



## **D I P L O M A R B E I T**

# **Trace Metal Speciation in Natural Aqueous Samples using a Solid Phase Extraction Flow Injection System on-line coupled to ICP - AES**

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## **Abstract**

Speciation analysis has gained in importance during the last years. This can be attributed to the increasing awareness of the influence of speciation of trace metals on their ecotoxic potential and their availability for biological and chemical processes like bioaccumulation or photooxidation. Therefore, analytical methods with which it is not only possible to quantify trace metals, but also to distinguish between different species, have to be developed.

Miscellaneous procedures for trace metal speciation have been previously described, but they share several drawbacks. Many methods are able to isolate one or more trace metal fractions, but for a determination of the total metal content supplemental measurements are inevitable. Furthermore, they consist of several steps necessitating complex sample pretreatment and thereby creating additional possibilities for contamination or handling errors.

The presented speciation method employs a Flow-Injection (FI) system consisting of three cartridges packed with different Solid-Phase-Extraction (SPE) sorbents, namely a reversed phase (RP) sorbent, a weak anion exchange (WAX) material and a strong cation exchanger (SCX). By passing the sample sequentially through these cartridges, the corresponding complexes are retained and thereby separated into a neutral, an anionic and a cationic fraction. Afterwards they are eluted directly into the nebulizer unit of the detecting Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES). The resulting transient signals of the elution profile are recorded and evaluated to acquire both the total metal concentrations and the distribution into the three mentioned classes in one measuring run. By aiming for a higher degree of automatization, the presented method minimizes potential sources of error. Due to the occurring preconcentration, enhanced detection limits could be achieved for the investigated elements Mg, Al, Ca, Cr, Mn, Fe, Co, Cu, Zn, Cd, Ni and Pb, which were analyzed simultaneously.

The accuracy of the method was verified using standard addition series and the applicability of the technique to molten snow samples was demonstrated. The results showed proper accordance to previously published values for the trace metal contamination of snow on urban sites.

## **Zusammenfassung**

In den letzten Jahren hat die Speziationsanalytik stark an Bedeutung gewonnen. Dies liegt an dem gesteigerten Bewusstsein darüber, dass das ökotoxische Potential und die Verfügbarkeit der Spurenmetalle für biologische wie auch chemische Prozesse wie Bioakkumulation oder Photooxidation stark von ihrer Speziation abhängen. Aus diesem Grund ist es notwendig analytische Verfahren zu entwickeln, die nicht nur imstande sind diese Spurenmetalle zu quantifizieren, sondern auch zwischen den verschiedenen Spezies zu unterscheiden.

Verschiedene Verfahren zur Speziation von Spurenmetallen wurden beschrieben, jedoch teilen sie alle diverse Nachteile. Viele Methoden sind zwar imstande eine oder mehrere Fraktionen zu isolieren, doch die Bestimmung der Gesamtgehalte erfordert ergänzende Messungen. Des Weiteren bestehen sie aus einer Vielzahl einzelner Arbeitsschritte und schaffen dadurch zusätzliche Möglichkeiten der Kontamination oder Bedienungsfehlern.

Das hier vorgestellte Speziationsverfahren benützt ein Fließinjektions- (FI) System, welches aus drei, mit verschiedenen Festphasenextraktions- (SPE) Materialien gefüllten Kartuschen besteht. Bei den eingesetzten SPE-sorbents handelt es sich um ein reversed-phase Sorbent (RP), einen schwachen Anionentauscher (WAX) und einen starken Kationentauscher (SCX). Durch das aufeinanderfolgende Durchpumpen der Probelösung durch die Kartuschen werden die entsprechenden Metallkomplexe adsorbiert und dadurch in die Fraktionen neutral, anionisch und kationisch aufgetrennt. Anschließend erfolgt die Elution direkt in den Zerstäuberteil des detektierenden ICP-AES (induktiv gekoppeltes Plasma – Atomemissionsspektrometrie) Gerätes. Das resultierende transiente Signal des Elutionsprofils wird aufgezeichnet und ausgewertet, wobei sowohl der Gesamtgehalt des entsprechenden Elements als auch die Verteilung in die drei oben genannten Fraktionen aus einer einzigen Messung bestimmt werden können. Durch den hohen Automatisationsgrad der Methode werden zudem potentielle Fehlerquellen eliminiert. Aufgrund der stattfindenden Aufkonzentrierung wurden verbesserte Bestimmungsgrenzen erreicht. In dieser Arbeit wurden die Elemente Mg, Al, Ca, Cr, Mn, Fe, Co, Cu, Zn, Cd, Ni und Pb simultan analysiert.

Die Richtigkeit der Methode wurde mittels Standardadditionsverfahren überprüft und die Anwendbarkeit des Verfahrens auf geschmolzene Schneeproben wurde dargestellt. Die Ergebnisse zeigten gute Übereinstimmung mit Literaturwerten.

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# 1 Introduction

## 1.1 General Background

The term “speciation analysis” is employed for a whole array of analytical targets and techniques ranging from the distinction between different isotopes of the same element<sup>[1]</sup>, different electronic and oxidation states<sup>[2]</sup>, inorganic compounds and complexes, complexes with organic ligands, organometallic compounds, to the discrimination of macromolecular complexes and proteins, such as heme proteins<sup>[3]</sup>.

In environmental and especially aquatic systems, the determination of trace metals and assessment of their toxic potential cannot be reduced to the measurement of their total concentration. The reason for this is that trace metals tend to be complexed by organic and inorganic ligands, changing their chemical form and thus affecting their properties, including the chemical reactivity and biological availability<sup>[4],[5]</sup>, *e.g.* by reducing the reactive, available species through complexation<sup>[6]</sup>. On the other hand, increased lipid solubility of hydrophobic complexes enhances the potential for bioaccumulation and -magnification. The main parameters influencing species distribution are the concentration and electronic state of the respective metal ions, the pH, ionic strength and redox potential of the solution and the concentration and type of available ligands, including dissolved organic carbon (DOC).

It is widely accepted that the most chemically reactive and biologically available form of the majority of trace metals (*e.g.* Cd, Cu, Pb, Zn) in aquatic environments is the free, or rather the hydrated ion<sup>[5],[7]</sup>. As a consequence, the presence of dissolved organic ligands (including humic substances) reduces the occurrence of free ions by forming soluble complexes with the respective ions, thus decreasing the amount of available reactive trace metal ions<sup>[8],[9]</sup>. On the other hand, due to their ability to permeate cell membranes, the methylated forms of Sn and Hg have been found to be more toxic than their ionic forms<sup>[10]</sup>.

In all cases however, the toxicity of trace metals is directly related to their chemical form and thus influenced by the ambient environmental conditions.

## 1.2 Current Status of Research

Numerous analytical techniques have been applied to determine the speciation of trace metals in different environmental matrices as soil<sup>[11]</sup>, coastal seawater<sup>[12],[13]</sup>, pore water from sediments<sup>[14]</sup> and natural waters<sup>[15],[16]</sup>, as well as different beverages as wine<sup>[17]</sup> and milk<sup>[18]</sup>.

The employed analytical approaches varied from electrochemical separation procedures, comprising voltammetric methods like anodic stripping voltammetry (ASV)<sup>[19],[20],[21]</sup> and potentiometric methods, including ion-selective electrodes<sup>[22],[23]</sup>, to separation techniques like solvent extraction<sup>[24],[25],[26]</sup> and chromatographic approaches like gas chromatography<sup>[27],[28]</sup> or liquid chromatography<sup>[29]</sup>.

In comparison among each other, the procedures based on gas or liquid chromatography are most simple and suitable for speciation studies in water<sup>[29]</sup>. Those investigations rely on the separation of the analytes on C18-bonded silica, ion-exchange resins or porous gels (involving size exclusion mechanisms to differentiate between organic and inorganic species, cationic and anionic ones and species of different molecular size). To ensure sufficient selectivity as well as sensitivity, the use of element-specific detection techniques, namely flame atomic absorption spectrometry (F-AAS), electrothermal atomic absorption spectrometry (ET-AAS). Multi-element detector systems, like microwave induced or inductively coupled plasma coupled with atomic emission spectrometry or mass spectrometry (MIP-AES, ICP-AES or ICP-MS) constitute encouraging approaches, as has already been demonstrated for individual species like butyltin or organic Pb-compounds<sup>[30],[31],[32],[33]</sup>.

Investigations, concerning the fate of elements in the mentioned different environmental compartments, require the development of analytical techniques able to both quantify the metal concentration in trace amounts and to determine the chemical form of said elements, preferably adopting a technique able to do both in one go. The chromatographic methods seem to represent a promising way, but since the variety of occurring metal species is very high and a full analysis would be rather complex and time-consuming it is necessary to develop analytical schemes that are simple, cheap, easily handled and provide an appropriate level of reproducibility, sensitivity and selectivity.

To achieve this goal, it is necessary to limit the required selectivity concerning the different chemical forms from isolating all differing compounds to the separation into operationally defined classes, reflecting their biological behavior and toxicity. A similar differentiation can be found in the extraction with 1-octanol, separating the extractable lipophilic fraction and the remaining hydrophilic free ion fraction<sup>[26]</sup>. As a consequence of the correlation of trace metal toxicity and biological availability to their respective fractions retained on different sorbent materials<sup>[34],[35],[36]</sup>, solid phase extraction (SPE) techniques have been widely employed, due to their potential to simultaneously perform a fractionation and an enrichment step.

### 1.3 Fractionation with solid phase extraction (SPE)

Due to the wide variety of available substrates and their various interactions with different metal species<sup>[37]</sup>, solid phase extraction has been employed for a whole line-up of speciation analysis techniques. Cation exchange materials, like Chelex-100<sup>[38]</sup> and a wide array of other SPE-sorbents with or without added chelating agents, have already been in use to achieve enrichment<sup>[39]</sup> and to group metals into classes according to their affinity to the used sorbent material<sup>[40]</sup>. Several resins have been employed for the separation or isolation of organic metal complexes (*e.g.* humic substances), including C18<sup>[41]</sup>, functionalized cellulose<sup>[42]</sup>, Amberlite XAD resins<sup>[43]</sup> and even baker yeast (*Saccharomyces cerevisiae*)<sup>[44]</sup>.

Numerous studies have applied different aliquots of the same sample to two or more different sorbents. In some of those, non-sequential approaches were utilized, using *e.g.* strong and weak acid cation exchangers and chelating resins to extract the according trace metals fractions from sediment suspensions at pH values ranging from 2 to 10, categorizing them into seven fractions<sup>[45]</sup>. Then again, sequential schemes were developed, employing series of different sorbents. The use of a cation and an anion exchange resin in series allowed, in combination with the determination of the unretained fraction, the differentiation into three fractions<sup>[16]</sup>. Other studies employed three columns, namely silica-C18, an anion exchanger (Dowex 1-X8) and a chelating sorbent (Chelamine) to separate the trace metals into the neutral hydrophobic, anionic and cationic fractions<sup>[46]</sup>. A similar three-column approach with an anion exchanger, reversed phase sorbent and cation exchanger (AG MP-1, silica-C18 and Chelex 100) was developed to study the effect of different parameters on the species distribution of standard solutions containing different complexing agents<sup>[47]</sup>.

Since most of the published fractionation procedures require extensive sample treatment and several separation and measurement steps, all of them are rather complex, time-consuming and have possible sources of contamination, the need for establishing automated procedures becomes apparent. The use of flow injection (FI) techniques for all kinds of automatization and their adaption to enhance sensitivity and precision of ultra trace elemental analysis has been widely demonstrated<sup>[48],[49],[50]</sup>. Due to the modular nature of FI-systems nowadays, the on-line coupling of a FI-sample-pretreatment system to an ICP-AES or ICP-MS detector is quite straightforward since the mentioned detectors are able to measure transient signals.

Several on-line systems for the enrichment of trace metals have been reported<sup>[51],[52],[53],[54],[55]</sup>. These consist of a column containing a sorbent material (cation exchanger, chelating resin or others) able to quantitatively retain metal ions. After passing the sample volume through the column, the retained fraction is eluted with an appropriate solvent and stored for quantification by F-AAS or ICP system. As the bed volume of a micro-column can be less than 1 ml, high enrichment factors can be achieved even if only small sample volumes are available and since the measurement of the transient signal gives a peak rather than a concentration average of the eluate, the signal intensity is generally higher than the one achieved by batch procedures. Finally, the risk of sample contamination is reduced and the precision improved by the use of fully automated on-line systems<sup>[56],[57],[58],[59],[60],[61]</sup>.

Even though both chemical speciation analysis by means of on-line FI-systems coupled to varying detector systems has been comprehensively studied<sup>[62],[63]</sup> and a few systems able to discriminate between organic and inorganic complexes as well as the free ionic form have been described<sup>[46],[47]</sup>, the existing methods have several drawbacks.

Most important is that most existing procedures can determine only some species occurring in natural samples, thereby requiring additional measurements to achieve a complete characterization of all physicochemical forms of the investigated trace metals. Further reasons for the necessity of additional measurements are the use of a single-element detection system like GF-AAS, necessitating one measurement per investigated element and off-line measurement of the isolated fractions, since even employing multi-element analysis each fraction has to be analyzed separately.

Due to the potential risk of contamination or operating errors posed by each additional measurement or other procedural step, on-line coupling of the separating FI-system to a multi-element detection unit is preferable, since it permits the recording of a transient signal containing information about all fractions. Although combinations of FI- and AAS-systems were realized for many applications, couplings of FI-systems to ICP-AES are rare since conventional ICP-AES measurements do not require processing of transient signals.

Therefore it is necessary to develop an analytical approach which incorporates the separating and preconcentrating power of an advanced FI-system, along with the multi-elemental detection of ICP-AES. This should be achieved in a way that eliminates largely the need for multiple analysis of a sample, thereby minimizing possible error sources through on-line coupling of separating and detecting components of the employed system.

#### **1.4 General Outline of this Project**

Aim of this diploma thesis was to develop an analytical technique with which is possible to determine not only the total trace metal concentration in aqueous samples, but also to differentiate between neutral hydrophobic complexes and the fraction including anionic complexes and the free hydrated ions (respectively cationic complexes). Isolation of the respective fractions shall be achieved by passing the liquid sample through a series of SPE cartridges which are sequentially eluted. The trace metal content of each obtained fraction will be quantified on-line by ICP-AES to achieve simultaneous multi-element quantification.

The need for manual handling has to be minimized, since any intervening manipulation constitutes a possible contamination risk. Furthermore, automatized processes tend to be more reproducible. This shall be achieved by incorporating the desired fractionation scheme into an adequate FI-system, which should also provide preconcentration of analytes to enhance the achievable limits of detection (LOD's). Direct elution of the retained trace metal complexes into the nebulizer unit of the ICP-AES should further improve the detection limits, since in this way the whole area of the elution peak is measured instead of the average concentration in 5 or 10 ml of eluant. Additionally, the intermediate step of manually bringing the eluted fractions off-line to a defined volume is eliminated and all acquired fractions can be

quantified in a single measurement. By maximizing the automatization grade of the developed SPE-FI-ICP-AES combination, highly reproducible results should be obtained.

To investigate the performance of the developed FI-system concerning separation and quantification of trace metal species as well as to determine operating parameters for the procedure, synthetic sample solutions containing a series of trace metals and differing organic ligands in varied concentrations and at different pH values will be selected and examined.

Applicability of the results obtained from the experiments with synthetic standard solutions to samples of natural origin will be verified by analyzing representative natural samples and by investigating possibly occurring interferences caused by matrix constituents.

The developed method will be applied to the study of an adequate set of natural samples. The attained results will be discussed and examined for their plausibility and compliance with literature data.

## 2 Experimental

### 2.1 Reagents and Materials

High purity water was obtained by double distillation of water which was previously deionized by a reverse osmosis/ion exchange combination (euRO 20 plus, SG Water Systems, Germany) and used throughout. All used chemicals were of p.a. grade purity or higher. Nitric acid (65%), hydrochloric acid (37%), ammonium carbonate, ammonia solution (25%), sodium hydroxide solution (50%) and methanol were purchased from Merck (Darmstadt, Germany). Reaction vessels used for preparation were decontaminated by soaking them for at least 24 hours in 5%  $\text{HNO}_3$  followed by another 24 hours in water, rinsing them again with high purity water and drying them under a VFT 1525 ultraclean laminar flow hood (WEISS Technik GesmbH, Austria).

Metal standard solutions were prepared by diluting a 100 mg/l custom assurance multielement standard solution (Ca, Mg, Cr, Mn, Fe, Co, Cu, Zn, Ni and Cd in 5%  $\text{HNO}_3$ ) from SPEX Certiprep (NJ, USA) and adding Al (1000 mg/l in 1%  $\text{HCl}$ ) and Pb (1000 mg/l in 1%  $\text{HNO}_3$ ) standard solutions (Merck).

Stock solutions containing hydrophobic ligands were prepared by dissolving 8-hydroxyquinoline (8-HQ), DL-tryptophan (TRP) (Fluka, Buchs, Switzerland) in 2.5%  $\text{HCl}$ , whereas nitrilotriacetic acid (NTA) (Fluka) was dissolved in water. A solution of the dinatrium salt of ethylenediamine tetraacetic acid (EDTA) was obtained from Merck.

Due to the need to reuse the solid phase extraction (SPE)-cartridges and -sorbents several times and the therefore inadequate chemical resistance of silica based materials to low pH, polystyrene-divinylbenzene (PS-DVB) based materials were preferred. These sorbents for the different fractions were kindly provided by Phenomenex (Aschaffenburg, Germany). All sorbents were delivered in cartridges containing 30 mg and space for 1 ml of sample solution. As shown in Figure 1, they were shortened and fitted with a male luer tip for incorporation into the FI system. Before first use as well as each measurement series, they were cleaned by passing 10 ml of 2 N  $\text{HNO}_3$  and 5 ml of pure methanol through each cartridge. The sorbent materials in use are listed in Table 1.

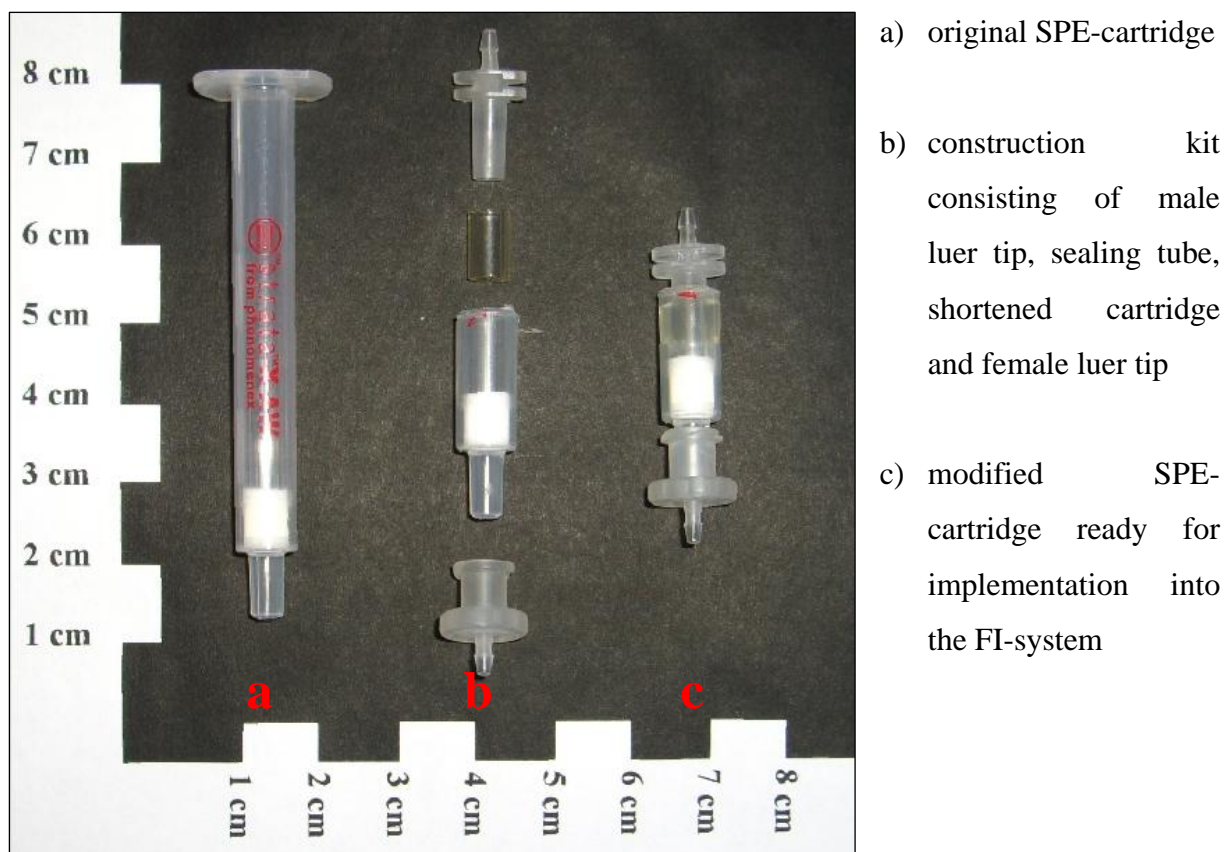
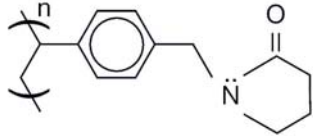
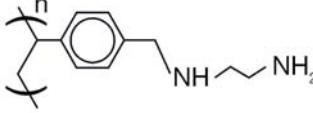
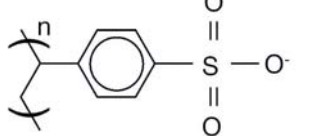


Figure 1: Adaption of sorbent cartridges.

Table 1: SPE sorbents used for fractionation.

Fraction	Sorbent name Part No.	Material	Formula <sup>[64]</sup>
Neutral Hydrophobic	Strata-X 33 µm Polymeric Reversed Phase 8B-S100-TAK-S	modified PS-DVB	
Anionic	Strata-X-AW 33 µm Polymeric Weak Anion 8B-S038-TAK-S	PS-DVB modified with amino-groups	
Cationic	Strata-X-C 33 µm Polymeric Strong Cation 8B-S029-TAK-S	PS-DVB modified with sulfonic acid groups	



## 2.2 Instrumentation

All metal determinations were performed with a Spectroflame P ICP–AES from SPECTRO A.I. (Kleve, Germany), equipped with an APEX E inlet system (ELEMENTAL SCIENTIFIC INC., Omaha, NE, USA) with an OpalMist PFA nebulizer (uptake 0.6 ml/min, from GLASS EXPANSION, West Melbourne, Australia). The spectrometer operated with the Smart Analyzer software (V.2.25, also from SPECTRO A.I.). Operating parameters are given in Table 2.

Table 2: Operating parameters for the ICP-AES.

Parameter	Value	Parameter	Value
RF Power	1440 W	Eluent Flow Rate	0.75 ml min <sup>-1</sup>
Nebulizer Flow	1.0 l min <sup>-1</sup>	Read Delay	0 s
Auxiliary Flow	0.5 l min <sup>-1</sup>	Integration Time	2 s
Coolant Flow	14 l min <sup>-1</sup>	Replicates	variable

The Spectroflame P ICP-AES is equipped with four simultaneous optical systems, of which three, including the UV optic, were used. The simultaneous optics operate on fixed wavelengths, given in Table 3. In addition a sequential optic is included in the spectrometer which can be set to any wavelength within its range. Due to the necessary time resolution and the low scanning velocity of the sequential optic, only one wavelength could be monitored at a time. In the course of this work, different elements, whose respective wavelengths are given in Table 4, were observed with the sequential system.

Table 3: Employed wavelengths of the simultaneous optics (SIM).

Element	Wavelength	Element	Wavelength
Magnesium	279.553 nm	Iron	259.940 nm
Aluminium	396.152 nm	Cobalt	228.616 nm
Calcium	317.933 nm	Copper	324.754 nm
Chromium	267.716 nm	Zinc	206.200 nm
Manganese	257.610 nm	Cadmium	226.502 nm

Table 4: Employed wavelengths of the sequential optic (SEQ).

Element	Wavelength	Element	Wavelength
Nickel	352.454 nm	Lead	283.306 nm

To achieve the time resolution necessary to record the elution peaks, a short integration time of 2 seconds was chosen. Furthermore the measurement had to be carried out without background correction, since the ICP-AES at disposal permitted only background measurement through a constant shift of the optical system, which was too time-consuming. By abandoning the spectral background measurement and selecting a 2 seconds integration time, a time resolution of 4.3 seconds per measurement could be attained. The discrepancy results from the time necessary for the computer system to calculate, transmit and store the recorded data.

Background correction was carried out by measuring the eluent before and after the peaks. This way, a subtraction of the unspecific background caused by plasma and solvent was possible, but spectral interferences occurring during the elution of the cartridges could not be accounted for.

The flow injection (FI)-system consisted either of the three SPE cartridges and three six-port two position valves or only two of each, depending on the setup employed. In the FI-manifold designed to separate two fractions, two Cheminert C22 valves with microelectric actuator from VICI (Schenkon, Switzerland) were used, both controlled by the serial communication program VCom v.1.1.01. The third available valve was a manually operated model from UPCHURCH SCIENTIFIC, INC. (Oak Harbor, WA, USA), which was integrated into the three cartridge setup. As a consequence, only the two Cheminert valves could be controlled by the employed communication program. Nevertheless, operating commands for the manually operated valve were included in the communication protocol.

All parts were connected with PTFE and TYGON pump tubings. The eluent and the sample flow were kept up through two channels of a SPETEC PERIMAX 12 peristaltic pump (Spetec GmbH, Erding, Germany).

All total organic carbon (TOC) values were measured employing a Shimadzu TOC 5000 equipped with an ASI 5000 autosampler system (both from Shimadzu, Duisburg, Germany).

### **3 The Two Cartridge FI-System**

#### **3.1 Preliminary Tests**

To ensure that the aspired species isolation of the metal complexes was attained, two sets of preliminary measurements were carried out. Both consisted of 5 ml aliquots of a trace metal standard containing 2 ppm of Mn, Fe, Cu and Zn each. Those aliquots were pumped consecutively through the RP- and the SCX-cartridge. To ensure the passage of the metal ions through the RP-sorbent, the first set of standards was buffered to a pH value of 5, while the second set of samples was spiked with 0.8 mM of 8-HQ and buffered to a pH value of 8.

Cleaning and conditioning the cartridges, as well as sample loading and washing step, were carried out according to chapter 3.2. Subsequently the setup was disassembled and the cartridges were eluted separately at a flow rate of 0.75 ml/min with 10 ml of 2 M HNO<sub>3</sub> each. The metal content of the eluates was then determined by ICP-AES. Results showed that complexing the metal ions caused them to be retained quantitatively on the RP-sorbent, while uncomplexed ions passed through this cartridge and were retained on the SCX-material. Furthermore, the chosen eluent proved to be effective.

#### **3.2 Procedure**

##### **3.2.1 Solid Phase Extraction**

The SPE process is generally divided into four steps:

1. Conditioning: Since the surface of SPE sorbents is often hydrophobic and therefore not easily compatible with aqueous solutions, it has to be pretreated. In case of the employed PS-DVB based materials this step was only necessary for the RP sorbent, since the ion exchange materials are modified with hydrophilic groups. Therefore, a brief flushing with methanol followed by equilibration of the pH by a buffer solution with the adequate pH value is considered as sufficient. For all measurements described in chapter 3, 2 ml of methanol was used to clean and condition the RP sorbent. To remove

residual methanol from the cartridges and to equilibrate the pH both SPE sorbents were flushed with 1 ml of 5 mM  $(\text{NH}_4)_2\text{CO}_3$  buffer solution.

2. Adsorption: During the adsorption step the sample is pumped through the SPE cartridges and the targeted analytes are retained by the corresponding material. The efficiency of the adsorption process is primarily dependant of the employed sorbent materials and the flow rate, since the contact time of the liquid to the solid phase has to be sufficient to ensure the mass transfer. The flow rate for conditioning, sample loading and washing for the presented setups was 1.7 ml/min.
3. Washing: Normally washing steps following the adsorption provide the opportunity to eliminate coadsorbed matrix constituents to remove residual air from the SPE cartridges. Since the presented setup consists of several cartridges in series, the main purpose of the washing with buffer solution is to ensure that the sample solution quantitatively passes through the whole setup. Without the washing step, the sorbents would either run dry, which should be avoided, or a significant portion of the sample volume would remain in the first cartridge, without ever passing the cation exchanger. The metal ions contained therein would in the following falsely contribute to the determined organic fraction. For the FI-setup with two SPE-cartridges, 2 ml of 5 mM  $(\text{NH}_4)_2\text{CO}_3$  buffer was used for washing.
4. Elution: Desorption of analytes from the SPE-sorbents is mostly accomplished by the use of organic solvents, *e.g.* methanol. But since these are mostly only marginally compatible with the employed measurement method, 2 M  $\text{HNO}_3$  is applied for this purpose. Unlike desorption using organic solvents, desorption of organically complexed metal ions by acids does not occur by removing the intact complex from the solid extractant, but rather by destroying the complex.

