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Extraction of Heavy Metals with Supercritical Carbon Dioxide

A novel approach to heavy metal analysis and decontamination

©Jin Wang

Department of Food Science and Agricultural Chemistry
Faculty of Agricultural and Environmental Science
McGill University, Montreal

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A thesis
submitted to the Faculty of Graduate Studies and Research in partial fulfillment
of the requirements of the degree of Ph.D.

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ABSTRACT

The technique of extraction with supercritical carbon dioxide (SC-CO_2), which is now used routinely for the recovery of non-polar analytes from a variety of liquid and semi-permeable solid media, was extended to the efficient extraction of metal ions. Measurements of the solubilities in SC-CO_2 corroborated the hypothesis that the non-polar character of a series of tetraalkylammonium dialkyldithiocarbamates ion pairs was influenced (*i.*) primarily by the chain length of the alkyl substituent(s) on the carbamate nitrogen and (*ii.*) to a lesser extent, by the chain length of the alkyl substituent(s) on the ammonium counter ion. In operation, analyte metal(s) in aqueous medium was derivatized by *in situ* complexation with the dithiocarbamate complexing reagent and the product complex(es) was partitioned into the SC-CO_2 mobile phase then purged from the extractor by replacing the headspace with fresh solvent. A novel silica flame-in-tube interface was developed for the sensitive detection, by atomic absorption spectrometry (AAS), of As, Cd, Cu, Mn, Pb, Se, or Zn in SC-CO_2 extractor eluate. For different analyte elements, the limits of detection (LODs) ranged from sub-nanogram to low picogram if standard was flow injected into the mobile phase. These sensitivities permitted differences in the rates of mobilization of different forms of the analyte metal from various media to be explored as a technique for probing the interaction of the analyte metal with the matrix. A portion of the Zn burden in fresh bovine liver was mobilized rapidly in the absence of complexing agent and the remainder was solubilized more rapidly than the Zn in a freeze-dried reference material of this tissue. The nebulizer assembly of a conventional flame-AAS was modified to extend the range of metals amenable to *on-line* detection. Supplemental heating of the inlet air to 200 °C resulted in an *on line* detector which provided approximately a ten-fold improvement over conventional operation. An alternate strategy employing a tetraalkylammonium halide (R_4NX) as an ion pairing reagent has been developed for the selective mobilization of anions including chromate, selenate and selenite from aqueous media. Finally, SC extraction was investigated as a method for mobilizing/extracting the Cd, Cu and Zn bound to metallothionein that had been isolated from rabbit liver.

RÉSUMÉ

Une extraction au dioxyde de carbone supercritique (CO_2-SC) a été développée et utilisée pour récupérer des ions metalliques dans divers milieux liquides et milieux solides semi-perméables. Des mesures de solubilité dans le CO_2-SC ont confirmé l'hypothèse voulant que la non-polarité de paires d'ions de dialkyldithiocarbamate de tétraalkylammonium était influencée (i) surtout par la longueur de la chaîne de substituant(s) alkyle(s) relié à l'azote de carbamate et, (ii) dans une moindre mesure, par la longueur de la chaîne de substituant(s) alkyle(s) relié(s) au contre-ion ammonium. En pratique, les métaux à analyser en milieux aqueux ont été obtenus par complexion *in situ* à l'aide du réactif de complexion dithiocarbamate et les complexes obtenus ont été séparés dans la phase mobile du CO_2-SC, puis purgés de l'extracteur par remplacement de l'espace libre par du solvant frais. Une nouvelle interface de type tube à flamme de silice a été conçue pour la détection fine, par spectrométrie d'absorption atomique (SAA), des métaux As, Cd, Cu, Mn, Pb, Se ou Zn dans l'éluat d'extraction de CO_2-SC. Pour divers analysats, les limites de détection (LD) allaient de la fraction de nanogramme à quelques picogrammes, après injection de l'étalon dans la phase mobile. Ces sensibilités ont permis d'étudier l'utilité de divers taux de mobilisation des diverses formes du métal à analyser à partir de milieux divers pour sonder l'interaction entre le métal à analyser et la matrice. Une portion du Zn de foie de bovin frais a été rapidement mobilisée en l'absence d'agent de complexion et le reste solubilisé plus rapidement que le Zn de la substance témoin séchée à froid provenant de ce tissu. Le montage en nébuliseur d'un SAA à flamme conventionnel a été modifié pour étendre la gamme de métaux susceptibles de détection *en direct* et a fourni un détecteur *en direct* environ dix fois meilleur que l'appareil conventionnel. On a conçu une autre stratégie utilisant le R_4NX comme réactif associateur d'ions pour la mobilisation sélective d'anions (chromate, séléniate, sélénite) de milieux aqueux. Enfin, on a étudié l'utilité de l'extraction SC pour la mobilisation/extraction du Cd, Cu et Zn liés à la métallothionéine isolée dans le foie de lapins.

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Chapter 1. Introduction

1.1 Supercritical Fluids

Over the past decade, the principles and practice of supercritical fluid (SF) technologies have experienced rapid advances. Supercritical fluids have been exploited as a medium for extraction, chromatography, and as a medium for chemical and enzymatic reactions.

More than a century ago, Hannay and Hogarth reported that supercritical fluids, or dense gases, have a surprisingly high solvating power (McHugh, 1986a). However, it is only in the past decade or so that numerous industrial and academic research and development laboratories have investigated the underlying fundamentals and process applications of supercritical fluid solvents. When a SF is used as an extracting solvent, it is possible to separate a multi-component mixture by exploiting both the differences in component volatility and the differences in the specific interactions between components of the mixture and the SF solvent. The application of SF solvents is based on the experimental observation that many gases exhibit enhanced solvating power when compressed to conditions slightly above their critical points. The motivation for the development of SF solvent technology (McHugh, 1986a) as the basis of a viable separation technique is a result of:

1) a sharp escalation in the cost of energy, which has increased the cost of traditional , energy-intensive separation techniques, such as distillation;
2) increased governmental scrutiny and regulation of common industrial solvents, such as chlorinated hydrocarbons, which has made non toxic, environmentally acceptable SF solvents, such as CO_2 , very attractive alternatives;

3) more stringent pollution-control legislation, which has caused industry to consider alternative means of waste treatment; and

4) increased performance demands on materials, which traditional processing techniques cannot meet.

One unique feature of SFs is that they display a wide range of solvating powers, which can be quantified by the "enhancement factor". The enhancement factor is the ratio of the actual solubility of a test solute in the SF solvent to its solubility in an ideal gas at the same temperature

$$E = \frac{Y_{actual}}{Y_{ideal}} \tag{1.1}$$

Enhancement factors of the order 10^5-10^7 are common. Since the solubility of a solute in a SF changes rapidly in the region near the critical point, the enhancement factor changes rapidly also. In addition to its unique solubility characteristics, an SF solvent possesses certain other physicochemical properties that include gas-like transport properties of diffusivity and viscosity. Additionally, the zero surface tension of supercritical fluids facilitates penetration into microporous materials.

In order to more fully understand the fundamental processes that govern the behavior of SF solvents, appreciable effort has been devoted to determining the fundamental aspects of solute-solute, solute-fluid, and solute-cosolvent interactions in SFs and SF processes. Some of these studies have used optical spectroscopy and others have used chromatographic methods as tools to probe these interactions. Many of these experimental studies have been supported by theoretical calculations and modeling (Petsche *et al.*, 1989). The general conclusion from these studies is that there is a localized density increase (*i.e.*, solvent clustering) about the solute near the critical point. In addition, it has been suggested that there are enhanced solute-solute interactions near the critical point. Spectroscopic investigations of SFs have demonstrated that changes in frequency, intensity, and/or phase can provide insight into the behavior of these fluids. Optical spectroscopy provides of a powerful battery of related methods for probing many aspects of a chemical system. Infra-red (IR) and Raman spectroscopy, for example, yield information about the vibration modes within a molecule. Insight into chemical bonding and the effects of the fluid on bonding is gained. Ultraviolet and visible (UV-Vis) absorption spectroscopy yield information the electronic structure within a molecule. If the valence electronic structure is altered by the interaction of the solute with the fluid, UV-Vis spectroscopy can be used to monitor and quantify these changes. Fluorescence spectroscopy permits one to probe the ground- and excited-states of the solute molecule and learn how each is influenced by local solvent composition.

The majority of the reports on SF solvation have used steady-state solvatochromic absorbance measurements. Initial SF work was on CO_2 and indicated weak interactions between the fluid and solute. Additional studies using Xe, SF_6, C_2H_6, and NH_3 have been reported. Johnston and co-workers (1989) demonstrated quantitatively that the local fluid density about the solute is greater than the bulk density. Local densities were determined by comparison of the observed spectral shift (or position) to the shift predicted for a homogeneous polarizable dielectric medium.

Vibrational spectroscopy, too, has been used to study SF systems. Buback (1991) reviewed this area, however, much of his discussions centered on fluid systems that were well removed from ambient conditions or that were difficult to handle in the lab. Yonker and co-workers (1988) used near- and mid-IR spectroscopy to study SC-CO_2 and binary supercritical fluid systems composed of CO_2/H_2O, Kr/H_2O, and Xe/H_2O. The CO_2 results were consistent with increased intermolecular interaction between CO_2 molecules, with increasing density. For an ideal gas/water system an Onsanger electrostatic model (dipole-induced-dipole) sufficed to describe the spectral shifts. In contrast, the CO_2/H_2O system exhibited density-dependent changes in specific intermolecular interactions. Smith and co-workers (1990) have employed dynamic light scattering small angle neutron scattering, and Fourier-Transform Infrared (FT-IR) spectroscopy to study AOT [sodium bis(2-ethylhexyl) sulfosuccinate; Aerosol-OT] reverse micelles in ethane, propane, and xenon. From their dynamic light scattering experiments, it was proposed that the average micelle size varied with fluid density. However, detailed FT-IR experiments demonstrated that the water core region remained insensitive to fluid density. This was confirmed by Johnston and co-workers (1989) using different spectroscopic methods.

Zerda and co-workers (1986) used Raman spectroscopy to study the naphthalene-CO_2 system under high pressure. The experimental results and model calculations indicated that quadrupole-quadrupole coupling is appreciable in this system. The preferred orientation of the O=C=O and naphthalene was determined to be face-to-face. A solvatochromic study of metal chelates formed in binary SF solvents was reported (Joe *et al.*, 1989). Metal chelates present the possibility of studying charge-transfer complexes in SFs over the range from gas-like to liquid-like densities. The metal chelates, $[Fe(1,10\text{-phenanthroline})_3]^{2+}$ and $[Fe(2,4\text{-pentanedioate})_3]$ were studied in SC-CO_2/methanol solutions as a function of pressure, methanol concentration, and temperature. The local solvation environment about the metal chelates was seen to be a function of methanol concentration and pressure. As the methanol concentration was increased, a red shift in the absorbance maximum occurred. This behavior contrasted with the effect of increasing pressure: increased pressure caused a slight blue shift. For both metal chelates as temperature increased, a blue shift was observed.

Steady-state solvatochromic techniques provide a convenient means of studying solvation processes in supercritical media. But, unless the interaction rates between the solute species and the supercritical fluid are slow, these "static" methods cannot be used to study solvation kinetics. Investigation of the kinetics requires an approach that offers inherent temporal resolution. Fortunately, time-resolved fluorescence spectroscopy is ideally suited to this task. To date, there have been only a few of time-resolved studies in dense fluid media. Of these, the majority focused on understanding a particular chemical reaction by adjusting the solvent environments. Only more recently have there been reports of experiments directed toward studying the peculiar effects of supercritical fluids on these solvation processes. The initial work (Lee *et al.*, 1985) showed that:

1) time-resolved fluorescence can be used to improve our understanding of salvation in supercritical fluids and
2) the local solvent composition, about a solute molecule, could change appreciably on a sub nanosecond time scale.

Eastoe and co-workers (1991) used time-resolved decay of anisotropy experiments to probe the AOT reverse micelle system in ethane and propane. These authors concluded that there was no local solvent density increase about the reverse micelle. In addition, the rotational dynamics of their probe was independent of fluid density. This observation was consistent with results from Smith and co-workers (1990).

1.2 Supercritical fluid extraction

Supercritical fluid extraction (SFE) has the potential to change dramatically the conventional extraction procedures in an analytical laboratory. SFE was introduced in 1963 (Kiyokatsu and Muneo, 1991). Since then, this method has developed rapidly, first on an industrial-scale, then as an analytical-scale technique and has been studied by many research groups. The following merits of SFs (compared with the properties of liquid solvents) demonstrate the potential for SFE to approach an idealized analytical extraction:

1) SFE is fast because of increased mass transfer characteristics (relative to liquids) caused by higher solute diffusivities and lower viscosities. Relative to conventional liquid extraction techniques, SFE can reduce the time required for the quantitative extraction and recovery of analytes (from a wide variety of samples) by as much as several orders of magnitude.
2) the solvating strength of a SF can be controlled easily since the density of the fluid can be modified appreciably (and predictably) by the regulating the pressure and temperature used for the extraction.
3) many SFs are gases under ambient conditions, so that recovery of the analyte from the SF extractor eluate is greatly simplified.
4) most SFs are relatively inert, pure, non toxic and inexpensive. The generation of liquid solvent wastes and the exposure of laboratory personnel to toxic solvents is minimized.

1.2.1 Theoretical Understanding of SFE

The theoretical principles of supercritical fluid extraction have been explored in depth. The most concise yet comprehensive presentation of SF phase diagrams to predict SFE processes is contained in the text book edited by Mchugh and Krukonis (1986b). Phase behavior, methods for determining solubility in SFs and a wide variety of SFE process application are discussed. This text provides a comprehensive review of supercritical phenomena for the novice as well as the experienced analyst. The optimization of SFE processes are considered from both a utility and cost point of view. Emphasis is placed on the development of strategies for optimizing steady-state flowsheets of supercritical fluid extraction processes (Mary *et al.*, 1992).

On an analytical scale, King considered how the knowledge of four basic parameters of SFE was helpful in predicting solute behavior in compressed gas media (King, 1989). These four parameters are the miscibility or threshold pressure, the solubility maximum, the fractionation pressure range and the cumulative physical properties of the solute. Specifically, the miscibility or threshold pressure is the pressure at which the solute starts to dissolve in the supercritical fluid. This pressure is technique dependent and varies with the sensitivity of the analytical method used to measure the solute concentration in the SF phase. Solubility maximum is the pressure at which the solute attains its maximum solubility in the SF. When the solvent strength of the extraction solvent is matched to the solute, the system is at the solubility maximum. The pressure range between the miscibility pressure and the solubility maximum is the fractionation pressure range. In this range, enrichment of one component over another in the SF is possible by varying the pressure or density. This technique of controlling solute solubility in the supercritical solvent is still not well developed. The fractionation is enhanced by differences in the solute physical properties. Two solutes of widely different vaporization or melting points can be more easily separated by temperature changes in the SFE process. Other solutes, with common properties are not fractionated as easily.

The matrix appears to control analyte extractability in a wide variety of samples. The effect of the water content of the matrix has been characterized. Onuska and Terry (1989) reported decreases in the extraction efficiency with SC-CO_2 for wet (19.8 % water) vs. dry (0.3 % water) sediment samples. McNally and Wheeler (1988) observed the opposite trend for the extraction of sulfonylurea molecules from a variety of soils; extraction efficiencies increased with the addition of water. The differences in these two studies was principally the polarity of the solute molecule and the age of the samples. Aged environmental samples, especially those that have been field weathered, present more difficult extraction problems. Presumably, solutes of interest become trapped in the interstitial volumes of the sample matrix. When these environmental matrices have polar or ionic character and the solute also shares these properties, strong chemical interactions can occur.

The solvating strength or polarity of a supercritical fluid can be enhanced by the addition of another component or even two or more additives. It has been suggested that solvent effects are more important than solute structure and that polar and hydrogen-bonding modifiers can have large effects on selectivity. Unfortunately, not only the strength, but also the phase behavior must be understood when attempting to predict the efficiency of mixed fluids as an extracting medium. Phase behavior is considerably more complicated for mixtures than for pure materials, especially for near critical conditions.

