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Occurrence of Synthetic Amino-Polycarboxylate Chelating Compounds in Oregon Surface Waters

Nathan A. Hersey

Portland State University, natehersey@gmail.com

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OCCURENCE OF SYNTHETIC AMINO-POLYCARBOXYLATE CHELATING
COMPOUNDS IN OREGON SURFACE WATERS

BY

NATHAN A. HERSEY

A research project report submitted in partial fulfillment
of the requirement for the degree of

MASTER OF SCIENCE
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Project Advisor:
James F. Pankow

Portland State University
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ABSTRACT

Cation sequestering compounds are contained in many industrial, commercial and personal care products. The most widely used chelating agents, EDTA and DTPA, are commonly found in natural and industrial settings. When chelating compounds are present, the activity and transport of metal cations in the environment and the uptake of metals in natural systems can be affected. Surface water samples from across the United States were analyzed, as well as local collection and analysis of surface waters and wastewater treatment plant effluent from around Northwest Oregon. Results indicate presence of synthetic chelating compounds in areas with human activity, and absence in areas that are less populated. As a stable and water soluble compound, monitoring for chelates could have potential use as an effective indicator of further anthropogenic effects.

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1.0 INTRODUCTION

Production of synthetic chelating agents on an industrial scale has been occurring since the 1930s (Knepper, 2003). In 2002, global consumption of synthetic aminocarboxylate chelates was in excess of 330 million pounds (SRI, 2012). Aminopolycarboxylate chelating agents (APCAs), such as EDTA, HEDTA, PDTA, and DTPA (Figure 1), play the role of cation sequestering compounds in many industrial, commercial and personal care products. They bind metal ions to form stable complexes. As the most widely used chelating agents, EDTA and DTPA are commonly found in natural and industrial settings (Table 1).

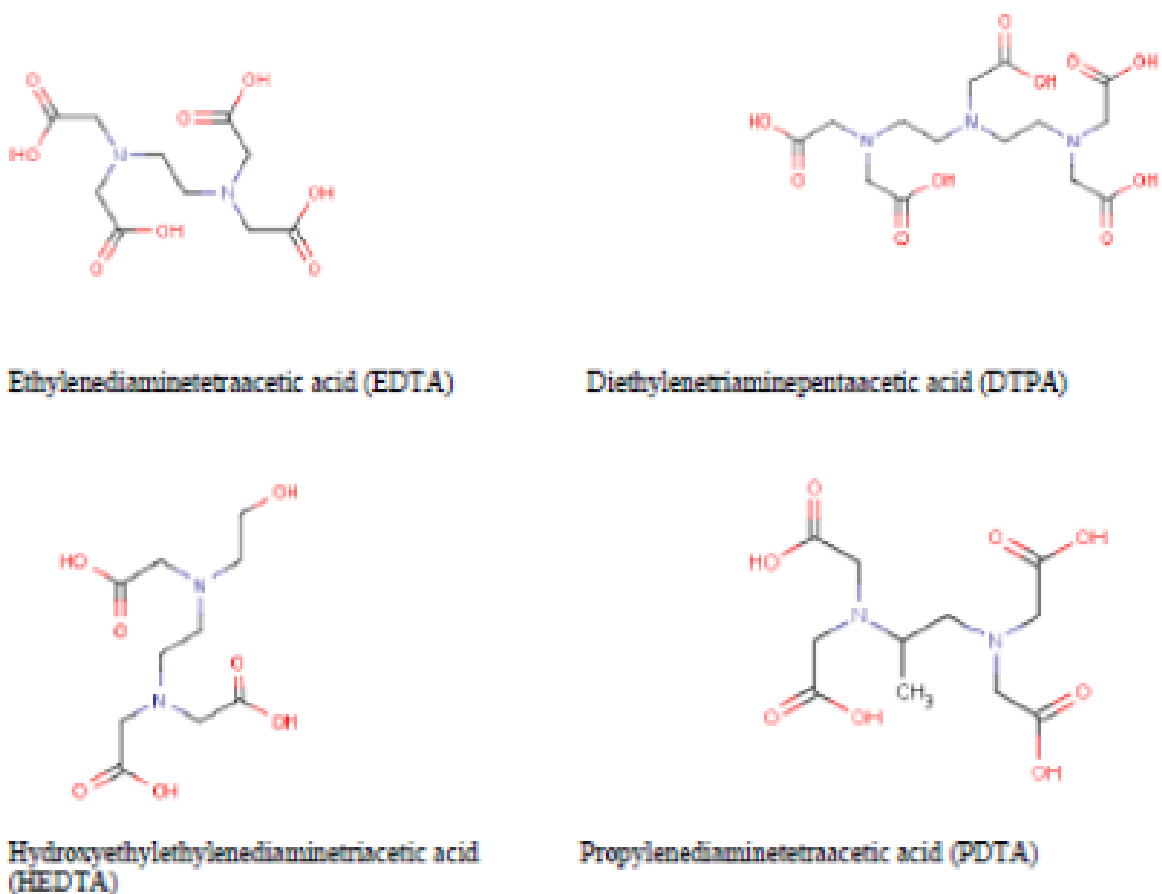


Figure 1. Synthetic Aminopolycarboxylate chelates. (Figures from ACTOR.EPA.gov)