### 3.2.2 FI – Setup

The FI-system for the isolation of hydrophobic organic metal complexes consisted of two six-port valves and two SPE cartridges.

Figure 2 depicts the configuration used for pH-equilibration of SPE sorbents. For previously conditioning the RP-sorbent with methanol, the Valve V2 was set to Wash position (*cf.* Figure 5). After conditioning and equilibrating the sorbents, the sample was pumped through the

cartridges (also employing the configuration shown in Figure 2), thus concentrating the analytes on the respective SPE sorbents, followed by the buffer solution used for washing.

Since only one peristaltic pump was used to drive both the eluent and buffer (respectively sample) through the FI-system, the pump tubing, used to maintain the buffer (P2) flow, was removed from the pump.

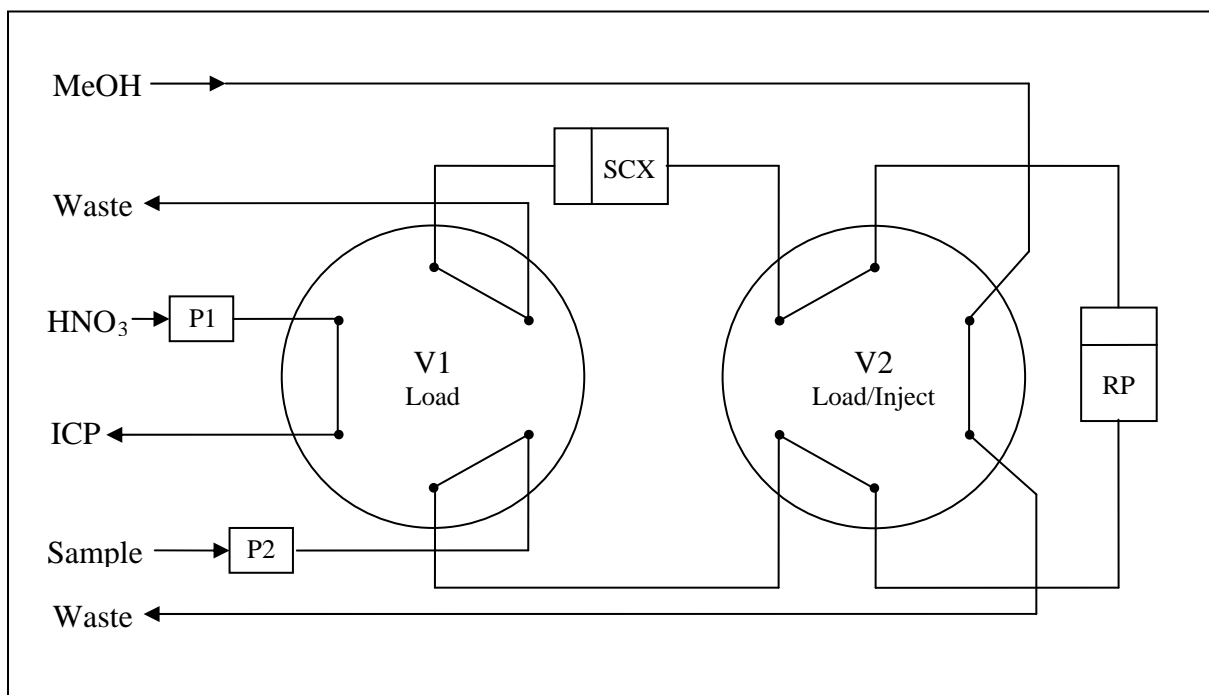


Figure 2: FI-setting for sample loading.

(P1) and (P2): peristaltic pump; (V1) and (V2): six-port valves; (SCX): strong cation exchanger; (RP): reversed phase sorbent.

After loading the SPE-sorbents, the cartridges were stripped of the bound trace metals with 2 M  $\text{HNO}_3$ . Elution of the cartridges was carried out consecutively in reversed order.

To initiate the measuring sequence, the VCom program and the ICP-AES data recording were started. The delay between the two program's starts was approximately one second, so that data-recording started simultaneously with switching of valve V1. The communication protocol valve interface is given in Table 5. In course of the measuring sequence 90 data points were collected, resulting in an average recording time of 6:26 min.

Table 5: Communication protocol for the FI-system, 2 cartridge setup.

Step	Time [min:s]	Command	Valve	Position	cf. Figure
Sample Load					Figure 2
	00:00	2cw	2	Wash	
	00:01	1cw	1	Inject	
Inject SCX					Figure 3
	03:00	2cc	2	Load/Inject	
Inject RP					Figure 4
	06:00	2cw	2	Wash	
	06:01	1cc	1	Load	
Wash RP					Figure 5
	manual	2cc	2	Load/Inject	

The elution sequence starts with switching valve V2 to the Wash position, to prevent the eluent from passing the reversed phase cartridge. Valve V1 is then set to Inject, enabling the eluent to flow through the SCX-cartridge and thus to strip the therein bound trace metal ions and complexes. Figure 3 reports the setting of the FI-system employed to strip the SCX-cartridge.

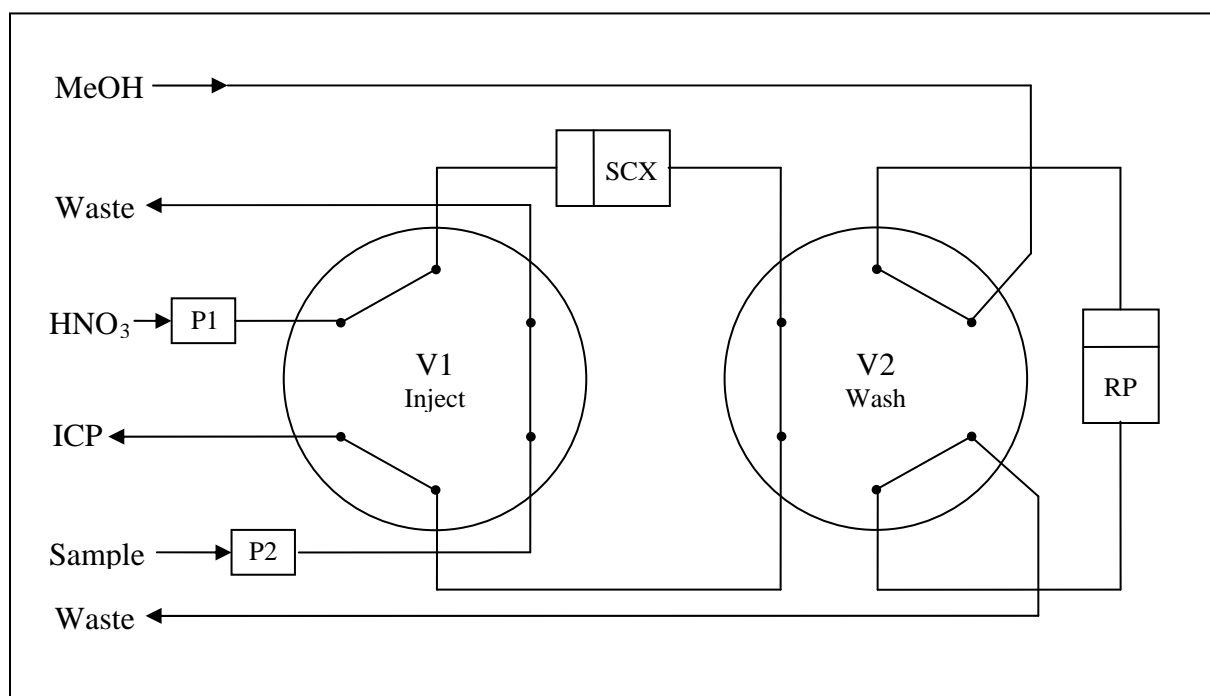


Figure 3: FI-setting for stripping the SCX-cartridge.

Subsequently to the elution of the SCX-cartridge, the RP-cartridge was inserted in the eluent flow by switching valve V2 back to the Load/Inject position as shown in Figure 4. Taking into account the eluent flow rate of 0.75 ml/min and duration of the elution steps of 3 minutes each, the eluent volume amounted to 2.25 ml per cartridge.

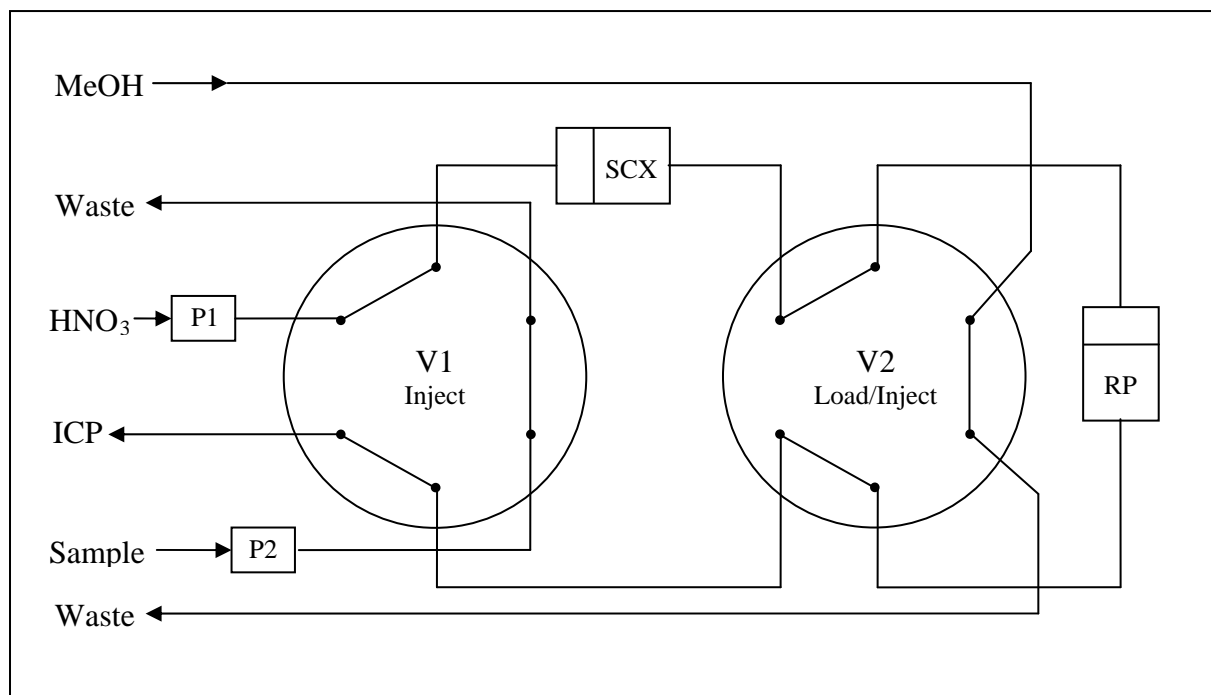


Figure 4: FI-setting for stripping the RP-cartridge.

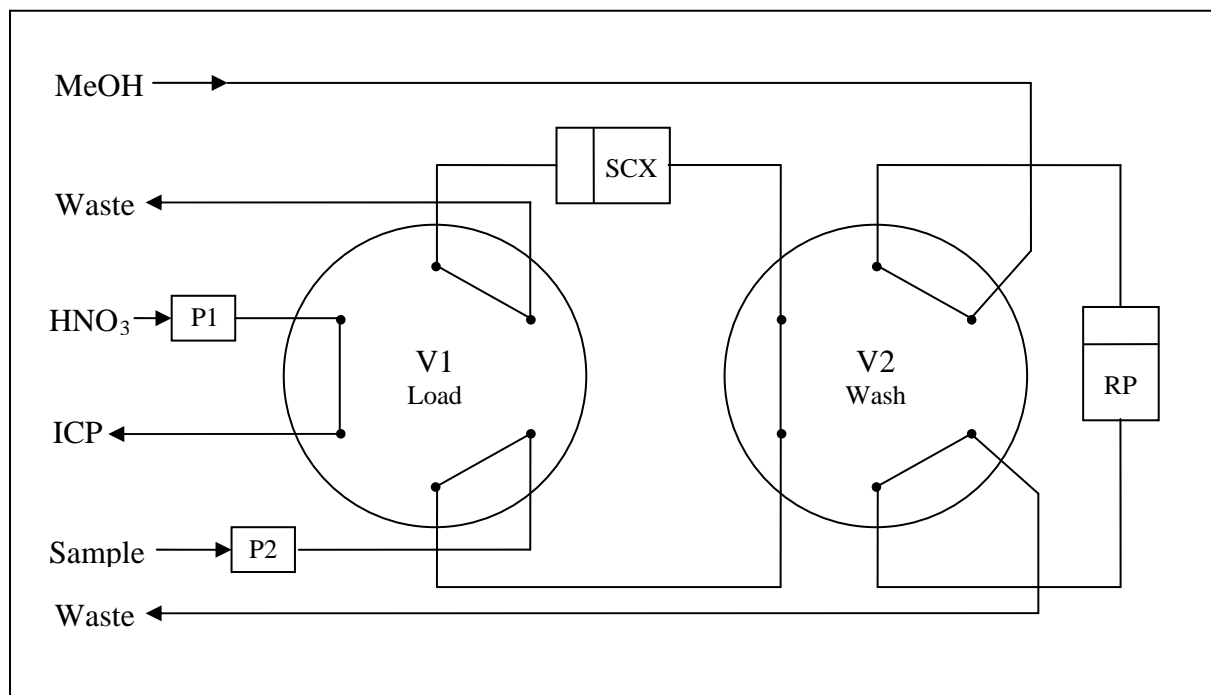


Figure 5: FI-setting for cleaning and conditioning the RP-cartridge.

At the end of the measuring sequence, valve V2 was reset to the Wash and valve V1 to the Load position (*cf.* Figure 5). After cleaning and conditioning the RP-cartridge with 2 ml of methanol and reinserting it into the loading cycle by manually setting valve V2 back to position Load/Inject, the FI-system was ready for the next sample.

### 3.2.3 Data Processing

Raw data received from the spectrometer consists of measured signal intensities and date/time indices of each replicate. To simplify the data evaluation, the date/time index of the first measured replicate was set to zero and deducted accordingly from all other indices. Moreover, an index representing the replicate number was added to each data set.

For calculation of the areas of the two elution peaks it is required to apply a background correction beforehand. Therefore, measurement points, situated apart from the peaks in the elution profile, were chosen. An average of these points was subtracted from all measured intensities to compensate an eventually occurring drift of the unspecific background. The points were selected from parts of the profile where only the pure eluent reached the spectrometer, namely the first measured points (measurements 1-7) where the eluent from the dead volume between FI-system and nebulizer is analyzed, also points between the peaks (43-49) and after the last peak (84-90) were considered applicable for this purpose.

This corrected data set was used for the integration of the peak areas. In doing so, adequate integration limits for each peak had to be applied. Figure 6 shows the background correction and integration limits by means of the Ca 317.933 nm elution profile observed for a standard solution containing 30 ppb Ca and 0.1 mM 8-HQ at a pH value of 10, using a sampling volume of 5 ml.



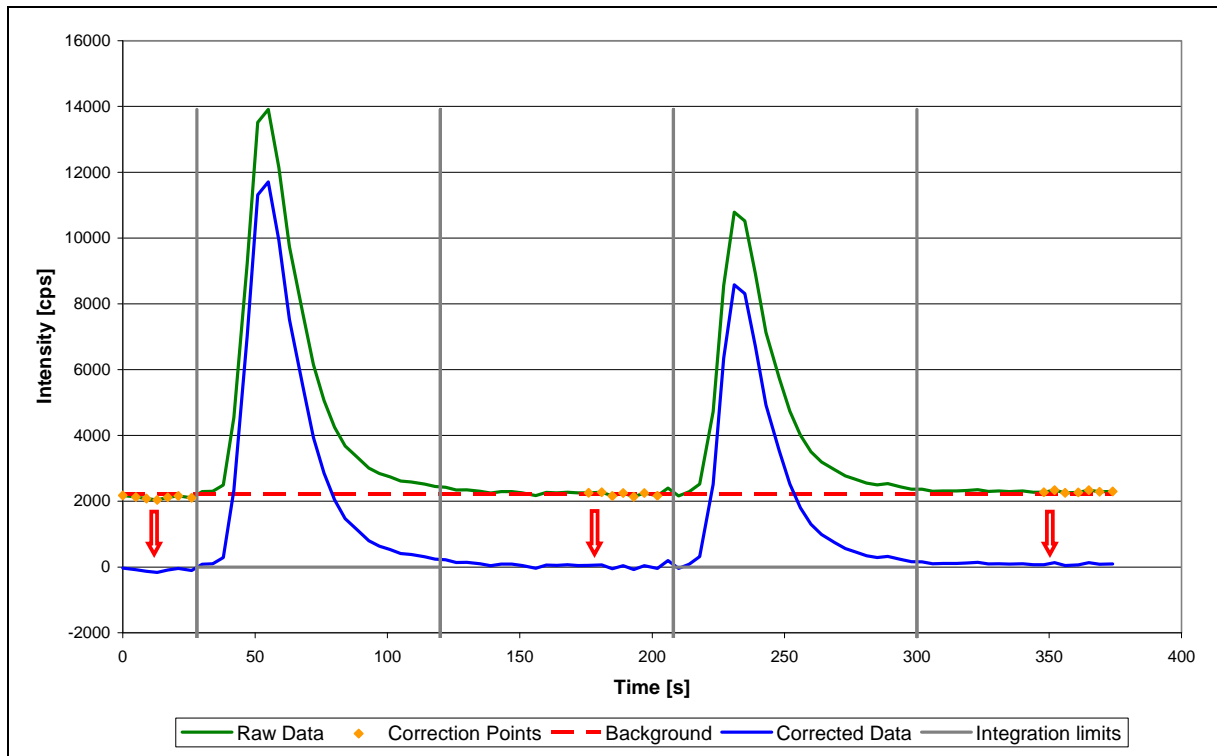


Figure 6: Background correction and data integration.

Total metal concentration was calculated by adding count values of the two peaks of the sample and comparing the sum to known values determined in calibration. Fractions of the different species were correspondent to the contributions of the respective peak area to that sum.

### 3.3 Results

#### 3.3.1 Separation of Species

To verify the separation of organic complexes from cationic species, the setup was tested using two different kinds of Cu standards. Neutral organic complexes were formed by the addition of 50  $\mu\text{l}$  of 0.01 M 8-HQ-solution to each 5 ml aliquot of standard and adding 25  $\mu\text{l}$  of 2 M  $\text{NH}_3$ . Thus, the samples contained 0.1 mM of ligand and had a pH value of 8. To ensure the quantitative presence of Cu as free ions or cationic complexes, the employed Cu solutions were brought to a pH of 3 adding 25  $\mu\text{l}$  of 2 M  $\text{HNO}_3$ . The Cu standard solutions used for these measurements contained 30 ppb and 100 ppb Cu. 5 ml of standard solution was used for each measurement. All measurements were carried out according to chapter 3.2 employing the operating parameters given in Table 2 and were performed in duplicate. The resulting elution profiles recorded at 324.754 nm are given in Figure 7.

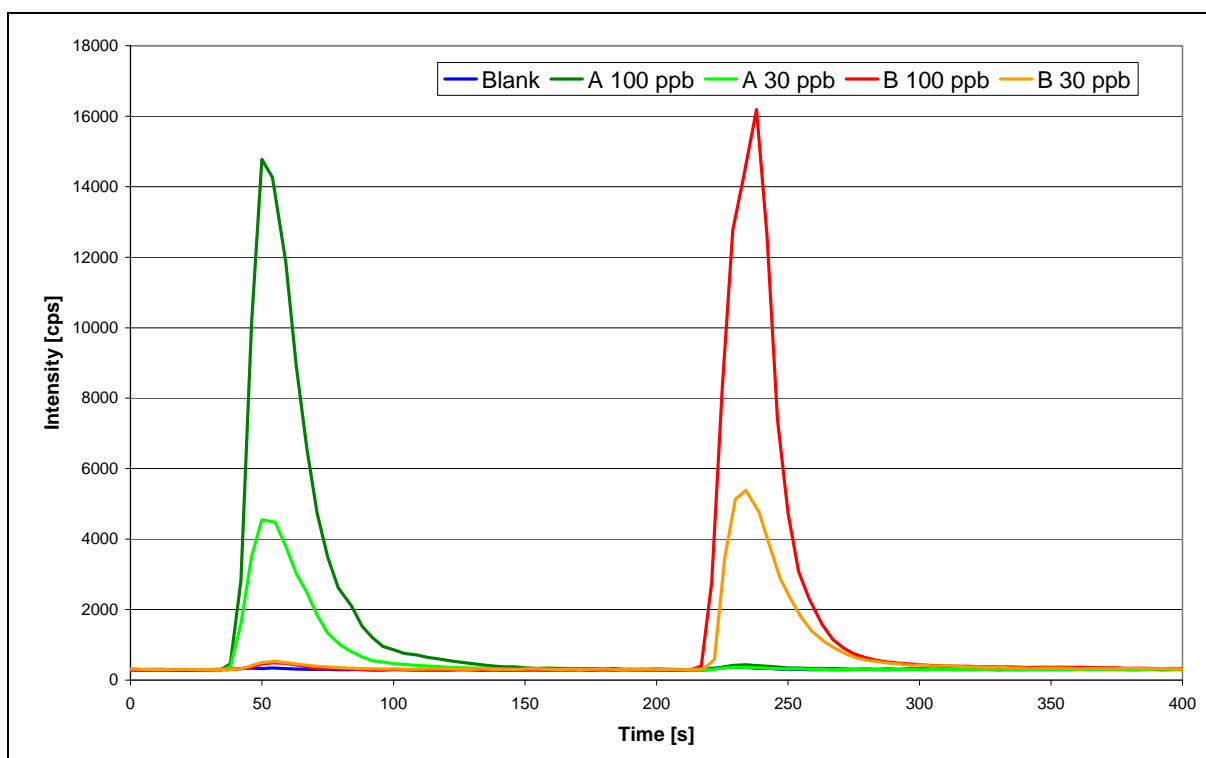


Figure 7: Separation of Cu species.

A: pH 3, no ligands; B: pH 10, 0.1 mM 8-HQ

### 3.3.2 Detection Limits

The limit of detection (LOD) of the two cartridge setup was determined by measuring a series of standards. These were prepared by diluting different aliquots of metal stock solutions with 5 ml of high purity water. To ensure that the metal ions were quantitatively retained by the SCX-cartridge, 20  $\mu$ l of 2 M  $\text{HNO}_3$  was added. Aliquots of the stock solution and the resulting concentration levels are given in Table 6.

Table 6: Concentrations of the standards used for calibration.

Volume of stock solution	Metal concentration of stock solution	Metal concentration in the standard
0 $\mu$ l	inapplicable	0 ppb (blank)
10 $\mu$ l	2.5 ppm	5 ppb
10 $\mu$ l	5.0 ppm	10 ppb
20 $\mu$ l	5.0 ppm	20 ppb
40 $\mu$ l	5.0 ppm	40 ppb
60 $\mu$ l	5.0 ppm	60 ppb

All calibration standards were measured in duplicate, employing standard procedure and parameters (*cf.* chapter 3.2 and Table 2).

The total intensity yield of these standard solutions was calculated according to chapter 3.2.3 and interpolated employing a linear function and exhibited good correlation over the observed range. Selected calibration curves are shown in Figure 8.

For definition of the detection limit, a blank solution was measured four times. The results were evaluated according to the  $3\sigma$  criterion and the sensitivity of the system was derived from the calculated calibration curves. Detection limits, sensitivities and correlation coefficients are reported in Table 7.

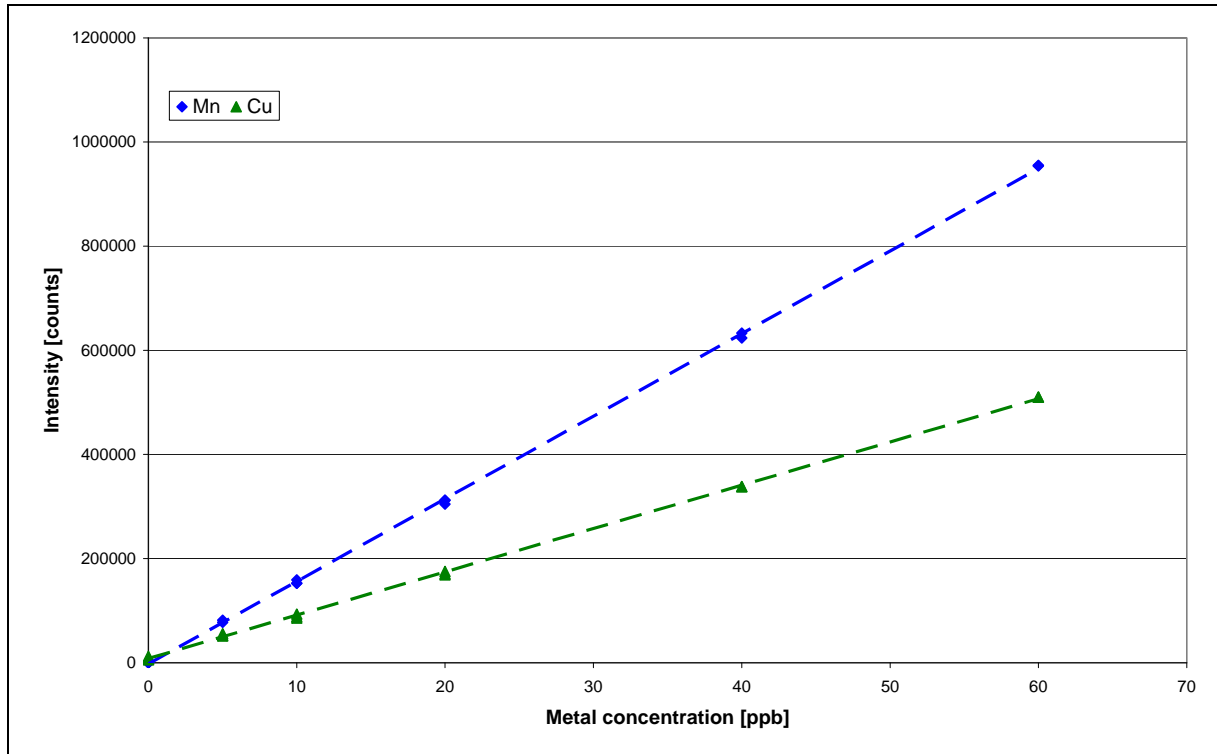


Figure 8: Selected calibration curves (linear).

Table 7: Calibration data for two-cartridge-setup.

Element	Sensitivity [counts/ppb]	Correlation ( $r^2$ )	Limit of Detection
Mg	32623.2	0.9938	0.9 ppb
Al	2421.7	0.9396	5.2 ppb
Ca	5826.9	0.9061	5.2 ppb
Cr	9540.7	0.9977	1.3 ppb
Mn	18899.6	0.9984	0.4 ppb
Fe	2120.1	0.9956	1.3 ppb
Co	351.2	0.9977	1.3 ppb
Ni	6456.8	0.9919	1.4 ppb
Cu	6654.7	0.9980	0.9 ppb
Zn	141.6	0.9954	1.9 ppb
Cd	508.1	0.9979	0.1 ppb

### 3.3.3 Standardization

Since sensitivity of an ICP-AES tends to show a certain daily variation, it was necessary to establish a standardization method to permit an accurate quantification of unknown samples. Due to the duration of a measurement, including the time needed for conditioning of the cartridges, the sample loading and the washing step, was rather long (compared to customary, that is to say non-transient, ICP-AES measurements) and the fact that each replicate requires a full measurement sequence, it was inefficient to run an extended calibration series on a daily basis, not to mention a calibration series for each of the two peaks of the elution profile. Furthermore, the quantitative conversion of different metal ions into another chemical form, an inevitable step in the preparation of standard solutions for the quantification of hydrophobic or anionic complexes, turned out to be a rather complex matter.

Therefore the established daily standardization routine consisted of measurements of several blanks and a duplicate of one standard solution containing all measured elements at concentrations depending on the estimated values of the samples.

### 3.3.4 Influence of Organic Ligands

To investigate the influence of organic ligands on fractionation, varied solutions (*cf.* Table 8) were added to 5 ml of a 100 ppb metal standard to achieve an approximately threefold excess of ligand and to adjust an adequate pH value.

Table 8: Composition of ligand-spiked standard solutions.