Recent studies, as well as work in progress, are aimed at developing the needed understanding. For example, Chester and Innis (1993) introduced a rapid method to determine the boundaries of the two-phase mixture region (in pressure and temperature dimensions) using chromatographic equipment. So SFE could clearly benefit from a better and more complete understanding of simple, commonly encountered mixtures. Once the phase information is adequately compiled, simplified extraction methods will surely follow, utilizing the safe zones in phase space for the particular technique and fluid mixture.

Langenfeld and co-workers (1992) determined the densities of three neat fluids (CO_2, N_2O, and Freon-22) and 5 % (v/v) methanol in CO_2 over a wide range of pressures (6-60.7MPa) and temperatures (40-150°C) using a simple device constructed from an SFE vessel. Chen and co-workers (1993) used a Mass spectrometry (MS) composition probe coupled with a batch cell to measure high-pressure phase equilibria at temperatures up to 200 °C and pressures up to 60 MPa. Maxwell and co-workers (1991) measured the equilibrium solubility of anthracene in CO_2 and methanol-modified CO_2. They used a modified supercritical fluid extractor fitted with pneumatic valves to inject the solute into a receiver which formed part of the mobile-phase stream. They discussed the effects of varying pressure and sample loop volume on the measurements.

1.2.2 Modeling of SFE

As novel applications of SFE continue to be reported, a theoretical model of SFE is beginning to emerge. Current thoughts on SFE involve both thermodynamic (*e.g.*, solvation, partitioning, and diffusion) and kinetic contributions. In the majority of thermodynamic descriptions of phase equilibria, equations of state (EOS) are used as the starting (empirical) information which must be converted to thermodynamic relationships. A generalized procedure using the Peng-Robinson (PR) EOS predicts either two- or three-phase equilibria for systems containing dense CO_2 with either well-defined hydrocarbon or continuous oil component(s). The predicted results are in very good agreement with experimental measurements for mixtures of CO_2 with naphthalene or methylnaphthalene or for CO_2-

ethylbenzene/systems (Tan *et al.*, 1991). Phase equilibrium of the system is described quantitatively and the PR EOS also provides a good prediction of molar volumes. However, the PR EOS with one adjustable parameter is not able to reproduce the phase behavior of systems in which the components differ appreciably in molecular size. Serious limitations were found for systems containing CO_2 and essential oils (Wells *et al.*, 1990).

The Redlich - Kwong (RK) EOS and many of its modifications have been applied widely as a very flexible correlation. For instance, by using the nonquadratic Panagiotopoulos - Reid mixing rules, systems containing CO_2 and fatty acids or esters can be well described (Zou *et al.*, 1990). The modification with properly adjusted parameters under reasonable assumptions can predict solubilities of solids in a supercritical solvent (Soave, 1991). The RK-Soave EOS was used successfully to predict the solubility of phenol and catechol in dense CO_2. The simplified perturbed hard chain theory (SPHCT) was applied to CO_2-hydrocarbon systems using a single interaction parameter and produced satisfactory estimates for 70 different multi-component systems (Ponce-Ramirez *et al.*, 1991). This approach proved to be superior to PR EOS for the tetratriacontane - propane system. The perturbed dipolar hard sphere EOS was also used for the description of the ethanol- water - CO_2 system (Martinez de la Ossa *et al.*, 1990).

Bartle and co-workers (1992) proposed a model for dynamic SFE which was based on the effect of solubility on extraction kinetics, as well as solute diffusion out of the sample matrix. Meanwhile, Pawliszyn (1993) presented a kinetic model of SFE which included several factors (*e.g.*, desorption kinetics, swelling, analyte diffusion in the organic component of the sample, and fluid/matrix distribution coefficients) that could potentially cause to slow rates of extraction. Cotton and co-workers (1993) evaluated the theoretical basis for observed extraction rates. They extracted a cyclic trimer from poly(ethylene terephthalate) and found that the extraction kinetics fit a model that presumed the extraction to be diffusion-limited. They also used a diffusion-limited model to describe the rate of removal of additives from polypropylene as a function of pressure, particle size, flow rate, and temperature. The variation of the extraction rate with pressure and flow rate confirmed the solubility limitation of the proposed model.

In recent work, Chunjie Zhang and co-workers (1993) studied the kinetics of supercritical fluid extraction of coal. This paper presented the results of a study of the kinetics of supercritical tert-butanol extraction of Illinois No. 6 bituminous coal. Extraction rates were estimated by continuously measuring the spectrophotometric absorbance of the effluent from a fixed-bed flow reactor. The experiments were conducted in the temperature range of 553-633°K and at 6.8 MPa constant pressure by programmed-temperature techniques. Lixiong and co-workers (1993) have developed a kinetic model for the wet oxidation of organic compounds in sub-critical or supercritical water. The methodology of developing a global kinetic model for wet oxidation of organic compounds is described. Simplified reaction schemes considering rate-controlling intermediates were used to develop this model. The selectivity of product vs. intermediate formation was considered to be a key model parameter to simplify calculations and characterize the "strength" of the feed stream. This model had been validated previously for hydrocarbons and oxygenated hydrocarbons using reported wet oxidation kinetic data obtained in the same temperature and pressure ranges.

Ramchandran and co-workers (1992) studied dynamic modeling and control of the supercritical fluid extraction process. This paper was concerned with the development of a dynamic simulator for the SFE process and presented a comparison of this simulation with operating data from a pilot-scale SFE process. In addition, the dynamic simulator was also used to study the process control aspects of the SFE process. Two different nonlinear process-model based control strategies were developed and tested for set-point tracking and disturbance rejection which resulted in good control performance.

1.2.3 *In Situ* Derivatization, Ion Pairing, and Complexation

An alternate and appealing approach to the recovery of polar analyte(s) by direct extraction, that of *in-situ* conversion of the analyte(s) to a less polar derivative(s), has also been successful. Extraction of polar, and even ionic, materials with nonpolar fluids, like CO_2, are becoming possible through the chemical derivatization of the analyt(s) either prior to or during the extraction. The approach has been to use a reactive modifier, such as hexamethyldisilazane or trimethylchlorosilane, to derivatize the active sites on the surface of the sample matrix, releasing the analytes for extraction. Rochette and co-workers (1993) performed initial experiments with silylation, ion pairing, methyl esterification, and ionic displacement for the SFE from soil matrices. Methyl esterification and ionic displacement proved to be the most promising approaches for quantitative recoveries. The research group of Hawthorne *et al.* (1992) used chemical derivatization of polar and ionic solutes during extraction to alter the extractability of the analytes. Trimethylphenylammonium hydroxide and boron trifluoride were used to derivatize and extract herbicides from soil and sediments, microbial phospholipid fatty acids from whole cells and wastewater phenolics from both water and octadecylsilyl (ODS) sorbents with greater than 90% recovery. The SFE approach and steam distillation were characterized by similar efficiencies. Methyl esters of seven chlorophenoxy acid herbicides were formed while these compounds were being extracted from soil (Lopez-Avila *et al.*, 1993). Trimethylphenylammonium hydroxide, benzyltrimethylammonium chloride, benzyltriethylammonium chloride, and tetrabutylammonium hydroxide/methyl iodide were evaluated as derivatization agents.

SFE with ion-pair reagents was used by Field (1993) and Fernandez *et al.* (1993). Both groups extracted cationic surfactants from sewage sludge. Field presented a broad range of analyte/ion-pair combinations with derivatization in heated GC injection ports during the subsequent quantitation procedure. However, the solubilization/mobilization of polar and ionic materials has remained somewhat elusive because of the non-polar character of those supercritical fluids which are readily generated in the laboratory.

1.3 SFE-Coupled Techniques

The direct combination of SFE with other analytical techniques can, in many cases, be a straightforward procedure, giving SFE an appreciable advantage over other extraction methods.

1.3.1. SFE-HPLC

Unger and Roumeliotis (1983) described the first coupling device which permitted *on-line* high performance liquid chromatography (HPLC) of SF extracts. The *on-line* system consisted of two high-pressure sample-injection valves connected in series. Later, Nair and Huber (1988) described the *on-line* HPLC analysis of ground tablets for ibuprofen. The SFE unit consisted of a constant-pressure pump to transfer the carbon dioxide to a preheater. This system was applied only for qualitative experiments. Recently Jeffrey and co-workers (1993) have described a membrane interface for *on-line* supercritical fluid extraction/flow injection analysis. A simple interface between the SC-CO_2 extractor and an *on-line* liquid-phase analytical system such as flow injection analysis (FIA) or HPLC was described. This interface used a membrane phase separator (*i.*) to remove CO_2 from the sample extract without the need for HPLC columns, and (*ii.*) to eliminate interference(s) while quantitatively transferring solutes of all types to the analytical system. The determination of chloramphenical and penicillin G solubilities in SC-CO_2 with a total analysis time of 2 min was demonstrated for an *on-line* SFE/FIA system utilizing this interface.

1.3.2 SFE-GC

Much of the development work for SFE-GC was performed early in the evolution of analytical scale SFE. Hawthorne and Miller (1986) were the first to couple SFE directly with GC, when they successfully performed a qualitative analysis of automobile-exhaust organics collected on Tenax. Since then, the number of publications involving *on-line* SFE-GC has continued to increase. Continuing with this methodology Wright and coworkers (1987) demonstrated that several modes of operation were possible, including the quantitative extraction of analytes from a sample matrix or selective extraction at various solvating powers to obtain specific fractions by pressure or density programming. A typical SFE-GC configuration is presented in Figure 1-1 (Vannoort *et al.*, 1990). The usefulness of *on-line* SFE-GC was well demonstrated for the extraction and selective fractionation of polynuclear aromatic hydrocarbon (PAH) mixtures. Recently a comprehensive SFE/GC analysis of organic compounds with an element-selective radio frequency plasma detector was reported by Mingin and co-workers (1993). The effect of the GC carrier gas (CO_2) and plasma reagent gas (oxygen) on the selectivity and sensitivity of the radiofrequency plasma detector (RPD) was studied to establish the optimal operating conditions. The results demonstrated that the (RPD) can substantially enhance the analytical utility of *on-line* SFE-high-speed GC for the analysis of selected target analytes. Recently Lou and co-workers (1993) examined the effects of CO$_2$ flow rate, interface temperature, and split ratio on the discrimination and reproducibility of *on-line* SFE-open-tubular GC with a conventional split/splitless injector as the interface.

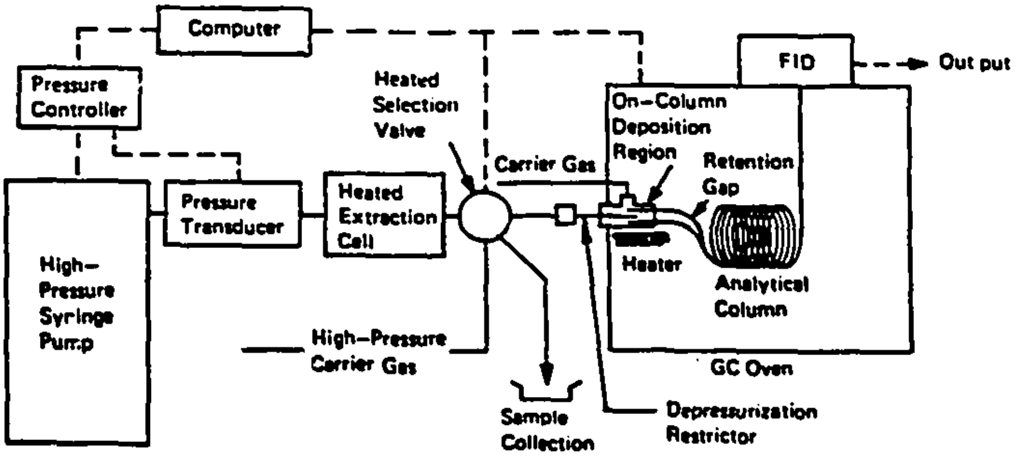


Figure 1-1. Schematic diagram of a SF extractor coupled directly to a capillary Gas Chromatography (Vannoort *et al.*, 1990).

SFE-GC with AE detection was reported by two groups (Casabianca, 1992; Liu *et al.*, 1993). In the first, halogenated and sulfur-containing pesticides in soils were analyzed. In the second, organotin compounds, some requiring *in situ* derivatization with n-pentylmagnesium bromide, were extracted from soil. Analyte/matrix interactions in environmental samples were studied by *on-line* SFE-GC (David *et al.*, 1992). *On-line* SFE-GC with derivatization was examined by King and co-workers (1992). Triglycerides extracted from seeds were transesterified to the methyl esters *in situ* over a solid catalyst.

1.3.3 SFE-SFC

Direct coupling of SFE with supercritical fluid chromatography (SFC) has been studied extensively, and has included theoretical models, interfacing techniques, instrumentation, and applications. Supercritical fluid extraction is an ideal way to introduce a sample into an SFC system. Because the injection solvent is the same as the mobile phase, an obvious advantage of *on-line* SFE-SFC over *on-line* SFE-HPLC and SFE-GC is that it is virtually impossible that sample constituents which are insoluble in the mobile phase will be introduced into the column. SFE can be combined with several forms of SFC including conventional packed columns, capillary columns and, as has been done more recently, with packed capillary columns.

Directly coupled laboratory-scale SFE-packed-column SFC was introduced in 1985 by Sugiyama and co-workers. Qualitative *on-line* SFE-packed-column SFC of powdered coffee beans was performed and monitored by multi-wavelength UV detection using a high-pressure cell. *On-line* SFE packed-column SFC has been compared with dichloromethane extraction followed by packed-column SFC for double-base propellants by Ashraf and Taylor (1989), using both FID and FT-IR detection. More than twice as many components were detected via packed-column SFC-FID coupled to SFE as with dichloromethane extraction. The conclusion was that either the SFE process dissolved a larger number of components or that the SFE extract was more concentrated than the dichloromethane extract.

Direct coupling of SFE to capillary SFC was systematically investigated by Gmur and co-workers (1987). The optimization of some important instrumental parameters such as internal diameter and length of the capillary pressure drop along the column, linear velocity and injection volume was studied. The development of an *on-line* SFE-capillary SFC system, with *off-line* FT-IR detection was reported by Raynor and co-workers (1988) for the separation and identification polynuclear aromatic hydrocarbans (PAHs) in coal pitch. An open-loop system was used. An SFE-capillary SFC fraction-collection system was developed to perform *on-line* extraction, separation and fraction collection of biologically important drugs. The SF extract was decompressed via a linear restrictor and deposited in a deactivated capillary concentrator within a cryogenic trap. This resulted in good chromatographic peak shapes. *On-line* SFE packed-capillary SFC is an interesting development in comparison with SFE capillary SFC, because of a higher loadability and shorter analysis times. In comparison with SFE packed column SFC, the advantages are a lower pressure drop, higher efficiency and lower flow-rates, resulting in an easier interfacing with FID or MS instruments. A similar system was also described by Hirata and co-workers (1988). They were able to confirm that the extracts were trapped efficiently in the first 5-cm section, even at an extraction temperature of 65°C.

Numerous other SFE-SFC applications have been reported. Berg and co-workers (1993) used an innovative SFE-SFC approach for the analysis of fatty acids and triglycerides. For their *on-line* procedure, they used an immobilized lipase on a catalyst bed to form methyl or butyl esters of fatty acids from triglycerides in edible fat. The determination of retinol palmitate and tocoperol acetate in a hydrophobic ointment by coupled SFE-SFC was also reported (Masuda *et al.*, 1993). Murugaverl and co-workers (1993) described a cleanup flow system for *on-line* SFE/SFC/MS analysis of carbamate pesticides from meat samples. Rocca and co-workers (1991) used *on-line* SFE-SFC to quickly and efficiently determine an animal feed marker used in a toxicological test. Perrigo and Joynt (1992) provided an impressive SFC database of over 100 compounds of forensic toxicological importance. As discussed earlier, Liebman and co-workers (1992) used SFE-SFC to characterize coatings designed for the fabrication of composite materials. Daimon and Hirata (1991) and Ashraf-Khorassani *et al.* (1991) used *on-line* SFE-SFC for the extraction and separation of polymer additives.

