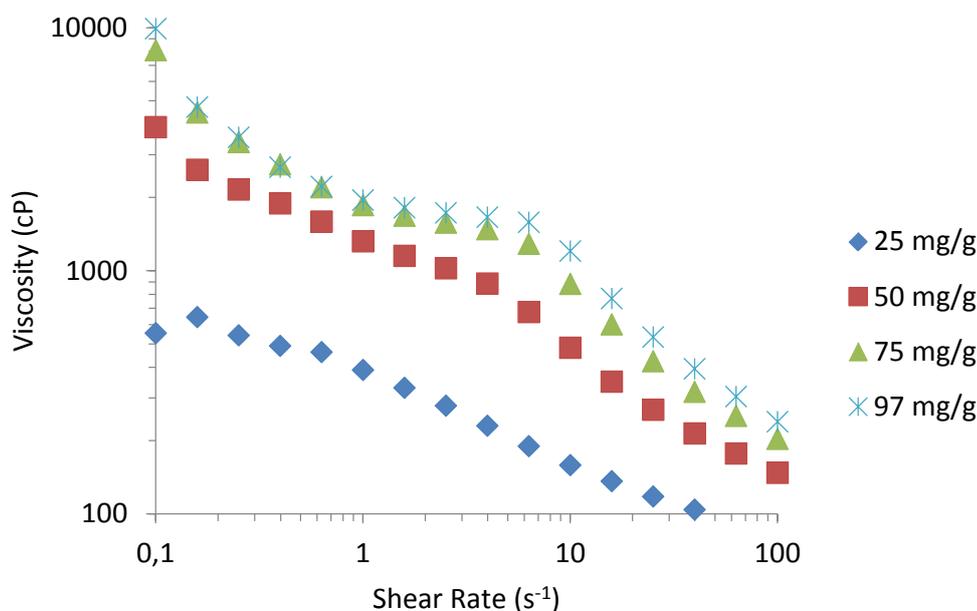
**Figure 1.** Phase 1 clinical lot ICH stability study preliminary results through 12 months: MMB4 assay values (top traces) and 4-PA primary impurity concentrations (bottom traces). 60 °C samples were held for a maximum of 3 months, and 50 °C for 6 months.

MMB4 assay and primary breakdown product (4-PA), are provided in Figure 1. Standard storage conditions for the drug product are 20-25°C.

Under the standard storage conditions of 25 °C and 60% relative humidity (RH), the drug product maintains its stability as indicated by the assay value, which remains approximately 100%. Additionally, degradation was captured by monitoring 4-PA concentration, which is less than 0.15%. Even under accelerated conditions, 4-PA levels remained below 0.4 % at 60 °C after 3 months, below 0.3 % at 50 °C after 6 months, and below 0.25 % at 40 °C after 9 months. Conclusions regarding shelf life and additional degradents will be made after stability testing is complete under this plan.

## Physical Properties

Enhanced Formulations have several properties that present challenges for an injectable drug product, all of which were overcome by controlling the physical properties of the mixtures. These potential challenges include needle occlusion and/or non-uniform dose delivery due to excessively large particles, aggregates, and/or sedimentation. The optimum formulation would have small particles which can flow easily through a needle, a high zero shear viscosity to resist particle sedimentation, and shear thinning to allow it to be injectable at the high shear rates induced by flow through the needle. As the particle size is reduced, the viscosity of the MMB4 DMS / cottonseed oil formulation increases for



**Figure 2.** Shear rate dependent dynamic viscosities of EF formulations at various concentrations. All formulations display shear thinning behavior. A large gap is evident between 25 mg/g and 50 mg/g, indicating a change in interparticle interactions.