Compound	Sampled Medium	Maximum Concentrations Observed	Analytical Method	Reference
EDTA	Surface Treatment Industrial effluent	1200 µg/L	HPLC and GC-MS	Pirkanniemi et al., 2008
	Groundwater	5.7 µg/L	GC-MS	Leenheer et al., 2001
	Industrial Discharge	> 50000 nM	HPLC-UV	Bedsworth and Selak, 1999
	Reclaimed Water	311 µg/L	GC-MS	Leenheer et al., 2001
	Surface Water	14 µM	GC-MS	Baken et al., 2011
	Surface Water	Detected	GC-MS	Vogel et al., 2003
	Surface Water	50 µg/L	GC-NPD	Sillanpää et al, 1996
	Surface Water	21 nM	HPLC-UV	Nowack et al., 1996
	Surface Water	3.7 µg/L (Median)	IC-MS	Reemtsma et al., 2006
	Surface Water	33 µg/L	IC-MS	Knepper et al., 2005
	Surface Water	5 µg/L	IC-MS	Bauer et al., 1999
	Surface Water	9.9 µg/L	LC-MS/MS	Quintana and Reemtsma, 2007
	Surface Water	5.2 µg/L	SPE with IC-MS	Kemmer et al., 2009
	Surface water, Downstream WWTP Effluent	210 µg/L	GC-MS	Barber et al., 2003
	WWTP effluent	240 µg/L	GC-MS	Barber et al., 2003
	WWTP effluent	702 nM	HPLC-UV	Nowack et al., 1996
	WWTP effluent	60 µg/L (Median)	IC-MS	Reemtsma et al., 2006
	WWTP effluent	970 µg/L	IC-MS	Knepper et al., 2005
	WWTP effluent	35 µg/L	LC-MS/MS	Quintana and Reemtsma, 2007
	WWTP influent	33 µg/L	LC-MS/MS	Quintana and Reemtsma, 2007
DTPA	Surface Treatment Industrial effluent	1400 µg/L	HPLC and GC-MS	Pirkanniemi et al., 2008
	Surface Water	20 µg/L	GC-NPD	Sillanpää et al, 1996
	Surface Water	1.1 µg/L	LC-MS/MS	Quintana and Reemtsma, 2007
	Surface Water	16 µg/L	SPE with IC-MS	Kemmer et al., 2009
	WWTP effluent	155 µg/L	IC-MS	Knepper et al., 2005
	WWTP effluent	13.3 µg/L	LC-MS/MS	Quintana and Reemtsma, 2007
	WWTP influent	20.3 µg/L	LC-MS/MS	Quintana and Reemtsma, 2007

Table 1. Past APCA Sampling Efforts Results

The application of APCAs in industrial processes and at the consumer level provides metal cation binding and sequestration necessary in industrial aqueous chemical processes and in household detergents (Oviedo and Rodríguez, 2003; Knepper, 2003). Industrial use of APCAs stems from the need to prevent metal precipitates in industrial processes (Knepper 2003). By sequestering the cations in a chelate complex, the negative effects of metals in process waters are generally minimized (Knepper, 2003). DTPA and EDTA are present in the industrial effluent of the electroplating and surface treatment industry, where they are employed as degreasing agents and to control metal precipitation during treatment (Pirkanniemi et al., 2008). In paper processing, APCAs (usually EDTA) are used to sequester transition metal ions prior to bleaching in order to decrease the use of hydrogen peroxide or other bleaching agents (Jones and Williams, 2002).

In cosmetic products, addition of chelates acts to enhance anti-microbial preservatives by interfering with the targets cell wall, allowing greater penetration of the active anti-microbial ingredients (Lundov et al., 2009). Similar use of EDTA in food products is effective in anti-bacterial applications; for instance the use of EDTA in conjunction with more conventional bactericides to prevent the growth of wide ranging bacteria types in meat and fish (Helander et al., 1997). By sequestering cations necessary for cellular function and cell wall growth, EDTA has been shown to have anti-fungal properties at levels around 1-2 mM (Holbein and Orduña, 2010; Brul et al., 1997). In food products where fatty acid oxidation is a concern, addition of EDTA in concentrations around 75 mg/kg can be added to prevent lipid oxidation (Nielsen et al., 2004). The mechanism of action as an anti-fungal agent is iron sequestration in the food product during storage (Nielsen et al., 2004).