1.3.4 Other SFE-Coupled Techniques

Other *on-line* combinations of SFE with analytical methods were also demonstrated. Stalling and co-workers (1992) showed the utility of *on-line* SFE of environmental and biogenic samples. Masuda and co-workers (1993) determined the efficiency of the method and studied the effect of extraction fluid flow rate and sample size on peak resolution. A high-pressure IR flow cell was used to directly couple SFE with FT-IR without the need for intermediate chromatographic separation. Quantitation was performed by integrating the IR signal area of the extracted analyte with time. The detection limit of n-tetracosane using this method was reported to be 74 ng.

Recently, SFE in combination with various bioassay procedures have been reported. France and King (1991) combined SFE with an enzyme assay to screen meat products for pesticides. The major benefit of this approach is that the analyst is not exposed to organic solvents during the procedure. In addition, the bioassay is capable of detecting chemically diverse pesticides. Wolfe and co-workers (1993) used SFE for the preparation of aqueous pesticide samples prior to scanning with a bioassay technique.

The coupling of SFE with an analytical technique provides the potential for combined sample preparation and analysis. In addition to completely automated operation, rapid analyses and high recoveries can be achieved. Although *on-line*

SFE has a number of advantages over the more classical approaches, there are certain limitations and problems inherent in this technique. For instance, the majority of *on-line* SFE-HPLC studies have been applied to measuring extraction efficiencies - typically, only qualitative data has been generated. For more polar analytes, the extraction efficiency depends strongly on the extraction conditions (*i.e.*, pressure and temperature) and the addition of an entrainer is often required. The addition of a suitable entrainer or the use of a more suitable SF (*e.g.*, ammonia) and/or the modification of the matrix (*e.g.*, pH) make the interfacing techniques much more difficult. For FT-IR detection, there is a requirement for extensive background correction because of CO_2 absorption problems which are exacerbated if pressure programming is used during the extraction process. In comparison with *off-line* extraction, a more complicated set-up is typically required.

Overall, supercritical fluid extraction is a promising technique for the isolation of analyte(s) from complex matrices. It can be both more efficient and faster than conventional liquid phase extraction. The ease of solvent removal, coupled with the case of controlling the extraction conditions, the appreciably shorter extraction time and the decreased risk of sample contamination are all important advantages of supercritical fluid extraction over conventional extraction procedures.

1.4 Applications of Supercritical Fluid Extraction

The attractive solvating properties of supercritical fluids have resulted in the application of these fluids to several different research fields. A very brief survey of some of the current applications are outlined below and include: applications in the fields of natural products, foods and pharmaceuticals, pollution abatement and enzymatic reactions.

1.4.1 Natural Products

The range of samples for which SFE has been applied continues to increase. One of the interests in natural products analysis is the determination of aflatoxin B_1. Engelhardt and Haas (1993) noted that with pure CO_2 they could extract lipid constituents from animal feeds, but required pressures of 83.1MPa at 40°C to extract the more polar aflatoxin. The use of a methanol modifier mobilized the aflatoxin analyte at lower extractor operating pressures, but decreased the selectivity of the extraction.

A great number of other natural products have also been extracted by SFE. Smith and Burford (1992a) used cellulose as a model matrix system to study the SFE of typical plant constituents. They observed that increased selectivity for moderately polar analytes, such as lactones, was obtained by solute trapping onto silica followed by selective elution from this medium. Methanol-modified CO_2 was used to mobilize tobacco-specific N-nitrosamines and transfer them to a Tenax trap. Subsequently, thermal desorption GC with detection using a thermal energy analyzer was used to quantify the analytes (Prokopczyk *et al.*, 1992). The fungal metabolite ergosterol was removed from flour, moldy bread, and mushroom samples by SFE with sensitivities of about 0.05 µg/g (Young *et al.*, 1993). Pressure, temperature, and solute collection methods were studied for the extraction of the sesquiterpene lactone parthenolide by Smith and Burford (1992b).

1.4.2 Food Products

Since the use of organic solvents and/or high temperatures can be circumvented using SFE, the technique is also gaining popularity for food and fragrance applications, particularly if fats or essential oils are the target solutes. Maxwell and co-workers (1993) described the efficient recovery of volatile nitrosamines from frankfurters, followed by GC with chemiluminescence detection. Recoveries ranged from 84.3 to 104.8% for samples spiked at the 20 ppb level. Marsili and Callahan (1993) compared SFE with an ethanol/pentane procedure for the extraction of carotenes from a number of vegetable samples. The SFE method proved superior to conventional extractions in terms of both extraction yield and speed.

Currently, the food industry is using supercritical fluid extraction to decaffeinate coffee, to remove oil from snack foods, to extract the flavor components of hops, and to extract oil from cottonseed. The process also has appreciable advantages for the production of flavor extracts from spices and herbs. High quality extracts are produced in high yield, by a process perceived as "natural" leaving no solvent residues. Froning and co-workers (1990) extracted of cholesterol and other lipids from dried egg yolk using supercritical carbon dioxide. Four different combinations of pressure and temperature were used. As the temperature and pressure were increased, more lipids and cholesterol were removed. Phospholipids and protein were concentrated in the residual sample under the supercritical extraction conditions used. Their results demonstrate that SFE has excellent potential for the production of lower cholesterol, lower fat egg products. Cholesterol extraction can be achieved without degrading the functional properties of the resultant dried egg yolk product.

Friedrich and co-workers (1982) successfully extracted oil from full-fat soy flakes using SC-CO_2. Soybean oil extracted with SC-CO_2 is lighter in color and contains less iron and about one-tenth the phosphorous of hexane-extracted oil from the same beans. Extraction with SC-CO_2 has also been used to separate lecithin from the triacylglycerol fraction of soy oil. Butterfat has been fractionated using SC-CO_2; the cholesterol was concentrated into selected fractions. The process has also been used to remove lipids and cholesterol from fish muscle. Wong and Johnston (1986) have investigated the solubility of cholesterol and other sterols in CO_2 under various conditions. Leiner and co-workers (1986) extracted egg powder with SC-CO_2 at 300 bar/40 °C. The extract contained triglycerides, free fatty acids, waxes and cholesterol whereas the phospholipids remained in the residue. They reported that the proteins of the extracted egg power were not denatured.

In another study, Egon and co-workers (1980) have reported that vegetable oils can be extracted from crushed seeds with SC-CO_2. The yields (recoveries) were dependant on the pressure and the temperature employed for the extraction as well as the size and shape of the seed particles. Oil fractions differing in color, taste, and odor can be recovered at various pressures and temperatures. Parameters influencing the extraction and fractionation of soybean, sunflower seed, and rapeseed oils were described.

Supercritical fluid extraction of essential oils from a number of plant species has been compared to other extraction methods. SFE was compared to hydrodistillation for the extraction of essential oils from savory, peppermint, and dragonhead (Hawthorne *et al.*, 1993a). A static SFE step with the addition of methylene chloride was necessary prior to dynamic extraction. Polesello and co-workers (1993) used SFE for strawberry aroma analysis. They found that SFE was more selective than liquid solvent extraction and was better able to recover the "character impact" compounds from the strawberries. Enantiomer ratios of carvone in essential oils extracted from caraway seeds and spearmint leaves were determined (Bounoshita *et al.*, 1993). Reverchon (1992) studied SFE mass-transfer characteristics for the fractionation of the essential oils from the cuticular waxes in marjoram leaves. Lembke and Engelhardt (1993) proposed an SFE method for the determination of the total fat content of meat and cheese products as an alternative to the official German solvent extraction method.

1.4.3 Pharmaceuticals

The most widely reported application of SFE to drugs and pharmaceuticals has involved the extraction of drug residues from animal feeds. A polar secoisolariciresinol and its diglucoside derivative were extracted from flaxseed using CO_2 modified with a tetrahydrofuran/water mixture (Wilson *et al.*, 1993). It was necessary to remove the fatty material from the sample with a preliminary extraction and then apply higher operating conditions to release the lignans from the cell wall material. Messer and Taylor (1992) also investigated the extraction a hypolipidemic compound from a drug formulation, from rat feed, and from feed/drug mixtures. They observed large extraction variabilities for the drug formulation indicating appreciable heterogeneity in this material. The extraction of polar drug residues (sulfonamide) required more rigorous conditions from animal tissues, such as chicken liver or swine muscle than from an inert matrix, such as sand. Higher recoveries were reported for extractions from the swine muscle than from chicken liver. The results obtained for these drug residues were in good agreement with reference values. Basic drugs of abuse have been extracted via SFE and analyzed by open-tubular SFC (Edder *et al.*, 1992). Following addition of the plasma matrix to a solid phase extraction (SPE) cartridge, the proteins were washed out, and subsequently, the drug of interest were eluted via SFE.

1.4. 4 Enzymatic Reactions

Supercritical fluids are a special class of nonaqueous media. Enzyme-catalyzed reactions in supercritical fluids can be particularly useful because there is no solvent contamination of the reaction products after depressurization. Additionally, the low viscosity, low surface tension, and high diffusivity of supercritical fluids increase mass transfer coefficients of solutes, thereby appreciably increasing the rate of diffusionally limited reactions. The rates of reaction involving gaseous reactant such as oxygen or hydrogen can also be enhanced because there is no limitation resulting from the lock of solubility of the gas in a liquid.

The first study of enzyme catalyzed reactions in a SF was reported by Randolph and co-workers (1985) who studied the hydrolysis of disodium p-nitrophenylphosphate hexahydrate using alkaline phosphatase. The disodium salt, enzyme, and a small amount of water were charged into an autoclave, and the extent of conversion determined as a function of reaction time. A subsequent paper reported on another enzyme catalyzed reaction, the oxidation of p-substituted phenols using polyphenol oxidase (Hammond and Karel, 1985). Polyphenol oxidase is found in high concentration in mushrooms, some fruits, and in tea and tobacco leaves.

Another enzyme catalysis study investigated transesterification reactions with lipases (Nakamura *et al.*, 1986). The reaction sequence involved loading triolein, free stearic acid, lipase, and a buffer solution of TES [N-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid] into a magnetically stirred autoclave. As an example of the case where deactivation is advantageous, moist CO_2 at conditions of 60.8 MPa was found to improve the flavor of soy bean protein and to deactivate peroxidase enzymes that can result in the undesirable oxidation of residual lipids in the protein meal. An Investigation of the stability of enzymes in supercritical CO_2 demonstrated that certain enzymes are temporarily deactivated during depressurization, presumably owing to the rapid release of CO_2 from the bound water around the protein. Loss of activity was also reported for *Rhizopus delemar* lipase that contained more than five times its own weight of water (Nakamura, 1990).

Following these reports, there has been steady progress in this area. The conformation of the monomeric enzyme trypsin has been studied in supercritical carbon dioxide (Zagrobelny and Bright, 1992). Steady-state fluorescence spectroscopy was used to follow the conformation of trypsin *in situ* as a function of CO_2 density. Their results demonstrated, for the first time, that protein denaturation can occur during the fluid compression step and that the native trypsin is only slightly more stable (1.2 kcal/mol) than the unfolded form. Russell and co-workers (1994) have demonstrated enzyme activity in carbon dioxide, ethylene, propane, fluoroform, sulfur hexafluoride, and ethane. They concluded that supercritical fluids are ideal solvents for developing and testing an understanding of the dependence of an enzyme's function on its environment. The unique pressure dependence of supercritical fluid physical properties presents many opportunities for the control of enzyme activity, specificity, and stability in biocatalytic reactors.

1.4.5 Pesticides, Herbicides and Polymers

Howard and Taylor (1992) studied the SFE of herbicides. They extensively evaluated solid-phase trapping conditions when using methanol-modified CO_2 for the extraction. They immobilized aqueous samples of the herbicides onto solid phase extraction (SPE) media prior to SFE. They also examined various trapping conditions using SFE to extract thiocarbamates from apples (Howard and Taylor, 1993). Subsequent analyses were performed by LC with UV or sulfur chemiluminescence detection. Ezzell and Richter (1993) also used SFE to recover pesticides from aqueous samples which had been applied to SPE media. The applicability of the methods at the 1 ppb range of analyte concentration was demonstrated. Pesticide recoveries increased with increasing density and pressure, but temperature had little effect on the extraction and even caused thermal degradation of certain analytes. Residues of fluazifop-butyl and its metabolites were extracted directly from onions without subsequent cleanup steps (Wigfield *et al.*, 1993). Quantitative recoveries were achieved and limits of detection of 0.02 ppm were reported.

The increased diffusivity of supercritical fluids over liquids, as well as the variable solvating strength, has made SFE attractive for polymer applications. Raynor and Bartle (1993) reviewed the use of SFC and SFE for the analysis of surface coatings. Kueppers (1992) dealt with the selective extraction of low molecular weight components of poly(ethylene terephthalate). A systematic study of the effects of temperature was used to develop a model for dynamic SFE which considered the temperature-dependent physical changes in polymers. Pressure and mobile-phase composition were also explored, and guidelines for estimating the optimum temperature for the SFE of polymers were presented. Venema and co-workers (1993) investigated the efficiency of SFE extraction of caprolactam and its oligomers from nylon-6 using methanol-modified CO_2. A silica column was used to selectively remove additives from all but the lowest molecular mass oligomers of liquid poly(alkylene glycol) lubricants (Hunt *et al.*, 1993). The approach was then used for a stepwise extraction of sorbitan ester formulations to result in a chemical class fractionation.

1.4.6 Environmental Decontamination(s)

SFE has gained its most rapid initial acceptance by the environmental community for the extraction of relatively non-polar analytes including polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and other compounds from soils, sediments, airborne particulates, and similar samples. Hawthorne and co-workers (1993b) discussed the development and evaluation of *off-line* and *on-line* SFE methods for products of the petroleum industry and other wastes. Myer and co-workers (1992) compared SFE to Soxhlet extraction, with an emphasis on environmental samples, for the sample preparation of herbicides, pesticides, hydrocarbons, waxes, and fats from a number of solid matrices. Furton and co-workers (1993) studied a variety of experimental parameters influencing the SFE of PAHs and PCBs from solid-phase sorbents. McNally and co-workers (1993) presented an in-depth investigation of the influence of soil type on the SFE of moderately polar compounds in soil samples. A study on the use of metal-containing selective sorbents for the determination of the constituents of cigarette smoke was performed (Wenzel *et al.*, 1993). The sorbents were thoroughly characterized; blanks observed for SFE were typically cleaner than those obtained with thermal desorption. Griest and co-workers (1992) recovered 2 ppm spikes of chemical warfare agents on soil in less than 2 min using 5% methanol-modified CO_2 at 30.4 MPa and 60 °C.

The determination of total petroleum hydrocarbons (TPH) by SFE, often using IR detection, is of great interest. Eckert-Tilotta and co-workers (1993) extracted soil samples contaminated by heavy fuel oil, diesel fuel, light crude oil, gasoline, or kerosene spills using both SFE and Soxhlet techniques followed by either IR or GC with flame ionization detection (FID). Two research groups were interested in extracting hydrocarbons from petroleum source rocks. Ashraf-Khorassani and co-workers (1992) reported that SFE of biological markers from sedimentary rock resulted in greater yields and shorter extraction times than Soxhlet extraction of the same material. The effect of temperature on the extraction efficiency of biomarkers was also studied. Brooks and Uden (1993) used SFE followed by either microbore SFC or GC-mass spectrometry (MS) (GC-MS), for the extraction and analysis of diesel fuel from soil. They reported extraction efficiencies of greater than 90% regardless of the organic content of the soil.