	<b>8-Hydroxy-quinoline</b>	<b>D,L-Tryptophan</b>	<b>Ethylene-diamine-tetraacetate</b>	<b>Nitrilo-triacetic-acid</b>
Abbreviation	8-HQ	TRP	EDTA	NTA
Metal Spike	100 ppb	100 ppb	100 ppb	100 ppb
Ligand Spike	0.1 mM	0.1 mM	0.1 mM	0.1 mM
other Additions	50 µl 2 M NH <sub>3</sub>	50 µl 2 M NH <sub>3</sub>	none	none
pH	10	10	5	5
Total Volume	5 ml	5 ml	5 ml	5 ml

To minimize losses of analyte to the vessel surface, the samples were prepared directly before the measurement. All measurements were performed in duplicate, employing standard procedure and parameters (*cf.* chapter 3.2 and Table 2) and quantified according to chapter 3.3.3.

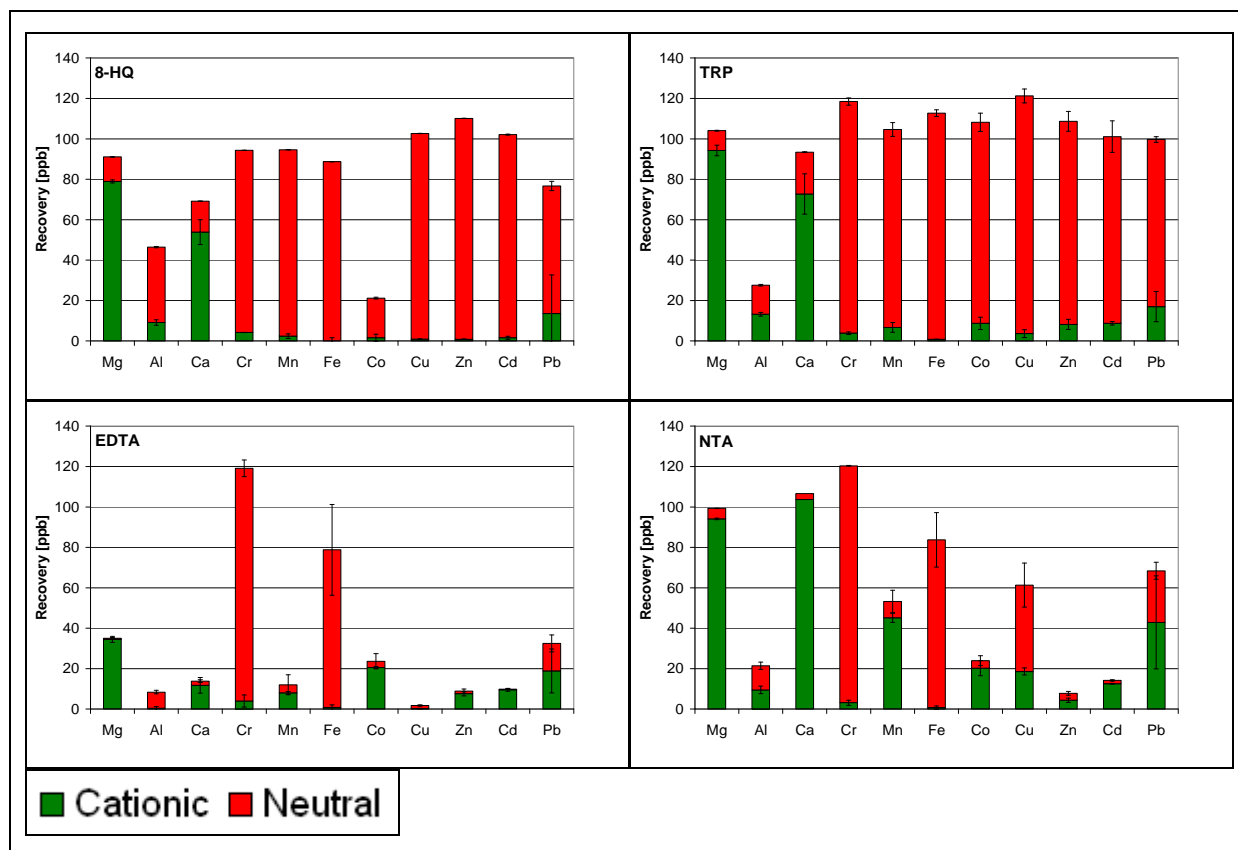


Figure 9: Fractionation of ligand-spiked standard solutions.

As apparent in Figure 9, recoveries for most elements differ from the target value of 100 ppb. In general, two types of deviation can be observed. Firstly, several elements yielded significantly higher recoveries, in most cases when complexed with TRP. Since the metal amount in those cases equaled the one used during standardization, three possible explanations can be given.

- Due to the rather low pH value of standards employed for standardization, potential losses could lead to an underestimation of the system's sensitivity. Standard measurements at higher pH values could not confirm this, since they showed no significantly higher response than the standards at pH 4.
- Another possible reason for the mentioned discrepancy is the calculation applied for background correction. If the recorded baseline was to indicate an upward drift, the

subtraction of a constant background value would not be applicable, since the later eluting RP-peaks would be overvalued compared to cationic bound metals. Close examination of the respective elution profiles however showed no such incline in the background line.

- The third possible reason for high recoveries is the occurrence of spectral interferences. High recoveries were all measured in samples exhibiting a distribution of complexes in favor of the neutral hydrophobic fraction. This means it is possible that during the elution of metals the complexing agents as well are washed from the reversed phase sorbent. These complexing agents are carried then by means of eluent flow to the ICP-AES and reach the nebulizer system exactly at the same time as the analytes. Since it is widely known that high concentrations of dissolved organic matter can affect the plasma conditions<sup>[65]</sup>, some sort of spectral background correction should be carried out during the measurements to compensate for occurring unspecific background changes. Unfortunately, the employed ICP-AES system could not provide an adequate background correction without sacrificing the ability for transient measurement.

Secondly, many experiments showed insufficient recoveries. These were mostly caused by addition of the aliphatic complexing agents EDTA and NTA to the sample; in case of Co, 8-HQ also caused the recovery to decrease notably. Al could not be satisfyingly recovered whenever any complexing agent was added.

Low findings, respectively the fact that too small amounts of the analyte in question reach the spectrometer and are detected, is most likely a consequence of a flaw in the FI-setup. Either the trace metals in question are not quantitatively retained by the applied SPE sorbents, or the elution process is not suited for quantitative desorption or decomposition of the retained metal complexes.

Since several studies<sup>[12],[47]</sup> confirm that the eluent in question, 2 M HNO<sub>3</sub>, is capable of stripping trace metals, respectively their complexes, quantitatively from both types of sorbents employed in this setup, it is assumed that formed metal complexes were not retained quantitatively. Reversed phase sorbents generally retain unloaded hydrophobic complexes, whereas strong cation exchangers should be able to break up most complexes, thus retaining the released free cations. Nevertheless, the obtained results indicate that anionic complexes

formed by both EDTA and NTA with most trace metals fit neither requirement and were therefore only partially retained on the columns.

### 3.3.5 Recovery of Anionic Complexes

The obvious solution for the loss of anionic complexes is to add another sorbent, namely a weak anion exchanger (WAX), to the existing FI-setup. Precluding extensive modifications of the FI-setup, there are two possible options for the incorporation of the WAX into the system.

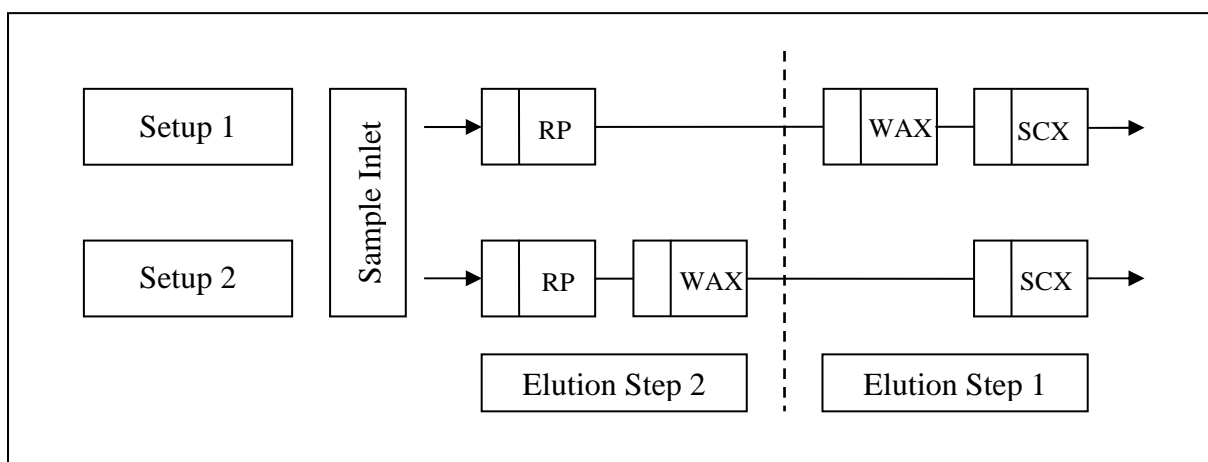


Figure 10: Possible WAX-Setups.

The additional SPE-cartridge containing the WAX-sorbent can be coupled either directly to the SCX-cartridge (*cf.* Figure 10, Setup 1), to enhance the recovery of total trace metal amount while separating, as carried out so far, the neutral hydrophobic complexes, or combined with the RP-cartridge (*cf.* Figure 10, Setup 2) to isolate the cationic fraction from anionic and neutral compounds.

To investigate the recovering ability of this setup, metal standards containing 100 ppb of each investigated trace metal were spiked with 0.1 mM of EDTA and adjusted to different pH values by adding small amounts of 2 M HNO<sub>3</sub> or 2 M NH<sub>3</sub> directly before measurement. 5 ml aliquots of the prepared samples were analyzed with each setup in duplicate, employing standard procedure and parameters (*cf.* chapter 3.2 and Table 2) and quantified according to chapter 3.3.3.



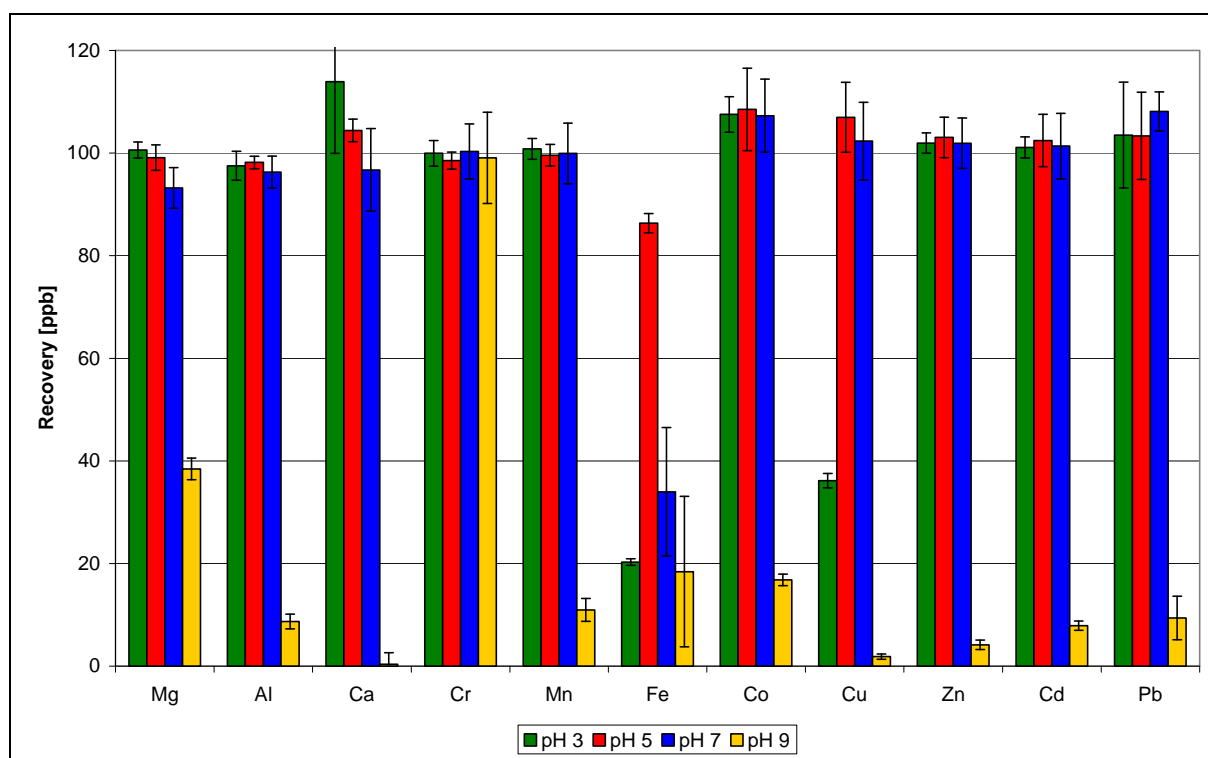


Figure 11: Recovery of EDTA-spiked standard solutions.

The achieved total recoveries, as shown in Figure 11, were quantitative for most elements at pH values ranging from 3 to 7, confirming prior expectations.

At pH 9 however, recoveries for all elements were significantly lower than expected. This decrease is probably due to the fact that at pH 9 EDTA is nearly completely in its  $\text{HY}^{3-}$ -form, thus strongly competing with the doubly charged EDTA- metal complexes for the available exchange sites. With the applied sorbent quantity of 30 mg, the estimated sorbent capacity is less than 0.1 milliequivalents and therefore 0.1 mmol of a triple charged ligand are likely to exhaust sorbent capacity.

Poor recoveries at low pH values, as present at pH 3 for Fe and Cu, can be attributed to insufficient retention of respective complexes or ions by either SPE sorbent.

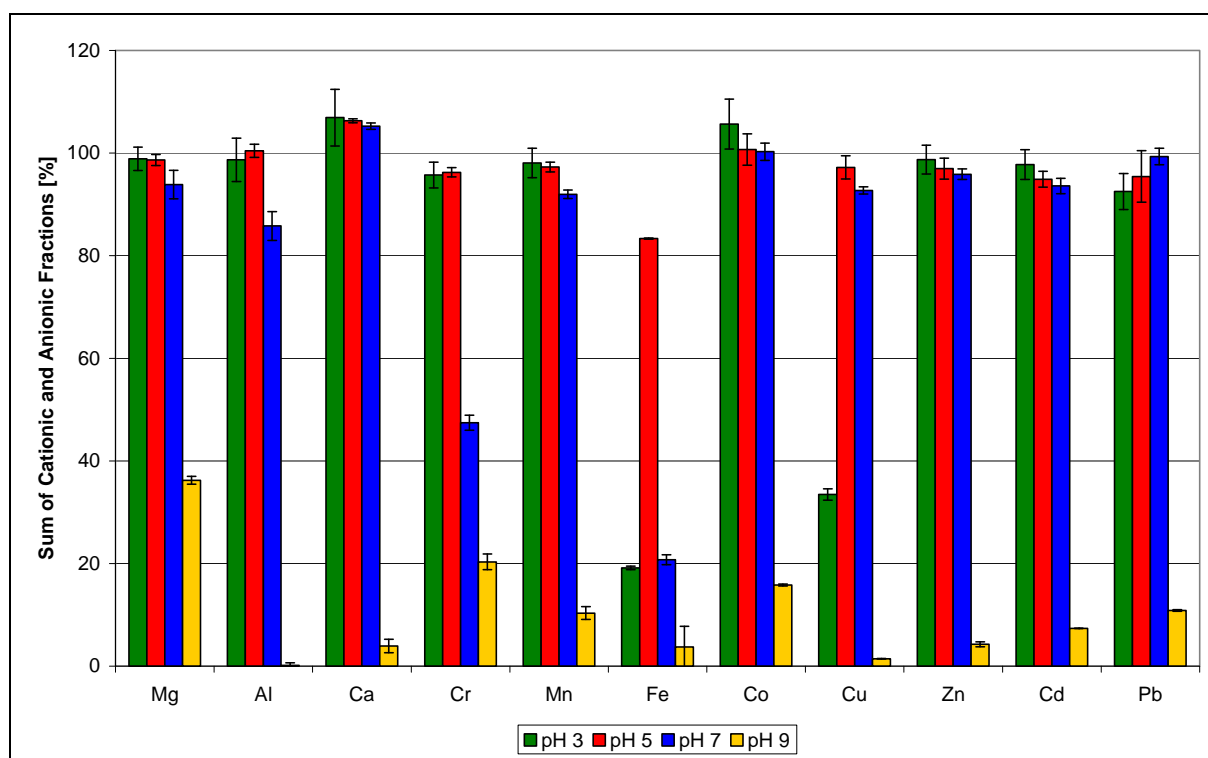


Figure 12: Charged fraction of EDTA-spiked standard solutions.

Figure 12 demonstrates that with use of both the SCX- as well as the WAX-cartridge for retaining the second fraction (*cf.* Figure 10, Setup 1), almost all present metal ions are detected either in cationic or in anionic form.

While the combination of a WAX-filled cartridge with the existing SCX-cartridge allows the separation of neutral hydrophobic complexes from charged species, the second setup (*cf.* Figure 10, Setup 2) permits the quantification of a fraction containing anionic as well as neutral complexes. A separation like this may be useful in case of trace metals complexed by *e.g.* humic acids or similar ligands, but with EDTA-spiked metal solutions the main focus should lie on quantification of the anionic fraction. Therefore, additional data, retrieved from the analysis of samples employing this second setup, can be used to achieve this.

The supplemental quantification of cations allows calculation of the anionic fraction without directly isolating the fraction in question, but by simply subtracting this amount from the previously determined sum of both species as demonstrated in Figure 13.

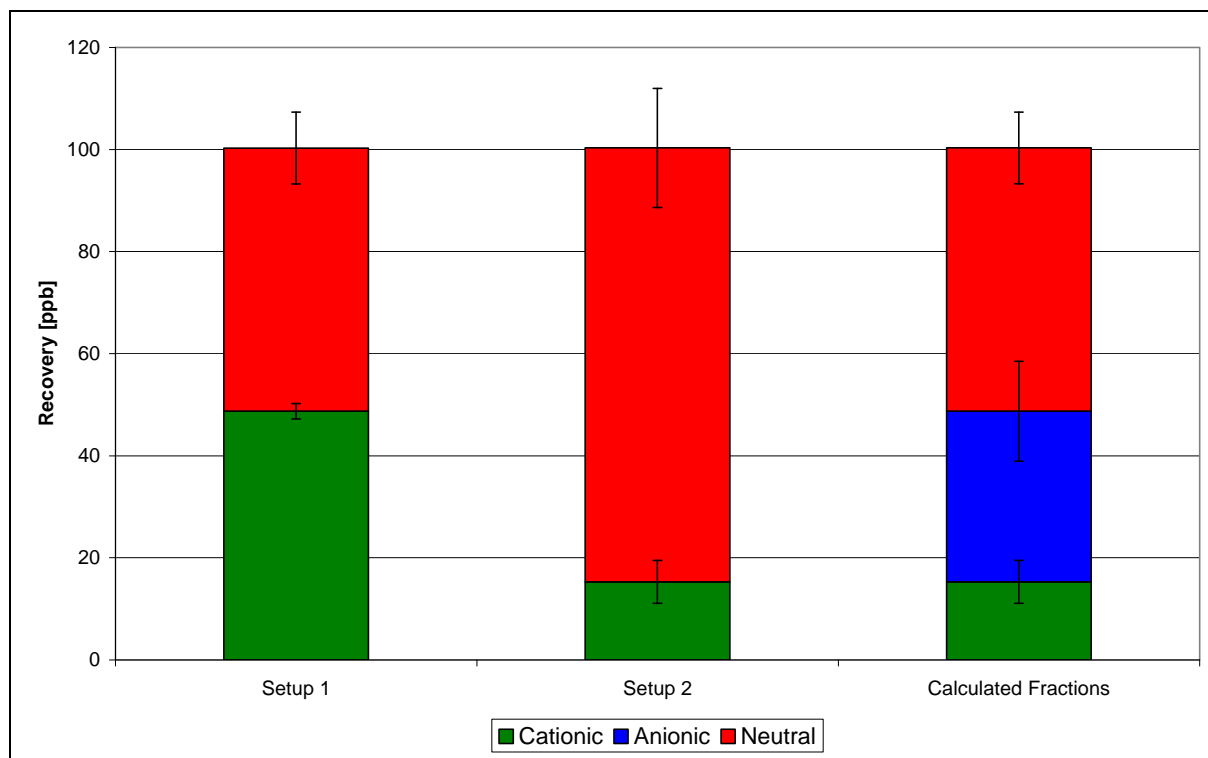


Figure 13: Calculation of Anionic Fraction.

Figure 14 gives the distributions of trace metals to the three analyzed fractions, calculated from results acquired by using the two-cartridge-setup with the two mentioned cartridge assemblies. Columns marked with \* represent calculated distributions of measurements yielding unsatisfactory recoveries and are therefore only quoted for the sake of completeness.

As expected, all monitored metal ions, except Mg and Ca, form anionic complexes with EDTA at the investigated pH values. Fe, Cu and Pb are even nearly quantitatively complexed from pH 3 to pH 7. Co, Zn and Cd, which are mostly cationic at pH 3, also form their anionic complexes at the pH values 5 and 7, whereas anionic complexes containing Mn and Al are mostly found at pH 7. A special case is Cr that, while being the only metal yielding outstanding recoveries at all investigated pH values, seems to form only limited amounts of anionic complexes. Instead of the anticipated  $\text{Cr(EDTA)}^-$ , a neutral complex is found with the described analysis system.

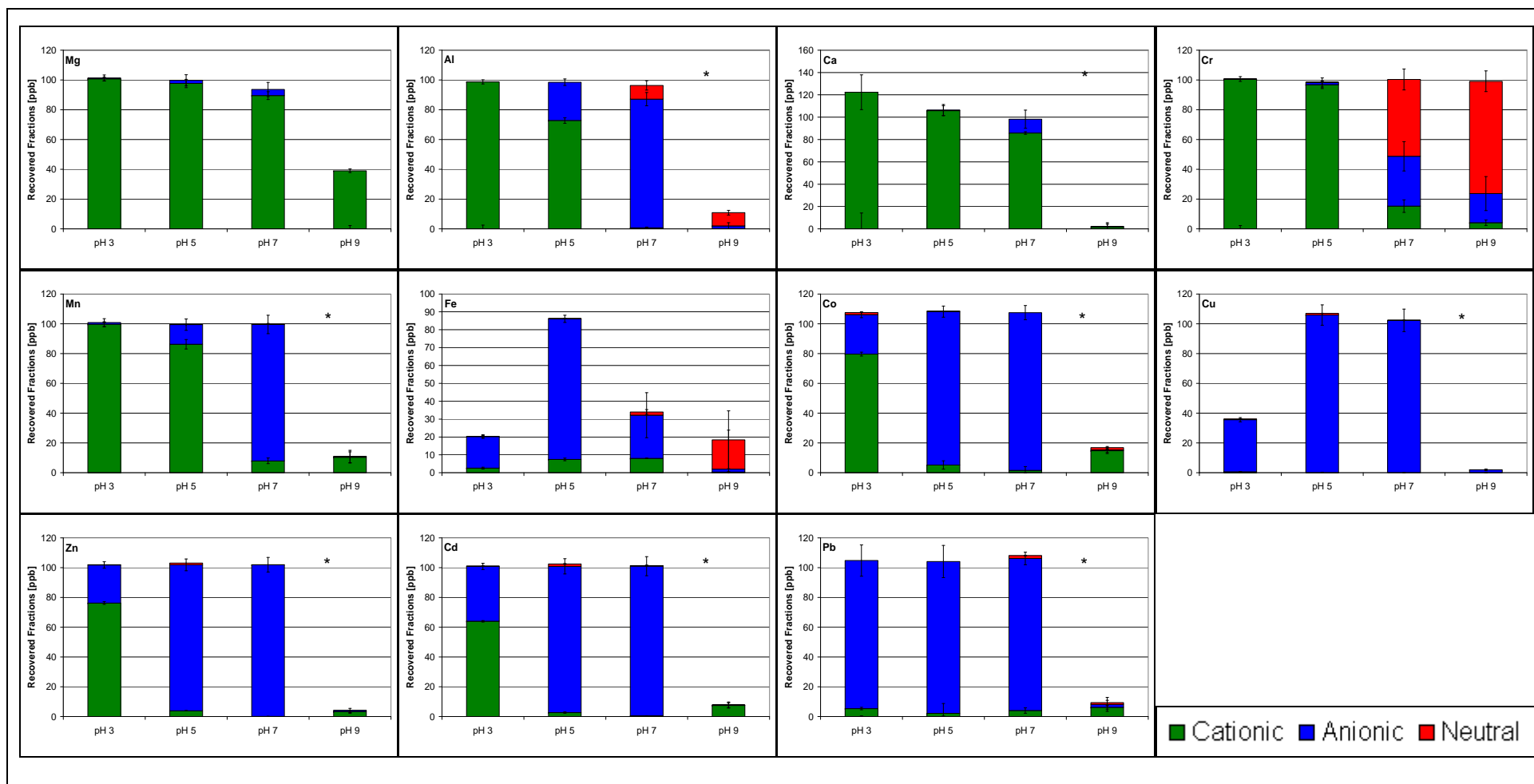


Figure 14: Fractionation of EDTA spiked standard solutions.

### **3.4 Conclusion**

The attempt to achieve a simultaneous quantification while fractionating trace metals into their different species with the described two-cartridge setup shows that it is crucial to incorporate an anion exchange material. In doing this, the loss of analytes in form of stable anionic complexes can be significantly reduced and the possibility to estimate a third fraction arises.

Since determination of three fractions in question necessitates a conversion of the FI-system and reanalyzing the samples, the advantages of this system, namely the low sample consumption and simultaneous detection of the fractionation as well as the total trace metal amount, is seriously compromised. Therefore the attempt was not considered as adequate to the aspired task.

Instead of abandoning the project completely, the FI-system was expanded in a way to allow determination of all three fractions in one measurement.

## 4 The Three Cartridge FI-System

### 4.1 Procedure

#### 4.1.1 Solid Phase Extraction

The general principle of solid phase extraction was already described in chapter 3.2.1. Several parameters had to be adapted to fit the requirements of the expanded FI-system. Since a third cartridge was included, the volume of 5 mM  $(\text{NH}_4)_2\text{CO}_3$  buffer solution used for pH-equilibration of the sorbents was raised to 2 ml. For the washing step after sample loading, an increased volume of 3 ml buffer solution was used.

The sequence of cartridges used for separation of different species is another crucial matter. Since the sorbent used for preconcentration of cations is a strong cation exchanger whose functional group is able to compete with many organic and inorganic natural ligands and to displace metals from the respective labile anionic or neutral hydrophobic complexes, it was placed last to avoid diminishment of the other fractions. The positions of reversed phase sorbent and anion exchanger also have an impact upon the achieved speciation, since many complexes have both anionic functional groups and hydrophobic regions. The employed setup, placing the RP-cartridge first and the WAX-cartridge second, was chosen because the main objective of this work was primarily to isolate the neutral hydrophobic species. Furthermore, the polystyrene-based bulk material of the WAX sorbent (*cf.* Table 1) is considered able to interact with hydrophobic species.

Nevertheless, several other possibilities of unintended interaction between metal complexes and the RP-sorbent placed in first position must be taken into account.

- The placement of the RP-cartridge in first position causes eventually present particular or colloidal matter, which can be retained by a simple physical filtration effect, to be attributed to the neutral hydrophobic fraction. While the particular fraction may be eliminated by filtration prior to analysis, colloidal matter cannot be removed this way. Furthermore, analyte losses caused by interaction of complexes with the filter may occur.
- Charged metal complexes containing extensive hydrophobic areas may be retained by the previously set RP-sorbent, since the hydrophilic effect of the functional group may not be dominant enough.

#### 4.1.2 FI – Setup

The FI-system for fractionation of metal species into hydrophobic organic complexes, anionic complexes and cations consisted of three six-port valves and three SPE cartridges as shown in Figure 15.

