Flammable Gas Safety Program

Analytical Methods Development: FY 1994 Progress Report

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Abstract

This report describes the status of developing analytical methods to account for the organic components in Hanford waste tanks, with particular focus on tanks assigned to the Flammable Gas Watch List. The methods that have been developed are illustrated by their application to samples obtained from Tank 241-SY-101 (Tank 101-SY). The analytical data are to serve as an example of the status of methods development.

Six samples of core segments from Tank 101-SY were analyzed for chelators, chelator fragments, and several carboxylic acids by derivatization gas chromatography/mass spectrometry (GC/MS). The major components detected were ethylenediaminetetraacetic acid (EDTA), nitrosoiminodiacetic acid (NIDA), nitrilotriacetic acid (NTA), citric acid, succinic acid, and ethylenediaminetriacetic acid (ED3A). The chelator of highest concentration was EDTA in all six samples analyzed. Liquid chromatography was used to quantitate low molecular weight acids (LMWA) including oxalic, formic, glycolic, and acetic acids, which are present in the waste as acid salts. The LMWA account for 23 to 61% of the total organic carbon (TOC) in the samples analyzed (Campbell et al. 1994). Normal paraffin hydrocarbon (NPH) was quantified in core segment waste samples from Tank 101-SY during FY 1994. Concentrations varied from 20 to 1440 ppm and 4 to 13% of the total organic carbon.

Recovery studies in the derivatization GC/MS procedure for the analysis of chelators, chelator fragments, and carboxylic acids using standard addition and deuterated internal standards were initiated. Deuterated EDTA was synthesized and the structure confirmed using high resolution mass spectrometry.

Nitroso compounds had been previously identified in tank waste samples using derivatization GC/MS. In addition, nitroso compounds were identified in tank waste under acidic conditions using liquid chromatography/mass spectrometry (LC/MS). Analysis of tank waste under strictly basic conditions using thermospray LC/MS indicated that formation of nitroso compounds is an artifact of the derivatization process.

The use of a cation exchange material was assimilated into the sample preparation procedure for tank waste analysis. Results of preliminary studies showed that the use of cation exchange to reduce the radioactivity levels did not introduce or reduce any organic carbon.

Analytical support was provided for the simulated waste studies under Task 04 to determine the extent of degradation and identify degradation products. An LC method for the analysis of chelators in simulated wastes was developed.

An LC method for analyzing of LMWA and another LC method for analyzing EDTA and N-(2-hydroxyethyl)ethylenediaminetetraacetic acid (HEDTA) were transferred to the 222-S laboratory. These methods were also transferred to the Waste Tank Organic Safety Program.

Summary

This report describes the status of developing analytical methods to account for the organic constituents in Hanford waste tanks, with particular emphasis on those tanks that have been assigned to the Flammable Gas Watch List. The methods that have been developed are illustrated by their application to samples obtained from Tank 241-SY-101 (101-SY). The marked improvement in ability to account for organic carbon in 101-SY samples, documented in this report, is a result of significant advancements in techniques for isolating organic constituents relatively free from radioactive contamination and for improving derivatization methodology.

The use of cation exchange was performed to reduce the radioactivity levels in tank waste samples prior to removal from the hot-cell facilities. This additional step to preparation was probably the single most important factor in increasing the percent TOC accounted for. This step allowed the drying and derivatization to be performed in a fume hood; parameters important for increased derivatization yields could be better controlled and monitored.

Six samples of core segments from Tank 101-SY, obtained during the window E core sampling, have been analyzed for organic constituents. Four of the samples were from the upper region, or convective layer of the tank, and two were from the lower, nonconvective layer. Normal paraffin hydrocarbon (NPH), C12 to C15, was identified in the waste samples from Tank 101-SY. Concentrations varied from 20 to 1440 ppm and were dependent on depth of the sample. The concentration of NPH was highest in core segment sample #4. Table S.1 summarizes the data for LMWA, chelators, chelator fragments, and NPH. The data presented in Table S.1 represent the most current results from Tank 101-SY waste samples. A letter report regarding the identity of the water-insoluble organic components was submitted. The results from analyses of the core segment sample from the bottom of the convective layer, segment sample 22R, have been delayed by the closure of the hot-cell facilities at the 325 building.

Nitroso compounds have been identified using GC/MS after derivatization of the waste extracts with boron trifluoride (BF_3) (Campbell et al 1994). The reaction of iminodiacetic acid (IDA) (spiked in a dried, simulated waste sample containing nitrate, nitrite, and hydroxide) with BF_3 /methanol produces nitrosoiminodiacetic acid (NIDA) as the major product. These results demonstrated that IDA, if present in the original waste, is probably a precursor for the production of NIDA during the boron trifluoride/ methanol derivatization. Under derivatization conditions, NIDA is stable. A probable precursor for two other nitroso compounds found in the derivatization product is ED3A. A procedure for direct analysis of the waste samples for nitroso compounds by LC/MS under basic conditions that do not involve derivatization was developed. Results obtained using thermospray LC/MS indicated IDA was present in the tank waste. However, based on the results using single ion monitoring, approximately 1 to 5% may exist as NIDA in the waste, based on the intensity of the base ion.

Recovery studies in the derivatization GC/MS procedure for the analysis of chelators and chelator fragments using standard addition techniques and deuterated internal standards were initiated. Results from the introduction of deuterated internal standards in the tank waste matrix showed no scrambling. Recoveries of derivatizable analytes based on results from spiked simulated wastes were estimated to be approximately 50 to 60%. Because the properties of simulated wastes are different than actual wastes,

recoveries in actual wastes are expected to be higher. During the deviltzation procedure the simulated waste dries into a cement-like material. In contrast, the actual waste dries into a paste-like material.

Analytical support was provided for Task 04 Gas Retention. Simulated wastes have been analyzed to determine the extent of component degradation and identify degradation products. Derivatization GC/MS was used very successfully for the analysis and quantitation of chelators, chelator fragments, and carboxylic acids. Because simulated wastes behave very differently from actual wastes, derivatization GC/MS was not successful. As a result, an LC method had to be used to analyze and quantitate HEDTA and EDTA in the simulated wastes. Further work is required in this area.

Transferring analytical methods is an important aspect of this project. An LC method for LMWA and for analyzing HEDTA and EDTA were transferred to personnel in the laboratory at 222-S. Techniques involving derivatization GC/MS and LC/MS for analyzing chelators, chelator fragments, and LMWA were transferred to personnel in the Analytical Chemistry Laboratory (ACL) and 222-S under the Organics Tanks Safety Program. In addition, a procedure for the use of cation exchange to reduce the levels of radioactivity has been transferred to both the ACL and 222-S laboratory.

Table S.1. Total Organic Carbon (TOC) Accounted for by Chelators, Chelator Fragments, Low Molecular Weight Acids, and Normal Paraffin Hydrocarbon (NPH) in Tank 101-SY Window E Core Segment Samples (mg C/g sample)

Sample ^(a)	Segment #	Chelators ^(b)	Low Molecular Weight Acids ^(c)	% TOC Accounted For
R4258/C	4	4.7 (44%) ^(d)	3.3 (30%)	74(87) ^(e)
R4259/C	6	3.3 (33%)	4.5 (44%)	77
R4260/C	9	4.8 (46%)	4.4 (41%)	87(91)
R4261/C	11	6.4 (52%)	2.5 (23%)	75(81)
R4262/NC	16	2.2 (20%)	5.8 (53%)	73
R4263/NC	17	3.6 (32%)	6.8 (61%)	93

(a) Sample R4264, a nonconvective layer sample, was lost during preparation; the analysis is in progress.

(b) Includes chelators, chelator fragments, nitrosated chelator material, and several carboxylic acids.

(c) Low molecular weight acids include acetic, glycolic, oxalic, and formic.

(d) Parentheses give percent of TOC accounted for by analyte category.

(e) Percent in parentheses includes NPH.

C Convective layer

NC Nonconvective layer

Glossary

ACL	Analytical Chemistry Laboratory
CA	citric acid
ED3A	ethylenediaminetriacetic acid
EDTA	ethylenediaminetetraacetic acid
FTIR	Fourier transform infrared
GC/MS	gas chromatography/mass spectrometry
HEDTA	N-(2-hydroxyethyl)ethylenediaminetriacetic acid
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
IDA	iminodiacetic acid
LC	liquid chromatography
LC/MS	liquid chromatography/mass spectrometry
LMWA	low molecular weight acids
NED3A	N-nitrosoethylenediaminetriacetic acid
NIDA	nitrosoiminodiacetic acid
NTA	nitrilotriacetic acid
NPH	normal paraffin hydrocarbon
PEG	polyethylene glycol
PFK	perfluorokerosene
SA	succinic acid
SIM	selected ion monitoring
SWM	simulated waste material
Tank	241-SY-101 Tank 101-SY
TOC	total organic carbon
UV	ultraviolet
WHC	Westinghouse Hanford Company

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1.0 Introduction

The objectives of this task are to develop and document extraction and analysis methods for organics in waste tanks, and to extend these methods to the analysis of actual core samples to support proposed mechanisms for gas production. This report documents progress at Pacific Northwest Laboratory^(a) during FY 1994 on methods development; on the analysis of Tank 241-SY-101 (101-SY) waste; on total organic carbon (TOC) accounted for; and on transfer of documented, developed analytical methods to personnel in the Analytical Chemistry Laboratory (ACL) and at the 222-S laboratory.

As reported in the FY 1993 Progress Report (Campbell et al. 1994) delivered to Westinghouse Hanford Company (WHC) on February 1, 1994, only 10 to 20% of the water-soluble organic carbon could be accounted for by derivatization gas chromatography/mass spectrometry (GC/MS) and liquid chromatography (LC) at the beginning of FY 1993. All of the sample preparation, including derivatization for chelator analysis, was performed in the hot-cell facilities. At the beginning of FY 1993, a set of eight core segment composite samples of Tank 101-SY from the window E sampling period were being analyzed for chelators, chelator fragments, and carboxylic acids. Four samples were from the convective layer, and four were from the nonconvective layer. Throughout FY 1993, the composite samples were used to refine the analytical methods. The use of simulated wastes was not as beneficial as real wastes for methods development; many of the properties of real waste are not duplicated in simulated wastes. Subsamples were used to determine the best sample size-to-reagent ratio in derivatization GC/MS, and to evaluate procedures for removing a major portion of the radioactivity (without loss of organic carbon) to enable the derivatization to be performed in the fume hood. Subsamples also were used to perfect LC methods for low molecular weight acids (LMWA). After the analytical procedures were in a final stage of development, additional subsamples of the composite samples were requested from the hot-cell facilities to perform analyses with the refined methods. Unfortunately, the segment composite samples were found to have been inadvertently discarded during a hot-cell cleanup. Additional samples from window E core sampling were requested from WHC archived samples. These core segments were analyzed using the improved analytical methods, and the results were reported in the FY 1993 Progress Report (Campbell et al. 1994).

Although tremendous progress was made during FY 1993 in terms of TOC accountability, there were still areas of concern. The sample preparation procedure could be streamlined and steps incorporated to remove the samples from the hot cell to the fume hood for derivatization. Questions regarding the presence or absence of nitroso compounds in the waste samples still remained unanswered. In addition, the concentration of water-insoluble organics had not been determined, and recovery studies during the derivatization GC/MS procedure were not complete. As a result, efforts for FY 1994 were focused in these areas.

Efforts for the remainder of FY 1994 were focused on developing a new sample-preparation procedure to include the use of cation exchange for reducing the levels of radioactivity. Removing most of the radioactivity with cation exchange without loss of organic carbon material permits

(a) Pacific Northwest Laboratory is operated for the U.S. Department of Energy by Battelle Memorial Institute under contract DE-AC06-76RLO 1830.

derivatization in a fume hood. Using a fume hood for sample preparation makes it much easier to control the temperature of the reaction and assure the dryness of the sample; both of these parameters are critical for the derivatization procedures. The fume hood also permits use of a much smaller sample size for derivatization. This improvement in the method allows the ratio of reagent to sample to be high enough to maximize the recoveries and minimize the matrix effect. The results not only gave much higher concentrations of derivatized components in the samples, but produced analyses that revealed sharp differences in component concentrations as a function of depth in the waste tank. Studies were initiated to determine the radionuclide species removed during the process. In addition, studies were started to compare the sodium and hydrogen forms of the cation exchange material.

Water-soluble organic components including chelators, chelator fragments, and carboxylic acids accounted for approximately 73 to 93% of the TOC (Campbell et al. 1994a). To improve the amount of TOC accounted for, studies were initiated to develop methods to identify water-insoluble organics such as normal paraffin hydrocarbon (NPH) that may be present in the waste.

Nitroso compounds were detected in the total ion chromatogram obtained from analyzing waste samples using derivatization GC/MS (Campbell et al.). This was the case in which the derivatizing reagent was either boron trifluoride (BF_3 , Lewis acid)/methanol or dimethyl sulfate under basic conditions. In addition, the results of LC analysis with an acidic mobile phase indicated the presence of nitroso compounds. As a result, efforts were undertaken to develop a LC/MS technique utilizing a basic mobile phase for analyzing waste samples to determine whether nitroso compounds existed in the waste or were artifacts of the derivatization procedures.

Studies of simulated wastes under Task 04 Gas Retention were undertaken to understand the mechanism involved in gas production and gas retention. Analytical support was provided to determine the amount of degradation and identify the degradation products. In addition, in view of the fact that simulated wastes are different from actual waste, methods development was required for some of the analytes.

Recoveries for the derivatized analytes were estimated to be 50 to 60% based on simulated waste studies. However, simulated wastes do not appear to behave similar to real wastes under derivatization conditions. As a result, studies have been initiated with both standard addition and the use of deuterated internal standards.

This report describes the progress made in FY 1994 in developing more effective procedures for separating and preparing samples, for isolating and derivatizing components, and for identifying components. In addition, several methods developed during this work either have been transferred or are in the process of being transferred to other laboratories for routine applications.

