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Study of the Hexamethylenetetramine, Ammonia, and Formaldehyde System: Quantitative Determinations

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STUDY OF THE HEXAMETHYLENETETRAMINE,

AMMONIA, AND FORMALDEHYDE SYSTEM:

QUANTITATIVE DETERMINATIONS

A DISSERTATION SUBMITTED TO

THE FACULTY OF THE GRADUATE SCHOOL

IN CANDIDACY FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

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BY

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LIST OF ABBREVIATIONS

AA   atomic absorption

abs   absorbance

ACS   American Chemical Society

Å   angstroms

BCG   bromocresol green

°C   degrees Celsius

cm   centimeters

conc   concentrated

D_g weight distribution coefficient

DMG   dimethylglyoxime

δ nuclear magnetic resonance chemical shift units

FID   free induction decay

FTNMR   fourier transform nuclear magnetic resonance

g   grams

GC   gas chromatography

GFAA   graphite furnace atomic absorption

H magnetic field strength

HMT   hexamethylenetetramine

HMX   high melting explosive

HPLC   high pressure liquid chromatography

Hz   hertz

I spin number

i.d.	inside diameter
M	molar
meq	milliequivalents
mg	milligrams
MHz	megahertz
mM	millimolar
mm	millimeter
mmol	millimoles
μg	micrograms
μL	microliters
nm	nanometers
μsec	microseconds
NMR	nuclear magnetic resonance
ppb	parts per billion
ppm	parts per million
RDX	research division explosive
rf	radio frequency
r^2	linear correlation coefficient squared
sec	seconds
USP	United States Pharmacopoeia

INTRODUCTION

Purpose

The goal of this study was to develop some novel techniques for the quantitative determination of aqueous hexamethylenetetramine (HMT) in microgram amounts in the presence of large amounts of formaldehyde. The determination of HMT has been accomplished by many other researchers at the milligram level but, there are relatively few methods for its determination at the microgram level. Hexamethylenetetramine is formed in slightly basic conditions by the condensation of ammonia and formaldehyde. The products are HMT and water. Hexamethylenetetramine is also quantitatively hydrolyzed to ammonia and formaldehyde in the presence of a strong acid. Since formaldehyde is commonly present as an interference in real life samples containing HMT, techniques to solve this problem were developed.

The spectrophotometric portion of this study was developed for the determination of HMT by employing a quantitative hydrolysis and subsequent determination of the formaldehyde released. The method which uses a technique developed for the determination of formaldehyde ultimately measures the absorbance of an iron-Ferrozine complex. If large amounts of formaldehyde are present in the sample, HMT determination cannot be accomplished. Consequently, techniques to reduce this interference were developed so that HMT can be determined in the presence of a large amount

of formaldehyde.   Then ammonia can be indirectly determined by its quantitative reaction with an excess of formaldehyde to form HMT.

The nuclear magnetic resonance (NMR) portion of this study was developed to provide a direct determination of HMT. A unique property of HMT is that it contains 12 equivalent protons that produce one single peak in the NMR spectra. With the use of a 300 MHz fourier transform-NMR (FT-NMR), methods have also been developed for the quantitative determination of microgram amounts of aqueous HMT in the presence of large amounts of formaldehyde. For the determination, aqueous samples are evaporated to dryness to remove water and formaldehyde and the residue is dissolved in an NMR solvent containing a reference standard. This method can then also be used to determine ammonia in the same manner as the spectrophotometric method.

Since HMT is a common urinary tract antiseptic, techniques were developed for both spectrophotometric and NMR determination of HMT in urine samples. Urine samples containing HMT were analyzed after the removal of interferences was accomplished.

Background and Applications

Structure:

Hexamethylenetetramine (HMT), $(CH_2)_6N_4$, also known as 1,3,5,7-tetraazatricyclo-$[3.3.1.1^{3.7}]$-decane,   metheneamine,   hexamine, hexamethyleneamine, formin, aminoform, and urotropin is a relatively old compound.   It was described in the literature as early as 1859 by Alexander Butlerow (1). Butlerow named this compound hexamethylenamin and

established the empirical formula in 1860 (*2*). Wilhelm Hofmann supported Butlerow's results through molecular weight determinations in 1869 (*3*).

Butlerow also proposed the molecular structure I in 1860 (*4*) but did not find agreement with other researchers on this proposal. Van't Hoff in 1881 (*5*) and Delépine in 1893 (*6*) proposed structure II. Von Lösekann proposed structure III in 1890 (*7*) and in 1895 Duden and Scharff (*8*) proposed structure IV. Other proposed structures include structure V by Guareschi in 1897 (*9*), structure VI by Cohn also in 1897 (*10*), and structure VII by Dominikiewicz in 1935 (*11*).

Proposed HMT Structures
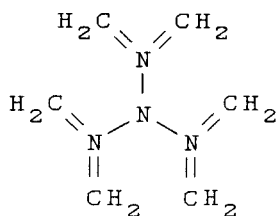
(I) Butlerow
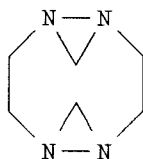
(II) van't Hoff
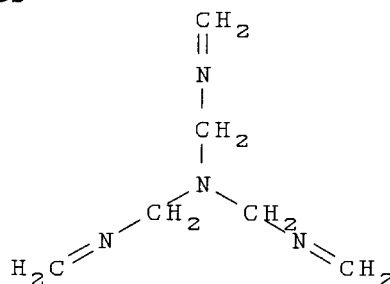
(III) Lösekann
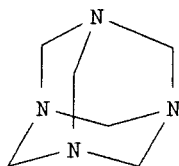
(IV) Duden and Scharf
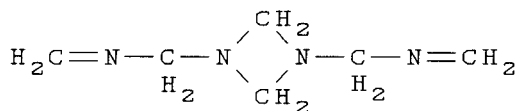
(V) Guareschi
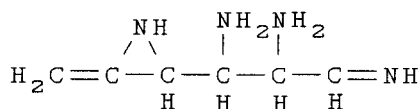
(VI) Cohn
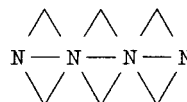
(VII) Dominikiewicz

The structure of Duden and Scharff (IV) is the generally accepted structure. It is reinforced by X-ray crystallography data first reported in 1923 by Roscoe Dickinson and Albert Raymond (*12*). Dickinson and Raymond reported that this data showed a regular tetrahedral symmetry arrangement with all carbon atoms equivalent and nitrogen atoms equivalent. These results are in agreement with structure IV and not with any other suggested structure. This work has been repeated for verification and refinement of bond distances and angles by Gonell and Mark, also in 1923 (*13*), Wyckoff and Corey in 1934 (*14*), and Hampson and Stosick in 1938 (*15*). It is interesting to note that the structure of Lösekann (III) explains the observation of HMT when it acts as a monobasic amine. Only one of the HMT nitrogen atoms would be expected to show monobasic characteristics. The Lösekann (III) structure obviously is not of proper symmetry to fit the X-ray data, but it may be the structure when one of the nitrogen atoms becomes pentavalent with the addition of a hydrogen atom (*16*). The physical properties of HMT are shown in table 1 (*17,18*).

Table 1   Physical properties of hexamethylenetetramine.

Molecular formula	$C_6H_{12}N_4$
Molecular weight	140.19 $^g/_{mole}$
Crystal appearance	colorless rhombic dodecahedrons
Melting point	263°C sublimation without melting with partial decomposition
Flammability	readily ignites
Solubility 25°C	water $^{1g}/_{1.5mL}$ chloroform $^{1g}/_{7.5mL}$ methanol $^{1g}/_{14mL}$ ethanol $^{1g}/_{35mL}$ acetone $^{1g}/_{150mL}$
pH	8-8.5 in water
pK$_a$ (19)	4.89 at 25°C

Formation:

The formation of HMT is accomplished by the condensation of 4 ammonia molecules and 6 formaldehyde molecules to produce 1 HMT molecule and 6 water molecules:

$$4\ NH_3 + 6\ CH_2O \longrightarrow C_6H_{12}N_4 + 6\ H_2O \tag{1}$$
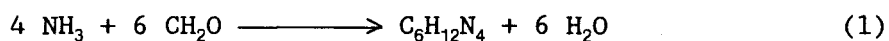
The mechanism of HMT formation has not been determined completely since this reaction has been found to be very complex with many possible byproducts and intermediates. The hypothesis of Duden and Scharff in

1895 (20) provided the main insight to the probable mechanism. They believed that formaldehyde and ammonia condensed to methyleneimine (VIII) and then trimerized to cyclotrimethylenetriamine (IX). Methylolation of this would then produce trimethylolcyclotrimethylenetriamine (X), which on condensation with ammonia would form HMT (IV) as shown below. Duden and Scharff believed the intermediate cyclotrimethylenetriamine (IX) formed quickly but that the HMT final product formation was slower. They showed that freshly prepared solutions containing ammonia and formaldehyde do not produce derivatives of aqueous HMT but do produce derivatives of the intermediate cyclotrimethylenetriamine (IX).

Duden and Scharff scheme

$$3\ CH_2O\ +\ 3\ NH_3\ \underset{}{\overset{fast}{\rightleftharpoons}}\ 3\ (HO-CH_2-NH_2)\ \underset{fast}{\overset{-3\ H_2O}{\rightleftharpoons}}$$

(VIII)

(IX)

(IX)   (X)   (IV) $+\ 3\ H_2O$
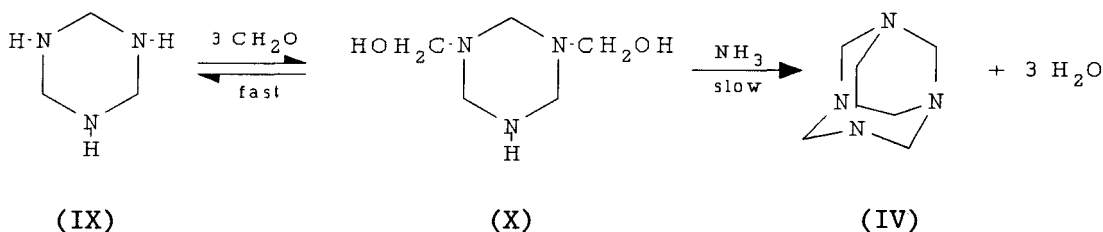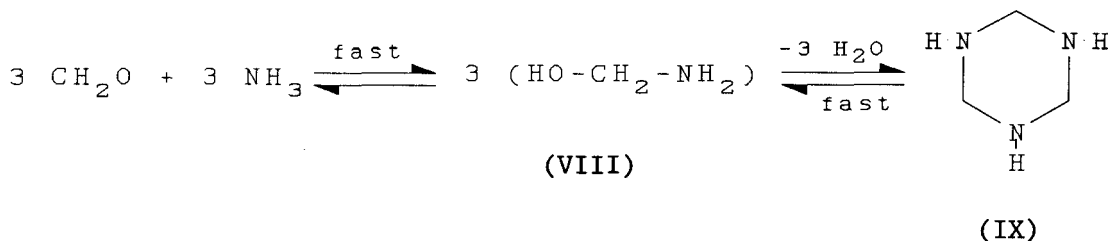
Baur and Ruetschi in 1941 (21) agreed with this mechanism hypothesis based on their kinetic studies on HMT formation. They also determined the

overall reaction order to be third order with respect to ammonia and formaldehyde in the molar ratio of 1:2.

Boyd and Winkler in 1947 (*22*) studied rate curves for the reaction formaldehyde and ammonia in aqueous solutions at 0° C and 35° C. The reactions were performed at various mole concentrations under different initial mole ratios ranging from an excess of formaldehyde to an excess of ammonia. At several points during the reaction, the concentration of formaldehyde, ammonia, and HMT were determined to see how much of the consumed ammonia and formaldehyde had formed HMT and how much had been tied up as intermediates. The results showed that, in general, more of the reactants were consumed during the reaction than HMT was formed. This fact led to the conclusion that a stable intermediate is formed. The results indicate that different intermediates may be formed depending on whether there is an excess of formaldehyde or ammonia. However, it is somewhat difficult to interpret some of these determinations since they may or may not also be including intermediates.

Richmond, Myers, and Wright in 1948 (*23*) re-examined the ammonia-formaldehyde system and agreed with the results of Duden and Scharf. This work also showed that cyclotrimethylenetriamine (IX) was the main intermediate in the eventual formation of HMT. There may also be other intermediates in the reaction system such as methylenediamine (XII) in equilibrium with substances having other formaldehyde-ammonia ratios as well as 1,5-endomethylene-1,3,5,7-tetrazacyclooctane (XX). It was also determined that the final stages of the HMT synthesis from cyclotrimethylenetriamine are not reversible in alkaline solution.

In 1957, Sameer Bose (24) performed half-life studies on solutions containing stoichiometric amounts of formaldehyde and ammonia at 15° C and 20° C. It was found that a dilute solution of acetic acid would arrest the reaction, hydrolyze the intermediate complex within 20 minutes, and did not have an appreciable effect on HMT in 20 minutes of standing. The intermediate was found to decompose completely into equimolar proportions of formaldehyde and ammonia. The half-life results obtained here reaffirm the contention that this is a third order reaction.

Atsushi Kawasaki and Yoshiro Ogata in 1967 (25) studied the kinetics of the reaction to form HMT in dilute aqueous solutions at 20° C in the pH range between 6.3 and 11.9. They also found that the reaction was first-order with respect to ammonia and second-order with respect to formaldehyde. This may be due to a rate-determining attack of methylolamine on free formaldehyde to form dimethylolamine (XIII). The pH studies showed a sharp rate increase with the increasing pH to a maximum between 9 and 10 followed by a gradual decrease. Reaction solutions tested for intermediates by decomposition in dilute acetic acid showed a formaldehyde to ammonia molar ratio of 2.0 as opposed to 1.0 reported by Bose (24). The unstable intermediates may include mono, di, and trimylolamine, cyclotrimethylenetriamine (IX), and 1,5-endomethylene-1,3,5,7-tetrazacyclooctane (XX). Methylenediamine (XII) may also exist but it probably does not decompose in dilute acetic acid as HMT with its N-C-N bonds is stable.

In 1979, Nielsen et. al. (26) examined the formaldehyde-ammonia reaction in D_2O solvent with the aid of ^{1}H and ^{13}C NMR spectroscopy. This reaction study at intervals at 25° C shows that there is rapid formation

of HMT and that reaction intermediates are present. The ^{1}H spectra reveals that the reaction intermediate 1,3,5-hexahydrotriazine (IX) (cyclotrimethylenetriamine) forms rapidly. It is initially higher in concentration than HMT, and is the main species present other than HMT. The 1,3,5,7-tetraazabicyclo[3.3.1]nonane (XX) (1,5-endomethylene-1,3,5,7-tetrazacycloöctane) is also determined here to be much lower in concentration than hexahydrotriazine (IX) except in the early stages where it appears to be nearly equal. The broad signals also present are attributed, principally, to *N*-methylol-*O-d* derivatives. The proton-decoupled Fourier transform ^{13}C spectra provide data are in agreement with that derived from the proton spectra. The main peaks shown near completion of the reaction belong to HMT and 1,3,5-hexahydrotriazine (IX). Since the ^{13}C acquisition required 10-15 minutes, the early and intermediate timed samples were very complex. The peaks were too numerous to allow structure assignments. The spectra obtained at later times are less complex, but also show weaker peaks which do not permit accurate structure assignments. These results and many of the previous studies including aldehyde-ammonia and aldehyde-amine reactions lead Nielsen *et. al.* to describe an "oversimplified" mechanism as one possible route for the reaction of formaldehyde and ammonia to form HMT.

Nielsen *et. al.* scheme

$$CH_2(OH)_2 \underset{}{\overset{-H_2O}{\rightleftharpoons}} CH_2O \xrightarrow{NH_3} HOCH_2NH_2 \underset{}{\overset{-H_2O}{\rightleftharpoons}} CH_2=NH \xrightarrow{NH_3} CH_2(NH_2)_2$$

(VIII)   (XI)   (XII)

$$HOCH_2NH_2 \underset{}{\overset{CH_2O}{\rightleftharpoons}} HOCH_2NHCH_2OH$$

(VIII)   (XIII)

$$HOCH_2NH_2 \underset{}{\overset{CH_2=NH}{\rightleftharpoons}} HOCH_2NHCH_2NH_2 \underset{}{\overset{CH_2=NH}{\rightleftharpoons}} HOCH_2NHCH_2NHCH_2NH_2$$

(VIII)   (XIV)   (XV)

$$CH_2(NH_2)_2 \underset{}{\overset{CH_2=NH}{\rightleftharpoons}} H_2NCH_2NHCH_2NH_2 \underset{}{\overset{CH_2=NH}{\rightleftharpoons}} H_2NCH_2NHCH_2NHCH_2NH_2$$

(XII)   (XVI)   (XVII)

$$H_2NCH_2NHCH_2NHCH_2NH_2 \underset{}{\overset{-NH_3}{\rightleftharpoons}} \text{(IX)} \underset{}{\overset{CH_2=NH}{\rightleftharpoons}} \text{(XVIII)}$$

(XVII)   (IX)   (XVIII)

$$\text{(XVIII)} \underset{}{\overset{CH_2=NH}{\rightleftharpoons}} \text{(XIX)} \underset{}{\overset{-NH_3}{\rightleftharpoons}} \text{(XX)}$$

(XVIII)   (XIX)   (XX)

$$\text{(XX)} \underset{}{\overset{CH_2=NH}{\rightleftharpoons}} \text{(XXI)} \underset{}{\overset{-NH_3}{\rightleftharpoons}} \text{(IV)}$$

(XX)   (XXI)   (IV)
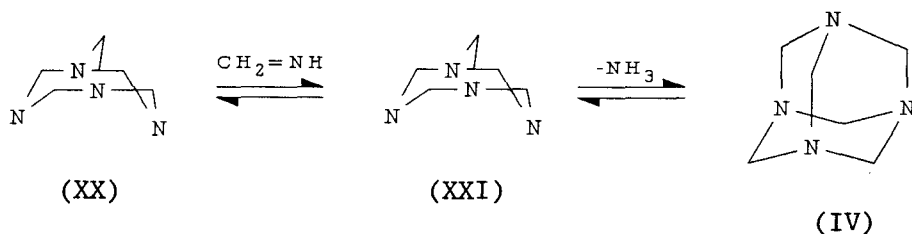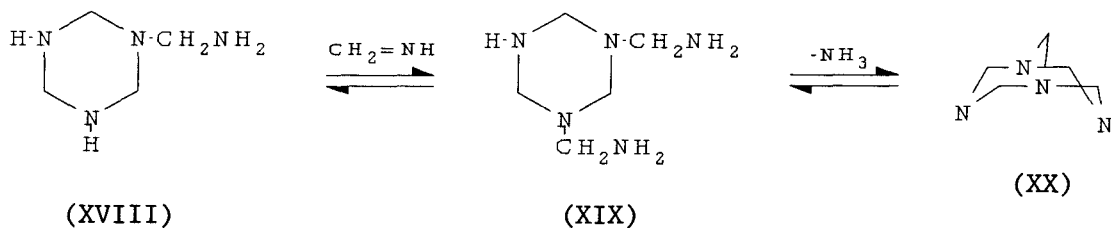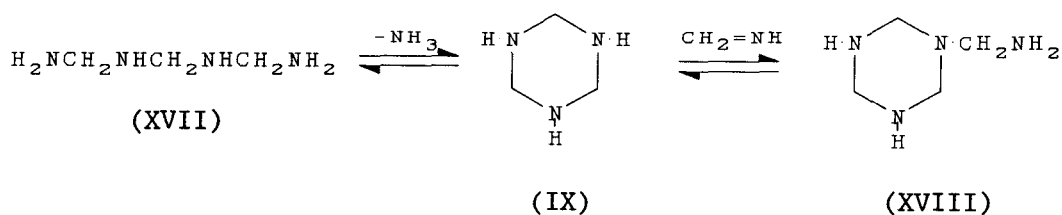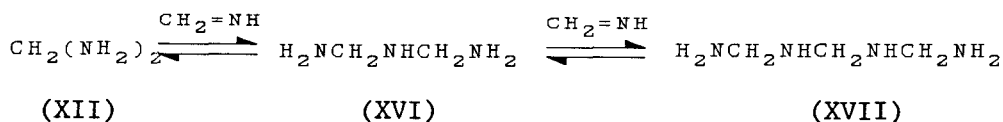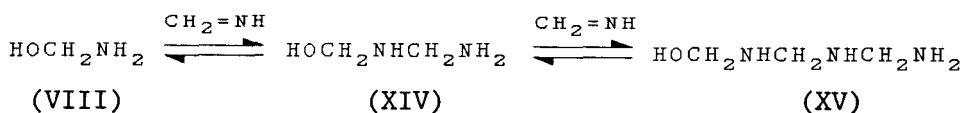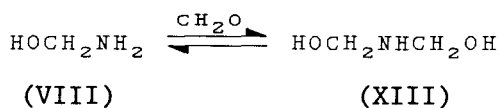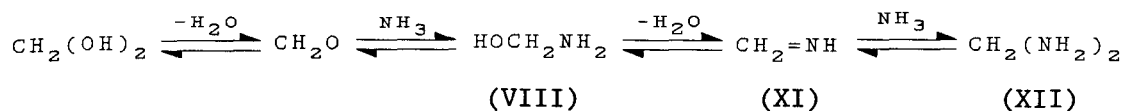
Hydrolysis:

Hexamethylenetetramine is hydrolyzed in aqueous solutions when heated in the presence of strong acids.

$$C_6H_{12}N_4 + 2\ H_2SO_4 + 6\ H_2O \xrightarrow{\text{heat}} 6\ CH_2O + 2\ (NH_4)_2SO_4 \qquad (2)$$
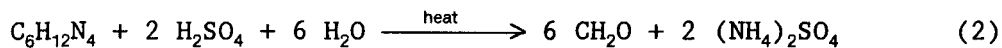
Early studies essentially determined reaction rates at pH's between 2 and 8 and concluded that the higher the H^+ concentration, the faster the reaction rate (27,28,29). In 1960, Hikoji Tada (30) determined that the decomposition reaction occurs with $HMTH^+$ and not HMT. HMT must first become $HMTH^+$ for decomposition to proceed. Also, in acid solution HMT gave mainly derivatives of 1,3,5-triazocyclohexane (IX). Tada interprets his results mechanistically as follows. The weakening of the C-N of $HMTH^+$ (XXII) produces a carbonium ion (XXIII). With the addition of H^+ to NH, the C-N bond is broken completely by the reaction of like charge. Since the carbonium ion (XXIII) is a derivative of 1,5-endomethylene-1,3,5,7-tetrazacyclooctane (XX) which decomposes faster than HMT, the formation of (XXIII) must be the rate determining step.

Tada scheme

(IV)   (XXII)   (XXII)   (XXIII)
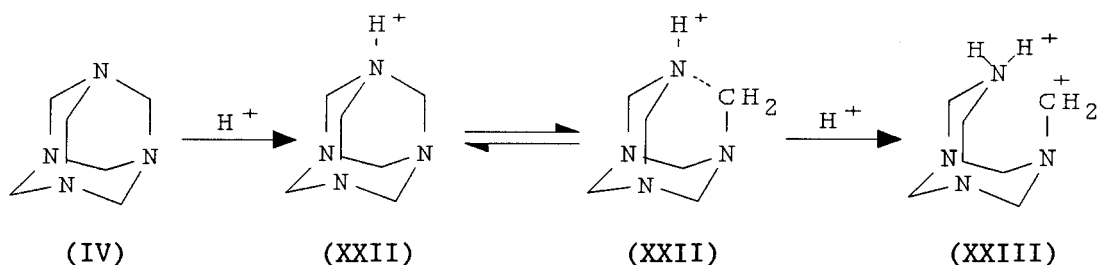
The acid hydrolysis using heat and strong acid is considered to be quantitative and is the basis for most methods of quantitative HMT determinations (31).

Applications:

Hexamethylenetetramine is used in many reactions, generally in one of two ways. It can be hydrolyzed, in many instances, as a source of anhydrous formaldehyde and it also functions in many reactions as a tertiary amine.

Commercially, HMT is usually used as a controlled source of anhydrous formaldehyde, an advantage over the use of paraformaldehyde. It's principal use is as a methylenating agent in the curing of phenol-formaldehyde resins. The compound is hydrolyzed thermally during the molding process with the methylene groups crosslinking to provide product strength. The release of ammonia acts as a catalyst.

The second large volume use is for the manufacture of the high explosives RDX (Dupont's "research division explosive", cyclonite, or 1,3,5-trinitro-1,3,5-triazacyclohexane) and HMX ("high melting explosive" or 1,3,5,7-tetranitro-1,3,5,7-tetra-azacyclooctane) during wartime operation. These nitration products are formed via reactions of concentrated nitric acid with HMT (32).

Other commercial uses of HMT include the hardening of proteins such as in glues, as a corrosion inhibitor, in fuel tablets for camping stoves, and as a preservative.

Medicinally, HMT is a common urinary tract antiseptic which was used for this purpose by Nicolaier (33) as early as 1894. This use is also

based on the release of the active ingredient formaldehyde, by hydrolysis in the bladder. An enteric tablet is used to bypass gastric acidity. Musher and Griffith (34) have reported that exposure in the urinary tract to $\geq$ 25 μg of formaldehyde per mL for $\geq$ 2 hr. causes a measurable delay in the growth of gram-negative bacteria. As a drug, HMT is administered as either the mandelate (4 grams/day) or hippurate (2 grams/day) salt. Hexamethylenetetramine is an ideal drug since it is relatively nontoxic, bacterial resistance to formaldehyde has not been shown to develop, significant levels of formaldehyde are not generated in the gut or body tissues, and it is relatively inexpensive (34).

Determinations:

Many methods have been developed for the determination of milligram (mg) amounts of HMT. This is largely due to the development of methods to assay prescription tablets which contain at least 250 mg. Table 2 lists many of the different types of methods available for the determination of HMT at this level and a reference example of each. The most common methods are titration.

Contrasted to the numerous determination methods available at the milligram level, there are relatively few methods available at the low microgram level as shown in table 3. Spectrophotometric methods are the most common and the chromotropic acid method is the USP XXII standard procedure. Most of these methods also determine HMT indirectly by quantitatively determining the formaldehyde liberated by means of acid hydrolysis. The most sensitive methods are chromatographic since they employ small sample sizes of 2-10 μL.

Table 2	Various types of methods available for milligram amount determinations of HMT.

Spectrophotometric	(35)
Infrared spectroscopy	(36)
Polarography	(37)
Specific gravity	(38)
Gravimetric	(39)
Bromatometric	(40)
Nuclear magnetic resonance	(41)
Acid-base	(42)
Amperometric	(43)
Potentiometric	(44)
Conductometric	(45)
Complexometric	(46)
Coulometric	(47)
Gas liquid chromatography	(48)

Table 3   Various methods reported for the determination of low microgram amounts of HMT.

Method/Reagent	ref	i/d[a]	Det. limit[b] HMT(μg/mL)	Final Vol(ml)
SPECTROPHOTOMETRIC				
1,2-Napthoquinone-4-sulfonate	(49)	(i)	7	25
Nash	(50)	(i)	6	10
Richter	(51)	(i)	1.7	5
Iodine charge transfer complex	(52)	(d)	1	10
Chromotropic acid	(53)	(i)	0.49	10
J acid	(53)	(i)	0.26	5
Phenyl J acid	(53)	(i)	0.11	12.5
Ag^+-Fe^{3+}-Ferrozine	proposed	(i)	0.04	10
POLAROGRAPHY				
Formaldehyde-ethanolamine	(54)	(i)	0.14	22
POTENTIOMETRIC				
Kinetic CN-selective electrode	(55)	(i)	2.6	19
FOURIER TRANSFORM NUCLEAR MAGNETIC RESONANCE				
d-Acetonitrile solv.	proposed	(d)	10	0.6
HIGH PRESSURE LIQUID CHROMATOGRAPHY				
CH$_2$O 2,4-dinitrophenylhydrazone	(56)	(i)	0.006	3
Ion pair	(57)	(i)	2	0.020
GAS CHROMATOGRAPHY				
Iodine charge transfer complex	(58)	(d)	0.005	1

[a]i/d = indirect or direct HMT determination
[b]Det. limit = Spectrophotometric at 0.1 Absorbance; others as reported

SPECTROPHOTOMETRIC DETERMINATION OF HEXAMETHYLENETETRAMINE

AND AMMONIA

Introduction

The most common methods used for the determination of microgram amounts of HMT are spectrophotometric methods. Table 3 lists the available methods. All but one of the methods listed use the indirect method of determination, i.e., by determination of hydrolyzed formaldehyde. Hexamethylenetetramine is completely hydrolyzed in the presence of heat and strong acid, usually sulfuric, to form formaldehyde.

$$C_6H_{12}N_4 + 6\ H_2O \xrightarrow[\text{heat}]{\text{acid}} 6\ CH_2O + 4\ NH_3 \qquad (3)$$
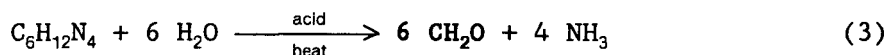
Formaldehyde is a very reactive compound. Many methods have been developed for its determination. Filipeva, *et. al.* (*50*) used an indirect method employing sodium 1,2-naphthoquinone-4-sulfonate. This method was developed for the determination of various drugs, in dosage form dissolved in an ethanol solvent. The absorbance was measured at 480 nm yielding a detection limit of 7 μg/mL at 0.1 absorbance.

The Nash procedure for the determination of formaldehyde was modified by Strom and Jun (*51*) for the determination of HMT. It is based on the Hantzsch reaction in which formaldehyde is reacted with ammonia and

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acetylacetone. The colored compound has an apparent molar absorptivity of 2,300 L/(cm mol) measured at 412 nm. The detection limit is 6 μg/mL at 0.1 absorbance.

Rizzoli (52) applied the Richter reaction to the determination of HMT as the picrate in chloroform and toluene-chloroform solutions. This HMT-picrate compound has an apparent molar absorptivity of 8,300 L/(cm mol) measured at 410 nm. The detection limit is 1.7 μg/mL at 0.1 absorbance.

Taha, El-Rabbat, Nawal, and Fattah (53) developed a direct determination of HMT by forming the intense charge-transfer band in the UV spectrum of 1:1 molecular complex with iodine in 1,2-dichloroethane or chloroform. This method was applied to prescription tablets and has an apparent molar absorptivity of 20,000 L/(cm mol) measured at 273 nm. The detection limit is 0.7 μg/mL at 0.1 absorbance.

The method using chromotropic acid (4,5-dihydroxynapthalene-2,7-disulfonic acid, disodium salt) is the USP standard (59) for HMT prescription tablets and is probably the most popular spectrophotometric method. This method has an apparent molar absorptivity of 28,800 L/(cm mol) measured at 578 nm. The detection limit is 0.49 μg/mL at 0.1 absorbance.

J acid (6-amino-1-napthol-3-sulfonic acid) and phenyl J acid (6-anilo-1-napthol-3-sulfonic acid) were described by Sawicki, Hauser, and McPherson (54) for the determination of formaldehyde and formaldehyde releasing compounds. For the determination of HMT, J acid has an apparent molar absorptivity of 54,000 L/(cm mol) at 468 nm and phenyl J acid has an apparent molar absorptivity of 122,500 L/(cm mol) at 660 nm. The

detection limits for J acid and phenyl J acid are 0.26 and 0.11 μg/mL at 0.1 absorbance.

Statement of Problem and Approach:

The proposed method is based on a spectrophotometric method developed in this laboratory by Al-Jabari and Jaselskis for the determination of formaldehyde (60). This formaldehyde method is based on the reduction of silver(I) by formaldehyde followed up by the oxidation of the metallic silver produced with iron(III) in the presence of 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid, monosodium salt monohydrate (Ferrozine). The method for the quantitative determination of ferric ions in the presence of Ferrozine was developed by Stookey in 1970 (61).

Ferrozine is one of the most sensitive spectrophotometric iron reagents available. The utility of this reagent is based on the selective reactivity of the ferroin grouping (shown below) which acts as bidentate ligands with certain metals to form colored complexes.

$$-\overset{|}{N}=C-C=\overset{|}{N}-$$
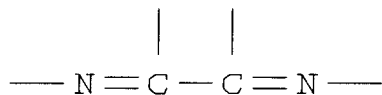
Ferroin group

The ferroin reaction with ferrous ion was first reported in 1898 by Blau (62) and thus has been given the trivial name of the ferroin group. These compounds also react with other metal ions such as cuprous and cobaltous to give colored complexes. The principal advantage of

Ferrozine, in respect to other ferroin compounds such as 1,10-phenanthroline, is the good solubility of this high molecular weight compound and its complexes in water. It also has a comparative low cost. Ferrozine has the structure shown below.
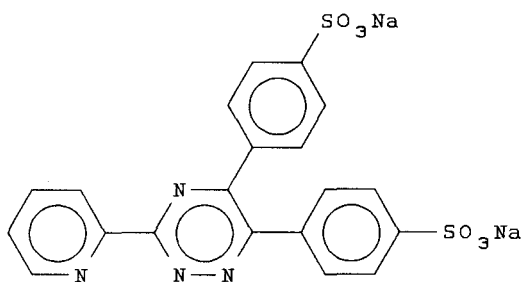
Ferrozine

The visible absorption spectrum of the ferrous complex of Ferrozine exhibits a single sharp peak with maximum absorbance at 562 nm. At this wavelength, the molar absorptivity is 27,900 L/(cm mol). The complex obeys Beer's law to approximately 4 ppm iron. The magenta colored $Fe(Fz)_3^{4-}$ species will form completely in aqueous solution between the pH values of 4 and 9. Once the complex is formed, it is very stable (50).

This iron-Ferrozine reaction was used by Al-Jabari and Jaselskis for the determination of micro amounts of silver(I), copper(II), and nickel(II) (63). In this determination, the metal ions are reduced to their metallic state and then reoxidized with added ferric ion. The ferrous ion produced was then quantitatively complexed by Ferrozine to produce the colored $Fe(Fz)_3^{4-}$ species previously described. The amount of $Fe(Fz)_3^{4-}$ formed was then measured at 562 nm and directly related to the amount of metal originally present in the sample.

Al-Jabari and Jaselskis then took this procedure one step farther with their method for the determination of micro amounts of formaldehyde (49). With this method, formaldehyde is quantitatively oxidized by an excess of hydrous silver oxide to form silver metal. Silver metal is reoxidized with added ferric ion and the ferrous ions produced are quantitatively determined as previously described. This determination is possible since formate ions and silver(I) ions do not produce colored complex ions with Ferrozine.

