Technical Notes

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Safety of Formazin and StablCal™ Stabilized Formazin as Primary Turbidity Standards

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SUMMARY

This paper reviews the previously-published, highly-controlled process required to synthesize accurate formazin standards repeatedly, the chemical reactions occurring during this well-defined process, and the theoretical chemical profile of the final formazin product. Further, this paper introduces new quantitative analysis information showing reactants hydrazine sulfate and hexamethylenetetramine (hexamine) to be present in considerably less quantities in the finished stock standard and its dilutions, compared to pre-synthesis concentrations. New single dose oral toxicity testing information reveals the LD_{50} of these products in rats is greater than 5000 mg/kg. New exposure testing information reveals no detectable hydrazine in the final product. Finally, regulatory classifications are reviewed, showing no regulations apply to the transport, shipment, handling, and disposal of these products. All evidence indicates that formazin and stabilized formazin are safe to use as primary turbidity standards when good laboratory practices are followed.

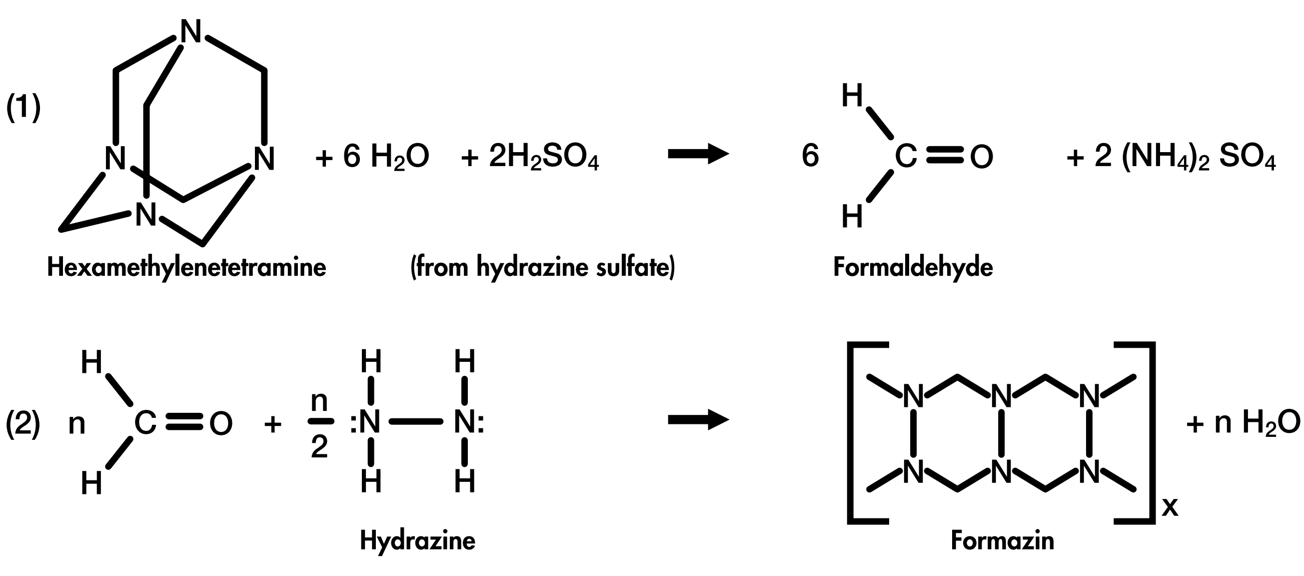
SYNTHESIS OVERVIEW

Formazin is chemically formed through a condensation reaction between formaldehyde and hydrazine. More specifically, two starting reagents, hydrazine sulfate and hexamethylenetetramine (also referred to as hexamine), are used. The formation of the formazin polymer at a concentration of 4000 NTU occurs in two subsequent steps:

(1) Hexamethylenetetramine reacts with water and sulfuric acid (from hydrazine sulfate) to form formaldehyde and ammonium sulfate.

(2) Formaldehyde reacts with hydrazine (from hydrazine sulfate) to form tetraformal triazine (TFTA) and water. The tetraformal triazine continues to polymerize with excess formaldehyde to form the gelatinous formazin precipitate.[1] Figure 1 summarizes these two formation steps.

Figure 1 Formazin Preparation[2]



Popular analytical references, such as *Standard Methods*[3], U.S. Environmental Protection Agency Method 180.1[4], and ASTM Annual Book[5], detail the instructions for preparing the stock 4000-NTU formazin standard. The 4000-NTU standard must be prepared exactly according to those accepted references, with reactants of high purity; any deviation in the amount of the reactants added or in the synthesis conditions will lead to variability in the polymer formation and will compromise the acceptability of the stock standard. The quantities of starting materials, intermediates, and final products described in the discussion are based on this precise methodology.