2.0 Summary of Results

At the beginning of FY 1993, a set of eight core segment composite samples of Tank 101-SY from the window E sampling period were being analyzed for chelators, chelator fragments, and carboxylic acids. Approximately 10-20% of the water-soluble organics were identified. Four samples were from the convective layer, and four were from the nonconvective layer. Throughout FY 1993, the composite samples were used to refine the analytical methods. The use of simulated wastes was not as beneficial as real wastes for methods development; many of the properties of real waste are not duplicated in simulated wastes. Subsamples were used to determine the best sample size-to-reagent ratio in derivatization GC/MS, and to evaluate procedures for removing a major portion of the radioactivity (without loss of organic carbon) to enable the derivatization to be performed in the fume hood. Subsamples also were used to perfect LC methods for LMWA. After the analytical procedures were in a final stage of development, additional subsamples of the composite samples were requested from the hot-cell facilities to perform analyses with the refined methods. Unfortunately, the segment composite samples were found to have been inadvertently discarded during a hot-cell cleanup. Additional samples from window E core sampling were requested from WHC archived samples. These core segments have been analyzed using the improved analytical methods, and the results were reported (Campbell et al. 1994.) At the end of FY 1993, approximately 73-93% of the TOC had been identified.

The new sample preparation procedure developed has resulted in a better accounting for the organic carbon. A 5-g aliquot of tank waste is stirred overnight with 10 mL of water. The filtered aqueous solution is then passed through a bed of cation exchange resin and rinsed with 10 mL of water. These procedures are performed in the hot cell, and the resulting solution is sufficiently low in activity to allow removal to a fume hood. An aliquot is concentrated to dryness under a stream of nitrogen. The dry material is derivatized with boron trifluoride BF_3 /methanol, and the derivatized material, in chloroform, contains less than 1% of the original radioactivity. This material then can be analyzed by GC/MS for derivatized components.

Table 2.1 shows the results from studies of the cation exchange material with Tank 101-SY samples. Using a fume hood for sample preparation facilitates controlling the temperature of the reaction and the dryness of the sample; both of these parameters are critical for the derivatization procedures. The fume hood also permits use of a much smaller sample size for derivatization. This improvement in the method allows the ratio of reagent to sample to be high enough to maximize the recoveries and minimize the matrix effect. This method yielded much higher concentrations of derivatized components in the samples, and produced analytical results that revealed component concentrations which varied sharply as a function of depth in the waste tank. Further studies are underway to determine the radionuclides removed from the leachate by elution through the cation exchange material.

Normal paraffin hydrocarbon was identified in chloroform extracts of Tank 101-SY waste samples. The concentrations varied from 20-1440 ppm and accounted for 4 to 13% of the TOC. The concentration of NPH varied as a function of depth of the sample, and was highest in the first, topmost, sample of the convective layer (Table 2.2).

The reproducibility of the results from derivatization GC/MS was estimated to be approximately 10% from duplicate manual injections of the same sample. Subsample variation was estimated to be

Table 2.1. Total Organic Carbon (TOC) Measurements With Cation Exchange

Sample	TOC ($\mu\text{gt/mL}$)	
	Trial #1	Trial #2
Waste Sample	2100	1995
After sepn thru Na+ form	2200	2170
After H+ form	470	490
Blank H+ form	9.5	8
Blank Na+ form	<0.5	<0.5

Table 2.2. Concentration (ppm) of Normal Paraffin Hydrocarbon (NPH) in Tank 101-SY Core Segment Samples

Sample	Segment #	NPH
R4258	4	1440
R4259 ^(a)	6	
R4260	9	410
R4261	11	670
R4262	16	20
R4263 ^(a)	17	

(a) Insufficient sample remaining to determine concentration of NPH.

approximately 14 to 20%, depending on the component. Recoveries of derivatized analytes were based on simulated wastes, and estimated to be 50 to 60%. However, the simulated wastes may not be similar to the actual wastes; it is estimated the recoveries would be higher with the actual waste samples, but the analytical results were not corrected for recoveries.

The reproducibility for the LC measurements of oxalic acid was estimated to be 5%. The determination of the other small acids was estimated to be approximately 10%, due to baseline drift. Recovery of oxalic acid from spikes in a simulated waste was 91%. The variability from sample to sample was a combination of sample heterogeneity and analytical irreproducibility. Recovery studies with actual waste samples from Tank 101-SY using deuterated EDTA and succinic acid (SA) as internal standards in the derivatization GC/MS procedure were initiated. In addition, recovery studies using tank waste in the LC method for the LMWA also were started.

Nitroso compounds were identified by analyzing waste samples using the following methods: derivatization with BF_3 and subsequent GC/MS analysis, LC/MS with an acidic mobile phase, and LC with an acidic mobile phase. Acid conditions favor the formation of N-nitroso compounds from amine-type groups in the presence of nitrite. The reaction of iminodiacetic acid (IDA) (spiked in a dried, simulated waste sample containing nitrate, nitrite, and hydroxide) with BF_3 /methanol produces nitrosoiminodiacetic acid (NIDA) as the major product. These results demonstrate that IDA can be a precursor for the production of NIDA. Other experiments indicate that NIDA is stable under derivatization conditions. In the same vein, a probable precursor for another nitroso compound, n-nitrosoethylenediamine triacetic acid (NED3A), is ethylenediaminetriacetic acid (ED3A). Boron trifluoride is a Lewis acid; even with the highly basic waste sample, the derivatization conditions promote the formation of the nitroso compounds. Components such as IDA and ED3A, both secondary amines, may react with the nitrate and nitrite under derivatization conditions to form these compounds. A direct-analysis procedure, using thermospray LC/MS with a mobile phase of pH 10.5, was developed. Results of analysis of IDA through the column showed a base peak at m/z 134 $(\text{M}+\text{H})^+$ with minor ions at m/z 151 $(\text{M}+\text{H}_2\text{O})^+$ and 152 $(\text{M}+\text{H}+\text{H}_2\text{O})^+$. A waste sample from Tank 101-SY was analyzed using single ion monitoring at m/z 134, 151, 152 for IDA and m/z 180 for NIDA. The preliminary results indicated the presence of IDA. A minor component at m/z 180, possibly indicative of NIDA, was present at approximately 5% based on the intensity of the base ion for NIDA.

During FY 1994, several analytical techniques developed by our group have been successfully transferred to personnel in the ACL and the 222-S laboratory. Liquid chromatographic methods for EDTA, HEDTA, and LMWA have been used by personnel in the ACL to analyze waste samples before grout treatment. A letter of transfer was signed on May 31, 1994 by both Jim Campbell and Kim Wehner of the 222-S laboratory for the LC methods. A letter of transfer was signed by both Jim Campbell, Kim Wehner (June 7, 1994), and Robert Stromatt of the ACL on May 24, 1994, for the LC/MS method for the analysis of LMWA, and a derivatization GC/MS method for the analysis of chelators, chelator fragments, and carboxylic acids.

3.0 Discussion of Methods

3.1 Cation Exchange for Reduction of Radioactivity Levels

All of the sample preparation, including derivatization for chelator analysis, was performed in the hot-cell facilities in FY 1993. Typically, a 5-g waste sample was stirred with 10 mL of doubly distilled water overnight. The solution was filtered through a 0.45 μm filter to remove any solid material. The aqueous solution was then dried using nitrogen-blow-down techniques, and the remaining residue was then heated with 2 mL of BF_3 /methanol for 1 h at 100°C to produce the methyl esters of chelators, chelator fragments, and carboxylic acids. After the solution cooled to room temperature, 1 mL of chloroform was added to the solution and the entire mixture was added to 3-5 mL of KH_2PO_4 adjusted to pH 7.0 with NaOH. The organic layer containing the methyl esters was separated and subsequently analyzed using GC/MS. At that point, only 10-20% of the TOC could be accounted for.

The dryness of the sample is critical to obtaining reproducible results from derivatization using BF_3 /methanol, as is monitoring the heating of the sample during derivatization. These parameters, essential for good recoveries from the derivatization procedure with tank waste samples, are not easily controlled with remote manipulators in the hot-cell facilities. The 101-SY matrix is laden with hygroscopic salts, which tend to crust over and encapsulate water in the drying process. Manipulation of the sample outside of the hot cell in a fume hood would allow sample monitoring and effective removal of residual water. However, in order to remove the samples from the hot cell, the radioactivity levels would have to be dramatically reduced. As a result, it was extremely important to develop a method for reducing the radioactivity levels without removing or introducing any organic carbon. In view of the fact that many of the suspected radioactive contaminants were cationic in nature, the use of cation exchange for the reduction of radioactivity was suggested.

The possible use of the cation exchange material for radioactivity removal was substantiated during LC analysis of a tank waste sample. A 1-mL aliquot of the water extract from a Tank 101-SY composite sample was diluted and analyzed using LC in a radioactive zone. After the analysis; a counter was used to check for radioactive contamination. The LC column was scanned, and most of the radioactivity was located within the guard column consisting of cation exchange packing material. As a result, a step involving cation exchange to remove the radioactivity was added to the separation procedure. The use of cation exchange resin to remove the radioactivity allowed the samples to be removed from the hot cell to a fume hood; this move was probably the single most important step in controlling the dryness of the sample prior to derivatization and heating during the derivatization procedure, and subsequently increased the TOC accounted for.

Initial tests were then performed to determine any losses of HEDTA and EDTA going through the Na^+ form of the cation exchange column. Preliminary results indicate the recoveries were approximately 90 and 95%, respectively; further studies are underway to substantiate these numbers. However, if one uses the hydrogen form of the resin, losses of EDTA do occur, presumably due to insolubility of the acid form of EDTA.

The current sample-preparation method for analysis of core samples of Tank 101-SY for organics is as follows. The TOC of an aliquot is determined on the starting material. A 5-g sample is stirred with 10 mL of doubly distilled water overnight. The solution is then filtered through a 0.45 μm filter,

and the TOC is measured on the remaining solid material. The filtered solution is then passed through a preconditioned Dowex 50 AGX cation exchange resin (Na^+ form, 10 g) and then rinsed with approximately 10 mL of doubly distilled water to ensure that no organic carbon is left on the column. The radioactivity level of the resulting solution is approximately 200 times less than the original sample, which allows removal of the aqueous sample from the hot cell to perform the derivatizations in the fume hood. The TOC was not changed by passing through the cation exchange column. The filtrate was analyzed for radionuclides after passing through the cation exchange column, and the results indicated it was primarily cesium. However, with the techniques used (Gamma Energy Analysis), Strontium would not have been detected. Further studies are underway to determine the identity of radioactive analytes removed. Transferring the derivatization procedure to the fume hood has improved the reproducibility and allows control of the parameters crucial to obtaining reproducible results, namely, temperature and dryness of the sample. Table 2.1 shows the results of TOC accountability from Tank 101-SY core segment samples with incorporation of the cation exchange technique for radioactive removal.

To determine if any TOC losses occur through the cation exchange column, TOC values were determined before and after the cation exchange procedure with tank waste samples. In addition, blanks were analyzed to determine if any TOC was introduced from the column. These results (Table 2.1) clearly indicate that the cation exchange column (Na^+ form) does not remove or introduce any TOC. This finding is extremely important; the sample was not compromised by eluting through the cation exchange resin. Additional studies are underway to support these results.

The incorporation of the cation exchange step to remove radioactivity was certainly successful in the analysis of samples from Tank 101-SY. However, the assumption cannot be made that it will work for every tank waste sample. For example, analytical results from T-111 samples subjected to cation exchange cleanup, indicate the possible loss of carbon. It is questionable whether it is in fact organic carbon that is lost. The pH of the T-111 sample was 9 and 13 for Tank 101-SY. The pH of the sample may be an important variable in using the exchange column for reduction of radioactivity. Studies are underway to examine this phenomenon. It is recommended that the TOC measurements be made prior to and after the cation exchange column to ensure that no TOC has been removed or added to the sample. In addition, it is unreasonable to expect that 73 to 93% of the TOC can be accounted for, as in the case of samples from Tank 101-SY, in every waste sample.

The method of reducing the radioactivity level in tank waste samples has been transferred to the ACL and is in the process of being transferred to the 222-S laboratory through Kim Wehner.

Other methods to separate the radioactive components from the organic constituents are also being explored. Recently, preliminary work has been done to determine the feasibility of extracting chelators from a complex matrix by supercritical fluid extraction (SFE). The chelators, however, are highly polar and, as such, are not amenable to SFE extraction even with a polar modifier like methanol. Several groups that have researched the extraction of acids from a complex media by SFE have successfully used *in situ* SFE methylation or silylation to reduce the polarity of the acids (Hawthorne 1992). This method involves adding the appropriate derivatizing reagent to the matrix and then using supercritical fluid modified with the commonly used derivatizing solvent. This approach has been applied to EDTA and IDA in sand, a test matrix, using BF_3 /methanol and was found to be successful (Campbell et al. 1994). Work is now being performed to refine this approach. If successful with tank wastes, the stability and lifetime of the derivatives may be increased. In addition, an approach to add BF_3 /methanol as a modifier will be investigated.

3.2 Determination of NPH

Initial efforts focused on the analysis of water-soluble organics. To improve the accountability of TOC, development of analytical techniques for water-insoluble organics were pursued. The procedure for extracting water-insoluble organic carbon was conducted in the hot cell. The sludge-like waste sample, at pH 13, was extracted with three equal volumes of chloroform, concentrated, and analyzed using GC/MS. The results are shown in Table 2.2; NPH is the primary constituent of the water-insoluble carbon. The concentration of NPH is highest in sample R4258; there is a strong correlation of NPH concentration with depth of the core sample, suggesting a density segregation of NPH. Table S.1 shows the TOC accounted for by chelators, chelator fragments, NPH, and LMWA. Figure 3.1 is a total ion chromatogram of the chloroform extract of the base-neutral fraction. The peaks indicated are NPH (C12 to C14). In view of the fact that 73 to 93 % of the TOC can be accounted for in samples from Tank 101-SY, very little, if any, water-soluble or water-insoluble carbon remains unidentified.