The determination of hexamethylenetetramine is based on this previous described work with Ferrozine. The determination is accomplished by hydrolysing HMT in strong acid solution, with heating, to produce formaldehyde and ammonia. The released formaldehyde is oxidized by hydrous silver oxide and the resulting metallic silver is quantitatively oxidized by iron(III) in the presence of Ferrozine to produce an iron(II)-Ferrozine complex as shown in the following reactions.

$$C_6H_{12}N_4 + 6\ H_2O \quad \xrightarrow[\text{heat}]{\text{strong acid}} \quad 6\ CH_2O + 4\ NH_3 \qquad (3)$$

$$6\ CH_2O + 12\ Ag^+ \quad \xrightarrow[\text{pH 12-13}]{(OH^-)} \quad 12\ Ag^0 + 6\ HCOO^- \qquad (4)$$

$$12\ Ag^0 + 12\ Fe^{3+} \quad \xrightarrow{H^+} \quad 12\ Fe^{2+} + 12\ Ag^+ \qquad (5)$$

$$12\ Fe^{2+} + 36\ Fz^{2-} \quad \xrightarrow[\text{Fz=Ferrozine}]{\text{pH 3-6}} \quad 12\ Fe(Fz)_3^{4-} \qquad (6)$$
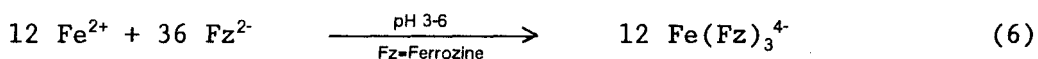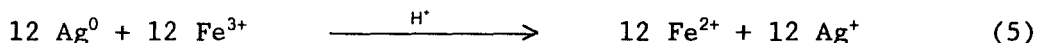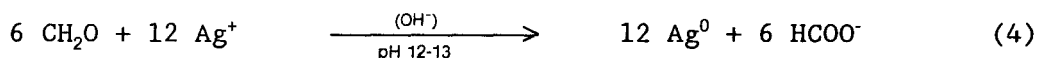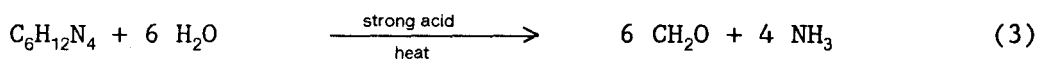
This $Fe(Fz)_3^{4-}$ complex has a molar absorptivity of 27,900 L/(cm mol) at 562 nm (50). Since each HMT molecule yields 12 $Fe(Fz)_3^{4-}$ complex ions, the apparent molar absorptivity for HMT should be 335,000 L/(cm mol).

Chemical amplification occurs in this process because one equivalent of formaldehyde reduces two equivalents of silver(I) and upon reoxidation of metallic silver with iron(III) two equivalents of iron(II) are produced.

The development of this procedure required investigation of a number of test parameters. Hydrolysis parameters were determined including choice of acid used for hydrolysis, acid concentration, reaction temperature, and time needed for completion of hydrolysis. Optimum reaction parameters for the formaldehyde determination were investigated including pH, silver(I) concentration, and time.

The method was also compared to the USP standard method using chromotropic acid (59). The USP standard method is also based on the indirect determination of HMT by the quantitative determination of formaldehyde resulting from the hydrolysis of HMT.

Once the optimum conditions had been established for aqueous samples of HMT alone, the method was applied to samples of HMT containing a large amount of formaldehyde. Because this method determines released formaldehyde from HMT, any formaldehyde present in the sample will also react and interfere. This method and most other indirect methods can successfully determine HMT in the presence of small amounts of formaldehyde. However, these indirect methods fail when there is a large amount of formaldehyde initially present, because the difference between the formaldehyde initially present and the total formaldehyde after hydrolysis is very small. Therefore, techniques were developed to remove or reduce the formaldehyde interference without affecting the HMT present. A number of procedures have been investigated to remove the formaldehyde

interference. The procedures found useful were evaporation and chemical reaction.

After developing this method to determine HMT in the presence of large amounts of formaldehyde, the procedure was used to study the determination of ammonia. Ammonia was reacted with a large excess of formaldehyde and heated to drive the reaction to completion and to form HMT. The large excess of formaldehyde was removed and the HMT present was quantitatively determined. The amount of HMT present was then related to the amount of ammonia originally present in the sample. The development of this procedure required optimization of the HMT formation reaction parameters. The reaction parameters studied include effect of pH, temperature, time, and the concentration of formaldehyde needed.

The determination of HMT in urine samples has not been accomplished very accurately with the many other available methods. Consequently, the feasibility of using this method to accurately determine HMT in urine was studied. Physiological concentrations of HMT range between 0.6 and 1.7 mg/mL (34). Urine samples contain many different compounds which can interfere with this method. Anything that reacts with the reagents used to form a precipitate, such as chloride ion with silver(I), interfere in the absorbance reading process. Compounds may also react to deplete or form an equilibrium with the reagents and thus interfere with the quantitative reaction sought. This interference would include any compound that can react with HMT, reduce hydrous silver oxide, reduce ferric ions, or complex with Ferrozine.

Experimental

Instrumentation:

All spectrophotometric measurements were obtained using a Cary 14 (Varian Instrument Group, Palo Alto) spectrophotometer with 1 cm pathlength quartz cells. The pH measurements were made using a Fisher Accumet model 830 pH meter. Constant temperatures were obtained using a Cole Parmer model 1266-00 immersion circulator water bath. Small amounts of reagents were weighed with a Sartorius semi-micro balance.

Reagents:

All chemicals used were analytical or primary standard grade. Hexamethylenetetramine was purified by recrystalization from absolute ethanol. Before using HMT, it was dried over phosphorous pentoxide for 4 hours as described in the USP XXII standard method (53). A 0.01 M Ferrozine,3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonicacid, monosodium salt monohydrate (Aldrich) was prepared by dissolving 0.511 g in 100 mL distilled water. A 0.004 M iron(III) in 0.09 M sulfuric acid solution was prepared by dissolving 1.929 g ammonium ferric sulfate dodecahydrate (Mallinckrodt) in 500 mL distilled water containing 5 mL concentrated sulfuric acid and then diluting it to 1 L. A 0.075 M silver(I) solution was prepared by dissolving 1.274 g silver nitrate (Fisher) in 100 mL distilled water. An acetate buffer solution of pH 3.5 was prepared by partially neutralizing a 1 L solution containing 29.4 mL glacial acetic acid with concentrated sodium hydroxide. Solutions of 0.025 M nickel(II), zinc(II), cobalt(II), calcium(II), and magnesium(II)

were prepared by dissolving the appropriate amount of nickel nitrate hexahydrate, zinc nitrate hexahydrate, zinc acetate dihydrate, cobalt nitrate hexahydrate, calcium nitrate tetrahydrate, and magnesium nitrate hexahydrate in 100 mL distilled water. Formaldehyde solutions were prepared by diluting an appropriate amount of 37 weight percent formaldehyde (~13.3 M) containing 10-15% methanol (Aldrich). Sodium borohydride solutions were prepared daily dissolving the appropriate amount of sodium borohydride (Aldrich) in 0.2% sodium hydroxide to decrease the decomposition rate (64). Sample evaporations were accomplished under aspirator vacuum in a vacuum desiccator containing Drierite. Chromotropic acid, 4,5-dihydroxynaphthalene-2,7-disulfonic acid, disodium salt dihydrate (Aldrich), reagent solution was prepared by mixing 100 mg chromotropic acid with 50 mL of distilled water in a 100-mL volumetric flask. The solution was cooled in an ice bath and, while cooling, 50 mL of concentrated sulfuric acid was added with mixing, slowly and cautiously The solution was cooled to room temperature, and dilute sulfuric acid (1 in 2) was added to volume. [Note-If excessive heat generated during mixing causes a violet color to appear in the solution, discard the solution and prepare another taking precautions to avoid excess heat.]

Development of Methods

Optimization of HMT Test Parameters:

The development of the spectrophotometric method for the determination of HMT required the optimization of several parameters: (i)

choice of the acid used for hydrolysis, (ii) acid concentration, (iii) hydrolysis temperature, and (iv) the time needed for the completion of hydrolysis. Once the conditions for the hydrolysis of HMT had been established, the resulting hydrolyzed sample was analyzed for formaldehyde. The optimized conditions for the determination of formaldehyde have been elucidated in this laboratory by Al-Jabari and Jaselskis, but for this application minor modifications were required. The following parameters were optimized: (i) amount of silver ion added, (ii) pH (the amount of sodium hydroxide added), and (iii) the amount of iron(III) and Ferrozine added.

The choice of a strong acid for HMT hydrolysis in our method is based on its effectiveness and noninterference during the procedure. In the chromotropic acid method 9 M sulfuric acid is used (59). However, sulfuric acid produces low results in this procedure. It appears that the sulfuric acid reacts with the added silver ions to form a precipitate of silver sulfate (K_{sp} ~10^{-5}), decreasing the amount of silver(I) available to reduce the formaldehyde. Similarly, halogen acids cannot be used since they also produce silver precipitates and turbidity. Nitric acid was not used since its nitration reaction with HMT forms the explosives RDX and HMX (32). Dilute perchloric acid has been chosen as the acid of choice. With perchloric acid, solutions remained clear and hydrolysis proceeded smoothly.

Once the choice of acid had been established, the following hydrolysis parameters were investigated: (i) the concentration of perchloric acid, (ii) hydrolysis temperature, and (iii) time required to accomplish the hydrolysis of HMT. Hydrolysis parameters were adjusted to

achieve maximum hydrolysis within a reasonable amount of time such as within ten minutes. Heating the hydrolysis sample dramatically increases the rate of hydrolysis. This step in the procedure has been carried out by using a thermostatically controlled heated water bath and adjusting the temperature to study its affects. In order to prevent the possible loss of formaldehyde, temperature studies did not exceed 60° C. Table 4 and figure 1 display the results for the time required to achieve maximum HMT hydrolysis at varied acid concentrations and reaction temperatures. These results show that the addition of 1.00 mL of 1.0 M perchloric acid to a 400 μL HMT sample heated at 60° C will provide quantitative results in about 5 minutes. The acid concentration in solution is 0.71 M. Samples larger than 400 μL can also be hydrolyzed as long as the acid concentration is at least 0.71 M.

Table 4   Time study for hydrolysis of HMT at various temperatures and perchloric acid concentrations.

TIME(min)	40°1M	40°2M	50°1M	50°2M	60°0.5M	60°1M	60°2M
0	0%	0%	0%	0%	0%	0%	0%
2	17%	34%	46%	72%	46%	73%	93%
4	31%	58%	69%	91%	73%	90%	95%
6	42%	72%	81%	95%	83%	93%	94%
8	51%	81%	87%	95%	90%	94%	95%
10	58%	84%	91%	95%	91%	94%	95%
12	65%	89%	94%	95%	93%	93%	
15	71%	92%	94%	95%	93%		
20	80%	95%	94%	96%	92%		

% = HMT hydrolysis yield
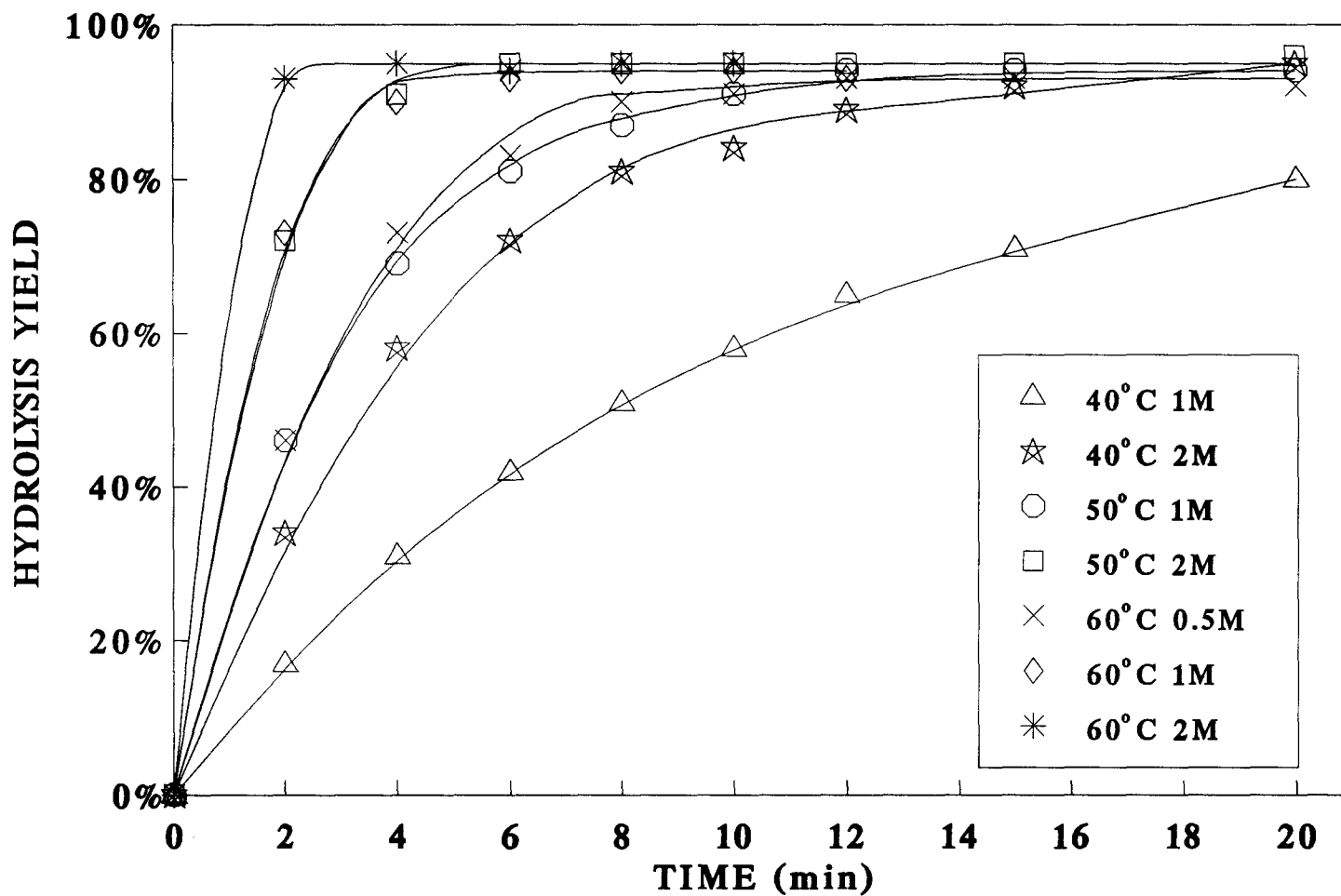
Figure 1   Time study for hydrolysis yield of HMT at various temperatures and perchloric acid concentrations.

Hydrous silver oxide iron(III)-Ferrozine parameters were adjusted by working with 400 μL samples hydrolyzed with 1.00 mL 1.0 M perchloric acid. The pH dependency of the reaction of hydrous silver oxide with formaldehyde was studied by varying the amount of 2.1 M sodium hydroxide added to the HMT sample after hydrolysis. The pH of the solution must be sufficiently high to form hydrous silver oxide but must not to exceed pH 13 because the precipitate becomes difficult to dissolve upon the addition of acidic iron(III) and the reaction of formaldehyde with hydrous silver oxide becomes slow. The reactivity of hydrous silver oxide with HMT hydrolyzed formaldehyde as a function of pH (OH^- concentration) is shown in figure 2. The optimum pH range is 12 to 13. The addition of silver(I) was studied by varying the amount of 0.075 M silver nitrate added. It was noticed that the smaller the amount of silver added, the quicker the colored iron(II)-Ferrozine complex was formed. The amount of silver ion added was varied between 0.005 and 0.025 millimoles (mmol). The results are shown in figure 3. The amount of silver added was chosen to be 0.015 mmol to provide rapid quantitative results. The addition of iron(III) and acetate buffer did not require modification and were used as described by Al-Jabari and Jaselskis. A diagram of the test parameters is shown in figure 4.

The expected apparent molar absorptivity of $12 \times 27,900 = 335,000$ (L/cm mol) was not obtained. The apparent molar absorptivity that this investigator obtained was between 312,000 and 322,000 (L/cm mol). Because of this slight variation, the most accurate determination results were obtained by preparing a standard calibration curve.
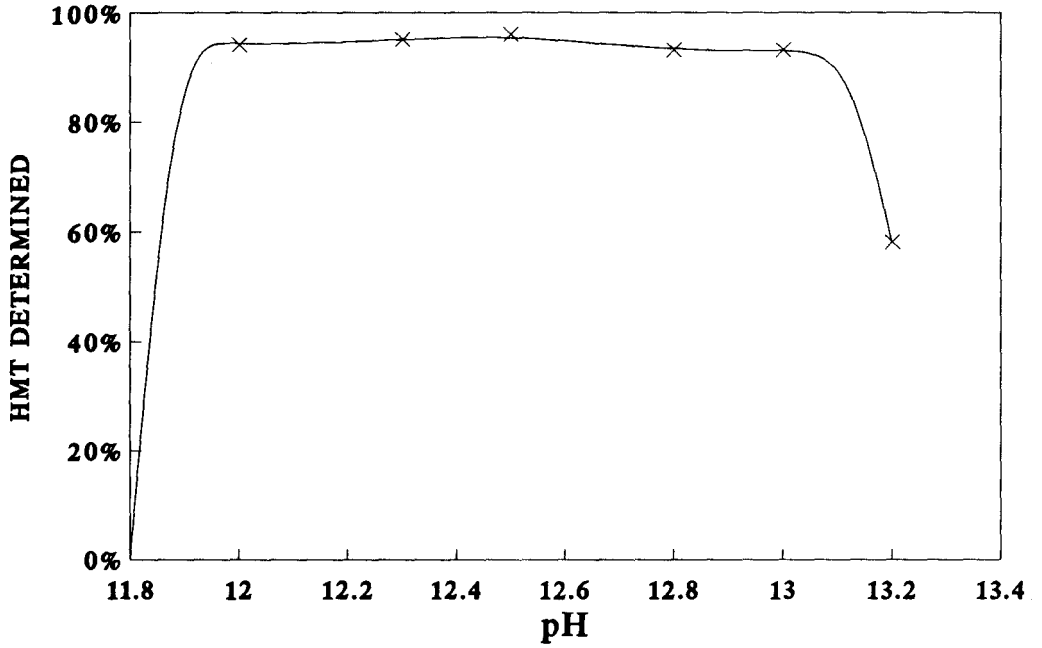
Figure 2   Study of pH effects on hydrous silver oxide reaction with HMT hydrolyzed formaldehyde.
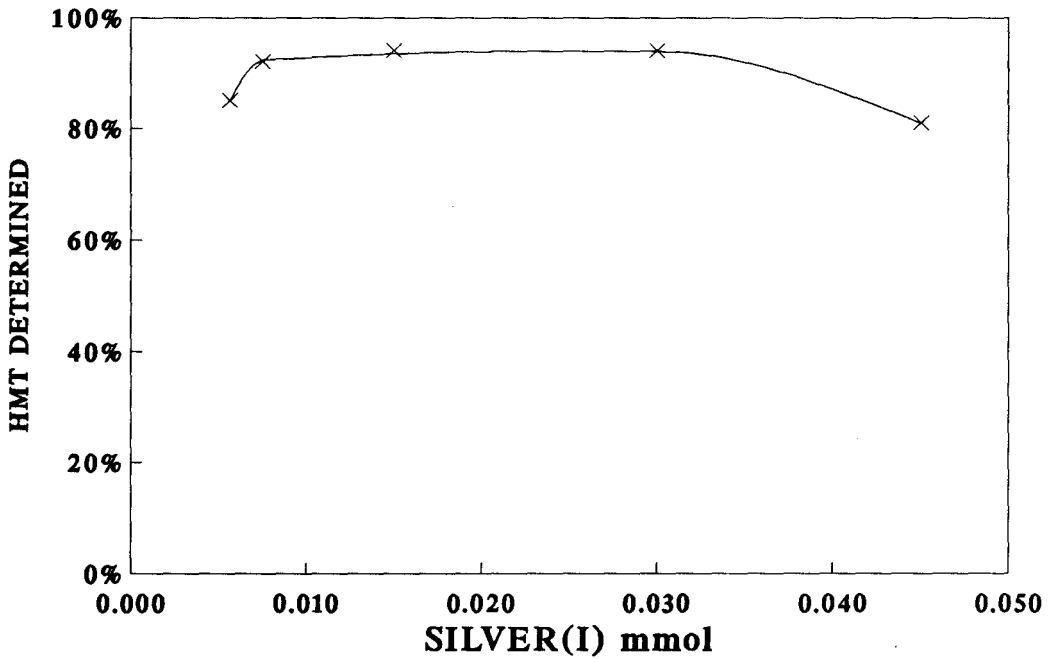
Figure 3   Study of the amount of silver(I) required for optimum results at pH 12.8.

HMT DETERMINATION

| HMT sample |

Hydrolyse HMT — add $HClO_4$; solution $\geq 0.71M$ heat $60^{\circ}C \geq 5$ min. chill to condense vapor

| CH_2O |

Oxidize CH_2O — add 0.015 mmol Ag^+ raise pH to 12-13 with NaOH vortex mix, react ≥ 5 min.

| Ag^o |

Reduce Fe^{3+} Form Complex — add 0.008 mmol Fe^{3+} add buffer pH 3.5 add 0.02 mmol Ferrozine dilute to vol., react ≥ 5 min.

| $Fe(Fz)_3^{4-}$ | measure abs. at 562 nm
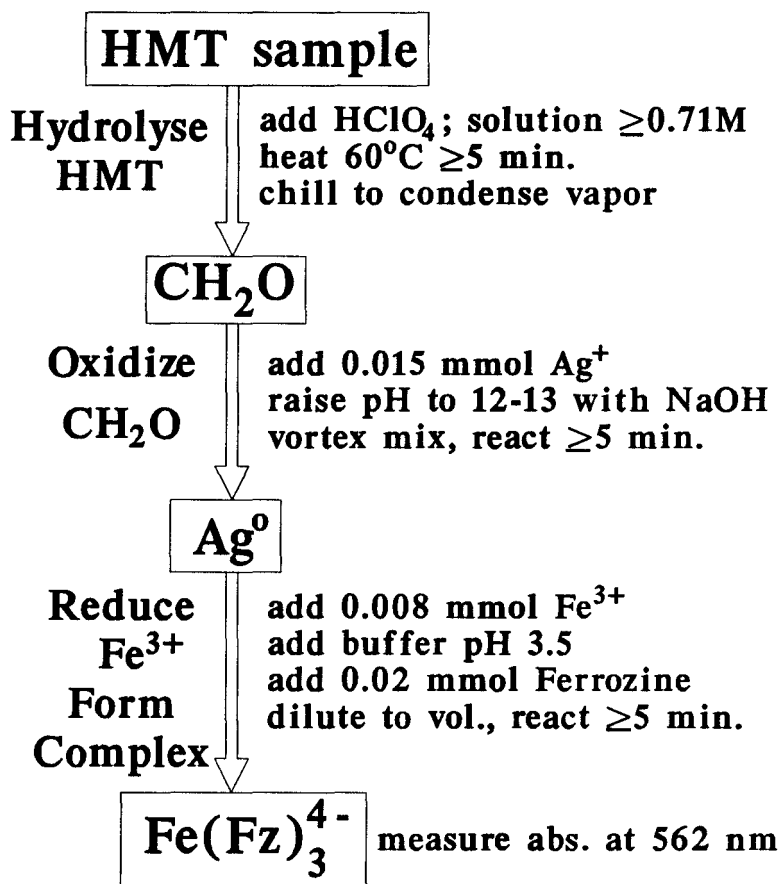
Figure 4   Flow diagram of the optimized HMT determination parameters.

Evaporation of Formaldehyde:

The removal of formaldehyde from HMT samples by evaporation appeared deceptively simple. Since formaldehyde has a boiling point of -20° C (65) and HMT has a melting point (sublimation point) at 260° C (66), formaldehyde should easily leave the sample with only solid HMT remaining after complete evaporation of the sample. However, this evaporation procedure produced two major problems. As the formaldehyde evaporated, it mainly polymerized into paraformaldehyde (67). Paraformaldehyde readily reacts under conditions of the determination in a manner equivalent to formaldehyde. Therefore, it is the source of a large interference. Also, it was found that HMT has a slight vapor pressure. When HMT is subjected to the sample evaporation conditions, sublimation occurs. To succeed with this method, inhibition of paraformaldehyde formation had to be achieved during evaporation of the sample, and the vapor pressure of the remaining HMT had to be decreased to prevent its sublimation.

The removal of the formaldehyde could be accomplished by adding dilute weak acid to the sample. The acid inhibits the polymerization and formation of paraformaldehyde during evaporation. Any paraformaldehyde formed in the weak acid is readily reconverted to formaldehyde (68).

$$\sim CH_2OCH_2OCH_2O\sim + H_2O \xrightarrow{\ H^+\ } CH_2O + 2 \sim CH_2OH \qquad (7)$$
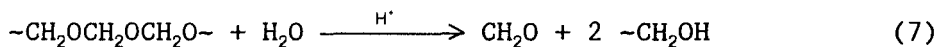
The acid added cannot be a strong acid because a strong acid will start to hydrolyze the HMT. Bose (24) described earlier that in a dilute solution of acetic acid HMT did not noticeably hydrolyze over a short period of time. Experiments confirmed that a small amount of glacial

acetic acid added to the sample did not decompose HMT. A sample containing 0.2 M formaldehyde could be evaporated when it contained approx. 0.1 M acetic acid. However, it was found that the acetic acid would decompose HMT if the solution was allowed to sit for long periods of time.

To prevent the sublimation of HMT, a salt was added to the sample before evaporation to complex and hold HMT as a less volatile complex. Sodium bisulfate was tested and appeared to have no affect. Metal salts were then tested. The results are shown in table 5. Zinc nitrate was tested and the retention of HMT decreased rapidly when the zinc to HMT ratio was greater than one. Cobalt nitrate and nickel nitrate, added in a 2:1 or greater ratio with respect to HMT, yielded reproducible results with HMT recovery between 97 and 103 percent. Other metals tested include calcium nitrate and magnesium nitrate. Both calcium and magnesium produced inconclusive results with varied ratios of the salt and HMT. All of the salts were also tested with a large amount of formaldehyde present and acetic acid added. All samples containing formaldehyde produced less accurate results than those without formaldehyde. Nickel nitrate produced the best results when the metal concentration was varied from a molar ratio of 1:1 to 16:1 Ni(II):HMT. Ni(II) also produced low blank values contrasted to Co(II). When the sample was evaporated to "dryness", small droplets still remain in the bottom of the test tube. This behavior also occurred when the sample was evaporated in a vacuum desiccator by either water aspirator vacuum or a vacuum pump. Most of the salts tested were chosen because of their high water of hydration which could possibly make the HMT complex more difficult to evaporate.

Table 5 also indicates a problem. The samples which contained 0.2 M formaldehyde produce higher blank values than the samples with no formaldehyde. This residual blank interference requires a correction when determinations are made using large amounts of formaldehyde in the sample. Fortunately, the blank increase is linear and proportional to the amount of formaldehyde originally present in the sample. A blank formaldehyde calibration curve must be determined along with the HMT sample determinations.

The formaldehyde concentration in the HMT sample can be determined using the spectrophotometric method by Al-Jabari and Jaselskis (49). Under these conditions, HMT does not interfere. Once the formaldehyde concentration in each sample is determined, the appropriate blank correction can be subtracted using the blank calibration curve.

| Table 5 | | | | ontaining metal salts to prevent sublimation of HMT. | | |

Table 5 — Aspirator vacuum evaporation study of 150 μL samples containing metal salts to prevent sublimation of HMT.

	HMT	CH_2O^a	*Metal*[b]	M:HMT[c]	A_{obs}	HMT
Nickel	0		10μL		0.013	blank
	0		20μL		0.013	blank
	LO		20μL	16:1	0.219	100%
	HI		20μL	4:1	0.800	99%
	HI		10μL	2:1	0.792	98%
	0	×	20μL		0.156	blank
	LO	×	20μL	16:1	0.367	102%
	HI	×	20μL	4:1	0.957	101%
Zinc	0		10μL		0.011	blank
	0		20μL		0.010	blank
	LO		20μL	16:1	0.050	19%
	HI		20μL	4:1	0.731	91%
	HI		10μL	2:1	0.781	97%
	0	×	20μL		0.042	blank
	LO	×	20μL	16:1	0.070	4%
	HI	×	20μL	4:1	0.716	85%
Cobalt	0		10μL		0.150	blank
	0		20μL		0.286	blank
	LO		20μL	16:1	0.499	103%
	HI		20μL	4:1	1.075	99%
	HI		10μL	2:1	0.941	99%
	0	×	20μL		0.332	blank
	LO	×	20μL	16:1	0.569	114%
	HI	×	20μL	4:1	1.128	100%
Calcium	0		10μL		0.008	blank
	0		20μL		0.008	blank
	LO		20μL	16:1	0.231	108%
	HI		20μL	4:1	0.827	103%
	HI		10μL	2:1	0.803	100%
	0	×	20μL		0.061	blank
	LO	×	20μL	16:1	0.259	96%
	HI	×	20μL	4:1	0.936	110%
Magnesium	0		10μL		0.008	blank
	0		20μL		0.010	blank
	LO		20μL	16:1	0.235	109%
	HI		20μL	4:1	0.794	99%
	HI		10μL	2:1	0.793	98%
	0	×	20μL		0.072	blank
	LO	×	20μL	16:1	0.260	91%
	HI	×	20μL	4:1	0.898	104%

[a] CH_2O = 0.20M solution [b] Metal = 0.025M

Reduction of Formaldehyde:

The removal of formaldehyde by reduction to methanol has many advantages over evaporation, the main advantage being a decrease in the amount of time needed to remove the formaldehyde. The evaporation method requires in excess of 2 hours to remove the water and formaldehyde. The reduction of formaldehyde to methanol reduces this time consuming step to an elapsed time of approximately 15 minutes.

Formaldehyde is readily reduced to methanol by reaction with sodium borohydride (69).

$$4 \ CH_2O + NaBH_4 \xrightarrow{\quad H_2O \quad} 4 \ CH_3OH + NaBO_2 + 2 \ H_2O \qquad (8)$$
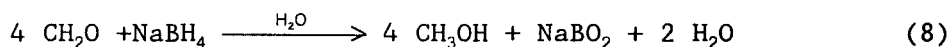
After borohydride reduction, the solution must be slightly acidified prior to HMT testing to destroy all remaining borohydride. Borohydride will interfere in this spectrophotometric method, but the remaining methanol and borate compounds do not interfere. It was found that a $NaBH_4:CH_2O$ mole ratio of 1:1 or greater produced analytically acceptable results. Sodium borohydride must be prepared daily in 0.2% sodium hydroxide. It remains stable for several hours.

The borohydride reduction of various amounts of formaldehyde also affects the spectrophotometric blank values. The residual interference is similar, but greater, than that observed for the Ni(II) evaporated samples previously described. As shown in figure 5, the blank absorbances increase as the original formaldehyde concentration increases. However, the blank increase is linear and relatively small for low formaldehyde concentrations. For accurate determinations of HMT at high formaldehyde concentrations, a blank calibration curve of known formaldehyde

concentrations must be employed as described for the Ni(II) evaporation method.
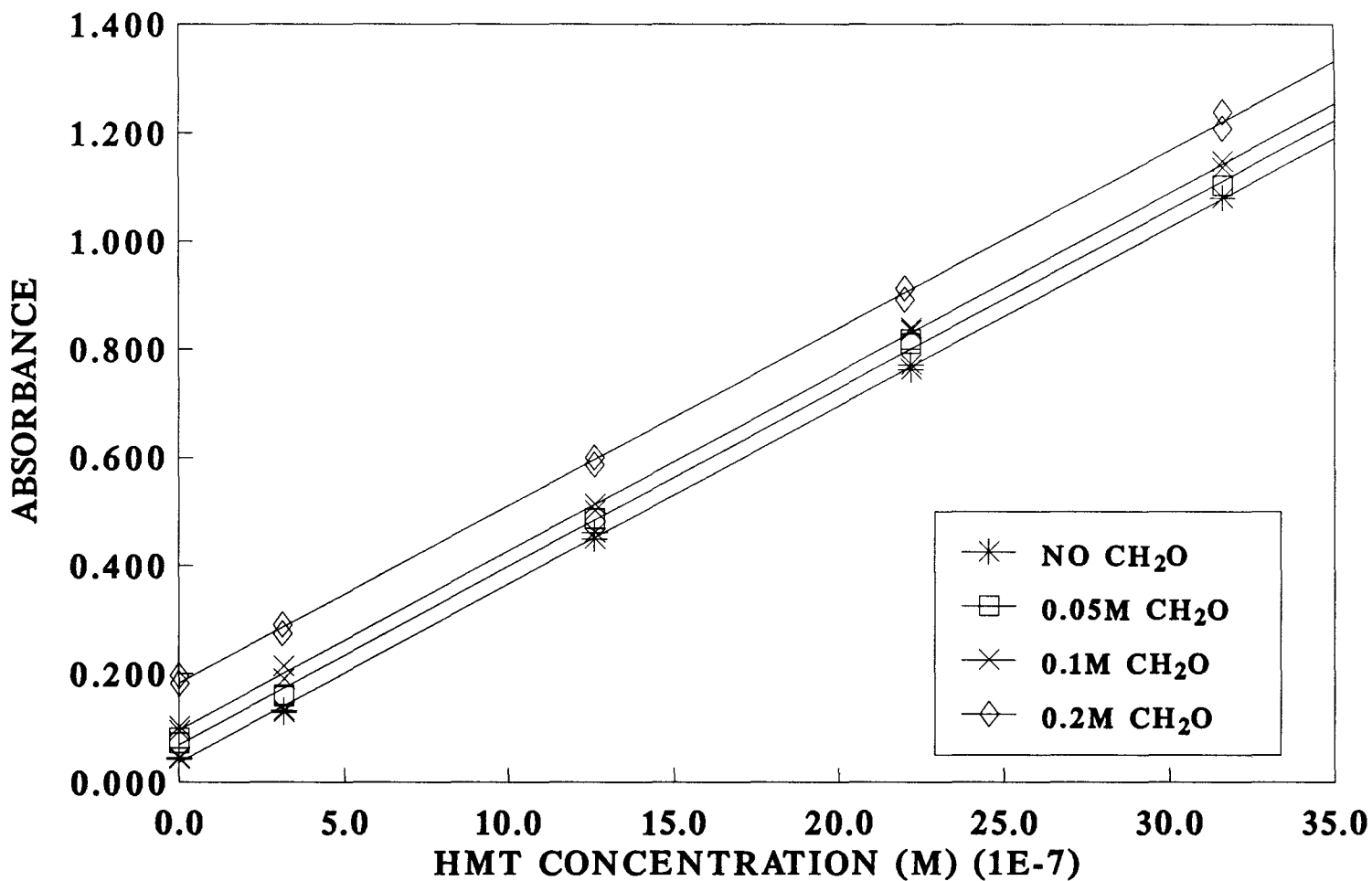
Figure 5   Study of blank residual absorbances in HMT standards following borohydride reduction. The standards were studied with formaldehyde at concentrations of 0 to 0.2 M.