In the human digestive system, excessive amount of EDTA may negatively impact necessary cation nutrient availability for absorption, however as an iron fortification in foodstuffs (administered as NaFe(III)EDTA), the effect on manganese and other nutrients uptake was not effected (Davidsson et al., 1998). The benefits and potential drawbacks employing APCAs in foodstuffs must be weighed when employing APCAs for iron fortification.

Chelation of heavy metals increases their uptake into agricultural plants (Vassil et al., 1998). Although this can be a positive effect when remediating soil, the use of chelates in agricultural applications for food crops could lead to increased concentrations of heavy metals in the food supply. Application of large quantities of chelates for soil remediation does not only remove the detrimental compounds, but iron as well (Sun et al, 2001). The longevity of EDTA in the environment is an undesirable characteristic in this use, but the changeover to more biodegradable options in this industry is slow (Jones and Williams, 2002).

Fe-HEDTA has been approved for use as a pesticide in the U.S. (EPA, 2008). Application against broad leaf plants, algae and mosses will allow for increased use at the commercial and consumer level. Although the mechanism of action is unclear, the possibility exists for Fe-HEDTA to increase iron uptake into plants in a less bio-available (chelated) form or excessive Fe may produce reactive oxygen species that lead to cellular damage (Environment Canada, 2010).

When chelating compounds are present, the activity and transport of metal cations in the environment as well as the uptake of metals natural systems is effected (Nowack, 2002). As the variety of applications for APCAs continue to grow, the concentrations in the natural environment can only increase. In order to facilitate and hasten bio-remediation of soils contaminated with divalent and trivalent cations, addition of EDTA to a remediation site has been employed (Wu et al., 2003). Soil washing (the practice of heavy metal removal by

application of chelates to the subsurface) involves concentrations of EDTA of up to 10^{-2} M (Kedziorek and Bourg, 2000). Although the majority of applied APCAs are removed following in-situ soil remediation, some will remain to eventually transfer into surrounding surface and ground waters. Application of synthetic chelates poses the possibility of altering subsurface transport of heavy metals long after any remediation effort has been completed (Wu et al., 2003). Land application of industrial wastewaters with high concentrations of APCAs may pose a public health risk (ATSDR, 2007). Even when low levels of heavy metals are present in the original discharge, the chelates complex with pre-existing sub-surface deposits. This re-suspension can increase concentrations of toxic heavy metals in drinking water sources down gradient from disposal sites in the same aquifer. Chelates are highly soluble, land application risks increased heavy metal runoff during storm events.

In 2008 the European Union issued their water framework directive and identified EDTA as substance under review for inclusion in their priority substance list (EU, 2008). A high add rate into the environment is only one side of the coin. The majority of the strongly-complexing chelating agents are resistant to biological degradation (Nörtemann, 2005; Lucena, 2003). Due to low rates of natural removal, presence of APCAs in natural waters has been demonstrated in numerous local studies (Vogel et al., 2003; Barber et al., 2003; Kari and Giger, 1996). These compounds are also stable in natural surface waters. Generally UV degradation is the main loss mechanism, although limited biotic degradation is possible. The main loss path for EDTA is through photo-degradation of the Fe-EDTA complex (Bucheli-Witchel and Egli, 2001).

With the low biological degradation rates of EDTA, it is no surprise to find no removal of EDTA in some WWTPs (Alder et al., 1990). EDTA in has been show to destroy some microbiota that normally would degrade organics in the environment (Oviedo and Rodriguez, 2003). The low

rates by biodegradation of other APCAs lead to slow removal of them as well. EDTA, DTPA and PDTA are resistant to removal in the secondary treatment systems of wastewater treatment plants (WWTPs) (Oviedo and Rodriguez, 2003; Nortemann, 2005). By increasing solubility of cations, the removal efficiency of potentially harmful heavy metals in WWTPs can be adversely effected (Bedsworth and Sedlak, 2001). Removal of EDTA from WWTPs requires specific additional actions in terms of microbial community and treatment processes (Nörtemann 1999). DTPA removal by biotic pathways (as a sole carbon source) has not been found to occur (Nörtemann 1999).

Environmental effects of chelates are independent of their low toxicity. Their solubility and environmental persistence are the characteristics that are relevant in the environment (Knepper 2003). The thermal and biotic stability of the chelates that are desirable during their employment in industrial and commercial use are negatives when the compounds are released to the environment (Knepper, 2003). When one population-centers wastewater becomes the next town's drinking water supply, the high polarity and solubility of these compounds and the low removal in conventional wastewater treatment allows passage into the drinking water supply (Knepper, 2003).