Figure 3.1. Total Ion Chromatogram of the Methylene Chloride Extract of Base-Neutral Fraction

3.3 Presence of Nitroso Compounds in Waste

Nitroso compounds such as NIDA have been previously identified in the waste samples using derivatization GC/MS, LC/MS with an acidic mobile phase, and LC under acidic conditions. The mass spectrum of the derivatized, synthesized NIDA standard was identical to that observed in the GC/MS total ion chromatogram, and the thermospray mass spectrum of the NIDA standard was identical to that of the major component in the total ion chromatogram of a waste sample from Tank 101-SY. However, in both of these instances, the separation or derivatization involved acidic conditions or reaction with a Lewis acid. Studies have shown that nitroso compounds will form when derivatized under acidic conditions (Campbell et al. 1994). In addition, the retention time of NIDA in the LC chromatogram (basic conditions) was identical to one of the unidentified peaks. This result is illustrated in Figure 3.2.

Figure 3.2. LC Chromatogram of Composite Waste Sample from Tank 101-SY

The ultraviolet (UV) spectrum of these two components is identical; the maximum absorbance was at approximately 240 nm.

Another promising procedure investigated involved derivatization under basic conditions with a methylating reagent, dimethyl sulfate. Under those conditions, results of the analysis of the derivatized simulated waste spiked with IDA indicated the presence of NIDA. Other studies indicated that NIDA was stable under derivatization.

A procedure was developed to utilize thermospray LC/MS with NH_4CO_3 as the mobile phase ($\text{pH} = 10.5$). Preliminary results are very encouraging. The thermospray mass spectrum of NIDA obtained under strictly basic conditions is shown in Figure 3.3. A similar experiment using thermospray LC/MS with a basic mobile phase was attempted to analyze a waste sample. The column appeared to have lost its retention capacity and everything eluted in the dead volume; no separation was obtained. The basic conditions may be too extreme for repeated analysis using a C18 HPLC column. The problem was corrected with a new column. The improved chromatography is illustrated in Figure 3.4(a-c). Figures 3.4(a) and 3.4(b) are chromatograms of NIDA under the separation conditions. Figures 3.4(c) and (d) are chromatograms of simulated waste spiked with NIDA and detected at 210 and 240 nm. However, the results of a selected ion monitoring of m/z 180 ($\text{M} + \text{H}_2\text{O}$) and 136 were

39310055.11

Figure 3.3. Thermospray Mass Spectrum of NIDA Obtained Under Basic Mobile Phase Conditions

(a) Standard of NIDA
Detected at 210nm

(b) Standard of NIDA
Detected at 240nm

(c) Simulated Waste Spiked
with NIDA at 210nm

(d) Simulated Waste Spiked
with NIDA at 240nm

Figure 3.4. LC Chromatograms for NIDA Obtained Under Basic Conditions

very interesting. The ratio of m/z 180 and 136 in simulated samples with NIDA spikes are practically

identical to the ratio found in the actual waste. A simulated waste sample without NIDA shows a very different ratio of m/z 180 and 136. The spectra are shown in Figure 3.5(a-c). The results were inconclusive.

Another attempt to analyze tank waste using thermospray LC/MS with a mobile phase of pH 10.5 was performed. Results of analysis of IDA through the column showed a base peak at m/z 134 $(M+H)^+$ with minor ions at m/z 151 $(M+H_2O)^+$ and 152 $(M+H+H_2O)^+$. This finding is illustrated in Figure 3.6. A waste sample from Tank 101-SY was analyzed using single ion monitoring at m/z 134, 151, 152 for IDA and m/z 180 for NIDA. The chromatogram is shown in Figure 3.7. The mass spectrum of the peak at retention time 5.0 is shown in Figure 3.8. The preliminary results indicated the presence of IDA; a minor component of m/z 180, possibly indicative of NIDA, was present at approximately 5% intensity.

The thermospray LC/MS results clearly indicate that the nitroso compounds identified in the derivatization GC/MS total ion chromatograms are primarily artifacts of the derivatization process. A possible mechanism involving the reaction of dimethyl sulfate and IDA under basic conditions is shown in Figure 3.9. This mechanism involves a tetracentric transition state cyclic intermediate similar to that suggested by Challis and Shuker (1980) and Casado et al. (1986). The first step involves the reaction of dimethyl sulfate with nitrite. A similar type of mechanism might be expected for the reaction of BF_3 /methanol and IDA to form the nitroso compound.

The presence of small quantities of nitroso compounds supports the mechanisms proposed by personnel at Georgia Institute of Technology (Barefield et al. 1994).

(a) In Simulated Waste Spiked with NIDA

Figure 3.5a. Selected Ion Monitoring of m/z 180 and 136

(b) In Simulated Waste without NIDA

Figure 3.5b. In Simulated Waste Without NIDA

(c) In Waste Tank Sample

Figure 3.5c. In Waste Tank Sample

Figure 3.6. Thermospray Mass Spectrum of IDA Obtained Under Basic Mobile Phase Conditions

Figure 3.7. Total Ion Thermospray Chromatogram of Waste from Tank 101-SY

Figure 3.8. Selected Ion Monitoring of Peak at 5.0 Minutes for m/z 134, 151, 152, and 180

3.4 Tracers for Determining Recoveries

3.4.1 Yield Tracers in Chelator Analysis

Of the available reagents, only BF_3 /methanol has been shown to give reliable derivatization with chelator materials contained in tank wastes. Even powerful methylating reagents such as dimethyl anion - CH_3I and diazomethane do not provide usable yields of the corresponding methyl esters of EDTA and nitrilotriacetic acid (NTA).

Results produced have quantified the relative amounts of chelators present in Hanford wastes *versus* the amount of carbon apparent in TOC analysis; however, quantification to determine the absolute conversion of these materials to methyl esters using Hanford tank matrix is being undertaken only now.

Initially, the direction taken to probe the overall yield from the BF_3 /methanol esterification was to add a known quantity of aliphatic monocarboxylic acid to the matrix and quantify the result obtained from the experiment for the aliphatic acid *versus* that obtained from a separate derivatization using reagent acids as the respective sodium salts. In order for this probe to be useful, the aliphatic acid chosen must not interfere with the chromatography of the known chelators and chelator fragments found in the matrix. The best candidate found for this application was myristic acid ($\text{C}_{13}\text{H}_{27}\text{COOH}$), the methyl ester of which eluted beyond that of NTA and prior to the methyl esters of ED3A and EDTA. A representative chromatogram is shown in Figure 3.10, with peak 5 being trimethyl NTA, peak 6 methyl myristate, and peak 7 tetramethyl EDTA.

Figure 3.9. Proposed Mechanism for Formation of Nitroso Compounds under Basic Conditions (Challis and Shuker 1980)

Our results indicated that 80 to 95% of the myristic acid added to Tank 101-SY matrix in an amount approximately that of the chelators present in the matrix was converted to the methyl ester using BF_3 /methanol reagent. This number may not accurately reflect the yield for chelators and chelator fragments in the matrix, because these materials contain at least two or as many as four carboxylic acid sites per molecule, and may not be methylated as readily as the singly carboxylated aliphatic acids. Further, steric influences in the case of NTA and EDTA may not allow these

Figure 3.9. (contd)

materials to methylate as readily as a straight chain material. The limited solubility of aliphatic acid sodium salts in water may also contribute to loss of the acids in stepwise manipulations involved with the analysis of the matrix. Thus, this material may not possess overall utility in probing conversion yields of chelators in the Hanford tank wastes.

3.4.2 Deuterated Chelator Probes

Deuterated chelators and/or chelator fragments have many distinct advantages over the introduction of other organic acid materials that might be used as yield probes. The methylation chemistry should be identical with that of the ordinary chelator found within the matrix, thus giving a direct measure of the efficiency of the methylation process for that particular chelator. If the labeled material is not subject to deuterium-exchange processes with the highly basic matrix, then the deuterated material can be used as a tracer; added at any point in the process, the probe acts as a monitor for loss of that material during any set of steps. The major criteria for use of these materials involves 1) deuterium stability, 2) well-defined amount/response ratios in quantitation, and 3) MS resolution for analysis of particular fragment ions. Unfortunately, the deuterated compounds either co-elute or overlap the parent unlabeled material to some degree.

D₄-succinic acid was purchased for initial trials. This material was found to withstand deuterium exchange if added directly to the highly basic Tank 101-SY matrix and derivatized immediately. The

Figure 3.10. Total Ion Chromatogram for Yield Tracer Study

BF_3 /methanol reagent is a strong Lewis acid, and the reaction produces hydrogen fluoride as a by product of reaction. Deuterium exchange on carbons *alpha* to carbonyl functions is expected to take place upon prolonged contact with base; thus d_4 -succinic acid is likely to suffer from some exchange during contact with the matrix. No hydrogen/deuterium exchange was observed in our initial experiments, which was somewhat surprising.

A better probe would not possess exchangeable deuteriums, and an attempt was made to obtain labeled EDTA with the deuteriums on the N-N' bridge carbons. This material was not readily available commercially, and quotes from a manufacturer of labeled materials indicated that purchase would be prohibitively expensive (\$3000/g, minimum 5g order). Synthesis of this material was accomplished by the method of V_{ny}_los (1955), using d_4 -ethylenediamine purchased from Isotech (Miamisburg, Ohio) and sodium chloroacetate. The structure is shown below.

The resulting simple synthesis produces gram quantities of the labeled EDTA in apparent high purity. ^1H nuclear magnetic resonance analysis of the bulk synthetic product displayed only the acetate H signal, along with a signal corresponding to partially deuterated water from exchange with the D_2O solvent used in the assay. Methylation using BF_3 /methanol and subsequent GC/MS analysis indicated

that the material was essentially free from chelator-type byproducts; a trace of the corresponding ED3A methyl ester was detected. High resolution MS performed with the JEOL High Resolution tandem mass spectrometer confirmed the expected deuterium content of this material. The mass spectrum is shown in Figure 3.11 and data from high resolution data is shown in Figure 3.12. The results of accurate mass measurements show the structure of m/z 352 is $C_{14}H_{20}D_4O_8N_2$. The structures of the major fragment ions m/z 293 and 176 are shown and help identify deuterated EDTA. The material produced appeared to be essentially pure d_4 -EDTA. Initial experiments with this material in simulated waste matrices indicated that the deuteriums were not exchanged even when the probe was exposed for long periods to basic media. Current plans are to incorporate this material into the standard addition recovery study outlined below. The d_4 -EDTA tetramethyl ester was found to practically co-elute with the unlabeled EDTA ester. Quantification of the two materials should coincide with comparison of corresponding ions for the two compounds at two points in the GC/MS trace of the combined eluted peak for the two materials, e.g., comparison of the total amount of ions 174 and 176 at two points in the GC peak. It appears that this will be a very effective probe for total EDTA derivatization in waste matrices.

The other major chelator components in Tank 101-SY matrix, namely, citric acid and NTA, suffer from the potentially exchangeable deuteriums *alpha* to the carboxyls on the molecule. The suitability of d_4 -succinic acid as a probe may dictate whether the corresponding deuterated forms of these materials might be pursued as yield probes.

Figure 3.11. High Resolution Mass Spectrum of Deuterated EDTA

Observed m/z	Int*	Err [ppm / mmu]	U.S.	Composition
352.1793	40.2	+22.6 / +8.0	8.0	C 18 1H 20 D 3 O 6 N
		+2.7 / +0.9	4.0	C 14 1H 20 D 4 O 8 N 2
		+7.1 / +2.5	4.5	C 14 1H 18 D 5 O 8 N 2
		+11.5 / +4.0	5.0	C 14 1H 16 D 6 O 8 N 2
		-9.3 / -3.3	8.0	C 17 1H 20 D 3 O 5 N 3
		-4.9 / -1.7	8.5	C 17 1H 18 D 4 O 5 N 3
		-0.5 / -0.2	9.0	C 17 1H 16 D 5 O 5 N 3
		+3.9 / +1.4	9.5	C 17 1H 14 D 6 O 5 N 3
		-17.2 / -6.1	0.0	C 10 1H 20 D 5 O 10 N 3
		-12.8 / -4.5	0.5	C 10 1H 18 D 6 O 10 N 3
		+22.0 / +7.7	8.0	C 16 1H 20 D 2 O 5 N 4
		-20.4 / -7.2	5.0	C 13 1H 16 D 6 O 7 N 4
		+14.1 / +5.0	0.0	C 9 1H 20 D 4 O 10 N 4
		+18.5 / +6.5	0.5	C 9 1H 18 D 5 O 10 N 4
293.1622	47.2	-23.6 / -6.9	7.5	C 17 1H 17 D 4 O 4
		-18.3 / -5.4	8.0	C 17 1H 15 D 5 O 4
		-13.0 / -3.8	8.5	C 17 1H 13 D 6 O 4
		+23.2 / +6.8	3.0	C 13 1H 19 D 3 O 7
		+8.7 / +2.6	7.0	C 16 1H 19 D 2 O 4 N
		+14.0 / +4.1	7.5	C 16 1H 17 D 3 O 4 N
		+19.3 / +5.7	8.0	C 16 1H 15 D 4 O 4 N
		+24.6 / +7.2	8.5	C 16 1H 13 D 5 O 4 N
		+9.8 / +2.9	0.0	C 9 1H 15 D 6 O 9 N
		-15.2 / -4.4	3.0	C 12 1H 19 D 3 O 6 N 2
		-9.9 / -2.9	3.5	C 12 1H 17 D 4 O 6 N 2
		-4.6 / -1.3	4.0	C 12 1H 15 D 5 O 6 N 2
		+0.7 / +0.2	4.5	C 12 1H 13 D 6 O 6 N 2
		-24.3 / -7.1	7.5	C 15 1H 17 D 3 O 3 N 3
		-19.0 / -5.6	8.0	C 15 1H 15 D 4 O 3 N 3
		-13.7 / -4.0	8.5	C 15 1H 13 D 5 O 3 N 3
		-8.5 / -2.5	9.0	C 15 1H 11 D 6 O 3 N 3
		+22.4 / +6.6	3.0	C 11 1H 19 D 2 O 6 N 3
		+8.0 / +2.4	7.0	C 14 1H 19 D 0 3 N 4
		+13.3 / +3.9	7.5	C 14 1H 17 D 2 O 3 N 4
		+18.6 / +5.4	8.0	C 14 1H 15 D 3 O 3 N 4
		+23.9 / +7.0	8.5	C 14 1H 13 D 4 O 3 N 4
		+9.1 / +2.7	0.0	C 7 1H 15 D 5 O 8 N 4
		+14.4 / +4.2	0.5	C 7 1H 13 D 6 O 8 N 4
192.1173	21.5	+11.6 / +2.2	5.0	C 12 1H 16 O 2
		+19.7 / +3.8	5.5	C 12 1H 14 D O 2
		+27.7 / +5.3	6.0	C 12 1H 12 D 2 O 2
		+35.8 / +6.9	6.5	C 12 1H 10 D 3 O 2
		-32.9 / -6.3	0.5	C 8 1H 18 O 4 N
		-24.8 / -4.8	1.0	C 8 1H 16 D O 4 N
		-16.8 / -3.2	1.5	C 8 1H 14 D 2 O 4 N
		-8.7 / -1.7	2.0	C 8 1H 12 D 3 O 4 N
		-0.7 / -0.1	2.5	C 8 1H 10 D 4 O 4 N
		+7.4 / +1.4	3.0	C 8 1H 8 D 5 O 4 N
		+15.5 / +3.0	3.5	C 8 1H 6 D 6 O 4 N
		-38.8 / -7.5	5.5	C 11 1H 14 D O N 2
		-30.7 / -5.9	6.0	C 11 1H 12 D 2 O N 2
		-22.7 / -4.4	6.5	C 11 1H 10 D 3 O N 2
		-14.6 / -2.8	7.0	C 11 1H 8 D 4 O N 2
		-6.5 / -1.3	7.5	C 11 1H 6 D 5 O N 2

