NIST Technical Note 1934

Cement Paste Reference Material (SRM 2492) Shelf-Life Extension

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Abstract

Cement-based materials (e.g. cement paste, mortar and concrete) are complex rheological fluids that display time-dependent and shear-dependent rheological behavior. Over the years, various concrete rheometers have been proposed and made available commercially; however, there is no method to calibrate them. Furthermore, typical calibration fluids used in commercial rheometers are not well suited for the concrete rheometer calibration due to their high cost and they are Newtonian (i.e., not a complex fluid). Therefore, there was a clear need for a reference material specifically designed for concentrated, granular suspensions, such as cement paste, mortar and concrete; this need led to the development of a new series of reference materials for calibration of devices used in cement-based suspension rheological testing. The reference material to simulate cement paste, SRM 2492, was composed of a fine limestone powder in a corn syrup matrix. While the ranking of mixtures tested using these rheometers tend to match, the absolute values for the rheological properties of the mixtures evaluated with different rheometers are not well correlated [1, 2]. Additionally, due to microbial growth in the paste matrix, the shelf life of standard reference material 2492 was limited to 7 d. This paper presents the results of a study to analyze how to minimize microbial growth within the paste matrix. Various biocides that extend the shelf-life of the reference material were examined, with the most promising method being sodium propionate, a non-toxic chemical. Furthermore, recommendations to improve the storage of SRM are provided to extend the usable shelf life.

Keywords: Rheology, Reference Materials, Biocide, Microorganisms, Microbes

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Acknowledgements

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1 Introduction

Rheology is a branch of science that deals with the study of the flow and deformation behavior of fluids. With the advances in the development of cement-based suspensions, such as self-consolidating concrete, underwater concrete, and 3-D printing slurries, there is an increasing desire to accurately determine the rheological properties. While great progress has been made in the field of concrete rheology, one aspect that has limited the broader adoption of rheological characterization in the concrete community is due to the fact the rheological properties extracted from the concrete rheometers lack good correlation amongst their absolute values. The American Concrete Institute (ACI) Committee 238 used two international testing campaigns to determine correlation between existing concrete rheometers and found that there was a need for a reference material to calibrate the rheometers [1, 2]. Discussions from ACI Committee 238 found that the reference material cannot be the traditional standard oils as they are likely to be too cost-prohibitive to fill a whole concrete rheometer (20 L); additionally, standard oils display Newtonian behavior, which is not representative of the rheological behavior of concrete. Therefore, there was a clear need for a reference material that could simulate paste, mortar and concrete with a large size distribution of particles. Additionally, the reference material needed to be a Bingham fluid since many cement-based materials exhibit Bingham behavior. This led to the development by National Institute of Standards and Technology (NIST) of a series of standard reference materials (SRM), composed of a corn syrup solution and fine limestone powder with glass bead inclusions.

SRM 2492 is a paste for calibration of rheometers used for cement paste measurements; a key attribute of this paste was that it was engineered to display Bingham behavior. It was found from preliminary studies that microorganism growth seems to be a cause of the deterioration of the SRM 2492, which limits the shelf life to 7 d. Therefore, efforts were focused in this study on finding an appropriate biocide that ensures the SRM 2492 is viable for a length of time that exceeds the certified 7 d shelf life [3, 4].

It was observed that after 7 d of storage microbial growth becomes noticeable, along with changes in the rheological properties of the SRM paste [5]. One method to destroy microbial growth would be heat treatment sterilization, but it is labor intensive since all instruments and containers need to be heat treated, as well as all materials such as the limestone and water. This is not an easy task in a commercial laboratory and especially in the field. The other solution would be to use chemical sterilization, such as a biocide. It is preferable that the biocides selected are not toxic to humans, so that no special precautions need to be implemented to use and for disposal. Therefore, this study was limited to the analysis of three biocides: grapefruit seed extract (GSE), Honey-B-Healthy (HBH) and sodium propionate (SP). The optimal biocide addition time was also examined: initially with the mixing water or later after the mixture is older than 7 d. A discussion is presented on the selection criteria of the biocides, as well as other factors that could contribute to the deterioration of the SRM and provides solutions to prolong the shelf life of the SRM. Furthermore, the rheological properties were monitored to ensure the selected biocide did not cause significant changes to the viscosity of the SRM.

2 Background

SRM 2492 [3] is a non-Newtonian reference material, with Bingham behavior composed of non-setting materials. The constituents are fine limestone, corn syrup and water, with the first constituents provided in the SRM box and the water provided by the user. The re-certification [4] of this SRM served as the matrix to create SRM 2493 [6], which is the mortar rheology SRM with the addition of 1 mm glass beads. SRM 2493 will then contribute as the stepping stone to develop a concrete SRM 2497 by the addition of 10 mm beads, thus completing the multi-phase series (expected in 2016).

It was observed during the development of SRM 2492 that the rheological properties are stable up until 7 d after mixing. **Figure 1** presents the plots showing the temporal evolution of yield stress and viscosity of the SRM paste [5]. Initially, the yield stress and plastic viscosity of the SRM were around 55 Pa and 6 Pa·s, respectively, when stored at 6 °C. Similarly, the yield stress and plastic viscosity of the SRM when stored at 23 °C, were around 62 Pa and 7 Pa·s, respectively. These values are higher than typical values measured for paste, and are likely due to the additional viscosity of the corn syrup which can be found in the original certification report [3]. Storing the SRM at a lower temperature, 6 °C, in between measurement was thought to inhibit the growth of microorganisms, but this had no significant effect on the results as shown in **Figure 1.** The increase in rheological properties after 10 d are apparent on both storage conditions.

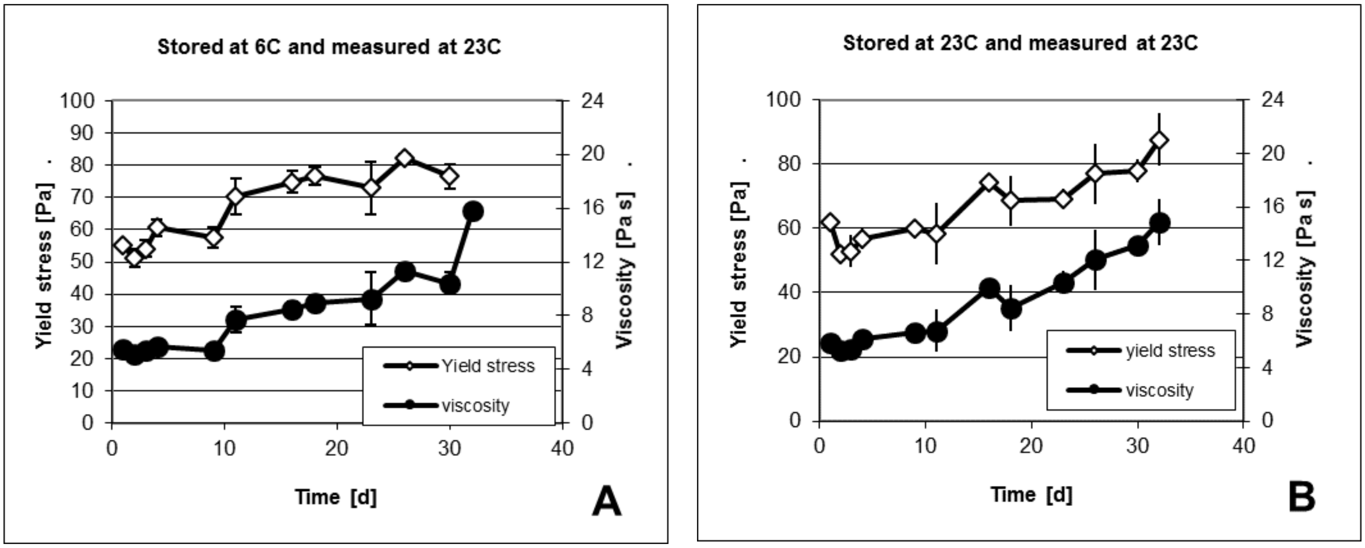


Figure 1: SRM 2492 rheological properties show stability until they exceed age = 7 days, the samples shown were stored at [A] 6 °C and [B] 23 °C. [5]. The standard deviation is shown in the figures.

The first leading factor causing the data divergence was discovered from observations of microbial growth on the SRM, left in the lab for two years, as shown in **Figure 2.** To verify this hypothesis, two mixtures were prepared: an aqueous solution of corn syrup and an SRM. The aqueous solution of corn syrup was placed in a closed, plastic container and after just 12 d of storage the microbial growth was already clearly visible on the side of the containers (**Figure 3**). On the other hand, the SRM placed in a similar container, showed (**Figure 4)** bacteria growth was visible after 9 weeks. The slow appearance of the bacteria might be due in part on the difficulty to detect the bacteria in the SRM, compared to the flask. It is speculated that the limestone had additional traces of minerals which could provide additional nutrients, the microorganisms feed on the sugars in the corn syrup, or that the limestone serves as substrate promoting the formation of microbial networks (e.g., biofilms), ultimately leading to further microbial growth.

To test the hypothesis that the microorganisms seen would be generated in a typical SRM mixture, small amounts of SRM mixture were placed in one form of accelerated growth medium (i.e., nutrient broth) in a culture flask. **Figure 5** shows two cases: a normally prepared SRM and a

SRM with all the ingredients sterilized by heat-treatment first (See section 3.2.1). Within 4 d, a green-yellow pubescence is visible in the normal sample but not on the sterilized sample**.** It was speculated that the presence of the microorganisms could be causing the increase in viscosity and yield stress observed in **Figure 1**.

Figure 2: Two-year old SRM stored in a closed container in the laboratory

Figure 3: Corn syrup + water solution showed signs of microbial growth after 12 d.

Figure 4: Microbial growth appears in top layer of SRM paste. Image taken at 9 weeks.

Figure 5: Accelerated microbial growth environments analyzing a (left) normal SRM sample and (right) a sterilized SRM sample.

These observations led to the evaluation of two possible approaches that limit microbial growth and control the degradation of the SRM after mixing: (1) heat treatment sterilization and (2) chemical sterilization. Heat treatments are very effective at deactivating/killing microorganisms, however the need for strict laboratory controlled conditions may hinder SRM users from being able to successfully implement this method. The efficacy of a particular biocide will vary depending on its concentration and the type of microorganism present, however chemical sterilization treatments using biocides are advantageous when there are concerns that the high temperatures from heat treatments may damage the material. Additionally, the simplicity of the chemical sterilization means that the SRM owner can introduce the biocide during initial mixing or at a later time period after the SRM is prepared.

Another interesting phenomena was also investigated. The rheological data shown in the SRM 2492 certificate show an increasing trend with time from 1 d to 7 d. All the values are still within the uncertainty posted, and can still be considered consistent with the certified values. One hypothesis, is that the plastic containers used to store the material were not adequate to avoid some water losses. Alternative containers were also examined and are discussed later in this study.

3 Experimental Procedure

3.1 Materials

3.1.1 SRM 2492

The main component in this study, SRM 2492 cement paste reference material, forms the matrix of all samples examined with the following composition, as per the certificate [7].

- Corn Syrup: 200.0 g
- Distilled water: 63.16 g
- Limestone: 458.1 g

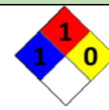
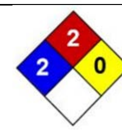
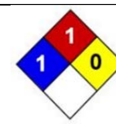
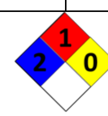
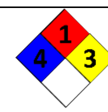
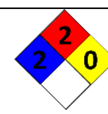
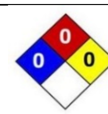
The constituents need to be prepared by the user according to the specification described in the certificate [7]. Further detail about the composition or properties of SRM 2492 can be found in the published certification report [4].

3.1.2 Biocides used

The selection of the biocides was restricted to those that were commercially available and inexpensive products and are listed in **Table 1**. On the other hand, a few non-commercial biocides were also analyzed for comparison and to offer a variety of solutions. These non-commercial biocides were prepared in-house at NIST. The biocides (with abbreviations[1] for future references) considered are listed below and followed by a more detailed description provided in the following sections.

[1] Abbreviations specified are solely for use during this report. They were created to ease references to the biocide name in and in no way represents any other brand or scientific name.

Table 1: Biocides used in this report

Biocide[1]	Preparation for usage	NFPA	
Grapefruit seed extract (GSE)	Use as purchased		
Honey-B-Healthy (HBH)	Use as purchased	Lemongrass Oil	
		Spearmint Oil	
		Sucrose	
Sodium propionate (SP)	Preparation needed		
Sodium azide (SA)	Preparation needed		
Cinnamaldehyde (CN)	Preparation needed		
Silver nanoparticle suspension (SNS)	Preparation needed		

3.1.2.1 Grapefruit Seed Extract (GSE)

The grapefruit seed extract (GSE**)** is a liquid extracted from a grapefruit's seeds and pulp. GSE is a great source of plant antioxidants which makes it rich in nutrients and phytochemicals [8]. There have been a number of reported uses of GSE in cosmetics and dietary supplements which claimed antioxidant and antibacterial effects [9].