Land application of industrial wastewater and bio-solids has leached APCAs into drinking supplies and led to the discontinuation of use of private wells in Sturgis, Michigan (ATSDR, 2007). The threat is not from the chelates themselves, but from the increased metal concentration in the effected drinking water supply (ATSDR, 2007). As a drinking water constituent, although not toxic, anthropogenic chelates should not be present (Nörtemann 1999). In locations where reclaimed wastewater is used to recharge aquifers, the presence of synthetic APCAs can be used as a tracer to determine the presence of anthropogenic compounds (Leenheer

et al, 2001). In drinking water, pretreatment with UV and hydrogen peroxide in the presence of EDTA may lead to the production of compounds that reduce the effectiveness of subsequent chlorination steps (Oviedo and Rodríguez, 2003).

Synthetic polycarboxylates chelates in water have been analyzed using derivatization followed by GC/MS (Yoshinori and Okumura, 1995; Sillanpää et al., 1996). The GC/MS method is sensitive, but requires a time consuming sample preparation process. High-performance liquid chromatography (HPLC) and ion chromatography (IC) are also popular method for chelating agent analysis, but due to high detection limits HPLC and IC methods are not directly used for environmental water samples. Solid-phase extraction and/or other concentration methods are required when HPLC and IC methods are employed for environmental relevant concentration detection. HPLC/MS and IC/MS are gaining in popularity over the past few years, especially by tandem mass spectrometry (Bauer et al., 1999; Knepper et al., 2005; Kemmei et al., 2009; Quintana and Reemtsma, 2007). Analysis by IC-ESI-MS of EDTA and DTPA is described by Knepper et al. (2005). The IC-ESI-MS method allows for analysis of sampled media that contain high salt concentrations and background organics and allows analysis down to 1µg/L with simple sample preparation (Knepper et al., 2005). Ion chromatography with electro-spray mass spectrometry for EDTA and other organics is also employed by Bauer et al. (1999).

In this study, water samples were collection from multiple surface water bodies throughout the United States as well as WWTP effluent and other water sources. EDTA, HEDTA, PDTA and, DTPA were analyzed using LC-ESI-MS/MS. Sample stability studies were conducted to determine the appropriate holding times for the sample and analysis methods, and samples were analyzed for concentration of common APCAs.

2.0 EXPERIMENTAL

2.1 MATERIALS AND STANDARDS

N-(2-hydroxyethyl)ethylenediaminetriacetate (HEDTA), 1,3-Propylenediamine-N,N,N',N'-tetraacetic acid (PDTA), Diethylenetriaminepentaacetic acid (DTPA) , Ethylenediaminetetraacetic acid tetrasodium salt dihydrate (EDTA), and Iron(III) chloride hexahydrate were obtained from Sigma-Aldrich (St. Louis, MO). EDTA-d₁₂ was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). All of the above chemicals have purity of 98% or better. Tributylamine at 99.5% and sodium thiosulfate pentahydrate at 99.5% purity were purchased from Sigma-Aldrich (St. Louis, MO). All solvents, acetic acids, and LC-grade water used were purchased from Honeywell Burdick & Jackson (Morristown, NJ). Water used to make standard and other solutions was from a Millipore system (Billerica, MA) at 18 MΩ.

Standard mixture stock solutions at 600 mg/L for each individual compound in water were prepared. Due to the solubility properties of the acid form of the chelates, they were dissolved in 0.1N NaOH solution before diluting to the volume. Stock solutions of EDTA, HEDTA, PDTA, and DTPA at 6 mg/L were diluted from the standard solutions. The six calibration standards between 1 and 100 µg/L were diluted from the 6 mg/L standard mixture. EDTA-d₁₂ at 5 mg/L was prepared by first dissolving in 0.1N NaOH solution and then diluting to the volume.

2.2 SAMPLING

Discrete field water samples were collected by USGS scientists from Rapid Creek, Lower French Creek, and Spring Creek in the Black Hills of South Dakota. Samples were also collected from the Tallapoosa and Schuylkill Rivers in Alabama and Pennsylvania, respectively during August 2009 to October 2009. Water samples from Mississippi river at 11 distinct sites from Minnesota

to Louisiana were collected in February, 2010. All samples were maintained in coolers with ice or blue ice and shipped to Portland State University by FedEx overnight service. Water samples from river, creeks, wastewater treatment plant effluent in Portland, Oregon and tap water from Portland State University were collected in July 2009. Sampling sites for river and creek samples were sites used by USGS for regular water quality monitoring. Samples were stored at 4 °C at Portland State University until analysis.

Samples were collected from the Tualatin River (Oregon) by weighted polyethylene bottle sampler in August, 2011. These samples and were collected over five transects along the width of the river and combined in a clean polyethylene churn. Following mixing the samples were placed in clean 40ml brown glass vials with Teflon caps for transport to the laboratory, where they were stored at 4 °C prior to analysis.