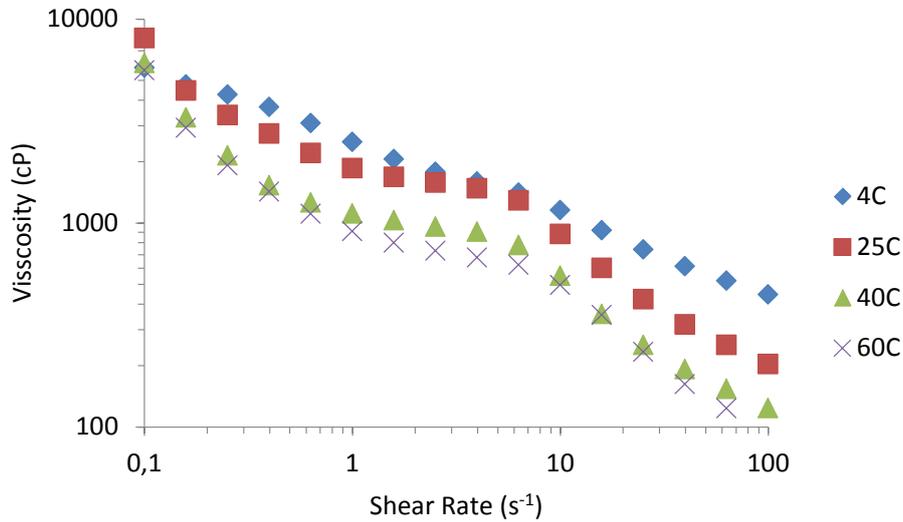
a given concentration (higher concentrations also increase viscosity). Therefore, the combination of particle size and concentration controls the rheology of the mixture.

The shear rate dependent viscosities of an Enhanced Formulation with various concentrations are shown in Figure 2. Shear thinning [9] is evident from the trend of decreasing viscosity with increasing shear rate. The effective shear rate of flow through

a needle during injection (approximately 10,000 – 30,000 s<sup>-1</sup>) [10] is at least an order of magnitude higher than the range of the rheometer, which can measure up to 1000 s<sup>-1</sup>. Therefore, the viscosity during injection would be significantly lower than the minima of the data shown in Figure 2. There is a large difference between the viscosities of the three highest concentrations and the lowest, indicating that there may be a threshold between 25-50 mg/g where there is a change in interparticle interactions, such

that the system is more Newtonian below the threshold and more structured above it. The high zero-shear viscosities of the higher concentrations (up to 10,000 cP) effectively limit the speed of particle sedimentation, but the 25 mg/g samples clearly

sediment more quickly. The temperature dependence of the viscosity is shown in Figure 3. Shear thinning is evident at all temperatures, indicating that EF should be injectable across a wide range of temperatures. Shear thinning is less pronounced at 4 °C, which is

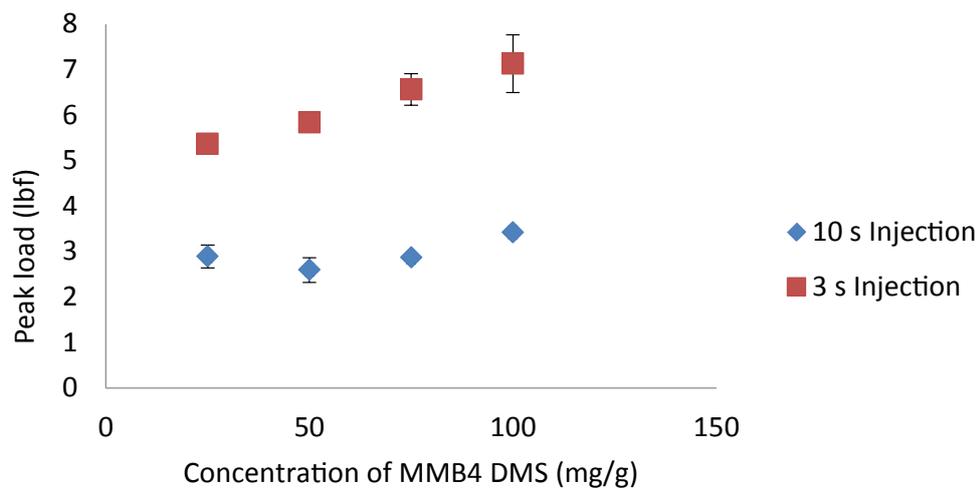


**Figure 3.** Shear rate dependent dynamic viscosities of EF formulations at various temperatures. Shear thinning is evident at all temperatures, maintaining injectability. Shear thinning is less pronounced at 4 °C, which is near the pour point of CSO.

near the pour point of CSO (-5 to 0 °C). The viscosity profiles shown in Figure 2 and Figure 3 suggest that EF formulations with a wide range of physical properties should be injectable over a wide range of environmental conditions.