Quantitative Ammonia Condensation With Formaldehyde to Form HMT:

The formation of HMT by the reaction of ammonia with formaldehyde has been previously described and is shown below.

$$4 \ NH_3 + 6 \ CH_2O \longrightarrow C_6H_{12}N_4 + 6 \ H_2O \tag{1}$$
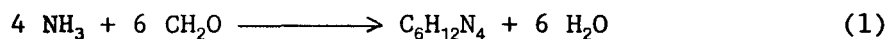
The quantitative conversion of ammonia to HMT requires optimization of the following variables: i) pH, ii) temperature, iii) time, and iv) formaldehyde concentration.

The pH of the reaction solution has been tested in various buffered and unbuffered solutions. Kawasaki and Ogata (25) reported that the maximum rate of formation occurred at a pH of 9.8. The initial pH of our unbuffered reaction solution with a 12:1 excess of formaldehyde was 9.8 and the final pH after reaction completion dropped to approx. 8.2. The reaction solutions were buffered at pH 8.5, 9.8, and 10.5 with borax buffers. However, the buffered solutions produced less satisfactory results than the unbuffered solutions. All subsequent reactions were carried out in unbuffered solutions.

The condensation of ammonia with formaldehyde was carried out with a sizable excess of formaldehyde. The reaction rate increased with the excess concentration of formaldehyde, and the condensation occurred within a reasonable amount of time. The parameters of formaldehyde concentration, temperature, and time were varied. Formaldehyde to ammonia mole ratios of 12:1 and 24:1 were investigated. The stoichiometric molar ratio of formaldehyde to ammonia is 1.5:1. In our experiments this ratio corresponds to a formaldehyde molar excess of 8 to 16 times. The mole

ratios $CH_2O:NH_3$ of 12:1 and 24:1 were found to be acceptable and enabled the quantitative removal of formaldehyde prior to the HMT determination. At formaldehyde concentrations higher than the 24:1 ratio, the remaining residual interference required additional estimation of the high blank values. Reaction temperatures were varied from 40° to 60° C. Aliquots from the reaction solutions were removed at timed intervals and the reaction stopped in ice water. The reaction was followed for 8 hours reaction time. The results of this study are shown in figure 6. These results show that an increase in reaction temperature of 10° C, reduces reaction completion time by about one half. The condensation of formaldehyde with ammonia (12:1 ratio) and the formation of HMT is complete after about 8, 4, and 2 hours at the temperatures of 40°C, 50°C, and 60°C respectively. When the ratio of formaldehyde to ammonia is increased to 24:1, the condensation of ammonia to form HMT is complete in about 4, 2, and 1 hour at 40°, 50°, and 60° C. At temperatures higher than 60° C the control of temperature was not as accurate and furthermore the loss of formaldehyde during hydrolysis was expected. Thus, 60° C was used for the hydrolysis of HMT.
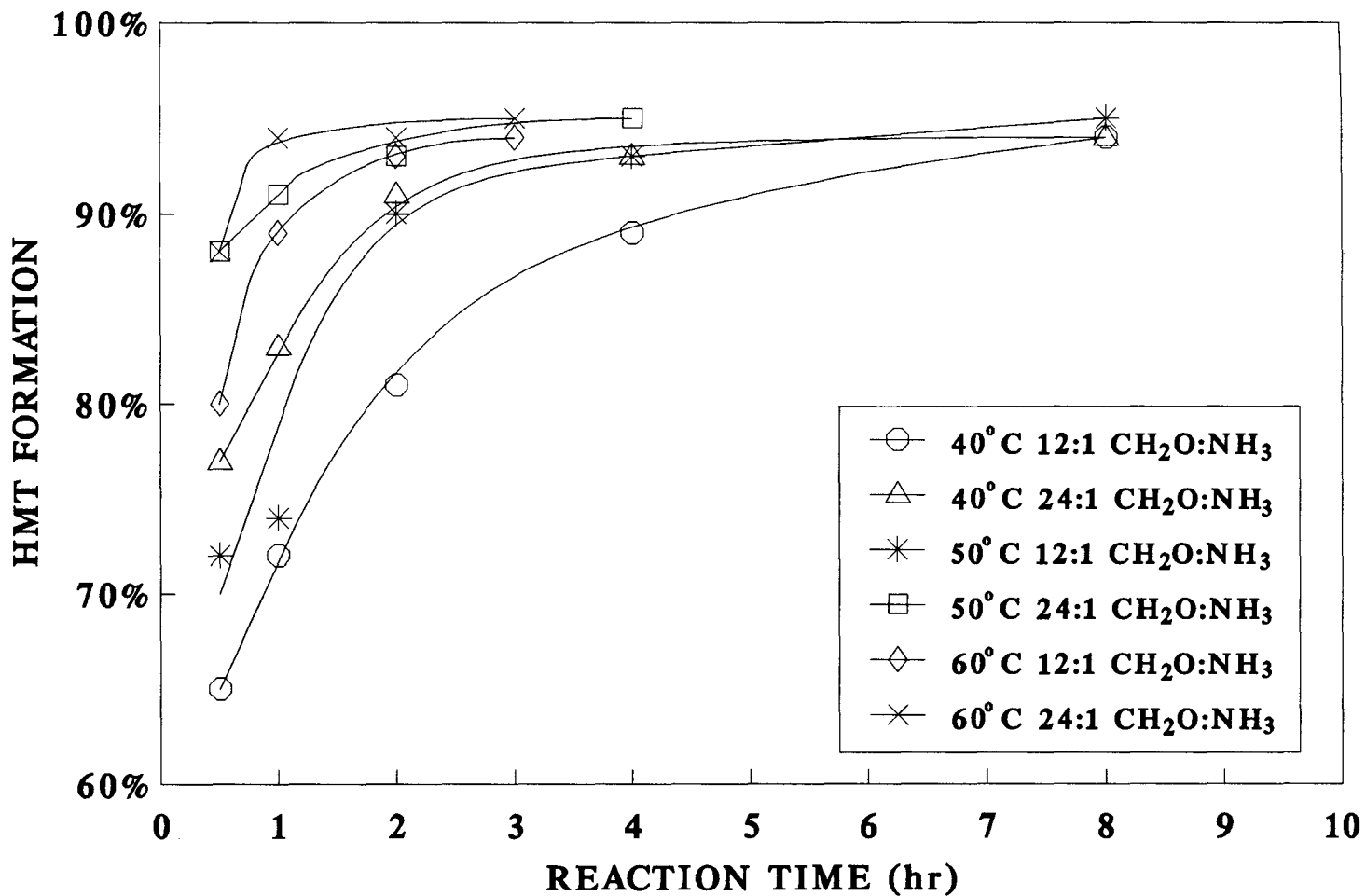
Figure 6   Time study of ammonia condensation with formaldehyde at various temperatures and formaldehyde:ammonia ratios.

<u>Urine Samples:</u>

Urine samples are complex and may contain many different interfering components. Tests performed on dilute urine samples without HMT produce colored interferences too large to subtract from samples containing hydrolyzed HMT. It was required that these interferences be eliminated before the HMT determination.

Removal of these interferences can be accomplished by chemical means. Richmond, Myers, and Wright (23) reported that HMT is very stable to hydrolysis in alkaline conditions. Thus, the interferences which are capable of reducing silver(I) in an alkaline media, including small amounts of formaldehyde, can be removed by "prereacting" the sample with hydrous silver oxide. Silver(I) and hydroxide were added to the sample and the sample heated at 60° C for 10-15 minutes to complete the reaction. The sample was then cooled, centrifuged, and filtered to remove all precipitated interferences. An aliquot of the alkaline supernatant was removed and analyzed for HMT as previously described.

Procedures

<u>Determination of Aqueous HMT:</u>

Hexamethylenetetramine in solid or aqueous samples was determined by hydrolysis to formaldehyde and subsequent reaction with hydrous silver oxide, iron(III), and Ferrozine.

Solid samples were weighed and dissolved with distilled water and treated in the same manner as aqueous samples. Sample aliquots of 400 μL containing 0.016 to 0.16 μmol HMT were pipetted into 50 mL volumetric

flasks using an Eppendorf pipette. The samples were hydrolyzed by adding 1 ml of 1 M perchloric acid and heating the stoppered flasks in a 60° C water bath for 10 minutes. After chilling in ice water for 1 minute, the samples were neutralized and made basic with 500 μL of 2.2 M sodium hydroxide. Silver(I) was added by pipetting 200 μL of 0.075 M silver nitrate into the sample to form hydrous silver oxide and allowed to react, after vigorous mixing, for 10 minutes. Two mL of 0.002 M acidic iron(III), 2 mL of 0.01 M Ferrozine, and 6 mL of pH 3.5 acetate buffer were added and the samples agitated and diluted to volume. The absorbance of the iron(II)-Ferrozine complex was measured at 562 nm after 10 minutes using a 1 cm pathlength cells. To obtain the high precision results, the amount of time required for each step must be closely monitored. The amount of time for each step was adjusted so as to allow the handling of 10 samples during a run. Each sample was given the same amount of reaction time in each step. A flow diagram of this procedure is shown in figure 7. Both standard samples and simulated unknown samples were determined by analyzing the samples in the same manner.

HMT DETERMINATION

$\boxed{\textbf{HMT sample}}$

Hydrolyse HMT $\Big|\Big|$ add 1 mL 1 M $HClO_4$
heat at $60^{o}C$ for 10 min.
chill for 1 min.

$\boxed{\textbf{CH}_2\textbf{O}}$

Oxidize CH_2O $\Big|\Big|$ add 200 μL 0.075 M Ag^+
add 500 μL 2.2 M NaOH
vortex mix, react for 10 min.

$\boxed{\textbf{Ag}^{o}}$

Reduce Fe^{3+} Form Complex $\Big|\Big|$ add 2 mL 0.004 M Fe^{3+}
add 6 mL buffer pH 3.5
add 2 mL 0.01 M Ferrozine
dilute to vol., react 10 min.

$\boxed{\textbf{Fe(Fz)}_3^{4-}}$ measure abs. at 562 nm
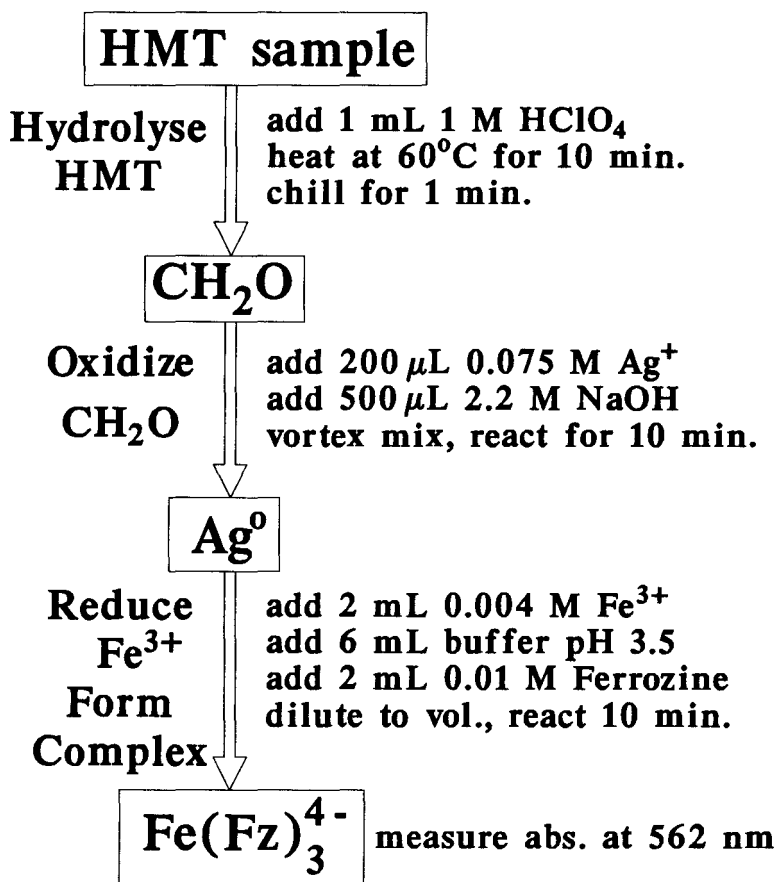
Figure 7   Flow diagram for the HMT determination procedure.

Determination of HMT by USP XXII Chromotropic Acid Method:

Hexamethylenetetramine in synthetic aqueous samples was determined by the USP XXII method (59). Sample aliquots of 80.0 μL containing 0.026 to 0.26 μmol HMT were pipetted into 10-mL volumetric flasks. Five mL of dilute sulfuric acid (1 in 2) and, 2.5 mL of chromotropic acid reagent solution were added and mixed. The 10-mL volumetric flasks were placed in a boiling water bath for 30 minutes, accurately timed, and then immediately cooled in ice water to room temperature. Dilute sulfuric acid (1 in 2) was added to volume, the solution mixed, and the absorbance measured at 570 nm against a blank.

Determination of HMT-monomandelate:

Samples of methenamine mandelate, the most common prescription form of HMT administered for urinary tract infections, in synthetic aqueous samples were tested by the same procedure as described for HMT. Mandelate does not interfere with any of the HMT tests and HMT-monomandelate produced the same results as pure HMT.

Determination of HMT in the Presence of a Large Amount of Formaldehyde:

Hexamethylenetetramine in the presence of a large excess of formaldehyde was determined using a modification of the hydrolysis and hydrous silver oxide iron(III)-Ferrozine method. The determination was accomplished by eliminating the large amount of formaldehyde before the hydrolysis of HMT. The elimination of formaldehyde was achieved by either evaporation or chemical reduction. To obtain the most accurate results for either method of formaldehyde elimination, a blank correction

calibration curve related to the original amount of formaldehyde in the sample was made. Formaldehyde blanks were determined along with the samples being tested. The blanks were linear and proportional to the original amount of formaldehyde present in the samples. If the concentration of formaldehyde in each sample was not known, the samples were tested for formaldehyde with the hydrous silver oxide iron(III)-Ferrozine method by Al-Jabari and Jaselskis (49). The samples were diluted 20 fold to obtain a formaldehyde concentration of ≤0.01 M. A 100 μL aliquot was introduced into a 50 mL volumetric flask containing 300 μL 0.075 M silver nitrate and was made alkaline with 100 μL 0.1 M sodium hydroxide. After vigorous mixing and 5 minutes standing, 2 mL 0.004 M Fe(III), 2 mL 0.01 M Ferrozine and 5 mL pH 3.5 acetate buffer were added and the contents diluted to volume. Absorbance of the iron(II)-Ferrozine complex was measured at 562 nm after 10 minutes.

Evaporation of formaldehyde containing samples and blanks was accomplished as follows. Sample aliquots of 50 μL containing 0.016 to 0.16 μmol HMT were pipetted into 30 mL test tubes. Twenty μL 0.025 M nickel nitrate and 10 μL glacial acetic acid were added and the sample was evaporated using aspirator vacuum in a vacuum desiccator containing Drierite. Samples were always kept to a maximum of 150 μL since larger volumes require too much time to evaporate. After evaporation was completed, 400 μL of distilled water were added and the samples were determined in the same manner as previously described for aqueous samples of HMT with a final diluted volume of 25 mL. A flow diagram of this procedure is shown in figure 8.

Reduction of the formaldehyde containing samples was accomplished as follows. Sample 50 μL aliquots containing 0.016 to 0.16 μmol HMT and up to 0.2 M formaldehyde were pipetted into 25 mL volumetric flasks. Twenty μL 2.40 M sodium borohydride was added and allowed to react for about 15 minutes. The samples were neutralized with 25 μL 2 M perchloric acid, diluted to about 400 μL, and determined as previously described for aqueous samples of HMT. A flow diagram of this procedure is also shown in figure 8.

ELIMINATION OF CH$_2$O

$$\boxed{\text{50 } \mu\text{L HMT} + \text{CH}_2\text{O}}$$

add 20 μL 0.025M Ni^{2+} add 20 μL 2.4 M BH$_4$

add 10 μL glacial acetic acid   react 15 min.

evaporate to dryness   add 25 μL 2 M HClO$_4$

Evaporation   *Reduction*

dilute to 400 μL   dilute to 400 μL

$$\boxed{\text{400 } \mu\text{L HMT}} \qquad \boxed{\text{400 } \mu\text{L HMT}}$$

Proceed with HMT determination
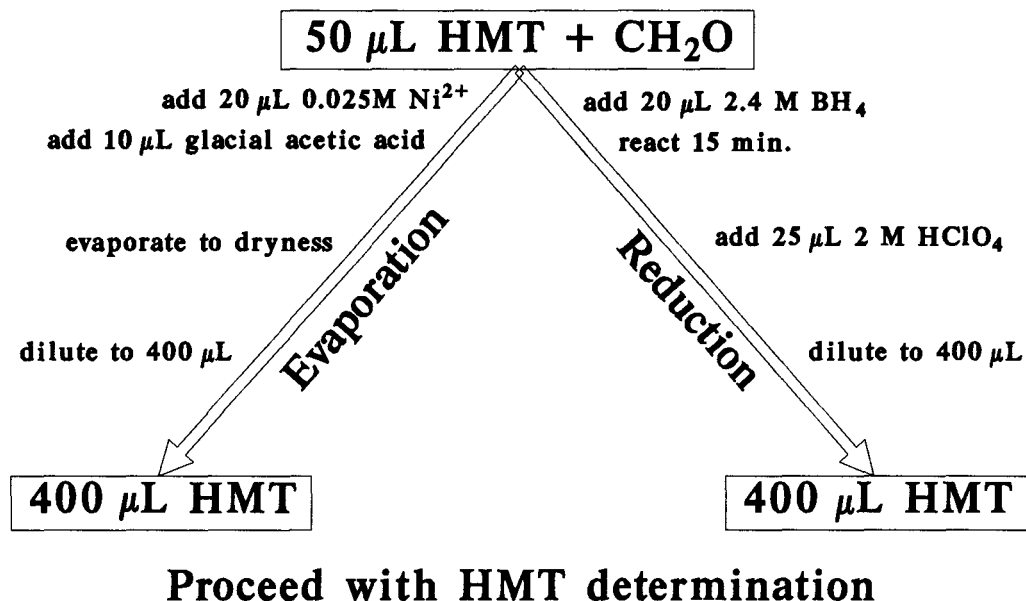
Figure 8   Flow diagram for formaldehyde interference removal by evaporation or reduction.

Determination of Ammonia:

The determination of ammonia was accomplished by first reacting the ammonia with an excess amount of formaldehyde. Once the reaction was complete, the formaldehyde was removed and the remaining HMT was determined and quantitatively related to the amount of ammonia originally present in the sample. A formaldehyde concentration of 0.1 M was used in these tests to make its removal for the HMT determinations easier.

Ammonia samples and standards were added quantitatively to volumetric flasks so that the final diluted ammonia concentration was in the range between 0.5 and 8.5 mM. Formaldehyde was quantitatively added to the flask to produce a diluted concentration of 0.10 M, the solution was diluted to volume with distilled water, and mixed. The flask was immersed in a 60° C water bath for 4 hours to insure complete HMT condensation. The sample was then cooled to room temperature and a 50 μL aliquot was removed to test for HMT formed. At this point the samples contained HMT and a large amount of formaldehyde. The samples were determined either using the evaporation method or the reduction method of formaldehyde removal. Both procedures were compared and HMT was determined as described previously for the determination of HMT in the presence of a large excess of formaldehyde.

Determination of HMT in Urine:

Urine samples spiked with HMT were determined for HMT using a modification of the previous methods to remove the interferences present. The removal of these interferences was based on the stability of HMT to hydrolysis in alkaline conditions.

Urine sample aliquots of 50 μL containing 0.096 to 0.48 μmol HMT were pipetted into 10 mL test tubes containing 20 μL 2.2 M sodium hydroxide and 550 μL 0.09 M silver nitrate. The test tubes were stoppered and heated at 60° C for 10 minutes to oxidize the interferences with silver oxide. The samples were chilled in ice water, centrifuged, and filtered through cotton plugged pasteur pipettes to remove all precipitated interferences. Aliquots of 100 μL from each sample were placed in 25 mL volumetric flasks containing 300 μL water and HMT was determined in the samples as previously described for aqueous samples of HMT.

A flow diagram of this procedure is shown in figure 9. Since interferences in urine vary, the method of standard additions was found to be best suited for determination of HMT present.

ELIMINATION OF URINE INTERFERENCE

50 μL HMT-urine

add 20 μL 2.2M NaOH
add 550 μL 0.09M Ag$^+$
heat 60°C 15 min.

chill to room temp.
centrifuge
filter supernatant

620 μL HMT-urine

remove 100 μL aliquot
add 300 μL H$_2$O

400 μL HMT(urine)

Proceed with HMT determination
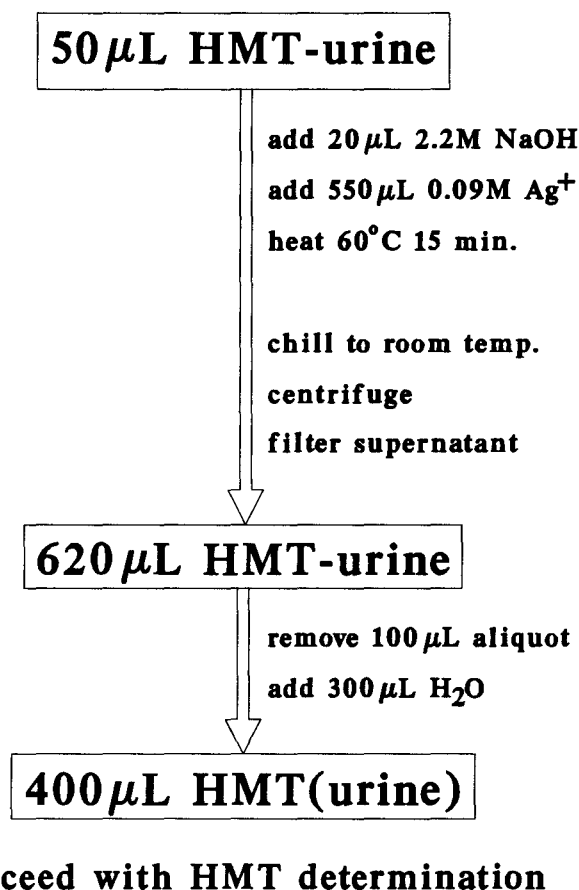
Figure 9   Flow diagram for the removal of urine interferences.

Results and Discussion

All the results of the various determinations in this study use the hydrolysis, silver oxide, and iron(III)-Ferrozine method unless otherwise noted. All calibration curves are shown with the 95% confidence intervals plotted as dotted lines. The simulated unknowns are plotted on the corresponding calibration curve as error bars of their standard deviations. For comparative purposes the simulated unknown results are also shown in tabular form.

Determination of Aqueous HMT Alone:

The standard calibration curve for the determination of HMT in aqueous samples is shown in figure 10. The unknown amount of HMT in synthetic samples is also determined using the standard calibration curve. The results of the unknown sample determinations are shown in figure 10 and table 6.

The calibration curve, figure 10, shows that the iron(II)-Ferrozine complex, formed after the hydrolysis of HMT, follows Beers law in the range 3.2×10^{-7} to 3.2×10^{-6} M HMT. This method has been found to be linear to at least 1.6 absorbance units. The theoretical apparent molar absorptivity should be $12 \times 27,900 = 335,000$ L/(cm mol) at 562 mn. However, the obtained apparent molar absorptivities are typically between 314,000 and 322,000, or equivalent to 94 to 96 percent of the expected 335,000 value.

This procedure requires about 40 minutes for the determination of 10 samples. The samples are run through each step at one minute time intervals between samples.

Table 6   Determination of HMT in simulated pure aqueous unknown samples and formaldehyde containing unknown samples.

Sample (ppb)	Formaldehyde conc. (M)	(M)det.	Absorbance[a] (A)	Amount determined (ppb)	(%)	R.S.D.[b]
			Pure aqueous HMT samples			
111			0.276 ±0.003	113	102	1.07
210			0.502 ±0.002	213	102	0.345
367			0.852 ±0.002	370	101	0.242
			Aqueous CH_2O-HMT samples: CH_2O removal by evaporation			
108	0.0331	0.0297	0.287 ±0.002	109	101	0.718
334	0.164	0.163	0.800 ±0.005	332	100	0.650
251	0.0943	0.0933	0.611 ±0.002	251	100	0.267
			Aqueous CH_2O-HMT samples: CH_2O removal by $NaBH_4$ reduction			
108	0.0331	0.0297	0.286 ±0.004	109	101	1.38
334	0.164	0.163	0.849 ±0.004	334	100	0.500
251	0.0943	0.0933	0.639 ±0.005	254	101	0.706

[a]Average of four samples and standard deviation
[b]R.S.D. = relative standard deviation

Regression Output:
Constant   0.0237
Std Err of Y Est   0.0115
R Squared   0.999
No. of Observations   16
Degrees of Freedom   14
Slope   314000
Std Err of Coef   2650
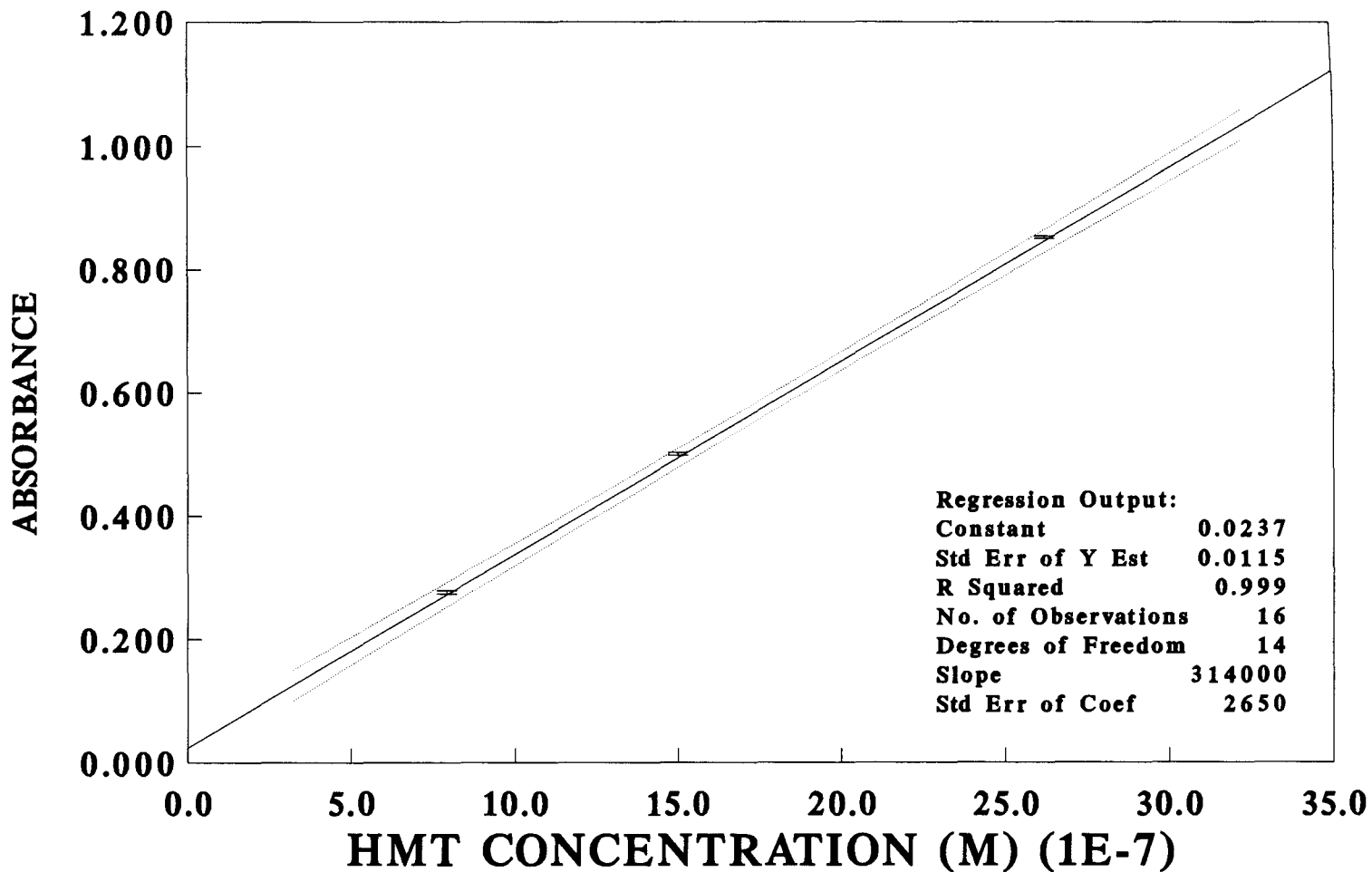
Figure 10   Calibration curve for the determination of pure aqueous HMT and synthetic unknown samples.

Determination of HMT by the USP XXII Chromotropic Acid Method:

The standard calibration curve for the determination of HMT in aqueous samples is shown in figure 11. Unknown synthetic samples were also run and HMT determination was accomplished using the standard calibration curve. The results of the unknown HMT sample determinations are shown in figure 11 and table 7.

The standard curve in figure 11 shows that the results are very linear with a correlation coefficient r^2 of 0.9998 for 6 samples. However, the apparent molar absorptivity of 41,400 L/(cm mol) is less than one half the theoretical apparent molar absorptivity of 15,900 X 6 = 95,400 L/(cm mol). Formaldehyde was tested along with the HMT samples for comparison. The results are shown in figure 12. The formaldehyde samples were tested by the same procedure as HMT and produced a molar absorptivity of 15,900 L/(cm mol). This good molar absorptivity agreement for formaldehyde demonstrates that the low HMT results are probably not due to formaldehyde lost during the 30 minute, 100° C hydrolysis step. The low results may be a consequence of the secondary reactions of HMT or decomposition of the chromotropic acid. Incomplete hydrolysis is probably not responsible for the low results.

Sulfuric acid was studied for the hydrolysis of HMT with the hydrous silver oxide iron(III) Ferrozine determination of formaldehyde. The results of 0.71 M sulfuric acid hydrolysis at 60° C ranged between 70 and 95 percent of the expected released formaldehyde. Since the chromotropic acid method uses 9 M sulfuric acid, lower results should not be expected.

The chromotropic acid method appears to be very time dependent. The USP procedure states that the 30 minute hydrolysis be accurately

timed. The measured absorbance of each sample decreased on repetitive observations. The absorbances may have stabilized after about 10 minutes after final dilution. It was also noted that the chromotropic acid reagent possibly degrades in solution after a few hours. Experiments with a fresh reagent produced apparent molar absorptivities of approx. 48,000 L/(cm mol). Six hours later the same reagent produced apparent molar absorptivities approx. 35,000 L/(cm mol). The results obtained with the 6 hour old reagent were also very erratic.

The USP method employs an HMT standard in the determination and does not list an expected apparent molar absorptivity. The use of the standard addition procedure accounts for any variation in the determination.

This procedure requires more than 1 hour for the determination of 10 samples. Each sample is analyzed separately which requires a minimum of a three minute interval between samples. A batch process of performing each step on all of the samples simultaneously was not used, because the absorbance decreased with time and must be closely monitored.