Paschke and co-workers (1992) extracted PAHs and nitrogenated PAHs from diesel exhaust particulates and diesel soot using CO_2 or solvent-modified CO_2 as the extracting fluid. The highest recoveries were achieved with 10% toluene in CO_2. Lopez-Avila and Beckert (1992) spiked and extracted sand samples with 36 nitroaromatics, and 42 organochlorine pesticides and also extracted a certified reference soil with supercritical CO_2 in a two- or four-vessel extractor to establish extraction efficiencies for samples extracted in parallel. They explored variables including flow rate, pressure, temperature, moisture content, extraction vessel volume, extraction time and modifier type. Dichloromethane was used as a static modifier in the SFE of PAHs in soils. Pyle and Setty (1991) described the use of a copper scavenger column (positioned downstream from the extraction vessel) to remove elemental sulfur from soils extracts during the SFE of PAHs. Without the copper scavenger, deposition of elemental sulfur in the restrictor blocked the CO_2 flow.

Advances in the supercritical fluid extraction of polychlorinated biphenyls, dibenzofurans, and dioxins were reviewed by Greibrokk (1991). His review covered SFE of polychlorinated organics from sediments, wastewater sludges, soils, plant and animal tissue, and SPE adsorbents. Kapila and co-workers (1992) concluded that the extraction of organochlorine compounds from environmental matrices by SFE is a promising method, but that it might be limited by the initial instrumentation costs and/or by impurities in commercial fluid sources. The selectivity of various adsorbents toward the fractionation of complex organic mixtures consisting of compounds having similar solubility parameters and containing polychlorinated dibenzo-p-dioxins (PCDDs) has been examined (Alexandrou *et al.*, 1992). These authors reported a Florisil cleanup method for the analysis of PCDDs and polychlorinated dibenzofurans (PCDFs). Full-scan mass spectra were obtained for picogram quantities of analyte.

Increased environmental concerns require that novel remediation technologies be developed/optimized for the efficient removal of heavy metal toxicants from contaminated processing media. To my knowledge, there are very few remediation procedures which can be optimized for natural matrices (soils, sediments, or natural waters) which have been contaminated with both organic and heavy metal toxicants. One promising technology which could be equally efficient for both toxicant types, is the use of supercritical fluid extraction. The mobilization of heavy metals by adding an appropriate sequestering agent into supercritical carbon dioxide (SC-CO_2) has been little studied. To date, studies have been restricted to extraction of performed acetylacetone complexes (Saito *et al.*, 1990), the chromatographic separation with SC-CO_2 of performed β-diketonate or dialkyldithiocarbamate complexes of certain heavy metal cations (Laintz *et al.*, 1992a and Ashraf-Khorassani *et al.*, 1987) and the spectroscopic characterization of metal chelates in SFs (Motley *et al.*, 1989). While the current studies were in progress, Laintz and co-workers (1992b) reported the use of lithium bis(trifluoroethyl)-dithiocarbamate for the partitioning into SC-CO_2 of Cu^{2+} from aqueous solution and from silica surfaces. The objectives of my studies were to assess the feasibility of recovering heavy metals from natural matrices.

1.5 Objectives of the Research

The overall objective of this thesis research was to develop procedures for the efficient recovery of transition metal ions from aqueous matrices. the strategy was to devise an automated extraction procedure in which ionic metal analyte, in aqueous medium, would be sequestered *in situ* by reaction with a complexing ligand and the non polar product complex would be transferred to supercritical carbon dioxide then purged from the reactor by replacing the headspace above the sample with fresh mobile phase. Initial efforts were to be directed to identifying a ligand (or ligand source) which (*i.*) would be appreciably soluble in the supercritical carbon dioxide and (*ii.*) would react rapidly with analyte(s) to form non-polar complex(es) also having appreciable solubility in the supercritical CO_2. An additional objective was to develop techniques for coupling supercritical fluid extraction with *on-line* detection by atomic absorption spectrometry so as to be able to monitor the course of the extraction with time. If successful, it was envisaged that different fractions of the analyte metal might be further characterized based on their relative rates of mobilization/purging from the extractor. The influence of extractor operating parameters and other components of the sample on the rate and efficiency of analyte purging was to be studied with a view to developing a mathematical model capable of predicting the course of the extraction. Finally, the extraction process was to be applied to increasingly complex natural matrices in an effort to validate the model.

Chapter 2

Supercritical Fluid Extraction of Heavy Metals from Aqueous Solution

2.1 Trace Metals Research

The sources of trace metals into the environment are both natural and anthropogenic. The rapid increase in the exploitation of metallic deposits in the earth's crust has resulted in increased exposure of living organisms to a wide variety of different metallic toxicants. Many of society's most useful metals are toxic. Even the so called essential elements are also characterized by a surprisingly narrow range of optimal biological activity. Because trace metals are widely distributed, vary in their behavior and fate in air, water, soil and within living systems, growing concerns have been expressed about the environmental contamination which has become increasingly severe in the past few decades.

The mobilization of heavy metal cations by adding an appropriate sequestering agent into supercritical carbon dioxide (SC-CO_2) has, to our knowledge, been little studied. The objectives of the current studies were to assess the feasibility of recovering trace heavy metals (*e.g.* Zn^{2+} , Cd^{2+}, Mn^{2+}, Cu^{2+} and Pb^{2+}) from aqueous matrices by extraction with SC-CO_2. The strategy was to saturate the mobile phase with a suitable ligand during a static equilibration phase of the process then to sequester heavy metal cations *in situ* and purge the product complexes from the aqueous matrix during a subsequent dynamic extraction. To achieve this, initial efforts were directed to identifying a ligand (or ligand source) which (*i.*) would have appreciable solubility in the supercritical fluid mobile phase and (*ii.*) would form a non-polar complex with the metal analyte also having appreciable solubility in the mobile phase.

These studies were undertaken with the realization that soluble carbonate salts have been used extensively as precipitating agents in the treatment of hazardous waste streams. Since the pH is decreased to approximately 3.5 for CO_2-saturated water at normal supercritical fluid extraction pressures (Hendrick and Taylor, 1992), it seemed possible although unlikely that insoluble carbonate precipitates of heavy metal analyte(s) might be formed during a static equilibration with SC-CO_2 prior to purging. It was anticipated that this transposition might be minimized by (*i.*) presaturating the SC-CO_2 with a soluble source of chelating agent rather than presaturating the aqueous sample and/or (*ii.*) intentionally reducing the pH of the sample matrix. The efficiency of transfer of an ionic metal species by complexometric extraction into a non-polar supercritical fluid mobile phase can be anticipated to vary as the (*i.*) thermodynamic stability constant, (*ii.*) the kinetic rate of formation and (*iii.*) the vapor pressure of the resulting complex. Dithio-acids (dialkyldithiocarbamates, alkylxanthates and dialkyldithiophosphates) have been used extensively as chelating agents for complexometric extractions because they form stable complexes with a wide variety of heavy metals.

For these ligand classes (dialkyldithiocarbamates, alkylxanthates and dialkyldithiophosphates) a linear relationship has been demonstrated between the log of the formation constant of the product Zn, Pb and Cd complex and the acidity of the dithioacid ligand (pKa) (Hayasi *et al.*, 1986). Since acidic conditions were to be used for the extraction, dithiocarbamates were chosen for further study because of (*i.*) the increased thermodynamic stability constants of dithiocarbamate relative to the corresponding xanthate or dithiophosphate complexes and (*ii.*) the apparent pseudo first-order rate constants for decomposition of the ligand were very similar in the pH range 1-4. Despite the fact that most dialkyldithiocarbamate complexes are only moderately soluble in non-polar organic solvents, they are formed very rapidly in the liquid phase and are generally characterized by high stability constants. It was postulated that an appreciable solubility of the product metal complexes in SC-CO_2 could be achieved by an appropriate choice of the alkyl substituent on the dithiocarbamate ligand and that a counter ion could be identified which, when combined with the dithiocarbamate ligand would form an ion pair with sufficient stability and volatility to be useful as an additive to SC-CO_2. The longer term objectives of these studies were to identify procedures for the efficient recovery of metal analytes from natural matrices.

2.2 Materials and Methods

2.2.1 Construction of a Supercritical Fluid Extractor

Supercritical fluid extraction (SFE): An analytical scale extractor (Figure 2-1), assembled in the laboratory, consisted of a compressor connected to the outlet siphon of a K-type cylinder of CO_2 which delivered the gas at a preselected pressure to a 25 x 1 cm (i.d.) HPLC column assembly filled with 4 mm stainless steel (ss) balls which acted as a temperature and pressure equilibration device (PTED). Mobile phase was transferred via a pressure transducer (PT) to a saturation vessel [SV, 25 x 1 cm (i.d.) ss HPLC column assembly] then to an extraction vessel (EV).

The extraction vessel (Figure 2-1) used for liquid-fluid extraction was a stainless steel column purchased from Chromatographic Science Company (Ville St

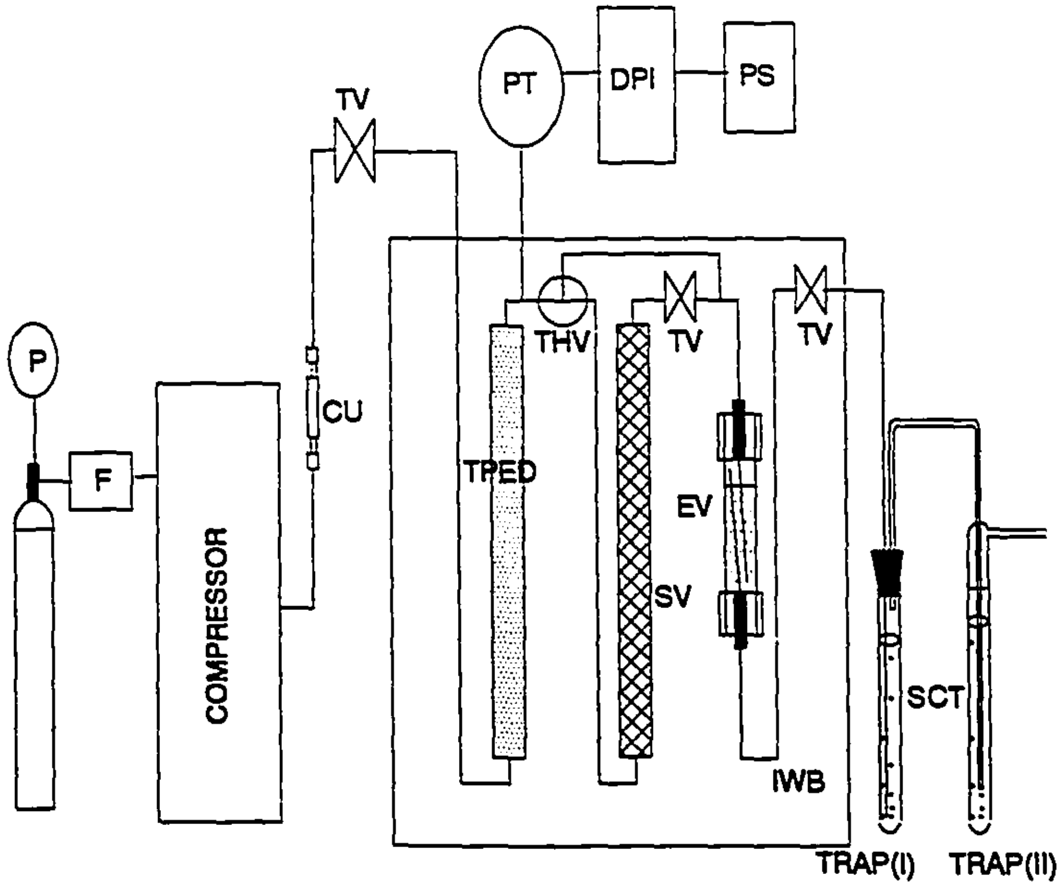


Figure 2-1. Supercritical fluid extractor consisting of compressor, a temperature pressure equilibration device (TPED), a saturation vessel (SV), and an extraction vessel (EV) immersed in a insulated water bath (IWB). Pressure was monitored with a pressure transducer (PT) mounted in series between the TPED and the SV and displayed on a digital pressure indicator (DPI). After passing through a capillary restrictor eluate from the extractor was decompressed into methanol contained in a sample collection tube (SCT). Two-way (TV) and a dual stem three-way (THV) high-pressure valves permitted static equilibration of the system prior to purging.

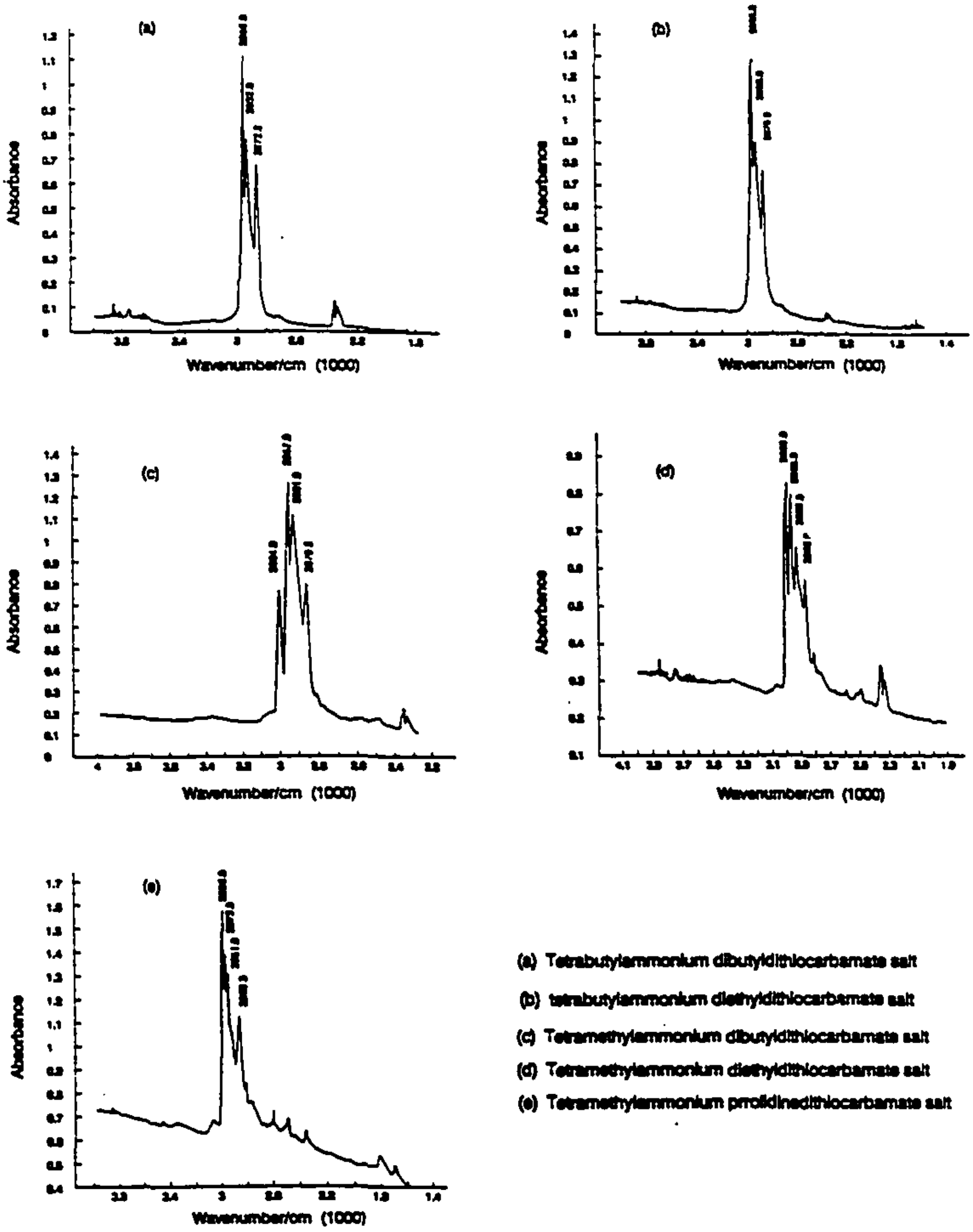
Laurent Que.) and measured 5 cm in length with an internal diameter of 0.98 cm (i.d.). The vessel was subsequently modified for use with liquid samples in the following manner. The zero dead volume channel at either end of the threaded fitting was drilled out to a diameter of 1/16". The high pressure finger tight fitting and ferrule purchased from Upchurch Scientific Inc. (Oak Harbor) were used as an integral part of the fitting so that a 1/16" stainless steel tube could pass freely through either end of the vessel. The stainless steel inlet and outlet tubes were 0.03" internal diameter and extended from opposite end to within 0.5 cm of the bottom and 0.7 cm of the top of the vessel. The PTED, SV and EV were immersed in a constant temperature water bath. Standard stainless steel HPLC grade fittings and 0.16 mm (i.d.) transfer lines (rated to 6,000 psi) were used throughout. A two-way flow through valve (TV), placed in series between the SV and the EV permitted the saturation of the mobile phase with the complexing reagent under static (no flow) conditions prior to dynamic complexation/purging of metal analyte(s) from the EV. After passing through a restrictor [a short length of narrow bore (0.025 mm i.d.) capillary quartz tubing (SGE, Houston TX)], the supercritical mobile phase was decompressed into a liquid trapping solution contained in a test tube which had been modified with a gas outlet for measuring the flow rate of decompressed gas. An appropriate choice of the length and diameter of the quartz tubing resulted in the desired flow of mobile phase.