Figure 3.12. Data from High Resolution (Accurate Mass Measurements) for Fragment Ions

		+1.5 / +0.3	8.0 C 11 1H 4 D 6 O N 2
		+32.6 / +6.3	1.0 C 7 1H 16 O 4 N 2
		+40.6 / +7.8	1.5 C 7 1H 14 D O 4 N 2
		+18.6 / +3.6	5.5 C 10 1H 14 O N 3
		+26.7 / +5.1	6.0 C 10 1H 12 D O N 3
		+34.7 / +6.7	6.5 C 10 1H 10 D 2 O N 3
		-25.9 / -5.0	1.0 C 6 1H 16 O 3 N 4
		-17.8 / -3.4	1.5 C 6 1H 14 D O 3 N 4
		-9.8 / -1.9	2.0 C 6 1H 12 D 2 O 3 N 4
		-1.7 / -0.3	2.5 C 6 1H 10 D 3 O 3 N 4
		+6.3 / +1.2	3.0 C 6 1H 8 D 4 O 3 N 4
		+14.4 / +2.8	3.5 C 6 1H 6 D 5 O 3 N 4
		+22.4 / +4.3	4.0 C 6 1H 4 D 6 O 3 N 4
176.0881	100.0	+24.6 / +4.3	6.0 C 11 1H 12 O 2
		+33.4 / +5.9	6.5 C 11 1H 10 D O 2
		+42.2 / +7.4	7.0 C 11 1H 8 D 2 O 2
		-42.7 / -7.5	4.0 C 8 1H 4 D 6 O 4
		-24.0 / -4.2	1.5 C 7 1H 14 O 4 N
		-15.2 / -2.7	2.0 C 7 1H 12 D O 4 N
		-6.4 / -1.1	2.5 C 7 1H 10 D 2 O 4 N
		+2.4 / +0.4	3.0 C 7 1H 8 D 3 O 4 N
		+11.2 / +2.0	3.5 C 7 1H 6 D 4 O 4 N
		+20.0 / +3.5	4.0 C 7 1H 4 D 5 O 4 N
		+28.8 / +5.1	4.5 C 7 1H 2 D 6 O 4 N
		-39.2 / -6.9	6.0 C 10 1H 12 O N 2
		-30.4 / -5.4	6.5 C 10 1H 10 D O N 2
		-21.6 / -3.8	7.0 C 10 1H 8 D 2 O N 2
		-12.8 / -2.3	7.5 C 10 1H 6 D 3 O N 2
		-4.0 / -0.7	8.0 C 10 1H 4 D 4 O N 2
		+4.8 / +0.8	8.5 C 10 1H 2 D 5 O N 2
		+32.2 / +5.7	6.5 C 9 1H 10 O N 3
		+41.0 / +7.2	7.0 C 9 1H 8 D O N 3
		-43.8 / -7.7	4.0 C 6 1H 4 D 5 O 3 N 3
		-35.0 / -6.2	4.5 C 6 1H 2 D 6 O 3 N 3
		-16.4 / -2.9	2.0 C 5 1H 12 O 3 N 4
		-7.6 / -1.3	2.5 C 5 1H 10 D O 3 N 4
		+1.2 / +0.2	3.0 C 5 1H 8 D 2 O 3 N 4
		+10.0 / +1.8	3.5 C 5 1H 6 D 3 O 3 N 4
		+18.8 / +3.3	4.0 C 5 1H 4 D 4 O 3 N 4
		+27.6 / +4.9	4.5 C 5 1H 2 D 5 O 3 N 4
148.0955	21.6	+45.4 / +6.7	5.0 C 10 1H 12 O
		-45.0 / -6.7	2.5 C 7 1H 6 D 5 O 3
		-34.6 / -5.1	3.0 C 7 1H 4 D 6 O 3
		-12.4 / -1.8	0.5 C 6 1H 14 O 3 N
		-1.9 / -0.3	1.0 C 6 1H 12 D O 3 N
		+8.5 / +1.3	1.5 C 6 1H 10 D 2 O 3 N
		+19.0 / +2.8	2.0 C 6 1H 8 D 3 O 3 N
		+29.4 / +4.4	2.5 C 6 1H 6 D 4 O 3 N
		+39.9 / +5.9	3.0 C 6 1H 4 D 5 O 3 N
		+50.3 / +7.5	3.5 C 6 1H 2 D 6 O 3 N
		-46.4 / -6.9	2.5 C 5 1H 6 D 4 O 2 N 3
		-36.0 / -5.3	3.0 C 5 1H 4 D 5 O 2 N 3
		-25.5 / -3.8	3.5 C 5 1H 2 D 6 O 2 N 3
118.0844	6.9	-61.0 / -7.2	2.5 C 6 1H 4 D 5 O 2
		-47.9 / -5.7	3.0 C 6 1H 2 D 6 O 2
		-20.1 / -2.4	0.5 C 5 1H 12 O 2 N
		-7.0 / -0.8	1.0 C 5 1H 10 D O 2 N
		+6.2 / +0.7	1.5 C 5 1H 8 D 2 O 2 N
		+19.3 / +2.3	2.0 C 5 1H 6 D 3 O 2 N
		+32.4 / +3.8	2.5 C 5 1H 4 D 4 O 2 N
		+45.5 / +5.4	3.0 C 5 1H 2 D 5 O 2 N

Figure 3.12. (contd)

3.4.3 Recovery Study

A recovery study experiment is underway to probe the derivatization efficiency of succinic acid, citric acid, NTA, EDTA, and HEDTA in Tank 101-SY matrix by adding known amounts of these materials as standards to matrix from the convective and non-convective regions of the tank. This study will also incorporate using d_4 -EDTA as well, since the signal from this component can be extracted from the nonlabeled EDTA present in the sample. The series of determinations will require derivatization of the native matrix and spiked matrix; we anticipate adding an amount of chelators as a mix that is approximately the level found within the matrix and at a level approximately twice that found in the matrix.

3.5 Identification of Water-Insoluble Organic Components in Waste From Tank 101-SY

At the beginning of FY 1993, only about 10 to 20% of the water-soluble organic carbon could be accounted for in core-composite samples from Tank 101-SY by derivatization gas chromatography/mass spectrometry (GC/MS)(Campbell et al. 1994). The core-composite samples consisted of four from the convective layer and four from the nonconvective layer. Principal constituents identified at that time included nitrosoiminodiacetic acid (NIDA), ethylenediaminetetraacetic acid (EDTA), and succinic acid. Liquid chromatography (LC) was used for the analysis of small organic acids, such as oxalic, acetic, formic, and glycolic. In addition, the results at that time indicated no variation in concentration of components as a function of depth of the sample. The question arose whether the poor accountability was due to (1) problems associated with the sample preparation procedure, (2) inadequate extraction of water-soluble organic carbon, (3) presence of water-insoluble carbon, or (4) the presence of high- molecular-weight material. Systematic studies were undertaken in each of these four areas to assess their contribution to the low total organic carbon (TOC) accountability.

Procedure Improvement Activities

All of the sample preparation in the early stages of methods development, including derivatization for chelator analysis, was performed in the hot cell facilities according to the following general procedure (Campbell et al. 1994). A waste sample was stirred with doubly-distilled water overnight at room temperature. The solution was filtered to remove any solid material. The aqueous solution was then reduced to dryness using nitrogen-blow-down techniques, and the remaining residue was then heated with boron trifluoride/methanol to produce the methyl esters of chelators, chelator fragments, and carboxylic acids. After the solution cooled to room temperature, 1 mL of chloroform was added to the solution. The organic layer containing the methyl esters was separated from the aqueous layer, removed from the hot cell, and subsequently analyzed using GC/MS. The problem associated with all of the sample preparation performed in the hot cell, contributing to low TOC accountability, was the inability to control the temperature of the reaction and dryness of the sample, parameters critical to obtaining complete derivatization.

An improved sample preparation procedure for the analysis of water-soluble organic carbon was developed to eliminate the difficulties involved in the hot cell. The procedure now involves weighing a 5-g sample, leaching, filtering, and eluting through a cation exchange column to reduce the radioactivity levels, followed by rinsing of the column. After radioassay confirmed that the

radioactivity level has been reduced, the sample is removed from the hot cell facilities to the fume hood for drying and derivatization, with subsequent analysis by GC/MS. The parameters critical for complete derivatization are more easily monitored and controlled. An aliquot of the aqueous extract is also analyzed by anion exchange and anion exclusion LC for low molecular weight acids (LMWA). The water-soluble organic carbon accounted for by LMWA, chelators, and chelator fragments using the improved sample preparation procedure was 73 to 93 % and fully documented (Campbell et al. 1994).

Investigation of Extraction Efficiency

It was hypothesized that one of the problems with poor TOC accountability in the early stages of methods development was partially due to low recovery of water-soluble organics. The samples were extracted at elevated temperatures to increase the amount of water-soluble organic carbon. A sample of the convective layer from Tank 101-SY was heated in the hot cell facility to approximately 50°C in doubly-distilled water while stirring. The amount of gas evolution was so large that the vial would not remain capped; the solution also foamed and resulted in spillage of the sample in the hot cell. The experiment was repeated with a smaller waste sample from the convective layer, but the results were the same. No quantitative TOC data could be obtained; the amount of entrained gas bubbles precluded obtaining any TOC data. The same procedure was performed with a sample of the nonconvective layer, and no gas evolution was observed.

Analysis of Solvent-Soluble/Water-Insoluble Carbon

In a further attempt to account for TOC, a procedure for extracting water-insoluble organic carbon was conducted in the hot cell. The sludge-like waste sample, at pH $\approx$ 13, was extracted with three equal volumes of chloroform, concentrated, and analyzed using GC/MS. The results are shown in Table 2.2; normal paraffin hydrocarbon (NPH), C12-C15, is the primary constituent of the water-insoluble carbon. The concentration of NPH is highest in sample R4258; there is a strong correlation of NPH concentration with the depth of the core sample, suggesting a density segregation of NPH. Table S.1 shows the TOC accounted for by the water-soluble organics including chelators, chelator fragments, LMWA and the water-insoluble organics, NPH.

High Molecular Weight Components

In view of the fact that 73 to 93 % of the organic carbon can be accounted for with chelators, chelator fragments, and carboxylic acids, very little contribution from higher molecular weight material is expected. In addition, there was no indication of higher molecular weight material observed with either derivatization GC/MS, LC, or liquid chromatography/mass spectrometry (LC/MS).

In summary, the potential contributions to low TOC accountability in the early states of analytical methods development were systematically examined. Primarily, the increase in TOC accountability was due to two factors: (1) reducing the radioactivity levels in waste samples using cation exchange resin so that the samples could be removed from the hot cell and further sample preparation performed in the fume hood, and (2) quantitating the water-insoluble organic carbon. Removing the samples from the hot cell and performing the derivatization in a fume hood allowed the critical parameters of temperature of the derivatization reaction and dryness of sample involved in the derivatization procedure to be more easily controlled. Furthermore, our experience has been that the net amount of chelator material found in a particular sample is actually a function of sample size to reagent ratio used

for derivatization. Large sample sizes were found to give a smaller concentration of chelators with the same amount of derivatizing reagent. It appears that the derivatizing reagent is consumed by the matrix. Thus, a judicious sample size to reagent ratio, based on knowledge of interferant content, has a large influence on the results obtained. In addition, determining the amount of NPH contributed to TOC accountability; NPH constitutes all of the water-insoluble organic carbon. In view of the fact that 73 to 93 % of the TOC can be accounted for in samples from 101-SY, very little, if any, water-soluble, water-insoluble, or higher molecular weight carbon remains unidentified.

3.6 Chemical Support for Simulated Waste Studies

Analytical chemical support has been supplied for Task 04, Gas Generation.

Samples containing organic analytes HEDTA, EDTA, citrate, formate, glycolate, IDA, and succinate have been irradiated and heated for various periods of time. These samples have been submitted under Task 04 to determine the amount of degradation and identify the degradation products.

Analysis of EDTA, HEDTA, and other chelators in actual waste samples was performed using derivatization GC/MS. However, simulated wastes do not behave like actual wastes during the dry-down procedure. Simulated wastes are cement-like after drying; this is not the case with actual waste. The recoveries of the chelators in simulated waste using derivatization GC/MS is very low, usually less than 50%. Further studies have been proposed to examine this phenomenon. As a result, EDTA, HEDTA, and IDA have been analyzed using LC techniques that have been developed.

Formate, citrate, and glycolate have been analyzed by anion exchange LC. Iminodiacetic acid has also been analyzed by anion exclusion LC. At this point, succinic acid has been analyzed by derivatization GC/MS.