2.3 ANALYTICAL METHODS

Prior to analysis, all samples were filtered using a 0.2 µm PTFE membrane, 15 mm syringe filter (Phenomenex, Torrance, CA). A 1mL sample or calibration standard was pipetted into a 2mL glass HPLC vial and spiked with 10 µL of HPLC grade concentrate acetic acid, 6 µL of 5 mg/L of EDTA-d12 (as internal standard), and 10 µL of 2 mmol of FeCl₃ solution. The vial was closed and mixed using a vortex mixer for 1min. The prepared samples or standards were stored in dark for 2 hours before LC/MS/MS analysis.

LC-MS/MS Apparatus and Analysis

The LC/MS/MS system for this study is a high-resolution Thermo LTQ-Orbitrap Discovery hybrid mass spectrometry coupled with an Accela auto-sampler and high speed LC system (San Jose, CA). It is equipped with an electrospray ionization source operating in positive or negative mode. The Orbitrap was externally calibrated prior to data acquisition allowing accurate mass

measurements to be obtained within 4ppm. Nitrogen for the instrument was provided by liquid N₂ tank (Airgas, Radnor, PA).

The Fe(III) complexes were separated using a Luna 3µm Phenyl-hexyl 2x150mm LC column (Phenomenex, Torrance, CA) at flow rate of 200µL/min and column temperature of 30 °C. A Phenomenex SecurityGuard guard column was used to protect the analytical column. Sample injection volume was 50 µL. The mobile phase is methanol/water with tributylamine as an ion-pair reagent. Both mobile phase eluents contained 2 mM of tributylamine and 2 mM of acetic acid. The gradient was adjusted as necessary to achieve separation. Analytes were detected as the Fe(III) complexes by a Thermo LTQ Orbitrap electrospray ionization mass spectrometry (ESI-MS) operated in the negative ion mode. The negative ESI-MS spectra of the Fe(III) complexes give the mono-valent molecular anions: [Fe(III)EDTA]⁻ (*m/z* 344), [Fe(III)HEDTA]⁻ (*m/z* 330), [Fe(III)PDTA]⁻ (*m/z* 514), and [Fe(III)DTPA]⁻ (*m/z* 445). The product ions are dominated by decarboxylations and loss of CO.

The method detection limits (MDLs) were evaluated by analysis seven replicates at 1.0 µg/L prepared in reagent water and run as samples. The MDL values were calculated as three times the standard deviation of this measurement. The method detection limits are 1 µg/L for the analysis of EDTA, HEDTA, PDTA, and DTPA.

3.0 RESULTS AND DISCUSSION

In order to allow for rapid analysis of low levels of these compounds in natural waters, an ion-pair liquid-chromatography tandem mass-spectrometry method for the synthetic polycarboxylate chelates EDTA, HEDTA, PDTA and DTPA adapted from Quintana and Reemsta (2007). The target APCAs are complexed with Fe(III) to form Fe(III)-chelate complex. The Fe(III)-chelate complex was separated and analyzed by ion-pair LC/MS/MS. The method is applied to analyze water samples of wastewater treatment plants, rivers, lakes, creeks, and finished drinking water.

Sample Stability Studies, Holding Time

The chelates in this study are generally subject to UV degradation. Bacterial degradation is not a major loss pathway for these compounds in the environment. When appropriate actions are taken to minimize light exposure, no significant loss of target analytes should occur. In order to determine the length of time a sample could be held prior to analysis, stability studies were undertaken in various media. Media included laboratory reagent water, groundwater, river water from the Willamette, wastewater effluent from a WWTP with conventional treatment processes and wastewater impacted samples from downstream of the effluent discharge in the mixing zone Tryon Creek WWTP on the Willamette River. All stability study media samples were collected as grab samples in clean one liter brown glass bottles with Teflon caps. Samples bottles were originally filled to no residual air volume and stored at 4 °C. For WWTP effluent treated with chlorine, sodium thiosulfate (80 mg/L) was added at the time of sample collection.

All media were spiked to known concentration levels, and the initial concentrations were the sum of spiked concentrations and occurrence of analytes in the collected media. Standard solution was spiked as either 4 mL or 8 mL of 6 mg/L standard mixture into each of the 1L samples bottles. Samples were shaken, mixed and allow to stand for two hour before filling samples into 40 mL brown glass sample vials with Teflon lined caps. Seven spiking replicates were prepared

for each sample media to determine their initial concentration. Fifteen 40 mL vials were filled for each media and stored at 4 °C for the analysis in the coming weeks. Three samples were analyzed each week for four weeks and three vials of samples were held as backup.

For EDTA and DTPA, the recoveries during the four weeks, range from 95 to 110%. The chelates are stable at 4 °C up to four weeks without preservatives for most water samples. Some measured increase in PDTA and DTPA concentrations over time in the wastewater effluent samples during the sample stability analysis was observed. The potential explanations are:

- a) since the wastewater effluent samples were not filtered before they were spiked, some of the PDTA and DTPA strongly partitioned to organic constituents of particulate matter and released back into the solution after period of time or
- b) Co-elution of other organics along with the target analytes may lead to suppression of the expected analyte response in the subsequent ESI-MS; the possibility exists that the organic matter in the sample breaks down or changes, which would lead to less matrix effect and an increase in observed concentrations.