The effectiveness of a processed GSE was investigated in the literature, as the commercially available biocide would also be processed into chemical (liquid) form by the biocide supplier. Results suggest GSE consistently contained antibacterial characteristics that are comparable to that of proven topical antibacterial. Although the GSE appeared to have greater inhibitory effect on gram-positive organisms than on gram-negative organisms, its comparative effectiveness against a wide range of bacterial biotypes is significant [10]. Gram-negative bacteria tend to have more longevity than gram-positive and are tougher to kill due to their structural differences, specifically in the thickness of the peptidoclycan layer(s). To account for the toughness of the gram-negative structure, the biocide of interest would preferably be a broad spectrum biocide.

The levels of toxicity for GSE concentrations [13] would signify both microbial and nontoxic threshold values. The usage of scanning transmission electron microscopy (STEM) revealed the mechanism of GSE's antibacterial activity. The results showed that GSE disrupts the bacterial cell membrane and liberates the cytoplasmic contents within 15 min after contact. However, tests indicate that GSE remains bactericidal but toxic at GSE solution concentrations of 1:1 through 1:128, while at a 1:512 dilution GSE is bactericidal but completely nontoxic.

3.1.2.2 Honey-B-Healthy

The Honey-B-Healthy (HBH) is a commercially available product and it was expected to serve as a preservation agent for the corn syrup solution. HBH uses lemongrass and spearmint oil concentrates for its naturally occurring pheromones. As shown in these studies, HBH concentrate [14] can be kept in syrup solutions with tightly sealed containers and fed to bees when needed during nectar shortages to help maintain healthy productive colonies for pollination and honey production.

The safety information of the main components for HBH (lemongrass oil, spearmint oil, and sucrose) were examined for possible hazards using available literature. The NFPA (National Fire Protection Association) ratings from the SDS (Safety Data Sheet) for all three components are summarized in **Table 1**, which show that the HBH is not a hazardous material for users due to its low NFPA ratings. The blue, red and yellow diamonds represent the health, flammability and instability hazard, respectively. Where the Health (blue) Rating is 2 indicates continued exposure can lead to temporary incapacitation unless given prompt medical attention. Also, Flammability (red) 2 indicates the material needs to be exposed to relatively high temperature before ignition can occur. A rating of 2 on the Instability (yellow) signifies risk of violent chemical change at high temperatures or pressures. Where Health 1 rating is indicated, only minor injury can occur if no treatment is given, and Flammability 1 indicates materials need preheating before ignition can occur. Yellow 1 indicates normally stable conditions, but high temperatures can make instability exist. Any ratings of 0 are considered normal or stable conditions.

3.1.2.3 Sodium Propionate

Sodium propionate (SP) is used as a food preservative in the United States, the European Union, and several other countries. Although SP is slightly toxic, it is generally safe in small amounts (less than 5000 mg per kg of total SRM mass). SP (also known as Mycoban) is commercially available but can also be easily prepared by the reaction of propionic acid with sodium carbonate or sodium hydroxide.

For this study the SP was prepared at NIST by the stoichiometric addition of 2 mol/L of sodium hydroxide into neat liquid propionic acid. A 10 % stock solution (by mass) of SP in deionized water was used as the working solution and diluted as needed.

3.1.2.4 Sodium Azide (NaN_3)

Sodium azide (SA) is a sodium salt of hydrazoic acid that is colorless, odorless, crystalline solid (salt-like) and is soluble in water or liquid ammonia. SA has a broad spectrum of applications as a biocide. The main applications of SA are as an additive to perishable food where it is used to prevent the growth of microorganisms. For this study, a 4 % solution (wt. /wt.) of Sodium Azide in deionized water was made as the primary working solution and diluted as needed.

However, SA can be highly toxic and the solid material can present an explosion risk when shocked or heated [15]. Nonetheless, SA is not harmful to humans if the dosage is between 0.3 mg to 150 mg dosage [16]. Since levels of toxicity typically depend on the concentration, previous studies have examined use of low concentrations of SA to minimize the effect of toxicity [17]. The analysis of this biocide found that a concrentration of 0.01 % of biocide per total SRM sample mass not only helps prevent the characteristic growth of microorganisms but it also inhibits common bacteria and other microorganisms and was a total dosage of 72 mg of biocide.

3.1.2.5 Cinnamaldehyde (C_9H_8O)

Cinnamaldehyde (CN) is a liquid, organic compound which aids in avoiding bacterial growth and mold formation. CN is typically used in the food industry, where it is often used as a fungicide and insecticide due to its low toxicity for human. Although this study used this product as produced synthetically, it should be noted that CN can be obtained naturally from the bark of cinnamon trees. It is used as flavor in the food industry in products such as chewing gum, candy and beverages.

3.1.2.6 Silver Nanoparticle Suspension

The silver nanoparticle suspension (SNS) is a biocidic suspension composed of silver (Ag) nanoparticles capped with citrate. The citrate is essentially the salt or ester of citric acid. The Ag nanoparticles have been applied in various fields such as electronics, optics, water treatment and biotechnology. SNS serves this wide range of industries due to Ag antimicrobial behavior. Recent [18] reports showed that Ag nanoparticles have potential risks to human health due to their “Trojan horse” mechanism where the silver ions are released via dissolution. The studies on the toxicity of SNS portray that the measurement of the Ag ion fraction is crucial for toxicity studies. The suspension examined here was composed of 60 mg Ag nanoparticles per liter of water which is a non-toxic level for humans. The Ag nanoparticles had a particle size of 20 nm in diameter.

3.2 Sterilization Approaches

3.2.1 Heat sterilization

Sterilization could be performed using a variety of methods, such as dry heat, autoclave or UV sterilization. To determine the effect of sterilization on the microbial growth, the easily available dry heat was deemed to be a good approach. This was based on the principle that microbial growth is improbable if there are no viable microbial cells in the system. This approach attempts to kill the microbial sources via heat treatment of the SRM constituents, instruments, and other laboratory equipment that comes in contact with the SRM.

The sterilization of the constituents began by heating the limestone at 150 °C for 48 h in order to ensure that devitalization of any bacteria or microorganisms in the limestone is reached. Then, a glass container is sterilized by cleaning it with an alcohol such as ethanol, and is used for storage after heating. The water was deionized and boiled at 100 °C, then stored in a sterile container at room temperature. There is no need to sterilize the corn syrup, as without dilution it does not allow microbial growth.

Then, all the instruments and any tool that came into contact with the SRM was sterilized. This was achieved by rinsing all parts repeatedly with ethanol. By sterilizing all instruments, external microbial contamination was avoided through pre-mixing and sampling of material which were to be done during all measurements. Alternatively, to use less ethanol, heat sanitation may be used for the metallic tools or instrument parts by placing the items in the oven with the limestone. Also, gloves and dust masks were used to help reduce opportunistic microbial contamination from the human body. Once the materials were cooled, they were mixed according to the mixing procedure in the certificate of analysis [7] using a high shear blender with temperature control. The storage containers were also sanitized with ethanol before storing the SRM paste. The storage conditions, such as room temperature and location, were the same as for the normal samples.

3.2.2 Chemical sterilization

Two approaches were used to introduce the biocides (as chemical sterilization agents): (1) immediate addition and (2) delayed addition. The immediate addition approach consisted of following the standard mixing procedure for SRM 2492 as per the certificate of analysis [7]. The only modification to the process is the inclusion of the biocide dosage with the mixing water. By adding the biocide into the water component during preparation, the water acts as the medium that the biocide particles can use to travel into the SRM paste.

The second approach adds the biocide chemical at a later age, after the SRM paste has already been used and stored. Each SRM 2492 batch contains enough material such that it was split in half and stored in two separate containers prior to testing. One half served as a control mixture while the other half received a dosage of a biocide. In this approach, the biocide is added into the SRM paste then re-mixed with a high shear blender for 2.5 min at 300 rpm (31.4 rad/s). This re-mixing step is highly recommended, as stated in the certificate of analysis, when re-using the SRM at a later age regardless if the biocide is added or not.

3.3 Measurement Devices

3.3.1 Rheometer

Since this study focuses on extending the life of SRM 2492, the rheometer protocol followed the exact procedure that was certified for SRM 2492 [4]. Therefore, serrated parallel plates of 35 mm in diameter were used to gather the rheological data. The gap between the two parallel plates was 0.600 mm ± 0.001 mm [4] and the temperature of the rheometer was maintained at 23 °C ± 0.5 °C [4] during all tests via controlled water bath.

The testing protocol consisted of shearing the material at 0.01 s^{-1} for 150 s before starting the Bingham test. The Bingham test consisted of increasing the nominal shear rate from 0.1 s^{-1} to 50 s^{-1} (15 points in total) and then decreasing shear rate from 50 s^{-1} to 0.1 s^{-1} (20 points in total). At each step, a time of 30 s was allowed for the torque readings to reach steady state behavior before the torque value was recorded. More details of the procedure could be found in [4].

3.3.2 Vibrational viscometer

This type of viscometer can only be used with a Newtonian fluid whose viscosity does not change with shear rate. The viscometer consists of a rod that is immersed in the fluid to be measured. The rod vibrates at a high frequency, and it measures the damping due to the fluid. The amplitude is small and the power consumed is then converted to viscosity [19]. The viscosity measured is the dynamic viscosity (μ) of the fluid, which is output by the viscometer in units of centipoise[2] [cP]. The instrument used also provides the temperature if needed.

3.4 Moisture Content Analysis

To determine the amount of water content in a prepared SRM batch, small samples at various time of storage (i.e. various sample ages) were placed in an oven in order to evaporate all the water, and the mass was measured before and after heating. Furthermore, in order to truly determine whether the containers were poorly sealed, just water was placed in various containers and the mass loss of the container was monitored over time.

[2] Unit Note: 1 cP = 1 mPa·s = 10^{-3} Pa·s

4 Results and Discussion

4.1 Heat Treatment Sterilization

The sterilization process yielded evidence of microbial depletion. As shown in **Figure 6**, there was a clear display of microbes in the normal SRM paste sample (N-S), while the heat treated sterilized sample (S-S) showed considerably less microbial growth [3] after 9 weeks. The only visible formation on the S-S was a layer of water due to bleeding and sedimentation, which were found to occur on SRM 2492 samples during storage. This phenomenon is further discussed in **Section 4.3**.

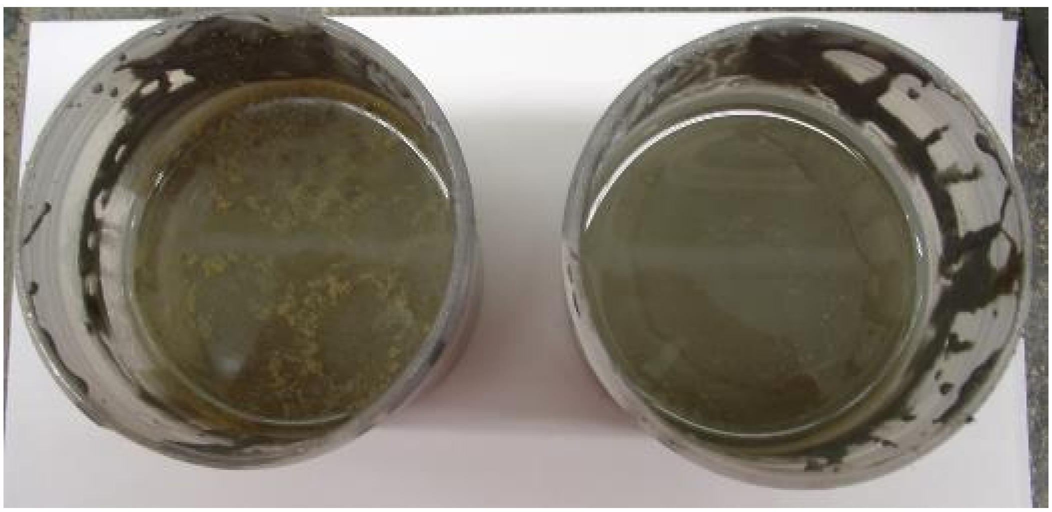


Figure 6: Comparison at 9 weeks of sterilized sample (S-S) on the right vs. normal sample (N-S) on left. N-S sample shows evidence of microbial activity on its surface.

Although the heat treatment sterilization method reduced the presence of microorganisms in the SRM paste, it is was deemed cumbersome as sanitation of parts for every rheological measurement will greatly increase the required time for testing. Nevertheless, this method is a non-toxic approach to extending the SRM paste shelf-life.