Three samples have been analyzed. However, analyses of all the samples are not complete. Examples of the chromatograms obtained using LC are shown in Figures 3.13-3.17. Figure 3.13 is a chromatogram of HEDTA and EDTA standards. Figure 3.14 is a chromatogram of a sample with no irradiation. Figure 3.15 is a chromatogram of a sample having undergone irradiation. All the peaks around HEDTA up to retention time of 5.0 min are thought to be degradation products. Figure 3.16 is a chromatogram of sample with heat and no irradiation. There is no indication of degradation. Figure 3.17 is a chromatogram of a simulated waste sample having undergone both irradiation and heat. The peaks around HEDTA appear to be degradation products. Further work is underway to identify the degradation products.

Figure 3.13. LC Chromatogram of EDTA and HEDTA Standards

Figure 3.14. LC Chromatogram of Simulated Waste Sample with No Irradiation

Figure 3.15. LC Chromatogram of Simulated Waste Sample with Irradiation

Figure 3.16. LC Chromatogram of Simulated Waste Sample with Heat and No Irradiation

Figure 3.17. LC Chromatogram of Simulated Waste Sample with Heat and Irradiation

4.0 Methods Development Transfer

The method, will be developed and transferred to designated personnel. A cover statement will be signed by both the transferor and the receiver. The test plan will then be developed, completed, and signed by the test plan author and technical group leader. The Advanced Organic Analytical Methods Group will provide training and consultation if necessary for the laboratories accepting the technology. The entire package, which includes the transferred method, cover statement, and test plan, will then be forwarded to the appropriate WHC project manager.

Various methods have been transferred to personnel in the ACL and the laboratory at 222-S, including the LC method for LMWA and the LC method for HEDTA and EDTA. A letter of transfer was signed May 31, 1994 by Kim Wehner. These methods are presently being used by the ACL in the analysis of tank waste samples before grout treatment. The derivatization GC/MS methods and the LC/MS methods for chelators, chelator fragments, and LMWA have been transferred to the ACL and 222-S during FY 1994 under the auspices of the Organic Tanks Safety Program. A transfer letter was signed by Jim Campbell and Kim Wehner of the 222-S laboratory on June 7, 1994. A similar letter of transfer was signed on May 24, 1994 by both Jim Campbell and Robert Stromatt of the ACL. Methods developed under the Flammable Gas Program that may be useful for organic analysis will also be transferred to the Organic Tanks Safety Program. Appendix C contains the methods that have been developed and transferred to both ACL and the 222-S laboratory.

5.0 Future Work

The large amounts of nitrate and nitrite in the waste samples pose a continuing problem in analyzing for organic species. Elimination of nitrate and nitrite would simplify what is clearly a very complex matrix and allow for more accurate and sensitive determination of the desired analytes. One method of removing these ions would employ an electrolytic reduction scheme. This technique would remove the nitrates and nitrites without altering the composition of the organic species.

Techniques will be developed for analyzing surfactants in tank waste.

Additional efforts will focus on the use of deuterated standards as internal standards during the derivatization process. Standards such as deuterated succinic acid and EDTA will be evaluated for stability and reactivity with the matrix in tank waste samples.

Efforts will be focused on determining what radionuclides are absorbed on the cation exchange material. Radionuclides will be measured before and after elution through the cation exchange column. The differences should represent the radionuclides absorbed onto the column.

Technology transfer also will be a major aspect of this program. Additional methods will be transferred to both ACL and 222-S laboratory personnel. The developed techniques also will be transferred to the Organics Tanks Safety Program. Techniques for direct analysis being developed under the auspices of the Organics Tanks Safety Program will be transferred to the Flammable Gas Safety Program as well.

Analytical support will be provided for the gas generation studies. For example, during an experiment to increase the amount of water-soluble organic carbon being leached into water, a sample of the convective layer was heated to approximately 60°C while being stirred with water. There was a significant amount of gas produced; a large enough volume of gas to blow the stopper off. A sample of the convective layer will be analyzed before and after heating to monitor the disappearance of organic carbon analytes. The results may indicate the analytes responsible for the gas production.

6.0 References

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Appendix A

Experimental Procedures

Appendix A

A.0 Experimental Procedures

A.1 Derivatization Gas Chromatography/Mass Spectrometry (GC/MS)- Determination of Chelators and Chelator Fragments

A.1.1 Waste Sample Extraction/Derivatization

The waste sample represented a composite of several segments of actual waste obtained by core drilling the waste in Tank 241-SY-101. This material was highly radioactive, contained a high concentration of nitrate and nitrite, and the OH^- concentration was 2 M. The total organic carbon (TOC) was 1.5 to 2.1 %.

Hot Cell Derivatization Procedure

Approximately 2 g of the solids from the composite sample were stirred overnight with 20 mL of doubly-distilled water. The solution was filtered, and the aqueous solution was evaporated down to dryness in Reactivials (VWR Scientific) using nitrogen blowdown techniques. This involves heating the sample while blowing a steady stream of nitrogen over the sample. Approximately 2 mL of 14% BF_3 /methanol (Aldrich) was added, and the sample was then heated to 100°C for 1 h. The solution was cooled, and 1 mL of chloroform was added. The entire solution was then poured into a test tube containing 2 mL of 0.1 M KH_2PO_4 adjusted to pH 7.0. The Reactivial was rinsed with 1 mL of chloroform, and this was also added to the test tube. The test tube was vortexed, and the aqueous and chloroform layers were allowed to separate. The chloroform layer, containing the derivatized organics, was retained, and the aqueous layer was discarded. At this point, approximately 90 to 95% of the radioactivity remains with either the aqueous layer or undissolved solid material. The chloroform solution was monitored for radioactivity and then removed from the hot cell for subsequent gas chromatography(GC)/electron impact mass spectrometry(MS) analysis. The derivatized waste sample was also analyzed using positive ion chemical ionization mass spectrometry to confirm parent ion assignment.

The above procedure was all done in the hot cell facilities. The hot cell facilities are used for the preparation, e.g., sample dissolution, dilution, and solvent extraction, and occasionally, the analysis of nuclear wastes. For highly radioactive wastes, e.g., 3 to 11 R/h, the hot cell must be used. This room is composed of thick walls (1.3 m) equipped with a sample entry port, viewing windows (lead glass and oil-filled), and remote manipulators, which are operated by a highly-trained specialist. When a radioactive sample is processed in the hot cell, the procedure is both time consuming and tricky. These procedures cannot be performed by just anyone, anywhere, in any laboratory, when highly radioactive samples are involved. Special training is required. Sample turnaround is much lower when using the hot-cell facilities. Wastes with low-to-moderate specific or total radioactivity, < 1 R/h, may be

prepared and analyzed outside the hot cell in a radiation hood or glove box. The actual cutoff levels which differentiate lab bench work, glove-box work, and hot-cell work is usually based upon local practice or the judgement of the resident health physicist.

Approximately 5 g of waste was weighed out and stirred with 10 mL of doubly-distilled water overnight. The aqueous phase was filtered through a 0.45 μm filter. The filtrate was then passed through a cation exchange column (10 g, AG50W-X8 resin), rinsed using an additional 10 mL of water to quantitatively remove the organic material from the resin. The resulting sample volume was 20 mL. After surveying the samples for radioactivity levels, the aqueous solution containing the organics was removed from the hot cell facilities.

Aliquots (2 mL) of the sample were transferred to teflon-sealed vials, evaporated to dryness using nitrogen blow down techniques, and derivatized with 2 mL of BF_3 /methanol. The sample was heated to 100°C for 1 h. The sample was then hydrolyzed with a KH_2PO_4 solution and extracted with chloroform as described previously. The chloroform extract was then analyzed by Gas Chromatograph/Mass Spectrometry (GC/MS).

In calculating the concentration of organic constituents, mechanical extraction loss experienced from the initial 10 mL extraction was estimated by determining the water present in the original samples, adding the 10 mL of water used for the extraction to that water, and then subtracting the volume of water recovered to determine the mechanical loss. Water content of samples was determined by measuring the percent solids.

Aliquots were also measured for LC analysis. Dilutions of the original solutions were usually necessary due to the concentration levels.

Methylation with BF_3 /methanol were carried out on chelator standards obtained from Aldrich and independent sources. All mass spectral analyses were performed on a Hewlett Packard HP 5988A GC/MS system.

Other experimental conditions are described below:

A.1.2 BF_3 /methanol Methylation

Approximately 1 mL of a 10% w/v solution of boron trifluoride (BF_3)/methanol (Aldrich) was added to about 5 mg of the chelator in a reaction vial, and the mixture was vortexed and heated for varying lengths of time (1 to 5 h) at 100°C, depending on the chelator being derivatized. One mL of chloroform was added to the cooled reaction mixture and vortexed. A buffer solution consisting of 1 M KH_2PO_4 was made, and the pH was adjusted to 6.92 using sodium hydroxide. When this buffer solution was added to the contents of the vial, the solution separated into two layers and an aliquot was pipetted from the bottom layer, the chloroform layer, for GC/MS analysis. Parent ion assignment was confirmed by using positive ion chemical ionization mass spectrometry.

A.2 Instrumentation

A.2.1 GC/MS Conditions

The instrument was equipped with a HP 5980 GC operated in the splitless mode. A fused silica column (DB-5, 30m X 0.25 mm i.d., 0.25 μ m film thickness, J & W Scientific) was used. The oven temperature was typically programmed in the following manner: 50°C for 1 min, 8°C/min to 300°C, and hold at 300°C for 5 min. The mass spectrometer was tuned daily with perfluorotributylamine (PFTBA). In these studies, the mass spectrometer was scanned from 50 to 500 amu and operated in the electron impact mode (70 eV). The source temperature was 200°C, the injector port temperature was 250°C, and the interfaces were also at 250°C.

Chemical ionization was carried out with both methane and isobutane in both positive ion and negative ion chemical ionization modes. The temperature of the source for positive ion chemical ionization MS was 200°C and 120°C for negative ion chemical ionization. The mass spectrometer was scanned from 100 to 600 amu in the negative ion mode and 70 to 500 in the positive ion mode.

A.2.2 Accurate Mass Measurements

Accurate mass measurements were performed on a VG 70-EHF double-focusing mass spectrometer equipped with a VG 11-250 data system. The instrument was tuned to a resolution of 5000 (10% valley definition). Data were acquired by scanning the magnetic field exponentially down over the mass range of 230 to 90 at a rate of 3 sec per mass decade. Instrument tuning and real-time mass measurements were performed by leaking perfluorokerosene into the electron impact ion source from the septum inlet reservoir. Computer-assisted accurate mass assignments and subsequent elemental compositions were made on data obtained from averaging four consecutive scans over the gas chromatographic elution profile of the analyte. The instrument was equipped with a Varian Model 3700 gas chromatograph. The GC was fitted with 30 m X 0.25 mm i.d. DB-5 capillary column (J & W Scientific, Folsom, California). The GC oven temperature was held at 50°C for 2 min, then programmed at 5°C/min to 250°C.

A.2.3 Analysis of Acid and Base-Neutral Solvent Extracts

A.2.4 Sample Preparation

A 2-g aliquot of a composite sample (pH 13) was mixed with 10 mL of methylene chloride and vortexed for three min. The solution was filtered and concentrated to approximately 100 μ L for GC/MS analysis. The pH of the original solution was then adjusted to 3 with the addition of HCl. The slurry was then mixed with 10 mL of methylene chloride and vortexed for 3 min. The solution was then filtered and concentrated to approximately 100 μ L for analysis using GC/MS.

A.2.5 Instrumentation

The extracts were analyzed using a JEOL SX 102/SX 102 double focusing tandem mass spectrometer in the electron impact mode and equipped with a JEOL UNIX data system. Instrument tuning and real-time mass measurements were performed by leaking perfluorokerosene (PFK) into the electron impact ion source from the septum inlet reservoir. The instrument was equipped with a

Hewlett-Packard 5890 gas chromatograph. The GC was fitted with a 30 m X 0.25 mm i.d. DB-5 capillary column (J & W Scientific, Folsom, California). The GC oven temperature was held at 50°C for 1 min, then programmed at 8°C/min to 300°C.

A.2.6 High Resolution Mass Spectrometry-Accurate Mass Determination, Empirical Formula

Accurate mass measurements were performed on a JEOL SX 102/SX 102 double focusing tandem mass spectrometer equipped with a JEOL UNIX data system. The instrument was tuned to a resolution of 10,000 (10% valley definition). Data was acquired by scanning the magnetic field exponentially down over the mass range of 30 to 600 at a rate of second per mass decade. Instrument tuning and real-time mass measurements were performed by leaking PFK into the electron impact ion source from the septum inlet reservoir. Computer-assisted accurate mass assignments and subsequent elemental compositions were made on data obtained from averaging four consecutive scans over the gas chromatographic elution profile of the analyte. The instrument was equipped with a Hewlett-Packard 5890 gas chromatograph. The GC was fitted with a 30 m X 0.25 mm i.d. DB-5 capillary column. The GC oven temperature was held at 50°C for 2 min, then programmed at 5°C/min to 250°C.

A.2.7 Thermospray LC/MS of Low Molecular Weight Acids

A.2.8 Reagents

Gluconic acid, sulfuric, formic, acetic, phosphoric, and hydrochloric acid were obtained from Aldrich. Gluconic acid was in the form of the potassium salt. Formic acid and acetic acid were diluted from the concentrated acid reagents. Tartaric acid and hexanoic acid were obtained from Chem Services. The sulfuric acid, acetic acid, phosphoric acid, and hydrochloric acid used in preparing the mobile phases were diluted from the concentrated acid reagents. Milli-Q water was used for all dilutions. N-Nitroso-iminodiacetic acid was synthesized in the following manner.

A.2.9 Synthesis of N-Nitroso-iminodiacetic acid (NIDA)

The synthetic procedure was similar to that reported in the literature (Warren and Malec 1972). To a 500-mL, four-necked flask—equipped with an overhead stirrer, an addition funnel, and a thermometer—were added 26.6 g (20 mmoles) of iminodiacetic acid (Aldrich) and 200 mL of 1 M phosphoric acid. The contents of the flask were heated to 50°C, and a solution of 27.6 g (400 mmoles) of sodium nitrite in 60 mL of water was added dropwise. The reaction was then stirred for 1 min and extracted with 5 100-mL portions of diethyl ether. The ethereal extract was dried (MgSO_4) and concentrated to yield a yellowish white residue, which after recrystallization from ethyl acetate-hexane afforded 13 g of nitroso acid.