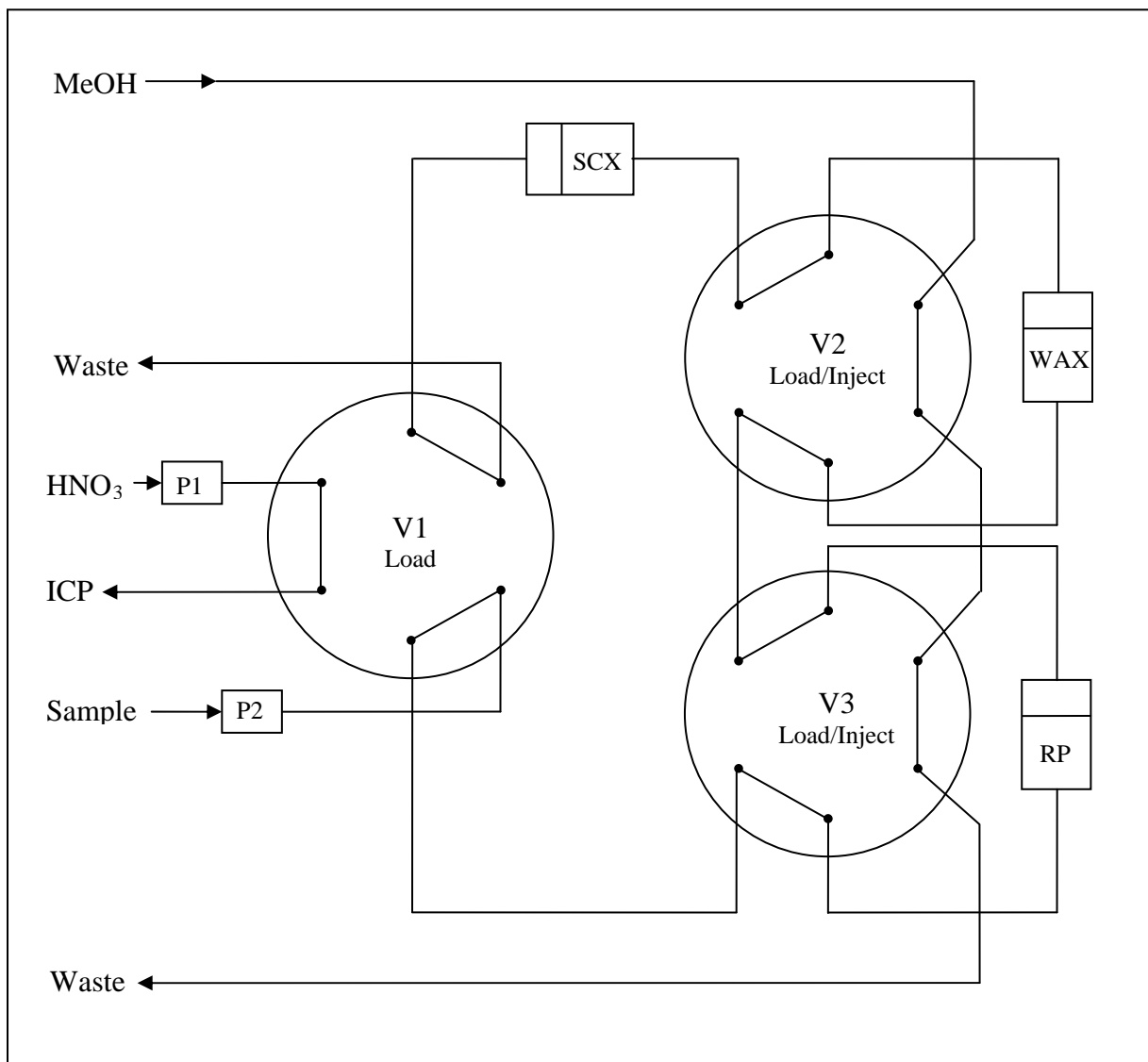


Figure 15: FI-setting for sample loading.

(P1) and (P2): peristaltic pump; (V1): six-port valve from Upchurch Scientific, (V2) and (V3): six-port valves from VICI; (SCX): strong cation exchanger; (WAX): weak anion exchanger, (RP): reversed phase sorbent.

The configuration employed for pH-equilibration of the sorbents, sample loading and washing step is shown in Figure 15. For the conditioning of RP- and WAX-sorbents with methanol prior to this, the Valves V2 and V3 were both set to Wash position (*cf.* Figure 19). After resetting the FI-system to Load configuration (*cf.* Figure 15) and equilibrating the pH of all

cartridges with buffer solution, the sample was pumped through the cartridges, thus concentrating the analytes on the respective SPE sorbents, followed by the buffer solution used for washing.

To ensure the passage of the whole sample volume through all three cartridges and to remove residual air from the FI-system, 3 ml of buffer solution was used for a washing step. The flow rate for conditioning, sample loading and the washing step was 1.7 ml/min. Since only one peristaltic pump was used to drive both the eluent and buffer (respectively sample) flow through the FI-system, the pump tubing, used to maintain the buffer (P2) flow, was removed from the pump.

After loading step, the cartridges were stripped of the bound trace metals with 2 M HNO<sub>3</sub>. Elution of the cartridges was carried out consecutively in reversed order.

Table 9: Communication protocol for the FI-system, three-cartridge setup.

Step	Time [min:s]	Command	Valve	Position	cf. Figure
Sample Load					Figure 15
	00:00	3cw	3	Wash	
	00:01	2cw	2	Wash	
	00:02	1cw	1 (Upchurch)	Inject	
Inject SCX					Figure 16
	02:30	2cc	2	Load/Inject	
Inject WAX					Figure 17
	04:59	3cc	3	Load/Inject	
Inject RP					Figure 18
	07:28	3cw	3	Wash	
	07:29	2cw	2	Wash	
	07:30	1cc	1 (Upchurch)	Load	
Wash RP					Figure 19
	manual	3cc	3	Load/Inject	
	manual	2cc	2	Load/Inject	



To initiate the measuring sequence the VCom program and ICP-AES data recording were started. The delay between the two program's starts was approximately one second, so that data-recording started simultaneously with switching of valve V2. It must be pointed out that although the commands for valve V1 were integrated into the communication protocol, the valve was operated manually at the time. The communication protocol for the serial valve interface is given in Table 9. In course of the measuring sequence 110 data points were collected, resulting in an average recording time of 7:52 min.

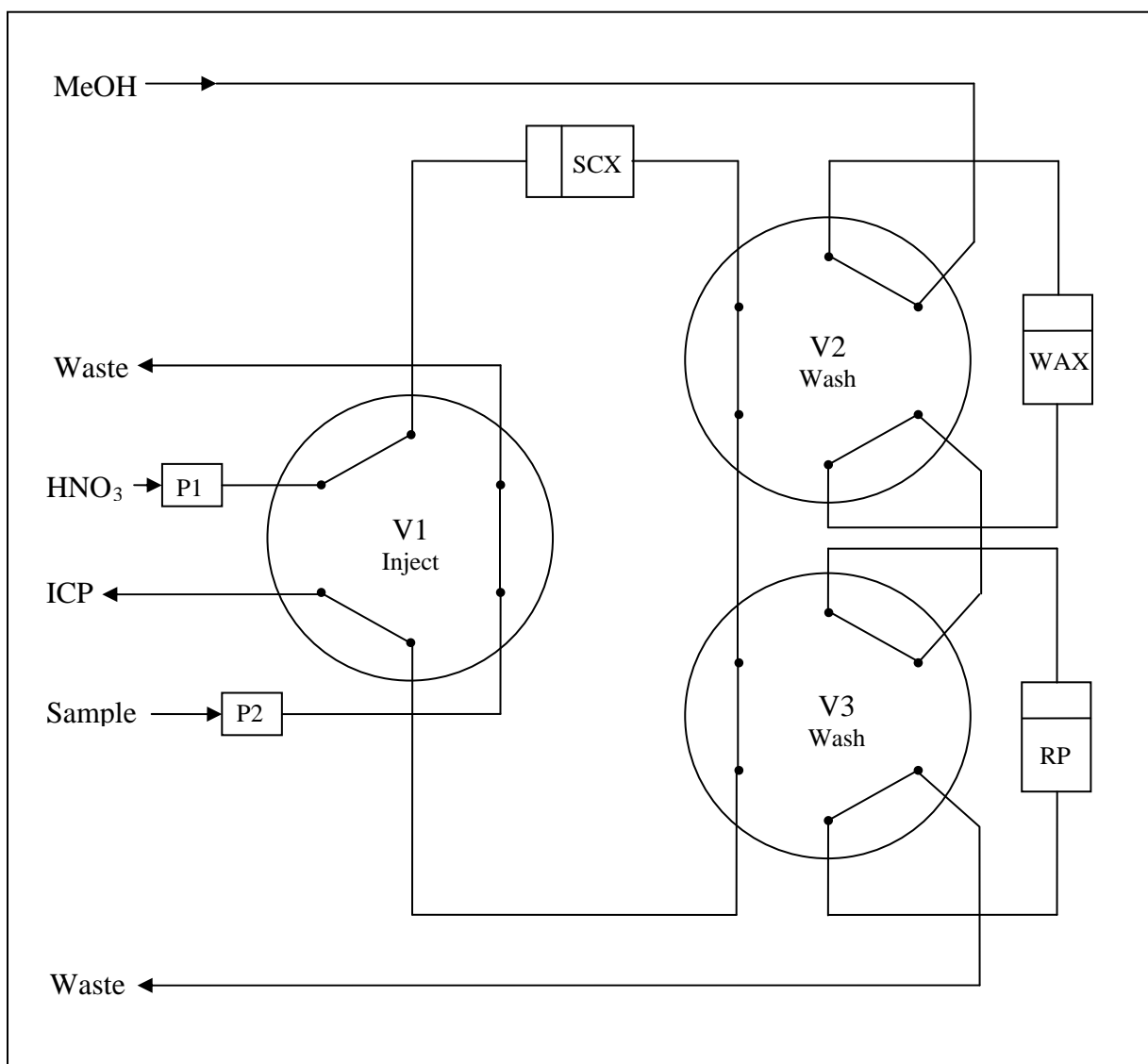


Figure 16: FI-setting for stripping the SCX-cartridge.

The elution sequence starts with switching valves V2 and V3 to the Wash position to prevent the eluent from passing the respective cartridges. Valve V1 is then set to Inject, enabling the eluent to flow through the SCX-cartridge and thus to strip the therein bound trace metal ions

and complexes. Figure 16 reports the setting of the FI-system employed to strip the SCX-cartridge.

Subsequently to the elution of the SCX-cartridge, the WAX- and RP-cartridges were inserted consecutively into eluent flow by switching first valve V2 and later on valve V3 back to the Load/Inject position as shown in Figure 17 and Figure 18.

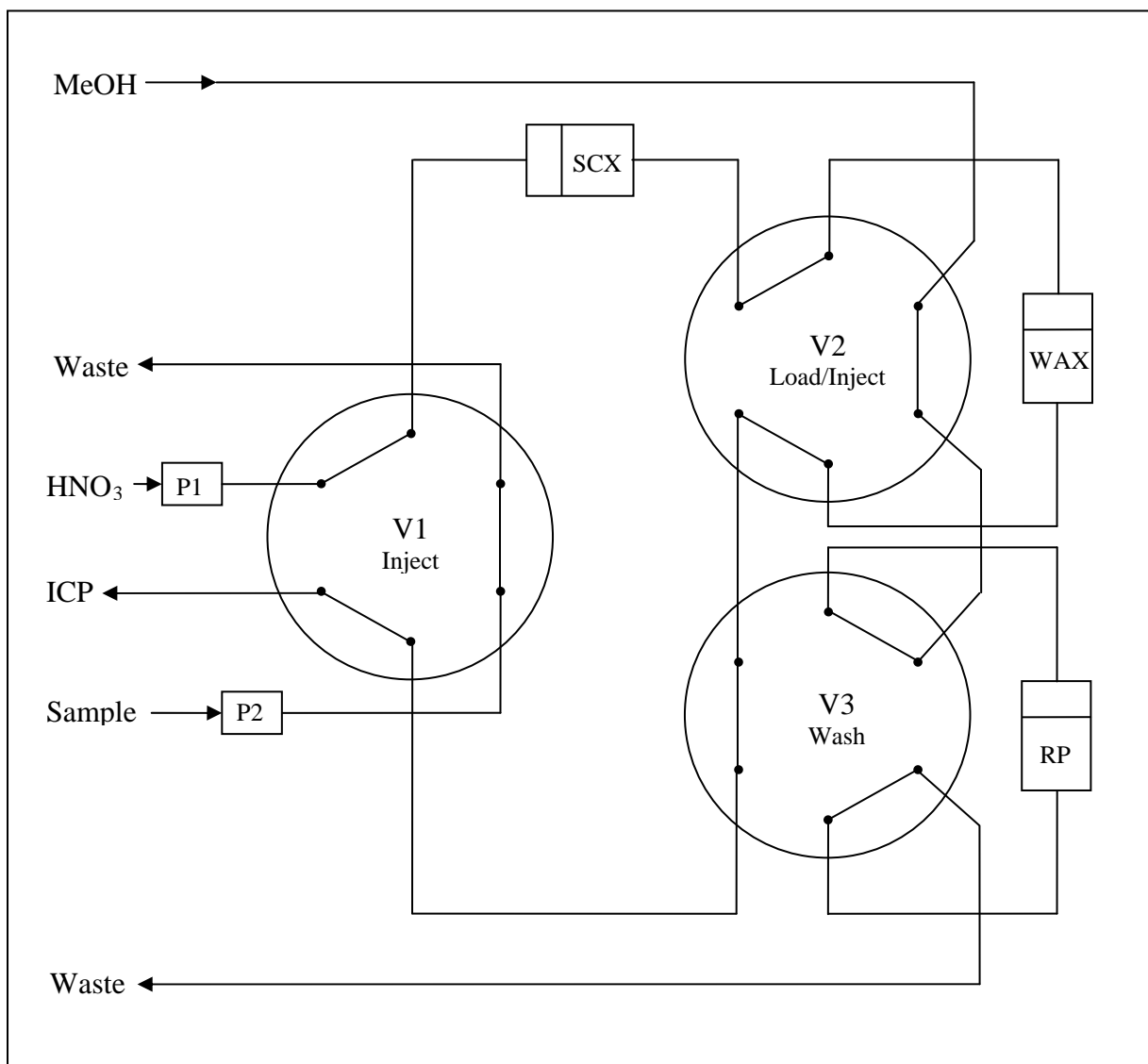


Figure 17: FI-setting for stripping the WAX-cartridge.

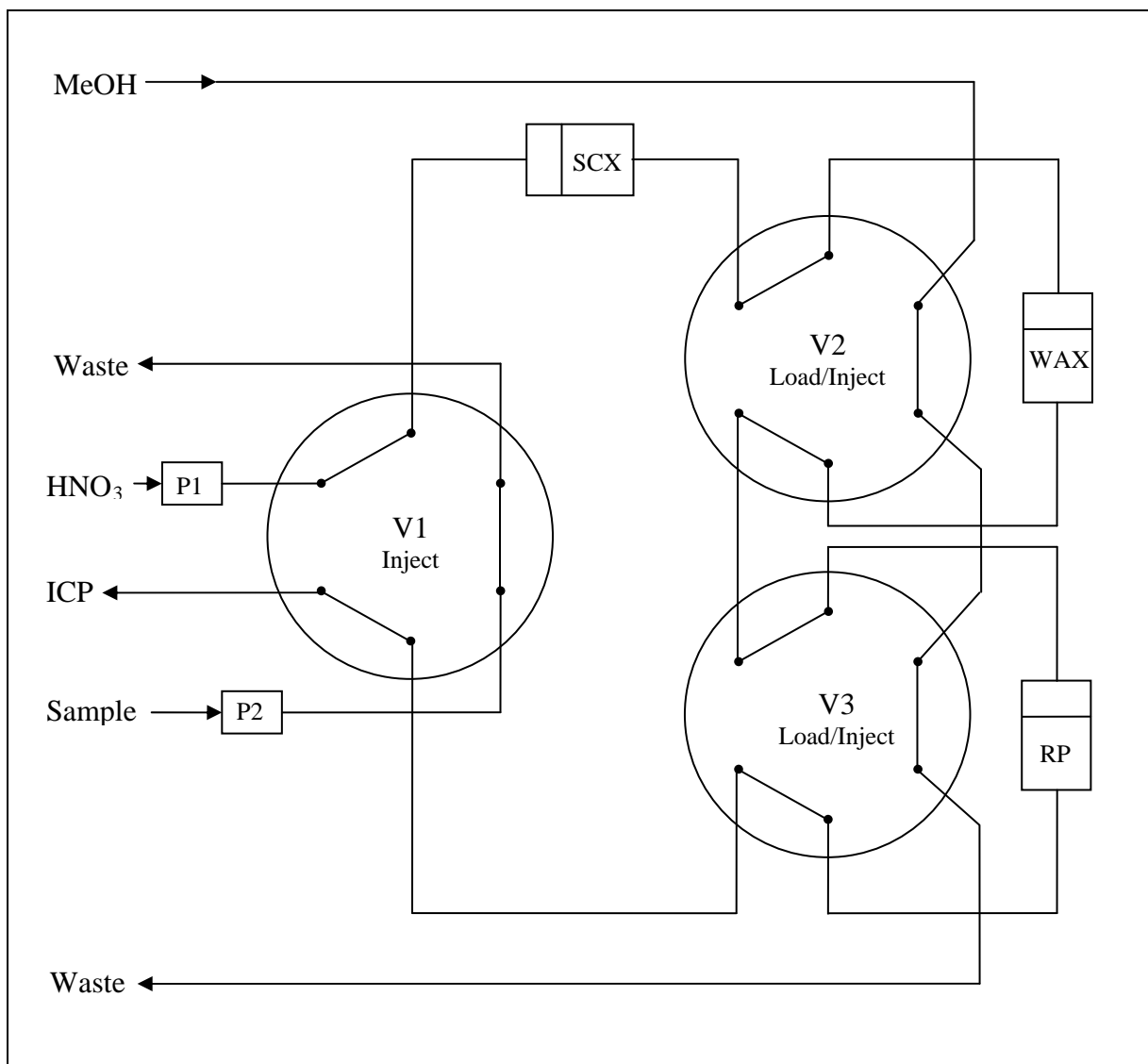


Figure 18: FI-setting for stripping the RP-cartridge.

At the end of the measuring sequence, valves V2 and V3 were reset to the Wash and valve V1 to the Load position. After cleaning the WAX- and the RP-cartridges with 2 ml of methanol and reinserting them into the loading cycle by manually setting both valves back to position Load/Inject, the FI-system was readied for the next sample.

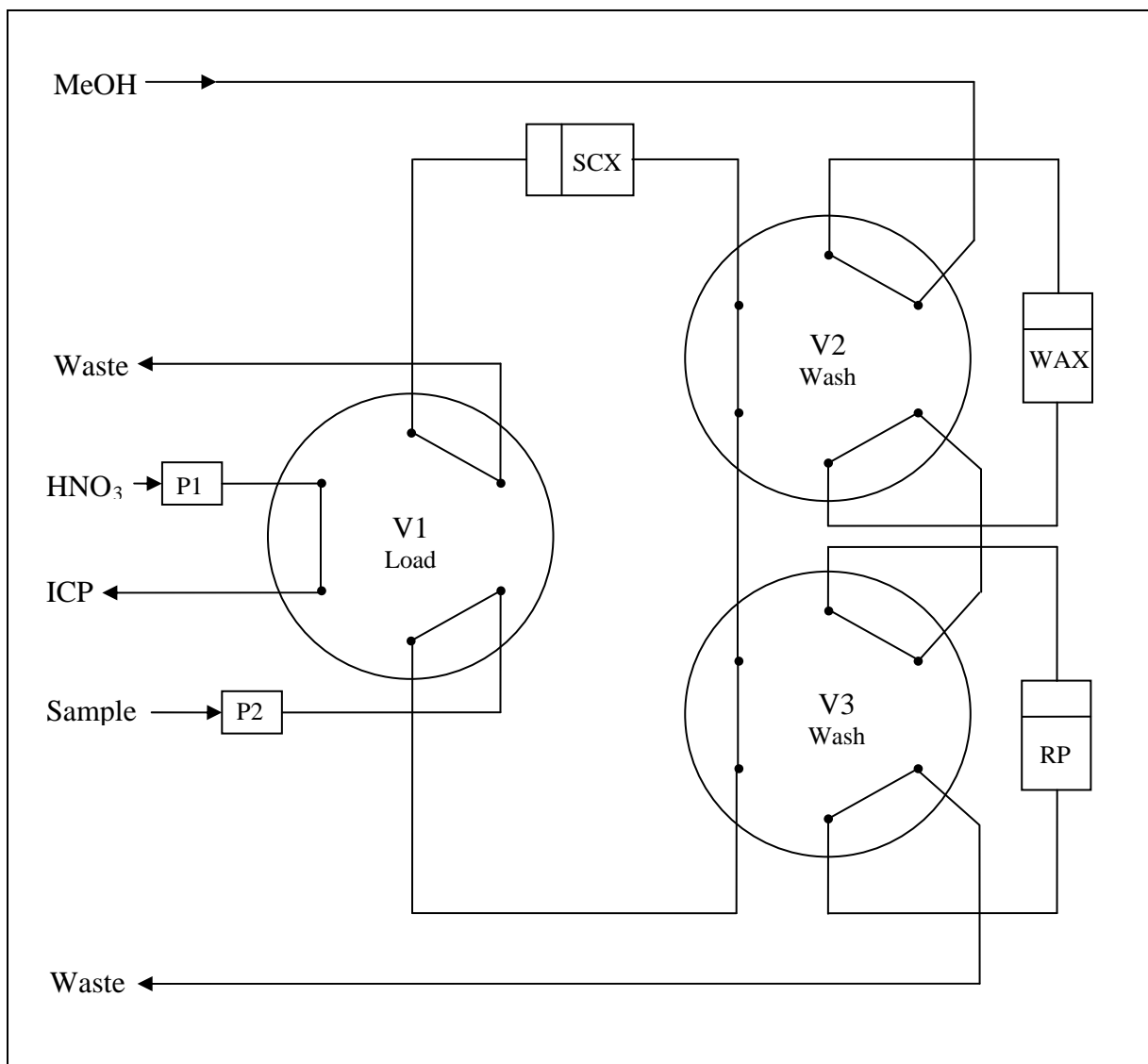


Figure 19: FI-setting cleaning and conditioning.

#### 4.1.3 Data Processing

Raw data received from the spectrometer consists of measured signal intensities and date/time indices of each replicate. To simplify data evaluation, the date/time index of the first measured replicate was set to zero and deducted accordingly from all other indices. Moreover, the replicate number was added to each data set.

To calculate the areas of the elution peaks it is necessary to previously apply a background correction. Differing from the method mentioned in chapter 3.2.3 the spectral background was calculated individually for each of the three peaks occurring in the elution profile.

Even though a rectifying method employing only one background value was selected for this purpose, series of five data points each from both sides of the peak were chosen. The average values of both data sets were calculated and after comparison, the smaller of the two values was subtracted from all measured intensities employed for the integration of the peak area.

This corrected data set was used for the integration of the peak areas. In doing so, adequate integration limits for each peak had to be applied. Figure 20 illustrates the background correction and integration limits by means of the Al 396.152 nm elution profile of 5 ml of a snow sample taken on 2005-12-30 at 15:30 on a traffic island in Vienna (Getreidemarkt, near Guermannngasse), containing 140 ppb Al (for further detail refer to chapter 5).

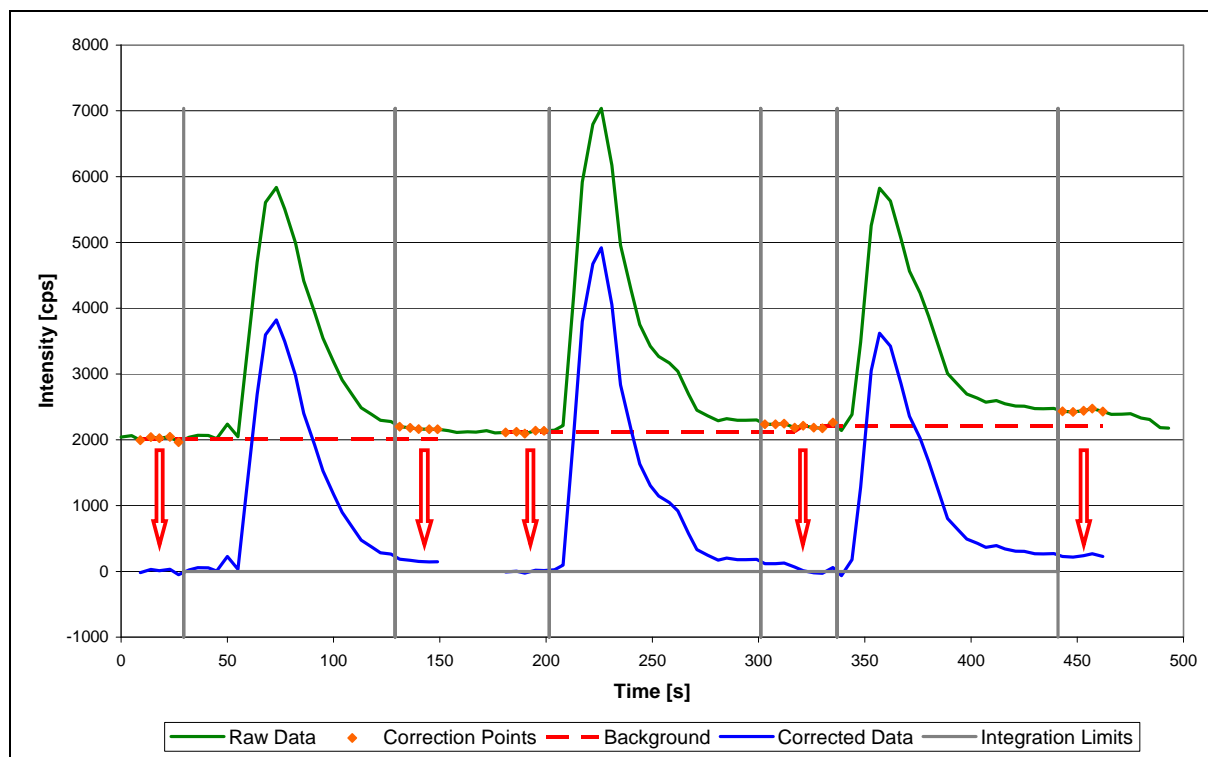


Figure 20: Background correction and data integration.

Total metal concentration was calculated by adding count values of the three peaks of the sample and comparing the sum to known values determined in calibration. Fractions of the different species were correspondent to the respective contributions of the respective peak areas to that sum.

## 4.2 Results

### 4.2.1 Enrichment factor

Taking into account the eluent flow rate of 0.75 ml/min and duration of the elution steps of approximately 2:30 minutes each, the eluent volume amounted to 1.875 ml per cartridge. Given an applied sample volume of 5 ml, theoretically a preconcentration factor of 2.67 can be achieved. Since the elution of the SPE-cartridges yields no square elution profile but rather a peak, the real preconcentration factor has more likely a value of 5. Figure 21 demonstrates the elution profile of 5 ml of a ligand-free 100 ppb Cd-standard measured with the FI-system employing standard procedure and parameters (*cf.* chapter 4.1 and Table 2) and the corresponding square functions of 2 ml and 1 ml of Cd-standards containing 250 ppb, respectively 500 ppb Cd, which both were conventionally measured with the ICP-AES using the same parameters (*cf.* Table 2).

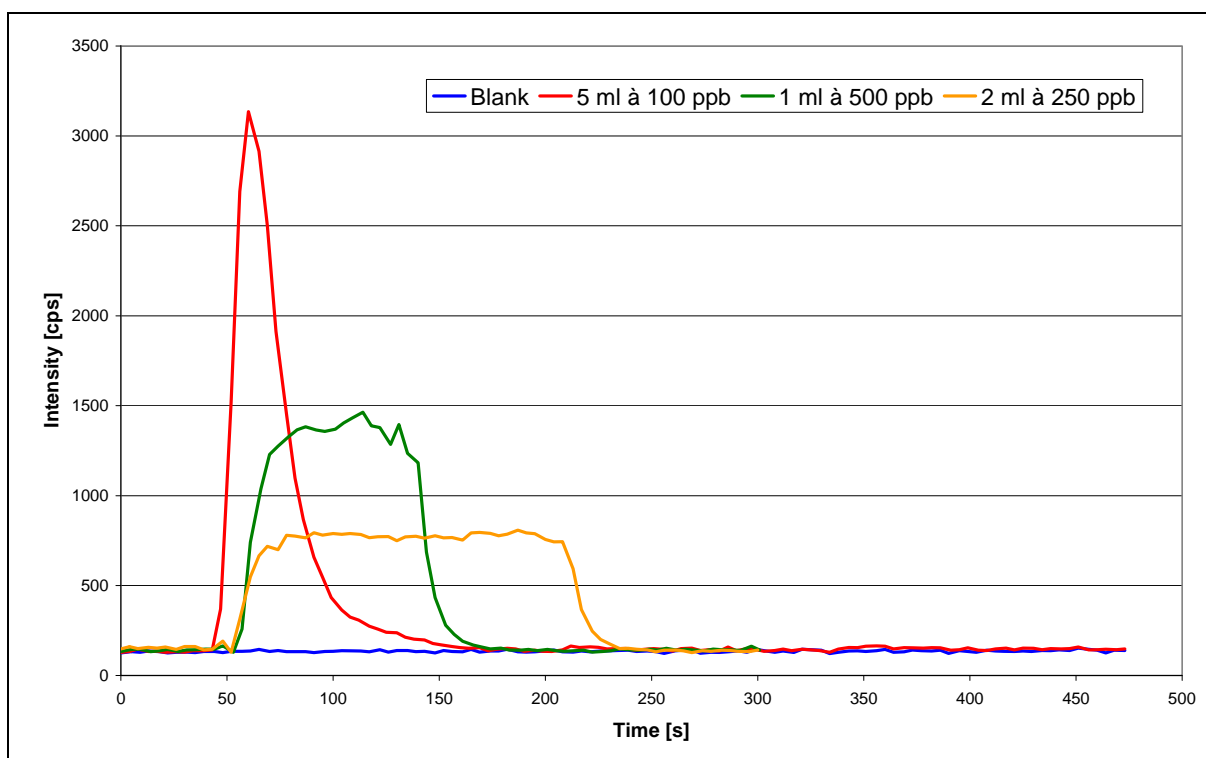


Figure 21: Preconcentration achieved with the FI-system.