FIRST FORMATION STEP

In order to produce one liter of 4000-NTU formazin, 5.000 grams of pure hydrazine sulfate are dissolved in approximately 500 mL of ultra-filtered deionized water in a one-liter class A volumetric flask. In addition, 50.00 grams of pure hexamethylenetetramine are dissolved in approximately 250 mL of ultra-filtered deionized water contained in a clean container. Next, the hexamethylenetetramine is quantitatively transferred into the flask containing the dissolved hydrazine sulfate. After the hexamethylenetetramine is transferred, the solution is diluted to one-liter using ultra-filtered deionized water. This solution is mixed for 15 minutes and then allowed to stand for at least 24 hours at 25 ±1º C. During this time, the formazin polymer will develop.

The limiting reagent in both synthesis steps cited above is hydrazine sulfate. In step 1, the hydrazine sulfate salt disassociates into hydrazine and sulfuric acid. The sulfuric acid is stoichiometrically quantified from the hydrazine sulfate, determining the exact amount of formaldehyde formed. Table 1 shows calculated amounts of reactants.

Synthesis step 1 summary: Stoichiometrically, one mole of hexamethylenetetramine plus two moles of sulfuric acid (from hydrazine sulfate) plus six moles of water react to form six moles of formaldehyde plus two moles of ammonium sulfate. Again, sulfuric acid from hydrazine sulfate is the limiting reactant of this first step. Table 2 lists the two products of formazin synthesis step 1. The formaldehyde product reacts in synthesis step 2.

Table 1: Formazin Synthesis Step 1, Reaction Summary (one-liter quantity)

Starting Material	Molecular Weight	Starting Quantity, in Moles (Grams)	Amount Used in Reaction, in Moles and Grams	Remaining Amount After Reaction, in Moles and Grams
Hexamethylene tetramine	140.19	0.356659 moles (50.00 grams)	0.019213 moles (2.69 grams)	0.337446 moles (47.31grams)
Hydrazine Sulfate ($N_2H_4 \cdot H_2SO_4$)*	130.12 (or 98.08 as H_2SO_4)	0.038426 moles (5.000 grams) as N_2H_4 or H_2SO_4	0.038426 moles (3.77 grams as H_2SO_4)	0.0 moles as H_2SO_4; 0.038426 moles as N_2H_4 (1.23 grams as N_2H_4)
Water	18.0	52.5 moles (945 grams)	0.115278 moles (2.08 grams)	52.38 moles (942.8 grams)

* Sulfuric acid from hydrazine sulfate only.

SECOND FORMATION STEP AND FINAL RAW MATERIALS QUANTITIES

In the second step of formazin synthesis, the generation of the formazin polymer is slightly more complex than the reaction shown in Figure 1. First, formaldehyde reacts with hydrazine to form tetraformal triazine (TFTA).[6] TFTA then polymerizes with excess formaldehyde to form poly tetraformal triazine, known as formazin. In this step, one mole of hydrazine reacts with two moles of formaldehyde to form the formazin polymer and two moles of water. The mole quantity of water produced equals the mole quantity of formaldehyde that is reacted. Hydrazine sulfate is the limiting reagent and theoretically will react completely during formazin synthesis; only very low residual concentrations—low ppm—should exist after polymer formation. The strong formation constant for the formazin polymer allows for the total consumption of hydrazine sulfate and for the accurate and repeatable synthesis of stock 4000-NTU standard. Table 3 summarizes the quantities of the reactants and products formed. The quantities in Tables 1, 2, and 3 are calculated based on the assumption that the reactions in steps 1 and 2 go to completion. In fact, trace amounts of residual hydrazine may be detected in solution.

Table 4 shows the quantities of material theoretically present in a one-liter quantity of 4000-NTU formazin standard. Hydrazine and sulfuric acid are present at mg/L levels and will have a minor effect on the quantities of the materials listed.

Table 2: Formazin Synthesis Step 1, Products Generated

Product	Formula Weight	Quantity of Product
Formaldehyde	30.03	0.115278 moles (3.4618 grams)
Ammonium Sulfate	132.14	0.038426 moles (5.08 grams)

Table 3: Formazin Synthesis Step 2, Reaction Summary

Starting Material or Product	Molecular Weight	Starting Quantity, in Moles (Grams)	Amount Used or Produced in Reaction, in Moles and Grams	Remaining Amount After Reaction, in Moles and Grams
Hydrazine (from hydrazine sulfate)	32.05 as hydrazine	0.038426 moles as hydrazine (1.2316 grams)	0.038426 moles as hydrazine (1.2316 grams) used	0 moles as hydrazine and 0 moles as hydrazine sulfate
Formaldehyde	30.03	0.115278 moles (3.4618 grams)	0.076852 moles (2.3079 grams) used	0.038426 moles (1.15393 grams)
Formazin	N/A	0 grams	2.1573 grams produced	2.1573 grams
Water	18.0	52.38 moles (942.8 grams)	0.0768 moles (1.3833 grams) produced	52.46 moles (944 grams)