4.2 Analysis of Biocides

To pre-screen the biocides effectiveness, they were tested using only the aqueous solution of corn syrup at first. To determine the effect of the addition of a biocide on rheological properties, the viscosity of the solution was monitored before and after addition of the biocide. The measurement was done by a vibrational viscometer.

To ensure that the viscosity measured was stable, the data were collected up to 20 min after mixing. Each mixture was measured three times to determine the uncertainty. **Figure 7** shows the data obtained. All three biocides and the control displayed decreasing viscosities until reaching a 15 min threshold, after which the viscosities stabilized from 15 min to 20 min. Therefore, it is recommended to wait 20 min between mixing end time and making rheological measurements in order to allow the viscosity of the liquid components to stabilize.

Table 2 only reflects the viscosity values of the biocide emulsions after 15 min of mixing as it is considered the stable value. The biocides did cause an increase in viscosity compared to the control, but none of them altered the changes in viscosity over time. The biocide dosages for measurements shown in **Figure 7** were specified at 0.2 % mass of biocide per mass of water-syrup solution. Significant alterations to the biocide dosage can lead to changes in SRM viscosity, therefore this study analyzes the optimal dosages for SRM 2492, as discussed in the following sections.

Table 2: Stable viscosity values determined using a vibrational viscometer for the biocide emulsions analyzed in this study obtained after 15 min post-mixing.

Biocide Emulsion Viscosities [mPa·s]				
Biocide	*Time after mixing*		**Average Viscosity**	Standard Deviation
	15 min	*20 min*		
SP	128.5	129.1	**128.8**	0.3
GSE	125.4	125.5	**125.4**	0.1
HBH	126.7	126.7	**126.7**	0.0
Control	124.9	124.9	**124.9**	0.0

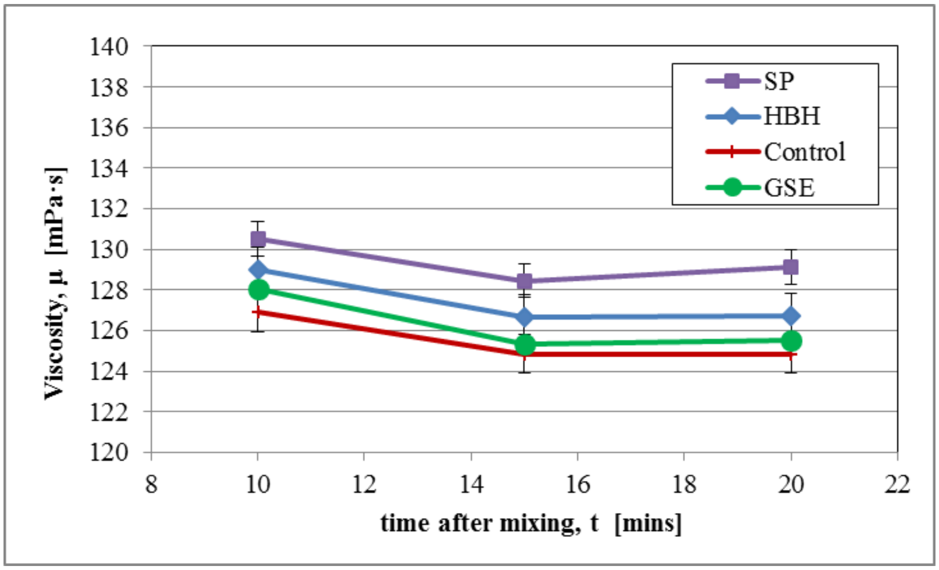


Figure 7: Viscosity behavior over time after mixing the biocide emulsions portray threshold value of 15 min prior to viscous stability. The uncertainty bar is one standard deviation calculated from 3 measurements on the same mixture.

4.3 Biocide Rheological Effects

The solution to use biocide to increase the shelf-life led to several application questions:

- Optimization of addition time: with the mixing water or later? How late to ensure that biocide addition is still effective?
- How to determine the correct dosage for each of the biocides selected?
- How the addition of the biocides would affect the rheological properties of the SRM 2492 as shown in the certificate?

In this section, the answer to these questions will be analyzed.

It should be noted that the biocides' effectiveness was tested using various methods, such as in the SRM or in a corn syrup solution kept in a closed container and examined periodically, or in a cell culture flask where nutrients were added to accelerate the bacteria or fungi growth. This latter method will be referred to as *accelerated method* in this report, and used a 0.5 percent agar growth medium enriched with: yeast extract (1 %), peptone (2 %), D-glucose (4 %), NaCl (0.5 %), and $CaNO_3$ (0.2 %).

The biocides discussed in the following sections are presented in the order that each series was analyzed logistically. **Table 3** displays the contents which will be discussed and the objective of each section. As well as nomenclature used throughout the following sections.

Table 3: Nomenclature and objective of biocide series results sections

Nomenclature	*Section*	Objecive
Biocide Series 1	*Section 4.3.1: Preliminary biocides*	Initial step or proof of concept towards finding the most suitable biocide for SRM users.
Biocide Series 2	*Section 4.3.2: Immediate addition of biocides*	Observe stability in rheological behavior of SRM paste when biocide is introduced at the beginning of SRM's life.
Biocide Series 3	*Section 4.3.3: Delayed addition of biocides*	Observe stability in rheological behavior of SRM paste when biocide is introduced later in the SRM's life.

4.3.1 Initial preliminary studies of the effect of biocides

This series of the biocide analysis consists of Cinnamaldehyde (CN), Sodium Azide (SA), and Silver Nanoparticle Solution (SNS). This set of biocides options were analyzed prior to the options presented in the previous section, and served as the initial step or proof of concept towards finding the most suitable biocide for SRM users. In this section, the results obtained from these preliminary studies [5] are presented. Such initial results inspired this study's selection of biocides (HBH, GSE, and SP) which are to be the focus of discussion throughout this study.

The **silver nanoparticle solution (SNS)** was prepared by replacing the distilled water component of the SRM with SNS (60 mg of silver nanoparticles per liter of water). The corn syrup and limestone components remained the same. The rheological behavior of SRM 2492 when SNS was added remains very similar to Bingham behavior, like the control sample, as shown in **Figure 8.** The viscosity appears to be slightly higher for the SNS sample since the slope is slightly steeper. However, microbial growth was confirmed when monitoring the SNS + SRM 2492 sample in the accelerated growth environment which provides excess nutrition for the bacteria to grow, see **Figure 9.**

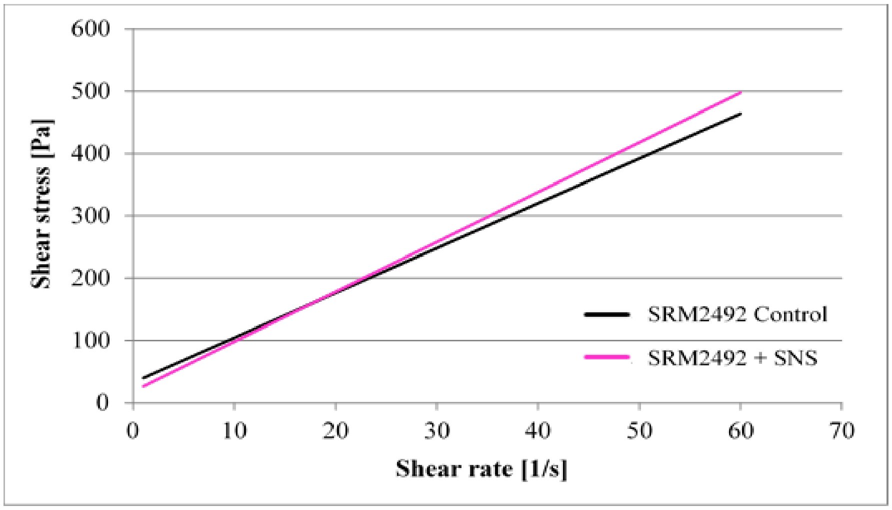


Figure 8: Addition of SNS to SRM 2492 slightly increases viscosity but Bingham behavior remains similar to control sample. [5] The uncertainty of the shear stress values is 1.69 Pa (obtained from the SRM 2492 certificate of analysis).

Figure 9: (Left) **SRM 2492 paste with SNS at 5 weeks.**

(Right) **Accelerated method showed signs of microorganisms at 2 weeks. [5]**

In this preliminary study, the addition of **Sodium Azide (SA)** was analyzed at a concentration of 0.08 %, where the dosage was calculated as mass of biocide per total SRM paste mass which was added and introduced with the water component of SRM 2492 during the standard mixing process. The viscosity and yield stress were monitored for 12 d and appeared to not change drastically with the exception of an outlier. The outlier occurs on the 4th day and is believed to be due to changes in lab room temperature during that period. Specifically, the room temperature would increase due to energy-saving procedures by NIST of shutting down the air conditioning during the weekend. However, the rheological properties returned to being close to the original value once normal conditions were reestablished, shown in **Figure 10**. Yield stress is represented by YS, and viscosity by μ in the following figures. The SA was analyzed in both the accelerated method and normal storage conditions, and both conditions lacked signs of microbes after several weeks (see **Figure 11**.).

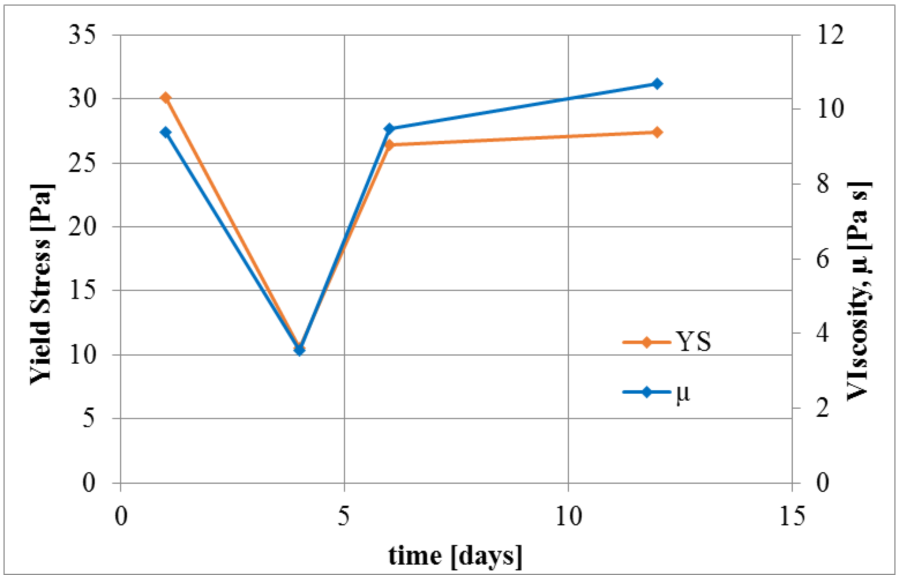


Figure 11: Use of <u>Sodium Azide (SA)</u> on SRM 2492 does not alter the rheological properties significantly. The 4th day values are extremely low due to higher temperatures during weekend. [5] The uncertainties are 1.69 Pa and 0.74 Pa·s for yield stress and viscosity, respectively (obtained from the SRM 2492 certificate of analysis).

Figure 10: (Left) **SRM 2492 paste with SA at 9 weeks.**
(Right) **Accelerated method showed no signs of microbes at 11 weeks. [5]**

The addition of **Cinnamaldehyde (CN)** was monitored at a dosage of 0.88 % (0.55 mL CN per 62.58 mL of water) [5]. The limestone and corn syrup components were not modified, and the standard mixing process was followed. The addition of CN to the SRM appeared to keep the viscosity slightly constant over time as shown in **Figure 12;** while the yield stress showed a slight increase. The use of CN in the accelerated and normal conditions yielded results of no microbial growth after several weeks, as portrayed in **Figure 13.** The measurements were collected at different time intervals for all biocides in this section.