Regression Output:
Constant   0.0267
Std Err of Y Est   0.00853
R Squared   1.00
No. of Observations   6
Degrees of Freedom   4
Slope   41400
Std Err of Coef   286
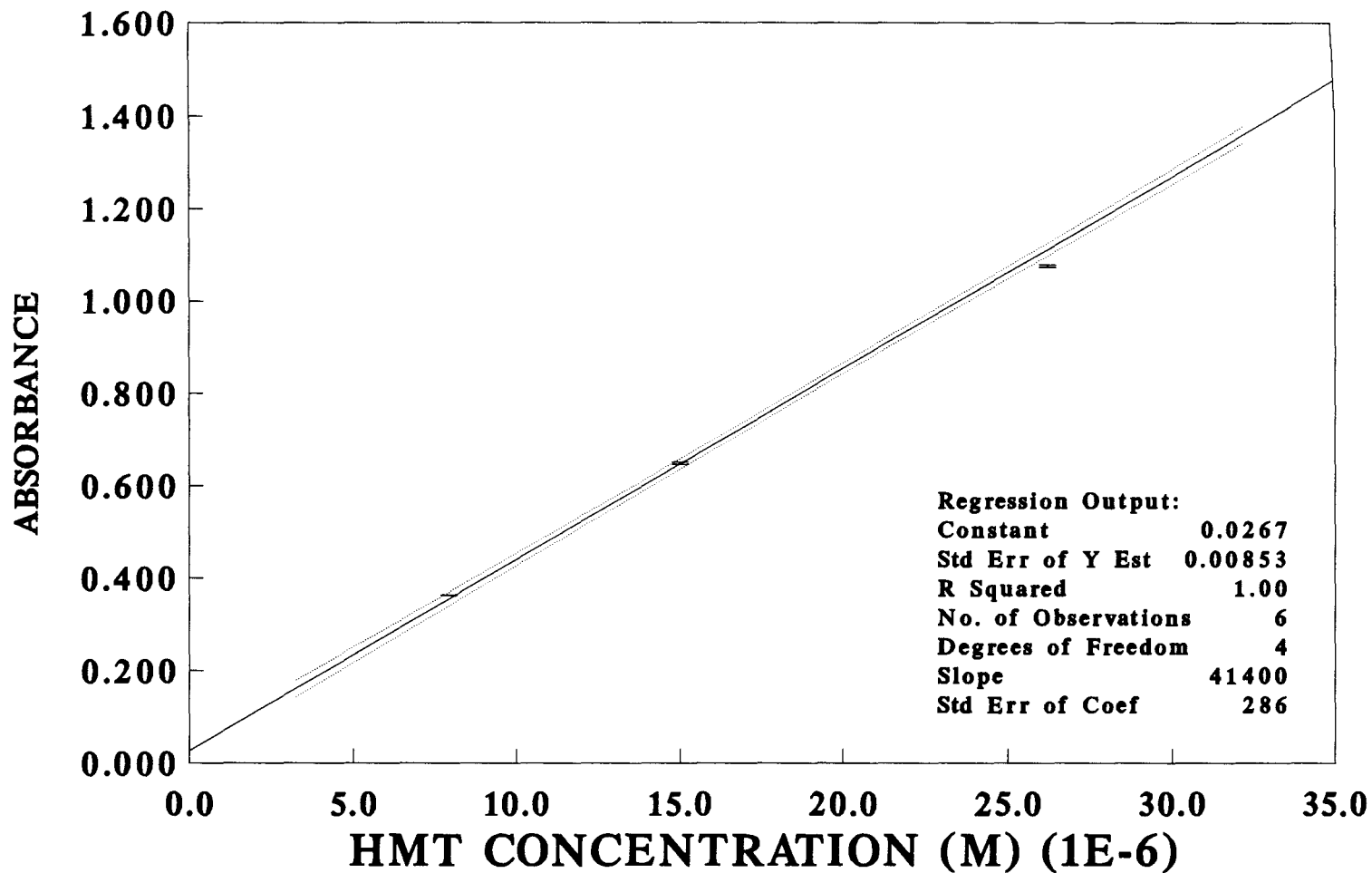
Figure 11   Chromotropic acid calibration curve for the determination of pure aqueous HMT and synthetic unknown samples.
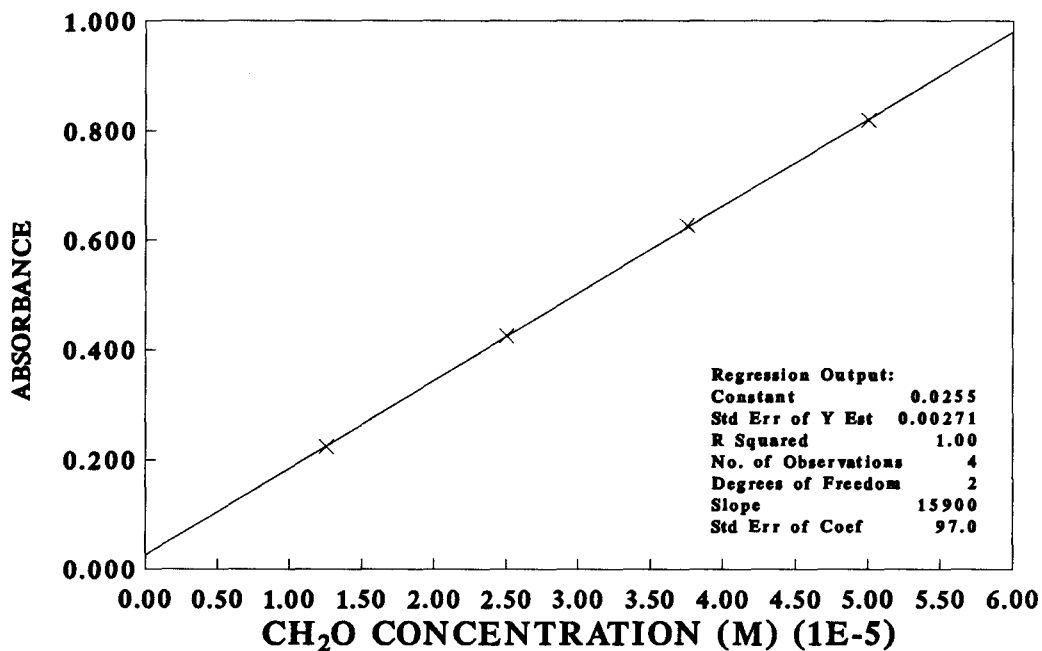
Figure 12   Chromotropic acid determination of formaldehyde standards.
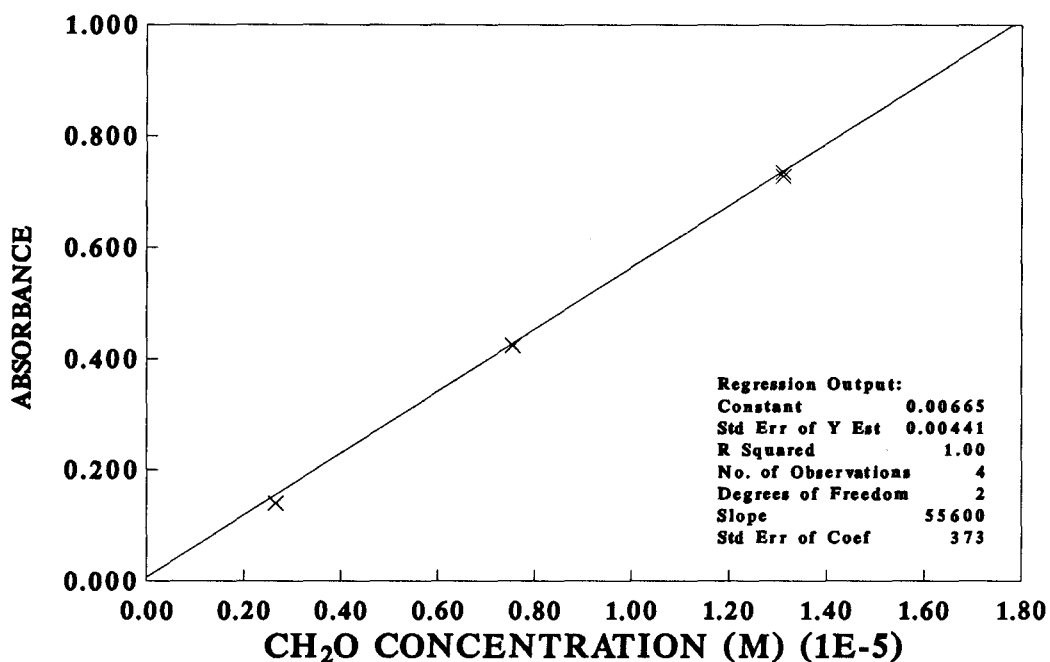
Figure 13   Hydrous silver(I) oxide iron(III)-Ferrozine calibration
              curve for formaldehyde in synthetic unknowns.

Table 7	Determination of HMT in simulated pure aqueous unknown samples by the USP chromotropic acid method.

Sample (ppm)	Absorbance[a] (A)	Amount determined		R.S.D.[b]
		(ppm)	(%)	
1.11	0.363 ±0.001	1.14	103	0.159
2.10	0.649 ±0.003	2.11	100	0.408
3.67	1.076 ±0.003	3.56	96.8	0.233

[a]Absorbance = average of three samples and standard deviation
[a]R.S.D. = relative standard deviation

Determination of HMT in the Presence of a Large Amount of Formaldehyde:

The determination of HMT in the presence of a large amount of formaldehyde was accomplished by first removing or greatly lowering the formaldehyde interference and then testing for the HMT as previously described. The formaldehyde interference was eliminated by either the method of evaporation in the presence of Ni(II) or by reduction to methanol with borohydride. Both methods were studied using the same samples for comparison. The standard calibration curves are shown in figures 14 and 15. The synthetic unknown sample results are shown in table 6 and figures 14 and 15.

To achieve the most accurate results possible, the amount of formaldehyde in each unknown sample was determined, using the method of Jaselskis and Al-Jabari (49). The formaldehyde standard calibration curve is shown in figure 13. The results of the diluted unknown sample are shown in table 6 and plotted as points on the calibration curve figure 13. The molar absorptivity for the formaldehyde determination corresponds to 55,600 L/(cm mol), which is very close to the theoretical value $2 \times 27,900 = 55,800$. However, this determination does not need to be very accurate because the residual absorbance corrections for initial formaldehyde concentration are relatively small.

The results of the evaporation procedure are shown in figure 14 and table 6. The apparent molar absorptivity obtained for the evaporated standards was 307,000 L/(cm mol). The obtained value was lower than the theoretical apparent molar absorptivity. The low value was probably due to a small loss of HMT during the evaporation step used in this procedure. Alternatively, a small amount of the HMT may have been hydrolyzed to

formaldehyde during evaporation in the presence of acetic acid or the HMT may have been sublimed when the sample reached dryness. This loss is proportional, thus the standard curve makes the appropriate correction. The time required for the formaldehyde removal was about 2 hours.

The borohydride reduction results are shown in figure 15 and table 6. The apparent molar absorptivity obtained for the reduction of standard HMT was 322,000 L/(cm mol). This apparent molar absorptivity indicates that there is no loss of HMT resulting from this procedure. The time required for the formaldehyde removal was about 15 minutes.

Both of these methods of formaldehyde removal work very well. However, these methods also have their disadvantages. The evaporation method requires a time interval corresponding to the amount of aqueous sample being evaporated. The time required to evaporate 10 to 20 samples containing 150 μL of aqueous solution is about two hours. Another problem with the evaporation method is residual formaldehyde interference. This interference corresponds to about 0.05 absorbance units per 25 mL final dilution of 50 μL 0.2 M formaldehyde. The borohydride reduction method requires only about 15 minutes time for reduction. However, there is a residual formaldehyde interference. This interference corresponds to about 0.18 absorbance unit per 25 mL final dilution of 50 μL 0.2 M formaldehyde sample as was shown in figure 5.
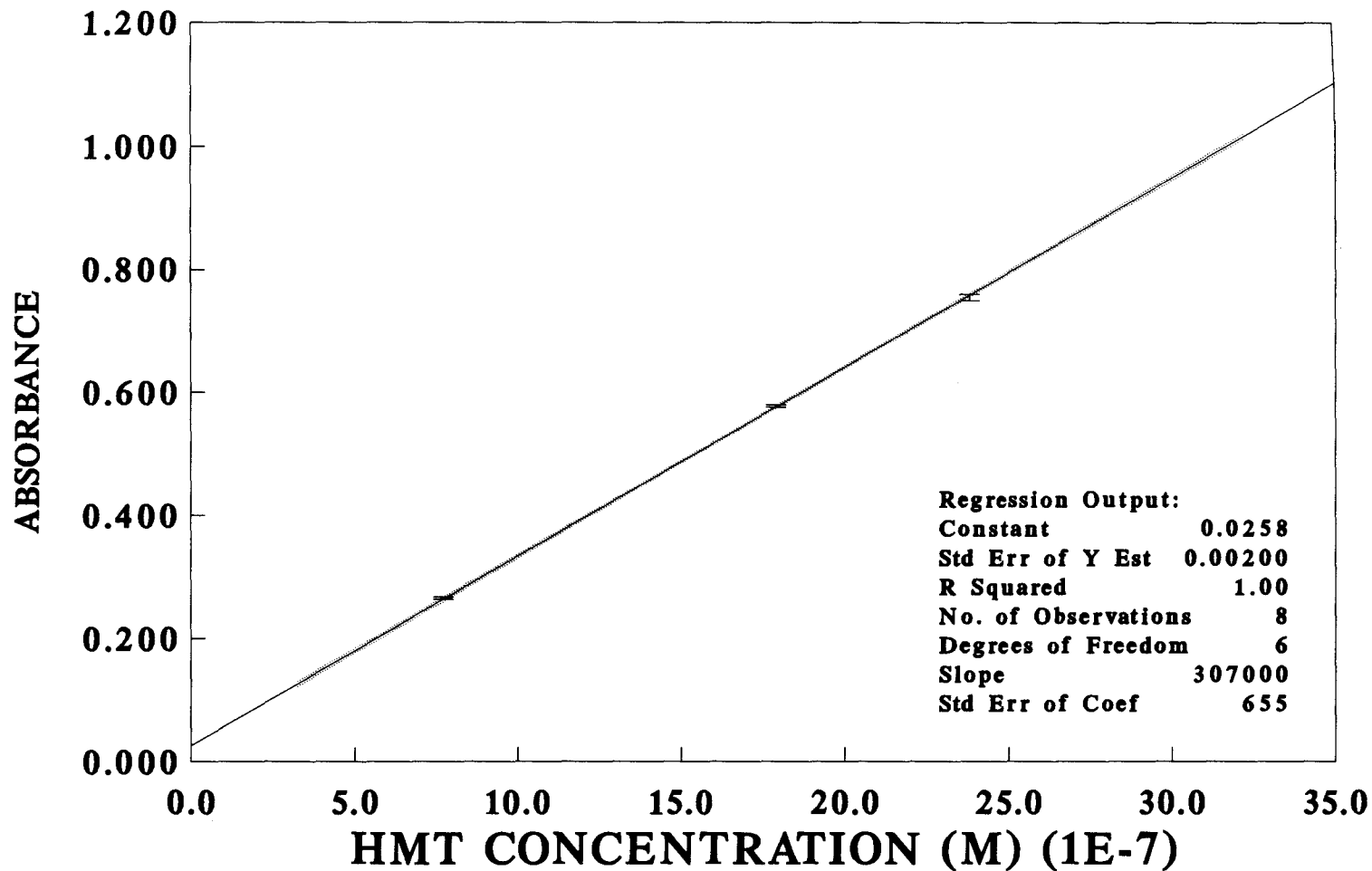
Figure 14   Calibration curve for the determination of HMT samples and CH$_2$O-HMT synthetic unknown samples after evaporation.
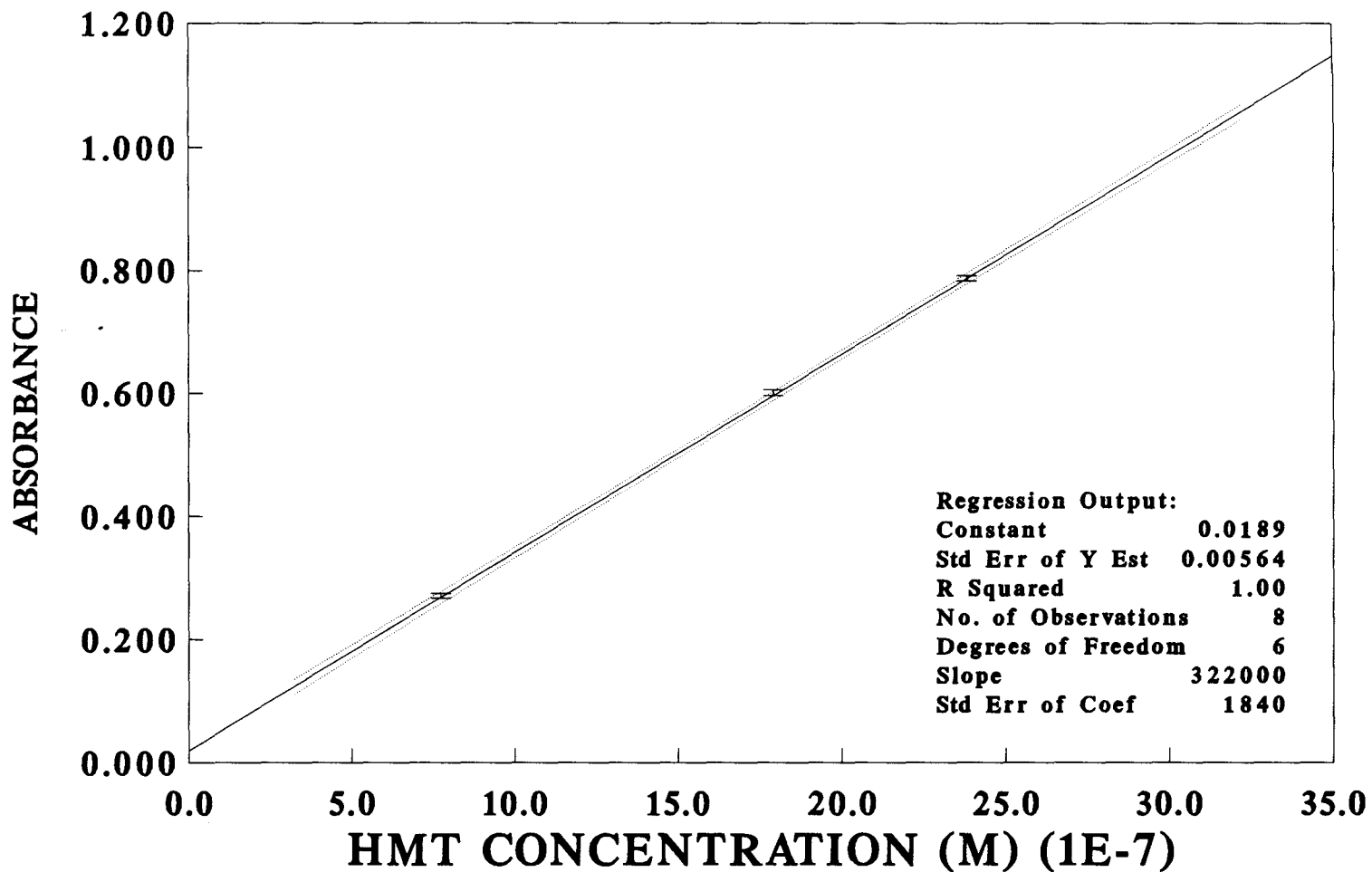
Figure 15   Calibration curve for the determination of HMT samples and CH_2O-HMT synthetic unknown samples after borohydride reduction.

Determination of Ammonia:

The determination of HMT formed was accomplished in a manner similar manner to the determination of HMT in the presence of a large amount of formaldehyde. The formaldehyde interference was first eliminated by evaporation or borohydride reduction and the remaining HMT was determined as previously described. Both methods of formaldehyde removal were used here for comparison on the same ammonia samples. The standard calibration curves are shown in figures 16 and 17. The synthetic unknown sample results are shown in table 8 and figures 16 and 17.

All of the standard and synthetic unknown reaction solutions were run at a formaldehyde concentration of 0.1 M formaldehyde. Thus all the samples were essentially the same in formaldehyde concentration after the condensation reaction was complete. The residual formaldehyde interference was the same in all samples and corrected for in the standard calibration curve.

The apparent molar absorptivities for these methods were both around 80,000 L/(cm mol). This falls in the expected range of 78,500 to 80,500, which corresponds to the aqueous pure HMT sample range. The ammonia sample concentrations ranged from 15 to 120 ppm.

Regression Output:
Constant   0.106
Std Err of Y Est   0.00404
R Squared   1.00
No. of Observations   7
Degrees of Freedom   5
Slope   80600
Std Err of Coef   312
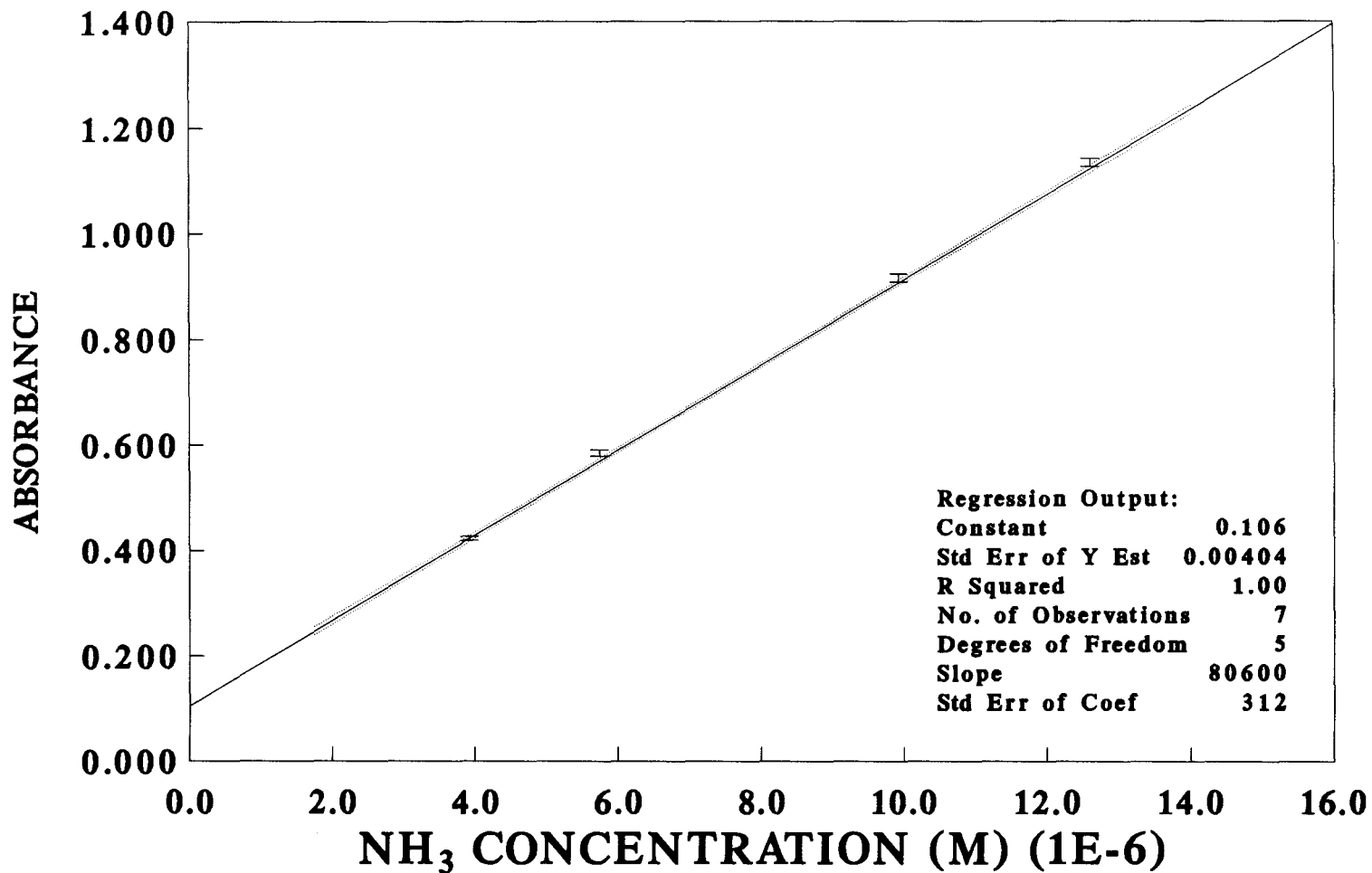
Figure 16   Calibration curve for the determination of ammonia samples and synthetic unknown samples after evaporation.
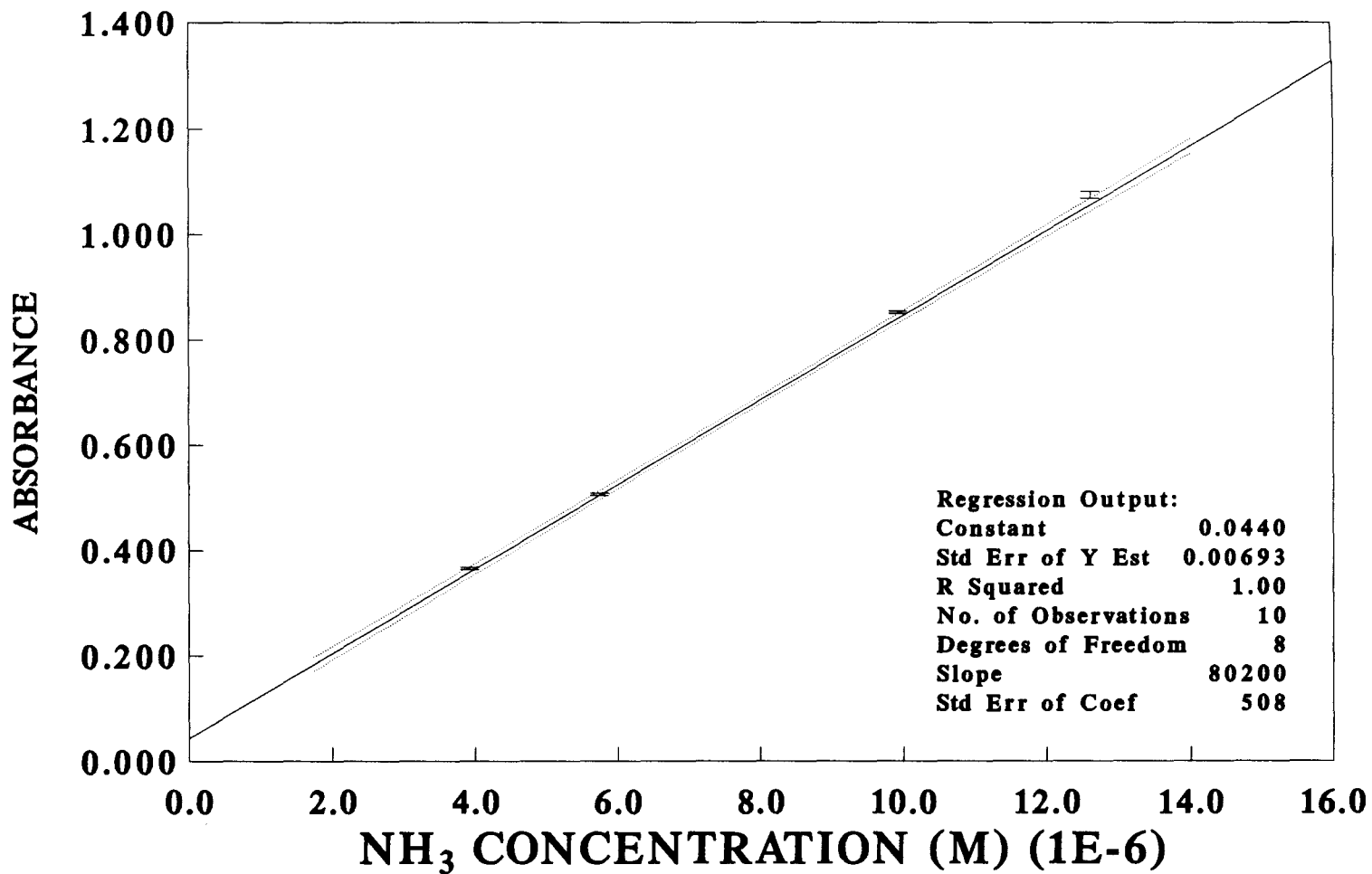
Figure 17   Calibration curve for the determination of ammonia samples and synthetic unknown samples after borohydride reduction.

Sample (ppb)	Formaldehyde removal	Absorbance[a] (A)	Amount determined (ppb)	(%)	R.S.D.[b]
33.3	evap.	0.422 ±0.004	33.4	100	0.948
48.9	evap.	0.584 ±0.006	50.5	103	0.969
84.5	evap.	0.915 ±0.007	85.5	101	0.790
107	evap.	1.133 ±0.008	109	101	0.685
33.3	reduct.	0.366 ±0.002	34.1	102	0.580
48.9	reduct.	0.507 ±0.002	49.1	100	0.419
84.5	reduct.	0.852 ±0.002	85.7	101	0.249
107	reduct.	1.074 ±0.007	109	102	0.658

Table 8   Determination of ammonia in simulated unknown samples.

[a]Absorbance = average of three samples and standard deviation
[b]R.S.D. = relative standard deviation

Determination of HMT in Urine:

The determination of HMT in urine was accomplished by first removing the urine interferences. Since HMT is stable to hydrolysis in alkaline solution, most of the urine interferences were removed by keeping the sample at high pH. However, a small residual amount of interference was at times still present after the interference removal procedure. This problem was overcome by using the method of standard additions. The standard additions curve is shown in figure 18.

The slope of the standard additions curve gave an apparent molar absorptivity of 314,000 L/(cm mol). This value fell within the expected range. Thus HMT was not lost during the interference removal process. The three points at 7×10^{-7} M HMT were separate samples run through the procedure and show high precision.

The diluted samples tested along the standard additions curve shown in figure 18 correspond to original urine-HMT concentrations of 0.27 to 1.35 mg/mL. Since physiologic concentrations of HMT range from 0.6 to 1.7 mg/mL (34), this method is sufficiently sensitive for clinical applications.

Other methods used for this determination have been criticized as being inaccurate, because they determine formaldehyde before HMT hydrolysis and the total formaldehyde after HMT hydrolysis. The formaldehyde difference is then due to HMT. The urine sample and formaldehyde determination are both in acidic solutions causing hydrolysis of HMT which results in a high "prehydrolysis" formaldehyde concentration. This results in inaccurate low values of HMT. The method described here with alkaline samples removes formaldehyde and interferences before HMT

hydrolysis. The problem of hydrolysis in acidic urine can be solved by making the solution alkaline after its collection. This does not interfere with the method described here.
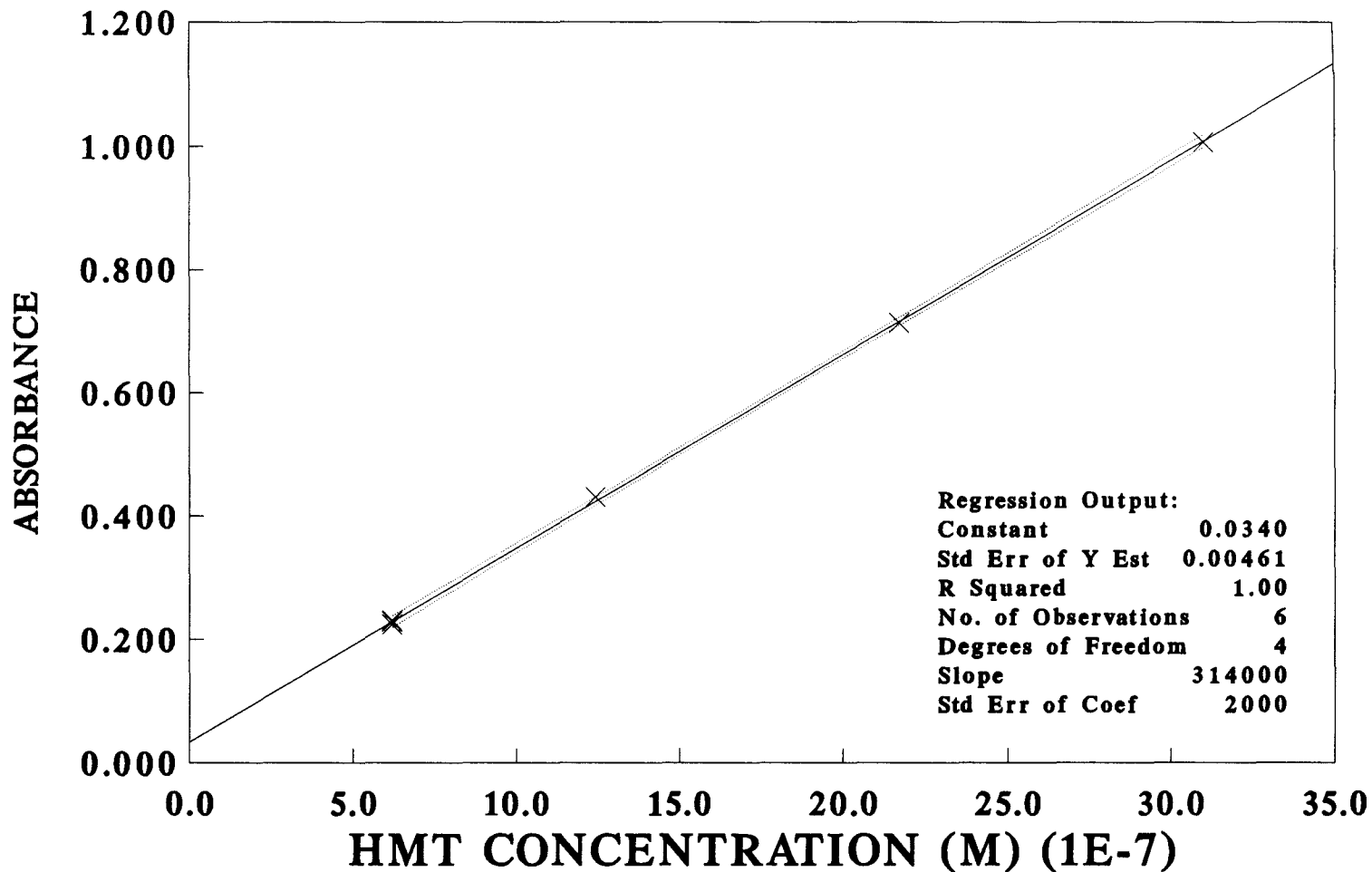
Figure 18   Standard additions curve for the determination of HMT in urine samples.

THE DETERMINATION OF HEXAMETHYLENETETRAMINE AND AMMONIA

BY NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Introduction

Background:

In NMR spectroscopy, a strong magnetic field causes the energies of certain nuclei to be split into two or more quantized levels, owing to the magnetic properties of these nuclei. In this magnetic field the nuclei orient themselves to populate the lower energy state to a greater extent than the higher energy state. At room temperature, only a small excess (<10 ppm) populates the lower energy state as compared to the higher energy state in accordance with the Boltzman distribution. Transitions among the resulting magnetically induced energy levels can be brought about by the absorption of radio frequency (rf) energy at the nucleus resonance frequency. The peak absorption of energy is observed at this resonance frequency.

Many atomic nuclei behave as if they were spinning and possess quantized spin angular momentum. If an atomic nucleus possesses either odd mass number or odd atomic number, or both, it has spin angular momentum. The angular momentum can be described in terms of spin number I. The number of allowed spin states is determined by its spin number and is a physical constant for each nuclei. In the presence of a magnetic

field, a nucleus will have $2I + 1$ discrete states. In the absence of a magnetic field, these states all have the same energy. The spin quantum numbers of several common nuclei are shown in table 9. A diagram of the proton splitting is shown in figure 19.

Conventional, or continuous wave NMR spectroscopy is not very sensitive. The spectrometer scans the spectrum at a slow rate in order to achieve a high signal for narrow absorbances. Therefore, time is wasted by recording mostly background and occasionally recording a signal. Efficiency and sensitivity of this system is far from optimum. The generation of good proton spectra for samples in microgram quantities is difficult, time consuming, and sometimes impossible.

Fourier transform (FT) NMR overcomes this time problem by operating in a different manner. A strong pulse of rf energy is applied to the sample for a very short time (1-1000 μsec). This pulse of energy contains a range of frequencies sufficiently great to excite nuclei with different resonance frequencies. Following the pulse, a rf emission signal due to the decay of the excited nuclei back to equilibrium in the magnetic field is recorded as a function of time. This free induction decay (FID) signal contains all the resonance frequencies of the excited nuclei present. The observation time is usually between 1 and 4 seconds. This process can then be repeated and the FID signals are averaged to give a vastly improved signal to noise ratio. This improvement occurs since the signals add linearly while the noise adds as the square root of the number of pulses. The FID signal contains all the information needed to produce a normal frequency domain NMR spectrum. A digital computer then performs

a fast Fourier transformation of the FID to produce the spectrum. A FID

and its transformed spectrum are shown in figures 20 and 21.