### Injectability

It is important to verify that EF suspensions with high viscosity are injectable through a typical syringe without requiring excessive force or time to inject.



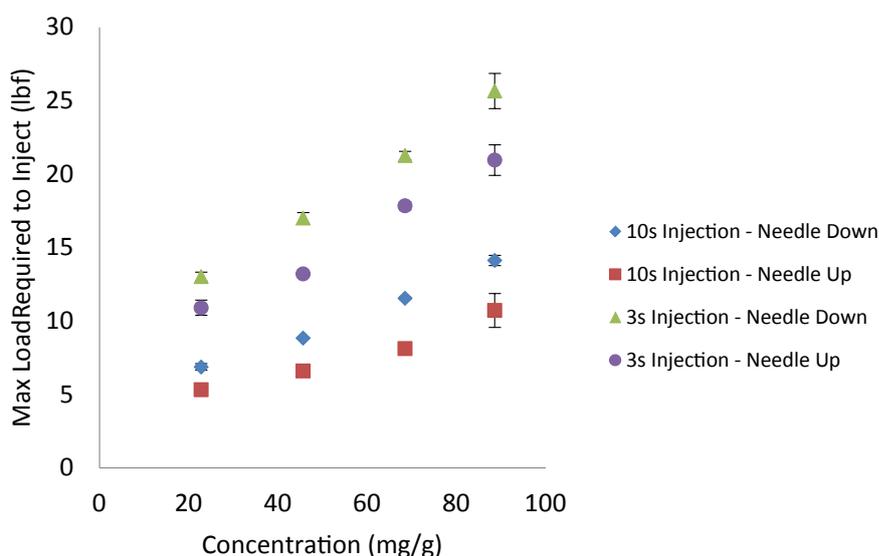
**Figure 4.** Average ( $n = 3$ ) peak force required to eject MMB4 DMS EF from a 1 mL glass syringe with staked 23 ga needle. The suspended nanoparticles were homogeneously distributed in the syringe prior to ejection. Error bars represent one standard deviation ( $n = 3$ ).

In a particulate suspension, the particles must be small enough to be injectable through the desired needle bore. As an example, the ATNAA autoinjector needle size is 23 ga, which has an inner diameter of approximately 340  $\mu\text{m}$ . As synthesized, MMB4-DMS powder has a mean particle size of approximately 400  $\mu\text{m}$ , clearly too large to be injectable. Various dry milling techniques which were originally investigated were able to reduce the mean particle size to less than 10  $\mu\text{m}$ , which is injectable. At the  $\sim 10\ \mu\text{m}$  size, the concentration of MMB4 DMS microparticles in CSO has to be very high ( $\sim 400\ \text{mg/g}$ ) for the EF to have a high enough viscosity to resist sedimentation. Reducing the particle size into the submicron regime by wet milling increased the viscosity dramatically, reducing the sedimentation velocity at the desired concentration of approximately 100 mg/g.

An electromechanical frame was equipped with a load cell and used to measure the amount of force required to eject various EF samples from 1 mL glass syringes with 23 ga staked needles. The syringes were supported in a manner similar to an autoinjector, such that the end of the barrel tapering into the hub was the load-bearing part, instead of the flange that would typically support the load when injecting manually. Figure 4 shows the peak loads (forces) required to eject a typical EF sample ( $\sim 300\ \text{nm}$  median particle size) with various

concentrations over different time periods. A slow injection (10 sec) required only  $\sim 3\ \text{lbf}$ , whereas a faster injection (3 sec) required  $\sim 5\text{-}7\ \text{lbf}$ . The concentration dependence was more pronounced for the faster injection, probably because the very high shear rate induced by the faster flow through the needle negated some of the shear thinning effect. Even so, all of these peak loads are well below the force required to break the syringes ( $>50\ \text{lbf}$ ).