#### 4.2.2 Separation of Species

To verify the separation of the different species, the setup was tested using different kinds of standards containing 100 ppb of copper. To ensure the quantitative conversion of free ions or cationic complexes of Cu, the employed solutions were brought to a pH of 3 by the addition of 25  $\mu$ l of 2 M  $\text{HNO}_3$ . For the formation of anionic complexes, 5 ml aliquots of Cu standard solution were spiked with 50  $\mu$ l of 0.01 M EDTA-solution, resulting in a ligand concentration of 0.1 mM. Due to the acid content of the metal stock solution the samples had a pH value of 5. Neutral organic complexes were formed by the addition of 50  $\mu$ l of 0.01 M 8-HQ-solution to each 5 ml aliquot of standard and adding 25  $\mu$ l of 2 M  $\text{NH}_3$ . Thus, the samples contained also 0.1 mM of ligand and had a pH value of 8. For each measurement 5 ml of standard solution was used. All measurements were carried out according to chapter 4.1, employing the operating parameters given in Table 2, and were performed in duplicate. The resulting elution profiles recorded at 324.754 nm are given in Figure 22.

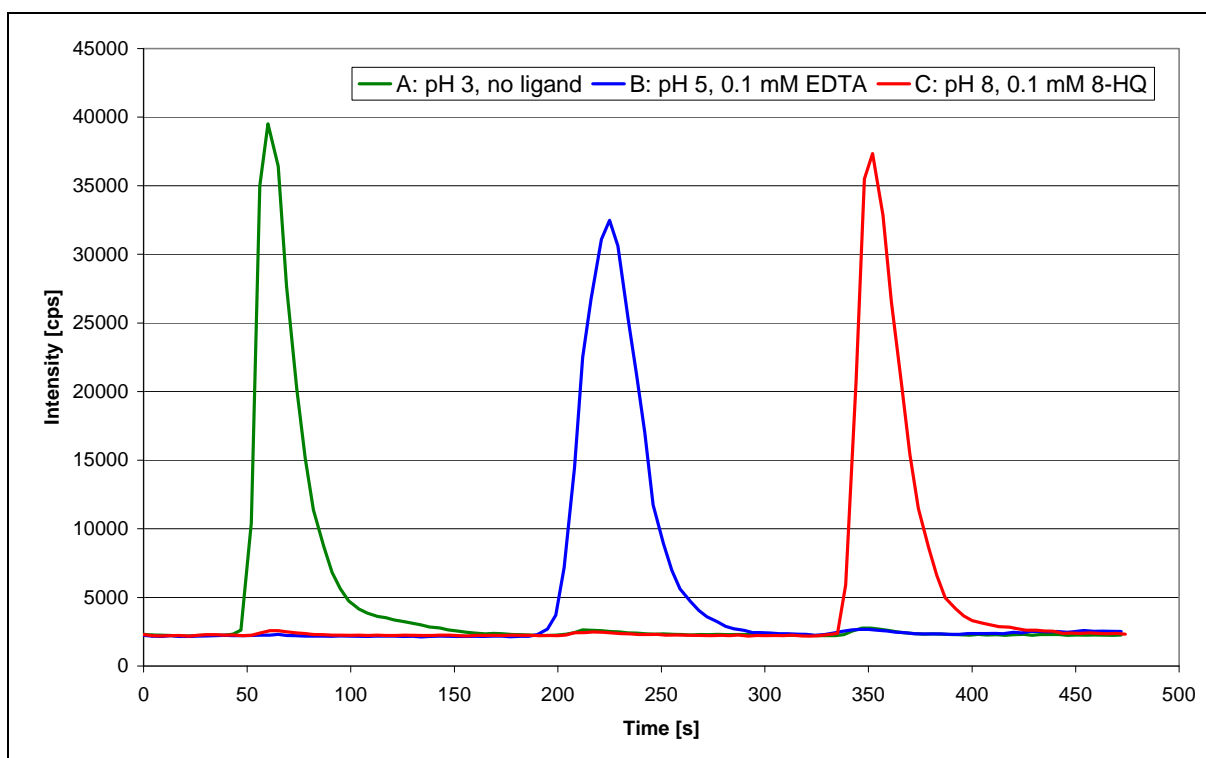


Figure 22: Separation of Cu species.

### 4.2.3 Detection Limits

For determination of the achievable detection limits and linear range of the developed system, three different types of calibration standards were used. Analog to the determination of the detection limit of the 2-cartridge setup (*cf.* chapter 3.3.2), ligand-free metal standard solutions were analyzed in a first series of measurements. The pH of those standards was brought to a value of three by addition of small amounts of 2 M HNO<sub>3</sub>. In two following series ligand stock solutions were added to the prepared standard. One of those series employed EDTA as complexing agent, the other D,L-tryptophan (TRP). In case of standards containing EDTA, no further reagents were added, thus solution pH was affected by the acid content of the metal stock solution. Standard solutions containing TRP were adjusted to a pH value of 10 by adding small amounts of 2 M NH<sub>3</sub>. The concentrations of the metal standards and added amount of ligand stock solution (when applicable) are given in Table 10. In all cases, the prepared standard volume for each measurement was 5 ml. All measurements were carried out according to chapter 4.1, employing the operating parameters given in Table 2, and were performed in duplicate.

Table 10: Standard composition for calibration.

<b>Metal concentration in the standard</b>	<b>Volume of metal stock solution</b>	<b>Concentration of metal stock solution</b>	<b>Volume of ligand stock solution (0.01 M)</b>	<b>Ligand concentration in the standard</b>
0 ppb (blank)	0 µl	inapplicable	0 µl	0.0 mM
3 ppb	15 µl	1 ppm	50 µl	0.1 mM
10 ppb	50 µl	1 ppm	50 µl	0.1 mM
30 ppb	15 µl	10 ppm	50 µl	0.1 mM
100 ppb	50 µl	10 ppm	50 µl	0.1 mM
300 ppb	30 µl	50 ppm	100 µl	0.2 mM
1000 ppb	100 µl	50 ppm	300 µl	0.6 mM
3000 ppb	300 µl	50 ppm	1000 µl	2.0 mM

After calculating total intensity yields of the standards according to chapter 4.1.3, the linearity of results was inspected and the operating range redefined correspondingly. The linear operating range was found to be strongly dependent of the monitored element and in some cases of the added ligands. As a consequence 300 ppb were estimated to be a reasonable value for the upper limit of linearity. At higher concentrations, the response of the system



diminishes due to tailing of elution peaks and the defined integration limits, but an extension of those would require an inappropriate prolongation of overall measurement duration.

Averaged sensitivities and correlation coefficients are given in Table 11, as well as the limits of detection calculated via the  $3\sigma$ -criterion from four blanks measured at each series. All other standards were analyzed in duplicate.

Table 11: Calibration data for three-cartridge setup.

<b>Element</b>	<b>Sensitivity [counts/ppb]</b>	<b>Correlation (<math>r^2</math>)</b>	<b>Limit of Detection</b>
Mg	36906.6	0.9986	1.2 ppb
Al	1813.8	0.9904	3.3 ppb
Ca	8079.2	0.9943	3.5 ppb
Cr	11219.5	0.9974	0.7 ppb
Mn	23270.5	0.9988	0.2 ppb
Fe	3130.6	0.9975	1.2 ppb
Co	104.9	0.9982	1.3 ppb
Cu	6799.6	0.9981	1.3 ppb
Zn	213.0	0.9989	1.4 ppb
Cd	743.1	0.9987	1.1 ppb
Pb	868.4	0.9973	12 ppb

Since the three measurement series employed for the calculation of the calibration data were carried out on different days, varying results concerning sensitivities were expected. Nevertheless, the slopes of the regression lines were noticeable in two cases, namely the Fe-calibration with EDTA and the Al-calibration at pH 10. Both results deviate significantly from the two corresponding results and were not included in the calculation of the average value. The reasons for this will be discussed in chapter 4.2.6.

#### 4.2.4 Standardization

As stated in chapter 3.3.3, neither a full calibration routine nor the quantification of three different standard solutions corresponding to the three peaks received in a measurement is practicable on a daily basis. Therefore the standardization procedure established for the two-cartridge setup was retained unchanged for the expanded system in order to permit

speciation and quantification of metals in samples, without spending too much time on the preparation for measurements.

#### 4.2.5 Reproducibility

For examination of reproducibility the standard solutions used for standardization were analyzed not only at the beginning of a measurement series, but also between measurements and at the end of the measurement series with an interval of two hours.

Table 12: Relative standard deviations (n=6).

Element	Mean [counts]	RSD [%]
Mg	3792204	4.8
Al	182352	3.1
Ca	914931	3.7
Cr	1215240	3.5
Mn	2469753	4.6
Fe	293158	4.5
Co	10161	2.9
Cu	625318	4.7
Zn	22516	5.9
Cd	76173	3.8
Pb	71334	6.4

Investigated was a ligand free standard solution containing 100 ppb of each analyzed trace metal of which an aliquot of 5 ml was employed for each measurement. Operating parameters are given in Table 2 and the followed procedure is specified in chapter 4.1.

The results shown in Table 12 were acquired from two sets of three standard measurements each, which were obtained on different days.

Accounting for the tendency of ICP-AES systems to show distinctive variations in their daily performance, the obtained relative standard deviations were definitely within an acceptable range.

#### 4.2.6 Influence of Organic Ligands

In order to investigate the influence of organic ligands, synthetic samples were prepared by spiking each aliquot of 5 ml high purity water with 50  $\mu$ l of metal stock solution. The stock solution contained 10 ppm of the investigated metal ions, thus the samples held an amount of 100 ppb of each. Furthermore, the samples were spiked with the different organic ligands and brought to different pH values by addition of different quantities of  $\text{NH}_3$ . In particular, the investigated ligands were 8-hydroxyquinoline (8-HQ) and D,L-tryptophan (TRP), which form neutral hydrophobic species, and ethylene-diamine-tetraacetate (EDTA) and nitrilotriacetic acid (NTA), which give rise to anionic complexes. The exact compositions of the samples are listed in Table 13.

Table 13: Composition of ligand-spiked standard solutions.

Ligand	$\text{NH}_3$ added	Ligand concentration	Resulting pH
8-HQ	50 $\mu$ l 2 M $\text{NH}_3$	0.1 mM 8-HQ	10
	25 $\mu$ l 2 M $\text{NH}_3$	0.1 mM 8-HQ	8
	15 $\mu$ l 2 M $\text{NH}_3$	0.1 mM 8-HQ	6
TRP	50 $\mu$ l 2 M $\text{NH}_3$	0.1 mM TRP	10
	15 $\mu$ l 2 M $\text{NH}_3$	0.1 mM TRP	7
	none	0.1 mM TRP	5
EDTA	50 $\mu$ l 2 M $\text{NH}_3$	0.1 mM EDTA	9
	15 $\mu$ l 2 M $\text{NH}_3$	0.1 mM EDTA	7
	none	0.1 mM EDTA	5
NTA	50 $\mu$ l 2 M $\text{NH}_3$	0.1 mM NTA	10
	15 $\mu$ l 2 M $\text{NH}_3$	0.1 mM NTA	8
	none	0.1 mM NTA	5

These samples were measured following the procedure given in chapter 4.1 employing standard operating parameters (*cf.* Table 2). The emerging species fractionation was quantified and the results of these measurements are given in Figure 23. According to chapter 4.2.4, a ligand-free trace metal standard, containing 100 ppb of each metal and adjusted to pH 3 with 50  $\mu$ l of 2 M  $\text{HNO}_3$ , was used for standardization. All measurements were performed in duplicate and 5 ml aliquots of the samples were used for each measurement.

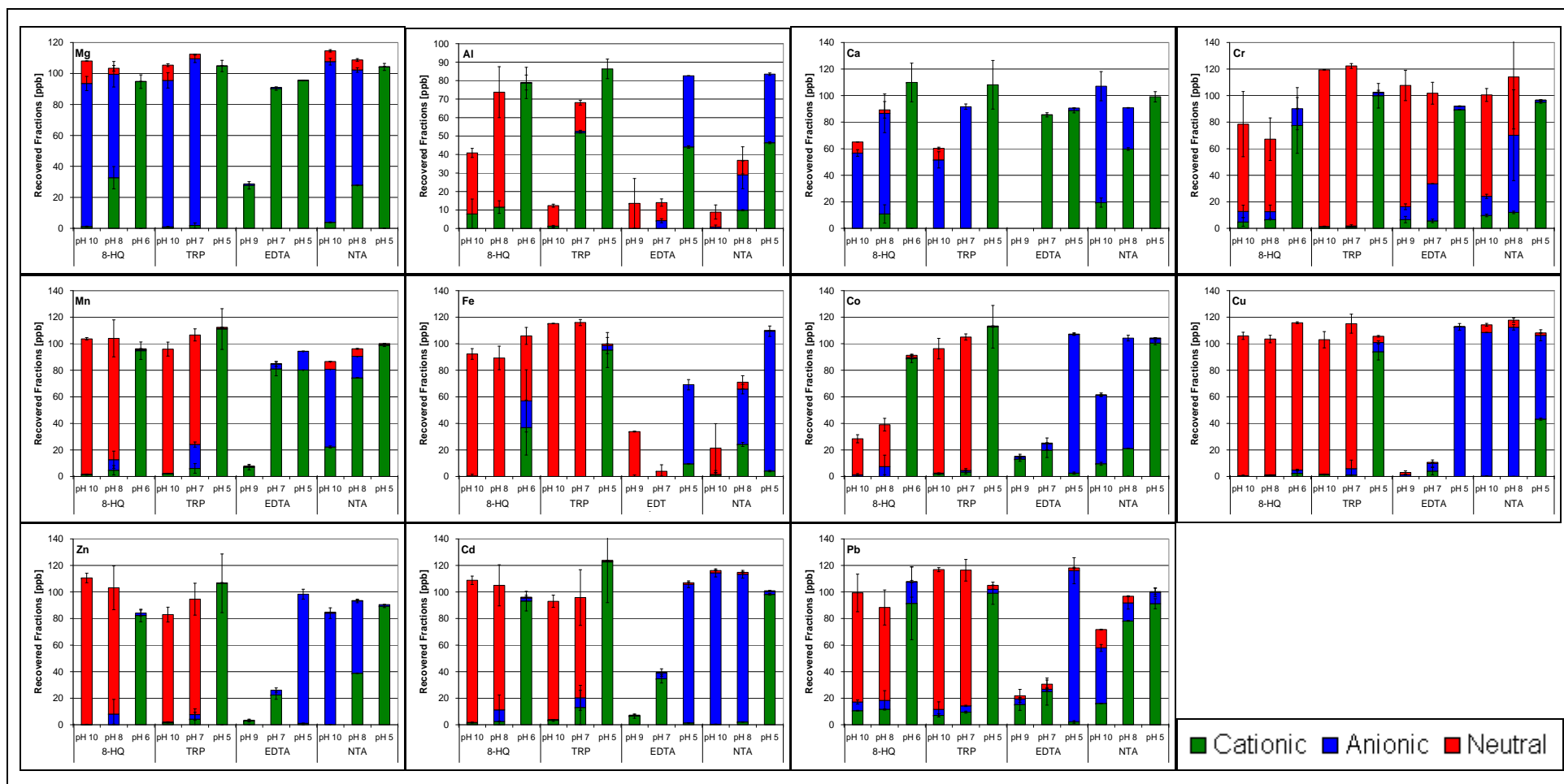


Figure 23: Fractionation and recovery of ligand-spiked standard solutions.

As apparent from the displayed data, trace metal speciation changes depending on the employed ligand and the pH value. Generally, fractions shift from cationic to anionic and neutral complexes with increasing pH. This was expected, since all investigated ligands are increasingly deprotonated and therefore better suited for complexation of trace metal ions. Furthermore, at neutral or high pH the ligands formed the expected species in nearly all cases.

Recoveries were satisfying for most of the investigated samples. Nevertheless, in several cases recoveries deviated significantly from the expected value of 100 ppb. Negative discrepancies from the targeted value can be mostly attributed to potential sources of error inherent in the SPE method.

- In sample preparation, losses of analyte can occur due to different reasons. A few elements, especially Al and Ca, exhibited decreasing recoveries at higher pH values, independent of the type of added ligand. This effect is more closely investigated in chapter 4.2.8. Furthermore, potential interactions of trace metals complexes, especially adsorption of hydrophobic metal species or hydroxocomplexes onto the walls of the sample vessels or the pump tubings, can lead to losses of analyte and so to reduced recoveries.
- Non-quantitative retention of complexes can result if either affinity or capacity of the SPE sorbents is insufficient. Since the employed array of sorbents, especially after the incorporation of the WAX-cartridge into the FI-system, should cover ionic as well as hydrophobic interactions, lack of affinity can be mostly ruled out. The dimension of the employed cartridges should also be sufficient to adsorb the analytes quantitatively. Nevertheless, additional possibilities have to be considered; e.g. distinctly diminished recoveries were found when employing EDTA for complexation, particularly at neutral and high pH values. This was also observed for NTA, in lesser extent and only for some elements, e.g. Fe. The reduction of recoveries by anionic ligands is further discussed in chapter 4.2.7.
- Another possible reason for low recoveries lies in the stability of organic complexes. Since the eluent employed is not an organic solvent like methanol but an acid, the complexes are not washed out of the cartridge during the elution process. Instead, the eluent rather destroys the complexes either by displacing the metal ion by means of its low pH value or, in case of  $\text{HNO}_3$ , it destroys labile ligands by oxidation. It is possible that very stable complexes, retained by an SPE sorbent, remain adsorbed and intact

during the whole elution step and are only washed out with methanol in the conditioning step for the next measurement cycle. Those complexes are not detectable with the presented FI-ICP-AES system. Experiments indicate that this case may occur when Co is complexed with 8-HQ at alkaline pH values.

Positive discrepancies, i.e. recoveries exceeding 100%, have to be related to other influences. As already discussed in chapter 3.3.4, the signal enhancement caused by the occurring carbon-provoked interference<sup>[65]</sup> can also be found in the measurement series employing the three-cartridge FI-setup. These high recoveries suggest, that, precluding a change of the detecting ICP-AES system to a model able to perform transient measurements while simultaneously allowing a background reading and correction, the scope of the presented system should only be limited to samples with low TOC content. Since the expected TOC-levels in atmospheric samples like snow, rain or fog water are significantly lower than the carbon content of the standards employed above, deviations of the recovery values caused by spectral carbon interferences are estimated to be not as evident as displayed.

Alternatively, the quantification routine should be adapted to the sample matrix, employing *e.g.* matrix matched standard solutions for external calibration or standard addition methods (*cf.* chapter 4.2.10).

#### **4.2.7 Recovery of Anionic Complexes**

Distinctly reduced recoveries were found when employing EDTA for complexation. This was also observed in lesser extent and only for some elements, especially Fe, for NTA. Especially at high pH values, all elements, with the exception of Cr, exhibited recoveries falling well below the 50 percent level, in some cases even lower than 10 percent. As already stated in chapter 3.3.5 this is probably due to the limited capacity of the WAX sorbent, which is overwhelmed by the high concentration of multiple-charged EDTA. To insure that the low recoveries concerning the investigated metals are caused by the excess of ligand, the measurements of samples with the pH values 7 and 9 were repeated employing reduced EDTA-concentrations. Added amounts of the EDTA stock solution per 5 ml sample are given in Table 14.

Table 14: EDTA-concentrations in the standard solutions.

<b>Volume of stock solution added</b>	<b>EDTA-concentration in the stock solution</b>	<b>EDTA-concentration in the sample</b>
50 $\mu$ l	0.01 M	0.10 mM
15 $\mu$ l	0.01 M	0.03 mM
7 $\mu$ l	0.01 M	0.014 mM

The EDTA-spiked samples were analyzed following the procedure given in chapter 4.1 employing standard operating parameters (*cf.* Table 2). All measurements were performed in duplicate and 5 ml aliquots of the samples were used for each measurement.

According to chapter 4.2.4, a ligand-free trace metal standard containing 100 ppb of each metal and adjusted to pH 3 with 50  $\mu$ l of 2 M HNO<sub>3</sub> was used for standardization. The emerging species fractionation was determined and is displayed in Figure 24.

As displayed, recoveries for most elements were near to 100% at a sample pH of 5, which is in agreement with expectations. The elements Co, Cu, Zn, Cd and Pb were quantitatively converted to anionic species under the investigated conditions, confirming the suitability of the employed setup for separation and recovery of anionic complexes.

At pH 7 however, recoveries are reduced for most elements. Least affected by the change in pH are the earth alkali elements Mg and Ca, as well as Mn. Neither recoveries nor species distribution of those elements change very much with the increase of the pH value from 5 to 7, indicating that their affinity to EDTA is insufficient to form complexes under the investigated conditions. Cr shows a shift in species distribution towards anionic and neutral complexes but is recovered quantitatively. When employing an EDTA-concentration of 0.1 mM, all other elements exhibit recoveries reduced to less than 50%, in some cases even down to 4%. With decreasing ligand concentration the total recoveries are gradually ameliorated, reaching values between 40% and 70% at an EDTA-concentration level of 0.014 mM. Notably Fe, while showing the lowest recovery at the highest ligand concentration, also exhibits the greatest increase of recovery.

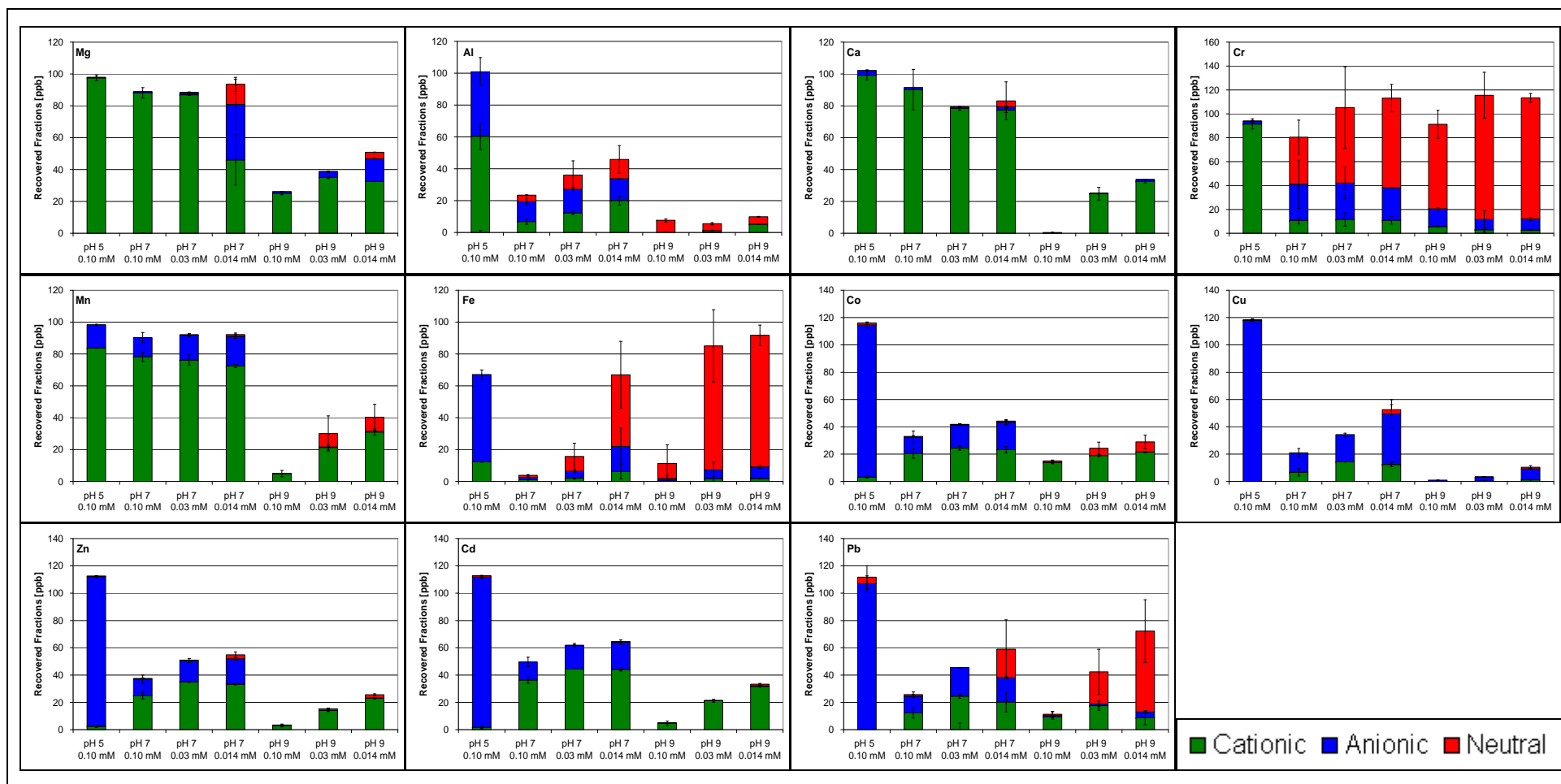


Figure 24: Fractionation and recovery of EDTA-spiked standard solutions.



Another unanticipated effect of the increase in pH is the appearance of the cationic fraction for the trace metals that were quantitatively present as anionic complexes at the lower pH of 5. Since higher pH values should in theory decrease the quantity of uncomplexed cations, this can not be due to shifts in speciation of the sample, but has to be caused by the employed separation process.

At a sample pH of 9, the described effects are even more prominent. Even the previously unaffected elements Mg, Ca and Mn exhibit significant losses of recovery and total recovery values of all metal ions, except Al and Cr, are inversely proportional to the added amount of EDTA and nearly no anionic complexes are detected. The cationic fraction found at pH 7 is still existent, but less pronounced.

All those observations can be explained by the previously made assumption that anionic complexes retained by the WAX-sorbent are displaced by the excess of negatively charged EDTA, which at pH 9 is almost quantitatively in its  $\text{HY}^{3-}$ -form. The competitive binding of multiply charged EDTA at high pH values on anion exchange sorbents has been observed previously by other authors<sup>[38],[47]</sup>. The earth alkali ions Mg and Ca, as well as Mn, do not follow this trend at pH 7, since the complexed fraction of these elements is comparatively low. Nevertheless it has to be assumed that the major part of the anionic fraction of these elements is displaced by the excess of negatively charged ligand ions, leading to poor recoveries, since anionic ligands are no longer quantitatively retained by the WAX-sorbent.

The cationic fraction of the elements Co, Cu, Zn, Cd and Pb emerging at pH 7 can be attributed to labile anionic metal-EDTA complexes that, if displaced from the retaining WAX-sorbent, decompose due to the competition with the functional groups of the following SCX-sorbent. With rising pH the binding of metal ions to the increasingly dissociated EDTA gets stronger, therefore the metal-EDTA complexes, while passing through the SCX-cartridge, are less likely destroyed.

#### 4.2.8 Influence of Sample pH

To differentiate between the influence of ligands and the discrepancy of recovery values caused by the pH value of the solution, synthetic samples containing trace metal amounts of 100 ppb of each analyte were brought to different pH values by addition of small amounts of 2 M HNO<sub>3</sub> or 2 M NaOH (*cf.* Table 15).

Table 15: pH-adjustment of ligand-free standard solutions.