Flow injection analysis atomic absorption spectrometry (FI-AAS): The content of metal analyte(s), recovered in the CH_3OH trapping solvent, was determined by flow injection analysis of 10-50 µL aliquots into a CH_3OH carrier solvent with detection by AAS. The residues of heavy metal analyte which remained in the water sample after extraction were also determined using this method with water as a carrier solvent. A Zeiss FMD-3 atomic absorption spectrometer equipped for deuterium background correction and fitted with an all-silica T-tube interface (Tan *et al.*, 1994) provided sub-ng limits of detection (LODs) for Cd-, Zn- and Pb-dialkyldithiocarbamate complexes in a methanolic carrier or residual analyte in the aqueous phase.

2.2.2 Synthesis and Identification of Complexing Agents and Metal Chelates

Carbon disulfide (50 mmol in 50 mL tetrahydrofuran) was added dropwise to an ice-cold stirred solution of tetraalkylammonium hydroxide (R_4NOH) and dialkylamine (R'_2NH, 50 mmol) in methanol (50 mL). When the addition was complete, the crude product mixture was allowed to warm to room temperature. The crude reaction mixture was evaporated to dryness to yield a pale yellow oil which crystallized rapidly on standing. Repeated recrystallization from isooctane-benzene or hexane-diethyl ether furnished analytical reagents. The identity and purity of the final products were corroborated by FT-IR spectroscopy and melting point determination. The FT-IR spectroscopic studies were carried out using a Nicole 740 FT-IR spectrometer. The sample prepared using Macro-Micro KBr Dye (Aldrich Chemical Co.). Figure 2-2 presents the FT-IR spectra of these tetraalkylammonium dialkyldithiocarbamate complexes.

Dialkyldithiocarbamate complexes of Zn^{2+}, Cd^{2+} and Pb^{2+} were prepared in aqueous media by reaction of sodium dialkyldithiocarbamate with a soluble salt (Cl^- or NO^-_3) of the metal cation. The product complexes were partitioned from the crude reaction mixture into $CHCl_3$. The organic washes were combined, dried over Na_2SO_4, filtered and the filtrate concentrated to dryness. The product complex was recrystallized from $CHCl_3/CH_3OH$. Following 30 min of static equilibration at 17.18 MPa and 45 °C, the solubility of ion pair complexing agents



(a) Tetrabutylammonium dibutyldithiocarbamate salt

(b) tetrabutylammonium diethyldithiocarbamate salt

(c) Tetramethylammonium dibutyldithiocarbamate salt

(d) Tetramethylammonium diethyldithiocarbamate salt

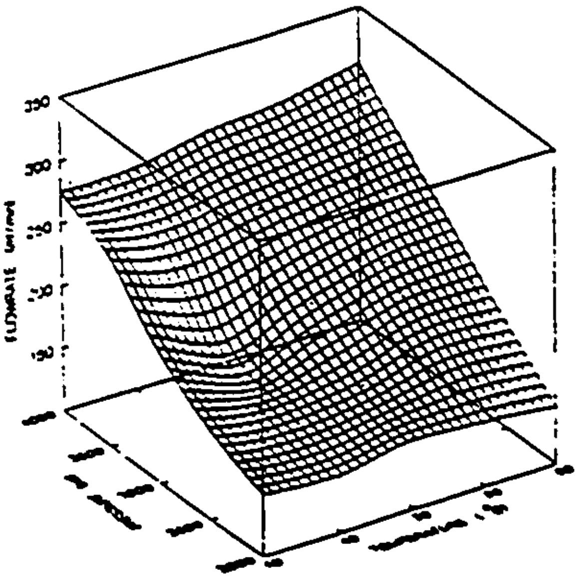
(e) Tetramethylammonium pyrrolidinedithiocarbamate salt

Figure 2-2. FT-IR spectra of metal complexing reagents recorded from KBr pellets (Macro-Micro KBr Die).

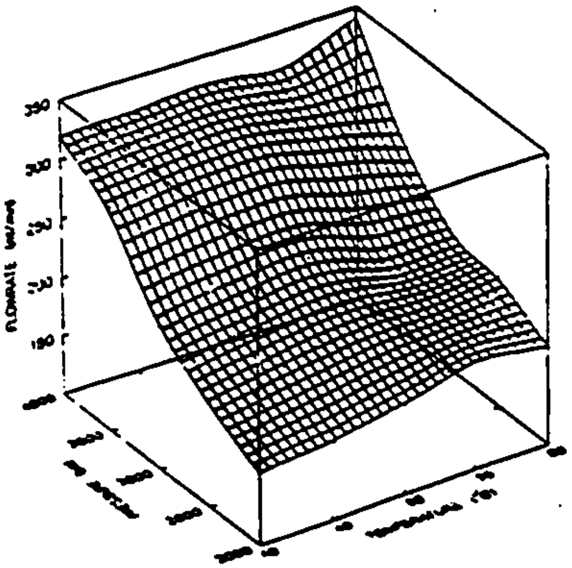
were estimated by trapping into methanol, material mobilized from the saturation vessel during 20 min of dynamic extraction. The quantity of complexing agent retained by the trapping solution was determined spectrophotometriclly at 298 nm by comparison with authentic standard. The surface response plots of depressurized CO_2 flow rate as a function of process pressure and temperature were developed using the LEAST SQUARE model and summarized as 3-D surface responses (software SYSTAT and SYGRAPHE, SYSTAT Inc., USA). Figure 2-3 presents the predicted flow rates of decompressed CO_2 eluting from the extractor, when using the silica capillary restrictor (a: 11 cm x 25 μm i.d., b: 9 cm x 25 μm i.d.) at process pressures between 14.48 MPa to 28.96 MPa and temperatures between 40°C to 60°C.

2.2.3 Solubility of Tetraalkylammonium Dialkyldithiocarbamates and Their Metal Complexes in SC-CO_2

A series of tetraalkylammonium (R_4N^+) salts of dibutyl (DBDTC), diethyl (DEDTC) or pyrollidinedithiocarbamate (PDTC) ion pairs were prepared. The trend in melting points of the products (Table 2-1) suggested that the vapor pressure of these products were influenced primarily by the chain length of the alkyl substituent on the N of the dithiocarbamate and, to a lesser extent, by the length of alkyl substituent on the ammonium counter ion. To the extent that non-polar character of the product ion pair remains the dominant parameter influencing its solubility in SC-CO_2, dibutyldithiocarbamate-metal complexes are anticipated to be appreciably more soluble than either the diethyl or the pyrollidine isologs. Additionally, for aqueous matrices, it was anticipated that residues of the tetraalkylammonium counter ion would be transposed to the bicarbonate ion pair. Since alkyl ammonium ions are disruptive of many biological membranes, residues of these toxic ions in the sample matrix would have to be minimized. An



(a) Restrictor: 11 Cm X 25 um i.d.



(b) Restrictor: 9 Cm X 25 um i.d.

Figure 2-3. Surface response plots of the predicted changes in the flow rate of decompressed CO_2 (at room temperature and atmospheric pressure) as a function of the extractor operating pressure and temperature.

Table 2-1. Melting points of tetraalkylammonium dithiocarbamate ion pairs.

Ion pair	**Melting point (°C)**[a]
$(C_4H_9)_4N^+[SC(S)N(C_4H_9)_2]^-$	82-84
$(CH_3)_4N^+[SC(S)N(C_4H_9)_2]^-$	105-106
$(C_4H_9)_4N^+[SC(S)N(C_2H_5)_2]^-$	120-122
$(CH_3)_4N^+[SC(S)N(C_2H_5)_2]^-$	162-163
$(CH_3)_4N^+[SC(S)NC_4H_8]^-$	200-202

[a] Melting points are uncorrected.

increasing chain length of the alkyl substituent on R_4N^+ would increase the SC-CO_2 solubility of the resulting ion pair formed with bicarbonate.

The relative polarity hypothesis was corroborated by determining the relative solubility of tetraalkylammonium-, ammonium- and sodium dialkyldithiocarbamate complexing agents in SC-CO_2. The saturation vessel of the extractor was charged with 0.5-1.0g of test complexing agent contained in a cardboard thimble which fit snugly inside the column assembly and equilibrated with the mobile phase for 30 min under static extraction conditions. The two-way ball valve was opened and the SC-CO_2 was transferred directly to the decompression assembly and the solute trapped in methanol. As recorded in Table 2-2, an appreciable variation in solubility was observed under arbitrarily chosen extractor operating conditions of moderate pressure and temperature. The tetrabutylammonium DBDTC was 8-times more soluble than the DEDTC isolog, 21-times more soluble than sodium DEDTC and 39-times more soluble than ammonium PDC under these conditions. The latter two reagents, which are available commercially, have been used widely for a variety of liquid phase complexometric extractions. Although not investigated in detail, the observed order of solubility in SC-CO_2 paralleled the intensity of the odor of CS_2 from the methanol trapping solution. Whereas a barely detectable odor was observed for the tetrabutylammonium DBDTC, appreciably stronger odors were observed for the methanol trapping solutions when the solubilities of the other chelating agents were examined.

Similarly, when Zn-DBDTC, Zn-DEDTC or Zn-PDTC standard, which replaced the complexing agent in the saturation vessel (55°C and 24.05 MPa), was equilibrated statically for 30 min followed by 20 min of purging, appreciable

Table 2-2. Solubilities of tetraalkylammonium-, ammonium-, and sodium dithiocarbamate ion pairs in SC-CO_2 at 17.18 MPa and 45 °C.

Complexing agent	**Solubility[a]** **μg/mL (SCF)**	**Ratio**
$(C_4H_9)_4N^+[SC(S)N(C_4H_9)_2]^-$	23.24	8 $[Bu_4NSC(S)NBu_2/Bu_4NSC(S)NEt_2]$
$(C_4H_9)_4N^+[SC(S)N(C_2H_5)_2]^-$	2.91	21 $[Bu_4NSC(S)NBu_2/NaSC(S)Et_2]$
$Na^+[SC(S)N(C_2H_5)_2]^-$	1.09	
$H_4N^+[SC(S)N(C_4H_8)]^-$	0.60	39 $[Bu_4SC(S)Bu_2/NH_4SC(S)C_4H_8]$

[a] Following 30 min static equilibration at 45 °C and 17.18 MPa, complexing reagent was purged from the saturation vessel during 20 min using the 11 x 0.025 mm i.d. restrictor.

variations in solubility were also observed for these complexes. Under these extractor operating conditions the Zn-DBDTC complex was some 20 times more soluble than the Zn-DEDTC and 139 times more soluble than the Zn-PDTC Complex (Table 2-3).

A subsequent series of trials were designed to test whether the mobile phase became saturated with Zn-complex under the extractor operating conditions. The mobile phase was equilibrated with test Zn-complex in the supercritical fluid for 30 min then three fractions corresponding to successive 20 min collections of solute contained in the extractor outflow were prepared. The two-way valve was closed, and mobile phase was again equilibrated for a further 30 min. The capillary restrictor tube was shortened from 11 to 9 cm and three more successive 20 min fractions were collected. Saturation of the mobile phase was indicated by the equivalent quantities of Zn in each of the six trapping solutions (Table 2-4). The operating conditions of the extractor apparently resulted in saturation of the mobile phase at both flow rates.

The solubility of a given compound in SC-CO_2 can be influenced by several parameters. Because CO_2 has a small polarizability and no net dipole moment, the solubility of both polar and nonpolar solutes with different functional groups in the SC-CO_2 varies as their polarizabilities. As the number of carbon atoms on the dialkyldithiocarbamate nitrogen is increased, the net polarity of the ligand is decreased, owing to an increase in its hydrocarbon character. Solubility can also be modified by the presence of functional groups capable of specific interactions with solvent molecules. It is noted that the solubility of a given compound in supercritical fluids can be influenced appreciably by the vapor pressure of the solute. The melting points of substance in SF can be depressed significantly. The

Table 2-3. Solubilities of Zn-dithiocarbamate complexes in SC-CO_2 at 24.05 MPa and 55 °C.

Zinc chelate	**Solubility**[a] **μg/mL (SCF)**	**Ratio**
$Zn[SC(S)N(C_4H_9)_2]_2$	248.4	
		20 (Zn-DBDTC/Zn-DEDTC)
$Zn[SC(S)N(C_2H_5)_2]_2$	11.9	
		139 (Zn-DBDTC/Zn-PDTC)
$Zn[SC(S)N(C_4H_8)]_2$	1.8	

[a] Following 30 min static equilibration at 55 °C and 24.05 MPa, Zn complex was purged from the saturation vessel during 20 min using the 11 x 0.025 mm i.d. restrictor.

Table 2-4. Solubilities of Zn complexes in SC-CO_2 at 24.05 MPa and 55 °C and 2 flow rates.

Zinc chelate	**Fraction**	**Solubility** **μg/mL(SCF)/min**	**Average** **Solubility ± RSD**[a]
11 cm x 0.025 mm i.d. restrictor			
	1	0.093	
$Zn[SC(S)N(C_4H_8)]_2$	2	0.090	0.089 ± 5.3 %
	3	0.084	
	1	0.634	
$Zn[SC(S)N(C_2H_5)_2]_2$	2	0.623	0.59 ± 10.4 %
	3	0.522	
	1	13.20	
$Zn[SC(S)N(C_4H_9)_2]_2$	2	12.08	12.42 ± 5.4 %
	3	11.98	
9 cm x 0.025 mm i.d. restrictor			
	4	0.098	
$Zn[SC(S)N(C_4H_8)]_2$	5	0.090	0.092 ± 5.8%
	6	0.089	
	4	14.62	
$Zn[SC(S)N(C_4H_9)_2]_2$	5	12.07	12.95 ± 11.2 %
	6	12.15	

[a] Relative standard deviation

lower-melting point of ligands and their chelates is still critical for solute enhancement, volatility and solubility in SC-CO_2. The melting points of these metal chelates are presented schematically in Figure 2-4.