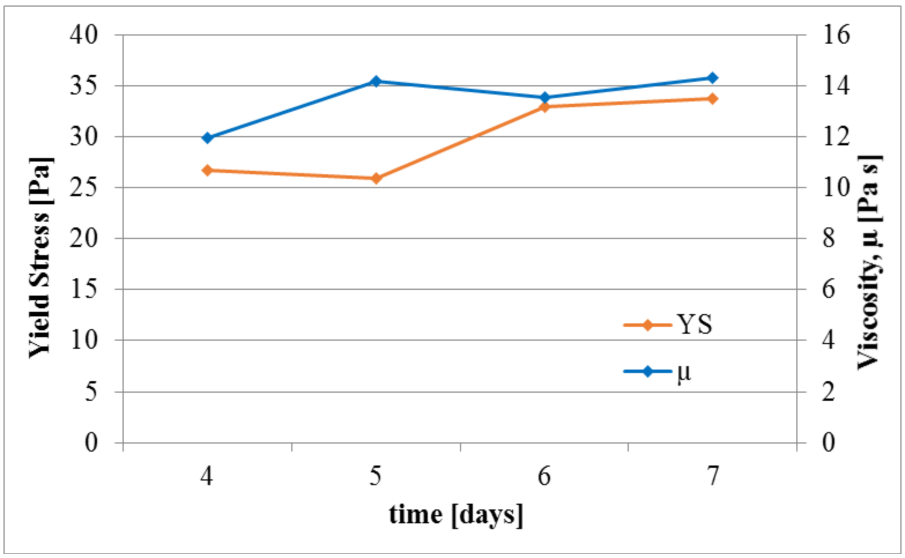


Figure 12: Cinnamaldehyde (CN) maintains SRM viscosity stable over few days, but has slight increase in yield stress. [5] The uncertainties are 1.69 Pa and 0.74 Pa·s for yield stress and viscosity, respectively (obtained from the SRM 2492 certificate of analysis).

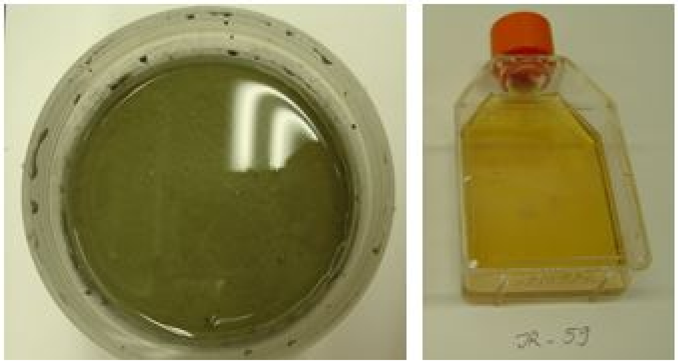


Figure 13: (Left) **SRM 2492 paste with CN at 5 weeks.**
(Right) **Accelerated method lacked signs of microbes at 8 weeks. [5]**

4.3.2 Impact of immediate introduction of biocide with the mixing water

The main purpose of this second series of biocide analysis was to observe the stability in rheological behavior of the SRM paste when the biocide was introduced at the beginning of the SRM's life. During this phase of the work, only the following biocides were evaluated: GSE, HBH, and SP. The GSE and HBH biocides were of main interest to analyze since they were selected based on the most recent investigations into good biocide candidates. SP was chosen based on previous work discussed in the previous section (4.3.1) where SA showed it was a promising candidate. Despite the good results from SA, another compound with similar effectiveness and less toxicity was needed; thus leading this study to evaluate SP.

The expectations set for biocides in this phase of the work were that they should extend the stability past 7 d (where stability is determined by no significant changes in rheological properties). The biocide selections were tested and compared to a control mixture (no biocide) in the viscosity curves shown in **Figure 14** and **Figure 15.** The biocide dosages were specified at 0.2 % for all three biocides in this first series, where the dosage was calculated as mass of biocide per total SRM paste mass. It was determined that adding the biocides was causing a reduction in viscosity of the SRM paste; however, the viscosity in mixtures that contained a biocide had better stability over time compared to the control which had no biocide. On the other hand, the yield stress of the SRM paste increased when HBH was added to the SRM, while addition of the other two biocides slightly reduced the yield stress of the control sample.

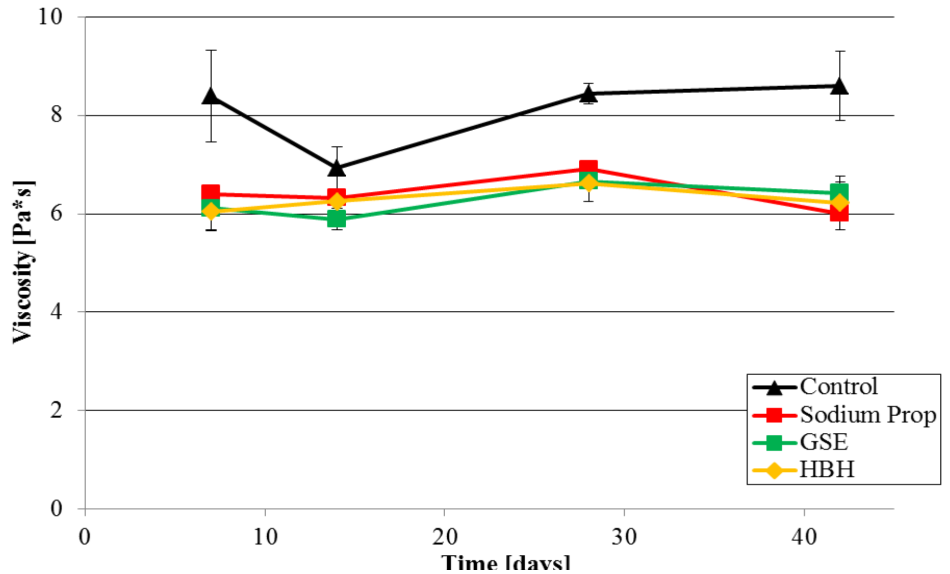


Figure 14: Effect of biocides on temporal evolution of viscosity when biocide is added at 0.2% dosage into freshly mixed SRM 2492. SRM 2492 is denoted as the "control" mixture in the figure. The uncertainty bar is one standard deviation calculated from 3 measurements on the same mixture.

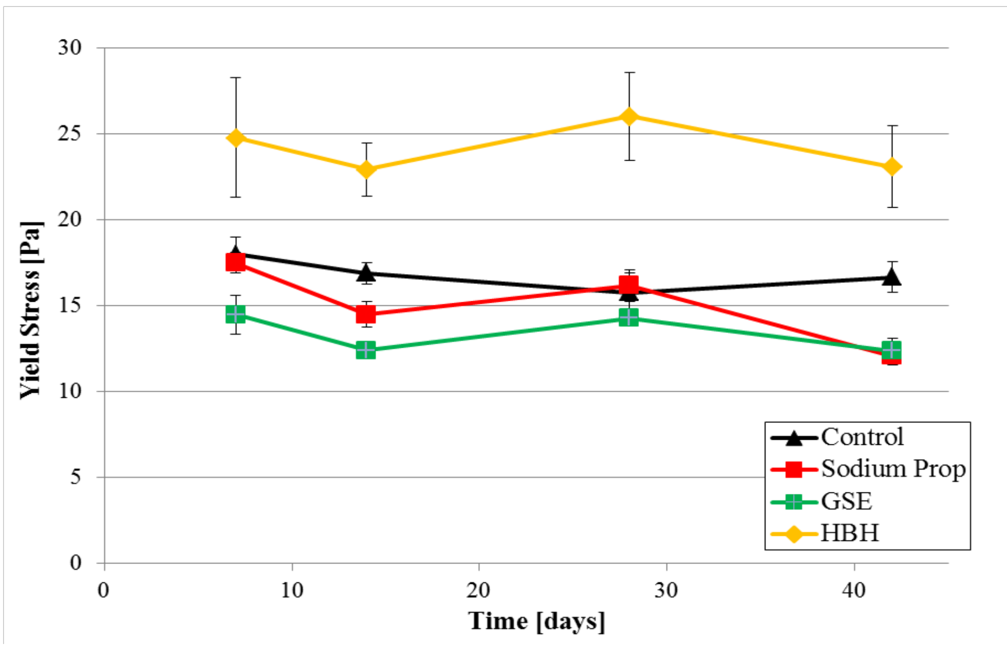


Figure 15: Effect of biocide on temporal evolution of yield stress when biocide is added at 0.2% dosage into freshly mixed SRM 2492 (denoted as "control"). The uncertainty bar is one standard deviation calculated from 3 measurements on the same mixture.

4.3.3 Impact of delayed addition of biocides

A third series of analysis was conducted on three mixtures of various ages from the SRM 2492; each mixture was split in half and stored in two separate containers prior to testing. One half served as a control mixture while the other half received a dosage of a biocide. Thus, a total of six samples were created which tested a total of three biocides. This second series of biocides were specified at 0.5 % for all three biocides in order to also gain insight on the impact of increasing the dosage from the previously observed 0.2 %, where the dosage was calculated as mass of biocide per total SRM paste mass. **Figures 16-18** show the viscosity and yield stress behavior of the mixes with and without delayed biocide additions. Viscosity is represented in the figures by red lines while yield stress is shown as blue lines. The lines with white-filled data points represent the SRM without any biocide (control or host mixture) which are shown having unstable behavior after 7 d for all three samples. The lines with color-filled data points represent the behavior of the host sample after receiving the biocide. The orange dashed line represents the time of biocide introduction to the host SRM sample.

The metric for biocides to be rated with a good performance was that the addition would stabilize the viscosity (and yield stress) readings beyond 7 d (i.e. the point where the original SRM rheological behavior begins to diverge), and ideally this stability would continue for an extended time. The addition of SP and HBH stabilized the viscosity of the mixture and nearly returned it to its original value (see **Figure 16** and **18**, respectively). The addition of SP decreased the yield stress while the addition of HBH did not result in any considerable changes to the yield stress. The GSE did not show promising results since it did not appear to stabilize the SRM viscosity nor yield stress when added to the host sample, see **Figure 17.**

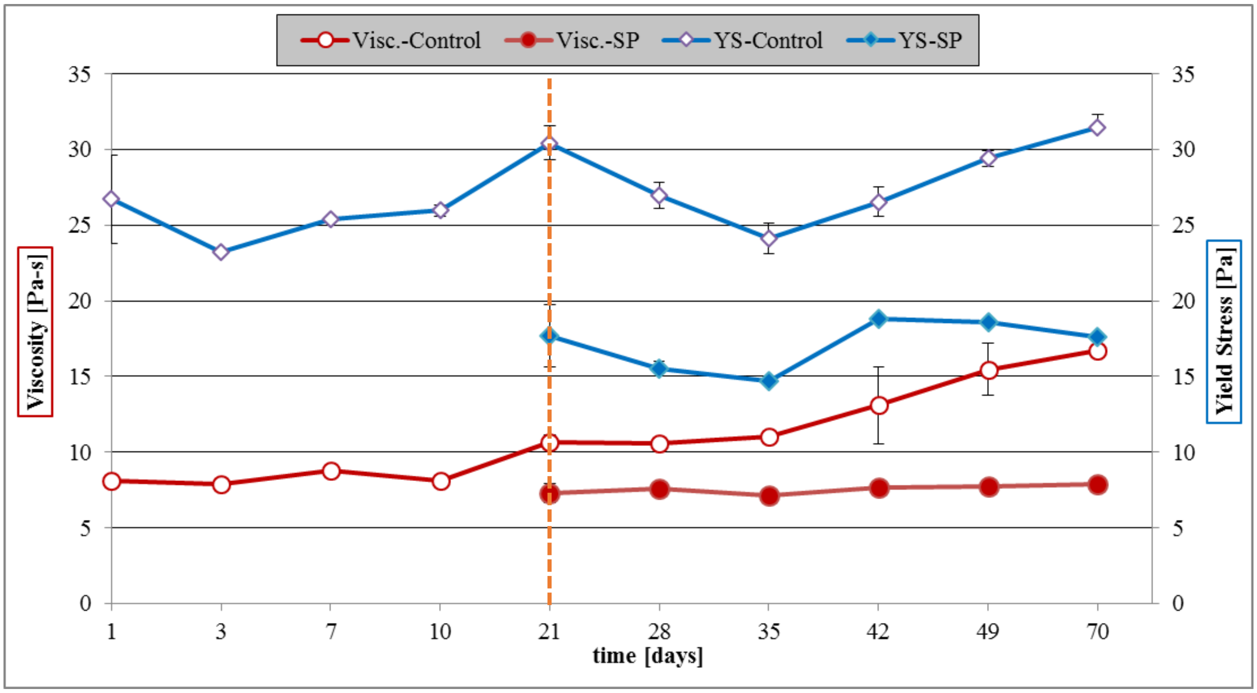


Figure 16: Effect of sodium propionate (SP) addition at 0.5% dosage on viscosity (Visc) and yield stress (YS) of SRM 2492. ***Orange vertical line represents time of biocide addition at age = 21 days.*** **The uncertainty bar is one standard deviation calculated from 3 measurements on the same mixture.**