Reagent	Added Volume	Resulting pH value
2 M HNO <sub>3</sub>	50 µl	3
none	none	5
2 M NaOH	15 µl	9
2 M NaOH	50 µl	11

The prepared samples were measured following the procedure given in chapter 4.1 employing standard operating parameters (*cf.* Table 2). According to chapter 4.2.4 a ligand-free trace metal standard containing 100 ppb of each metal and adjusted to pH 3 with 50 µl of 2 M HNO<sub>3</sub> was used for standardization. All measurements were performed in duplicate and 5 ml aliquots of the samples were used for each measurement. The emerging species fractionation was quantified and the results of these measurements are given in Figure 25.

Apparently evaluation of the total recoveries in this series shows that the pH value does not significantly influences the performance of the employed system, except in case of Ca and Al. Since these elements are known to form and to be precipitated as insoluble hydroxocomplexes, this effect was to be expected.

However, the analysis of the partitioning between cations, anionic and neutral complexes shows, that Ca and Al are not the only elements affected by the increase of the pH value. As shown in Figure 25, all investigated elements are influenced. At acidic pH values trace metals tend to occur, as expected, in cationic form, assumedly as free hydrated ions. Higher alkalinity, as caused in this case by addition of 2 M NaOH, induces the formation of other metal species. The trace metal ions, if not precipitated, are found nearly quantitatively in the anionic or even in the neutral fraction. This indicates that all metals in question form either anionic or neutral hydroxocomplexes, or that the increase of the pH value enhances the

formation of aggregates of colloidal size. Those colloids are found in the neutral fraction due to a simple physical filtration effect, independent of their ionic charge or hydrophobicity.

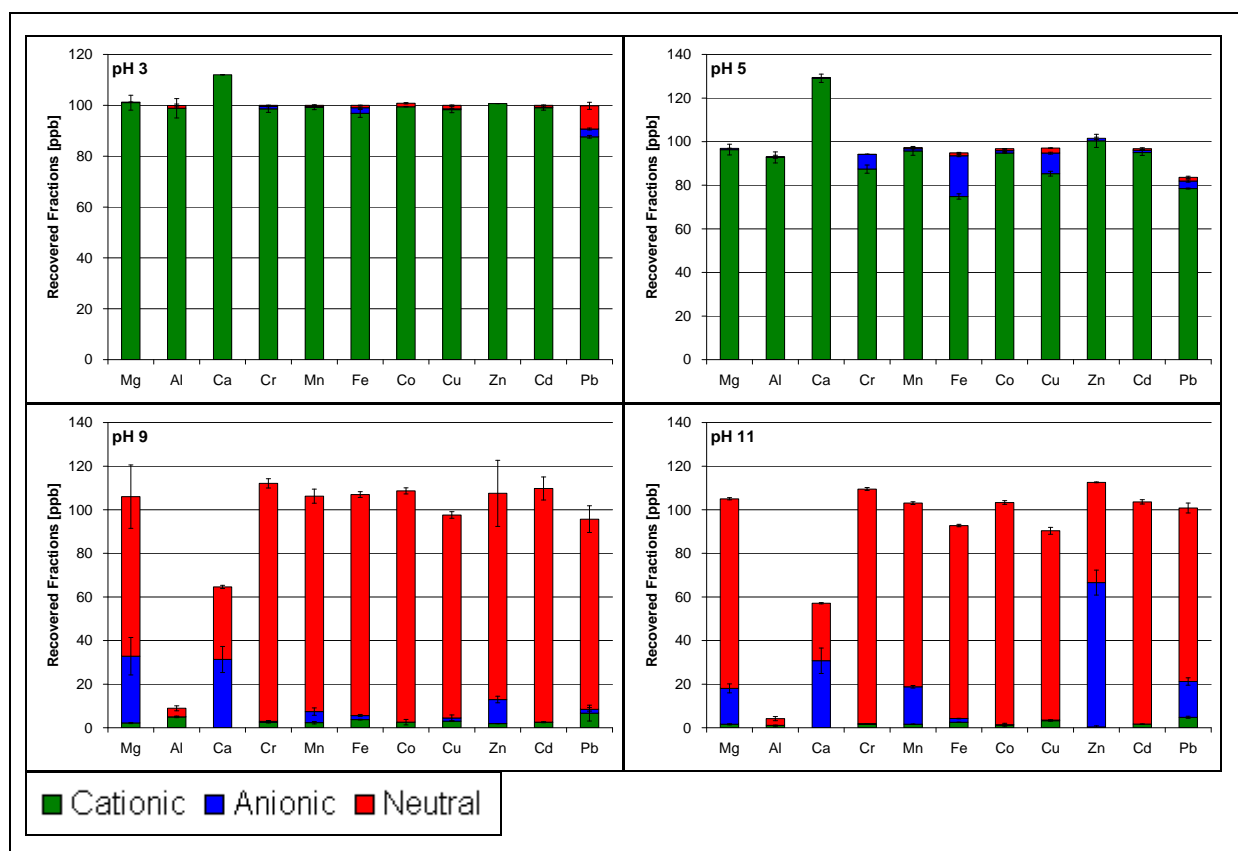


Figure 25: Fractionation of ligand-free standard solutions.

#### 4.2.9 Influence of Sample Volume

The limited linear range of the analytical method and the preconcentration step inherent in the fractionation scheme suggest an expansion of the upper limit of quantification by decreasing the sample volume. On the other hand, an increase of the sample quantity should lead to an enhancement of the limit of detection, since the preconcentration factor would be affected in the same way. Nevertheless, the washing step of the presented analytical scheme is rather suitable to ensure completion of sample transport through the FI-setup than to eliminate any matrix constituents adsorbed alongside the analytes. Therefore any change of the applied sample volume leads to a corresponding alteration of the adsorbed and possibly interfering matrix.

Since the investigation of interfering matrix effects is best carried out with a sample containing a broad range of naturally occurring constituents, a surface-water sample was taken from the Donaukanal near Schwedenbrücke (48°12'42.9" N, 16°22'41.3" O at 176 m a.s.l.; cf. Figure 26) in Vienna, Austria on May 29, 2007.

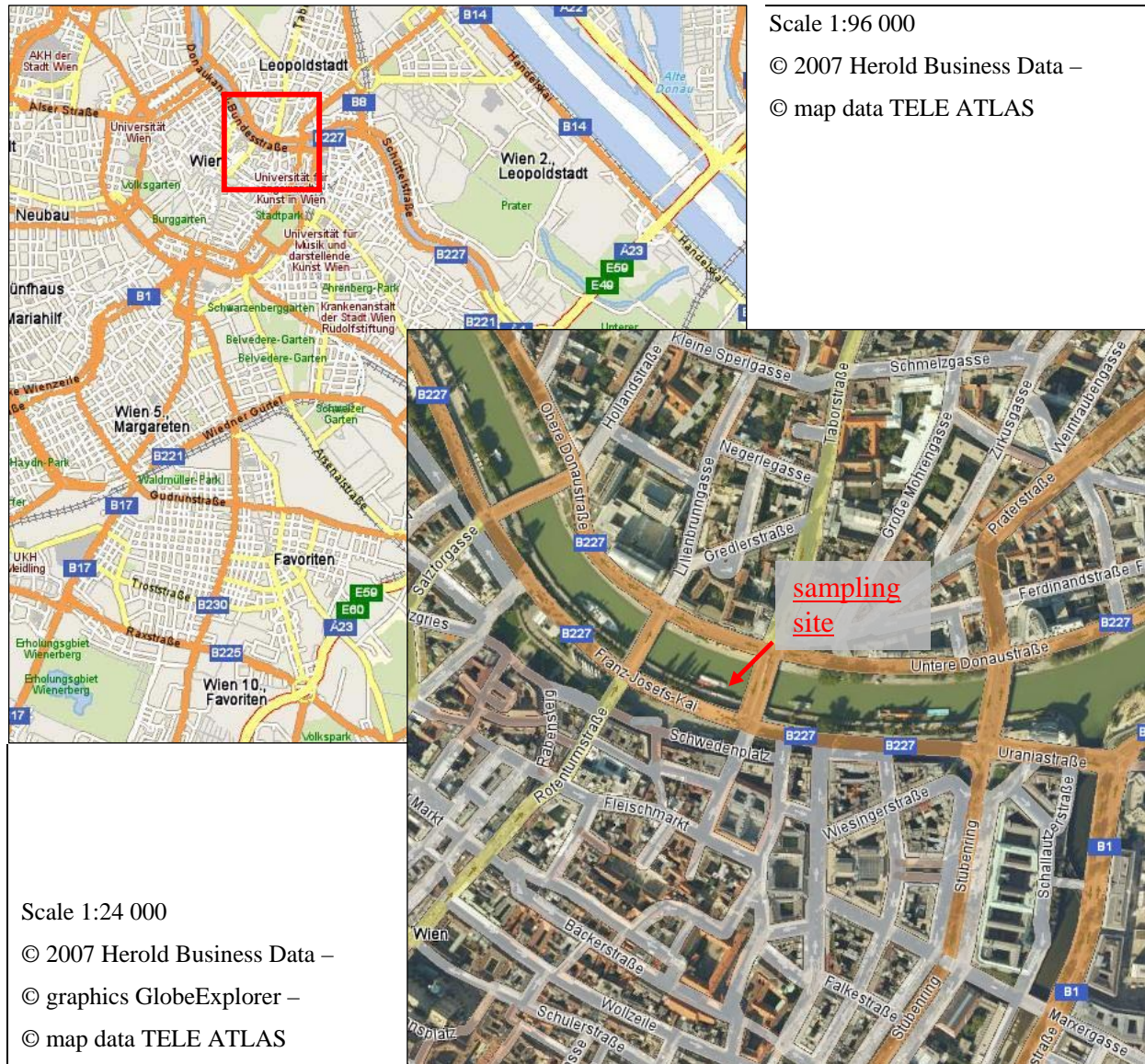


Figure 26: Location of sample drawing (Donaukanal).

To remove particular matter, the sample was filtered through an acid washed frit. Preliminary measurements showed that the original sample contained only small amounts of the trace metals in question, therefore it was modified better to fit the requirements for the intended experiments. Therefore, the sample was spiked with a small amount of the multielement and the Pb standard solutions to provide a reasonable amount of trace metals. Since the total sample volume was unknown, the resulting concentrations can only be estimated. To compensate for the acid content of the standards, approximately 200 ml of the sample was

mixed in a ratio of 4:1 with a 0.025 M  $\text{NH}_4\text{CO}_3$  buffer solution and spiked with 250  $\mu\text{l}$  of a 50 ppm trace metal standard containing all investigated elements except Al. The resulting reagent concentrations amounted to approximately 5 mM  $\text{NH}_4\text{CO}_3$  and 50 ppb of the added trace metals. The content of total organic carbon (TOC) was found to be 62.6 mg/l. The fractionation of trace metals in the modified Donaukanal sample is shown in Figure 27.

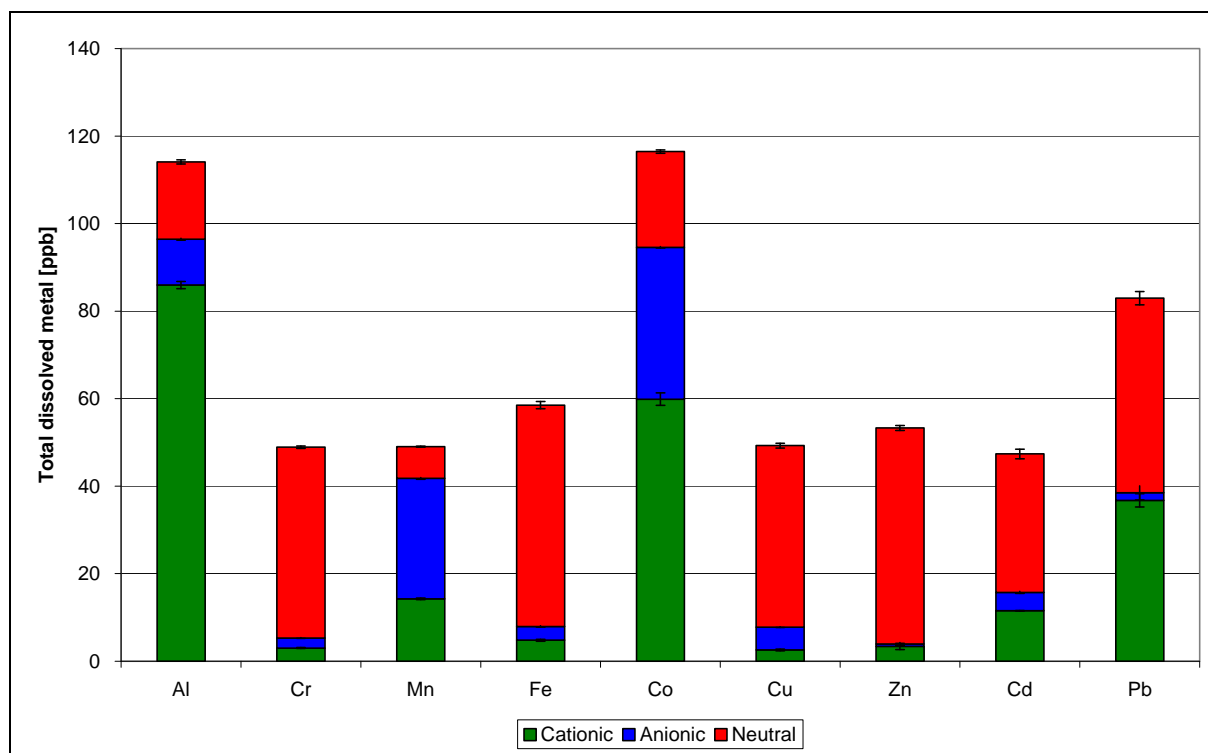


Figure 27: Fractionation of the modified Donaukanal sample (original).

The results shown in Figure 27 have been acquired using 5 ml of the sample solution. The gained response has been evaluated in comparison to a 100 ppb standard, of which equally 5 ml was used. The alkaline earth metals Mg and Ca could not be quantified with this sample volume, since the appropriate detectors were overloaded.

The series used for evaluation of the influence of sample volumes deviating from the standard volume consisted, apart from the standardization routine, of repeated measurements of the described Donaukanal sample. The only varied parameter was the sample volume, *i.e.* the volume loaded onto the SPE-cartridges. Employed sample volumes were 1, 2, 3, 5, 7 and 10 ml. The prepared sample was measured following the procedure given in chapter 4.1 employing standard operating parameters (*cf.* Table 2). All measurements were performed in duplicate.

Provided that both retention and elution of analytes are quantitative, the total response, *i.e.* the sum of all three peak areas, of the system should be directly proportional to the employed sample volume. Evaluation of these integrated areas shows that results differ for the different trace metals.

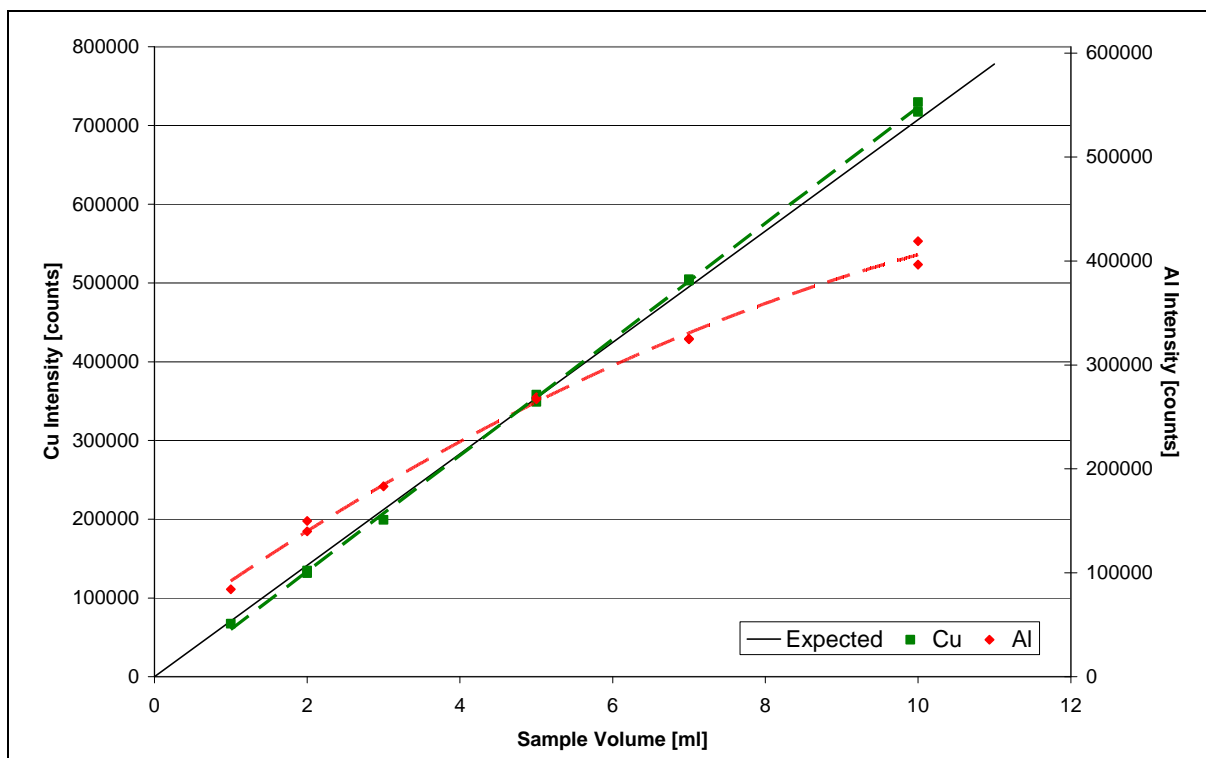


Figure 28: Relation of sample volume and response signal.

Typical examples of this relation are shown in Figure 28. While the assumption is confirmed for several elements, including Cu, other trace metals exhibit a deviation from this ideal case as it is demonstrated for the case of Al.

Nevertheless, fractions were quantified according to chapter 4.2.4. 5 ml of a ligand-free trace metal standard containing 100 ppb of each metal and adjusted to pH 3 with 50  $\mu$ l of 2 M  $\text{HNO}_3$  was used for standardization. Divergent sample volumes were accounted for using a suitable factor accounting for the varied preconcentration. Results are displayed in Figure 29.



# Trace Metal Speciation in Natural Aqueous Samples using a SPE-FI-System on-line coupled to ICP-AES

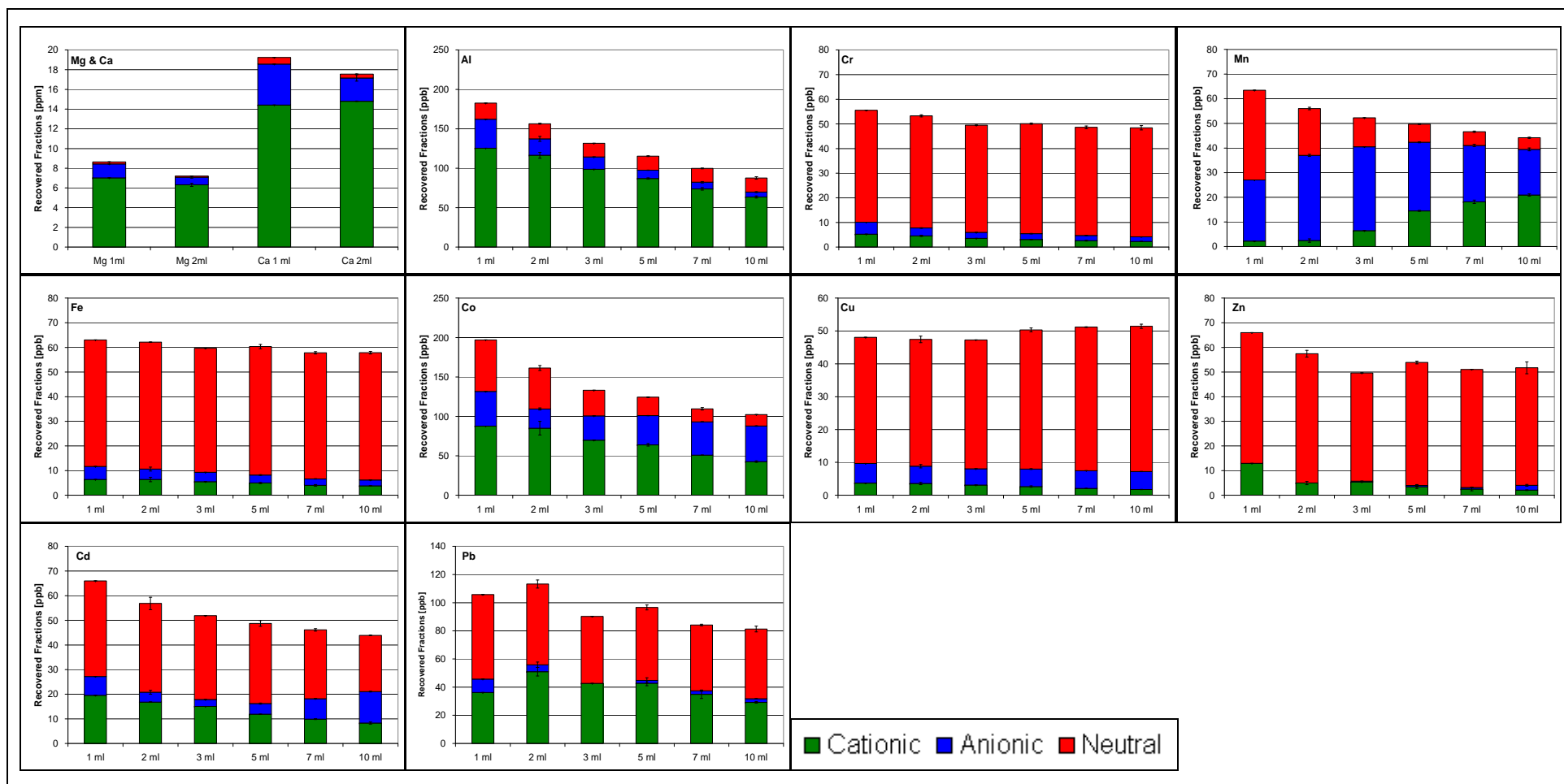


Figure 29: Influence of sample volume.

Dependent on the examined element, different conclusions can be reached concerning the influence of varying sample volumes.

- Headmost, the earth alkali elements Mg and Ca occurred in high quantities of  $7.9 \pm 0.8$  ppm respectively  $20.2 \pm 1.0$  ppm. Since the applied metal standard had only a metal content of 100 ppb each, these values have to be considered as a rough approximation. A reduction of the applied sample volume was necessary to allow quantification since the measured signal exceeded the detector capacity if sample volumes of more than 2 ml were employed. Another possible method to extend the analytic range of the ICP-AES is the change of analytic wavelength for the respective element. Due to technical limitations of the employed spectrometer the wavelengths could not be changed but the possibility could arise if an adequate spectrometer is used.
- The quantification of the remaining metals Cr, Fe, Cu, Zn and Pb showed no distinct tendency concerning the change in sample volume. Neither the total concentration nor the determined speciation change significantly, but volumetric errors in sample preparation and proportionally lower signal intensities lead to higher statistical variations.
- A special group of elements (Al, Mn, Co and Cd) followed a distinctive trend. Their specific response increased significantly with decreasing of the sample volume. The distribution into fractions remained largely unaffected, except in the case of Mn. The change in Mn speciation exhibited in Figure 29 indicates a shift in direction of the latter cartridges caused by larger sample volumes.

There are several possible explanations for this discrepancy. One is again the lack of spectral carbon interference correction. This may cause an overestimation of the metal content which will be, due to the applied factor, most prominent when low sample volumes are applied. This theory is supported by the fact that the influenced elements, especially Al, are known to be susceptible to carbon interferences.

Another explication is supported by the detected change in speciation of Mn (*cf.* Figure 29). Since the overall ion content, especially concerning Mg and Ca, was rather high, ineffectual retention due to displacement of the targeted analytes by competing ions is also a possible explanation. Due to the limited capacities of the employed sorbent quantities, increased sample volumes and the corresponding increase of the total amount of analytes, the retention of metal complexes may not be quantitative. Consequently, complexes that ought to be



retained on either sorbent are either washed out of the cartridges during the sample loading or the subsequent washing step of the SPE-procedure. Since these analytes are not registered by the ICP-AES, the premature elution leads to insufficient recoveries, as in the cases of Al, Co and Cd. Additionally, the species distribution may not be representative for the sample, since the displacement of analytes is not necessarily equal for all employed sorbent materials and occurring complexes. Furthermore, successive SPE-sorbents with free capacities may retain the metal complexes prematurely eluted from preceding cartridges, either because of unintended affinities between sorbent and metal complex (cf. chapter 4.1.1) or by displacing the metal ion from the ligands. This would cause a distinctive shift from the neutral to the anionic and on to the cationic fraction, as exhibited by Mn in Figure 29.

In conclusion, the variation of the employed sample volume, while necessary to expand the analytical range of the method in both directions, causes several problems. Since the determination of the total metal content is affected by spectral carbon interferences<sup>[65]</sup>, the disturbance caused by varying levels of TOC transported into the system cannot be neglected. This results in insufficient comparability between both, the synthetic standards and samples of deviant sample size, and the samples of varying volume among each other. Especially at low sample volumes the tendency for overestimation of the metal content seems evident.

Since the possibility for extending the upper limit for quantification by lowering the sample volume and to improve the limits of detection by increasing the same cannot be disregarded, the standard solutions used for quantification should be adopted for the employed strategy in size and composition. For all cases, an optimization of the operating parameters, including an adaption of the SPE-sorbent quantity to fit the required capacities, should be performed. This is even recommended if the employed ICP-AES system is able to perform the mentioned interference correction without abandoning the ability to register transient signals, *i.e.* to simultaneously record peak and background signals with the necessary temporal resolution.

#### 4.2.10 Standard Addition

To examine the influence of a natural matrix on the presented analysis, again the modified “Donaukanal” sample (*cf.* chapter 4.2.9) was used. In addition to the modification specified in chapter 4.2.9, the sample was spiked with aliquots of a 10 ppm metal standard corresponding to added metal amounts of 0, 50, 100 and 150 ppb of each element. The prepared samples were measured following the procedure given in chapter 4.1 employing standard operating parameters (*cf.* Table 2). All measurements were performed in duplicate and 5 ml aliquots of the samples were used for each measurement.

An example for the acquired elution profiles is shown in Figure 30. In this case the added Cu-spike was found nearly quantitatively in the neutral fraction.

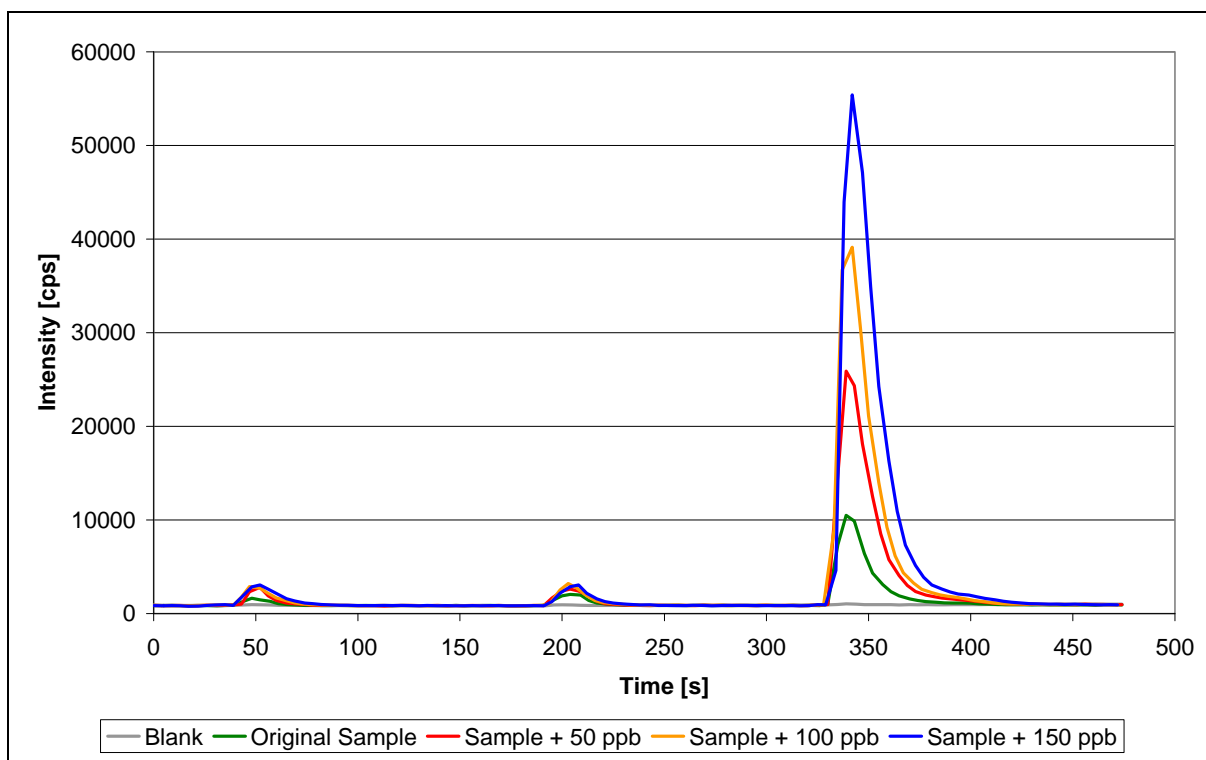


Figure 30: Standard addition of Cu.