2.3 Recovery of Metal Cations (Cd, Zn, and Pb) from Aqueous Media with SC-CO_2

2.3.1 Extraction Procedures

The analytical-scale supercritical fluid extractor (Figure 2-1.), which was constructed in house, consisted of a compressor which delivered mobile phase to three stainless steel (ss) HPLC column assemblies which were connected in series. The initial column was filled with ss balls and acted as a temperature and pressure equilibration device (TPED). The second column assembly (saturation vessel, SV) was charged with complexing agent and served to saturate the mobile phase and the final column was the extraction vessel (EV). In operation, the 4.5 mL capacity EV was partially filled with an aqueous sample (3 mL) containing the analyte(s), the mobile phase was saturated with complexing agent for 20 min under static conditions (no net flow) and then the analyte(s) were chelated *in situ* and the product complexes were purged from the EV during a dynamic phase of the extraction. Whereas the operating pressure of the extractor was controlled by the compressor, the flow rate of the mobile phase was determined by the length and diameter of the silica capillary tube restrictor which vented the outflow from the extractor to atmospheric pressure. The tip of the capillary tube was immersed in liquid trapping solution. Analyte heavy metal (Cd, Zn, and Pb) which had been transported to the trapping solution or which remained in the aqueous phase was determined by FI-AAS. Sub-ng limits of detection (LODs) were obtained for each analyte metal and the AAS response was independent of the identity of the DTC

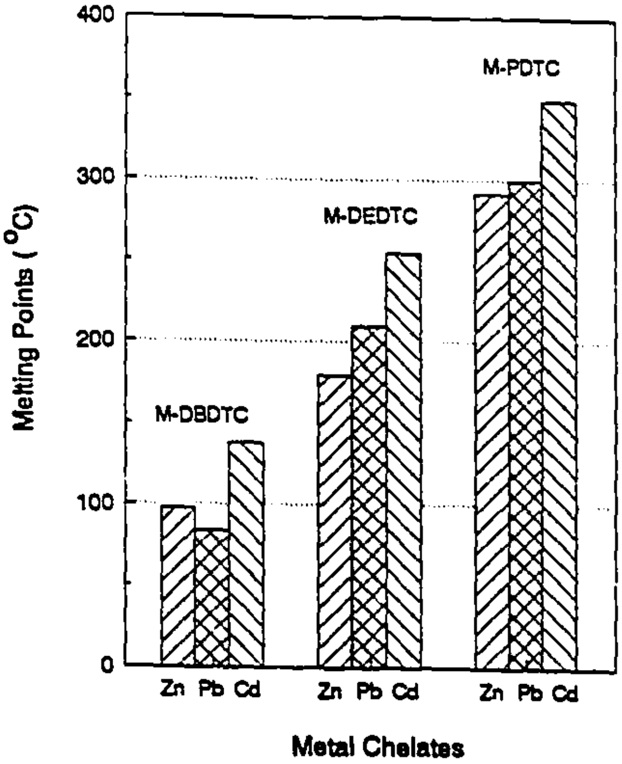


Figure 2-4. Melting points of zinc, lead and cadmium dibutyldithiocarbamate (DBDTC), diethyldithiocarbamate (DEDTC) or pyrolidinedithiocarbamate (PDTC).

ligand or the composition of water or methanol carrier solvent. Background correction proved to be unnecessary for these analytes in the flow injection mode.

2.3.2 Efficiency of Recovery

Two fundamental parameters in SFE are the efficiency of recovery (the proportion of the amount of solute extracted relative to amount of analyte initially present in the sample, typically expressed as a mass percentage) and the extraction rate (extraction recovery per unit time at a given flow velocity of the supercritical fluid through the extraction). The former parameter was chosen as the initial parameter to evaluate the experimental results.

A preliminary study of the dynamic purging of metal cations from aqueous solution involved the extraction of Cd^{2+}, Zn^{2+} or Pb^{2+} (10-20 µg/mL) added as soluble salts to distilled deionized water. $Bu_4NSC(S)NBu_2$ served as the ion pair source for the DTC ligand. Separate aliquots (3 mL) of the same sample added to a 4.5 mL capacity sample vessel were extracted for up to 60 min using a mobile phase flow rate which was four times greater than the studies above (0.050 mm i.d. restrictor vs 0.025 mm i.d. above). FI-AAS determination of the residual levels of analyte in the extracted sample indicated a rapid removal of the metals ($\geq$ 75% after 5 min and $\geq$ 94% within 15 min (declination curves of Figure 2-5). For these trials, quantitation was performed by both the method of external standards and/or by the method of standard additions. However, a portion of the volatilized metal was apparently "held up" within the extractor transfer lines. The corresponding cumulative recovery of Zn with time is presented in Figure 2-5 D. Typically, one of two different methods are used for collecting the analyte when the supercritical phase is decompressed; either a liquid or a solid phase trapping can be used. In this study two liquid traps were placed in series at the exit of the extractor. Over

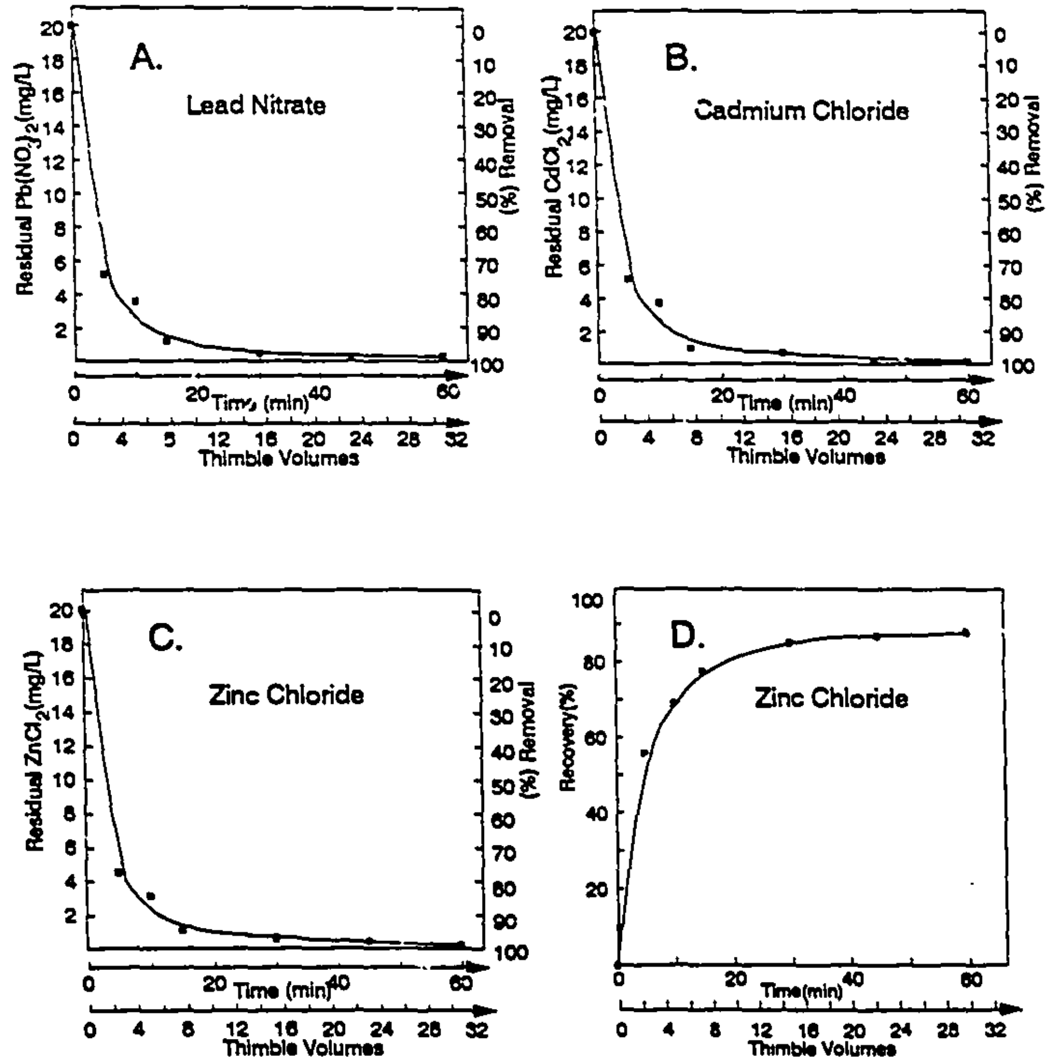


Figure 2-5. Declination curves with time (or exchange volumes of the head space) from solutions of A, lead; B, cadmium or C, zinc which had been added, as soluble salts, to distilled water. Curve D represents the corresponding cumulative recovery with time of zinc in the methanolic trapping solution. Extractions were performed at 50 °C and 24.05 MPa (CO_2 density, 0.825).

the course of the extraction, the cumulative quantity of Zn in the second trap never exceeded 2% of the quantity added initially to the sample. Moreover declination curves, when transformed to plots of the log of the fraction of analyte metal remaining ($[M]/[M_0]$) in the sample aliquot vs duration of extraction were distinctly biphasic. To the extent that an appreciable excess of complexing agent was anticipated to be present in the mobile phase entering the extraction vessel, pseudo-first order kinetics for the rate of depuration of metal analyte from the sample solution might have been anticipated. As suggested by the plots of Figure 2-6, which record the average of three replicate extraction for each data point, a biphasic response provides an appreciably better predictive model for the observed extraction efficiencies with time. In this Figure error bars represent the 95% confidence interval about the mean (± 1.96 relative standard deviations).

2.3.3 Insoluble Carbonate(s)

A possible explanation for the biphasic behavior was that a portion of the analyte metal was transposed to an insoluble carbonate salt during the extraction. To test this hypothesis, separate aliquots of the aqueous solutions (10-20 μg analyte metal/mL) were acidified to pH 2-3 with nitric acid and the extraction (up to 20 min duration) was performed using operating conditions which were identical to the operating conditions above. Appreciable increases in the apparent rates of analyte purging from aliquots of the acidified samples were evident (Figure 2-6). Greater than 95% removal of each metal analyte from the acidified samples was achieved within 10 min. However, as suggested by the plots of Figure 2-6, a biphasic model provided an appreciably better fit to the observed data even for the acidified samples. It must also be recognized that this model assumes that excess complexing agent is always present in the aqueous phase which cannot be true at time zero.

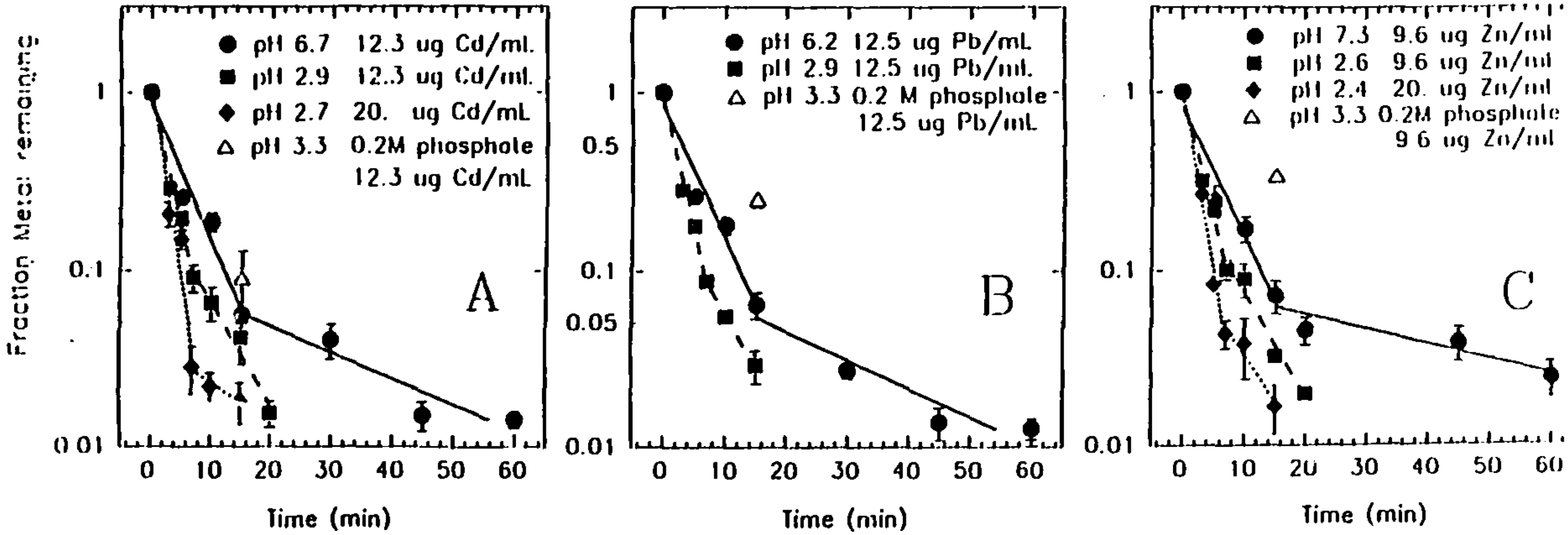


Figure 2-6. Logarithmic-linear plots of the fraction of metal $[M]/[M_0]$ remaining in the aqueous phase vs purging time for aqueous samples of **A**, cadmium; **B**, lead or **C**, zinc which had been dissolved in pH 3.3 phosphate buffer (hollow symbol) or acidified to various pHs prior to extraction. Error bars reflect the 95% confidence interval about the mean of three replicate extractions performed at 50 °C and 24.05 MPa (CO_2 density, 0.825).

To further assess the possibility of carbonate formation, the three-way valve was mounted between the saturation vessel (SV) and the equilibration device (TPED). With this design, the three-way valve could serve two purposes, (*i.*) introduction of SC-CO_2 into the extraction vessel for static equilibration at the chosen extraction operating conditions. and (*ii.*) redirection of the flow of SC-CO_2 to the saturation vessel so as to saturate the mobile phase with the dithiocarbamate reagent. Aliquots of the same aqueous Zn sample (9.6 μg/mL, initial pH 7.3) were presaturated with SC-CO_2 for 30 min then dynamically extracted for various periods of time using the same process operating conditions as before. The pretreatment with SC-CO_2 had no apparent effect on either the levels of residual Zn in the aqueous phase or on the rate of purging of analyte Zn from these solutions. However, this pretreatment accelerated the decomposition of the ion pair contained in the SC-CO_2 and resulted in intermittent blockage of the silica restrictor. The cause of the biphasic kinetic behavior remains to be identified. Given the paucity of data points and the experimental difficulties of generating appreciably greater numbers of data points, a more rigorous approach to modeling the kinetic data was not feasible with *off-line* experimental protocol.

2.3.4 Influence of Anion(s)

The apparent influence of anion(s) on the relative rates of purging prompted a further series of exploratory experiments. Soluble metal salt [($Zn(NO_3)_2$, $Cd(NO_3)_2$, or $Pb(NO_3)_2$] was added to a phosphate buffer (pH 3.3; ionic strength (I), 0.2M) to result in samples containing 9.6-12.5 μg metal/mL. Three aliquots of each solution were separately extracted for 15 min. The repeatability of the extraction as measured by the relative standard deviation (≤ 2%) associated with the residual analyte levels was excellent. The mean extraction efficiencies from the phosphate buffer (together with error bars representing the 95% confidence interval about the mean) have been added as hollow triangle symbols to each graph of Figure 2-6. Despite the lack of knowledge of the pH and the relative proportions of the various phosphate species present when CO_2 saturation of the aqueous matrix is achieved, it seems probable that at least a portion of the metal cation was transposed to an insoluble form. Clearly, the buffer constituents appreciably retarded the mobilization of analyte(s). None the less, more than two thirds of the analyte was purged from the aqueous matrix within 15 min.

In total, these results indicate that metal analytes can be rapidly and efficiently recovered from aqueous matrices by *in situ* chelation followed by dynamic mobilization of the resulting complex into SC-CO_2 and subsequent trapping. The influence of phosphate anionic species on the rates of metal analyte extraction suggests that by monitoring the changes in rates of mobilization with time it will be possible not only to quantify total levels of extractable analyte but also to distinguish between different analyte species based on their rates of transposition to an extractable form.

Chapter 3

Interfacing Techniques for Coupling SFE with *On-line* Detection by AAS

3.1 Analytical Methods Development

Many advances have been made over the past two to three decades in the extraction and detection of trace elements. One of the principal advantage of SFE is the possibility of coupling this extraction technique with *on-line* methods of detection. Because atomic spectroscopy especially atomic absorption has become the most widely used technique for trace metal detection, the objectives of the current study were to develop interfaces for coupling SF extraction with *on-line* detection by atomic absorption spectrometry (AAS). The availability of an *on-line* metal selective detection system for the SC-CO_2 extraction would (*i.*) appreciably simplify and accelerate the analysis procedures and (*ii.*) permit process optimization during the course of the extraction. By venting the extractor effluent to atmospheric pressure, the SC-CO_2 spontaneously reverts to a gas, which facilitates detector interfacing; however, the attendant adiabatic cooling can cause problems. An inherent difficulty with linear restrictors used to achieve a controlled depressurization is the possibility of reduced or interrupted flow due to plugging, which can occur when the mobile phase contains a high concentration of solute and/or water. Typically, these problems are circumvented by heating the restrictor or by maintaining the collection solvent at a constant temperature. The required detection system was to be capable of operation at conventional extractor flow rates and to provide sensitivities that were at least equivalent to or better than conventional flame AAS. In addition, the possibility of performing chemical speciation based on differences in the kinetic rates of purging was to be explored.