Table 9   Spin quantum numbers of some common nuclei.

ELEMENT	$^{1}_{1}H$	$^{2}_{1}H$	$^{12}_{6}C$	$^{13}_{6}C$	$^{14}_{7}N$	$^{16}_{8}O$	$^{17}_{8}O$	$^{19}_{9}F$	$^{31}_{15}P$	$^{35}_{17}Cl$
SPIN NO.	$\frac{1}{2}$	1	0	$\frac{1}{2}$	1	0	$\frac{5}{2}$	$\frac{1}{2}$	$\frac{3}{2}$	$\frac{1}{2}$
SPIN STATES	2	3	0	2	3	0	6	2	2	4
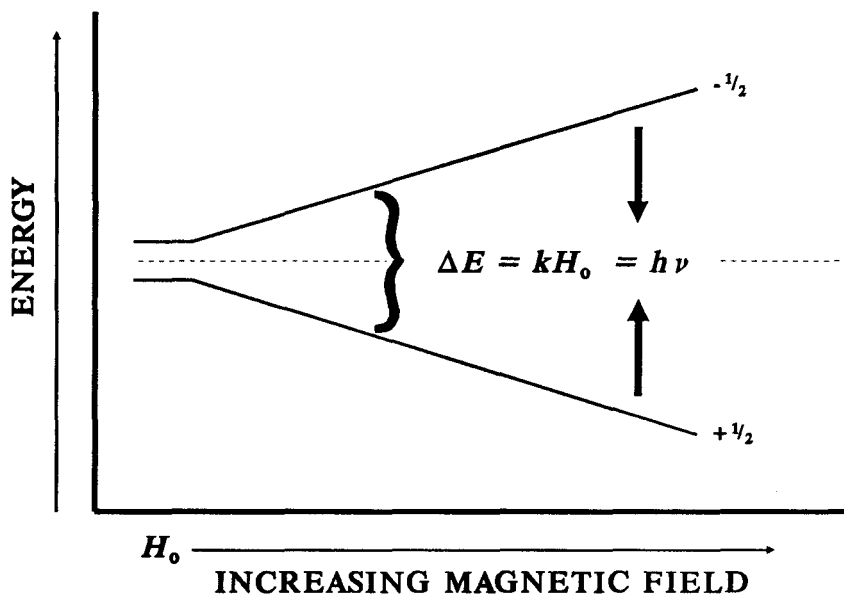
Figure 19   The spin state energy separation as a function of the strength of the applied magnetic field (H_o).
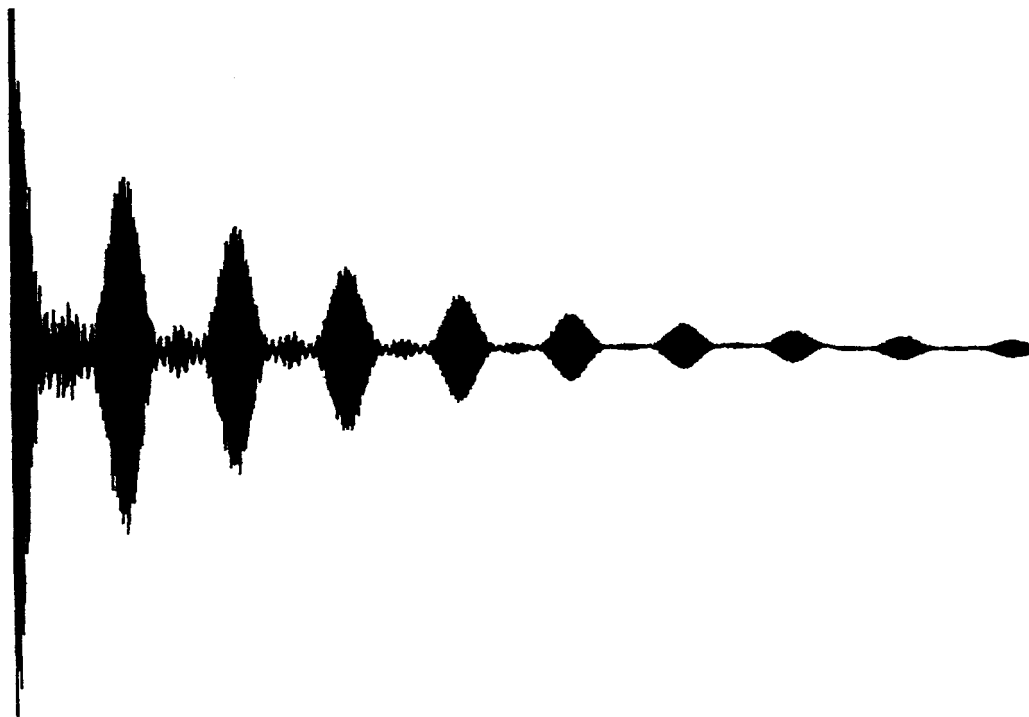
Figure 20   Example of FID signal containing all proton resonance signals.
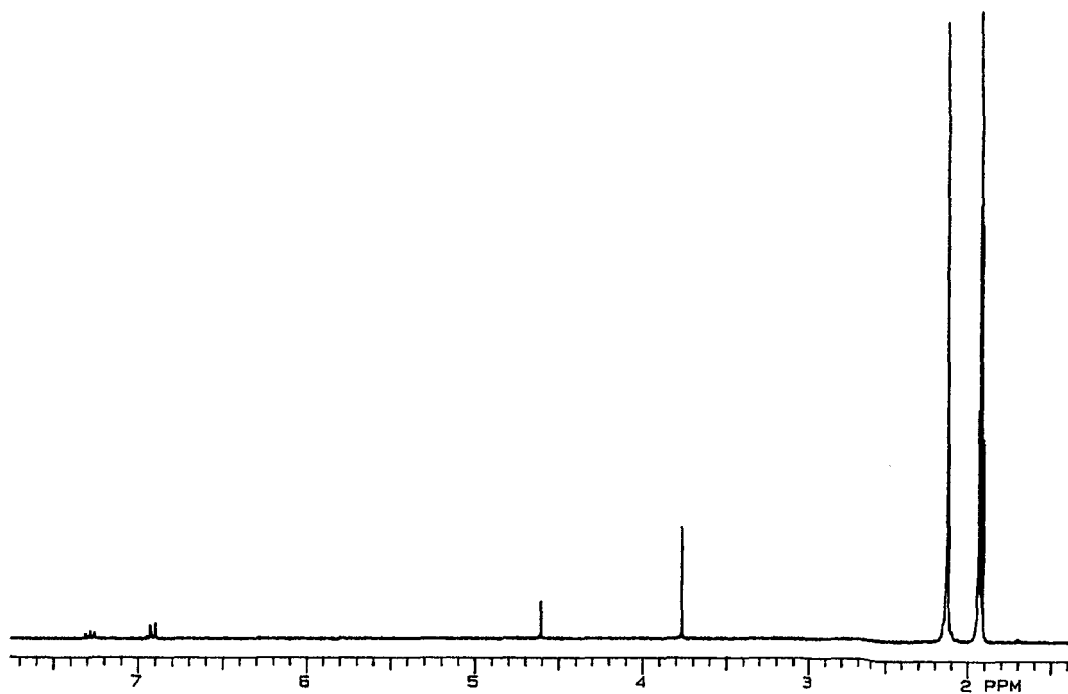
Figure 21   Transformed FT-NMR frequency domain spectrum.

The resolution and sensitivity are critically dependent on the strength and quality of the magnet. A stronger magnetic field produces better line separation of nuclei in the spectrum. This higher sensitivity is due to a more populated lower energy state resulting from greater splitting of the energy states. This splitting due to magnetic field strength is shown in figure 19. The magnet also must be highly homogeneous and reproducible within the sample area.

Nuclear magnetic resonance methods are not widely used for quantitative analyses. The most common and important applications of NMR are for the identification of atomic configurations of organic, metal-organic, and biochemical molecules. In NMR spectroscopy, the area under an absorption band is proportional to the number of nuclei responsible for the absorption. A quantitative determination does not require a pure sample but the peaks of interest in the spectra must not overlap other peaks present.

Two methods have been reported in the literature for the quantitative NMR determination of HMT at the milligram level using a 60 MHz continuous wave NMR. Baum and Goodman (70) in 1970 determined HMT in urea-formaldehyde molding compounds. Deuterated chloroform was used to extract the HMT from a dried finely ground sample. The HMT containing sample was evaporated to about 10 mL and cyclohexane was added as the reference standard. The spectrum was then determined and integrated. Losses were reported to occur if there was moisture in the sample and if the sample was heated during the drying process.

In 1973, Turczan and Goldwitz (41) reported a method for the determination of HMT in methenamine and methenamine mandelate

pharmaceutical tablets. At least 20 tablets were finely ground. About 70 mg HMT or 150 mg HMT-mandelate and 350 mg maleic acid reference standard were added to 3 mL NMR solvent. To overcome the problems of solubility, overlapping of the resonance signals, and potential decomposition of HMT, the deuterated NMR solvent was formulated to contain 65% acetonitrile, 25% acetone, and 10% dimethylformamide. The sample was mixed, centrifuged, and 0.4 mL was transferred to a NMR tube. Between 94 and 100 percent HMT were obtained.

Statement of Problem and Approach:

The goal of this study was to develop a moderately sensitive proton NMR method for the determination of HMT in aqueous solutions alone and in the presence of large amounts of formaldehyde. Determination of HMT in the presence of a large amount of formaldehyde will also allow the indirect determination of ammonia, via its quantitative reaction with excess formaldehyde to form HMT. Application of this method to the determination of HMT in urine was also studied.

The proposed method is based on the use of a 300 MHz FT-NMR to provide high resolution and sensitivity. The 300 MHz FT-NMR has a magnet operating at 7.05 tesla as compared to a basic 60 MHz NMR magnet operating at 1.409 tesla. High resolution and sensitivity are critically related to the strength of the magnetic field. This high field strength allows the determination of low microgram amounts of HMT within a reasonable amount of NMR operating time. The working determination range for HMT in 0.6 mL NMR solvent is 5 to 35 μg. This amount of HMT cannot be detected using a continuous wave 60 MHz NMR.

The determination of HMT is based on the quantitative integration of its proton resonance peak area. Hexamethylenetetramine is a symmetrical "adamantane like" molecule containing 12 equivalent protons as shown below.
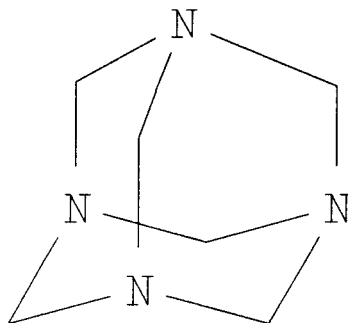
HMT

These 12 equivalent protons produce one large singlet proton NMR signal. This signal can be used for the quantitative measurement of HMT concentration as shown in figure 22.

The development of this procedure required investigation of experimental and instrumental parameters. The experimental parameters included: i) a suitable reference standard employed for quantitative measurements, ii) appropriate solvent in respect to solubility and reactivity of HMT and the reference standard, iii) elimination of resonance peak interferences. Instrument parameters were optimized including: i) spectral width, ii) pulse width, and iii) pulse delay.

Once the optimum conditions were established for solid HMT, the method was applied to various aqueous samples. Water cannot be present

in the NMR determination. Water was replaced with a deuterated NMR solvent. Methods were developed to remove water either by evaporation or extraction into a more volatile solvent for evaporation. Extraction of HMT into a more volatile organic solvent was investigated to reduce the time required for evaporation. Extraction of HMT in a deuterated NMR solvent would eliminate the need for evaporation but the amount of NMR solvent needed would be cost prohibitive. After evaporation, the residue was dissolved in an NMR solvent containing a reference standard, transferred to a 5 mm NMR tube, and run on the FT-NMR.

After developing the procedure to determine aqueous HMT samples, methods were investigated to determine HMT in the presence of a large amount of formaldehyde. The evaporation of formaldehyde leaves paraformaldehyde, which yields resonance signals that interfere with the HMT signal of interest. Therefore, techniques were needed to reduce the formation of this formaldehyde residue. A number of approaches were investigated, and satisfactory modified evaporation and extraction techniques were developed.

After developing the method to determine HMT in the presence of large amounts of formaldehyde, the procedure was used to study the determination of ammonia. Ammonia was reacted with a large excess of formaldehyde and heated to drive the reaction to completion and to form HMT. The large excess of formaldehyde was removed and the HMT was quantitatively determined. The amount of HMT was then related to the amount of ammonia originally in the sample. The development of the quantitative ammonia condensation parameters was described previously for the spectrophotometric determination.

This method was then applied to the study of spiked urine samples. Urine samples contain many different compounds which may produce proton resonance signals which interfere with the HMT signal. Extraction methods were developed to remove the urine interferences and to leave the HMT in a volatile organic solvent suitable for evaporation.

Experimental

Instrumentation:

All fourier transform nuclear magnetic resonance experiments were performed on an 300 MHz Varian VXR-300 FT-NMR using a Varian 300 MHz Generation III switchable 5 mm probe. All continuous wave NMR experiments were performed on an 60 MHz Varian EM-360 NMR. Constant temperatures were obtained using a Cole Parmer model 1266-00 immersion circulator water bath. The pH measurements were made using a Fisher Accumet model 830 pH meter. Solid and liquid samples were weighed with either a Sartorius analytical balance or semi-micro balance.

Reagents:

All chemicals used were analytical or primary standard grade. Acetonitrile-d_3 and chloroform-d_3 NMR solvents were purchased from Aldrich and Isotech. Formate buffer, pH 4.0, was prepared by titrating 0.1 M formic acid (3.93 mL concentrated formic acid/1 L) with 50% sodium hydroxide. Bromocresol green, 3',3",5',5"-tetrabromo-m-cresolsulfone-phthalein, sodium salt (Aldrich), was dissolved in pH 4.0 formate buffer to produce a 0.0025 M solution (0.180 g/100 mL). Silica gel columns were

prepared by placing 900 mg silica gel, grade 60, 230-400 mesh, 60 Å (Aldrich), into a 7.0 mm i.d. glass column plugged with glass wool. All other reagents used are described in the previous sections.

Development of Methods

Solvent Solution:

The best solvents for proton NMR spectroscopy contain no protons, such as carbon tetrachloride. However, many compounds are not soluble in carbon tetrachloride. Thus, a variety of deuterated solvents are used instead. The choice of NMR solvents took into account price and ready availability. Since HMT is very highly soluble and stable in water, D_2O appeared to be a good choice. However, D_2O presented a problem because its HDO solvent impurity resonance signal occurred at the chemical shift of δ 4.61 and the HMT signal at δ 4.69. These signals were sufficiently close together to interfere with each in the quantitative integration measurements. The solvent of preference was deuterated chloroform, probably the most common and least expensive deuterated NMR solvent. Chloroform produced very good spectra because its solvent impurity resonance signal, at δ 7.13, did not interfere. However, chloroform will slowly decompose amine. A fine white precipitate has been observed forming in deuterated chloroform after the dissolution of HMT. The next choice was deuterated acetonitrile since its solvent impurity resonance signals were produced at δ 1.93, and the H_2O impurity resonance signal was at δ 2.13. In this solvent the HMT resonance signal was observed at δ 4.60 and the HMT compound was stable. These signals are labeled and shown

in figure 22. The solubility of HMT in acetonitrile was limited but at the low microgram per mL concentrations, solubility is not a problem.

Reference Standard:

Quantitative measurement of HMT concentration requires the use of an internal standard of known concentration. The proton signal area of the compound of interest can then be quantitatively related to the proton signal area of a known concentration of the reference standard. The internal standard should preferably produce a strong singlet resonance signal close to the proton resonance signal of interest, but it must not interfere. The internal standard also should not react with HMT or the NMR solvent. Knowing the amount of the internal standard present and the ratio of the two proton signals, one can calculate the amount of HMT present using equation 9

$$\text{mass A} = \text{mass B} \times \frac{\#\text{B protons in signal}}{\#\text{A protons in signal}} \times \frac{\text{M.W. A}}{\text{M.W. B}} \times \frac{\text{A signal area}}{\text{B signal area}} \quad (9)$$

where A is the unknown HMT and B is the internal reference standard.

Anisole ($C_6H_5OCH_3$) was chosen as the internal standard, since it is soluble in deuterated acetonitrile, and its resonance signals do not interfere with the HMT resonance singlet signal. The anisole methoxy group produces a resonance singlet at δ 3.77 and the benzene group resonance signals are around δ 7 in deuterated acetonitrile as shown in figure 22.

NMR Instrument Parameters:

To produce the best possible signal, instrumental parameters have been optimized. Spectral width was narrowed from 4000 Hz to 2510 Hz giving a greater number of data points for more precision in measurement. The transmitter offset was adjusted from 400 Hz to 100 Hz to produce a spectral range of δ 0.3 and δ 8.0 which contained all the resonance signals in the sample. Care had to be taken in the adjustment of spectral width and transmitter offset, because any peak occurring outside of this narrowed range would become folded and could possibly produce a poor spectrum. A pulse width of 17.0 μsec was used for increased signal to noise which is near the 90° pulse width of about 20 μsec. A pulse delay of 1 sec was used to ensure that the FID signal had returned to equilibrium before the next pulse occurred. Longer pulse delays were investigated, and no signal to noise improvement occurred. Acquisition times were adjusted for each sample to obtain an acceptable signal to noise ratio for quantitative integration of the signal peaks. Thirty minutes was considered the longest reasonable amount of time to be used for a low concentration sample acquisition. Exact adjustment of the phase for each spectrum was crucial for quantitative integration and small adjustments had a large effect on the integration and the results for low concentration samples. Electronic integration results were checked with hand measured results to verify the accuracy of the electronic integration. The best integration data were obtained when the reference resonance signal was similar in area to the HMT signal. For each group of samples tested, a constant concentration of reference standard was used so that the only variable was the concentration of HMT. Optimized

instrumental settings used are shown in table 9.   An example of the spectra obtained using these optimized parameters is shown in figure 22.

Table 10	Instrument parameters used for FT-NMR determinations.	
	d-acetonitrile	*d*-chloroform
Nucleus	1.250	1.750
Frequency	300 MHz	300 MHz
Spec. Width	2510.0 Hz	4000.0 Hz
Acq. Time	3.697 sec	3.752 sec
Pulse Width	17.0 μsec	17.0 μsec
Offset	100 Hz	700 Hz
Pulse Delay	1.000 sec	1.000 sec
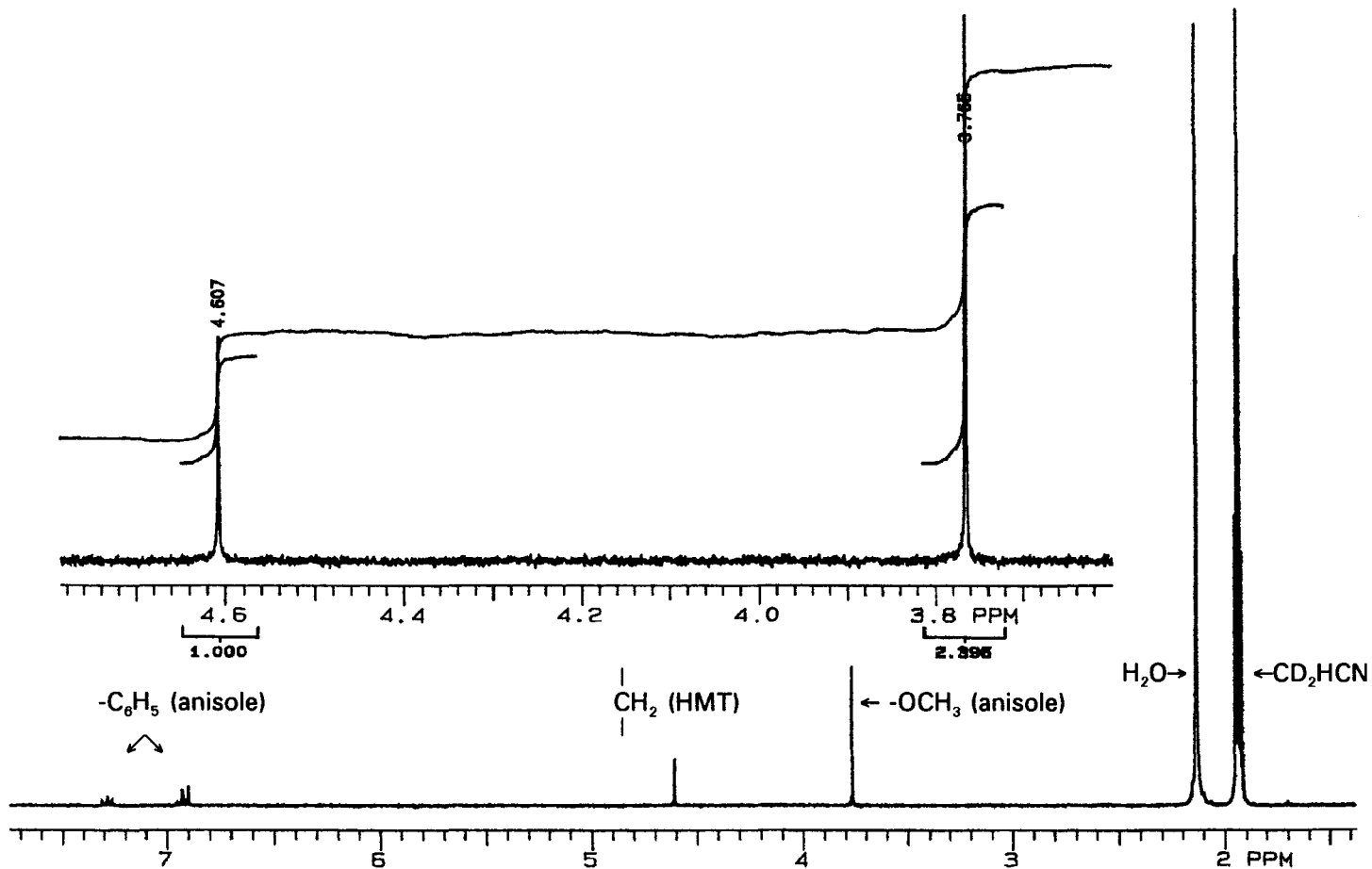
Figure 22 Example FT-NMR spectrum of HMT and anisole in *d*-acetonitrile solvent using the optimized instrumental parameters. The inset spectrum shows the HMT and anisole peaks integrated in the standard manner.

Evaporation of Water:

Aqueous samples of HMT required the removal of solvent (water) and its replacement with deuterated acetonitrile. The evaporation of aqueous HMT samples described earlier for the spectrophotometric method presented similar problems here. Evaporation of the samples in a vacuum desiccator had to be stopped immediately when the water was evaporated. Sublimation of the solid HMT began to take place at this point. To prevent the loss of HMT due to sublimation, metal salts were added to the sample as described for the spectrophotometric method. Nickel nitrate and cobalt nitrate were tested. They greatly broadened the HMT peak to near baseline height because of complexation with HMT and their paramagnetic properties. Potassium cyanide was added to the NMR solvent to tie up the metal as a stable complex thus releasing the HMT, but it produced a fine precipitate which was difficult to work with. Dimethylglyoxime (DMG) was added to nickel containing samples and produced a very large fluffy precipitate which was simple to filter through cotton. Nickel-DMG results were sometimes good but not reproducible, because the HMT peak signal rapidly decreased after DMG addition and filtration. A small amount of strong acid ion exchange resin was added to the solvent in an attempt to adsorb nickel, but positive results were not obtained. Calcium nitrate, magnesium nitrate, and zinc nitrate were tested, since they are not paramagnetic. These metals did not broaden the HMT peaks to near baseline height, but varied amounts of these metals slightly broadened and shifted the HMT peak downfield in the NMR spectra due to complexation. The amount of shift varied with the amount of metal present. Unfortunately, these metals also produced inconsistent results and began to decompose HMT.

However, it was found that zinc acetate added in the ratio of 0.5:1 to 2:1 Zn(II):HMT would yield reproducible results. If zinc acetate was added in amounts greater than 2:1 Zn(II):HMT, similar decomposition problems would occur.

Evaporation of Formaldehyde:

The removal of formaldehyde by evaporation was accomplished in the manner described earlier for the spectrophotometric method. Glacial acetic acid was added to the sample to inhibit formaldehyde polymerization to paraformaldehyde during the evaporation. When this interference was not completely removed from the sample, the remaining interference resonance peaks obstructed the integration of the HMT peak as shown in figure 23. As was described for the spectrophotometric method, samples containing up to 0.2 M formaldehyde required approx. 0.1 M acetic acid for the evaporation. Zinc acetate was also added before evaporation to help prevent sublimation. The sample evaporation was carried as described previously for the evaporation of water.
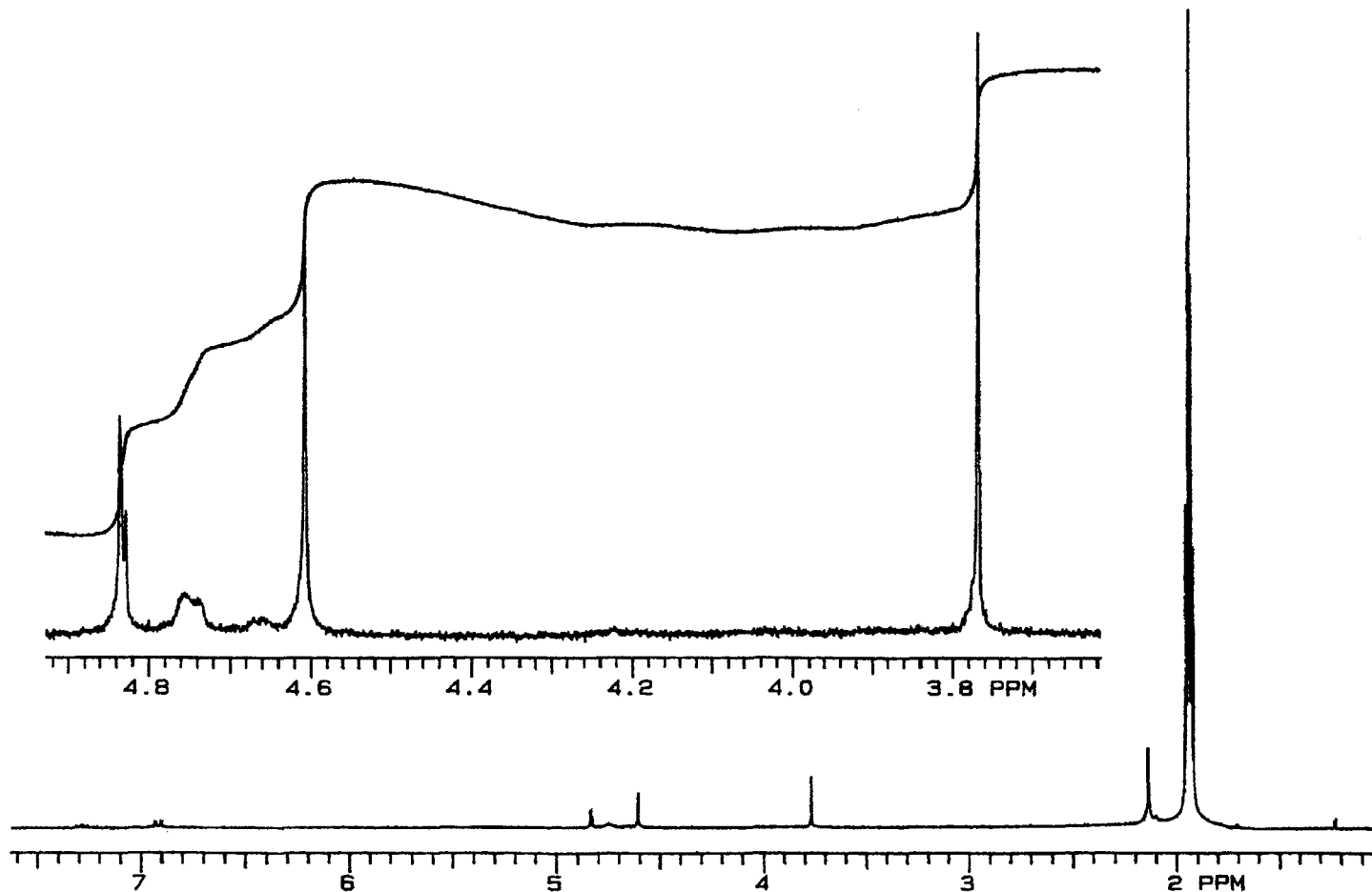
Figure 23   Example of FT-NMR spectrum determination of HMT with formaldehyde residue interference remaining after evaporation of formaldehyde.

Extraction of HMT:

The removal of formaldehyde from HMT samples may also be accomplished by extraction of HMT from the aqueous sample leaving behind formaldehyde and other interferences. Attempts were made to extract HMT as a picrate into methylene chloride, chloroform, and isobutyl ketone, but the results were not very promising. Liquid-liquid extraction of HMT is not very efficient, because HMT is highly water soluble and weakly basic.

However, Strom and Jun (51) developed a method in which HMT was adsorbed on a silica gel cartridge from aqueous solutions as an HMT-bromocresol green ion pair. Thus, the interfering substances were allowed to pass through. The technique involved equilibrating the column with 5 mL of a pH 4 citrate buffer. One mL of the HMT sample was mixed with 1 mL of bromocresol green (BCG) in citrate buffer to form an ion pair which was adsorbed on the silica gel cartridge. The ion-pair was then eluted with 5 mL methylene chloride:1-pentanol (95:1). The ion-pair was extracted from the eluate, and the HMT was freed from the ion pair by a 5 mL solution of tetrabutylammonium iodide in 0.1 M HCl. An aliquot was removed and the HMT was spectrophotometrically determined by the Nash method (51) as described earlier. The silica gel cartridge could be reused by rinsing with 5 mL anhydrous methanol and reequilibrated with 5 mL buffer solution. This method was applied to the determination of HMT in prescription compounds (51) and also in urine samples containing HMT and a small amount of formaldehyde (50).

The application of this technique for the NMR procedure required altering some of the parameters. The parameters were also adjusted to allow the developed spectrophotometric method to monitor the results.

The citrate buffer was replaced with formate buffer to eliminate spectrophotometric interferences caused by citrate. The formate buffer was prepared using 0.1 M formic acid and adjusting the pH to 4.0 with sodium hydroxide. Rinsing the column after loading was not specified by Strom and Jun, presumably because they removed all the liquid in the cartridge with air. Rinsing the column was desirable to be sure all interfering substances were removed before eluting the HMT. Rinsing the column with buffer solution, water, and methanol slowly eluted the ion-pair. However, acetonitrile apparently left the ion pair on the column while eluting interfering substances. Elution of the ion pair with methylene chloride:1-pentanol (95:1) was not desireable since 1-pentanol has a boiling point of 136-138° C making it difficult to evaporate to dryness. Methylene chloride was investigated as a means of eluting the ion pair, but the results were not favorable. The ideal solvent for elution should have a low boiling point and should decrease the amount of time required for the evaporation required for the NMR determination. Methanol appeared ideal since it was used to "clean off" the column and has a boiling point of 65° C. However, methanol lowered the spectrophotometric determination results proportionally to the amount of methanol in the aliquot tested. Methanol also "cleaned" off unwanted urine interferences which had adsorbed when this method was applied to urine samples. Acetonitrile containing 0.01 M perchloric acid was chosen, because it did not interfere with the spectrophotometric procedure, and it can be easily evaporated to dryness for the NMR determination. The perchloric acid was added to break up and elute the HMT-BCG ion pair. A profile of this elution with 0.01 M perchloric acid in acetonitrile is

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shown in figure 24. The eluted HMT solution was immediately made basic, because HMT would be hydrolyzed by the perchloric acid. An aliquot of this basic eluate was removed and evaporated with added zinc acetate as described previously for the evaporation of water. A flow diagram of this procedure is shown in figure 25.
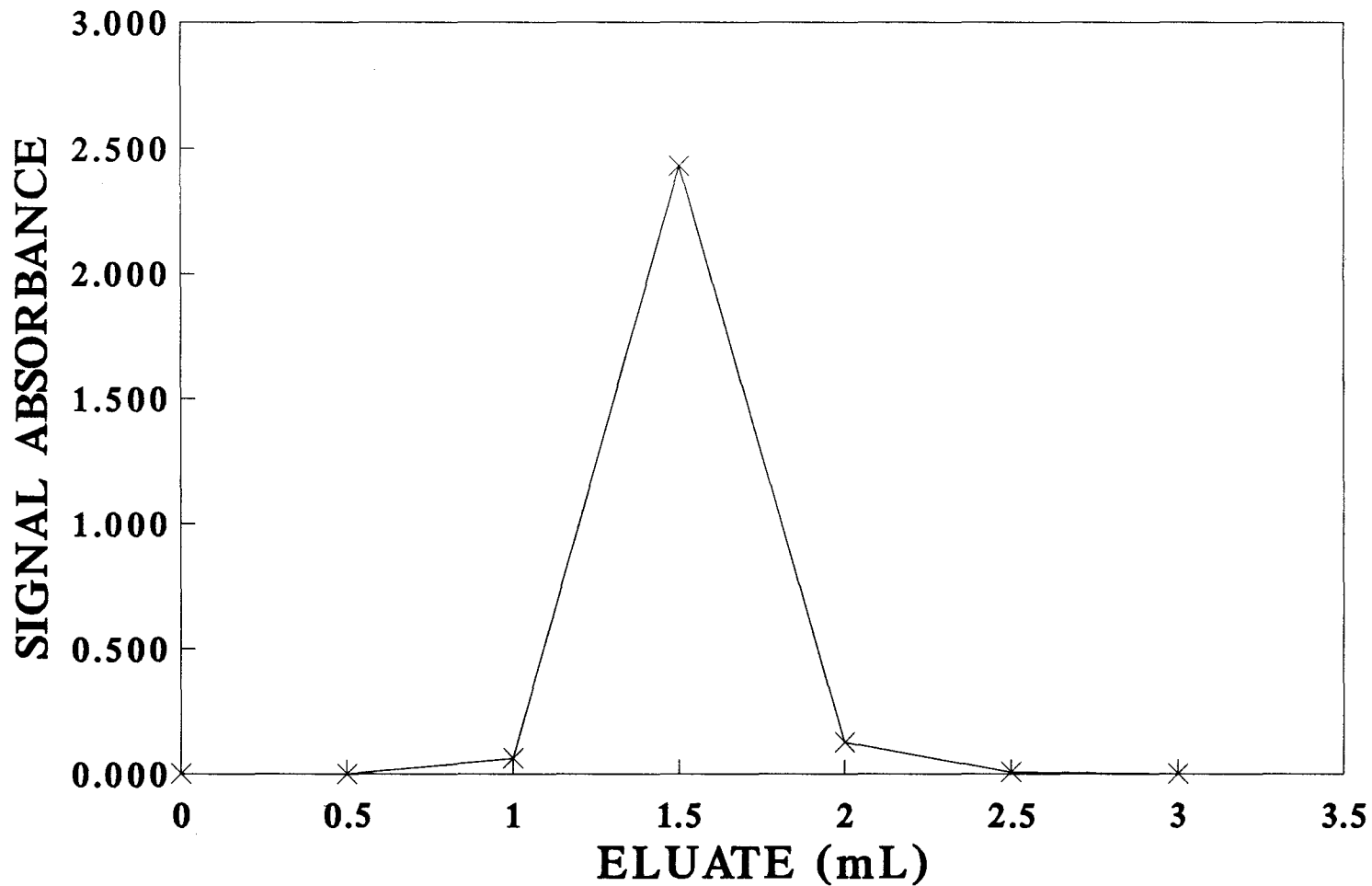
Figure 24   Elution profile for break up and extraction of HMT-BCG ion pair using 0.01 M perchloric acid in acetonitrile.