Table 4: Final Quantities of Materials in 4000-NTU Formazin (one-liter quantity)

Product	Quantity	Percent
Formazin	2.1573 grams	0.216%
Formaldehyde	1.1539 grams	0.115%
Ammonium Sulfate	5.08 grams	0.508%
Hexamethylenetetramine	47.31 grams	4.731%
Water	944 grams	94.40%

PREPARATION OF StablCal™ STABILIZED FORMAZIN STANDARDS

StablCal™ Stabilized Formazin Turbidity Standards contain the same formazin polymer that is present in 4000-NTU stock formazin standard solution. In fact, all StablCal™ Stabilized Formazin Standards are prepared from the same 4000-NTU stock formazin standard as are traditional dilute formazin standards. For this reason, StablCal™ Stabilized Formazin Turbidity Standards are included in the present discussion of formazin attributes. Yet, stabilizing the dilutions requires further production consideration.

The instability of traditionally-diluted formazin standards can be predicted by Le Chatelier's Principle which states: *When more reactant is added to, or some product is removed from, an equilibrium mixture, thereby changing the concentration of the reactant or product, the net reaction will move from the left to the right to yield a new equilibrium and more products are produced.* In formazin synthesis, there is an equilibrium between reactants and products, evidenced by residual hydrazine--the theoretical limiting reagent in the condensation reaction--still being present at low ppm levels at the end of synthesis. This residual hydrazine indicates the reaction proceeds to near (99%) completion but not to full completion. Low-level dilution with high quality water not only removes the turbidity-causing formazin polymer but also removes other matrix components, shifting the equilibrium back to the left toward the reactants. The matrix characteristics of the dilution are very different compared to those of the original 4000-NTU stock formazin standard. The diluted formazin polymer is more likely to degrade to compensate for this equilibrium shift.

In order to stabilize the formazin polymer, the reaction equilibrium must be forced to the right as far as possible. Matrix component concentrations should remain unchanged during dilution of the polymer. StablCal™ Stabilized Formazin Turbidity Standards are prepared through volumetric dilutions of the stock standard using a diluent formulation that closely represents the matrix present in the 4000-NTU stock formazin standard. (U.S. Patent pending). Maintaining a consistent matrix, regardless of dilution level, keeps the formazin polymer reaction equilibrium from shifting back to the left and allows formazin polymer stability in even the lowest turbidity standards.

PRODUCT ANALYSIS

Quantitative analysis of hydrazine (hydrazine sulfate) in formazin or stabilized formazin is very difficult. Traditional colorimetric analysis methods are subject to positive interference from excess hexamethylenetetramine and residual formaldehyde present in these standards. Ion chromatography coupled with electrochemical detection has been shown to accurately identify hydrazine quantities in these standards.[7]

Using this methodology, independent investigation identified hydrazine sulfate concentrations in prepared formazin calibration standards. Analysis was conducted as described by Hoekstra and Johnson.[8] Correlation of hydrazine standard concentration and peak area was completed by using a series of standard solutions prepared by serial dilution. This calibration provided evidence of recovery and a basis for determining hydrazine concentration in the formazin and stabilized formazin samples. A signal-to-noise ratio of three (S/N = 3) allowed an estimated detection limit of 0.03 ppm hydrazine. Hydrazine sulfate concentration was calculated from the hydrazine determinations. Table 5 lists analysis results.

Table 5: Hydrazine Sulfate Concentration in Formazin Standards and StablCal™ Stabilized Formazin Turbidity Standards

Solution	Dilution	Hydrazine Peak Area	Peak Area Adjusted for Dilution, Where Applicable	Hydrazine Sulfate Concentration
50 µM Hydrazine (calibration)	1	1536.0	1536.0	6.5 ppm
4000-NTU Formazin	1:10	75.3	753	3.2 ppm
20-NTU Formazin	1	98.2	98.2	0.4 ppm
1800-NTU StablCal™ Standard	1:10	163.8	1638	6.9 ppm
20-NTU StablCal™ Standard	1:10	below detection limit	—	<0.03 ppm
1-NTU StablCal™ Standard	1:10	below detection limit	—	<0.03 ppm
1800-NTU StablCal™ Standard spiked with 6.5 ppm Hydrazine Sulfate (after dilution)	1:10	unable to resolve overlapping peaks	—	not determined

EXPOSURE LEVELS

Hach Company examined the head space of formazin standards bottled product as it is available to the end-user. Fifteen bottles each of 1800-NTU StablCal™ Stabilized Formazin Turbidity Standard* and 4000-NTU Formazin Turbidity Standard** were examined, using a method recommended by National Institute for Occupational Safety & Health (NIOSH) as a standard method for detecting a test article in air. In this analysis, the head space of each of the bottles was pumped with an Accuro pump 20 times onto a single Drager detection tube for hydrazine in air.*** The tube showed no discoloration, indicating hydrazine was too low to detect or not present.