3.2 Materials and Methods

3.2.1 Quartz Tube Interface Design

A silica flame-in-tube interface was designed for the detection, by AAS, of As, Cd, Cu, Mn, Pb, Se, or Zn in supercritical fluid extractor eluate. The three-component all silica interface (Figure 3-1) consisted of (a) an optical tube [13 x 1.1 cm inner diameter (i.d.)], (b) a flame tube (3 x 0.8 cm i.d.) fitted with (d) gas entry ports (6 x 0.64 cm o.d., 0.4 cm i.d.) for O_2 and H_2, and (c) a sample introduction tube (10 x 0.64 cm o.d. 0.4 cm i.d.). The optical tube (a) was positioned within the optical beam of the AA spectrometer and heated with a 12-turn coil of 22-gauge Kanthal A-1 heating wire, 4- Ω/m. Oxygen or hydrogen was delivered to the appropriate gas inlet port of the flame tube via a flow controller (0-500 mL or 0-2 L capacity, respectively, Matheson, Whitby, ON) connected to (e) quartz insert tubes (8 x 0.16 cm o.d.) which extended concentrically inside the gas entry ports to within 1 cm of the center of the Y-shaped flame tube. The insert tube (e) was constricted to a 2-mm orifice at the exit end, positioned within the entry port with a modified Swagelok 0.64-0.32 reducing union and made gas-tight with Vespel ferrules. The fitting had been drilled out to permit the insert tube to pass through this fitting. Teflon tubing, which connected the flow controller with the appropriate gas inlet, was heat shrunk around the entry of the insert tube to produce a gas-tight seal. The sample introduction tube, which met the optical tube at an angle of 30 °C relative to the plane formed by the optical tube and flame tube assemblies, was gently heated with a 15-turn coil of the same heating wire. The heating coils (positioned around the optical and the sample introduction tubes) were separately energized by alternating currents rectified by variable transformers (Variacs). Extractor eluate contained in a 23 cm x 50 μm i.d. fused silica transfer line was vaporized by thermospray effect into a diffused flame maintained within

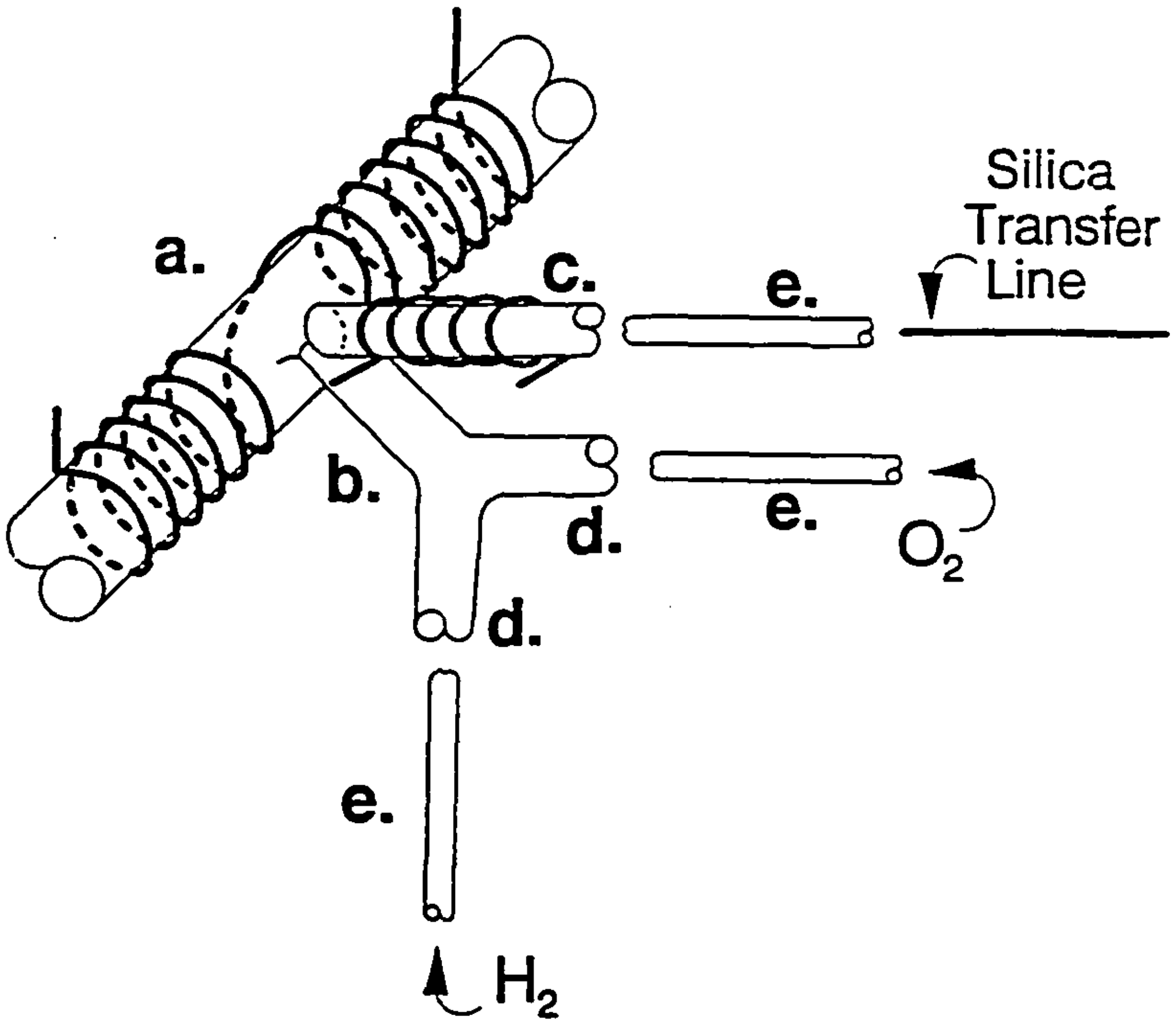


Figure 3-1. Silica T-tube interface (prototype 3) consisting of: (a.) an upper optical tube (13 x 1.1 cm i.d.) mounted within the optical beam of the spectrometer and heated with a 12-turn coil of high resistance heating wire; (b.) a flame tube (3 x 0.8 cm i.d.) fitted with O_2 and H_2 gas inlet ports [(d.) 6 x 0.64 cm o.d. (0.4 cm i.d.)], and (c.) a sample introduction tube [10 x 0.64 cm o.d. (0.4 cm i.d.)]. Quartz insert tubes (e., 8 x 0.32 cm o.d.), constricted to a 1 mm orifice at the exit end, were positioned within the gas entry ports and the sample introduction tube with modified Swagelok 0.64-0.32 reducing unions and made gas-tight with Vespel ferrules.

the upper region of the flame tube (b). The gaseous combustion products subsequently were entrained through the optical tube of the assembly.

To initiate operation of the interface, the optical tube and the sample introduction tubes were heated to their normal operating temperatures (with a direct current of 5 and 1.5 A, respectively). The flow of H_2 to the interface was increased to 700 mL/min, during which the gas autoignited within the optical tube. The flow of O_2 was increased slowly until it autoignited (typically at 50 mL/min) and then increased further to attain the normal operating flow rate. Visible flames emanating from both ends of the optical tube resulted in maximal response with the detection system. The capillary transfer line was extended into the sample introduction tube to a point just upstream from the optical tube, and the two-way flow-through valve from the EV was opened to permit the flow of eluate to the interface. To extinguish the diffused flame, the two-way flow-through valve from the extractor was closed, the capillary transfer line was withdrawn from the sample introduction tube, and the flow of O_2 to the interface was closed. Finally, the H_2 flow was interrupted and the interface was permitted to cool to room temperature.

Caution. The operator should be protected from possible injury caused by an explosion-induced shattering of the interface. The interface should be positioned directly below an efficient hood capable of venting the exit gases directly to the outside. The order of procedural steps for igniting and extinguishing the diffused flame must be followed.

3.2.2 Complexing Agent and Metal Chelates

Tetrabutylammonium dibutyldithiocarbamate and metal-DBDTC complexes were prepared as described in Chapter 2. Repeated crystallization from isooctane, benzene, or hexane diethyl ether furnished analytical standards.

3.2.3 FI-AAS

Residual analyte metal in the aqueous sample solution was quantified by flow injection atomic absorption spectrometric (FI-AAS) analyses of 10-50 μL aliquots injected into the aqueous carrier solvent. Quantitation was performed using the method of external standards and/or standard additions.

3.3 Results and Discussion

3.3.1 *On-line* SFE-AAS System

Supercritical fluid extraction with *off-line* quartz-tube atomic absorption spectrometry was used for the studies described in Chapter 2 to monitor the quantity of analyte metal in the SC-CO_2 extractor eluate. Generally, for *off-line* SFE, more attention has to be paid to such phenomena as aerosol formation and precipitation of the analyte in the restrictor which are common problems with *off-line* procedures. These phenomena can result in poor analyte recoveries for very volatile analytes because of the difficulties in simultaneously controlling the temperatures of both the restrictor and the trapping solution. Dilution of analyte can also be a serious problem with solvent trapping; the use of solid sorbents for either *on-line* or *off-line* modes of operation requires testing to define optimal conditions for both quantitative trapping and efficient elution of each analytes. Other components of the sample such as water can affect analyte trapping efficiencies adversely. In addition, to obtain an extraction recovery curve at specified extraction operating conditions (pressure, temperature, sample size), several samples have to be determined in order to define a single recovery curve. By contrast, with *on-line* detection variations in operating conditions for a single sample can often suffice to define optimal conditions.

A schematic diagram of the *on-line* SFE-AAS system assembled for this study is presented in Figure 3-2. The supercritical fluid extractor was essentially as described in Chapter 2 but with the exception that eluate was channeled via a 0.5 m x 0.16 mm (i.d.) stainless steel heated transfer line to a capillary fused silica restrictor (23 cm x 50 µm i.d., SGE, Houston, TX), which was positioned within the interface of the atomic absorption spectrometer (Philips Model PU 9100, equipped with a deuterium background correction system). Whereas pressure within the extractor was controlled by the compressor, the mobile phase flow rate was controlled by an appropriate choice of the length and inner diameter of the restrictor. The analog signal from the AAS was captured with a chromatographic data reduction software package (Turbochrome 3, Perken-Elmer Corp., Wilton CT). The distribution of chemical species at thermodynamic equilibrium were predicted using MINEQL^{+}, Version 3.0, (Environmental Research Software, Hallowell, MA, USA). In order to operate the SFE-AAS system successfully, the temperature and pressure decrease of the SC-CO_2 had to be kept to a minimum during the transfer of the extract from the extraction vessel to the quartz tube interface. The quartz-tube interface AAS system was set up and stabilized at the optimal operating parameters before the dynamic stage of the extraction was initiated.

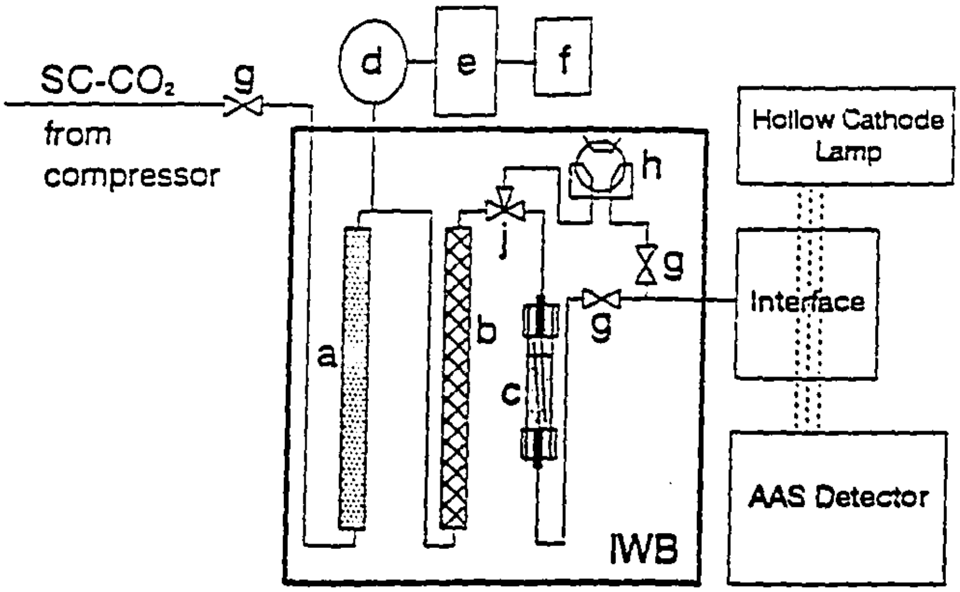


Figure 3-2. Supercritical fluid extractor consisting of: (**a.**) a temperature and pressure equilibration device (TPED); (**b.**), a saturation vessel (SV); and (**c.**), an extraction vessel (EV) immersed in an insulated water bath (IWB). Pressure was monitored with (**d.**) a pressure transducer mounted in series between the TPED and the SV and displayed on (**e.**) a digital pressure indicator powered via (**f.**), a power supply. Two way flow through valves (**g.**) positioned after the EV and after a six-port rotary injection valve (**h.**) in combination with a dual stem three way selection valve (**j.**) permitted the mobile phase to be directed to the EV or to the injection valve. Extractor eluate was channeled via a 30 cm x 0.05 mm i.d. silica transfer line to the interface mounted within the optical beam of the AA spectrometer.

3.3.2 Optimization of SFE-AAS Quartz Tube Interface

In order for an SFE-AAS method to be attractive, it should (*i.*) exhibit a high level of repeatability as measured by a low relative standard diviation (RSD), (*ii.*) respond rapidly and (*iii.*) be easy to control. The interface operating parameters were optimized in the current studies by installing a manual HPLC rotary injection valve (between the extraction vessel and the AAS interface) so as to be able to: (*i.*) calibrate the AAS detection signal by injecting standard directly into the mobile phase, (*ii.*) optimize the response of the detection system by modifying the decompressed CO_2 flow rate, or by changing gas flows and operating temperatures of the interface and (*iii.*) to estimate the limits of detection for different analyte(s) in a flow injection mode. The repeatability of the extraction (three replicates performed on separate aliquots of the sample) was also assessed. To study the capacity, various dilutions of the same standard solution were extracted and the changes in the AAS response with time were compared. To determine analyte recoveries, residual analyte in the extracted aliquots were determined by FI-AAS.