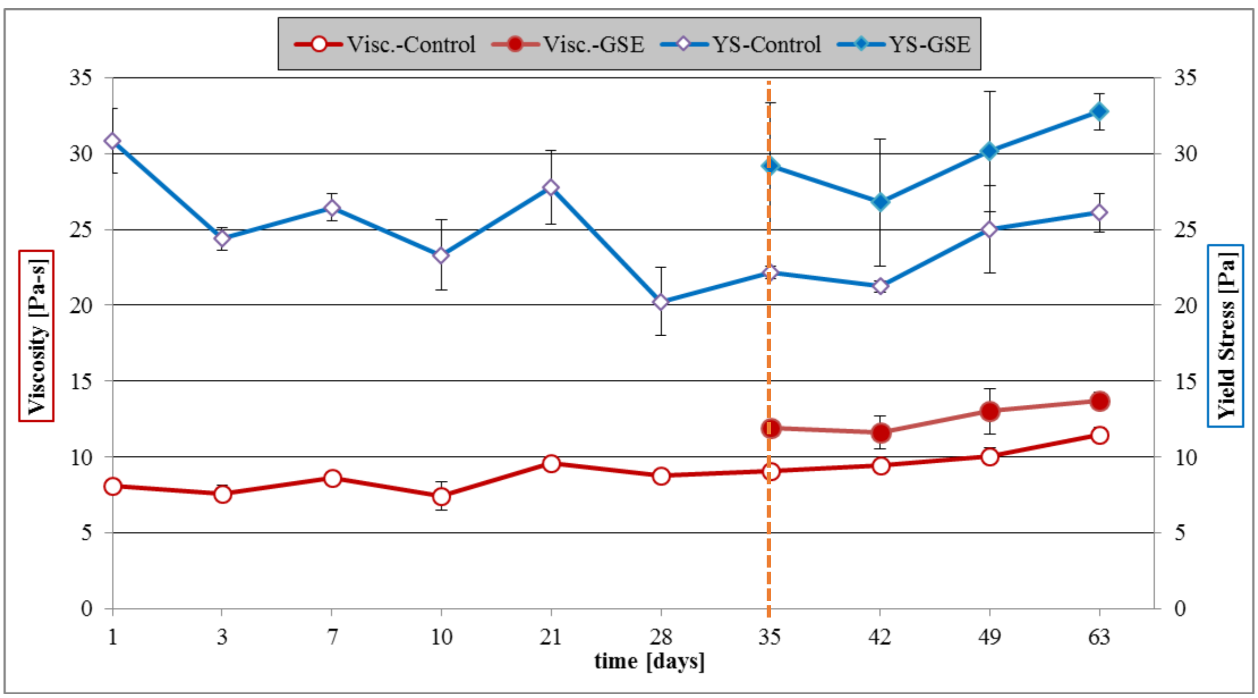


Figure 17: Effect of grapefruit seed extract (GSE) addition at 0.5% dosage on viscosity (Visc.) and yield stress (YS) of SRM 2492. ***Orange vertical line represents time of biocide addition at age = 35 days.*** **The uncertainty bar is one standard deviation calculated from 3 measurements on the same mixture.**

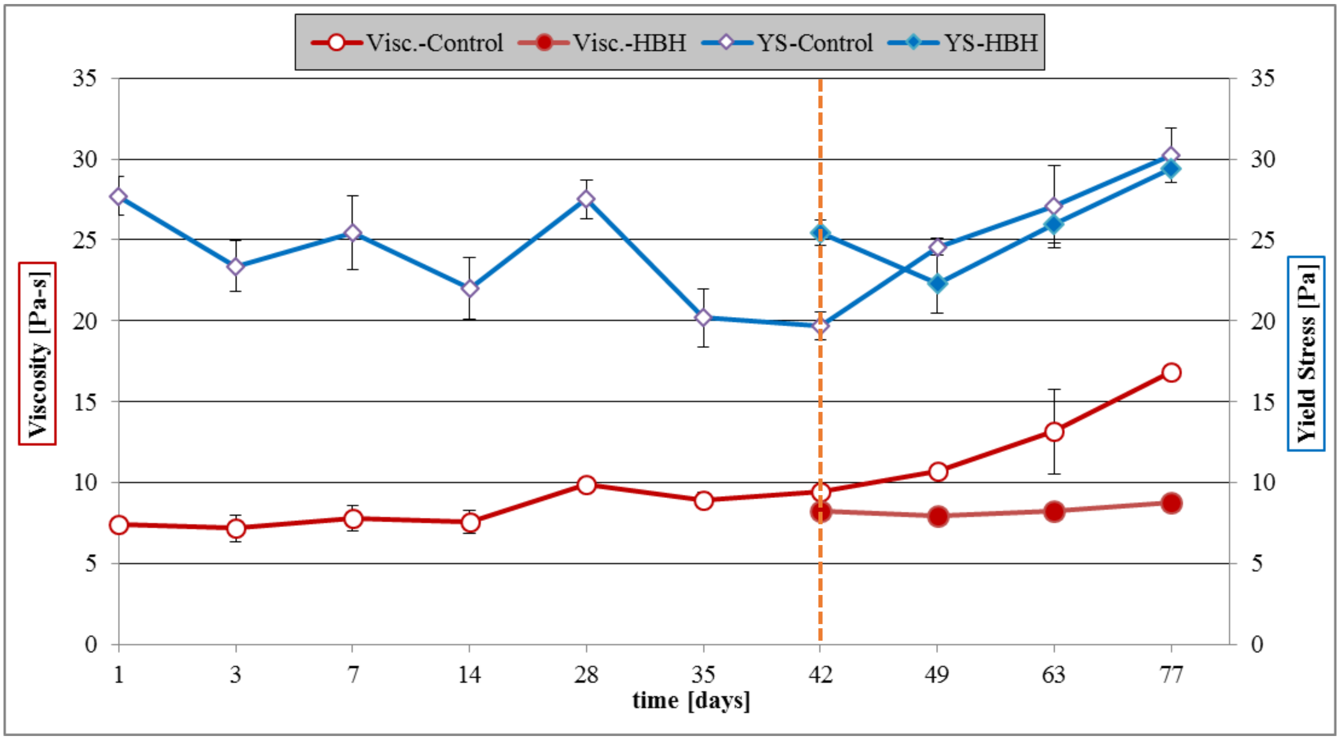


Figure 18: Effect of Honey-B-Healthy (HBH) addition at 0.5% dosage on viscosity (Visc) and yield stress (YS) of SRM 2492. *Orange vertical line represents time of biocide addition at age = 42 days.* The uncertainty bar is one standard deviation calculated from 3 measurements on the same mixture.

4.4 Biocide Optimization

The following discussions on biocide optimization will use the nomenclature to the biocide series discussed in **Table 3.** Results from the preliminary biocide series (section 4.3.1) indicated that SNS was not a good candidate for further analysis since it was the only biocide of its series that showed microbial growth with the accelerated method. The SNS is also an expensive solution, thus not the best for practical use either. On the other hand, the SA and CN both yielded positive results in the accelerated and normal condition monitoring since no microbial growth was observed. However, CN showed that the rheological properties were not maintained over 2 months. The plastic viscosity of a sample with CN tends to drop significantly after 2 months as can be seen from the slopes of the lines in **Figure 19.** The large viscosity change deemed the CN solution not desirable since the predictability of the data is affected. The SA resulted being the most promising option from this series of biocides, but another compound with similar effectiveness and less toxicity than SA was needed; thus leading this study to observe Sodium Propionate (SP).

Based on the second series (Biocide Series 2, section 4.3.2), the SP and GSE biocides were deemed to be more promising than the HBH biocide in applications where the biocide will be mixed into freshly prepared SRM mixtures. Addition of SP and GSE biocides do not show considerable change on the yield stress, and provide a lower but more stable viscosity if added to SRM 2492.

The results from the third analysis series (Biocide Series 3, section 4.3.3) showed that GSE was to be discontinued from future research in this study since it failed to comply with the acceptable requirements of both Biocide Series 2 and 3. The HBH showed better results in Series 3 than it did prior in Series 2, since it helped stabilize the viscosity and didn't affect the yield stress of SRM 2492 when added into the mix at a later age. Also the SP helped in stabilizing the viscosity even if added at a later age.

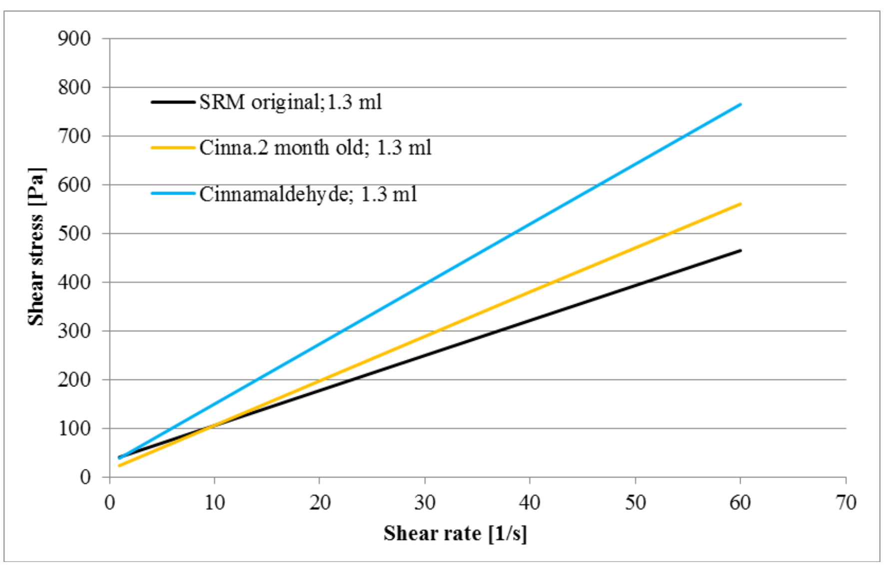


Figure 19: Effect of CN on viscosity of SRM 2492 decreases after 2 months. The uncertainty is 1.69 Pa for shear stress measurements up to 7 days (obtained from the SRM 2492 certificate of analysis).

Based on the results discussed above, further analysis was conducted in order to optimize the standard practice of using the better biocide selections: SP and HBH. This analysis only focused on optimizing results based on viscosity stabilization; yield stress was not observed since the HBH doesn't affect it, and the SP lowers the yield stress initially but keeps it constant at the lower value. This would be acceptable as it could be conceived that the SRM certificate be modified to include a different lower yield stress, if that would increase significantly the shelf-life. Different dosages of SP and HBH were analyzed with the goal of stabilizing viscosity at a value close to the original certified SRM 2492 values. As shown in **Figure 20** and **Figure 21**, some dosages were added at the initial sample mix (shown by filled circles to represent biocide dosage is present in sample) or introduced at a later age (shown by white circles to represent empty biocide dosage). The dosages are also labeled along the lines.

Thus, optimization of the biocide requires a dosage that is not too small such that it doesn't take effect, like the 0.18 % SP dosage. Also, the dosage shouldn't be so high that there is too much liquid content added since that would cause a decreased viscosity as shown by the 0.35 % SP dosage. All dosages mentioned in this section onward are shown as percentages (%) which were calculated as mass of biocide per mass of total SRM paste. Therefore, the target dosage of SP could balance the loss of moisture which resulted being around 0.26 % as shown by the blue line. This dosage appears to stabilize viscosity effectively enough to bring the viscosity value close to the desired baseline value. Furthermore, the stability is maintained even if added at later age. However, for HBH all dosages appeared to behave very similar to the control by gradually increased viscosity; therefore, is not as reliable a biocide as the SP. Once this instability was noticed, the observation of that biocide dosage was discontinued.

For best practice when using SRM 2492, SP should be used as the biocide to account for microbial growth activity that has been noticed to form on the paste after 7 d of storage. As shown in this study, the SP can be added at a later age (up to 70 d shown in this study) and still stabilize the viscosity to the paste viscosity value certified for SRM 2492.