EXTRACTION PROCEDURE

900 mg silica gel

Prepare Column add MeOH
add buffer

Load Column add HMT-BCG ion pair

Rinse Column acetonitrile

Elute HMT $HClO_4$ in acetonitrile

HMT
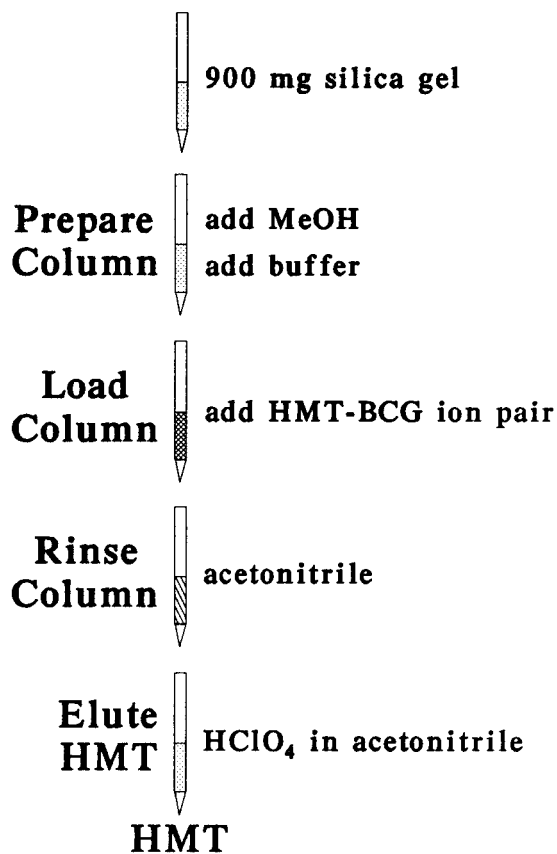
Figure 25 Flow diagram for HMT extraction from formaldehyde containing sample.

Reduction of Formaldehyde:

The removal of formaldehyde may also be accomplished by borohydride reduction followed by evaporation. Sodium borohydride was added to the formaldehyde sample to reduce the formaldehyde to methanol. However, after evaporation, the resulting precipitate was difficult to dissolve in either deuterated acetonitrile or deuterated chloroform and produced large hydride peaks between δ 0 and δ -1. The experimentally determined HMT recoveries were not good, possibly because of HMT trapped in the solid matrix. Various acids other than perchloric were added to neutralize the borohydride, but they produced new problems which also resulted in low HMT recoveries. It appeared that borohydride reduction of formaldehyde created new problems which were not solved.

Urine Samples:

Urine samples are complex and contain many different interfering components. Figure 26 shows the NMR spectrum of an evaporated HMT spiked urine sample with no pretreatment for the removal of interferences. The sample was not very soluble in d-acetonitrile which may explain why the obtained spectrum was less complicated than expected. However, a large interference "hump" can be seen at the expected location of the HMT peak. This broadened peak was probably due to nuclear qudrupole broadening of -NH_x compounds present in urine. Because of this problem, the interferences had to be removed.

The extraction method proved to be the best method for the removal of these interferences. The procedure used for this extraction was the same procedure described earlier for the extraction of HMT. The HMT-urine

sample had to be diluted at least 10:1, because apparently some of the interference was also adsorbed onto the silica gel and eluted with the 0.01 M perchloric acid in acetonitrile. However, the interference appeared to elute at a slower rate than the HMT so that most of the interference could be left on the column after the HMT had eluted.

Since some of the interference shown in figure 26 remained with the HMT after the extraction procedure, a small and very broad interference peak below the HMT peak remained as shown in figure 27. The problems associated with this interference can be seen most clearly in the integration. Small HMT peaks on top of this broad peak could not be integrated in the standard manner as is demonstrated in figure 27. Because the signal peak due to HMT was very narrow, the broad peak could be used as the baseline, since it added only a very small amount of area. The HMT peak was expanded in the plot and integrated as shown in figure 28. The anisole reference standard peak was also integrated in exactly the same manner. Since the scale adjustments for expanding the plot and the height of the plot were not changed between the integration of the two peaks, this integration technique worked very well. This integration technique was tested on previous spectra which did not contain interferences and no significant differences in the results were obtained when contrasted to the standard integration technique. The expanded spectra containing the interference was also plotted and integrated by hand to verify the validity of this technique.

2/28 URINE + HMT + ANISOLE

EXP1 PULSE SEQUENCE: STD1H
DATE 02-26-91
SOLVENT CD3CN
FILE URINE

ACQUISITION		DEC. & VT	
TN	1.250	DN	1.250
SW	4000.0	DO	406.2
AT	3.696	DM	NNN
NP	29568	DLP	20
PW	17.0	HOMO	N
P1	0		
D1	1.000	PROCESSING	
D2	0	MATH	F
TO	700		
NT	128	DISPLAY	
CT	128	SP	372.3
PW90	20.0	WP	1991.3
BS	64	VS	1031
SS	0	SC	0
IL	N	WC	230
IN	N	IS	412
DP	Y	RFL	841.1
HS	NN	RFP	578.9
ALOCK	S	TH	93
		INS	1.000
		DC	
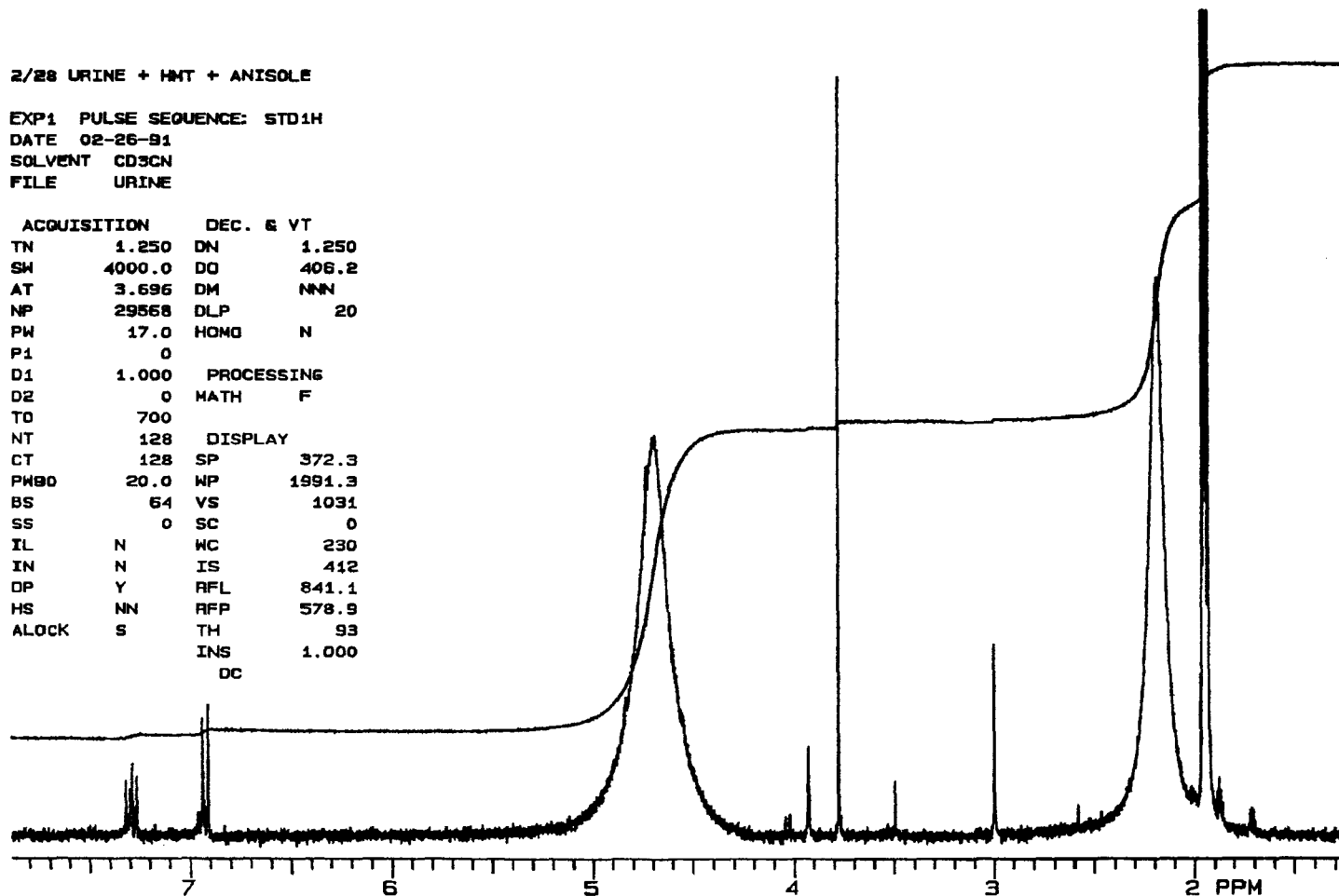
Figure 26   NMR plot of evaporated urine sample with no pretreatment to remove interferences.

2/26 COLUMN URINE 1

EXP1 PULSE SEQUENCE STD1H
DATE 02-26-91
SOLVENT CD3CN
FILE HMT1

ACQUISITION   DEC. & VT
TN   1.250   DN   1.250
SW   4000.0   DO   406.2
AT   3.896   DM   NNN
NP   29568   DLP   20
PW   17.0   HOMO   N
P1   0
D1   1.000   PROCESSING
D2   0   MATH   F
TO   700
NT   256   DISPLAY
CT   256   SP   1074.3
PW90   20.0   WP   437.3
BS   64   VS   2314
SS   0   SC   0
IL   N   WC   230
IN   N   IS   18052
DP   Y   RFL   841.1
HS   NN   RFP   578.9
ALOCK   S   TH   53
                 INS   1.000
                  OC
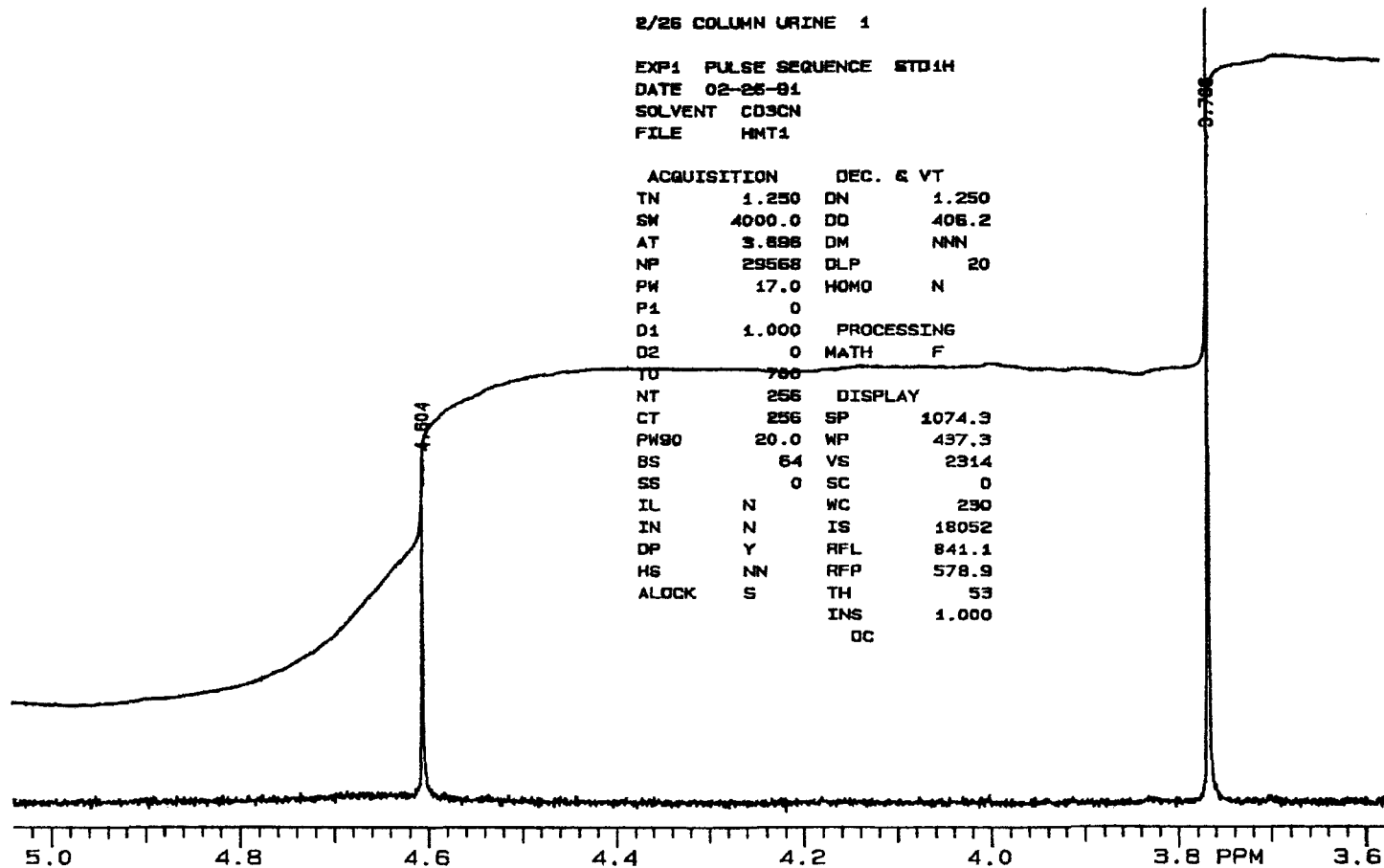
Figure 27   NMR plot with standard integration showing broad interference remaining in sample after the extraction procedure.
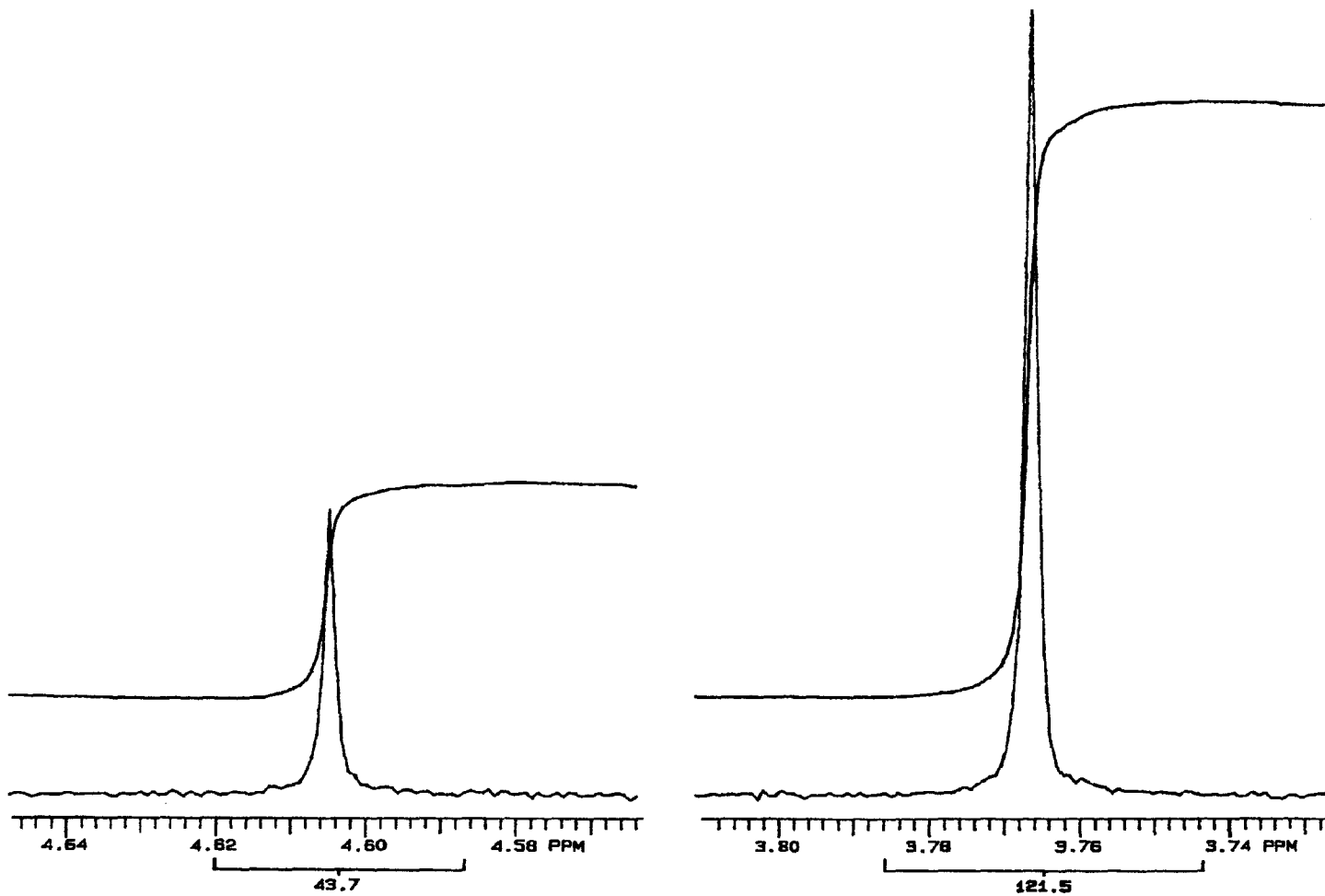
Figure 28   Expanded plots of HMT and anisole peaks from figure 27 using the alternate method of signal area integration.

Procedures

Determination of Solid HMT:

Hexamethylenetetramine in pure solid samples is determined with the use of a 300 MHz FT-NMR. The solid HMT determinations were performed mainly for the optimization of instrumental and solvent parameters.

All of the following weighings are made to 0.01 mg. A stock solution of HMT in d-acetonitrile was prepared by accurately weighing 2-3 mg of HMT into a 5 mL volumetric flask. The solution was diluted to volume with d-acetonitrile and weighed. Aliquots corresponding to 10-50 μg HMT were removed and weighed into 5 mm NMR tubes. A stock solution of anisole reference standard was prepared by weighing pure anisole into a 5 mL volumetric flask containing 5 mL of weighed d-acetonitrile. The amount of anisole in 0.6 mL samples was calculated to produce a signal peak area equivalent to the middle of the HMT range being tested. Aliquots containing 0.6 mL of stock anisole solution were then weighed into the NMR tubes. The samples were run on the 300 MHz FT-NMR to develop the optimized parameters as shown in table 10. The amount of HMT present in each sample was calculated by either using equation 9 or a standard calibration curve using the integration ratios of HMT and anisole.

Determination of Aqueous HMT:

Aqueous samples of HMT were determined in a similar manner as solid samples of HMT after the removal of the solvent water. The determination was accomplished by first evaporating the water with zinc acetate added to prevent sublimation of HMT. The sample was then dissolved in d-

acetonitrile containing the reference standard anisole and transferred to a 5 mm NMR tube to be run on the FT-NMR.

Sample aliquots of 75 or 150 μL, corresponding to a working range of 7 to 35 μg HMT, were pipetted into 10 mL test tubes. An 11.0 μL aliquot of 0.0089 M zinc acetate was added to each sample making sure that the mole ratio of Zn(II):HMT is less than 2 for the dilute samples. The samples were evaporated just to dryness in a vacuum desiccator, containing Drierite, using water aspirator vacuum. Samples were always kept to a maximum 200 μL since larger volumes require more than a reasonable amount of time to evaporate. Samples can be evaporated without zinc but must be removed from the vacuum precisely at the time when the water has been removed. After evaporation of the water was complete, 0.6 mL deuterated acetonitrile containing 46 μg anisole was weighed into each sample test tube. The amount of anisole used was calculated to produce a signal peak area equivalent to the middle of the HMT range being tested, which is about 15 μg HMT. The samples were vigorously mixed and briefly sonicated to dissolve the solid HMT. After the solid samples were dissolved, the solutions were transferred to 5 mm NMR tubes and tested as described for the solid HMT samples using the FT-NMR parameters in table 10.

Determination of HMT in the Presence of a Large Excess of Formaldehyde:

Hexamethylenetetramine in the presence of a large excess of formaldehyde was determined after the removal of both water and formaldehyde. The formaldehyde concentrations were in the range of 0 to 0.2 M. The removal of water and formaldehyde was achieved by either evaporation or extraction followed by evaporation.

Evaporation of water and formaldehyde was accomplished in a manner similar to that given in the spectrophotometric method and the aqueous HMT method just described. Sample aliquots of 75 or 150 μL, corresponding to a working range of 7 to 35 μg HMT, were pipetted into 10 mL test tubes. An 11.0 μL aliquot of 0.0089 M zinc acetate was added to each sample to help prevent HMT sublimation. Aliquots of 10 μL glacial acetic acid for 75 μL samples and 20 μL glacial acetic acid for 150 μL samples were added for formaldehyde removal. The samples were evaporated just to dryness in a vacuum desiccator containing Drierite and using water aspirator vacuum. An 0.6 mL aliquot of deuterated acetonitrile containing 46 μg anisole was weighed into each sample test tube. A calculated amount of anisole was used so as to produce a signal peak having an area equivalent to the middle of the HMT range being tested, i.e., about 15 μg HMT. The samples were vigorously mixed and briefly sonicated to dissolve the solid HMT. After the solid samples had dissolved, the solutions were transferred to 5 mm NMR tubes and tested as described for the solid HMT samples using the FT-NMR parameters in table 10.

The removal of formaldehyde by extraction of HMT onto silica gel was carried out as follows. A small extraction column was prepared by adding 900 mg silica gel into a glass wool plugged 7.0 mm i.d. extraction column. The column was cleaned and equilibrated by passing 5 mL anhydrous methanol and 5 mL pH 4 formate buffer through the column. All solutions were forced through the column by exerting gentle air pressure from the top of the column. One mL BCG solution and one mL HMT-formaldehyde solution containing between 45 and 150 μg HMT was added and mixed in the column space above the silica gel to form the ion pair. The mixture was

then passed through the silica gel. Five mL of acetonitrile were passed through to rinse the formaldehyde and water from the column. The HMT was eluted with 0.01 M perchloric acid in acetonitrile and 5 mL of the eluate was collected in a 5 mL volumetric flask. A 1 mL aliquot was removed and weighed into a 10 mL test tube containing 10 μL of 0.0089 M zinc acetate to prevent HMT sublimation during evaporation and 10 μL of 2.2 M sodium hydroxide to neutralize the perchloric acid. The sample was then sonicated for a few minutes to get the aqueous sodium hydroxide into the acetonitrile solvent. The samples were evaporated just to dryness in a vacuum desiccator containing Drierite and using water aspirator vacuum. An 0.6 mL aliquot of deuterated acetonitrile containing 46 μg anisole was weighed into each sample test tube. A calculated amount of anisole was used so as to produce a signal peak having an area equivalent to the middle of the HMT range being tested, i.e. about 15 μg HMT. The samples were vigorously mixed and briefly sonicated to dissolve the solid HMT. The solutions were then transferred to 5 mm NMR tubes and tested as described for the solid HMT samples using the FT-NMR parameters in table 10. The column used for the extraction can be reused a number of times as long as the column is "cleaned" with anhydrous methanol as described in the first step of this procedure. A flow chart for this procedure is shown in figure 29.

EXTRACTION PROCEDURE

900 mg silica gel

Prepare Column
pass 5mL MeOH
pass 5mL buffer

Load Column
add 1mL BCG solution
add 1mL HMT sample
mix and pass through

Rinse Column
pass 5mL acetonitrile

Elute HMT
pass $HClO_4$ in acetonitrile
collect 5mL eluate

HMT
remove 1mL aliquot
neut. with NaOH
add zinc acetate
evaporate to just dry
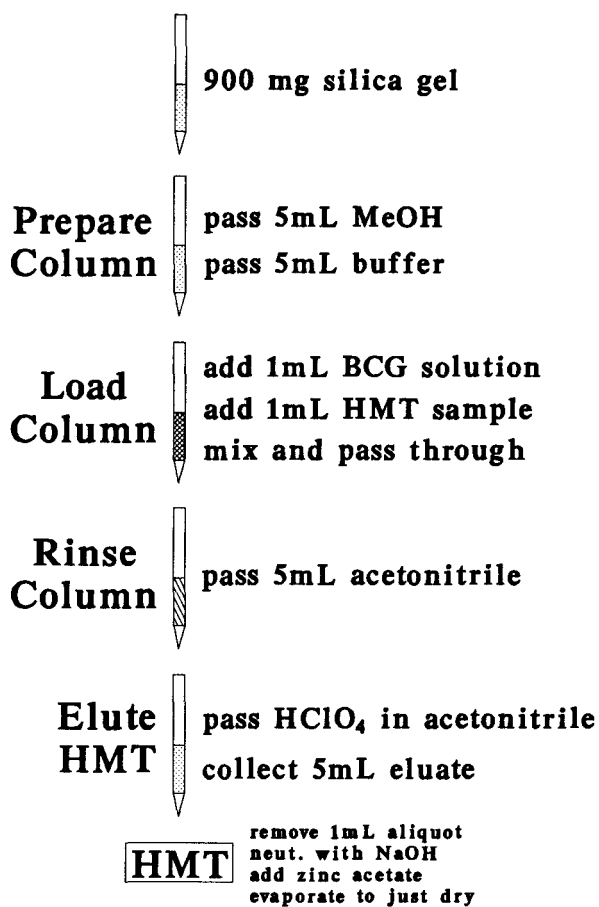
Figure 29   Flow diagram for extraction procedure.

Determination of Ammonia:

The determination of ammonia was accomplished by first reacting the ammonia with an excess amount of formaldehyde to quantitatively form HMT. Once the reaction was complete, the formaldehyde was removed and the remaining HMT was determined and quantitatively related to the amount of ammonia originally present in the sample. The parameters developed for this quantitative reaction were described previously in the spectrophotometric section under quantitative ammonia condensation with formaldehyde to form HMT. A formaldehyde concentration of 0.1 M was selected for the reaction to make its removal for the HMT determination easier.

Ammonia samples and standards were added quantitatively to volumetric flasks so that the final diluted ammonia concentrations were in the range of 0.5 to 8.5 mM. Formaldehyde was quantitatively added to each flask to produce a diluted concentration of 0.10 M, the solution diluted to volume with distilled water, and mixed. The flasks were immersed in a 60°C water bath for 4 hours to insure complete HMT condensation. The samples were then cooled to room temperature. At this point the samples contained HMT and a large amount of formaldehyde. The samples were then analyzed for HMT by using either the evaporation method or the extraction method of formaldehyde removal. Both procedures are compared here.

Determination of HMT in Urine:

Urine samples, spiked with HMT, were analyzed for HMT by using the extraction method described previously for the removal of a large excess

of formaldehyde. Most of the urine interfering substances were removed by this procedure.

The HMT spiked urine samples were diluted 1:10 or greater to bring the sample HMT concentration into the HMT determination range of 25 to 150 μg/mL. The procedure described previously for the extraction separation of HMT from formaldehyde was followed. A residual precipitate remained in the sample after the dissolution of HMT in *d*-acetonitrile which required that the samples be centrifuged and filtered before their transfer to 5 mm NMR tubes. After each sample was run, the spectrum was integrated by expanding the HMT and anisole reference peak in the plot and integrating each peak separately.

Determination of HMT-Monomandelate:

Samples of HMT-monomandelate was tested by the same procedures as described for HMT and similar results were obtained.

Results and Discussion

All the results of the various determinations shown here were run on a Varian 300 MHz FT-NMR. The acquisition times ranged between 1 minute for the more concentrated samples to 20 minutes for the very dilute samples. All samples were phase adjusted electronically or manually depending upon which produced a better phased spectra. Integrations were plotted and electronically determined in the standard manner except for the urine samples which were integrated as described previously. The

amount of HMT present in each sample was calculated using equation 9. However, the samples which employed the extraction method for interference removal showed some loss of HMT. The loss for each set of determinations was proportional within each set. Therefore, calibration curves were plotted for each set of determinations to obtain the most accurate results. All standard calibration curves are shown with the 95% confidence intervals plotted as dotted lines. The simulated unknowns are plotted on the corresponding calibration curve as error bars denoting their standard deviation. The simulated unknown results are also shown in tables for comparison.

Determination of Aqueous HMT:

The standard calibration curve for the determination of HMT in aqueous samples is shown in figure 30. The unknown amount of HMT in synthetic samples was determined using the standard calibration curve and by calculation using equation 9. The results of the unknown sample determinations are shown in figure 30 and table 11.

The calibration curve in figure 30 shows a very good correlation for the standards in the range between 7 and 34 μg HMT. The standards and unknowns were determined from aliquots of either 75 or 150 μL of sample. This difference in sample volume had no affect on the sample results. The calibration curve results were only about 1% higher than the results calculated from the reference standard using equation 9. This correlation indicates that virtually all of the HMT was accounted for in the determination and no HMT had sublimed during the evaporation procedure.

Table 11   Determination of HMT in aqueous samples.

Sample[a] (µL)	HMT (µg)	Signal ratio (HMT/anisole)	Amount determined				R.S.D.[b]
			Std[c](µg)	(%)	Calc[d](µg)	(%)	
150	16.7	1.085 ±0.004	16.9	102	16.8	101	0.326
75	15.8	1.011 ±0.005	15.8	100	15.7	99	0.490
75	27.6	1.748 ±0.033	27.2	99	27.1	98	1.86

[a]Sample = sample aliquot evaporated
[b]R.S.D. = relative standard deviation
[c]Std = determination by standard calibration curve
[d]Calc = determination by reference standard calculation
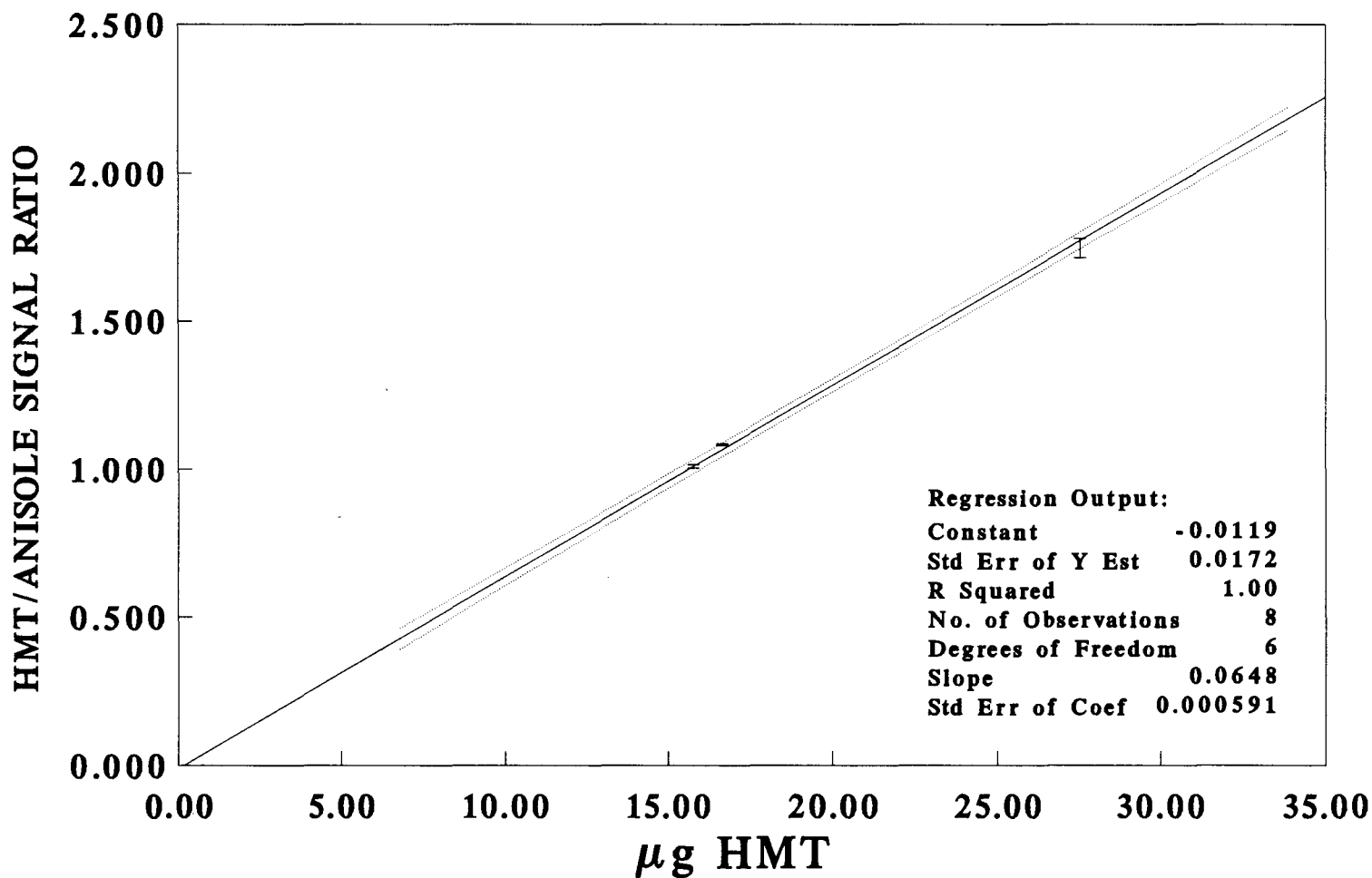
Figure 30   Calibration curve for the determination of pure aqueous HMT and synthetic unknown samples.