It should be noted that the wastewater effluent was spiked and then allowed to sit for a 5 week period. Visible particulate was noted in the bottom of some vials of wastewater effluent. Large standard deviations for DTPA and PDTA during analysis are partially the result of poor peak shape. These poor peak shapes are seen most often in sample media with low concentrations and high organic content, mostly wastewater effluent for PDTA and DTPA.

Standard Addition Recovery and Standard Stability

In various collected samples and tested media, standard addition recovery over time was investigated. Standards of all analytes in concentrations of 25ppb or 50 ppb were spiked into the samples. The concentration of both spiked and the original media were determined. Some

standards at 25ppb and 50 ppb were also analyzed one week following preparation to evaluate the stability of the chelate complex. This investigation demonstrated the Fe(III)-chelate complexes are stable for at least one week.

PH and Ion Strength Effect

Reducing sample pH to 2.0 by addition of HCl is a common preservation method for water samples by USGS and EPA. At sample pH of 2, very poor peak shape and significantly shifted retention time were observed during initial sample analyses. In some initial investigations, the chromatographic base line was found drift as well. When the low PH samples were neutralized to PH=7 using NaOH, the determined concentrations were similar to those observed at low PH with a more well defined peak shape. Retention time shift was observed for samples neutralized with NaOH. Since the prepared samples contained 10 μ L of concentrated acetic acid, the pH value for the prepared samples were lower than the received samples. The observed less than ideal peak shape and retention time shift may not have been caused directly by low pH, but higher ionic strength of the sample. Three 40mL volumes of reagent water samples spiked with 25 ppb EDTA were treated with 25mg, 50 mg, and 100mg of sodium chloride respectively. When they were analyzed under the same conditions as the samples, the same poor peak shape and retention time shift was seen. This indicates the stronger ion strength due to the sample acidification and/or neutralization is the actually cause. If the sample contains large amount of electrolytes, it may cause signal suppression for MS (Garcia, 2005). Salts may also deposit in the ion source, which would cause diminishing accuracy of the analysis (Garcia 2005). Based on the results of this study, samples for the analysis of chelates should not be preserved by adding strong acid when employing this analytical method.

Matrix Impact

High organic matter (OM) concentration in samples may lead to poor LC performance and also cause MS signal suppression. Quintana and Reemtsma (2007) found strong matrix effects from co-elution of unknown matrix constituents. They adapted a slower gradient and used ^{14}C labeled analytes as internal standard. By analyzing diluted samples, OM impact was minimized. The analyte concentrations in diluted samples were found to be higher than the non-diluted results. For water samples with high organic carbon content, poor peak shapes and base line were also observed. For the original WWTP effluent samples, 10-50% signal suppression was detected, especially for DTPA and PDTA. Following dilution by a factor of two, signal suppression was still observed for some WWTP effluents.

Two possible solutions were proposed for high organic matter samples. The sample can either be run through a C18 SPE cartridge to remove the organic matters or diluted with reagent water. In media with high expected concentrations of the target analyte, dilution is preferred. A 10 to 1 dilution is sufficient for accurate measurement in WWTP samples. For environmental samples, 1:2 dilutions with pure water are sufficient. For samples with low chelate concentration and high organic content, C18 SPE was used to remove the organic content. C18 pre-treatment has been used as successfully when dealing with naturally occurring organic acids (Kim et al., 2003; Hogendoorn et al., 1999; Ahmad et al., 2002).

For C18 pre-treatment, water samples were first passing a C18 cartridge at 1 to 2mL/min. The collected samples were then prepared and analyzed as regular samples. If SPE is the chosen sample pre-treatment, additional time and material expense are required. In order to ensure the final results are not affected, care must be taken in the SPE and the samples must be subjected to the same driving force and time spent traversing the SPE cartridge. However, if the expected

environmental concentrations are low, SPE pre-treatment is advisable, especially for the analysis of DTPA and PDTA.

During Environmental sampling, stream conditions at the sample site were noted in terms of general turbidity. For clear running streams and analytical waters, the samples were analyzed without dilution or SPE filter. WWTP samples were either diluted or filtered through a C-18 SPE cartridge. SPE minimized the matrix effects on the sample resulting from dissolved carbon, especially in the form of compounds containing carboxylic acid that interfered with analysis of the compounds of interest. For wastewater effluents, dilutions of 1:10 were necessary. This did not significantly degrade analytical capability, since the analyte concentrations in these sampled media are very high. For surface waters, generally 1:2 dilutions were employed.