Even though EF samples with nano-sized MMB4 DMS particles are highly resistant to sedimentation, some sedimentation does occur over long periods of time, especially at elevated temperatures. Therefore, another experiment was conducted to determine if sedimented samples were injectable. Filled syringes were centrifuged for at least 12 hours at 500 g, in two orientations (“needle down” and “needle up”), resulting in a worst case scenario of sedimentation far exceeding any conditions that could be encountered in a fielded device. The direction of the needle signifies the orientation in which the syringes were centrifuged: for “needle down” samples, the gravitational force vector was pointed toward the needle, resulting in syringes containing sedimented particles closer to the needle end of the syringe. For “needle up” samples, the gravitational force vector was pointed away from the needle, resulting in sedimented particles closer to the plunger end of the syringe.



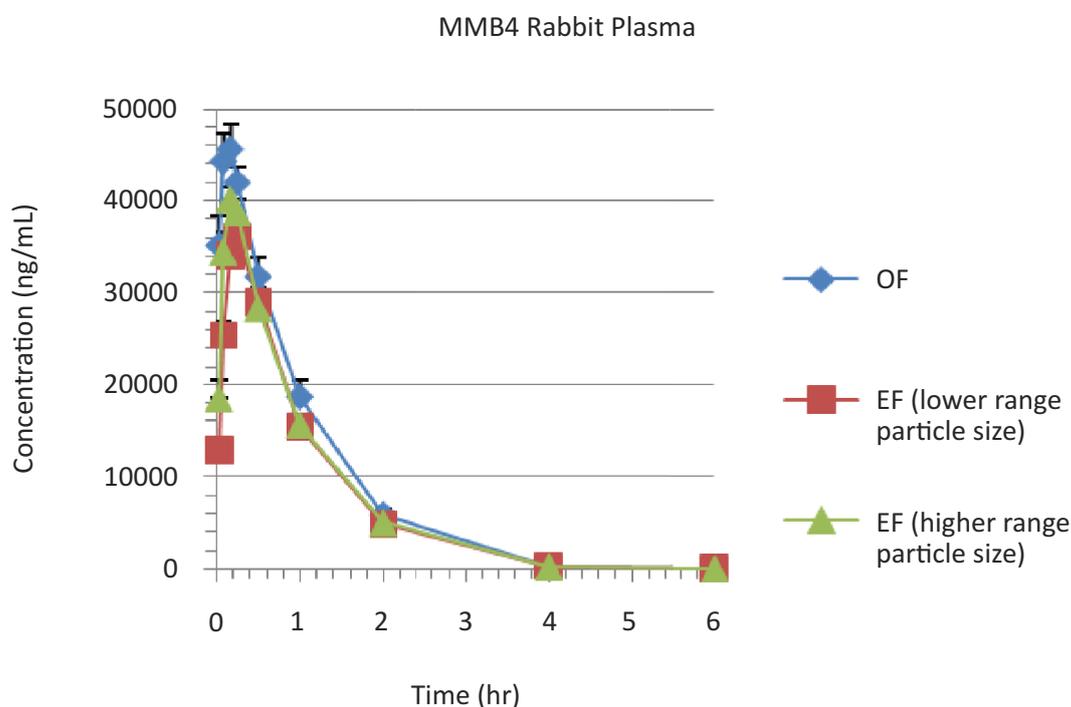
**Figure 5.** Average ( $n = 3$ ) peak force required to eject MMB4 DMS EF from a 1 mL glass syringe with staked 23 ga needle. To force sedimentation of the suspended nanoparticles, the filled syringes were centrifuged in two different orientations prior to ejection: “needle down” (gravitational force vector pointed toward the needle) and “needle up” (gravitational force vector pointed away from the needle). Error bars represent one standard deviation ( $n = 3$ ).