Total metal content was estimated according to chapter 4.1.3 employing a ligand-free trace metal standard containing 100 ppb of each metal and adjusted to pH 3 with 50  $\mu$ l of 2 M  $\text{HNO}_3$  for standardization (*cf.* chapter 4.2.4). Since the concentrations of Mg and Ca in the investigated sample were 9 ppm respectively 20 ppm and therefore far out of range for quantification as well as in another magnitude than the added spikes, the obtained results were not taken into consideration. Appearing shifts in speciation towards the cationic fraction

induced by the addition of the standard solution were noted and can be attributed to the acid content of the spike, but were not relevant for evaluation of this measurement series, since only total metal contents were of interest.

The principle of standard addition is demonstrated in Figure 31. After determination of the total metal concentrations of the original as well as the spiked samples, the acquired concentration differences were compared to the nominal values of the spikes. These deviated significantly from expectations, thereby prohibiting the application of external standards for quantification without further adjustment. After ascertaining the linearity of the deviation, alternative calibration functions were calculated, employing the original and the spiked samples, thereby accounting for matrix effects.

Due to the linear nature of the standard addition procedure, recovery factors could be derived from the slopes of the acquired external and matrix matched calibration functions. These recovery values can be adopted as correction factors for similar samples, enabling the use of aqueous standard solutions.

The results of the quantification employing external calibration standards and by standard addition, as well as the correlation coefficients and calculated recovery factors are given in Table 16.

Table 16: Evaluation of standard addition (Donaukanal sample).

<b>Element</b>	<b>External Calibration</b>	<b>Standard Addition</b>	<b>Correlation <math>r^2</math></b>	<b>Recovery</b>
Al	$88.4 \pm 3.3$ ppb	$99 \pm 13.6$ ppb	0.9785	$89.3 \pm 11.9$ %
Cr	$30.2 \pm 0.4$ ppb	$29.5 \pm 1.8$ ppb	0.9939	$102.6 \pm 6.4$ %
Mn	$41.8 \pm 2.5$ ppb	$58.3 \pm 3.9$ ppb	0.9929	$71.7 \pm 4.4$ %
Fe	$35.7 \pm 0.7$ ppb	$30.1 \pm 1.7$ ppb	0.9944	$118.8 \pm 6.5$ %
Co	$114.6 \pm 6.9$ ppb	$108.1 \pm 10.9$ ppb	0.9885	$106 \pm 7.7$ %
Cu	$37.4 \pm 1.8$ ppb	$32.2 \pm 3.8$ ppb	0.9817	$116.1 \pm 13.7$ %
Zn	$32.9 \pm 2.7$ ppb	$26.9 \pm 1.7$ ppb	0.9944	$122.3 \pm 7.1$ %
Cd	$39.6 \pm 2.2$ ppb	$34.1 \pm 1.9$ ppb	0.9941	$116.1 \pm 5.9$ %
Pb	$61.3 \pm 5.0$ ppb	$52.7 \pm 4.9$ ppb	0.9875	$116.3 \pm 9.1$ %

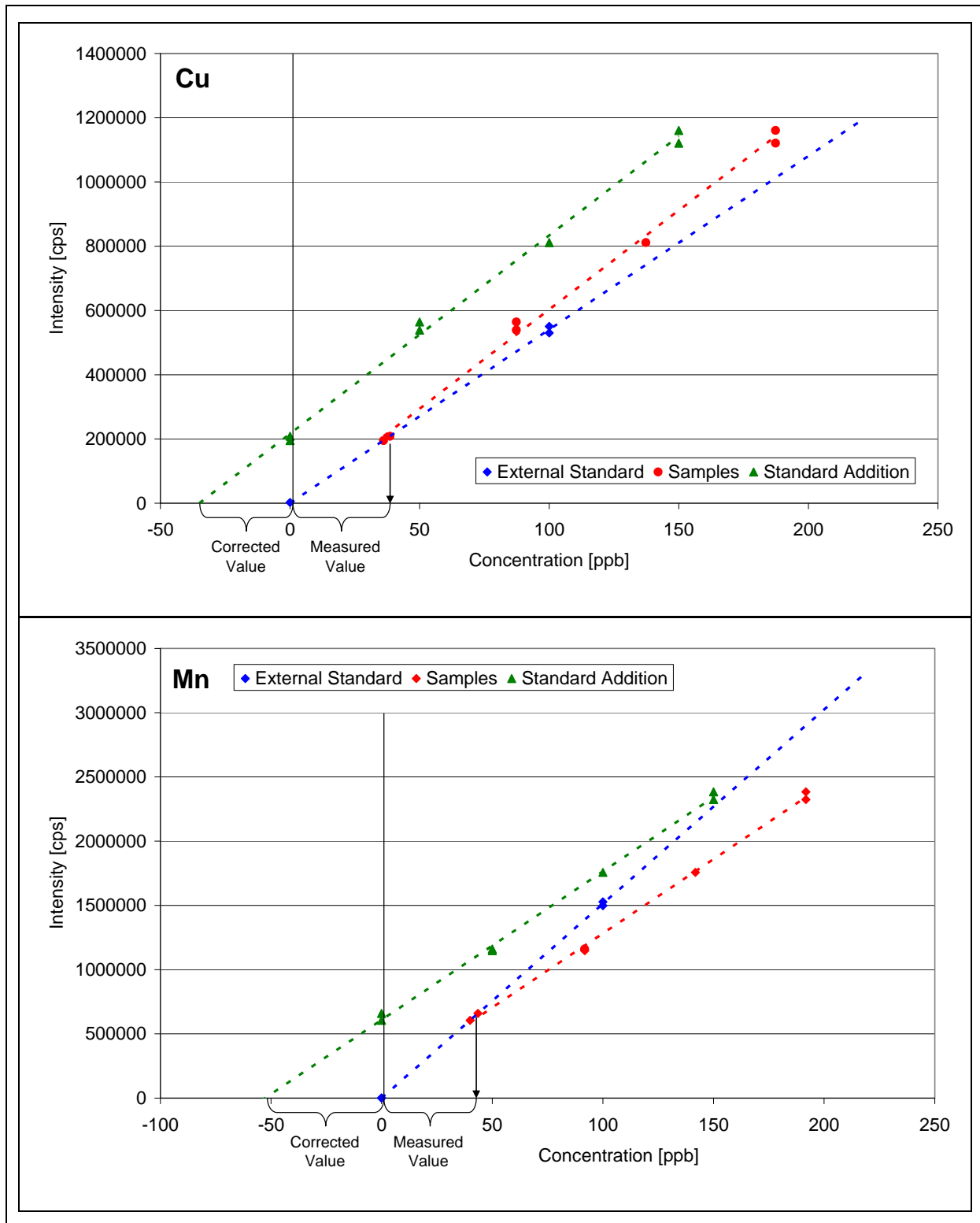


Figure 31: Principle of standard addition.

Cu: matrix causes overestimation of Cu-content, recovery > 100%.

Mn: matrix dampens response, recovery < 100%.

Application of the recovery factors to fractions derived from the total concentration values to the individual fractions allows the determination of the sample's speciation, including the

correction for matrix effects. The total amounts and the fractionation of trace metals as shown in Figure 32 deviated from the designated value (as reported in Figure 27 in chapter 4.2.9).

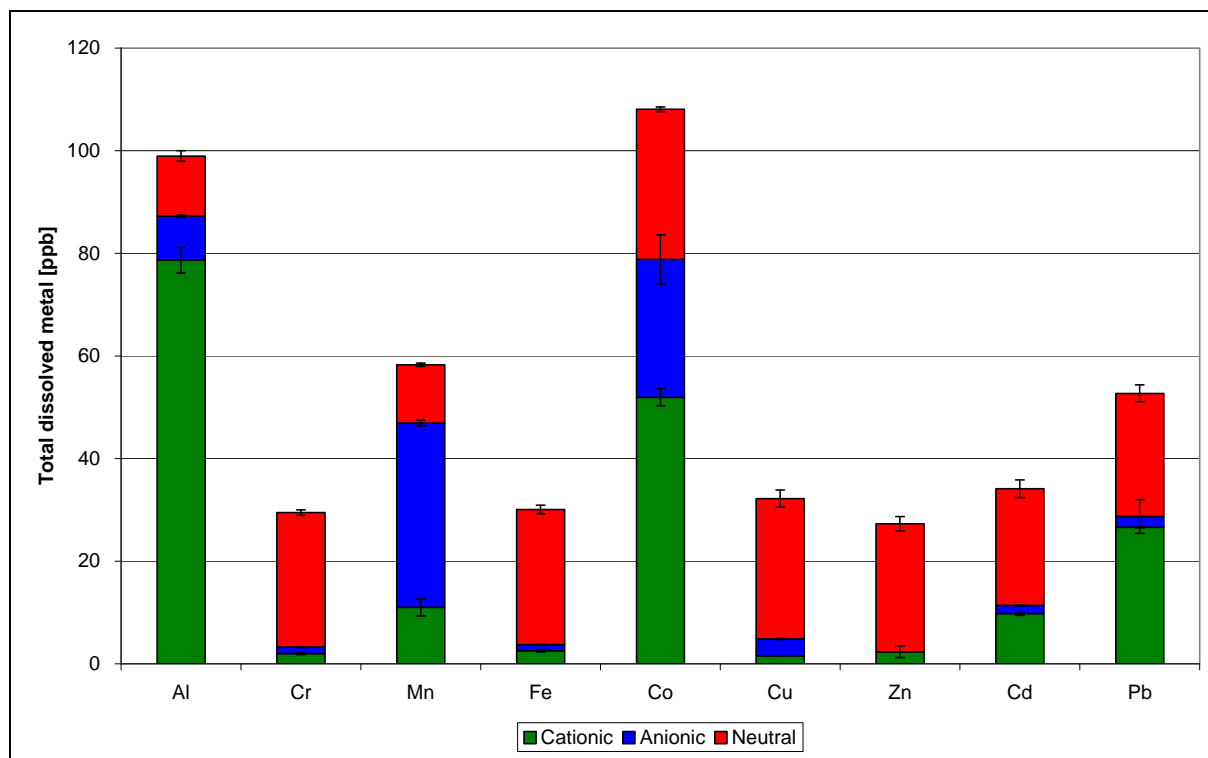


Figure 32: Fractionation of the modified Donaukanal sample for standard addition.

This discrepancy is due to insufficient conservation of the employed sample. Since preservation of the trace metals by acidification with nitric acid would destroy the natural equilibrium in the sample, it was stored frozen at -12 °C. Nevertheless, the sample seems to have been altered, possibly due to multiple freezing/thawing, which each time needed several hours at room temperature to complete.

## 5 Analysis of Snow Samples

Due to the susceptibility of the system to carbon induced interferences, snow samples were used to demonstrate the application of the system on environmental samples, since snow is unlikely to exhibit high carbon levels.

### 5.1 Sample Collection

The samples were chosen to exhibit certain variability and were taken therefore at three different urban locations near the Vienna University of Technology and one remote sampling site at the Fanningberg ski resort. This array included remote and urban background samples as well as samples polluted by city traffic. The samples were stored frozen at -12 °C and were transferred to 50 ml PE bottles for melting at room temperature before analysis.

Table 17: Sampling sites, dates and TOC of snow samples.

Sample	Sampling Site	Date	Time	TOC [mg/l]
FB1	Fanningberg	27.03.2007		12.2
FB2	Fanningberg	27.03.2007		8.2
FB3	Fanningberg	27.03.2007		6.0
1A	University Courtyard	27.01.2004		6.9
1B	Traffic Island	27.01.2004		6.0
2A	University Courtyard	19.01.2005		6.1
2B1	Traffic Island	19.01.2005		6.0
2C	Traffic Island, heavily polluted	19.01.2005		11.0
2B2	Traffic Island	20.01.2005		9.1
3A	University Courtyard	28.12.2005	08:30	3.8
3B1	Traffic Island	28.12.2005	08:30	5.2
3B2	Traffic Island	28.12.2005	13:30	7.9
3B3	Traffic Island	28.12.2005	18:30	7.3
3B4	Traffic Island	29.12.2005	08:30	4.3
3B5	Traffic Island	30.12.2005	07:45	3.7
3B6	Traffic Island	30.12.2005	15:30	6.7

TOC levels were measured from the liquid samples and ranged from 3.7 mg/l to 12.2 mg/l. An overview of the samples concerning date, time and location of sample drawing as well as the determined TOC values is given in Table 17.

The exact coordinates of the sampling sites are given in Table 18, whereas Figure 33 and Figure 34 show the maps indicating the sampling locations.

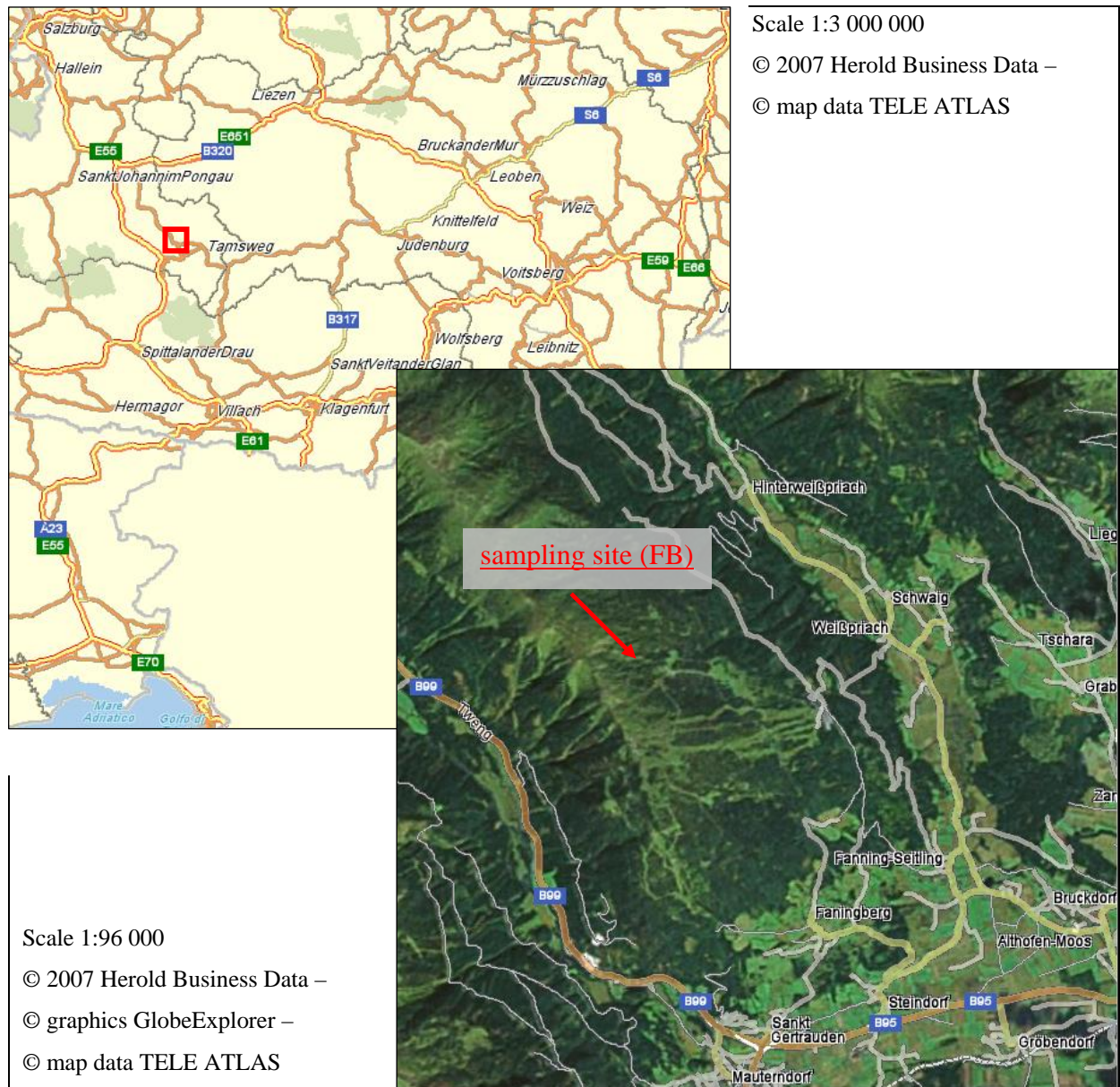


Figure 33: Location of sample drawing (Fanningberg).



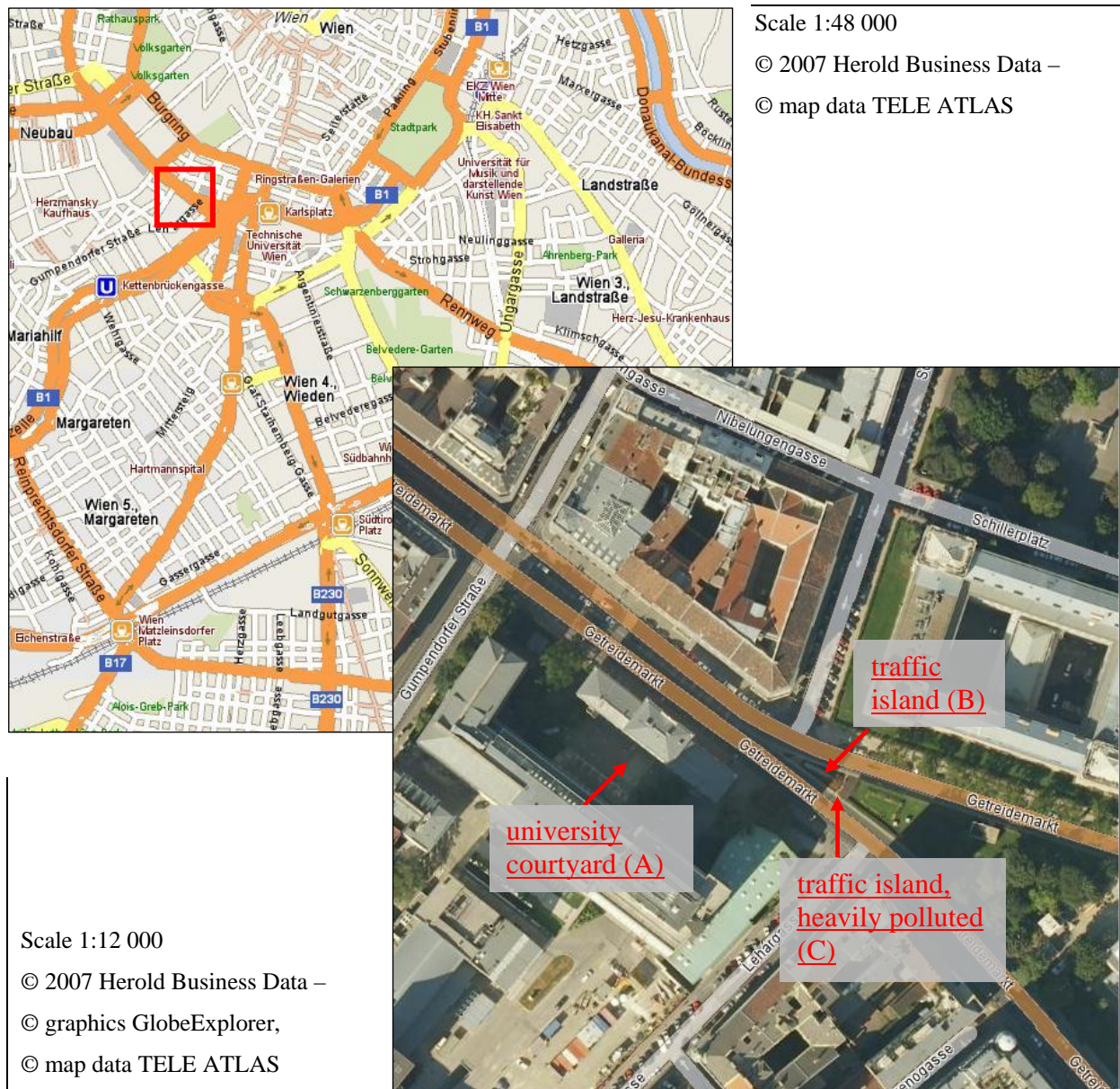


Figure 34: Locations of sample drawing (Vienna University of Technology).

Table 18: Coordinates of sampling sites.

Sampling Site	Latitude	Longitude	Altitude
Fanningberg (FB)	47°10'34" N	13°39'55" O	1800 m a.s.l.
University Courtyard (A)	48°12'03.5" N	16°21'47.2" O	182 m a.s.l.
Traffic Island (B)	48°12'03.9" N	16°21'49.7" O	181 m a.s.l.
Traffic Island, heavily polluted (C)	48°12'03.6" N	16°21'50.1" O	181 m a.s.l.



## 5.2 Standard Addition

To ensure the accuracy of the analysis, a standard addition series was performed with a selected sample (sample 2C, *cf.* Table 17). The series was carried out by spiking each 5 ml aliquot of the sample with a small amount of a 5 ppm standard solution in order to reach added concentrations of 0, 50, 100, 200 and 400 ppb. The prepared samples were measured following the procedure given in chapter 4.1 employing standard operating parameters (*cf.* Table 2); each measurement was performed in duplicate. In analogy to the standard addition procedure described in chapter 4.2.10 the results were compared to an external calibration consisting of blanks and standard solutions of 100 and 400 ppb, adjusted to pH 3 with 50  $\mu$ l of 2 M HNO<sub>3</sub> each.

As shown in Table 19, recoveries for most elements were within an acceptable range, confirming the assumption that the primary interference originates from the TOC content of the sample. Due to the high Ca content of the sample (*cf.* Table 21), the standard addition series was not evaluated for this element.

Table 19: Evaluation of the standard addition (Snow).

Element	External Calibration	Standard Addition	Correlation $r^2$	Recovery
Mg	$232 \pm 11$ ppb	$215 \pm 31$ ppb	0.9921	$108.0 \pm 9.5$ %
Al	$78.6 \pm 21.0$ ppb	$51.0 \pm 13.7$ ppb	0.9871	$154 \pm 26$ %
Cr	$1.1 \pm 0.5$ ppb	$1.11 \pm 0.04$ ppb	0.9995	$98.1 \pm 3.3$ %
Mn	$8.03 \pm 0.04$ ppb	$7.8 \pm 0.1$ ppb	0.9995	$102.7 \pm 1.8$ %
Fe	$22.5 \pm 2.5$ ppb	$21.9 \pm 1.2$ ppb	0.9997	$102.6 \pm 5.1$ %
Co	$12.8 \pm 0.9$ ppb	$13.4 \pm 0.7$ ppb	0.9994	$96.2 \pm 5.1$ %
Cu	$30.9 \pm 3.3$ ppb	$25 \pm 1.9$ ppb	0.9995	$123.5 \pm 8.3$ %
Zn	$13.6 \pm 2.8$ ppb	$14.3 \pm 2.2$ ppb	0.9967	$95 \pm 14$ %
Cd	$3.6 \pm 0.2$ ppb	$4.0 \pm 0.1$ ppb	0.9997	$90.0 \pm 2.1$ %
Pb	$12.4 \pm 1.5$ ppb	$12.3 \pm 2.2$ ppb	0.9895	$101 \pm 17$ %

The recoveries for Al and Cu still deviate from the target value of 100%, indicating that in those cases even the low carbon content of the employed sample causes interferences at the applied wavelengths. In case of Al this seems to be correct, but further evaluation of the data acquired by direct measurement of the spiked sample suggests that this discrepancy is not

occurring exclusively when applying the FI-system, but exists in the same manner if the sample is analyzed conventionally by ICP-AES.

### 5.3 Measurement

All snow samples were analyzed following the procedure given in chapter 4.1 employing standard operating parameters (*cf.* Table 2). All measurements were performed in duplicate. Since the primary objective of the experiment was to quantify the trace metals occurring in rather low abundance, the chosen values for standardization were 20 and 100 ppb, again adjusted to pH 3 by addition of 50  $\mu$ l of 2 M HNO<sub>3</sub> each. To compensate for occurring matrix interferences without using matrix-matched standard solutions, the recovery values obtained from the standard addition (*cf.* Table 19) were considered as representative for molten snow samples and therefore included into the calculation of the results for all elements except Ca, for which no recovery factor was determined (*cf.* chapter 5.2).

### 5.4 Results

As a consequence of the chosen standardization values, the calculated concentrations for several elements are to be considered as rough estimates, since they exceeded the upper limit of the operating range. The elements in question are Mg and Zn, of which several samples contained more than 100 ppb, and especially Ca, occurring in amounts of several ppm. Furthermore, due to the demonstrated influence of the carbon content, Al and Cu values are to be considered with caution.

The determined metal concentrations for each sample are presented in Table 20. The contributions of the three analyzed fractions were calculated according to chapter 4.1.3 for each element and are shown in Figure 35.

Table 20: Trace metal concentrations of individual snow samples.