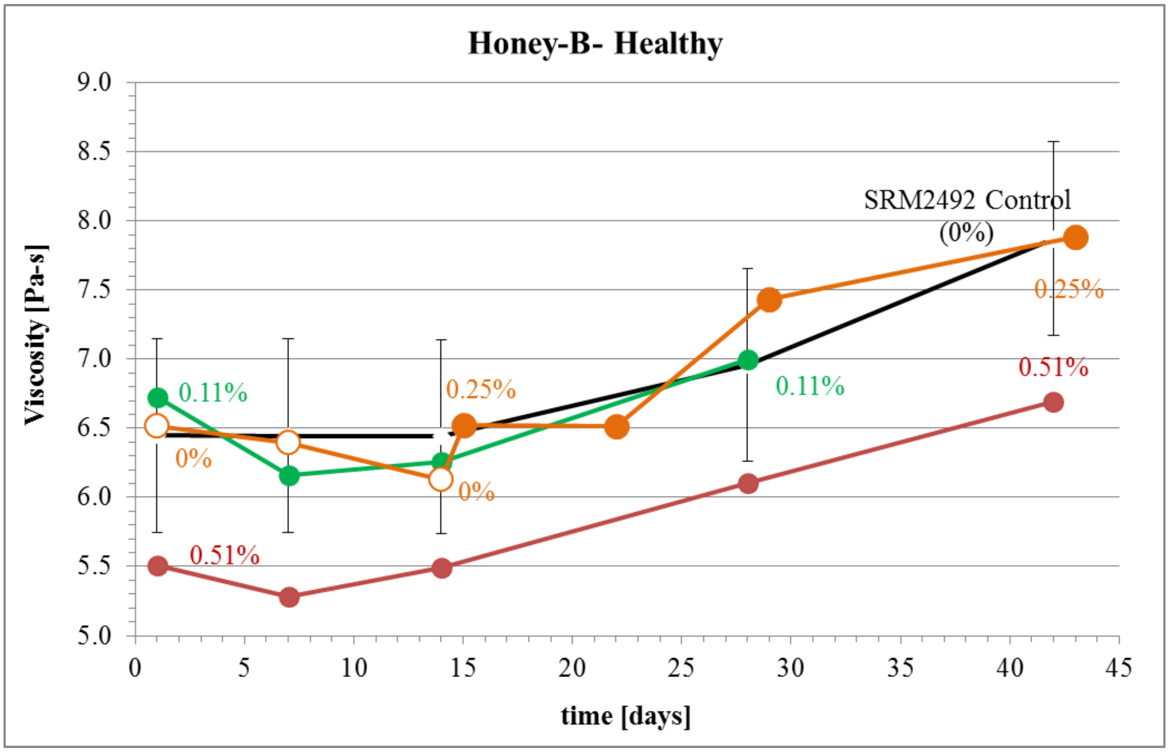


Figure 20: Effects of different dosages of Honey-B-Healthy (HBH) on viscosity of SRM 2492. Uncertainties shown for SRM 2492 are based on the certificate of analysis.

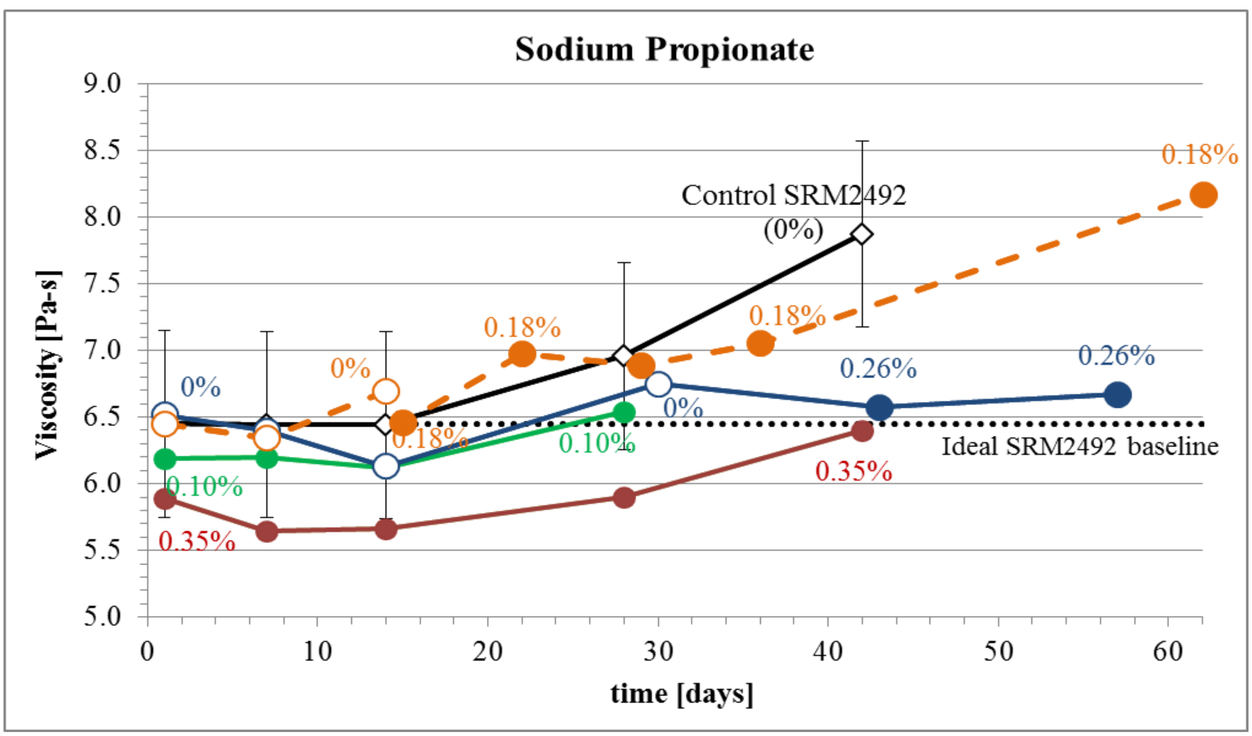


Figure 21: Effects on viscosity of SRM 2492 by different dosages of sodium propionate (SP). Uncertainties shown for SRM 2492 are based on the certificate of analysis.

4.5 Effect of Moisture Content on SRM 2492 Shelf-life

During this study an observation was made of water bleeding to the top of the SRM paste when stored for an extended period of time. Many samples showed a thin layer of water atop the paste surface after at least a day of resting; the longer the samples were left untouched yielded thicker layers of bleeding water. This layer does not directly affect the rheological properties of the SRM 2492 as it is protocol to mix it for 30 s at 300 rpm (31.4 rad/s) prior to any rheological tests. However, bleed water is problematic from two aspects: (1) bleed water on the surface of the SRM mixture may promote an environment for microbial growth (2) the plastic storage containers are not 100 % resistant to moisture evaporation. Thus, the bleed water may slowly evaporate which ultimately is reducing the water content in the mixture composition and could result in increased viscosity. Nevertheless, a trend of increased viscosity in the certified values was observed despite the fact that the values are still within the statistically certified uncertainty. Thus, an investigation into the storage containers (aspect 2) was conducted to determine if the reason for the increasing viscosity trend was due to water loss during storage.

4.5.1 Moisture content of SRM 2492

The analysis for moisture loss began by observing the moisture content conducted with traditional tests of oven drying over time. **Figure 22** shows the original composition of SRM 2492 compared to its solely liquid composition (excluding the limestone). Samples of SRM 2492 and its liquid portion were both monitored over time for changes in moisture content. By independently analyzing the liquid portion only we are able to observe the contribution that the corn syrup solution has on the SRM's moisture losses.

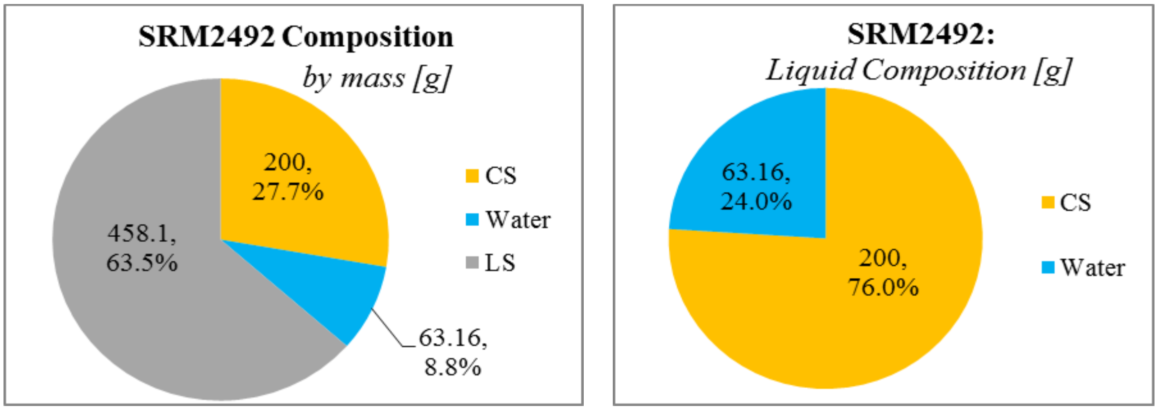


Figure 22: Composition of SRM 2492 shown in (left) its entirety and (right) with only the liquid components.

The moisture content tests were conducted on SRM 2492 samples of three different ages: 5 weeks, 17 weeks and 35 weeks old. Each age was represented by 3 test samples which yielded a mass loss uncertainty of 1 %. Furthermore, the samples were tested by drying them at two temperature conditions: 40 °C and 100 °C as shown in **Figure 23** and **24,** respectively. Thus, each temperature condition contained nine total SRM 2492 samples of various ages which were placed in the oven and weighed periodically for 14 d. The data points shown in the figures are the periods when the samples were weighed. Due to operator schedule constraints the 100 °C test presents more data points than the 40 °C test. It was found that after 14 d the moisture loss reaches a plateau value, as shown by the slope of the curves. **Figures 23 and 24** both highlighted the moisture content of the 5 weeks old SRM 2492 sample is higher than the water content at 17 weeks and 35 weeks old, regardless of temperature condition tested. This is assumed to be caused by the moisture losses due to evaporation which is primarily occurring in the first weeks.

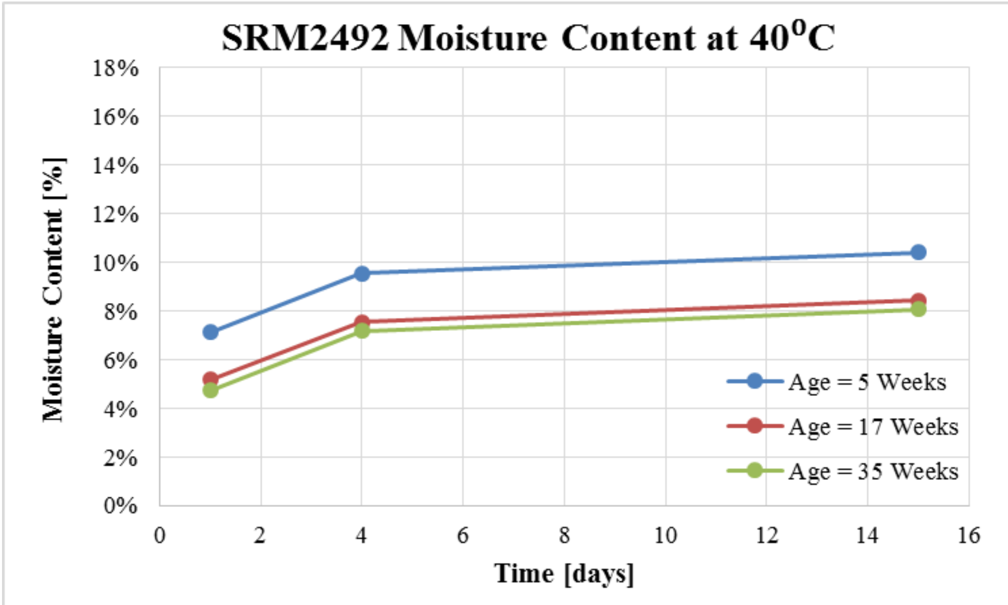


Figure 23: Moisture content available in SRM 2492 samples of different ages, observed via continuous oven-drying at 40 °C. Moisture contents have an uncertainty of 1 % calculated from 3 samples measured at each point.

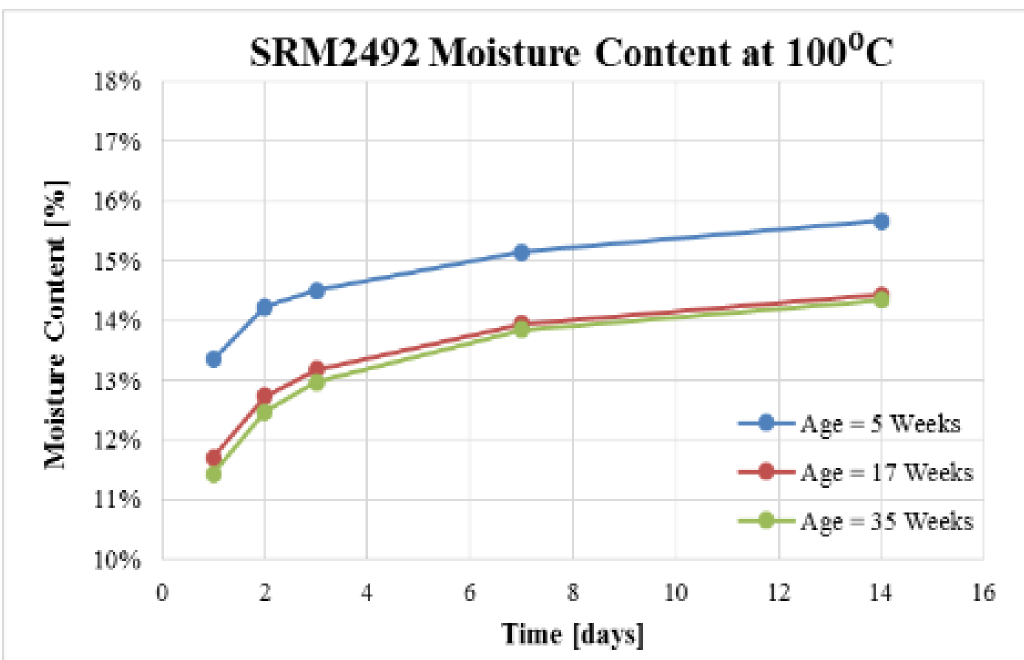


Figure 24: Moisture content available in SRM 2492 samples of different ages, observed via continuous oven-drying at 100 °C. Moisture contents have an uncertainty of 1 % calculated from 3 samples measured at each point.