Environmental and WWTP Effluent Sampling Occurrence Data

Generally, the chelate concentration is a function of addition from anthropogenic sources and the dilution in the water body. Since addition near point sources are limited to the immediate area, samples taken further downstream contain lower chelate concentrations. The two samples near Hastings, MN, are illustrative (Table 2). The samples were analyzed at 30.6 and 3.5 ppb respectively. The flow in the river at these two sample times was a factor of 10 greater for the lower concentration (81,500 CFS vs 8,000 CFS) as measured at the nearby USGS gauging station.

Synthetic Chelate Concentrations Mississippi River ($\mu\text{g/L}$)						
Sample Location	EDTA	HEDTA	PDTA	DTPA	Flow (CFS)	EDTA (kg/hr)
Mississippi river at Clinton, IA	11.7	ND	1.2	0.9	42300	51
Mississippi river at Hastings, MN	30.6	ND	ND	0.9	8000	25
Mississippi river at Hastings, MN	3.5	ND	ND	ND	81500	30
Mississippi river at Thebes, IL	6.3	ND	ND	ND	487000	310
Mississippi river below Grafton	8.4	ND	ND	ND	300000	260
Mississippi river at Memphis, TN	5.6	ND	ND	ND	-	-
Mississippi river at Nodena, AR	4.1	ND	ND	ND	-	-
Mississippi river at Luxora, AR	4.8	ND	ND	ND	-	-
Mississippi river at Baton Rouge, LA	5.7	ND	ND	ND	578200	340
Mississippi river at Belle Chasse, LA	5.2	ND	ND	ND	1020000	540
Mississippi river at St Francisville, LA	3.3	ND	ND	ND	632800	210

Table 2. Samples retrieved from the length of the Mississippi River.

Although it may be possible to achieve input into streams from runoff, the majority of input is likely wastewater treatment effluent discharge. For EDTA, all of the wastewater treatment effluent measured was in the hundreds of parts per billion (Table 3). Samples taken downstream of WWTP effluent discharge points generally reflect this anthropogenic input (Tables 3, 4). In random sample locations across the country, EDTA was noted between non-detectable levels to nearly 100ppb (Tables 2,3,4). For HEDTA, PDTA and DTPA the concentrations were less, which reflects their lower usage rates in consumer products.

Synthetic Chelates Occurrence Portland Area (µg/L)				
Sample Location (Lat, Long)	EDTA	HEDTA	PDTA	DTPA
WWTP A Effluent	210.7	4.2	3.2	33.7
WWTP B Effluent	600.8	4.1	1.7	59.1
WWTP C Effluent	214.5	2.9	3.1	30.5
Lab tap water at PSU	ND	ND	ND	ND
Drinking water at SB2, PSU	ND	ND	ND	ND
East Fork Dairy Creek (45.501N, -122.995W)	ND	ND	ND	ND
Rock Creek (45.497N, -122.949W)	4.5	ND	ND	0.8
Beaverton Creek (45.496N, -122.844W)	5.6	ND	ND	ND
Oswego Dam (45.411N, -122.665W)	28.3	ND	ND	1.1
Oregon City, 30cm (45.359N, -122.610W)	ND	ND	ND	ND
Mciver Park, 30cm (45.300N, -122.353W)	ND	ND	ND	ND
Willamette River, 30cm (45.512N, -122.669W)	3.8	1.8	ND	3.1
Upstream Cook Park (45.394N, -122.771W)	9.4	ND	ND	ND
Downstream Cook Park (45.393N, -122.764W)	36.3	2.0	ND	1.6
Commonwealth Lake (45.513N, -122.806W)	1.3	ND	ND	1.1
Tualatin Hills Nature Park (45.498W, -122.847N)	2.8	ND	ND	0.8
Smith Lake, Winter (45.614N, -122.719W)	0.8	ND	ND	0.6
Columbia Slough Summer (45.611N, -122.755W)	ND	ND	ND	ND
Blind Slough Summer (45.610N, -122.750W)	ND	ND	ND	ND
Smith Lake, Summer (45.611N, -122.741W)	ND	ND	ND	ND
Reagent Water	ND	ND	ND	ND
OR Well Water	1.3	ND	ND	ND
Willamette River, Winter (45.512N, -122.669W)	3.0	ND	ND	ND
WWTP D Effluent Sample 0	203.7	ND	ND	3.0

Effluent Samples Were analyzed following a factor of 10 Dilution.
Table 3. Samples From the Portland Metropolitan Area.

Synthetic Chelate Concentrations National Sampling (µg/L)				
Sample Location	EDTA	HEDTA	PDTA	DTPA
Tallapoosa River near Montgomery, AL	ND	ND	ND	ND
Site 5 Rapid City, SD	ND	ND	ND	ND
Site 4 Rapid City, SD	10.4	ND	ND	ND
Site 3 Rapid City, SD	0.9	ND	ND	ND
Site 2 Rapid City, SD	95.4	ND	2.2	4.1
Site 1 Rapid City, SD	ND	ND	ND	ND
Schuylkill River, PA	10.8	ND	1.9	1.1
Finished Water, Schuylkill River, PA	4.3	ND	ND	ND

Table 4. Samples Obtained throughout the US by the USGS.