	<b>FB1</b>	<b>FB2</b>	<b>FB3</b>	<b>1A</b>	<b>1B</b>	<b>2A</b>	<b>2B1</b>	<b>2C</b>
Mg	91.0 ± 4.5 ppb	86.6 ± 1.0 ppb	66.0 ± 0.6 ppb	78.9 ± 3.9 ppb	115 <sup>+</sup> ± 1 ppb	90.6 ± 0.7 ppb	129 <sup>+</sup> ± 2 ppb	295 <sup>+</sup> ± 12 ppb
Al	26.8 ± 0.9 ppb	21.8 ± 0.2 ppb	21.8 ± 0.3 ppb	28.3 ± 0.4 ppb	30.1 ± 1.2 ppb	31.3 ± 0.4 ppb	50.9 ± 2.2 ppb	79.3 ± 8.8 ppb
Ca	4.8 <sup>+</sup> ± 0.2 ppm	4.8 <sup>+</sup> ± 0.1 ppm	3.7 <sup>+</sup> ± 0.1 ppm	3.6 <sup>+</sup> ± 0.2 ppm	4.1 <sup>+</sup> ± 0.1 ppm	3.8 <sup>+</sup> ± 0.1 ppm	4.7 <sup>+</sup> ± 0.1 ppm	7.4 <sup>+</sup> ± 0.1 ppm
Cr	0.9 ± 0.1 ppb	1.3 ± 0.6 ppb	<0.7 <sup>*</sup> ppb	0.8 ± 0.4 ppb	0.8 ± 0.2 ppb	<0.7 <sup>*</sup> ppb	<0.7 <sup>*</sup> ppb	1.2 ± 0.03 ppb
Mn	2.7 ± 0.03 ppb	1.3 ± 0.1 ppb	1.8 ± 0.01 ppb	3.4 ± 0.1 ppb	5 ± 0.2 ppb	3.3 ± 0.02 ppb	7.0 ± 0.2 ppb	17.5 ± 0.6 ppb
Fe	11.3 ± 0.6 ppb	6.1 ± 1.6 ppb	6.8 ± 0.9 ppb	10.9 ± 0.3 ppb	17.9 ± 2.8 ppb	10.8 ± 0.9 ppb	30.3 ± 0.9 ppb	70 ± 15 ppb
Co	13.4 ± 2.2 ppb	13.2 ± 1.1 ppb	11.2 ± 0.7 ppb	9.1 ± 3.3 ppb	13.4 ± 1.3 ppb	9.6 ± 0.4 ppb	14 ± 2.1 ppb	20.7 ± 1.7 ppb
Cu	<1.3 <sup>*</sup> ppb	<1.3 <sup>*</sup> ppb	<1.3 <sup>*</sup> ppb	1.8 ± 0.2 ppb	3.1 ± 0.3 ppb	<1.3 <sup>*</sup> ppb	7.0 ± 0.7 ppb	23.1 ± 0.8 ppb
Zn	365 <sup>+</sup> ± 5 ppb	231 <sup>+</sup> ± 34 ppb	245 <sup>+</sup> ± 11 ppb	239 <sup>+</sup> ± 6 ppb	244 <sup>+</sup> ± 17 ppb	203 <sup>+</sup> ± 0.3 ppb	130 <sup>+</sup> ± 7 ppb	124 <sup>+</sup> ± 6 ppb
Cd	2.6 ± 0.01 ppb	2.7 ± 0.03 ppb	2.0 ± 0.05 ppb	2.2 ± 0.01 ppb	2.5 ± 0.2 ppb	1.4 ± 0.2 ppb	2.4 ± 0.2 ppb	3.2 ± 0.01 ppb
Pb	<12 <sup>*</sup> ppb	<12 <sup>*</sup> ppb	<12 <sup>*</sup> ppb	<12 <sup>*</sup> ppb	<12 <sup>*</sup> ppb	<12 <sup>*</sup> ppb	<12 <sup>*</sup> ppb	15.6 ± 3.6 ppb
	<b>2B2</b>	<b>3A</b>	<b>3B1</b>	<b>3B2</b>	<b>3B3</b>	<b>3B4</b>	<b>3B5</b>	<b>3B6</b>
Mg	239 <sup>+</sup> ± 0.4 ppb	46.6 ± 2.0 ppb	77.9 ± 1.8 ppb	246 <sup>+</sup> ± 7 ppb	158 <sup>+</sup> ± 3 ppb	202 <sup>+</sup> ± 8 ppb	259 <sup>+</sup> ± 6 ppb	317 <sup>+</sup> ± 5 ppb
Al	97.2 ± 0.4 ppb	19.3 ± 2.4 ppb	27.2 ± 0.1 ppb	82.2 ± 1.4 ppb	56.4 ± 2.3 ppb	61.9 ± 1.0 ppb	78.1 ± 9.4 ppb	91.5 ± 2.0 ppb
Ca	6.0 <sup>+</sup> ± 0.1 ppm	2.1 <sup>+</sup> ± 0.1 ppm	3.2 <sup>+</sup> ± 0.1 ppm	5.8 <sup>+</sup> ± 0.2 ppm	4.8 <sup>+</sup> ± 0.1 ppm	4.9 <sup>+</sup> ± 0.1 ppm	8.8 <sup>+</sup> ± 0.2 ppm	7.2 <sup>+</sup> ± 0.1 ppm
Cr	1.0 ± 0.1 ppb	<0.7 <sup>*</sup> ppb	<0.7 <sup>*</sup> ppb	0.8 ± 0.1 ppb	1.2 ± 0.2 ppb	<0.7 <sup>*</sup> ppb	1.3 ± 0.9 ppb	<0.7 <sup>*</sup> ppb
Mn	10.7 ± 0.1 ppb	1.7 ± 0.6 ppb	2.6 ± 0.03 ppb	12.1 ± 0.2 ppb	11.7 ± 0.3 ppb	7.7 ± 0.01 ppb	14.3 ± 1.1 ppb	12.3 ± 0.2 ppb
Fe	55.1 ± 0.5 ppb	17.1 ± 3.9 ppb	14.3 ± 0.9 ppb	72.5 ± 3.0 ppb	58.0 ± 2.6 ppb	41.1 ± 1.1 ppb	49.3 ± 6.8 ppb	72.8 ± 0.3 ppb
Co	21.2 ± 4.7 ppb	10.1 ± 1.5 ppb	9.4 ± 1.1 ppb	17.4 ± 1.9 ppb	14.8 ± 2.3 ppb	15.1 ± 4.6 ppb	25.2 ± 0.1 ppb	21.1 ± 0.6 ppb
Cu	23.6 ± 0.2 ppb	<1.3 <sup>*</sup> ppb	<1.3 <sup>*</sup> ppb	11.3 ± 0.5 ppb	11.0 ± 0.8 ppb	6.6 ± 0.6 ppb	8.0 ± 1.4 ppb	9.9 ± 0.4 ppb
Zn	64.7 ± 1 ppb	113 <sup>+</sup> ± 14 ppb	179 <sup>+</sup> ± 9 ppb	91 ± 13 ppb	65.3 ± 3.6 ppb	36.4 ± 0.2 ppb	74.6 ± 6.9 ppb	71.6 ± 1.7 ppb
Cd	3.5 ± 0.1 ppb	<1.1 <sup>*</sup> ppb	2.0 ± 0.3 ppb	3.5 ± 0.2 ppb	2.7 ± 0.6 ppb	2.7 ± 0.3 ppb	4.7 ± 0.3 ppb	3.7 ± 0.1 ppb
Pb	13.4 ± 1.9 ppb	<12 <sup>*</sup> ppb	<12 <sup>*</sup> ppb	<12 <sup>*</sup> ppb	16 ± 10 ppb	<12 <sup>*</sup> ppb	<12 <sup>*</sup> ppb	<12 <sup>*</sup> ppb
*: below detection limit (LOD), cf. Table 11				+: exceeds upper operational range				

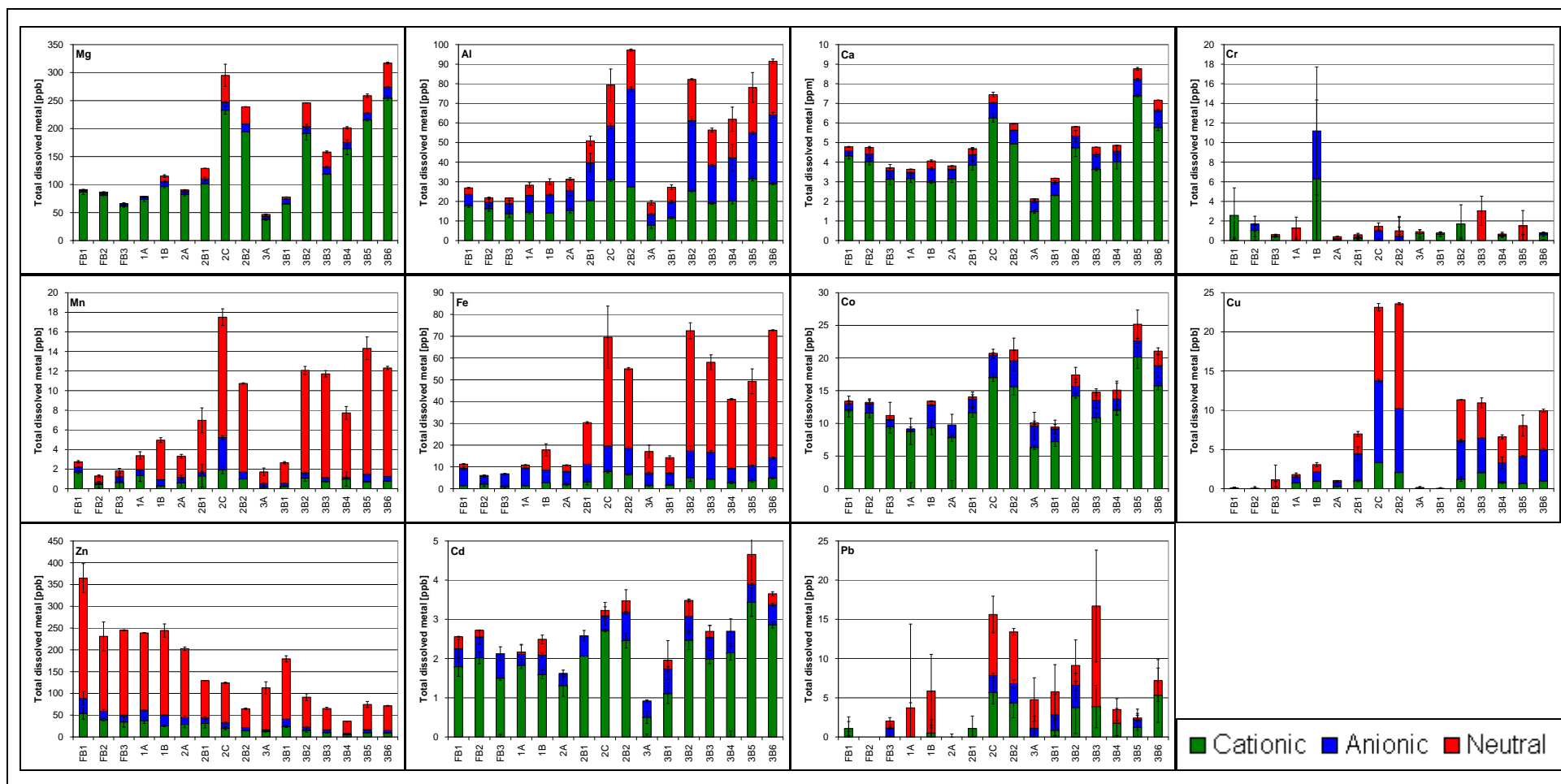


Figure 35: Fractionation of trace metals in snow samples.

## 5.5 Discussion

The results indicate that the investigated elements can be divided into several classes according to their occurrence and speciation.

- The alkaline earth metals Mg and Ca are found nearly quantitatively in cationic form, presumably as free ions. The Ca concentration in molten snow samples ranges from 2 ppm up to 9 ppm, whereas Mg concentration is considerably lower, ranging from 45 ppb to 320 ppb. Nevertheless, despite their difference in content and due to their resembling chemical and physical properties, their speciation is similar, exhibiting a dominating cationic fraction.
- Similarly occurring but mainly in cationic form are the trace metals Co and Cd, although in much lower concentrations. Co content in samples ranges from 9 ppb to 25 ppb and Cd from 0.8 ppb (below detection limit) to 5 ppb.
- In contrast to the aforementioned elements Mn (1.3 ppb to 18 ppb), Fe (6 ppb to 75 ppb) and Zn (35 ppb to 400 ppb) occur mostly as neutral hydrophobic complexes and only relatively small portions belong to the anionic or cationic fraction.
- The fraction distribution of Al (10 ppb to 100 ppb) and Cu (up to 25 ppb) indicates a relatively high percentage of anionic species. Unlike all other examined elements there is no distinct tendency towards one of the other fractions.
- The two elements Cr and Pb could not be quantified, since the concentrations were below the limit of detection.

The impact of an urban environment can be distinguished by comparing the trace metal content and speciation according to the sampling location. Classification of the samples into the categories remote background, urban background and urban polluted samples, and detailed analysis of the results (*cf.* Table 21) shows that the urban impact differs depending on the examined element.

Direct comparison of the snow samples taken as background samples at the Fanningberg sampling site (FB) and in the courtyard of the Vienna University of Technology (A) exhibits only slight differences, contradicting the assumption that the Fanningberg site is uninfluenced by urban pollution.

- For the elements Mg, Al, Cr, Mn, Cu and Pb no significant difference in the results for remote and urban background samples could be found.
- Only the Fe values for the samples taken at site A were distinctly higher than those found at sampling site FB.
- Contrary to the previously made supposition of an unpolluted site, the levels of Ca, Co, Cd and especially Zn in FB samples were elevated in comparison to samples taken at an urban, but rather traffic-unaffected site (A).

Possible sources for trace metals in the snow at Fanningberg are local and regional traffic and industry. Additionally, traffic and maintenance work caused by tourism near the sampling site have to be taken into account.

In contrast, snow samples taken near a heavily frequented street showed considerably increased trace metal concentrations.

- The mean values of Mg, Al, Mn, Fe and Cu were significantly elevated. Mean contents of Mg, Al and Mn for the polluted samples were by a factor of 3 higher than for both types of background samples, whereas Fe-values were increased by a factor of 5 and Cu by a factor of 10.
- Results for Ca, Co and Cd also showed increased mean values compared to the determined background values, but not as distinctive as in the case of the aforementioned elements. Still, concentrations measured in the polluted snow samples exceeded the background values by 50%.
- No impact caused by urban traffic could be determined for Zn. The highest concentrations were found in the FB samples and, contrary to expectations, the lowest concentrations were found in the polluted samples. The high background value is presumably of different origin.
- No increased values could be found for Cr and Pb, since the measured values were in most instances below the limit of detection.

Nevertheless, for all samples observed concentrations were within the typical range reported for trace metals in snow or rain (*cf.* Table 22).

Table 21: Trace metal concentrations of different snow sample types.

	Remote Background (FB)		Urban Background (A)		Urban Polluted (B, C)	
	Mean	Range	Mean	Range	Mean	Range
Mg	81.2 ppb	66.0 – 91.0 ppb	72.0 ppb	46.6 - 90.6 ppb	204 <sup>+</sup> ppb	77.9 - 317 <sup>+</sup> ppb
Al	23.5 ppb	21.8 - 26.8 ppb	26.3 ppb	19.3 - 31.3 ppb	65.5 ppb	27.2 - 97.2 ppb
Ca	4.4 <sup>+</sup> ppm	3.7 <sup>+</sup> - 4.8 <sup>+</sup> ppm	3.2 <sup>+</sup> ppm	2.1 <sup>+</sup> - 3.8 <sup>+</sup> ppm	5.7 <sup>+</sup> ppm	3.2 <sup>+</sup> - 8.8 <sup>+</sup> ppm
Cr	0.9 ppb	<0.7* - 1.3 ppb	<0.7* ppb	<0.7* - 0.8 ppb	0.9 ppb	<0.7* - 1.3 ppb
Mn	2.0 ppb	1.3 - 2.7 ppb	2.8 ppb	1.7 - 3.4 ppb	10.1 ppb	2.6 - 17.5 ppb
Fe	8.1 ppb	6.1 - 11.3 ppb	12.9 ppb	10.8 - 17.1 ppb	48.1 ppb	14.3 - 72.8 ppb
Co	12.6 ppb	11.2 - 13.4 ppb	9.6 ppb	9.1 - 10.1 ppb	17.2 ppb	9.4 - 25.2 ppb
Cu	<1.3* ppb	<1.3* ppb	<1.3* ppb	<1.3* - 1.8 ppb	10.4 ppb	<1.3* - 23.6 ppb
Zn	280 <sup>+</sup> ppb	231 <sup>+</sup> - 365 <sup>+</sup> ppb	185 <sup>+</sup> ppb	113 <sup>+</sup> - 239 <sup>+</sup> ppb	108 <sup>+</sup> ppb	36.4 - 244 <sup>+</sup> ppb
Cd	2.4 ppb	2 - 2.7 ppb	1.5 ppb	<1.1* - 2.2 ppb	3.1 ppb	2.0 - 4.7 ppb
Pb	<12* ppb	<12* ppb	<12* ppb	<12* ppb	<12* ppb	<12* - 15.8 ppb
*: below detection limit (LOD), cf. Table 11			+: exceeds upper operational range			

Table 22: Literature values for trace metal concentrations (in ppb).

	<b>Western Massachusetts, US</b> <sup>[66]</sup>	<b>Higashi- Hiroshima, JP</b> <sup>[67]</sup>	<b>Innsbruck, AT</b> <sup>[68]</sup>		<b>Amman, JO</b> <sup>[69]</sup>
<b>Type</b>	wet deposition	urban background	urban background	high traffic	urban polluted
Mg	20	2.14 - 842			42 - 3675
Al	53	0.24 - 142			6.1 - 395
Ca	130	8.74 - 2304			806 - 19954
Cr	0.14				0.1 - 4.6
Mn	1.2	0.16 - 29.4			0.07 - 12
Fe	65				4 - 88
Co	0.045				0.03 - 0.51
Cu	0.95	0.08 - 10.4	10 - 40	10 - 4290	0.7 - 17
Zn	3.7	0.71 - 65.6	50 - 300	10 - 3170	1.4 - 370
Cd	0.31	0.006 - 1.23	<0.25 - 11.54	0.72 - 25.76	0.05 - 1.9
Pb	4.5	0.02 - 25.15	0.2 - 33	0.4 - 525	1.98 - 3.6

Concerning the speciation of trace metals in the investigated samples, elements can be grouped into three categories.

- The alkaline earth metals Mg and Ca, as well as Co and Cd, were found in all samples nearly quantitatively in cationic form, presumably as free ions.
- In contrast, the elements Mn, Fe and Zn mostly occur as organic neutral complexes and only relatively small portions belong to either the anionic or cationic fraction.
- The fraction distribution of Al and Cu indicates a relatively high percentage of anionic species. Unlike all other examined elements there is no distinct tendency towards one of the other fractions.
- Due to the low total concentrations, no fraction distribution could be determined for Cr and Pb.

A more detailed analysis of the results acquired for sample series 3, taken during a snowfall period from 28.12.2005 to 30.12.2005, *cf.* Table 17, and linking of the results to meteorological data (*cf.* Table 23) allows further interpretation of the findings. A more detailed presentation of the concentration and fractionation profiles for selected elements



(Al, Cu, Mn, Fe, Co and Cd) is shown in Figure 36. The column marked “background” represents sample 3A, the other shown samples are 3B1 to 3B6, arranged in chronological order.

Table 23: Meteorological data (Vienna, Hohe Warte station).

Date	Mean Temperature	Precipitation	Snow Depth	Fresh Fallen Snow
28.12.2005	-1.7 °C	6.5 mm	8 cm	7 cm
29.12.2005	-1.6 °C	0.4 mm	18 cm	12 cm
30.12.2005	-3.5 °C	1.3 mm	15 cm	1 cm
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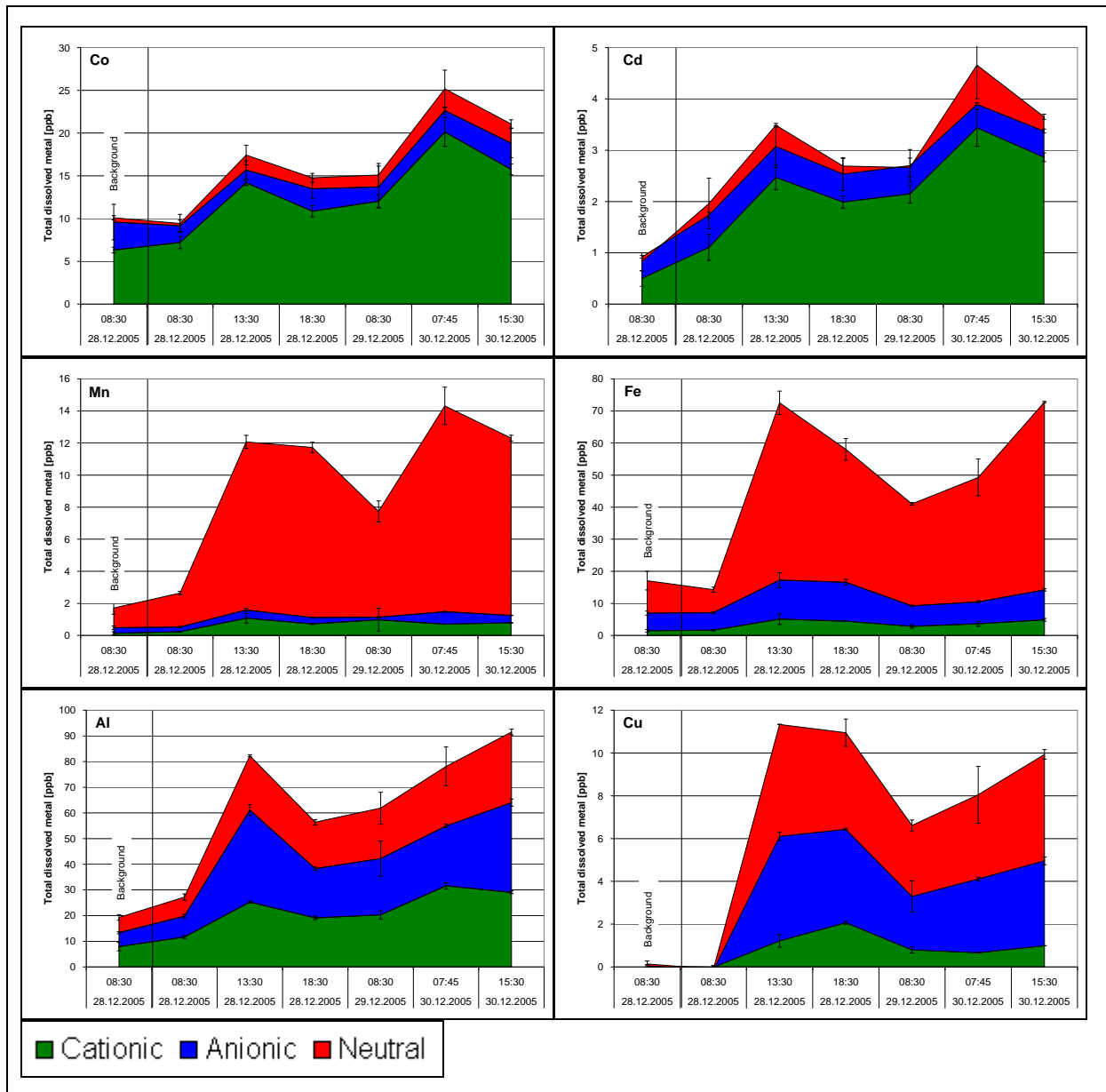


Figure 36: Selected concentration and fractionation profiles.

As it is apparent in Figure 36, most trace metal concentrations increase with continued exposure to traffic.

While sample 3B1, taken in the early morning at the beginning of the snowfall event shows little difference to 3A, the background sample taken at the same time, concentrations in the samples 3B2 and 3B3, collected over the day, are significantly higher. On the two following mornings the samples 3B4 and 3B5 were gathered, but unlike the first morning, trace metal concentrations were still elevated. Nevertheless, due to continuing snowfall and reduced traffic during the night, trace metal contents were diminished compared to the samples collected the day before.

Speciation analysis of the snow samples reveals the different properties of the accumulated trace metals.

- Co and Cd (as well as Mg and Ca, not shown in Figure 36) are nearly quantitatively found in the cationic fraction. Nevertheless, both anionic and neutral species are present to a minor degree and it is noticeable that the quantity of these non-cationic fractions remains nearly unchanged during the whole sampling period. Therefore it can be assumed that the concentration increase induced by urban traffic is mostly caused by cationic trace metal species.
- The opposite is the case for Mn, Fe and Zn: while the sum of cationic and anionic fractions ranges from 0.5 ppb to 1.6 ppb for Mn, 7.0 ppb to 17.2 ppb for Fe and 7.7 ppb to 40.6 ppb for Zn, the concentration ranges of neutral complexes are 1.2 ppb to 12.8 ppb, 7.2 ppb to 58.6 ppb and 28.6 ppb to 138.7 ppb. Accordingly the increase can be attributed nearly quantitatively to neutral trace metal complexes. Solely the Zn concentration showed no clear dependence of the exposition to traffic.
- In contrast, Al and Cu are almost equally distributed among the three fractions, independent of the total concentration.

## 6 Conclusion

The aim of this work was the development of an analytical technique able to quantify trace metal concentrations in aqueous samples while simultaneously distinguishing neutral hydrophobic complexes from the anionic and cationic fraction. To avoid potential contamination risks and to maximize reproducibility sample preparation was to be mostly incorporated into an automatized FI-system, thereby minimizing intervention by the operator during measurements.

The FI-system should provide the necessary separation of species and provide preconcentration to enhance sensitivity, allowing enhanced detection limits. Furthermore, by on-line coupling the FI-system to ICP-AES in order to elute the retained trace metal complexes directly into the nebulization unit of the detection system and to record and evaluate the resulting transient signal the whole process consisting of separation, preconcentration and quantification of trace metal complexes should be accomplishable in a single step.

The two-cartridge FI-system presented in chapter 3 consists of two cartridges containing a reversed-phase (RP) sorbent and a strong cation exchanger (SCX) and two six-port two position valves. It is able to discriminate between neutral hydrophobic complexes and cationic trace metals and provides acceptable preconcentration. Nevertheless, measurements of synthetic standards containing different ligands exposed severe drawbacks of the developed system.

First and foremost, while neutral complexes and cations were retained quantitatively by the respective sorbents, several ligands formed anionic complexes that were retained by neither sorbent. Therefore an additional cartridge containing a weak anion exchanging material (WAX) was incorporated into the existing FI-system, enabling it to retain the previously undetected stable anionic complexes. Since the positioning of this WAX-cartridge was crucial for the apportionment of anionic complexes to a fraction, differing setups permitted the quantification of a third species. As this constituted a reasonable extension of the developed method, but would require additional measurements with the existing FI-system, it was decided to abandon the two-cartridge setup in favor of an expanded three-cartridge system.

The FI-system presented in chapter 4, employing a third six-port valve, incorporates the added WAX-cartridge as an independent component. With this expansion, it is now able to separate neutral hydrophobic and anionic complexes from the cationic constituents by retaining and simultaneously preconcentrating each of the three fractions separately on the corresponding SPE-sorbent.

Experiments involving synthetic ligand-spiked standard solutions confirmed the achieved separation efficiency and the enhanced capability of the system to retain and preconcentrated anionic complexes. Nevertheless, the achieved separation is dependent on the employed SPE materials, the employed mass of sorbents and sequence. The retaining capacity of low sorbent amounts may be exhausted by the presence of large quantities of matrix components competing with the analytes.

Polyfunctional complexes matching several fractionation attributes, *e.g.* hydrophobic charged complexes, can interact with several of the substrates and cannot be easily assigned to a category. Therefore it is necessary to record not only the employed sorbents, but also their sequence. This information is crucial, since the fractionation achieved by the system depends mostly on those parameters.

Due to the inherent preconcentration abilities of the presented FI-system the achievable limits of detection for the quantification of the total metal content in aqueous samples by ICP-AES were drastically improved in comparison to conventional ICP-AES measurement techniques. The possibility to further enhance detection limits by increasing the employed sample volume was discussed.

On-line coupling of the FI-system to the nebulizer of the ICP-AES and automatized control of the employed valves minimized contamination risks and led to good reproducibility of determined results.

The study of both synthetic standards containing model compounds and natural samples of differing matrices indicate that the main interference originates from the carbon content of the specimen. Since these carbon interferences originate from matrix compounds eluting from the sorbent materials simultaneously to the analytes and the ICP-AES unit at disposal is unable to provide spectral background correction as well as transient data recording at the required

resolution, it is not possible to compensate for these interferences with the employed background correction routine. Preceding matrix elimination would destroy the natural balance of the sample; therefore the data processing routine had to be adapted to at least partially compensate for matrix interferences.

To adjust the quantification routine to take account for matrix specific deviation, use of matrix matched calibration method would be the method of choice. Since the exact composition of most natural samples is rather difficult to duplicate, standard addition is used to determine the deviation of results in comparison to the use of conventional acid-stabilized trace metal standard solutions. For samples with low carbon content, close study of the standard addition series yielded constant recovery factors for each investigated element, enabling a matrix-corrected quantification routine while employing conventional standard solutions.

To demonstrate the applicability of the developed method to snow samples, samples collected at different sites were analyzed. The investigated snow samples contained up to 12 mg/l TOC, necessitating the correction of results with recovery factors derived from a standard addition series employing a representative snow sample. Observed total metal concentrations were within the range reported previously for snow samples collected at urban sites. By interconnecting the acquired results with date, time and location of sampling, the influence of urban traffic on trace metal concentrations and speciation in snow could be pointed out. Apparently the increase can be accounted nearly quantitatively to the fraction which is already dominant in the background samples.

The tendencies of trace metals concerning the formation of complexes could be shown by fractionation. While Mg, Ca, Co and Cd occurred mainly in cationic form, Mn, Fe and Zn tended to form neutral complexes. Al and Cu were distributed nearly even between the three separated fractions. Concentrations of Cr and Pb in the investigated snow samples were too low to determine speciation. The demonstrated occurrence of different metal species may facilitate the understanding concerning differing properties of the investigated trace elements concerning chemical and photochemical reactivity, as well as tendencies to accumulate in particular environmental, especially biological compartments.

## 7 Outlook

Further automatization would reduce the need for intervention by the operator and thereby eliminate potential sources of contamination or handling errors. Foremost, the valve V1 (*cf.* chapter 4.1.1) should be changed to a model that can be controlled by the employed serial communication program to remove the necessity for manual switching. Provided the new valve responds to the same commands as the employed VICI models, the relevant commands have already been incorporated into the control program (*cf.* Table 9).

To minimize possible mishandling during the sample loading, the uptake of buffer solution for conditioning and washing of the cartridges could be controlled by an adequate autosampler system. Alternatively, since the sample volume needs to be constant for every measurement, an improvement in precision could be achieved by the incorporation of a sample loop into the FI-system. Of course, this extension would require another six-port two position valve to be controlled by the operator program.

The increasing complexity of the FI-system would require extensive modifications of the employed soft- and hardware. To ensure smooth execution of the operating sequence, a linking of all necessary control programs for synchronization of the start signals or the implementation of controlling software for the whole setup seems advisable, preferably including control options for the employed pumping systems.

The incorporation of more SPE units into the existing system for further discrimination between different kinds of complexes should be easy, since FI-systems are generally of a modular nature. In case of the presented system, any additional SPE cartridge requires, if it is to be eluted separately, an appropriate six port valve.

Spectral interferences originating from the presence of matrix constituents, particularly carbon compounds, are a main cause for the need to carry out post-measurement correction calculations. A change of the ICP-AES towards a system providing spectral background correction is the primary step to enhance the accuracy of the presented system. It would provide the possibility to broaden the scope of analysis to samples carrying higher carbon charges as well as reduce the potential of interelemental interferences.

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