SINGLE DOSE ORAL TOXICITY

An independent laboratory investigation determined single dose oral toxicity of 4000-NTU Formazin Turbidity Standard and 1800-NTU StablCal™ Stabilized Formazin Turbidity Standard. This study, intended to determine the potential for toxicity of the test articles when administered orally, was designed to comply with the standards set forth by the Organization for Economic Cooperation & Development (OECD)[9] and was conducted according to Good Laboratory Practice requirements of USEPA 40 CRF Parts 792 and 160 and FDA 21 CFR Part 58.

In the first study using 1800-NTU StablCal™ Standard* as the test article, five healthy male and five healthy female Wistar albino rats were dosed at 5000 mg/kg of body weight. The rats were observed 1, 2, and 4 hours post-dose and once daily for 14 days for toxicity and pharmacological effects. The animals were observed twice daily for mortality. Body weights were recorded immediately pretest, weekly, at death, and at termination in the survivors. All animals were examined for gross pathology.

All animals survived the 5000 mg/kg oral dose. There were no abnormal systemic signs noted during the observation period. Body weight changes were normal. Necropsy results were normal. The LD_{50} was determined to be greater than 5000 mg/kg.

* Hach Company catalog number 26607.

** Hach Company catalog number 2461-49.

***Obtained from Lab Safety Supply, Inc., Drager part number 6733121, batch KF-0121, detection range of 0.2 ppm to 10 ppm hydrazine.

In the second study using 4000-NTU Formazin Standard* as the test article, five healthy male and five healthy female Wistar albino rats were dosed orally at 5000 mg/kg of body weight. The rats were observed 1, 2, and 4 hours post-dose and once daily for 14 days for toxicity and pharmacological effects. The animals were observed twice daily for mortality. Body weights were recorded immediately pretest, weekly, at death, and at termination in the survivors. All animals were examined for gross pathology.

All animals survived the 5000 mg/kg oral dose. There were no abnormal systemic signs noted during the observation period. Body weight changes were normal in 8 of 10 animals Two females lost weight during the second week of the observation period. Necropsy results were normal. The LD_{50} was determined to be greater than 5000 mg/kg.

NO REGULATIONS APPLICABLE

No regulatory restrictions have been instituted regarding the handling of formazin during more than 20 years of its use as a primary turbidity standard. The United States Occupational Safety and Health Administration (OSHA) and Canada's Workplace Hazardous Materials Information System (WHMIS) both regulate for toxicity those chemicals with LD_{50} values up to 500 mg/kg. The more-stringent European Union's Classification and Packaging of Dangerous Substances regulates chemicals with LD_{50} values up to 2000 mg/kg. With an LD_{50} value greater than 5000 mg/kg, Formazin 4000-NTU Turbidity Standard and StablCal™ Stabilized Formazin Turbidity Standard are not toxic according to OSHA, WHIMIS, and European Union dangerous substances regulations.

Formazin 4000-NTU Turbidity Standard, StablCal™ Stabilized Formazin Turbidity Standard, and dilutions thereof, do not meet the definition of a hazardous waste per United States Environmental Protection Agency regulations, which require a hazardous waste fit into one of the following categories: (a) flammable waste (D001), (b) corrosive waste (D002), (3) reactive waste (D003), (4) toxic waste (D004 through D043), and (5) hazardous waste listed in Tables F, K, P or U.[10]

Formazin is not explosive, flammable, toxic or corrosive; it is not an oxidizer, nor does it possess any other properties that would regulate it under 49 CFR, Hazardous Materials Transportation.[11] It may be shipped by all means to all locations worldwide in normal packaging with no hazardous marking, special documentation or fees applied.

CONCLUSION

While the raw materials of formazin are suspect, the residuals of these materials in the final formazin polymer suspension, including Formazin 4000-NTU Turbidity Standard and StablCal™ Stabilized Formazin Turbidity Standards, are less than 3.5 ppm (Table 5). Consequently, no federal regulations apply toward the transport, shipment, and disposal of these products. Quantitative analysis, user-exposure monitoring, and single oral dose toxicity data support that, as prepared 4000-NTU stock standard and subsequent dilutions, formazin products should be considered safe to use as primary turbidity standards.

* Hach Company catalog number 2461-49.

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