The results yielded larger moisture contents when oven-dried at 100 °C compared to conducting the test at 40 °C. SRM 2492 samples dried at 100 °C yielded moisture contents of about 14 % ± 1 % while the 40 °C samples had moisture contents of about 9 % ± 1 % This occurrence is also portrayed in the testing of the liquid composition (corn syrup + water only). As shown in **Figure 25** the moisture contents for the liquid portion when oven-dried at 100 °C and 40 °C differ from 37 % ± 0.5 % to 30 % ± 0.5 %, respectively. These differences of moisture content calculations can either be due to the higher heat loads at 100 °C being able to drive out amounts of water from the samples, possibly some decomposition of corn syrup, or a combination of the two.

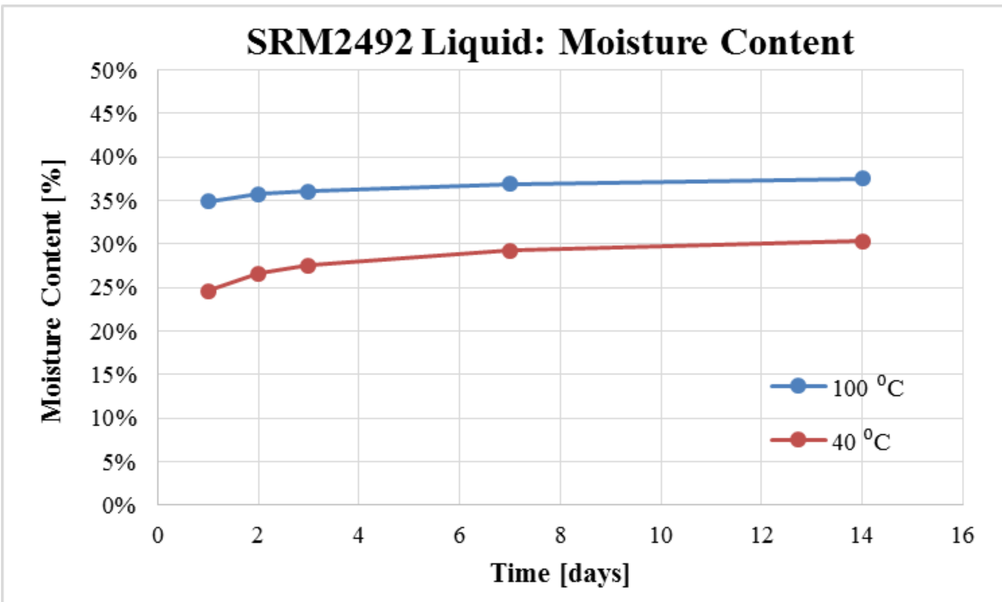


Figure 25: Moisture content observed for liquid composition (diluted corn syrup only) of SRM 2492 at oven-drying conditions of 40 °C and 100 °C. These moisture contents have an uncertainty of 0.5 % calculated from 3 samples measured at each point.

4.5.2 Storage container analysis

A series of tests were conducted to determine the effectiveness of the containers in which the SRM 2492 samples were stored. The analyses measured the loss of mass over time when storing SRM 2492 paste and compared those results to only storing water in similar containers. This study analyzed different methods of sealing the storage containers with the intention of finding the most effective method to retain the moisture content when stored long periods of time. These analyses were also studied because if moisture can escape out of the container then air can enter, which increases the likelihood of microbial growth.

4.5.2.1 Container and sealant types

A variety of containers, lids and sealing methods were analyzed in order to find the sealing system with the least moisture loss during storage. This analysis looked into altering not only the material type for the containers and lids, but also observed different methods of sealing the containers. All the types of components that were analyzed are listed in **Table 4** in no particular order, from which various combinations were observed during this study.

Table 3: Listing of all components analyzed for most effective container sealing system.

Container Types	Lid Types	Sealant Types
Polypropylene (PP)	Normal	Plastic film (e.g. Saran Wrap)
Polystyrene (PS)	Lined	Paraffin wax film
-	Alt. Lined	Plastic zip bag (e.g. Ziploc bag)

The original container from SRM 2492 certification testing is composed of polypropylene (PP) with a normal lid, where normal signifies the lid has no special performance of moisture retention. The alternate container type analyzed in this study differs from the normal container by the polymer type which is polystyrene (PS). The main difference between PP and PS that will impact this study is the Moisture Vapor Transmission Rate (MVTR) of each polymer. MVTR is a measure of the passage of gaseous H_2O through a barrier. Thus, a lower MVTR rating represents a better retention of moisture content. As shown in **Table 5**, the PS has a high MVTR of 10 while the PP only has a 0.5 rating. The only advantage a PS container has in this comparison over a PP container is that PS has better clarity, but impact strength wouldn't be significantly different between the two polymers. The increased clarity could allow a better observation of sedimentation and microbial growth. But the increase in impact strength is more important that the increase in clarity, thus PP container is preferred to the PS container. The table also compares the MVTR of those polymers used as sealant systems such as the paraffin wax film. Also shown is the plastic film (Saran wrap) which is composed of PVDC, and the plastic zip bag (Ziploc bag) which is composed of polyethylene film.

Table 4: Moisture Vapor Transmission Ratings (MVTR) and basic matter properties of common polymers. *MVTR values are in g-mil/100in^2/24h.* **[20, 21]**
(Typical SI units: g-mil/m^2/day = 15 g-mil/100in^2/24h.)

Polymer Type	MVTR	Clarity	Impact Strength
Polypropylene (PP)	0.5	Poor	Fair
Polystyrene (PS)	10.0	Excellent	Poor
Paraffin Wax Polybutene-1	2.7	Poor	N/A
Polyvinylidene Chloride (PVDC)	0.2	Poor	N/A
Polyethylene film (LDPE)	0.6	Fair	N/A

Other than changing the container's polymer type, this study also observed the use of an alternative lid labelled in this study as "lined lid". Both the normal and lined lid were obtained from the same manufacturer and are composed of Polypropylene. However, the lined lid represents a lid that is more air-tight since it comes with the addition of a PTFE-Faced Foamed Polyethylene liner adhered on the underside of the lid surface (PTFE: Polytetrafluoroethylene). The polymer pad fits snugly between the lined lid and the container once closed, thus making the container more air-tight.

Furthermore, various methods of sealing the containers with materials that are commonly available were also analyzed, such as plastic film and plastic zip bags. This study also investigated the use of paraffin wax film wrap since it is a more adhesive type of material. The application of the paraffin film wrap was executed in two ways, either (1) placed under the cap when closed or (2) placed on the entire circumference of the container lid (as if taping the lid shut around its edge). The moisture retention performance for various combinations of the components listed in this section are discussed in the following sections.

4.5.2.2 Performance of sealing systems

The effectiveness of the lids was analyzed by observing the evolution of SRM 2492 mass loss when stored, and assuming some mass loss is due to moisture evaporation. This section purpose was to explore the limits of the various type of sealing system. Thus, just few tests were performed and they should be considered more so as a trend rather than definitive values with a clear uncertainty. First, an analysis was conducted to measure the difference in moisture loss prevention between the normal and lined lid on a normal container. During this first analysis, parallel samples were analyzed to observe the effect of mixing protocol on the SRM 2492 sample when making measurements of mass loss. The parallel analysis allowed us to highlight mass loss behavior in a more practical scenario since an SRM user would in fact be required to re-mix their sample prior to making rheological measurements. As shown in **Figure 26**, all samples experienced mass loss due to evaporation, as expected. When interpreting the figure, the lines with circle data points signify a normal lid was used during storage while square data points represent a lined lid was applied. In regards to mixing protocol, the solid data points mean the samples were re-mixed prior to measuring such data point, while open data points represent a sample that was never re-mixed prior to mass measurements. These preliminary single data point results highlight a reduction in moisture loss when using the lined lid given that both of the samples with lined lids (samples C and D) showed the lowest mass losses regardless of mixing protocol. The effect of re-mixing a sample stored in a normal, unlined container (sample A) showed reduction in moisture loss compared to not re-mixing the sample (sample B). This supports the idea that water evaporates more easily (moisture is lost) from the bleed layer formed during storage then from a well-mixed specimen, because re-mixing prevents the amount of water in the bleed layer to grow. On the other hand, when re-mixing an SRM sample stored in a lined container (sample C) the opposite effect occurs, and an increase in moisture loss compared to not re-mixing (sample D) occurs. This is likely due to the paste's exposure to air when opening the container to conduct the re-mixing and rheological testing. Furthermore, the lined container is able to maintain its original composition fairly well, so any exposure to air will result in more moisture loss compared to keeping the lined lid closed by not re-mixing. When measuring the specimen for mass loss and the container was remixed, the mass was measured before and after to ensure that any material lost on the mixing blade was considered.

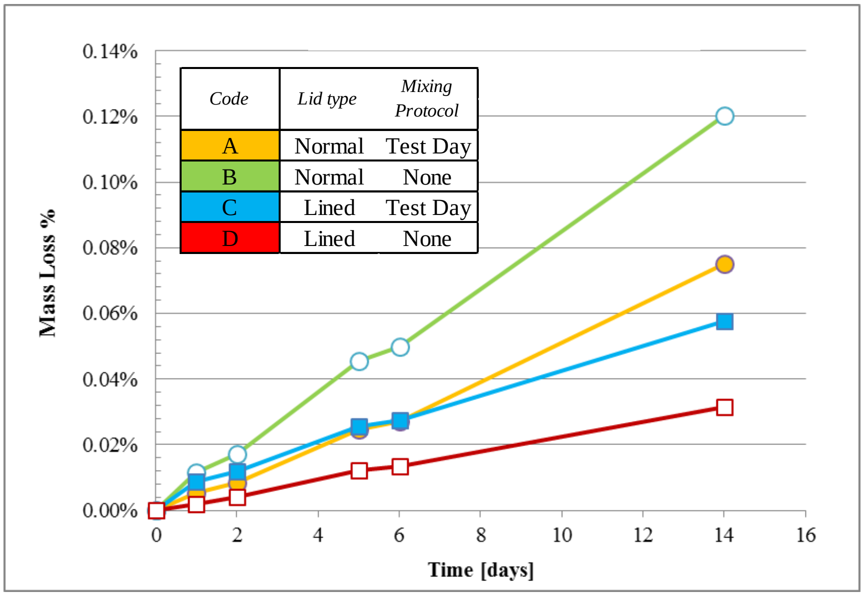


Figure 26: Mass losses of SRM 2492 samples recorded over time to show water content evaporated when stored increases with time. These are single measurements, the uncertainty is estimate from the balance precision at 0.01%.

Circles *represent normal lid was used during storage;* ***squares*** *represent lined lid.*

Solid *data were re-mixed pre-measurement;* ***open*** *data were unmixed samples.*

It is evident from the **Figure 26** that the use of a lined lid is recommended for standard practice when using SRM 2492 and intending on storing for future use even for less than 7 d. Nonetheless, a second analysis was conducted on two unmixed samples to verify the effectiveness of using a lined lid versus a normal lid. As shown in **Figure 27,** the use of a lined lid can reduce the loss of moisture from being nearly 3 % in 20 d down to under 0.5 %.