Determination of HMT in the Presence of a Large Excess of Formaldehyde:

The determination of HMT in the presence of a large amount of formaldehyde was accomplished by removing both the formaldehyde and the solvent. The formaldehyde concentrations studied were in the range of 0.03 to 0.16 M. The formaldehyde interference was removed by either evaporation or by HMT ion pair extraction from the sample. Both methods were studied here for comparison. The standard calibration curves are shown in figures 31 and 32. The synthetic unknown sample results are shown in table 12 and figures 31 and 32.

The evaporation procedure used was identical to the aqueous HMT sample procedure except for the addition of acetic acid in the case of evaporation of formaldehyde. The calibration curve used for this determination was the same calibration curve used for the aqueous HMT samples. The evaporation results for these formaldehyde containing samples were generally the same as those for samples without formaldehyde. There was also no significant difference between the results using the calibration curve or the reference standard results as calculated from equation 9.

The ion pair extraction procedure required the use of a calibration curve since the reference standard calculated results were well below 100%. This difference was probably due to the sublimation loss during the evaporation step. Most of the salts present in the extraction eluate were not very soluble in acetonitrile. As the acetonitrile evaporated, these salts began to precipitate out of solution and dry on the sides of the test tube. If HMT was trapped in the formation of this precipitate, there was a chance for sublimation to occur. A small amount of water was added

to the samples to keep the salts in solution but less than 100% results were still obtained. However, the losses within each batch of evaporated samples were proportional so that a standard calibration curve correcting for this problem could be used.

Table 12   Determination of HMT in the presence of a large amount of formaldehyde by evaporation and extraction-evaporation methods.

Formaldehyde conc.(M)	Sample[a] (µL)	HMT (µg)	Signal ratio (HMT/anisole)	Std[b] det. (µg)	(%)	Calc[c] det. (µg)	(%)	R.S.D.[d]
Formaldehyde removal by evaporation								
0.0331	150	16.3	1.015 ±0.022	15.8	97	15.7	97	2.16
0.164	75	25.0	1.653 ±0.006	25.7	103	25.6	102	0.385
0.0943	75	18.8	1.197 ±0.012	18.7	99	18.5	98	1.00
Formaldehyde removal by extraction								
0.0331	1020	11.1	0.479 ±0.015	10.9	98	7.42	67	3.20
0.164	1010	16.9	0.761 ±0.015	17.2	102	11.8	70	2.02
0.0943	1010	25.3	1.112 ±0.015	25.2	99	17.3	68	1.37

[a]Sample = sample aliquot evaporated
[b]Std = determination by standard calibration curve
[c]Calc = determination by reference standard calculation
[b]R.S.D. = relative standard deviation
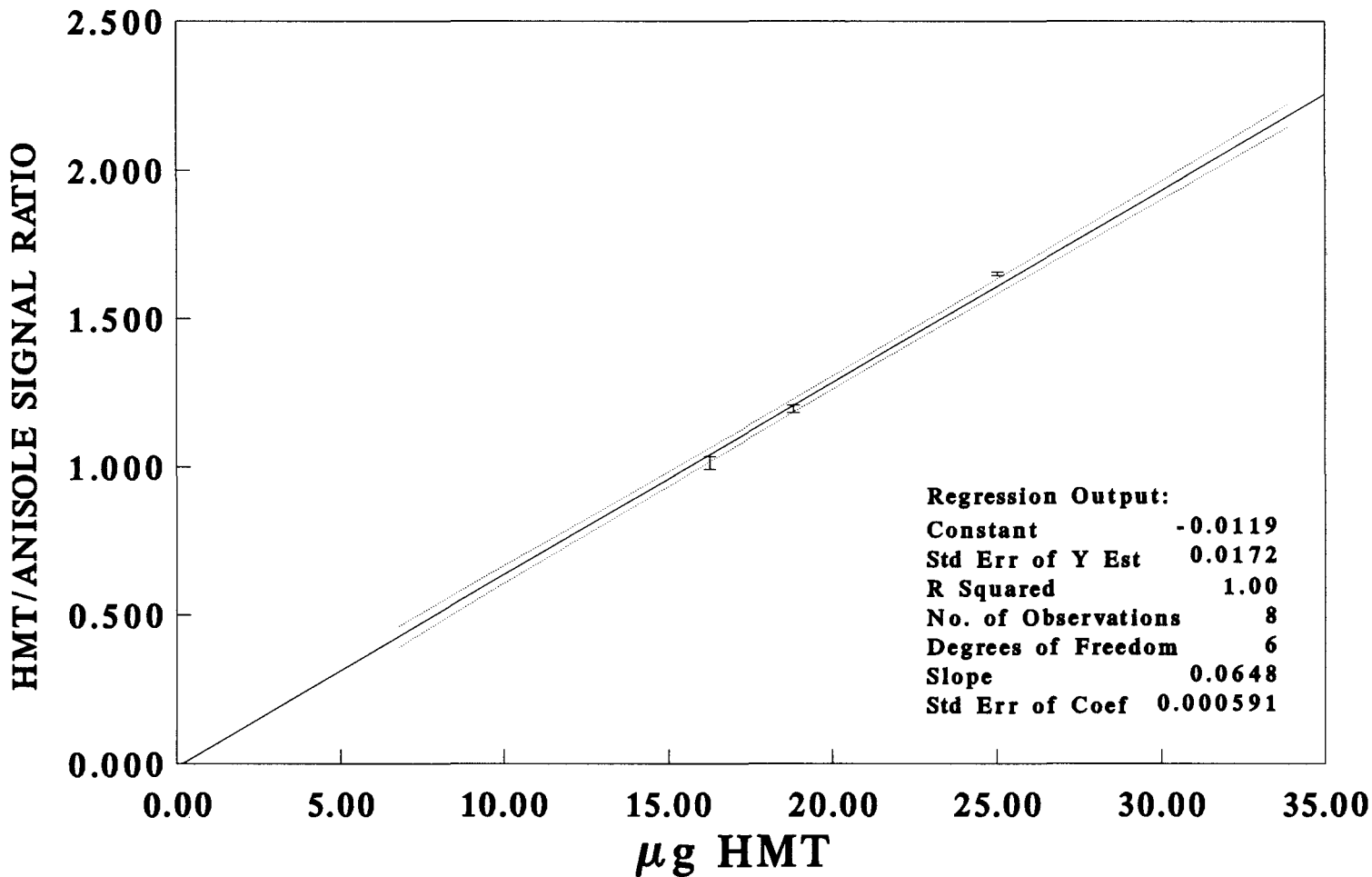
Figure 31   Calibration curve for the determination of HMT samples and formaldehyde-HMT synthetic unknown samples after evaporation removal of formaldehyde.

Regression Output:

Constant	-0.0105
Std Err of Y Est	0.0153
R Squared	0.998
No. of Observations	5
Degrees of Freedom	3
Slope	0.0449
Std Err of Coef	0.00103
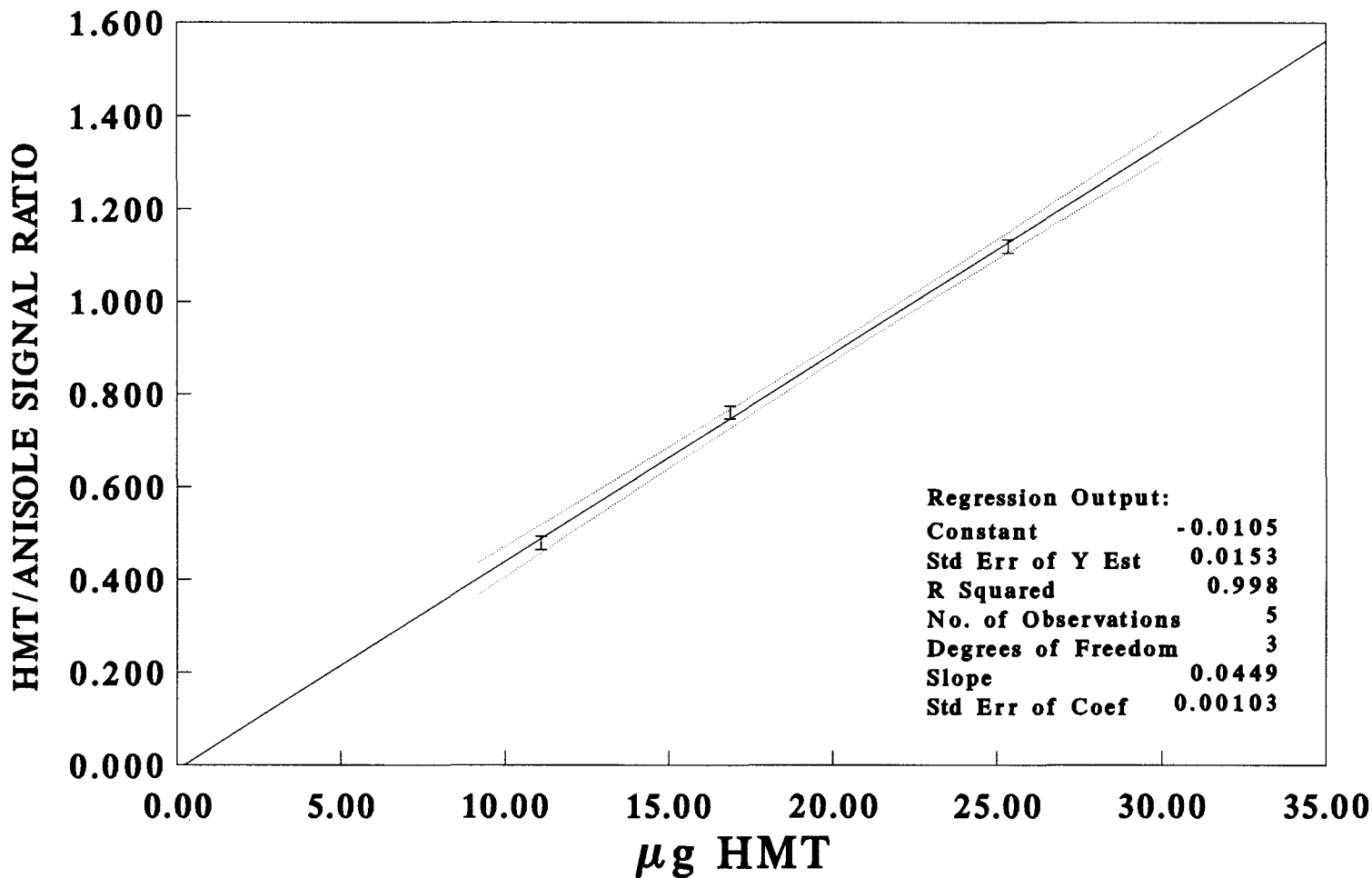
Figure 32   Calibration curve for the determination of HMT samples and formaldehyde-HMT synthetic unknown samples after HMT-BCG ion pair extraction.

Determination of Ammonia:

The determination of ammonia was accomplished by its quantitative reaction in a large excess of formaldehyde to form HMT. The HMT was then determined in a manner similar to the determination of HMT in the presence of a large amount of formaldehyde. The interfering formaldehyde was evaporated along with the water or removed by extracting the HMT from the aqueous formaldehyde sample. Both methods were investigated and the results are shown for comparison. The standard calibration curves are shown in figures 33 and 34. The results for synthetic unknown samples are shown in table 13 and figures 33 and 34.

All of the standard and synthetic unknown ammonia reactions were carried out in a formaldehyde concentration of 0.1 M. The ammonia concentration in the samples ranged from 15 to 120 ppm. These were the same samples tested by the spectrophotometric procedure. Sample aliquots were adjusted for use within the desired determination range. The evaporation method required 75 and 150 μL aliquots and the extraction method required 500 or 1000 μL aliquots of sample.

The evaporation procedure used was exactly the same as that used for the determination of HMT in the presence of a large amount of formaldehyde. The samples could be determined directly using equation 9 and the reference standard to calculate the amount of HMT present. However, the results were less accurate than those obtained using the standard calibration curve. Slight losses could have resulted from hydrolysis, as the water evaporates and the acetic acid is concentrated, or by sublimation when the sample reaches dryness. The losses were

proportional and the standard calibration curve was used to make the corrections.

The extraction procedure was also exactly the same as that used for the determination of HMT in the presence of a large amount of formaldehyde. These results also required the use of a standard calibration curve since the reference standard calculated results were below 100%. At least part of this loss appeared to be due to sublimation.

Table 13   Determination of ammonia in synthetic unknown samples by the evaporation and extraction-evaporation methods.

Sample[a] (μL)	NH_3 (μg)	Signal ratio (HMT/anisole)	Std[b] det (μg)	(%)	Calc[c] det (μg)	(%)	R.S.D.[d]
		Formaldehyde removal by evaporation					
150	5.00	0.899 ±0.053	5.05	101	4.75	95	5.91
75	8.06	1.531 ±0.053	8.20	102	8.08	100	3.47
		Formaldehyde removal by extraction					
996	6.64	0.576 ±0.033	6.55	99	4.79	72	5.79
995	10.7	1.020 ±0.033	10.9	102	8.53	80	3.27

[a]Sample = sample aliquot evaporated
[b]Std = determination by standard calibration curve
[c]Calc = determination by reference standard calibration
[d]R.S.D. = relative standard deviation

2.000

1.500

1.000

HMT/ANISOLE SIGNAL RATIO

0.500

0.000

0.00   2.00   4.00   6.00   8.00   10.00

μg NH$_3$

Regression Output:
Constant   -0.114
Std Err of Y Est   0.0532
R Squared   0.997
No. of Observations   4
Degrees of Freedom   2
Slope   0.201
Std Err of Coef   0.00797
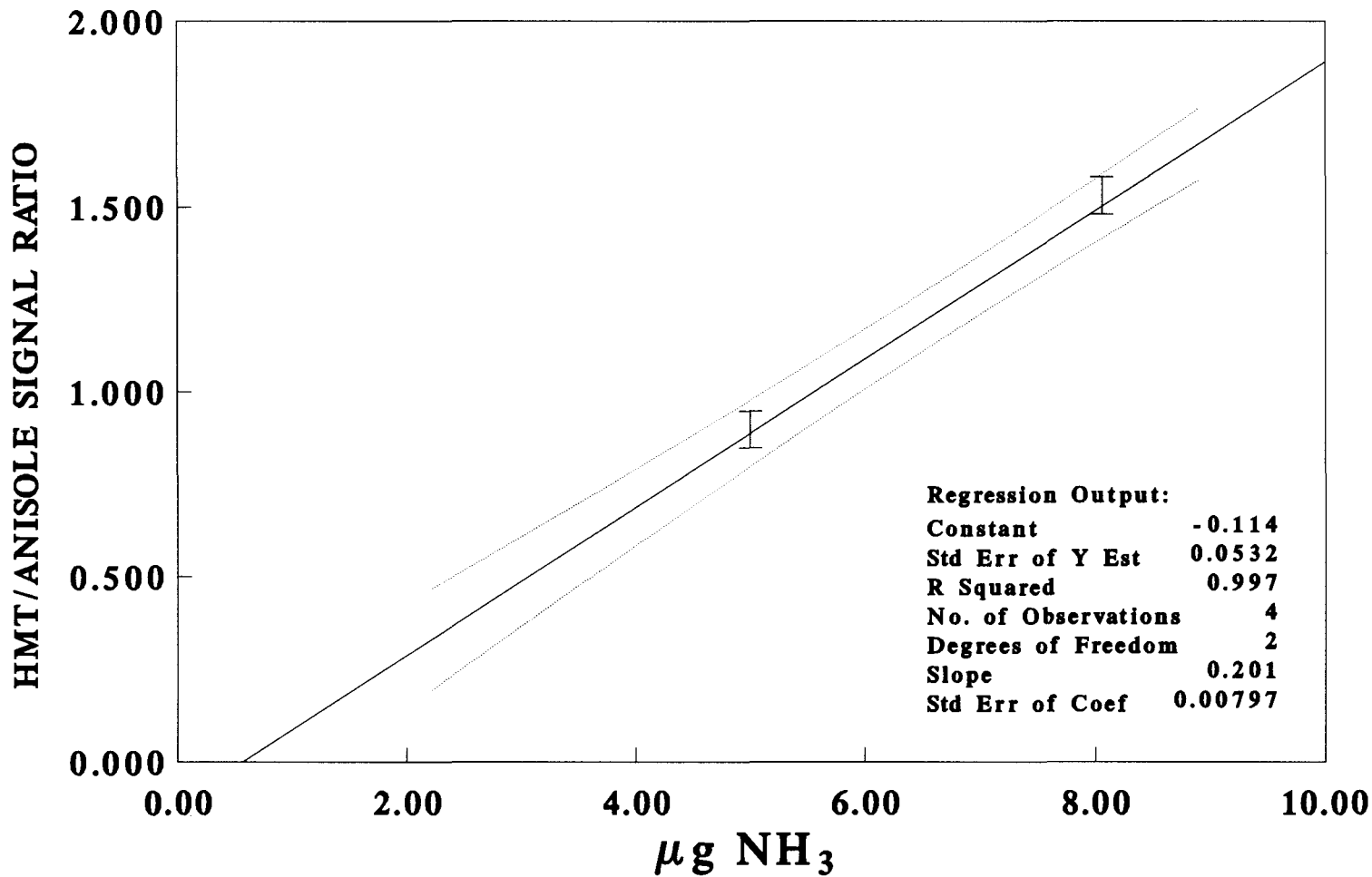
Figure 33   Calibration curve for the determination of ammonia samples and synthetic unknown samples after evaporation removal of formaldehyde.

Regression Output:

Constant	-0.0862
Std Err of Y Est	0.0334
R Squared	0.997
No. of Observations	3
Degrees of Freedom	1
Slope	0.101
Std Err of Coef	0.00520
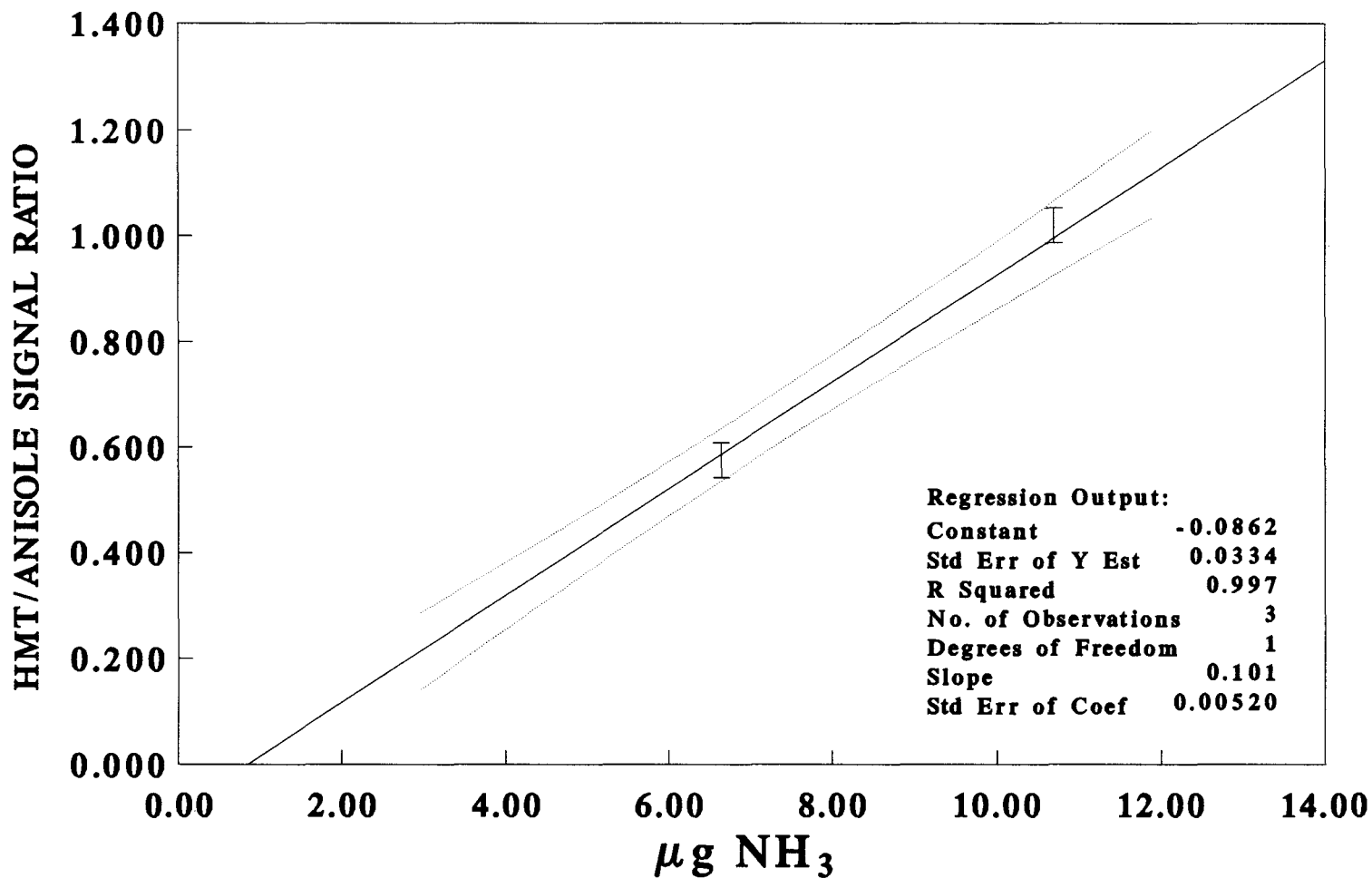
Figure 34   Calibration curve for the determination of ammonia samples and synthetic unknown samples after HMT-BCG ion pair extraction.

Determination of HMT in Urine:

The determination of HMT in urine was accomplished using the extraction-evaporation technique for the removal of interfering substances. The standard addition curve is shown in figure 35. These results show that the method of additions can be used for the determination of an unknown urine sample and that the results are linear in the range of 5 to 30 μg HMT.

If the urine sample was unknown and the first four determinations shown in figure 35 were standard additions of 0, 5.00, 11.2, and 18.6 μg HMT, the extrapolated value for the unknown would be 5.01 μg HMT. This result would be 101% of the actual 4.98 μg HMT present.

Since the extraction-evaporation results tended to produce results that were less than 100 percent, when calculated using the reference standard and equation 9, the method of standard additions was considered to be most accurate. The results for this determination calculated from the reference standard were between 87 and 93 percent. In contrast to the previous extraction-evaporation determinations, these results were consistent at around 90 percent. The loss was probably not due to evaporation because evaporation losses tend to produce results proportional to the amount of HMT present. A standard HMT sample was not determined during the procedure verifying the amount of anisole reference standard present. The exact amount of reference standard is critical for the calculation results. The standard additions method requires only that the amount of anisole reference standard present be constant in each sample.

Regression Output:

Constant	-0.0307
Std Err of Y Est	0.0302
R Squared	0.999
No. of Observations	6
Degrees of Freedom	4
Slope	0.0733
Std Err of Coef	0.00129
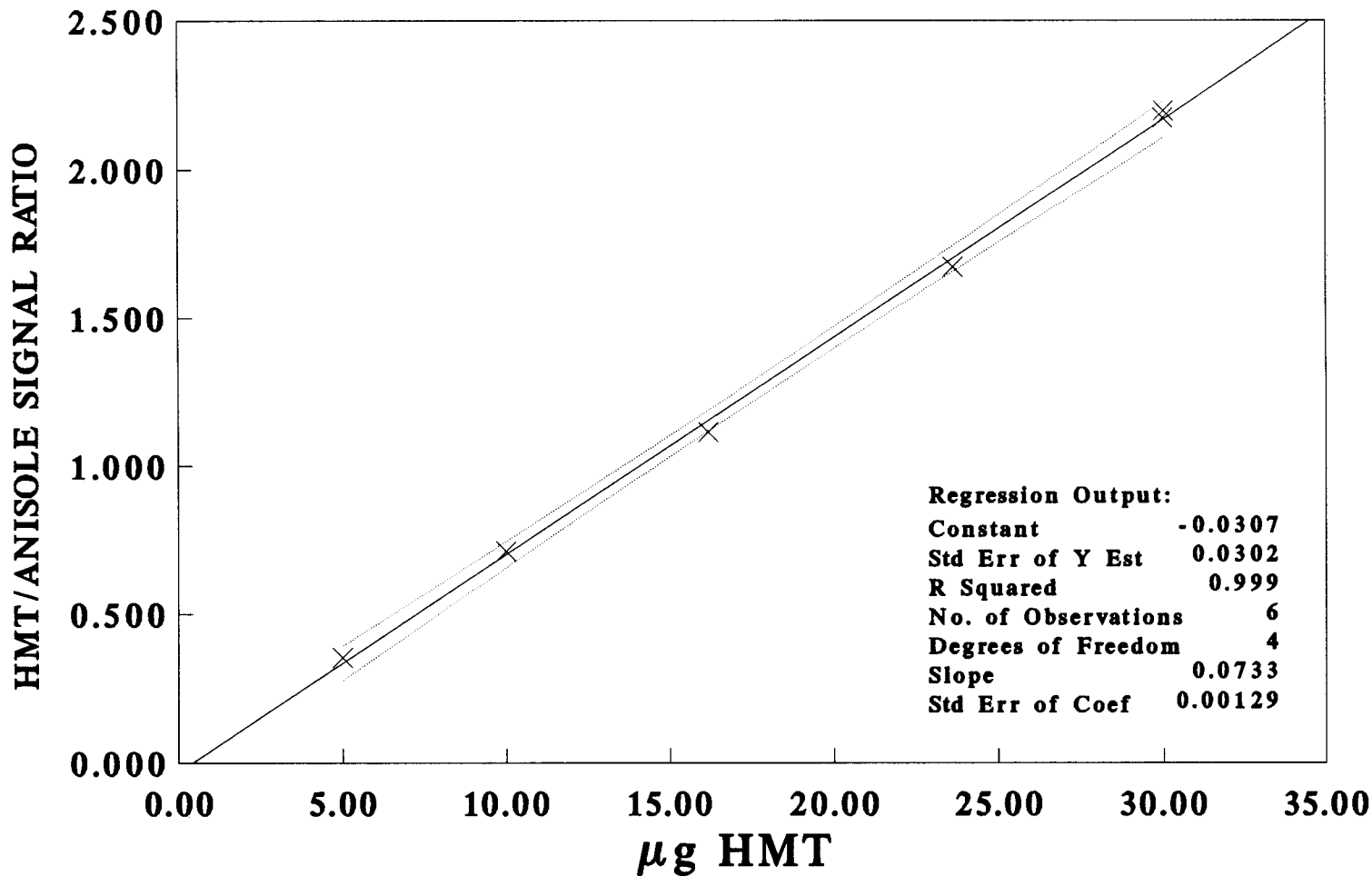
Figure 35   Standard additions curve for the determination of HMT in urine samples.

ION EXCHANGE DETERMINATION OF AMMONIA

Introduction

Ion exchange chromatography is based on the exchange of ions of like sign between a solution and the ions of a solid-insoluble resin in contact with it. The solid resin contains a number of positively or negatively charged ionic groups that are fixed in a three-dimensional cross-linked polymeric hydrocarbon network. These charged ionic groups are surrounded by oppositely charged ions that are free to move inside the resin but cannot leave the resin particle unless replaced by another ion of equal charge, since electrical neutrality must be maintained. The inside of the resin particle is very similar to a concentrated solution of a strong acid or base except that the negative or positive charges are held firmly in place instead of being free to move as they are in solution.

When an ion exchange resin is placed in solution, ions may enter the solid particle and may be exchanged for ions of like charge. Ions of the same charge as the fixed ionic groups are excluded from the resin particle. Thus, a cation resin charged with hydrogen ions is in contact with a solution of sodium chloride, ion exchange occurs. The equilibrium established can be represented by equation 10

$$Na^+ + H^+R^- \rightleftharpoons Na^+R^- + H^+ \tag{10}$$

where R⁻ represents the fixed negative sites on the resin. The exchange is a reversible reaction that attains equilibrium and follows the law of mass action to a first approximation. Consequently, if a solution containing a small amount of sodium ion is passed through a large amount of cation resin charged with hydrogen ions, the sodium will be quantitatively removed from the solution and replaced with an equivalent amount of hydrogen ions. In a like manner, if a concentrated acid is passed through a cation resin charged with sodium ions, the sodium ions will be completely removed and replaced with hydrogen ions. This regeneration of the resin can be repeated many times without apparent damage to the resin.

Ion exchange resins show a degree of selectivity in respect to different ions. They have been found to follow some general rules which are summarized as follows: The affinity of various ions to the same resin increases with the ionic charge of the ion. Under the same conditions, polyvalent ions are attached to a resin more strongly than monovalent ions. For ions of the same charge, affinities are inversely proportional to the radius of the hydrated ions. The extent of resin cross linkage affects selectivity as a function of ion size. Greater amounts of cross-linking, result in pore sizes in the resin which in turn inhibit the movement of water and ions. A highly cross-linked resin can exclude large organic ions.

The measure of strength with which an ion is held by a resin is expressed by the distribution coefficient (D) shown in equation 11.

$$D = \frac{\text{amount of component in exchanger phase at equilibrium}}{\text{amount of component in liquid phase at equilibrium}} \quad (11)$$

If the equation is related to unit weight, the results are expressed as the weight distribution coefficient (D_g) defined according to equation 12.

$$D_g = \frac{\text{conc. of ion in resin}}{\text{conc. of ion in solution}} \times \frac{\text{mL of solution}}{\text{grams of dry resin}} \quad (12)$$

The larger the distribution coefficient, the more strongly the ion is held by the resin.

The use of ion exchange for the determination of ammonia has been reported by other researchers. Bouyoucos (71) separated and determined ammonia directly by ion chromatography. Samples containing 300-600 ppm ammonia and various amounts of methylamines were injected (50 μL) onto a strong acid exchange resin in 0.01 M HCl. The ions were separated by the exchange column, passed through a stripper column, and detected by a conductivity measurement.

Jaworski (72) separated and determined ammonia directly by ion exchange chromatography. Samples containing 0.1-1.0 mg ammonia and ethylene diamine-urea reaction products were injected onto a strong acid exchange resin in 2 M HCl. The ions were separated by the exchange resin and detected directly by a thermal detector.

Moreno et al. (73) determined ammonia indirectly by forming indophenol with the ammonia in the solution. This chromophore was concentrated by ion exchange and determined spectrophotometrically. The procedure was as follows: Samples of 2-200 mL containing 10-50 ppb ammonia were reacted for 40 minutes to form indophenol. The samples were then passed through an Amberlite XAD-7 ion exchange resin, eluted with 2

mL acetone, evaporated to dryness, the residue redissolved in 2 or 10 mL distilled water, and the absorbance measured at 640 nm.

Statement of Problem and Purpose of Research:

The goal of this study was to develop an ion exchange method for the determination of ammonia in solution with the use of atomic absorption (AA) determination of the exchanged ion. Since ammonia is a difficult ion to determine at low concentrations, it is convenient to quantitatively exchange it for an ion which is easier to determine. Since AA is a relatively sensitive method of determination, the exchanged ion can usually be determined by this method.

Ammonia is volatile and commonly distilled from a sample and trapped in an acid, such as boric acid or sulfuric acid. The proposed method is based on the quantitative ion exchange of ammonium in boric acid for lithium by means of a strong acid cation exchange resin. The exchanged lithium is then quantitatively determined by AA.

The development of this procedure required the investigation of several test parameters. The resin parameters that were investigated include choice of resin material, amount of resin required, and optimum solvent system. The AA parameters were adjusted, using a lithium hollow cathode lamp, with both a graphite furnace and an acetylene-air flame for the atomization of the sample. The graphite furnace AA (GFAA) determinations required optimizing the following parameters of both time and temperature for solvent evaporation, pretreatment removal of interfering elements, and atomization temperature. The flame AA (FAA) determinations required adjusting the following parameters of gas

mixtures, sample aspiration rate, and burner head position. Once the optimum conditions were established, small amounts of ammonia were determined in boric acid as ammonium ions.

Experimental

Instrumentation:

All atomic absorption measurements were obtained using a Perkin Elmer model 5000 AA equipped with a Perkin Elmer Model 500 Automatic Burner Control and a Perkin Elmer Model 400 Graphite Furnace Control. The analytical wavelength used for lithium was 670.8 nm. The slit width for the GFAA determinations was 1.4 nm, low, and for the FAA determinations the slit width was 0.4 nm, high. Ten microliters of sample solution were injected by Eppendorf pipette into pyrolytic coated graphite furnace tubes for GFAA.

Reagents:

All chemicals utilized were analytical or primary standard grade. The strong acid cation exchange resin was Dowex 50W-X8 100-200 mesh resin purchased from Bio-Rad. All acetone used was distilled before its use. Stock ammonium solutions were prepared from ACS grade ammonium chloride which had been dried at 110° C for 2 hours.

Development of Method

The development of this method for the determination of ammonium ion required optimization of resin, solvent, and AA parameters. An ammonium sample in 0.1 M boric acid was passed through an ion exchange column charged with lithium. The eluate of exchanged lithium was then quantitatively determined by atomic absorption.

Optimization of Exchange Parameters:

The development of the ion exchange method for the determination of ammonia required optimization of several parameters: (i) choice of resin type, (ii) amount of resin required, (iii) and nature of the solvent. The first tests with resins employed 3 cm of resin in an 8 mm i.d. glass tube. The resin was then charged with lithium. In this lithium charging procedure, the resin was first charged with H^+ by passing a large excess of 3 M HCl through the column. Excess HCl was removed by rinsing the column with distilled water until the eluate did not test acidic using litmus paper. Then, 0.2 M LiOH was passed through the column to exchange Li^+ for H^+. The completion of this charge was also monitored using litmus paper by testing the eluate for basic solution. Solvent containing 0.1 M boric acid was then passed through the column until the excess lithium was rinsed from the column. Ideally there would be no lithium elution from the resin due to the solvent at this point.

Of the available strongly acidic cation exchange resins tested, Dowex 50W-X8 produced the best results. All of the resins investigated slowly exchanged lithium via solvent exchange. The boric acid

concentration was reduced but no decrease in solvent exchange was observed.