As shown in Figure 5, the peak loads required to inject the sedimented samples were significantly higher than the homogeneous samples shown in Figure 4. Nevertheless, all samples remained injectable without breaking the glass syringes. The concentration effect is also more pronounced, and the “needle down” syringes required more force to inject, probably because the plug of particles at the needle end of the syringe required a greater peak force to start the injection than the “needle up” samples which expelled the particle plug at the end of the injection. Expelled samples were collected and assayed to determine whether sedimentation affected the dose delivered. All assay values were within approximately 6% of the expected value (data not shown), which is within the error of the HPLC method.

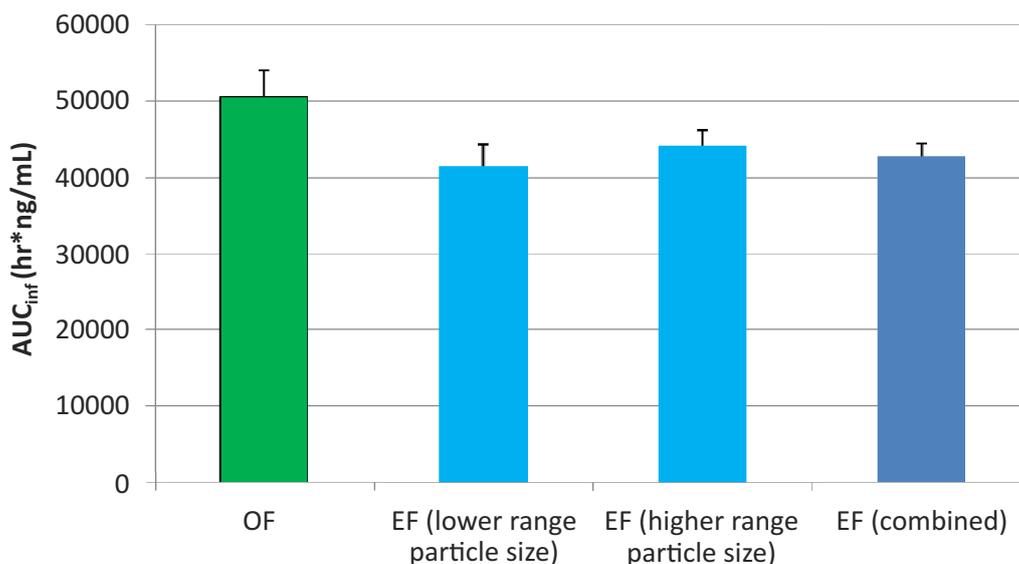
### Bioavailability

Range-finding preclinical studies were conducted to determine the dose proportionality of multiple combinations of particle sizes and concentrations of MMB4 DMS EF by evaluating PK parameters following a single IM injection in male Sprague-

Dawley rats and in male New Zealand White rabbits. Three dose levels were varied using different combinations of concentrations and particle sizes as follows: 1.25 mg/kg (5 mg/mL at 200, 600, and 6000 nm), 15 mg/kg (60 mg/mL at 200 and 600 nm), and 30 mg/kg (120 mg/mL at 400 and 6000 nm). The goal of the study was to test the effects of particle size and concentration of the micro/nanoparticle suspension formulation of MMB4 DMS on bioavailability. Establishing bioavailability across a large range of particle size distributions would allow for a robust manufacturing process by enabling a wide specification range for particle size. These range-finding preclinical studies showed that the MMB4 DMS EF nanoparticle suspension formulations were bioequivalent across a particle size range of 200 to 6000 nm, measured at concentrations that ranged from 5 to 120 mg/mL [5]. The systemic exposure level was also found to be proportional to the dose across this concentration range. Therefore, the particle size range of samples for further studies was selected so as to optimize the physical stability and injectability of the suspensions at the chosen concentration of 100 mg/g.