A.2.10 Instrument Conditions

A.2.11 Liquid Chromatography

A Bio-rad HPLC Organic Acids column, Aminex Ion Exclusion, HPX-87H, 300 X 7.8 mm, with compatible guard column was used. The mobile phase for this part of the work was 0.001 N HCl (isocratic). The LC oven temperature was 40°C, and the flow rate was 0.6 mL/min. A typical run

time was 25 min. Standards of organic acids were analyzed using direct flow injection and after introduction onto the analytical column. A mixture of organic acids was also analyzed after separation by the analytical column.

A.2.12 Mass Spectrometry

The thermospray analyses were performed using the HP 5988A LC/MS in the isocratic mode. The ion source was 275°C, and the stem initial temperature was 100°C. Typical values for the tip and the vapor were 230°C and 272°C, respectively. Filament operation was in both the on-and-off modes. The multiplier voltage was 2692 V, and the scan range was either 79 to 500 or 92 to 300 amu, depending on the mixtures being analyzed. The injection volume was either 10 µL or 20 µL, depending on the concentration of the sample.

The instrument was tuned with polyethylene glycol (PEG) tuning solution to give maximum abundance of ions. It should be noted that the thermospray ion source should be cleaned approximately once a week to remove nonvolatile chloride salts resulting from the use of HCl as the mobile phase. Also, since HCl possesses corrosive effects on metal surfaces, water was flushed through both the HPLC and thermospray system at the end of the day. As long as these cleaning and flushing procedures were followed, no detrimental effects of the thermospray or chromatographic equipment were observed.

A.2.13 Actual Waste Sample

The waste sample represented a composite of several segments of actual waste obtained by core drilling the waste in Tank 101-SY. This material was highly radioactive, contained a high concentration of nitrate and nitrite, and the OH⁻ concentration was 2 M. The TOC was 1.5 to 2.1 %.

The actual waste sample was passed through a Bio-Rad analytical grade cation exchange column (AG 50W-X8, 50-100 mesh, hydrogen form) in a remote handling hot cell which removed almost all of the radioactivity due to primary fission products in the waste (sometimes down nearly background levels) before being brought to the instruments for analysis. The hot-cell facilities are used for the preparation, e.g., sample dissolution, dilution, and solvent extraction, and occasionally, the analysis of nuclear wastes. For highly radioactive wastes, e.g. 3 to 11 R/h, the hot cell must be used. This room is composed of thick walls (1.3 m) equipped with a sample entry port, viewing windows (lead glass and oil-filled), and remote manipulators, which are operated by a highly-trained specialist. When a radioactive sample is processed in the hot cell, the procedure is both time consuming and difficult. These procedures can not be performed by just anyone, anywhere, in any laboratory, when highly radioactive samples are involved. Special training is required. Sample turnaround is much lower when using the hot cell facilities. Wastes with low-to-moderate specific or total radioactivity, < 1R/h, may be prepared and analyzed outside the hot cell in a radiation hood or glove box. The actual cutoff levels that differentiate lab bench work, glove-box work, and hot-cell work is usually site specific, based upon local practice or the judgment of the resident health physicist.

A 1-g aliquot of the waste sample was weighed and placed in a 25.0 mL volumetric flask. The sample was then neutralized with 0.001 N HCl to the pH of the mobile phase using an Orion extended scale pH meter. The solution was then diluted to volume using Milli-Q water. An aliquot of the solution was filtered into an LC vial using a Gelman Acrodisc LC13, 0.45 µm syringe-tip filter. Approximately 20 µL was used for HPLC or thermospray LC/MS analysis.

A.2.14 Liquid Chromatography-Determination of HEDTA and EDTA

A.2.15 Chemicals and Reagents

Tetrasodium ethylenediaminetetraacetic acid (EDTA) dihydrate and trisodium hydroxyethylenediaminetetraacetic acid (HEDTA) hydrate were obtained from Aldrich (Milwaukee, Wisconsin). Dodecyltrimethylammonium bromide was obtained from Kodak Chemicals (Eastman Kodak Co., Rochester, New York). The water was purified by a Milli-Q system. All other chemicals were reagent grade.

A.2.16 High Performance Liquid Chromatography

A model 510 solvent delivery pump, a model 712 WISP autosampler, and a model 484 variable wavelength UV absorption detector (Waters Associates, Milford, Massachusetts) were used. The column was an Adsorbosphere C-8 (25 cm x 4.6 mm, particle size 5 μ m) equipped with an Adsorbosphere C-8 guard column cartridge (Alltech, Deerfield, Illinois).

The mobile phase was 0.002 M dodecyltrimethylammonium bromide, and 0.05 M potassium dihydrogen phosphate (pH 6.5, adjusted with 6N sodium hydroxide). The chromatography was performed at ambient temperature with a flow rate of 2 mL/min. The eluant's absorbance was monitored at 280 nm. The chromatographic data were collected and processed by Maxima 820, v3.02, software (Dynamic Solutions, Ventura, California) on a model APC IV NEC computer (Boxborough, Massachusetts).

A.2.17 Calibration Standards

To 129 mg of tetrasodium EDTA dihydrate and 117 mg of trisodium HEDTA hydrate in a 10 mL volumetric flask were added water, 3 mL, and 0.5M copper(II) sulfate, 2 mL. After total dissolution of the chelators, the volume was taken to 10 mL with water. This gave a stock solution of 0.03 M EDTA and 0.03 M HEDTA. Further serial dilutions gave solutions of 0.012M, 0.006 M, and 0.003 M of both chelators. Another calibration standard for HEDTA was similarly prepared from 87.5 mg of trisodium HEDTA hydrate. Serial dilutions of this standard gave solutions of 0.009 M, 0.018 M, and 0.023 M of HEDTA.

To one gram of simulated waste mixture in a 25 mL volumetric flask were added water, 5 mL, and 0.5 M copper(II) sulfate, 2 mL. The pH was adjusted to 6.5 by the dropwise addition of 1.5 N phosphoric acid. The pH was monitored by means of pH indicator strips (Baxter, McGaw Park, IL). Typically one gram of simulated waste mixture required about 7 mL of acid. After the desired pH had been attained, the sample was diluted to 25 mL with water. A 5-mL aliquot of solution was placed in a centrifuge tube and spun in a centrifuge (IEC, model HN-S, Needham Heights, Massachusetts) for 10 min at 1500 rpm. This was necessary to remove a precipitate, probably the hydroxide or oxide of aluminum, that formed upon addition of the phosphoric acid. After centrifuging, a portion of the supernatant was transferred to a WISP autosampler vial for analysis.

A.2.18 Quantitation

Calibration Curves for both chelator were obtained by plotting the peak areas of the calibration standards against the concentrations of the calibration standards. The data were then fitted to a straight line by linear regression.

Concentrations of each chelator in simulated waste mixture (SWM) were calculated based on the corresponding calibration curve.

A.2.19 Liquid Chromatography for Low Molecular Weight Acids

A.2.20 Apparatus

Anion exchange chromatography was performed with a model 510 solvent delivery pump, a model 712 WISP autosampler, and a model 484 variable wavelength UV absorption detector (Waters Associates, Milford, Massachusetts). The column was an Ion Pac A54A (250 mm x 4.0 mm) equipped with an Ion Pac AG4A guard column (50 mm x 4.0 mm) (Dionex, Sunnyvale, California). The chromatographic data were collected and processed by Maxima 820, v3.02, software (Dynamic Solutions, Ventura, California) on a model APC IV NEC computer (NEC, Foxborough, Massachusetts). The mobile phase was 1.3 mM NaHCO_3 and 1.4 mM NaCO_3 at a flow rate of 2 mL/min. Detection was accomplished at 210 nm. The sample volume injected for analysis was 10 μL .

Ion exclusion chromatography was performed on a model 1090 high performance liquid chromatograph (HPLC) equipped with a diode array detector, and a data collection system (Hewlett-Packard, Palo Alto, California). The column was an IC-PAK 50, 300 x 7.8 mm, 7 μm , equipped with a guard column (Waters Associates). The mobile phase was 0.01 N H_2SO_4 at a flow rate of 0.6 mL/min. Detection was accomplished at 210 nm. The typical sample volume injection was 20 μL .

The cleanup procedure used Poly prep, 40 mm x 8 mm, disposable fritted columns packed with AG50W-X8 cation exchange resin, hydrogen form (Bio-Rad, Hercules, California). The cleanup procedure was performed within hot-cell facilities on the Hanford Reservation.

A.2.21 Reagents

Citric, formic, gluconic, glycolic, gluconic, oxalic, and tartaric acids were all purchased from Aldrich Chemical Company (Milwaukee, Wisconsin). Acetic acid was purchased from Baker. Sodium bicarbonate and sodium carbonate were purchased from Fisher Scientific. Sulfuric acid was from Baker. Water was purified with a Milli-Q water system (Millipore, Bedford, Massachusetts).

A.2.22 Sample Preparation

Aliquots of the samples were dried into 5-mL reaction vials for storage. Because of the alkalinity of the samples ($\text{pH} > 13$), the acids were in non-volatile salt forms, and therefore were not lost on drying. Water, 5 mL, was added to a dried sample, and the sample was sonicated for 1 h. After sonication, a 2.5 mL aliquot, of sample was withdrawn. The other half, also 2.5 mL, was retained for different experiments. The aliquot was loaded onto water rinsed AG50W-X8 resin, 2 mL bed, in a Poly-prep column. The eluent was captured in a scintillation vial, and the resin was rinsed with 1 mL

of additional water. The eluent was transferred to a secondary scintillation vial, and its radioactivity was measured. If the sample's radioactivity was below 1 to 2 mR, then it was removed from the hot cell. If it was above that level, it was run through fresh ion exchange resin, and its radioactivity was tested again.

With the radioactivity level diminished, the sample could be handled in fume hoods. The contents of the scintillation vial were transferred to a 5-mL volumetric flask and taken to the mark with water. Samples were then withdrawn from this volumetric flask for chromatographic analyses.

Appendix B

List of Waste Tank 101-SY Window E Core Segment Samples and Location Within Tank

Appendix B

B.0 List of Waste Tank 101-SY Window E Core Segment Samples and Location Within Tank

Segment	
R2458	From Segment 4, convective layer
R4259	From Segment 6, convective layer
R4260	From Segment 9, convective layer
R4261	From Segment 11, convective layer
R4262	From Segment 16, nonconvective layer
R4263	From Segment 17, nonconvective layer
R42664	From Segment 22R, nonconvective layer and bottom most segment

Appendix C

Test Plans for Methods Developed and Transferred to both ACL and 2220-S Laboratory

Appendix C

C.0 Test Plans for Methods Developed and Transferred to both ACL and 2220-S Laboratory

TEST PLAN, GROUT ANALYTICAL SERVICES PROJECT, 7/19/93

ANALYSIS FOR EDTA AND HEDTA IN GROUT SAMPLES BY
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

1.0 INTRODUCTION

This Test Plan (TP) is for the analysis for ethylenediaminetetraacetic acid (EDTA) and N-(2-hydroxyethyl) ethylenediaminetriacetic acid (HEDTA) by High Performance Liquid Chromatography (HPLC or LC), in particular in samples from the Grout Characterization Project. The plan also describes the preparation of samples in 325B hot cells or in the organic analysis sample preparation laboratory (lab 306), depending on the radionuclide content.

2.0 RESPONSIBILITIES

2.1 Analysis

The cognizant scientist for this analysis implemented the method, and will initially perform the sample analyses. As experience is gained with the method, additional analysts. As experience is gained with the method, additional analysts will be trained. Hotcell sample preparations will be conducted by personnel previously trained in the solid phase extraction (SPE) cleanup of waste tank 101-SY samples. Training for the sample preparation in lab 306 will be provided by the cognizant scientist and/or the experienced hotcell operator.

2.2 Test Modifications

The cognizant scientist will be responsible for modifications to the test plan that may be required. The modifications will be documented by memoranda to the program manager and group leader, and will be included in the report narrative.

2.3 Data Review

Upon completion of the analysis of a group or batch of samples for inclusion in a report, the group leader of the Organic Analysis Group of the Analytical Chemistry Laboratory, or designee, will review the data and narrative.

3.0 PROCEDURE

3.1 Objective and Scope

The objective of this TP is to describe the procedure for the analysis of Grout samples for EDTA and HEDTA using reverse-phase ion-pair LC with ultraviolet (UV) diode array spectrophotometric detection (DAD). The procedure is primarily for the determination of EDTA and HEDTA in solutions at high pH that contain relatively high concentrations of nitrate, nitrite and radionuclides; and includes a sample preparation step to reduce radioactivity through solid phase extraction (SPE). However the procedure is not limited to analysis of these samples.

3.2 Discussion

The procedure is tentative as presented in this TP. Normally important variables were evaluated in its implementation; but because of potential complexity of the samples, modifications of both the sample preparation and LC analysis may be necessary. Because of the anticipated high radionuclide content of the sample, a very large dilution and/or sample cleanup is necessary. The cleanup method for this procedure is SPE using a sodium form cation exchange column. Hotcell experience with this method for 101-SY sample cleanup has been successful, and should result in a sample dilution of no more than about 10. After extraction, the sample can be transferred to lab 306, and sample preparation completed.

Copper (II) sulfate is added to the decontaminated sample to complex the EDTA and the HEDTA, and the pH is adjusted to 6.5. This will cause aluminum, expected to be in some grout samples, and copper reagent to precipitate leaving in solution the copper complexes of the EDTA and HEDTA. The sample, after dilution to volume, is ready for the HPLC analysis.

The mobile phase in this procedure is 0.05M KH_2PO_4 and 0.002M dodecyltrimethylammonium bromide at pH 6.5. The DAD is set to detect at 260 nm, the absorbance maximum of both complexes, and at 280 nm in the event of interference UV absorbance at lower wavelengths. The linear analysis range is at least from 10 to 1000 $\mu\text{g/mL}$. The estimated instrument detection limit (dl) is 1.0 $\mu\text{g/mL}$ for both complexants. The actual sample dl will depend on the dilution and sample matrix, but will not likely be lower than 10 $\mu\text{g/mL}$.