The Tualatin River flows from the coast range through the western suburbs of the city of Portland. There are multiple uses along the river including agricultural, commercial, and residential as well as multiple discharges from municipal wastewater treatment plants. Samples were collected during the low flow season (late summer) (Table 2). Minimum flows on the river at this time of year are as low as two CFS. In order to minimize nutrient input into the river at this time, the discharge permits of the WWTPs indicate that tertiary (advanced) treatment is required for nutrients. The Tualatin receives municipal wastewater treatment with periodic effluent volume changes, due to the smaller size and residential nature of the communities served by the WWTPs. Transit times along the river are slow, due to the extremely low summertime flows, allowing for significant diffusion of added waters.

Discharges from the WWTPs without advanced treatment are routed to the facilities that provide these advanced treatments. During the low flow season only Rock Creek and Durham WWTP discharge to the Tualatin River. The nature of the river at these two discharges is different in relation to the flow at the discharge points. Both are discharged to the middle of the river at the bed through diffusers. Upstream at the Rock Creek plant, the output from the WWTP effluent is a significant source of flow into the river. Flow from the outlet diffuser is obvious to the casual observer and the difference between flow from effluent and from the river is distinct for at least 200 meters. The river eventually mixes after two shallow reaches, where the increase velocity creates turbulence that enhances the mixing. The volume in the river and the river depth at the Durham outlet is greater. There is no visible effluent; however there are two shallow reaches downstream that serve to enhance mixing. Two S bends serve to mix longitudinal segments of the Tualatin downstream of the Durham effluent. Samples collected to represent the river downstream of both WWTPs were taken after sufficient distance to assume complete mixing.

Chelates are highly soluble, and studies in natural waters show less than 4% of EDTA is sorbed to sediment (Nowack et al., 1996). Sample collection methods were such that only occurrence data at the sample locations is known. Although the streams sampled can be assumed to have good mixing, without flow values at the sample sites, the overall environmental concentrations in the rivers and streams are not calculated nor reported.

The most upstream sample location (Gaston) in this study yielded low but measureable chelate concentrations (Table 3). There are little industrial or other inputs in this reach of the river; however there is some agricultural and residential activity. Further downstream the Scoggins creek was sampled. This water is fed by a reservoir at Henry Hagg Lake and there is little activity, save a lumber mill and some limited agriculture in the area. The absence of chelates indicates that with reduced anthropogenic activity, there can be no measurable chelate addition. The highest observed concentrations are observed downstream of the discharging WWTPs. Upstream and downstream locations for these areas were sampled. The residential area around Rock Creek showed low incidence, as well as the upstream area at Rood Bridge. Significant measurable concentrations of synthetic chelates are demonstrated downstream of the Rock Creek WWTP effluent discharge. Similar, but less pronounced effects are observed for the Durham plant. By the outlet of the Tualatin at the Willamette (Weiss Bridge), chelate concentrations were observed at lower concentrations due to dilution.

4.0 CONCLUSIONS

Due to the anthropogenic nature of synthetic APCAs, they offer the possibility as a human activity indicator when detected in surface water bodies. In areas with no surface sources, the possibility of subsurface sources must be considered. Leaking septic systems in shallow aquifers can contribute to potential human health hazards (Swartz et al., 2006). As groundwater makes its way through to surface water and to potential drinking water supply, an efficient method for determining the potential for contamination from these sources is valuable. With the declining state of municipal infrastructure, the possibility for infiltration from rainfall into sewerage exists (Shelton et al., 2011). By using chemical markers in sewage, Shelton et al. (2011) demonstrate the ability to determine these phenomena on a large scale. The research community has the potential to use APCAs as wastewater indicator compounds in surface waters. The need for this is demonstrated by attempts to use all manner of organic anthropogenic compounds such as artificial sweeteners, caffeine, and pharmaceuticals. An inexpensive analysis for an anthropogenic tracer compound in similar studies would be a boon to the research community. Analysis of synthetic aminocarboxylate chelates EDTA, HEDTA, PDTA and DTPA in natural waters by LC-MS/MS has been presented. Sample pre-treatment by dilution or SPE can minimize the effects of high organic content in the sampled media. The compounds amenable to this method are not in and of themselves a threat to human health or the fishability or swimability of a given river reach. It is their nature as indicators of anthropogenic activity that makes them valuable. The low cost and ease of analysis can inform researchers of the likely presence of wastewater or agricultural runoff that may contain compounds that threaten the health of a stream. The relatively long half life and high solubility of the synthetic aminocarboxylates mimics the nature of compounds that are of greater interest to the regulatory and scientific community.

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