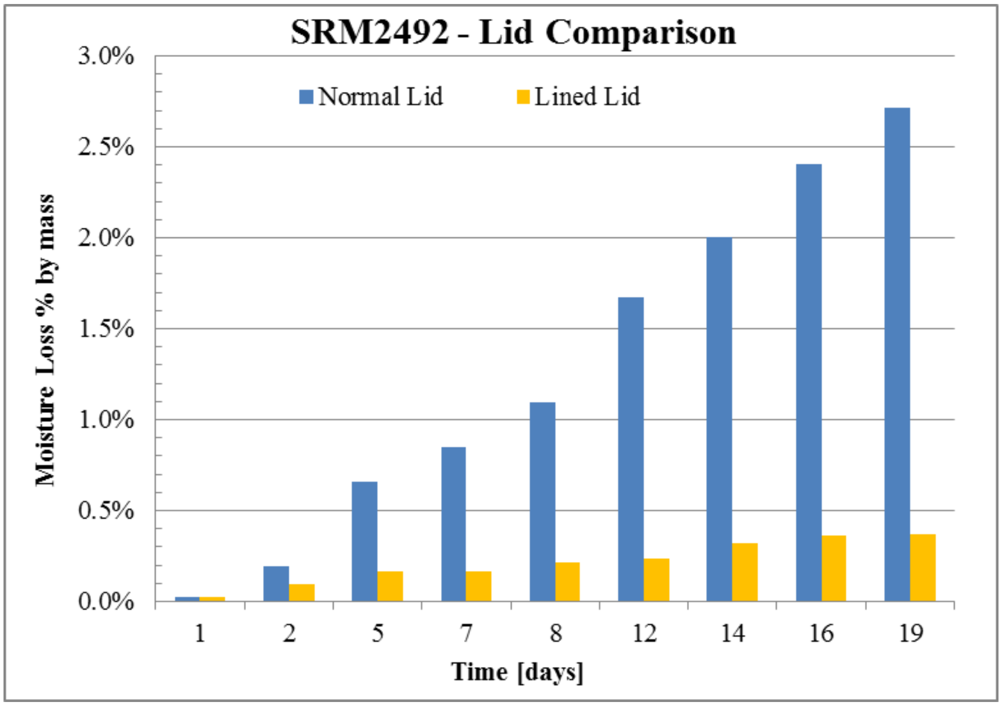


Figure 27: Moisture loss when storing SRM 2492 reduced significantly by application of lined lid. These are single measurements, the uncertainty is estimate from the balance precision at 0.01 %.

Next, an analysis on containers with only water was conducted in order to focus the results on the effectiveness of moisture loss prevention, since it is safe to assume from previous results that much of the mass loss during SRM 2492 storage is due to moisture evaporation. This set of analysis compares the use of the normal lid, lined lid, plastic zip bag and plastic film to seal the water-filled containers. The lined lid yielded the lowest moisture losses, keeping the loss under 0.05 % mass loss at 21 d (see **Figure 28)**. The application of a plastic zip bag and/or plastic film caused a higher loss in water compared to the normal container.

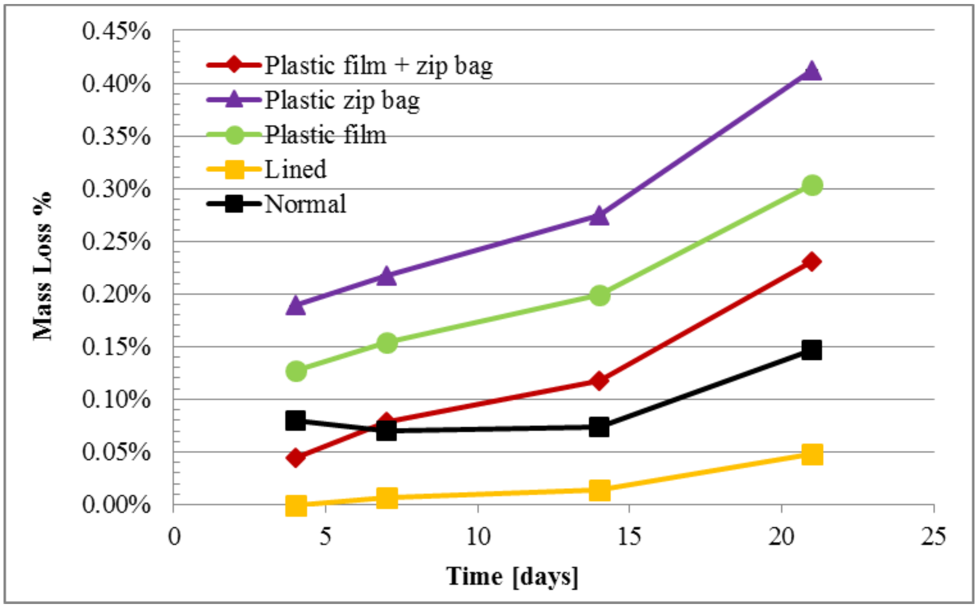


Figure 28: Various methods of sealing the containers were analyzed using plastic film, plastic zip bag or a combination. None of which retained moisture more effectively than the normal or lined lid. These are single measurements, the uncertainty is estimate from the balance precision at 0.01 %.

A final series of moisture loss analysis was conducted in which the two container types (PP and PS) were also included in the combination of sealing systems analyzed, shown in **Figure 29.** Furthermore, an additional lid type was included during this final series which was labelled "Alternative Lined Lid" since it is also a PVC lid, however from a new source whose identity is irrelevant to the results. The original Lined Lid is still included in the following analysis, but the Alternative Lined Lids were ordered due to the effectiveness shown from the original's results. Furthermore, this final set of sealing system analysis replaced the use of Saran wrap for a more adhesive paraffin film wrap.

Refer to the legend below Figure 29 for important notes on interpreting the results. The PS container resulted having higher losses of moisture compared to the original PP container, as expected due to PS having a higher MVTR. On the other hand, the use of paraffin film wrap resulted in good retention of moisture. In fact, the samples with paraffin film wrap around the lid's circumference yielded the lowest moisture losses (gold-bordered circles) similar to the PP container with a lined lid (purple line). These results were promising for the paraffin film wrap since it performed as well as the lined lid, of which the latter was expected to be the best sealing system based on the previous results discussed. In conclusion, the use of a lined lid or paraffin wax film are able to keep moisture losses under 0.05 % by mass.

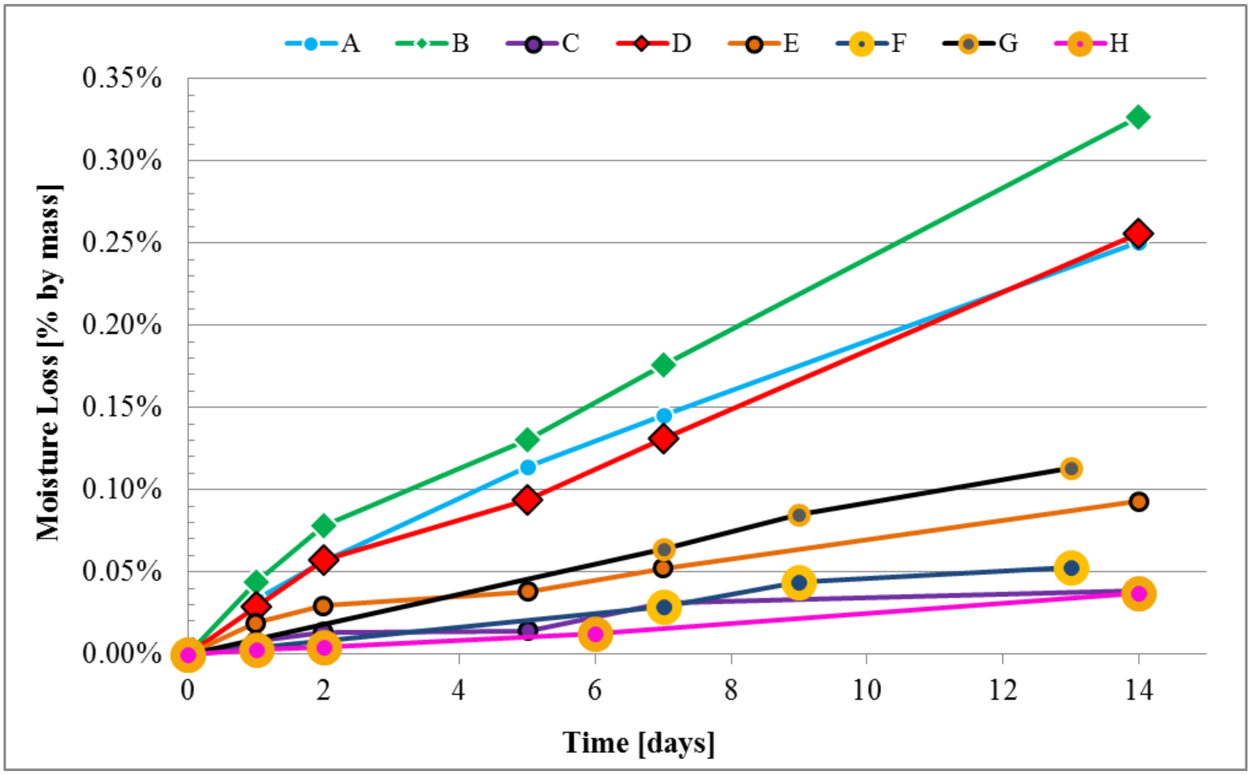


Figure 29: Evolution of moisture loss (by % mass) show high losses in the PS containers. These are single measurements, the uncertainty is estimate from the balance precision at 0.01 %.

Code	**Container type**	**Lid type**	*Film wrap*
A	PP	Unlined	*None*
B	PS	Unlined	*None*
C	PP	Lined	*None*
D	PS	Alt Lined	*None*
E	PP	Alt Lined	*None*
F	PP	Alt Lined	*Circumference*
G	PP	Alt Lined	*Under cap*
H	PP	Unlined	*Circumference*

Legend (Fig 29)*: Codes shown on table represent the colors for lines portrayed in figure above.*

- *PP types are represented by the circles, while PS are shown as squares.*
- *Containers with lined lids have black borders around the circle/square, while unlined lids shown by white borders.*
- *Containers with paraffin wax film wrap shown with gold borders around circles.*

4.5.2.3 Summary of storage container analysis

The high MVTR of the PS container caused higher losses of moisture compared to the original PP container, therefore the former is not recommended for use with SRM 2492. The original PP containers resulted being the best choice in terms of having one of the lowest MVTRs, and are recommended for storage of SRM 2492. The results from the moisture analysis enabled this study to recommend that SRM 2492 should be stored in polypropylene (PP) containers, and preferably in combination with a lined lid and possibly with the addition of paraffin film wrap to seal the lid. More importantly, the finding of this recommended practice helped explain the cause of increased viscosities found at later ages of the SRM 2492 life (during storage), in addition to the microbial activity visible after 7 d. The use of lined lid can serve as the solution to restraining evaporation from the bleeding layer which forms due to the natural occurrence of particle sedimentation caused by gravity. In conclusion, the use of a lined lid helps minimize moisture loss, minimizes changes of SRM2492 moisture content during storage and could prevent undesired viscosity increases.

5 Recommended Standard Practice for Storage of SRM 2492

Before the microbial activity visible at 7 d, another issue dominates the paste behavior which is the bleeding of water due to particle sedimentation; this phenomenon creates a layer of water at the top surface of the SRM 2492 sample. The formation of such bleeding layer could be providing an environment for microbial activity to grow; whose presence then increases the viscosity of the sample as well as makes the rheological data less predictable. Furthermore, the container in which the samples are stored appear to lose moisture over time, which depletes the water in the bleeding layer. The moisture loss of the aqueous layer results in increased viscosity of the entire SRM mixture. These issues encouraged this study to develop methods that minimize moisture loss.

The most effective method evaluated of minimizing moisture loss during storage resulted being the use of a low MVTR plastic, like polypropylene (PP), combined with a lined lid. Applying this as standard practice for storage of SRM 2492 increases the likelihood that the water content won’t change significantly when the paste is re-used past the sample’s certified age of 7 d. The bleeding of the water alone does not affect the rheological properties as the SRM needs to be remixed for 30 s at 300 rpm (31.4 rad/s) prior to every usage.

This study provided mitigation issues for the moisture factor which occurs in the first 7 d, and solutions to microbial activity which occurs after 7 d. While this study showed that sterilization of all equipment and materials worked well in keeping viscosity stable, it was deemed a complex process for user-friendliness. Therefore, based on the results from the Biocide Series, sodium propionate (SP) is recommended for use during future work on this SRM since it showed the most promising results.

This study will allow some changes in the certificate of the SRM 2492 to include how to better store the material after mixing by using the proper container and lid. Nevertheless, more tests would be needed before recommending the use of a biocide, as full statistical analysis would need to be conducted.

6 Conclusion

An analysis on various methods to extend the shelf life and reduce variability of paste reference material, SRM 2492 was conducted. It was observed throughout this study that microbial activity is visible at about 7 d after mixing a SRM 2492 sample. Therefore, an analysis on various methods to extend the shelf life of the paste reference material were conducted. Heat treatment sterilization was found to be adequate in reducing microbial growth, but was deemed too complex for user-friendliness. Chemical stabilization through use of biocides, particularly sodium propionate (SP), was found to be effective in extending the shelf life of SRM 2492 by preventing microbial growth. This study focused not only on considering the effectiveness of the biocides to stabilize rheological results (for better predictability), but also considered the simplicity of practicing these methods at any sample age. Therefore, the use of SP proved to be the most effective method to account for microbial growth and extend the shelf life of SRM 2492. As for the moisture loss prevention, it is recommended to store the SRM 2492 in polypropylene containers with lined lids. Alternatively, the application of paraffin film wrap around the circumference of the closed lid provides an effective method to minimize moisture loss.

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