3.3 Personnel Qualifications

The cognizant scientist implemented the procedure and investigated analytical variables, and is qualified to perform the analyses. The hotcell sample preparation operations have been performed on similar samples, and the hotcell operator received training by the developer of the cleanup procedure. Training of other analysts, for both sample preparation and analysis, will be performed by the cognizant scientist and/or the experienced hotcell operator as needed.

3.4 Documentation

The analysis data will be generated on both disk and hard copy. The raw data generated by the HPLC will be stored on 3.5 inch floppies, and the hard copy will be filed and delivered to WHC as described in the Technical Project Plan (TPP 20749) and in the test instructions (TI). An injection log will be maintained that will record sample and data file information.

3.5 Apparatus

- 3.5.1 HPLC - Hewlett-Packard HP-1090, or equivalent. As an absolute minimum, an LC pump and controller with appropriate injector and other valves, solvent degasser, analyte separation column, UV detector, and a printer/plotter are required. However, for maximum flexibility for the analysis and data handling, the equivalent of the HP-1090 with DAD and computer controller/data processor are recommended, especially in the analysis of samples with complex matrices. In addition, an autosampler is recommended so that samples can be injected from septum-sealed vials to help maintain control of radioactive samples.
- 3.5.2 HPLC column - Hewlett-Packard LiChrospher 100 RP-8 (5 μ m) 4 x 250 mm column, or equivalent. Use of an appropriate guard column is highly recommended.
- 3.5.3 Balance - capable of weighing to 0.1 mg.
- 3.5.4 Volumetric flasks - 1000, 100, 25, and 10 mL.
- 3.5.5 Syringes - 1000, 500, 250, 100, and 25 μ L.
- 3.5.6 Graduate cylinder - 100 mL.
- 3.5.7 SPE cartridges - Bio-Rad Poly-Prep columns filled with 2-mL AG 50W-X8 resin (106-250 μ m) or equivalent. The amount of resin may need to be increased for samples with high concentrations of strongly bound cations.
- 3.5.8 Magnetic stirrer with magnets.
- 3.5.9 pH meter with pH probe covering the pH range of at least 2 to 12.
- 3.5.10 VOA vials - 40-mL and 20-mL.
- 3.5.11 Disposable graduated centrifuge tubes - 15-mL capacity, capped, clear.
- 3.5.12 Syringe filter - 13 mm diameter, 0.46 μ m pore size.

3.5.13 Wide range pH paper.

3.6 Reagents

- 3.6.1 Mobile phase - 0.002M dodecyltrimethylammonium bromide, 0.05M KH_2PO_4 , 100 mL of X10 concentrated stock solution (0.617 g dodecyltrimethylammonium bromide and 6,804 g KH_2PO_4 in 100 mL reagent water), measured in a 100 mL graduate cylinder, is brought to volume in a volumetric flask.
- 3.6.2 0.5M CuSO_4 - 12.48 g/100 mL in reagent water.
- 3.6.3 Reagent water - deionized water using a deionizer with an organic removal cartridge.
- 3.6.4 H_3PO_4 - typically 1:10 dilution. The concentration of the acid for the pH adjustment may be different depending on the sample and its basicity.
- 3.6.6 Stock standards - 10000 $\mu\text{g/mL}$: 1.274g di-sodium di-hydrate of EDTA and 1.367g tri-sodium monohydrate of HEDTA dissolved in 100 mL reagent water. Individual 10000 $\mu\text{g/mL}$ standards can be prepared for individual spikes as needed. Lower concentration standards for calibration purposes are prepared by appropriate dilution of the stock standards.
- 3.6.7 pH standards - commercial pH 4, 7, and 10.
- 3.6.8 Dilute NaOH - 1N and 0.1N. Dissolve 4.0 ($\pm .05$)g in 100 mL water for the 1N base, and dilute the 1N base 1 to 10 for the 0.1N base.
- 3.6.10 Compressed air - breathing air quality, 75 psi, for autosampler operation.

3.7 Safety

- 3.7.1 Observe general laboratory safety rules.
- 3.7.2 Follow requirements of applicable RWP's.

3.8 Quality Control

This method is preliminary, and as such, quality control parameters have yet to be developed. As a minimum, matrix spike (MS) and matrix spike duplicate (MSD) samples spiked with EDTA and HEDTA (see section 3.11.1.2), and duplicate samples will be analyzed at a frequency required by the TPP or TI. In addition, a reagent blank will be analyzed with each group of samples. Initially, MS/MSD and sample duplicated will be analyzed for each sample. As experience is gained with the method on the Grout samples, the number of analyses will be

reduced as appropriate, or as required by the TP and/or TPP. Acceptance criteria for the blank, the sample/sample duplicate RPD (relative percent difference), and the MS/MSD RPD will be initially based on the "normality" of the results: for example, a blank chromatogram with a co-eluting peak, RPD value that are grossly different (one of the duplicates with no analyte response), and no spike response. Such situations will require evaluation and re-analysis.

The instrument will be calibrated daily using a single concentration calibration. The calibration will be repeated after each 4 or 5 sample determinations. This calibration frequency may be changed once experience with the method is gained. An initial calibration will be performed for the instrument using multiple concentration levels to define analytical linearity and instrument quantitation range.

3.9 Calibration

3.9.1 Daily calibration will be performed using a single concentration level standard prepared by dilution of the stock standard and following the procedure described in section 3.10.1. Initially, the concentration of this standard will be 300 $\mu\text{g/mL}$ for both the EDTA and HEDTA. After experience with the Grout samples is gained, different concentrations may be used.

3.9.2 Initial calibration is multiple concentration level calibration performed using standards prepared by appropriate dilutions of the stock standard following the procedure described in section 3.10. For this TP, a 6-level calibration was prepared: 25, 50, 100, 300, 600, and 1000 $\mu\text{g/mL}$.

3.10 Calibration standards preparation

3.10.1 Daily calibration standard.

3.10.1.1 Pipet an appropriate amount of the stock standard for the desired concentration of the daily calibration standard to a 40-mL VOA vial. A final volume of 25-mL for the standard is convenient.

3.10.1.2 Assuming a 300 $\mu\text{g/mL}$ concentration and 25 mL volume, add about 0.4 mL of 0.5M CuSO_4 to the standard in the beaker.

3.10.1.3 Add about 15 mL reagent water to the standard, and mix on a magnetic stirrer.

3.10.1.4 Slowly add 1N NaOH (the more dilute base is used for the final pH adjustment) to adjust the pH to 6.5 (± 0.2).

3.10.1.5 Centrifuge the solution, and transfer the supernate into a 40-mL VOA vial. Wash the precipitate with reagent water, centrifuge the solution if necessary, and transfer the rinse solution into the VOA vial. Repeat the wash, centrifuge and transfer steps twice more.

3.10.1.6 Check the pH and adjust to 6.5 (± 0.2) if necessary. Transfer the solution to a 25-mL volumetric flask and dilute to volume.

3.10.2 Initial calibration standards - these are prepared as described in section 3.10.1 except as noted below, but with final concentrations of 25, 50, 100, 300, 600, and 1000 $\mu\text{g/mL}$. Use 200 μL of the copper stock solution in preparation of the 25, 50, and 100 $\mu\text{g/mL}$ standards; 400 μL of the copper solution for the 300 and 600 $\mu\text{g/mL}$ standards; and 1000 μL of the copper solution for the 1000 $\mu\text{g/mL}$ standard.

3.11 Sample Preparation

3.11.1 SPE cleanup - this can be performed in the hotcells or in the sample preparation lab depending on the sample radionuclide content.

3.11.1.1 Convert the as-received acid resin form SPE column to the sodium form by passing 1N NaOH through the column until the eluate pH is > 9 . Wash the column with reagent water until the eluate pH is 7-9. The SPE columns can be made up in the laboratory using the specified resin and appropriate columns.

3.11.1.2 Pipe 1.0 (± 0.05) mL, or other volume specified by the cognizant scientist, of the sample onto the column. The pH of most samples will be > 9 . This can be verified using the pH paper. If it is not, adjust the pH maintaining sample volume accountability. Record the sample volume on the sample bench sheet, or equivalent form. The sample volume used may need to be reduced for some samples because of the amount of

precipitate formed in the pH adjustment and the radioactivity of the sample. Catch the eluate in a 20 or 40-mL VOS vial.

Prepare a reagent water blank consisting of 50 μL 1N NaOH in 1.0 (± 0.05) mL reagent water. Transfer the blank to the SPE column, and catch the eluate in a 20 or 40-mL VOA vial.

For preparation of the NS/MSD samples, add an appropriate amount of stock standard to the column just prior to addition of sample. The amount of standard to add will depend on the analyte concentration in the sample. The MS/MSD analyses will be used both as a method to estimate reproducibility, and to correct for matrix effects and analyte losses in sample preparation. Consequently, the

spike concentrations are recommended to be no lower than the lowest calibration level and at least as high as the analyte concentration, but no greater than 2 or 3 times the analyte concentration within the linear portion of the calibration range. Circumstances may make it impractical to spike according to the preceding recommendation. In such cases, add 3000 μg EDTA and HEDTA (300 μL of the 10000 $\mu\text{g}/\text{mL}$ stock) to the column just prior to addition of the sample. Record the amount of spike standard and its concentrations on the sample bench sheet, or equivalent form.

3.11.1.3 Wash the column into the collection vial with 2-column volumes of reagent water. Cap the vial, and transfer it to the organic group for further processing.

3.11.2 Copper complexation and pH adjustment.

3.11.2.1 Add 0.5 (± 0.05) mL 0.5M CuSO_4 to the sample solution and mix.

3.11.2.2 Adjust the sample pH to 6.5 (± 0.2) by slowly adding 1:10 H_3PO_4 while mixing over a magnetic stirrer.

3.11.2.3 Centrifuge the sample, transfer the supernate into a 15-mL disposable centrifuge tube and wash the precipitate (the standards will have only a copper precipitate while precipitates from some samples may also have aluminum). Repeat the wash, centrifuge and transfer steps twice more.

If the sample precipitate is so voluminous that this step is difficult to accomplish, either add additional reagent water and dilute to 25 mL in step 3.11.2.5, or repeat the cleanup with less sample. In order to maintain the dl as low as possible and improve decontamination, the preferred action is to repeat the process with less sample starting at step 3.11.1.

3.11.2.4 Check the pH and adjust to 6.5 (± 0.2) if necessary.

3.11.2.5 Dilute the supernate the 10-mL in the centrifuge tube, and cap the tube. Record the final volumes on the sample bench sheet.

3.12 HPLC Measurement

3.12.1 HPLC conditions - set up the HPLC under conditions described below. Start the instrument, de-aerate the mobile phase, allow the column to come to equilibrium, and allow the DAD to stabilize. Operation of the instrumentation is described in the system manuals.

Column: HP LiChrospher 100 RP-8 4 x 250 mm
Column temperature: 40° C
Flow rate: 2 mL/min
Mobile phase: 0.002M dodecyltrimethylammonium
bromide - 0.05M KH₂PO₄ - pH at 6.5
Injection volume: 25 µL
Detector wavelength: 260 nm with 280 nm as a backup

- 3.12.2 Transfer about 10mL of processed sample from step 3.11.2.5 to a 1.7-mL auto-sampler vial through the 0.45 micro syringe filter, and seal the vial with a crimp septum cap.
- 3.12.3 The sealed standard and sample vials are loaded into the HPLC autosampler, and a sequence programmed such that a single point calibration is performed prior to every 4 or 5 sample analyses. Provide each sample with a unique data file identity and record this identification along with other sample information in the LC injection log. The instrument manual provides a data file naming protocol. As experience with the analysis is gained with these samples, the calibration frequency may be increased or decreased.
- 3.12.4 Start the analysis as described in the system manuals.
- 3.12.5 Upon completion of the analysis sequence, review and correct the data as necessary.
- Manual integration of chromatographic peaks may be necessary for peaks in complex chromatograms. Manual integration is described in the system manuals.
- 3.12.6 Determine that all raw data are stored on the hard disk, and transfer pertinent data to the 3.5 inch disks for archive. Generate necessary hard copy of the data.
- 3.12.7 Calculate final results based on sample size and all dilutions and other sample manipulations performed.

4.0 REFERENCES

1. Grout Analytical Services Technical Plan, Revision 0, TPP 20749, March 1993.
2. J. A. Campbell, R. B. Lucke, K. E. Grant, S. A. Clauss: Application of LC and Thermospray LC/MS to the Study of Small Organic Acids in Radioactive Mixed Hazardous Wastes: Presented at the 48th Northwest Regional Meeting of the American Chemical Society, Laramie, Wyoming, 17 June, 1993. (Manuscript for publication in preparation.)

3. HEWLETT-PACKARD, HP 1090 Series II/L and /M Liquid Chromatograph, Part 1 and Part 2, HP Part No. 01090-90210, April 1990.
4. HEWLETT-PACKARD, HPLC Chemstation (Pascal Series), Fact Files and Handbooks, HP Part Nos. 79994-90031 through -90035, April, 1990.

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TEST PLAN GROUT ANALYTICAL SERVICES PROJECT, 7/19/93

ANALYSIS FOR CITRATE IN GROUT SAMPLES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

1.0 INTRODUCTION

This Test Plan (TP) is for the analysis of citrate by High Performance Liquid Chromatography (HPLC), in particular in samples from the Grout Characterization Project. The plan also describes the preparation of samples in 325B hotcells or in the organic analysis sample preparation laboratory (lab 306), depending on the radionuclide content.

2.0 RESPONSIBILITIES

2.1 Analysis

The cognizant scientist for this analysis implemented the method and will initially perform the analyses. As experience with the method is gained, additional analysts will be trained. Hotcell sample preparations will be conducted by personnel previously trained in the solid phase extraction (SPE) cleanup of waste tank 101-SY samples. Training for samples preparation in lab 306 will be provided by the cognizant scientist and/or the experienced hotcell operator.

2.2 Test Modifications

The cognizant scientist will be responsible for modifications to the test plan that may be required. The modifications will be documented by memoranda to the program manager and group leader, and will be included in the report narrative.