**Figure 6.** Rabbit Plasma Concentration of MMB4 Following IM Injection. MMB4 DMS OF and MMB4 DMS EF show a similar  $T_{max}$  and  $C_{max}$  for a 25mg/kg dose delivered at a concentration of 100 mg/g. Reprinted from “Good Manufacturing Practice: Manufacturing of a Nerve Agent Antidote Nanoparticle Suspension,” by A. P-Z. Clark, H.Dixon, N. L. Cantu, L. A. Cabell, and J. A. McDonough, 2013, *Int. J. Toxicol.*, 32(S2), p. 6S.



**Figure 7.** Comparison of MMB4 DMS AUC<sub>inf</sub> for MMB4 DMS OF versus MMB4 DMS EF. Reprinted from “Good Manufacturing Practice: Manufacturing of a Nerve Agent Antidote Nanoparticle Suspension,” by A. P-Z. Clark, H.Dixon, N. L. Cantu, L. A. Cabell, and J. A. McDonough, 2013, *Int. J. Toxicol.*, 32(S2), p. 7S.

A GLP bridging study was performed with two different samples of EF (median particle sizes of approximately 260 and 290 nm) to determine the PK and relative bioavailability of EF compared to OF. The object of this GLP bridging confirmatory study was to compare the systemic exposure of MMB4 DMS OF, an aqueous MMB4 DMS formulation which possesses acceptable  $C_{max}$  and  $T_{max}$  values for treatment of nerve agent intoxication, versus MMB4 DMS EF, a nanoparticle suspension formulation, in male New Zealand white rabbits following an (IM) injection [6]. The primary goals for this study were to confirm that systemic exposure was independent of particle size and to compare the systemic exposure of MMB4 DMS OF to MMB4 DMS EF following an (IM) injection. All formulations were prepared at MMB4 DMS concentration of 100 mg/g and dosed at a level of 25/mg/kg for the rabbit.

The results shown in Figure 6 and Figure 7 indicate no apparent formulation effects on the MMB4 DMS PK parameters related to formulation type (OF or EF). They also confirm that exposure was independent of particle size, within experimental error attributed to variability between animals. Observed and predicted  $C_{max}$  and  $T_{max}$ , AUC values, absorption half-life and elimination half-life values, clearance and apparent volume of distribution are similar across all three groups. An important manufacturing outcome

of these studies was the confirmation that there was no effect on the MMB4 DMS pharmacokinetics due to particle size. This information will be valuable when setting acceptable particle size specifications for the manufacture of the MMB4 DMS EF drug product.

## CONCLUSION

SwRI has developed a novel formulation technology for autoinjectable drug products that solves many of the problems of traditional therapeutics for emergency treatment of nerve agent intoxication using MMB4 DMS Enhanced Formulation (EF), which is a suspension of MMB4 DMS API nanoparticles in a biocompatible cottonseed oil vehicle. This suspension in hydrophobic CSO minimizes exposure to water, which causes rapid hydrolysis of the bis-pyridinium oxime in traditional aqueous formulations. The relatively high loading of nanoparticles (10% or more) gives the formulation non-Newtonian rheological properties. The high zero-shear viscosity makes the suspension resistant to sedimentation, while shear thinning causes the viscosity to drop when flowing through a needle during injection. This allows the drug product to be injectable by standard autoinjectors and needles.

The vastly improved stability of Enhanced Formulation drug products increases shelf life dramatically, particularly under operationally relevant storage conditions of high temperature and/or high humidity. EF technology will significantly reduce the cost required to maintain stockpiles of CBRN antidotes, and it will be applicable to a diverse range of drug products and applications. Any API that is subject to hydrothermal degradation is a good candidate for EF, and multi-drug cocktails are also simplified, because of the limited interactions between insoluble nanoparticles in suspension together. In summary, EF nanoparticle suspension technology has the potential to transform the future of autoinjectable drug delivery.

#### ACKNOWLEDGEMENT

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