According to Marhol (74) selectivity generally decreases in the series (Dowex 50W-X8): $Ag^+ > Cs^+ > Rb^+ > K^+ > NH_4^+ > Na^+ > H^+ > Li^+$ for dilute solutions. This series showed that H^+ in the solvent should readily exchange Li^+. The pH of the solvent was raised to 7.00 with tetrabutyl-ammonium hydroxide in an attempt to reduce the solvent-Li^+ exchange. However, the amount of solvent-Li^+ exchange increased. This increase may have been the result of sodium impurity from tetrabutylammonium hydroxide preparation or of the tetrabutylammonium ion exchanging with lithium.

At this point the exchange required large amounts of solvent. A decrease in the column size to a 6 mm i.d. pasteur pipette and a reduction in the amount of resin to about 0.25 g, or 2 cm height, lowered the exchange volume to about 1 mL for 0.25 mL of 0.01 M ammonium chloride in 0.1 M boric acid. Changing the resin particle size from 200-400 mesh to 100-200 mesh increased the solvent flow through the column. Resin of 50-100 mesh was found to be too large because the solvent passed through the column too quickly, requiring very quick manipulation of the collection glassware. The change in resin size did not have an effect on Li^+ elution. Resin of 100-200 mesh was the size of choice.

The nature of the solvent was investigated with the purpose of reducing the amount of solvent exchange in the column. Since the affinity of the alkali-metal elements increases with decreasing radius of its hydrated ion, the hydrated ion radius can be decreased by changing solvent composition (75). Various solvent mixtures with water were tested in the range of 20-90 percent methanol or acetone. Various amounts of methanol

had little affect on solvent exchange, but acetone greatly reduced the solvent exchange as shown in figure 36. To demonstrate the acetone-water results, weight distribution coefficients (D_g) for H^+-Li^+ were determined according to equation 12. The weight distribution coefficient was determined by equilibrating 250 mL of a 0.1 M boric acid solution containing 5 meq of Li^+ with 2.500 g equivalent of dry resin (76). A mechanical shaker was used. The dry resin weight was determined by first vacuum pumping a weighed sample for 24 hours and then drying it in an Abderhalden at 65° C over phosphorous pentoxide for 24 hours. The Dowex 50W-X8 100-200 mesh resin in the proton form contained 52.86 percent water. Acetone-water solvent mixtures of 0, 10, 30, 50, and 70 percent acetone with 0.1 M boric acid were investigated. The Li^+ eluent concentrations were determined by AA. The concentration of Li^+ on the resin was obtained by subtracting the Li^+ eluate concentration from the initial Li^+ concentration. The results are shown in figure 37. They fit a third order regression curve very well. Figure 37 shows that there is a dramatic increase in the resin affinity for Li^+ as the percentage of acetone is increased. An acetone-water mixture of 50 percent was chosen as the mixture of choice to decrease solvent Li^+ exchange.

After charging the column with lithium, a determination of the point at which the solvent exchange equilibrium was required. Solvent was passed through the column with the collection of 1 mL samples every 2 mL. The collections were tested for lithium. The "baseline" equilibrium appeared to be established after about 13 mL. All subsequent experiments were performed after the passage of about 15 mL of solvent.
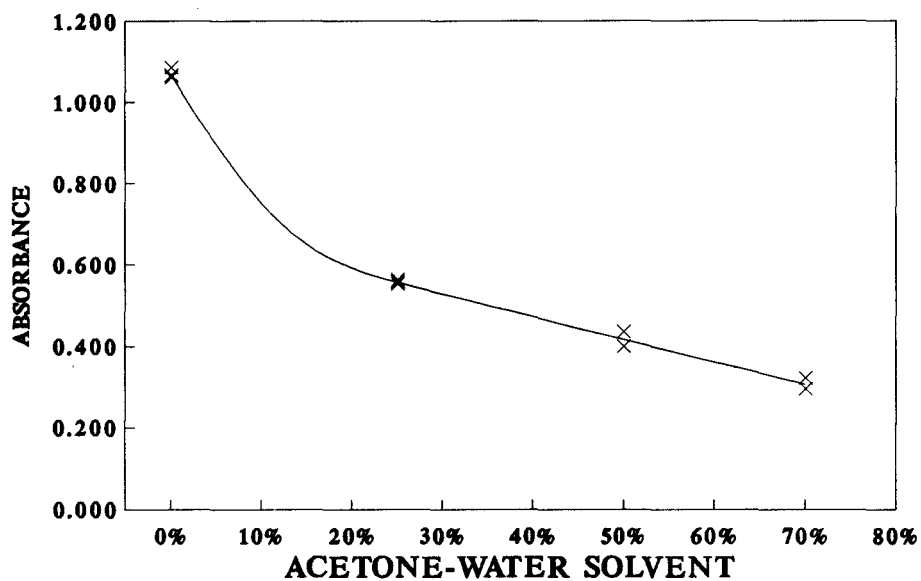
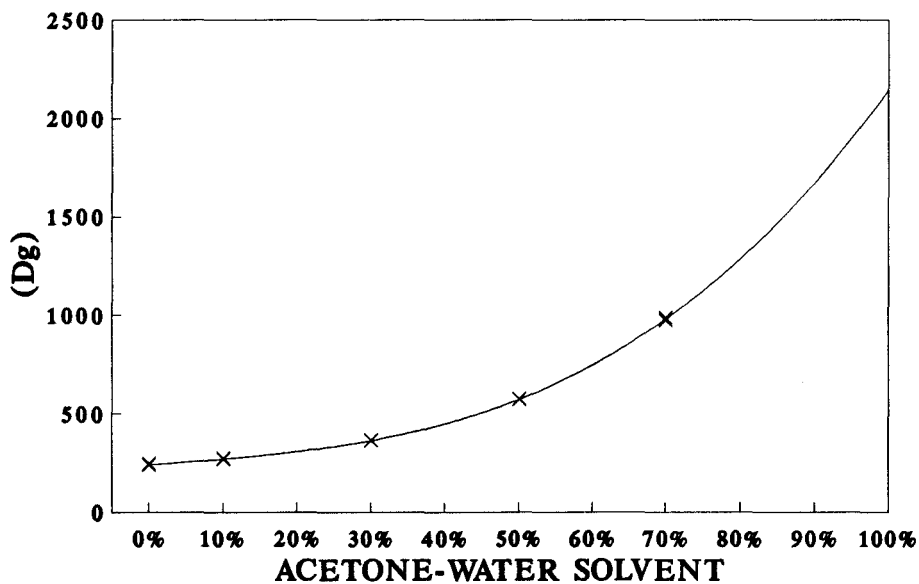**Figure 36**   Solvent-Li$^+$ exchange at various acetone-water mixtures.

Figure 37   Determination of weight distribution coefficient (D_g) at various acetone-water mixtures.

Optimization of Atomic Absorption Parameters:

To obtain optimum conditions for the graphite furnace determinations, the following parameters were studied with each solvent system: (i) time and temperature for solvent evaporation, (ii) pretreatment removal of interfering elements, and (iii) atomization temperature. The graphite furnace control box used was programmable and each step in the procedure was adjusted. The solvent evaporation step was adjusted by temperature and time to evaporate the sample droplet slowly and constantly without spattering. The second step of pretreatment was adjusted by temperature and time to remove as much of the interfering sample matrix as possible without atomizing any Li^+. The third step of atomization was adjusted to the lowest atomization temperature that produced a maximum signal. A fourth step was used after atomization with a high temperature and full gas flow through the graphite tube to remove any remaining sample and prevent carryover problems.

The parameters used for the determinations are shown in table 14. High concentrations of acetone in the solvent caused large errors in delivering 10-20 μL samples into the graphite furnace. The acetone did not adhere to the plastic tip very well and a portion would leak out before transfer in the furnace tube. The correlation coefficient for a 50 percent acetone-water solvent sample run 35 consecutive times was only 0.701. The correlation coefficient for an aqueous solvent sample run 18 consecutive times was 0.977. Therefore, lower acetone concentrations were highly desireable for reproducible graphite furnace determinations. However, higher water concentrations in the solvent exchanged more Li^+ from

the resin resulting in high blank signal. Despite these problems, acceptable results were obtained when great care was used.

Table 14   Optimized graphite furnace parameters programmed into the furnace controller.

Step	Temp °C	Ramp sec	Hold sec	inner gas flow mL/min
Aqueous Samples				
Evaporation	110	2	30	310
Pretreatment	900	3	20	310
Atomization	2600	1	5	25
Cleaning	2700	1	3	310
50% Acetone-Water Samples				
Evaporation	80	5	20	310
Pretreatment	1300	3	20	310
Atomization	2600	1	5	60
Cleaning	2700	1	3	310

The problems of sample delivery were eliminated by using the flame for atomization. The flame was set for the acetylene-air gas mixture which required adjustment of the following parameters for each solvent system: (i) gas mixture, (ii) aspiration rate, and (iii) burner head position. When analyzing samples in organic solvents, adjustments had to be made in the acetylene-air flow mixture to compensate for the flammability of the solvent. The fuel flow was reduced until maximum

sensitivity was obtained. The aspiration rate was adjusted to the point where the maximum absorbance was obtained. However, the aspiration rate had to be decreased to 2-4 mL/min when small volume samples were tested. This lowered the sensitivity. The lower aspiration rate was required in order to obtain the Li^+ atomization equilibrium in the flame before the whole sample had been aspirated. The burner head was adjusted for maximum sensitivity while aspirating a standard solution of Li^+. The optimum position varied according to the solvent composition being aspirated and adjustments to the burner head position were made in every direction. The standard AA parameters were used; high 0.4 nm slit width, continuum source background correction, and 3 times 3 sec signal averaging.

Procedure

The 6 mm i.d. columns charged with 0.25 g Dowex 50W-X8 100-200 mesh resin were cleaned and regenerated by passing 3 M HCl through the column. The excess HCl was removed by rinsing the column with distilled water until the eluate did not test acidic with litmus paper. Then, 0.2 M LiOH was passed through the column to exchange Li^+ for H^+. The completion of the charge was monitored using litmus paper to test the eluate for a basic solution. Fifteen mL of 0.1 M boric acid in 50 percent acetone-water solvent were then passed through the column to remove the excess lithium from the column.

All of the samples were analyzed for lithium by acetylene-air flame atomic absorption. Lithium standards were tested continuously to correct

for any drift in the determinations. Graphite furnace AA also could have been used for the lithium determinations by using the parameters listed in table 14. However, the samples containing 50 percent acetone were more accurately determined by the FAA technique.

Determination of Ammonia by Quantitative Lithium Ion Exchange:

Aqueous ammonia samples in 0.1 M boric acid were determined by pipetting an aliquot onto a small ion exchange column charged with lithium and measuring the exchanged lithium collected in the eluate.

After the exchange column had passed 15 mL of solvent, 1 to 2 mL of eluate was collected for a blank determination. Aqueous samples were prepared from a stock solution to produce samples containing between 0.19 to 3.2 mM NH_4Cl in 0.1 M H_3BO_3. A sample aliquot of 200 μL was added to the column followed by the solvent. The eluate was collected in a 2 mL volumetric flask and another sample of the eluate was collected for the blank determination. A second 200 μL sample was loaded onto the column and 2 mL of the eluate was collected. A third eluate blank was collected and a third sample was added and collected from the column. The 3 samples and blanks were analyzed for lithium by FAA with parallel lithium standards. The sample absorbances were subtracted from the blank absorbances and the amount of lithium present in the samples was calculated from the standards. The column was regenerated and used again.

Determination of Ammonia by Ammonium-Lithium Ion Exchange Equilibrium:

A more sensitive method of determination was attained by continuously passing an ammonium ion sample through the column to exchange

the lithium at a constant rate. The amount of lithium exchanged was equivalent to the concentration of ammonium ion in the sample.

Aqueous ammonium samples were prepared from a stock solution to produce samples containing between 0.038 to 0.31 mM NH_4Cl in 0.1 M H_3BO_3. After rinsing the column of excess lithium with 15 mL solvent, 1 mL of the ammonium ion sample was passed through the column and discarded. The ammonium ion sample exchange was continued and 3 collections of about 2 mL eluate were taken for the determination of lithium.

Results and Discussion

The calibration curves are shown with the 95% confidence intervals plotted as dotted lines. The actual points are also plotted as error bars of their standard deviations.

Determination of Ammonia by Quantitative Lithium Ion Exchange:

Aqueous samples of ammonium ions were determined and are shown in figure 38. The results were very linear in the determination range between 0.13 and 2.2 ppm Li^+ producing a linear correlation coefficient r^2 of 0.999. However, results corrected for the blank are about 90 percent of the expected exchange. Standardized LiCl samples were passed through the column in the same manner as the NH_4Cl samples and similar results were obtained. If the exchange resin was not 100 percent charged with lithium, an equilibrium could have occurred as the sample passed through the column. Ten percent of the ammonium ions or lithium ions may not have

exchanged in the column or may have exchanged with H^+ present and therefore were undetected in the eluate.

Regression Output:
Constant   -0.0502
Std Err of Y Est   0.0291
R Squared   0.999
No. of Observations   18
Degrees of Freedom   16
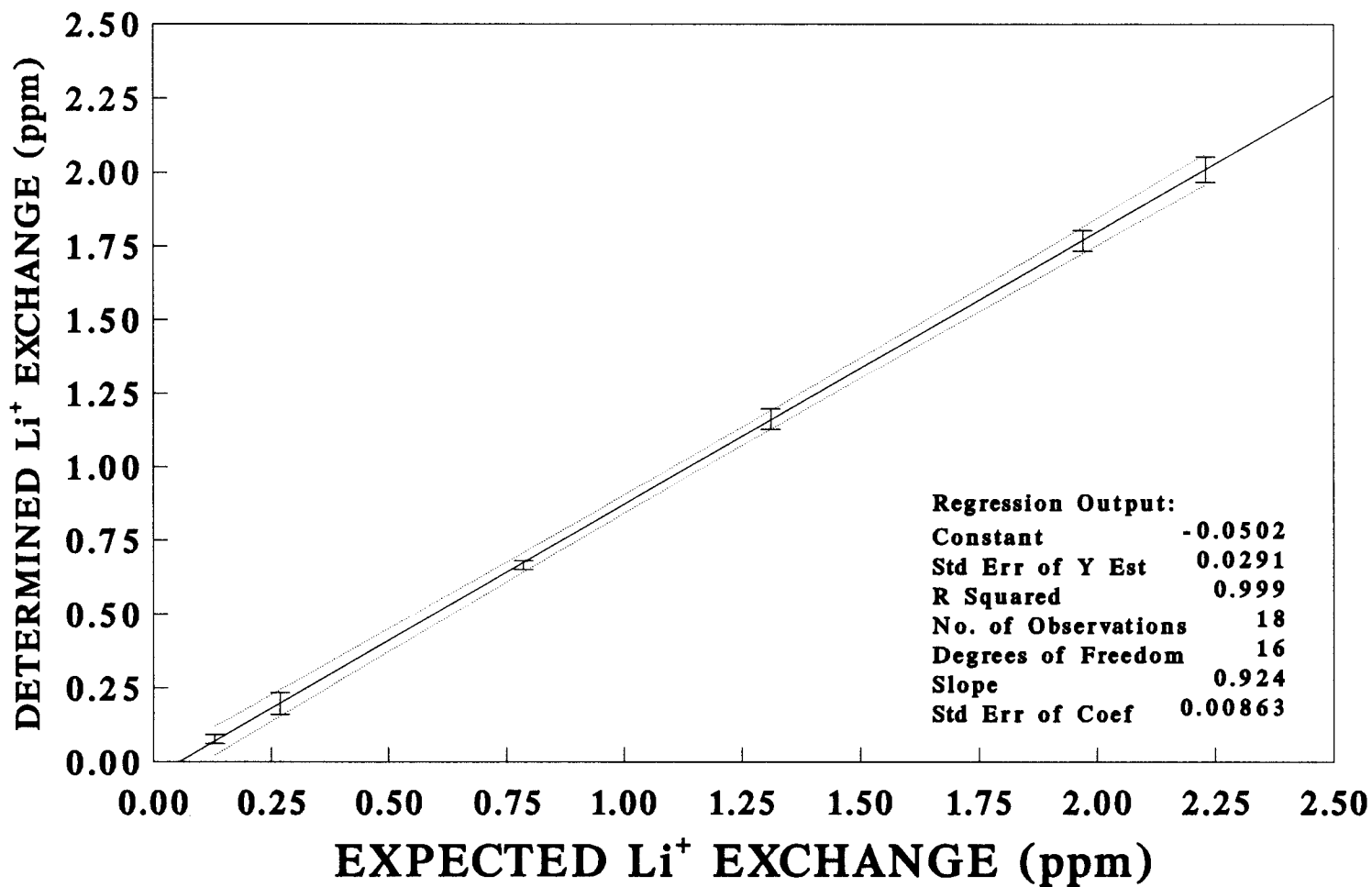Slope   0.924
Std Err of Coef   0.00863

Figure 38   Calibration curve for the determination of ammonium ion samples in 0.1 M boric acid by quantitative lithium ion exchange.

Determination of Ammonia by Ammonium-Lithium Ion Exchange Equilibrium:

   Aqueous samples of ammonium ions were determined and are shown in figure 39. The results produced a linear correlation coefficient of 0.998 in the determination range between 0.27 and 2.7 ppm Li^+. The results shown in figure 39 are not corrected for the blank. An exchange equilibrium resulted when each sample passed through the exchange column. The use of standards eliminates the need for blank determinations. When the blanks were subtracted, the results were about 85 percent of the expected exchange results. The blank subtracted results were also more erratic due to the blank determination results for each sample. Standardized LiCl samples were passed through the column in the same manner as the NH_4Cl samples and similar results were obtained. These results suggest that the column may not have been fully loaded with lithium.

Regression Output:

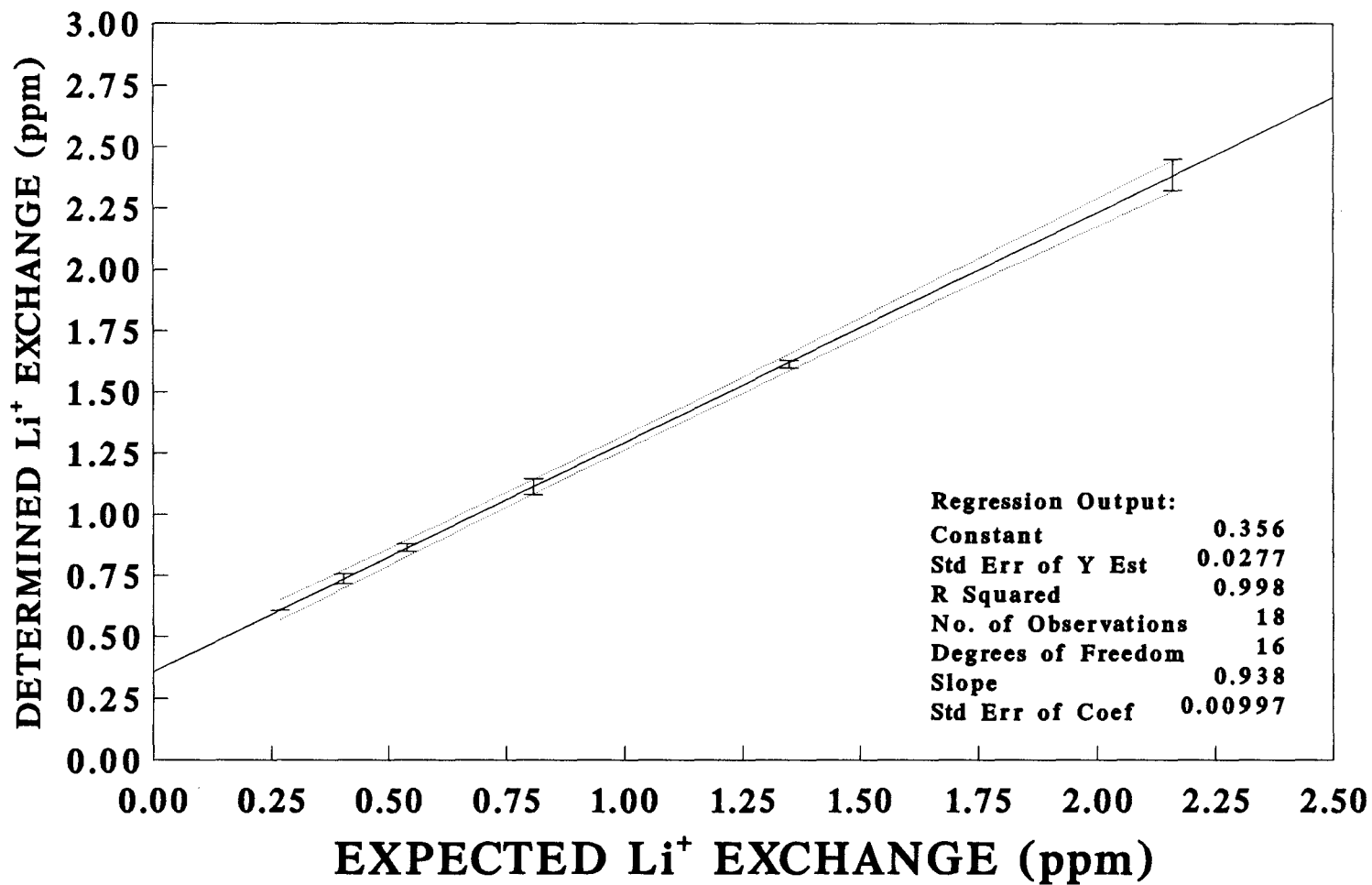|	
Constant	0.356
Std Err of Y Est	0.0277
R Squared	0.998
No. of Observations	18
Degrees of Freedom	16
Slope	0.938
Std Err of Coef	0.00997

Figure 39   Calibration curve for the determination of ammonium ion samples in 0.1 M boric acid by quantitative lithium ion exchange equilibrium.

SUMMARY AND SUGGESTIONS FOR FUTURE WORK

In this study, methods were developed for the quantitative determination of hexamethylenetetramine (HMT) and ammonia present in various matrices. In particular, the determination of HMT in the presence of a large amount of formaldehyde was investigated. Techniques were developed to eliminate or reduce the interferences present and allow the quantitative determination of HMT.

A spectrophotometric method was presented for the quantitative determination of hexamethylenetetramine and ammonia present in various matrices. Hexamethylenetetramine was determined indirectly, after the separation from interferences, by its quantitative hydrolysis with perchloric acid and subsequent determination of the released formaldehyde. The formaldehyde was quantitatively oxidized by an excess of hydrous silver oxide to form silver metal. Then silver metal was reoxidized with added ferric ion and the resulting ferrous ions were quantitatively complexed with Ferrozine. The iron(II)-Ferrozine complex absorbance was measured and related to the amount of HMT originally present in the sample.

Hexamethylenetetramine was determined by this spectrophotometric method in the range of 3.2×10^{-7} to 3.2×10^{-6} M. Pure aqueous samples were determined, producing an apparent molar absorptivity of 314,000 L/(cm mol). Hexamethylenetetramine samples containing up to 0.2 M formaldehyde

were determined after first removing or decreasing the large formaldehyde interference. The formaldehyde was removed by either evaporation or borohydride reduction. Hexamethylenetetramine was also determined in urine. Interferences in urine were removed by oxidation with silver(I) in basic conditions leaving HMT unreacted. Hexamethylenetetramine was quantitatively determined in the supernatant after removal of the resulting precipitate.

Future work could include investigation of established indirect HMT determination methods coupled with the developed techniques for the removal of formaldehyde interference. These other indirect methods can tolerate only small amounts of formaldehyde present in the samples.

A NMR technique also was developed for the quantitative determination of hexamethylenetetramine and ammonia present in various matrices. Hexamethylenetetramine was quantitatively determined directly using its 12 equivalent proton resonance signal. A 300 MHz FT-NMR instrument was used with *d*-acetonitrile as the solvent and anisole as the reference standard.

Hexamethylenetetramine was determined in the range of 3 to 35 μg in 0.6 mL of solvent. All samples were evaporated to dryness for the removal of the interfering solvent and redissolved in *d*-acetonitrile containing anisole. Hexamethylenetetramine in samples containing up to 0.2 M formaldehyde was determined after first removing or decreasing the large formaldehyde interference. The formaldehyde was removed either by the evaporation procedure or by silica gel extraction of an HMT:bromocresol green ion pair and subsequent elution in a more volatile acetonitrile eluant. Hexamethylenetetramine was also determined in the presence of

urine. The urine interferences were decreased by using the ion pair extraction technique.

Future work could include investigation of the extraction technique using *d*-acetonitrile to elute and free the extracted HMT:BCG ion pair from the column. This eluate could then be scanned on the NMR eliminating the need for evaporation and reducing sample total analysis time to approx. 15 minutes.

An ion exchange technique for the determination of ammonia trapped in boric acid was investigated. Dowex 50W-X8 strong acid cation exchange resin charged with lithium was used for the exchange. Atomic absorption was used for the determination of the exchanged lithium.

Exchanged lithium ions were determined in the range of 0.15 to 2.25 ppm. Lithium analyzed in 2 mL eluate samples corresponded to 38 to 640 μmol of ammonium ions. The exchange was made more sensitive by continuously passing the sample through the column to form an exchange equilibrium rather than using the singular sample elution. The lithium analyzed eluate samples corresponded to 0.038 to 0.31 mM ammonium ions.

REFERENCES

1. Butlerow, A. *Liebig. Ann. Chem.* **1859**, *111*, 249.

2. *ibid.*, **1860**, *115*, 322.

3. Hofmann A. W. *Ber. dtsh. chem. Ges.* **1869**, 2153.

4. Butlerow, A. *Liebig. Ann. Chem.* **1860**, *115*, 322.

5. Van't Hoff, J. *Ansichten über organische Chemie*, I; Braunschweig, 1881; p 121.

6. Delépine, M. *C. R. hebd. seances Acad. Sci.* **1893**, *120*, 197.

7. Lösekann, G. *Chem. Zeit.* **1890**, *14*, 1409.

8. Duden, P.; Scharff, M. *Liebig. Ann. Chem.* **1895**, *288*, 218.

9. Guareschi, J. *Einführung in das Studium Alkaloide*; H. Kunz-Krausse: Berlin, 1897; p 620.

10. Cohn, G. *J. prakt. Chem.* **1897**, *56*, 345.

11. Dominikiewicz, M. *Arch. Chem. Farm.* **1939**, 4, 1.

12. Dickinson, R. G.; Raymond, A. L. *J. Am. Chem. Soc.* **1923**, *45*, 22.

13. Gonell, H. W.; Mark, H. *Z. physik. Chem.* **1923**, *107*, 181.

14. Wyckoff, R. W. G.; Corey, R. B. *Z. Krist.* **1934**, *89*, 462.

15. Hampson, G. C.; Stosick, A. J. *J. Am. Chem. Soc.* **1938**, *60*, 1814.

16. Walker, J. F. *Formaldehyde*, 3rd ed.; Reinhold: New York, 1964; p 514.

17. *Kirk-Othmer Encyclopedia of Chemical Technology*, 2nd ed.; Standed, A., Ed.; Interscience: New York, 1966; p 96.

18. Walker, J. F. *Formaldehyde*, 3rd ed.; Reinhold: New York, 1964; p 522.

19. Cooney, A. P.; Crampton, M. R.; Golding, P. *J. Chem. Soc., Perkin Trans. 2* **1986**, *6*, 835.

20. Duden, P.; Scharff, M. *Liebig. Ann. Chem.* **1895**, *288*, 218.

21. Baur, E; Ruetschi, W. *Helv. chim. Acta* 1941, *24*, 754.

22. Boyd, M. L.; Winkler, C. A. *Can. J. Research* 1947, *25B*, 387.

23. Richmond, H. H.; Myers, G. S.; Wright, G. F. *J. Am. chem. Soc.* **1948**, *70*, 3659.

24. Bose, S. *J. Indian chem. Soc.* 1957, *34*, 663.

25. Kawasaki, A; Ogata, Y *Mem. Fac. Eng., Nagoya Univ.* **1967**, *19*, 1.

26. Nielsen, A. T.; Moore, D. W.; Ogan, M. D.; Atkins, R. L. *J. Org. Chem.* 1979, 44, 1678.

27. Duden,P.; Scharff, M. *Liebig. Ann. Chem.* **1895**, *288*, 218.

28. Philippi, E.; Löbering, J. *Biochem. Z.* 1935, *277*, 365.

29. Slowick, E.; Kelley, R. *J. Am. Pharm. Assoc.* 1942, *31*, 15.

30. Tada, H. *J. Am. Chem. Soc.* 1960, *82*, 255.

31. Smolin, E. M.; Rapoport, L. *s-Triazines and Derivatives*; Reinhold: New York, 1964; p 552.

32. Epstein, S.; Winkler, C. *Can. J. Chem.* 1952, *30*, 734.

33. Nicolaier, A *Zbl. Med.Wiss.* 1894, *32*, 897.

34. Musher, D. M.; Griffith, D. P. *Antimicrob. Agents Chemother.* **1974**, *6*, 708.

35. Stankovic, V. *Chem. Zvesti* 1963, *17*, 274.

36. Pawelczyk, E.; Marciniec, B. *Acta Pol. Pharm.* **1978**, *35*, 643.

37. Tokes, B.; Suciu, G. *Chim. Anal. (Bucharest)* 1972, *2*, 193.

38. Sandu, M. A.; Lozan, R. M.; Ropot, V. M. *Izv. Akad. Nauk Mold. SSR, Ser. Biol. Khim. Nauk* 1988, *2*, 71.

39. Slusanschi, H. *Z. Lebensm.-Untersuch. u. -Forsch.* **1960**, 112, 390.

40. Koszegi, D.; Salgo, E. *Magyar Kem. Folyoirat* 1960, *66*, 142.

41. Turczan, J. W.; Goldwitz, B. A. *J. Ass. Off. Anal. Chem.* **1973**, *56*, 669.

42. Pentegova, I. A.; Dubrovina, G. I. *Metody Anal. Kontrolya Proizvod. Khim. Prom-sti.* **1977**, *8*, 34.

43. Maruna, A. T. *Tr. Komis. po Analit. Khim., Akad. Nauk SSSR, Inst. Geokhim. i Analit Khim.* **1963**, *13*, 320.

44. Fishman, G. I.; Pevzner, I. D. *Zavod. Lab.* **1970**, *36*, 926.

45. Stransky, Z.; Dolinek, J.; Pavlica, J. *Acta Univ. Palacki. Olomucl, Fac. Rerum Nat.* **1978**, *57*, 197.

46. Blazsek, A.; Biro, Z. *Rev. Med. (Targu-Mures)* **1968**, *14*, 70.

47. Nikolic, K.; Velasevic, K. *Arh. Farm.* **1985**, *35*, 215.

48. Strom, J. G.; Jun, H. W. *J. Pharm. Sci.* **1977**, *66*, 589.

49. Filipeva, S. A.; Strelets, L. N.; Petrenko, V. V.; Buryak, V. P. *Zh. Anal. Khim.* **1989**, 44, 131.

50. Strom, J. G.; Jun, H. W. *J. Pharm. Sci.* **1986**, *75*, 416.

51. Rizzoli, C. *Boll. soc. ital. biol. sper.* **1949**, *25*, 433.

52. Taha, A. M.; El-Rabbat, N. A.; Fattah, F. A. *Analyst* **1980**, *105*, 568.

53. Sawicki, E.; Hauser, T. R.; McPherson, S. *Anal. Chem.* **1962**, *34*, 1460.

54. Zhantalai, B. P.; Ruch'eva, N. I. *Zh. Prikl. Khim.* **1966**, *39*, 2339.

55. Koupparis, M. A.; Efstathiou, C. E.; Hadjiioannou, T. P. *Anal. Chem. Acta* **1979**, *107*, 91.

56. Levin, J. O.; Faengmark, I. *Analyst* **1988**, *113*, 511.

57. Nair, J. B.; Delaney, M. F.; Combs, K. J. *Ana. Lett.* **1983**, *16*, 711.

58. Koga, M.; Shinohara, R.; Akiyama, T. *Anal. Sci.* **1985**, *1*, 381.

59. *The United States pharmacopeia. The national formulary*; United States Pharmacopeia Convention, inc.; Rockville, Md., 1990; p 846.

60. Al-Jabari, Ghazi; Jaselskis, Bruno *Talanta* **1988**, *35*, 655.

61. Stookey, L. L. *Anal. Chem.* **1970**, *42*, 779.

62. Blau, F. *Monatsh.* **1898**, *19*, 666.

63. Al-Jabari, G.; Jaselskis, B. *Talanta* **1987**, *34*, 479.

64. *The Merck Index*, 9th ed.; Windholz, M., Ed.; Merck: Rahway, N.J., 1976; p 1110.

65. *CRC Handbook of Chemistry and Physics*, 65th ed.; Weast, R. C., Ed.; CRC Press: Boca Raton, Florida, 1984; p C-301.

66. *ibid.*, p C-326.

67. *The Merck Index*, 9th ed.; Windholz, M., Ed.; Merck: Rahway, N.J., 1976; p 545.

68. Morrison, R. T.; Boyd, R. N. *Organic Chemistry*, 4th ed.; Allyn and Bacon: Boston, 1983; p 759.

69. Morrison, R. T.; Boyd, R. N. *Organic Chemistry*, 4th ed.; Allyn and Bacon: Boston, 1983; p 754.

70. Baum, D. E.; Goodman, K. D. *Brit. Polym. J.* **1970**, *2*, 81.

71. Bouyoucos, S. A. *Anal. Chem.* **1977**, *49*, 401.

72. Jaworski, M. *Chromatographia* 1980, *13*, 101.

73. Moreno, P.; Sáchez, E.; Pons, A.; Palou, A *Anal. Chem.* **1986**, *58*, 585.

74. Marhol,M. *Ion Exchangers in Analytical Chemistry. Their Use in Inorganic Chemistry*; Svehla, G., Ed.; Wilson and Wilson's Comprehensive Analytical Chemistry vol. XIV; Elsevier: Amsterdam, 1982; p 41.

75. *ibid.*, p 171.

76. Strelow, F. W. E.; Victor, A. H.; van Zyl, C. R.; Eloff, C. *Anal. Chem.* 1971, *43*, 870.

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In September, 1980, Gary Madsen entered Bradley University in Peoria Illinois. He worked as an undergraduate teaching assistant and received the degree of Bachelor of Arts with a major in chemistry in May, 1984.

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The final copies have been examined by the director of the dissertation
and the signature which appears below verifies the fact that any necessary
changes have been incorporated and that the dissertation is now given
final approval by the Committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy.

7/12/91
———————————
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Bruno Jaselskis
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