2.3 Data Review

Upon completion of the analysis of a group or batch of samples for inclusion in a report, the group leader of the Organic Analysis Group of the Analytical Chemistry Laboratory, or designee, will review the data and narrative.

3.0 PROCEDURE

3.1 Objective and Scope

The objective of this TP is to describe the procedure for the analysis of Grout samples for citrate using ion exclusion chromatography with detection by ultraviolet (UV) diode array spectrophotometry (DAD). The procedure is primarily for the determination of the citrate in solutions at high pH that contain relatively high concentrations of nitrate, nitrite and radionuclides; and includes a sample preparation step to reduce radioactivity through solid phase extraction (SPE). However the procedure is not limited to analysis of these samples, and is applicable to other small organic acids after suitable calibration.

3.2 Discussion

The procedure is tentative as presented in this TP. Normally important variables were evaluated in its implementation; but because of potential complexity of the samples, modifications of both sample preparation and LC analysis may be necessary. Because of the anticipated high radionuclide content of the sample, a very large dilution and/or sample cleanup is necessary. The cleanup method for this procedure is SPE using a sodium form cation exchange column. Hotcell experience with this method for 101-SY sample cleanup has been successful, and the cleanup portion of the sample preparation should result in a sample dilution of no more than about 10.

Nitrate and nitrite at high concentrations, as expected in some of these samples, will interfere with the citrate to a degree depending on their concentrations. A total dilution factor of about 50 is expected to be necessary for some of the samples.

After extraction, the sample will be transferred to lab 306. Samples with moderate radionuclide concentration will be prepared entirely in lab 306. The pH of the decontaminated sample is adjusted to 2.0, and the sample is diluted to volume. The sample, after dilution, is ready for the HPLC analysis.

The mobile phase in this procedure is 0.01N H_2SO_4 . The DAD is set to detect at 210 nm, the absorbance maximum of the citrate. The linear analysis range is at least from 10 to 600 $\mu g/mL$. The 1000 $\mu g/mL$ standard is not on the linear curve. The r^2 value for the second order curve including the 1000 $\mu g/mL$ standard is 0.991 as opposed to 1.000 for the 10 to 600 $\mu g/mL$ linear curve. The estimated instrument detection limit (dl) is 2.2 $\mu g/mL$. The actual sample will depend on the dilution and sample matrix, but will not likely be lower than 110 $\mu g/mL$ for many of the samples.

3.3 Personnel Qualifications

The cognizant scientist implemented the procedure and investigated analytical variable and is qualified to perform the analyses. The hotcell sample preparation operations have been performed on similar sample, and the hotcell operator received training by the developer of the cleanup procedure. Training of other analysts, for both sample preparation and analysis, will be performed by the cognizant scientist and/or the experienced hotcell operator as needed.

3.4 Documentation

The analysis data will be generated on both disk and hard copy. The raw data generated by the HPLC will be stored on 3.5 inch floppies, and the hard copy will be filed and delivered to WHC as described in the Technical Project Plan (TPP 20749) and in the test instructions (TI). An injection log will be maintained that will record sample and data file information.

3.5 Apparatus

- 3.5.1 HPLC - Hewlett-Packard HP-1090, or equivalent. As an absolute minimum, an LC pump and controller with appropriate injector and other valves, solvent degasser, analyte separation column, UV detector, and a printer/plotter are required. However, for maximum flexibility for the analysis and data handling, the equivalent of the HP-1090 with DAD and computer controller/data processor are recommended, especially in the analysis of samples with complex matrices. In addition, an autosampler is recommended so that samples can be injected from septum-sealed vials to help maintain control of radioactive samples.
- 3.5.2 HPLC column - Bio-Rad Aminex ion exclusion organic acid column, HPX-87H with an appropriate guard column. Two other ion exclusion columns were tested. Resolution between the citrate and nitrate/nitrite peaks was very poor with one of the columns, and there was co-elution of a column degradation product with the citrate. Resolution with the second column was improved; but there were several column degradation peaks, and the baseline was poor.
- 3.5.3 Balance - capable of weighing to 0.1 mg.
- 3.5.4 Volumetric flasks - 1000, 100, 25, and 10 mL.
- 3.5.5 Syringes - 1000, 500, 250, 100, and 25 μ L.
- 3.5.6 Graduate cylinder - 100 mL.
- 3.5.7 SPE cartridges - Bio-Rad Poly-Prep columns filed with 2-mL AG 50W-X8 resin (106-250 μ m), or equivalent. The amount of resin may need to be increased for samples with high concentrations of strongly bound cations.
- 3.5.8 Magnetic stirrer with magnets.
- 3.5.9 pH meter with pH probe covering the pH range of at least 2 to 12.
- 3.5.10 VOA vials - 20 and 40-mL.
- 3.5.11 Syringe filters - 13 mm diameter, 0.46 μ m pore size.
- 3.5.12 Disposal graduated centrifuge tubes - 15-mL capacity, capped clear.
- 3.5.13 Wide range pH paper.

3.6 Reagents

- 3.6.1 Reagent water - deionized water using a deionizer with an organic removal cartridge.
- 3.6.2 Mobile phase - 0.01N H_2SO_4 . 1.00 (± 0.02) mL of 10N H_2SO_4 (28 ± 1 mL conc H_2SO_4 diluted to 100 mL with reagent water) diluted to 1000 mL.
- 3.6.3 H_2SO_4 - typically 1:10 dilution. The concentration of the acid for the pH adjustment may be different depending on the sample and its basicity.
- 3.6.4 Stock standards - 10000 $\mu\text{g/mL}$: 1.000 (± 0.002)g citric acid dissolved in 100 mL reagent water. Lower concentration standards for calibration purposes are prepared by appropriate dilution of the stock standards.
- 3.6.5 pH standards - commercial pH 4, 7, and 10.
- 3.6.6 Dilute NaOH - 1N and 0.1N. Dissolve 4.0 g NaOH in 100 mL reagent water for the 1N base and dilute the 1N base 1 to 10 for the 0.1N base.
- 3.6.7 Helium - 99% or better, at 0 to 7.5 psi, purge gas for mobile phase.
- 3.6.8 Compressed air - breathing air quality, 75 psi, for autosampler operation.

3.7 Safety

- 3.7.1 Observed general laboratory safety rules.
- 3.7.2 Follow requirements of applicable RWP's.

3.8 Quality Control

This method is preliminary, and as such, quality control parameters have yet to be developed. As a minimum, matrix spike (MS) and matrix spike duplicate (MSD) samples spiked with citric acid (see section 3.11.1.2), and duplicate samples will be analyzed at a frequency required by the TPP or TI. In addition, a reagent blank will be analyzed with each group of samples analyzed. Initially, MS/MSD and sample duplicates will be analyzed for each sample. As experience is gained with the method on the Grout samples, the number of analyses on each sample will be reduced as appropriate, or as required by the TP and/or TPP. Acceptance criteria for the blank, the sample/sample duplicate RPD, the MS/MSD RPD will be initially based on the "normality" of the results: for example, a blank chromatogram with a co-eluting peak, RPD values that are grossly different (one of the duplicates with no analyte response), and no spike response. Such situations will require evaluation and re-analysis.

The instrument will be calibrated daily using a single concentration calibration. The calibration will be repeated after each 4 or 5 sample determinations. This calibration frequency may be changed once experience with the method is gained. An initial calibration will be performed for the instrument using multiple concentration levels to define analytical linearity and instrument quantitation range.

3.9 Calibration

3.9.1 Daily calibration will be performed using a single concentration level standard prepared by dilution of the stock standard and following the procedure described in section 3.10.1. Initially, the concentration of this standard will be 100 $\mu\text{g/mL}$ in citric acid. After experience with the Grout samples is gained, different concentrations may be used.

3.9.2 Initial calibration is a multiple concentration level calibration performed using standards prepared by appropriate dilutions of the stock standard following the procedure described in 3.10. For this TP, a 6-level calibration was prepared: 25, 50, 100, 300, 600, and 1000 $\mu\text{g/mL}$. However, linearity extends, within this data set, only to 6000 $\mu\text{g/mL}$.

3.10 Calibration standards preparation

3.10.1 Daily calibration standard.

3.10.1.1 Transfer 250 μL of the stock standard to a 40-mL VOA vial, add about 18 mL reagent water and mix.

3.10.1.2 Adjust the pH to $2.0 \pm .1$, transfer the standard to a 25 mL volumetric flask, dilute to volume with reagent water and mix.

3.10.2 Initial calibration standards - these are prepared as described in section 3.10.1, but with final concentrations of 25, 50, 100, 300, 600, and 1000 $\mu\text{g/mL}$.

3.11 Sample Preparation

3.11.1 SPE cleanup - this can be performed in the hotcells or in the lab 306, depending on the sample radionuclide content. The dilution that will be required is primarily dependent on the nitrate/nitrite concentrations. To minimize the effect of matrix on citrate response and column stability, the combined concentration of the nitrate and nitrite should not exceed about 0.1M. For some samples, the dilution resulting from the SPE cleanup will suffice. If possible, obtain an estimate of the nitrate/nitrite concentration so that a reasonable dilution can be made.

- 3.11.1.1 Convert the as-received acid resin form SPE column to the sodium form by passing 1N NaOH through the column until the eluate pH is > 9 . Wash the column with reagent water until the eluate pH is < 9 . The SPE columns can be made up in the laboratory using the specified resin and appropriate columns.
- 3.11.1.2 Pipet 1.0 (± 0.05) mL, or other volume specified by the cognizant scientist, of the sample onto the column. The pH of most samples will be > 9 . This can be verified using pH paper. If it is not, adjust the pH to > 9 maintaining sample volume accountability. Catch the sample eluate in a 40-mL VOA vial. Record the sample volume on the sample bench sheet, or equivalent form. The amount of sample to use will depend on the sum of concentrations of nitrate and nitrite. If their concentrations are known, an appropriate sample volume can be calculated so that the final nitrate/nitrite concentration is $\leq 0.1M$.

For preparation of the MS/MSD samples, add an appropriate amount of the stock standard to the column just prior to addition of sample (the pH of the stock citric acid is satisfactory). The amount of standard to add will depend on the concentration of the citrate in the sample. The MS/MSD analyses will be used both as a method to estimate reproducibility, and to correct for matrix effects and analyte loss in sample preparation. Consequently, the spike concentration is recommended to be no lower than the lowest calibration level and at least as high as the citrate concentration, but no greater than 2 or 3 times the citrate concentration within the linear portion of the calibration range. Circumstances may make it impractical to spike according to the preceding recommendation. In such cases, add the citric acid spike (1000 μg spike if the dilution factor is 10 or 5000 μg spike if the dilution factor is 50) to the column just prior to addition of the sample. Record the amount of spike standard added and its concentration on the sample bench sheet, or equivalent form.

- 3.11.1.3 Wash the SPE column into the collection vial with 2-column volumes of reagent water. Cap the vial, and if the SPE operation is performed in the hotcells, transfer it (without dilution) to the organic group for further processing.
- 3.11.1.4 Adjust the sample pH to 2.0 (± 0.2).
- 3.11.1.5 Transfer the solution to a 15-mL disposable centrifuge tube and dilute to 10 mL. A greater dilution may be necessary. Record the final volume on the sample bench sheet.

If a precipitate persists at pH 2, transfer the supernate to the centrifuge tube, wash the precipitate with a small amount of reagent water, centrifuge the mix, and transfer the wash solution to the centrifuge tube. Repeat the wash, centrifuge, and transfer cycle twice.

3.12 HPLC Measurement

- 3.12.1 HPLC conditions - set up the HPLC according to conditions described below. Start the instrument, de-aerate the mobile phase, allow the column to come to equilibrium, and allow the DAD to stabilize. Operation of the instrumentation is described in the system manuals.

Column:	Aminex ion exclusion, HPX-87H
Column temperature:	40° C
Flow rate:	0.6 mL/min
Mobile phase:	0.01N H ₂ SO ₄
Injection volume:	25 µL
Detector wavelength:	210 nm

- 3.12.2 Transfer about 1-mL of processed sample from step 3.11.1.5 through a 0.45 micron syringe filter to a 1.7-mL autosampler vial and seal the vial with a crimp septum cap.

If the nitrate and nitrite concentrations are unknown and a relatively low dilution factor resulted (about 10 for example), a screen can be performed by injecting 2.5 µL of one of the samples prior to transferring all of the samples to the vials. If additional dilution is needed, dilute the samples appropriately, and transfer about 1-mL of the processed sample to autosampler vials. Record the dilutions used on the sample bench sheet.

- 3.12.3 The sealed standard and sample vials are loaded into the HPLC autosampler, and a sequence programmed such that a single point calibration is performed prior to every 4 or 5 samples. Provide each sample with a unique data file identity, and record this identification along with other sample information in the LC injection log. The instrument manual provides a data file naming protocol. As experience with the analysis is gained with these samples, the calibration frequency may be changed be increased or decreased.

- 3.12.4 Start the analysis as described in the system manuals.

- 3.12.5 Upon completion of the analysis sequence, review and correct the data as necessary.

- 3.12.6 Determine that all raw data are stored on the hard disk, and transfer pertinent data to the 3.5 inch disks for archive. Generate necessary hard copy of the data.

- 3.12.7 Calculate final results based on sample size and all dilutions and other sample manipulations performed.

4.0 REFERENCES

1. Grout Analytical Services Technical Project Plan, Revision 0, TPP 20749, March 1993.
2. J. A. Campbell, R. B. Lucke, K. E. Grant, S. A. Clauss: Application of LC and Thermospray LC/MS to the Study of Small Organic Acids in Radioactive Mixed Hazardous Wastes: Presented at the 48th Northwest Regional Meeting of the American Chemical Society, Laramie, Wyoming, 17 June, 1993. (Manuscript for publication in preparation.)
3. HEWLETT-PACKARD, HP 1090 Series II/L and /M Liquid Chromatograph, Part 1 and Part 2, HP Part No. 01090-90210, April 1990.
4. HEWLETT-PACKARD, HPLC Chemstation (Pascal Series), Fact Files and Handbooks, HP Part Nos. 79994-90031 through - 90035, April, 1990.

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