To optimize the detector response to analyte metals, different interface configurations were evaluated. Initial feasibility trials were conducted with a silica T-tube interface (prototype 1, Figure 3-3, Momplaisir *et al.*, 1994) composed of an upper optical tube and a lower sample introduction tube. The downstream portion of the sample introduction tube had been expanded to form a combustion chamber that housed a diffused flame supported by O_2 and H_2 which were added, via separate gas entry ports, to the base of the chamber. In operation, mobile phase was nebulized by a thermospray effect into the third entry port also located at the base of the chamber. For these studies, SC-CO_2 at 50 °C and 24.05 MPa was delivered from the extractor via the rotary injection valve. Despite the fact that the strategy of thermospraying analyte metal, in either an aqueous or

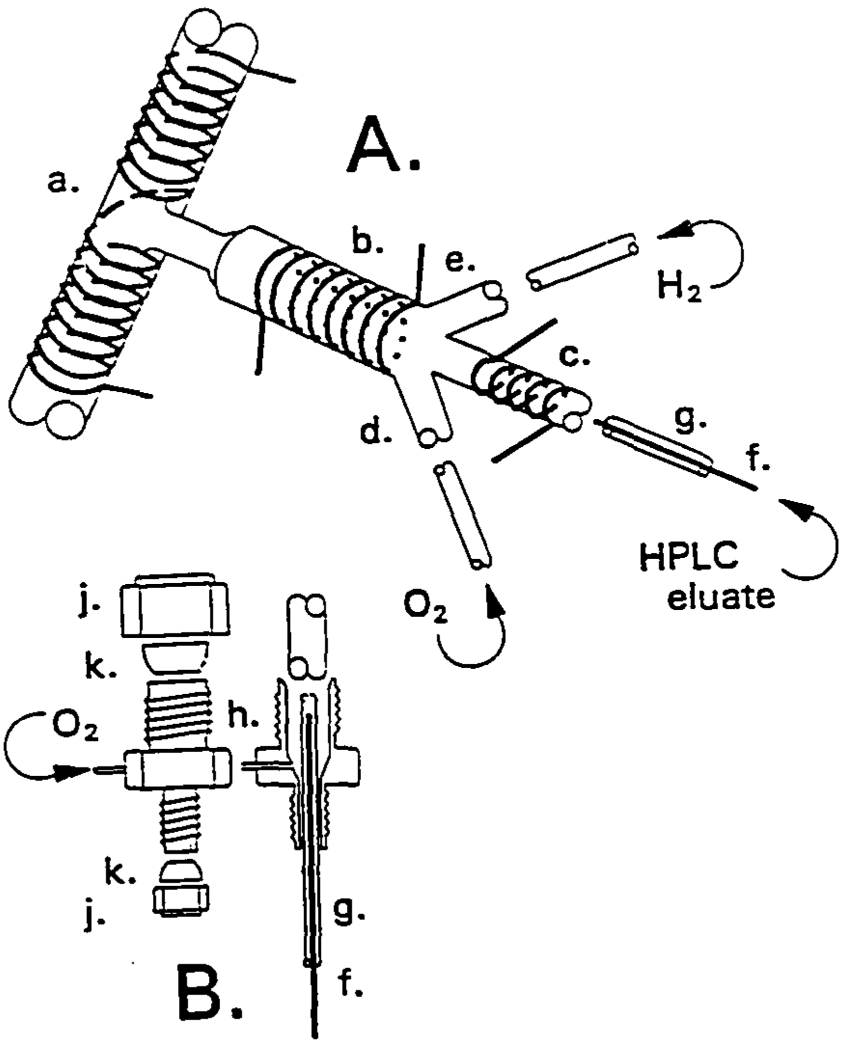


Figure 3-3. (A.) Silica T-tube interface (prototype 1) consisting of an optical tube (a.) and a lower sample tube containing a pyrolysis chamber (b.) surrounded by a 10-turn coil of high-resistance heating wire; gas inlet ports for O_2 (d.) and H_2 (e.); a fused silica capillary transfer line (f.); and a 0.32-cm o.d. guide tube (g.). (B.) Close up and cross sectional views of the 0.64-0.32 stainless steel reducing union (h.) which was made gas-tight with ferrules (k.) and nuts (j.) and modified to accept a 0.16 o.d. stainless steel tube which served as the entry port for supplementry O_2 (Momplaisir *et al.*, 1994. A somewhat similar design has also been described by High *et al.*, 1992.).

methanolic mobile phase, into the diffused hydrogen/oxygen flame had resulted in sensitive responses to six analyte elements (Tan *et al.*, 1994), this design had only a limited tolerance for CO_2. For flow rates of decompressed CO_2 corresponding to greater than 13.74 MPa extractor operating pressure, it was not possible to maintain a flame within the interface. To increase the pressure operating range of the detection system, several design modifications were evaluated. These modifications included increasing the inner diameters of both the optical tube and combustion chamber from 0.9 to 1.7 cm and moving the O_2 and H_2 gas entry ports from the base to new positions on opposite sides of the chamber and located approximately midway between the entrance and exit of the combustion chamber. With this new design (prototype 2, Figure 3-4), only the region of the combustion chamber between the thermospray tube and the gas entry ports was heated. Although the prototype 2 design supported the requisite flow rates of decompressed CO_2, the principal shortcoming proved to the limited range of analyte metals that could be detected with sufficient sensitivity to be useful for characterizing the metal burdens of biological/environmental samples. Only cadmium- or zinc- DTC proved to be sufficiently volatile to provide sensitive responses. In addition, the prototype 2 interface was not compatible with either aqueous or methanolic liquid mobile phases.

3.3.3 Calibration and Limits of Detection (LODs)

To calibrate and determine the limits of detection, an alternate strategy for introducing mobile phase to the interface was evaluated. The major interface modification (prototype 3, Figure 3-1) involved the introduction of the superheated SC-CO_2 extractor eluate into the upper region of the diffused flame rather than thermospraying liquid eluate into the base of the flame. In operation, extractor eluate contained in a 30 cm x 50 μm i.d. silica transfer line was

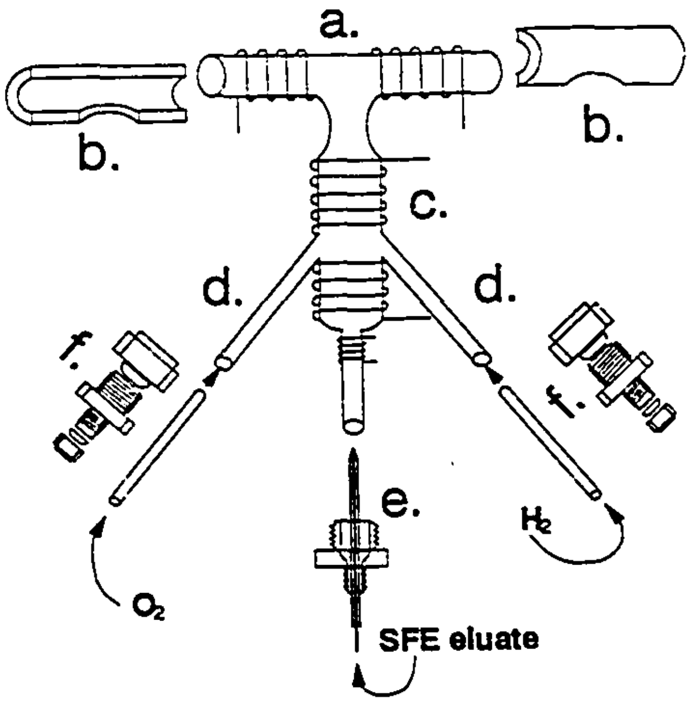


Figure 3-4. Silica T-tube interface (prototype 2) consisting of: (**a.**) an upper optical tube (13 x 1.5 cm i.d.) mounted within the optical beam of the spectrometer and heated with a 12-turn coil of high resistance heating wire; (**b.**) shaped firebrick insulation; (**c.**) a pyrolysis chamber surrounded by a 9-turn coil of high-resistances heating wire; (**d.**) gas inlet ports for O_2 and H_2; (**e.**) a fused silica capillary transfer line (25 cm x 0.05 mm i.d.); and (**f.**) a 0.64-0.32 stainless steel reducing union which was made gas-tight with ferrules.

superheated and thermosprayed into the tip of a diffused flame maintained within the upper region of the flame tube and in the optical tube. Whereas the operating pressure of the extractor was maintained by the compressor, the flow rate of mobile phase was controlled by the length and inner diameter of the silica transfer line. Typical flow rates of decompressed mobile phase at the exit of the extractor were in the range of 600-800 mL/min (corresponding to 0.5-0.7 mL/min compressed fluid). The displacement of the sample introduction tube from the base to the tip of the combustion chamber appreciably improved the sensitivity of the detection process to zinc or cadmium. As well, the new design permitted the detection of number of analyte metals that could not be detected efficiently with the previous prototype design. The detector response (peak area) to seven elements entrained by flow injection into an aqueous, methanolic, or SC-CO_2 mobile phase was maximized by optimizing the flow rates of H_2 and O_2 to the flame tube using of a univariate optimization procedure. Whereas soluble nitrate salts or acidified solutions of selenate or arsenite served as calibration standards for the aqueous mobile phase, metal-dibutyldithiocarbamate (M-$DBDTC_2$) complexes were used for either the methanolic or SC-CO_2 mobile phase. Optimized interface operating parameters and the estimated limits of detection (LODs) for these analyte elements are presented in Table 3-1.

For the aqueous mobile phase, a maximum response to the analyte metal was obtained with slightly reducing atmospheres (mean ratio for 7 analyte metals, H_2/O_2: 3.2 ± 25%). However, the optimized ratios of gas flow rates for this interface design were considerably less than the optimum ratio for As or Se (12 and 32 respectively) or the mean ratio for the maximum response to Cd, Cu, Pb or Zn (4.9 ± 15%) observed for the previous interface design (prototype 1.). By contrast, optimized gas flow rate ratios for the maximum response to these

Table 3-1. Estimated limits of detection[a] (LODs) for the AAS determination of analyte element in different mobile phases

		H_2O			CH_3OH			SC-CO_2[b]		
Analyte[c]	λ(nm)	Optimized flow rate (mL/min)		LOD (pmol)	Optimized flow rate (mL/min)		LOD (pmol)	Optimized flow rate (mL/min)		LOD (pmol)
		H_2	O_2		H_2	O_2		H_2	O_2	
As	193.7	1050	460	5.42						
Cd	228.8	1450	325	0.14	210	245	0.04	1010	455	0.13
Cu	324.7	2200	645	0.96	220	525	0.73	1460	525	2.83
Mn	279.5	2200	685	2.00	150	585	1.91			
Pb	217.0	1350	485	0.27	540	325	0.28			
Se	196.0	1050	460	9.31						
Zn	213.9	1250	325	0.17	150	285	0.13	1105	455	0.46

[a] Limit of detection for FIA-AAS were estimated as 3 (peak to peak baseline noise)/slope of the calibration plot for standards.

[b] Operating conditions were 50 °C and 24.05 MPa with a decompressed CO_2 flow rate of 500 mL/min.

[c] Analytical standards were nitrate salts, H_3AsO_4 or H_2SeO_4 in H_2O mobile phase or M-DBDTC standard in a methanolic or SC-CO_2 mobile phase.

analyte-dithiocarbamate complexes in the methanolic mobile phase were distinctly oxidizing (H_2/O_2, 0.74 ± 75%) and only slightly reducing (2.5 ± 12%) for the SC-CO_2 mobile phase.

In all cases, the corresponding limits of detection of these analyte metals ranged from subnanogram to low picogram. The flow injection of Zn, Cu, Pb, Mn, Cd, Se or As in each of the mobile phases are presented in Figure 3-5, 3-6, 3-7. Whereas the injection of mobile phase resulted in no detectable instrumental response for either the water or methanol mobile phase, the injection of methanol into the SC-CO_2 phase resulted in a minor blank signal equivalent to approximately 20% of the lowest quantity of standard injected into this carrier. The use of the deuterium background correction system appreciably degraded the performance of the detection system (increased background noise) and was not used for these studies. For the SC-CO_2 mobile phase, the flow injection of methanolic standards of either Mn-DBDTC or Pb-DBDTC at levels corresponding to sub-microgram quantities of analyte metal did not result in any detector response. By contrast, the mobilization of 30 µg of either Pb or Mn from distilled water (3 mL) by complexation with DBDTC and mobilization into SC-CO_2 (28.86 MPa, 50 °C) did produce an appreciable detector response (Figure 3-8). A possible explanation for this phenomena is that methanol somehow accelerated the formation of analyte metal oxide(s).

The repeatability of the extraction process was tested by performing three replicate extractions on 3-mL aliquots from solutions containing 5 µg of Cu/mL, 10 µg of Pb/mL or 10 µg of Mn/mL. The AAS declination curves corresponding to the three replicate extractions of separate aliquots of the Cu solution are presented in Figure 3-9. For these trials, no static pre-equilibration of the mobile phase with

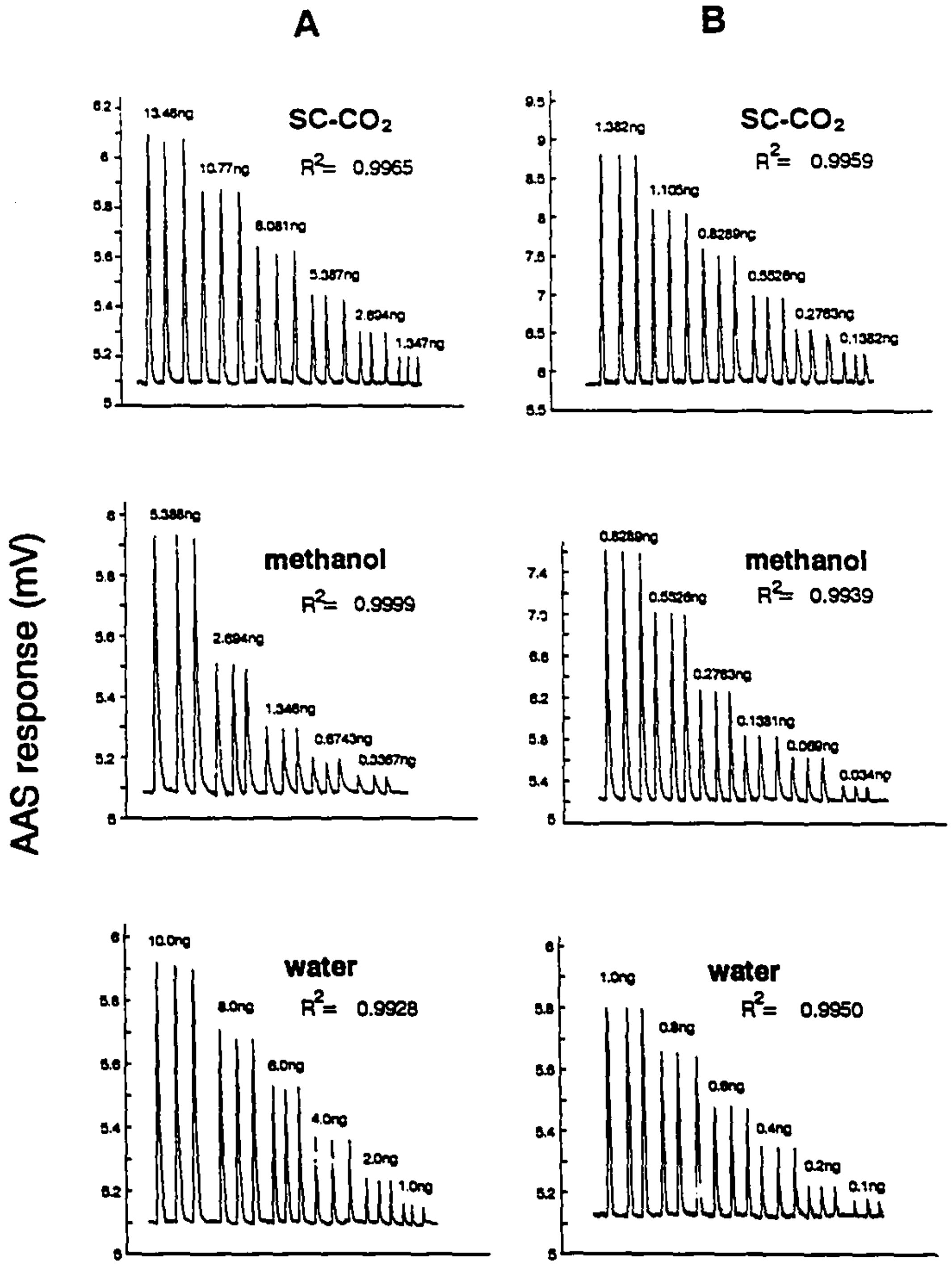


Figure 3-5. Typical AAS responses for the flow injection of various quantities (as metal analyte) of Cu (A.) or Zn (B.) standard into a SC-CO_2, methanolic, or an aqueous mobile phase.

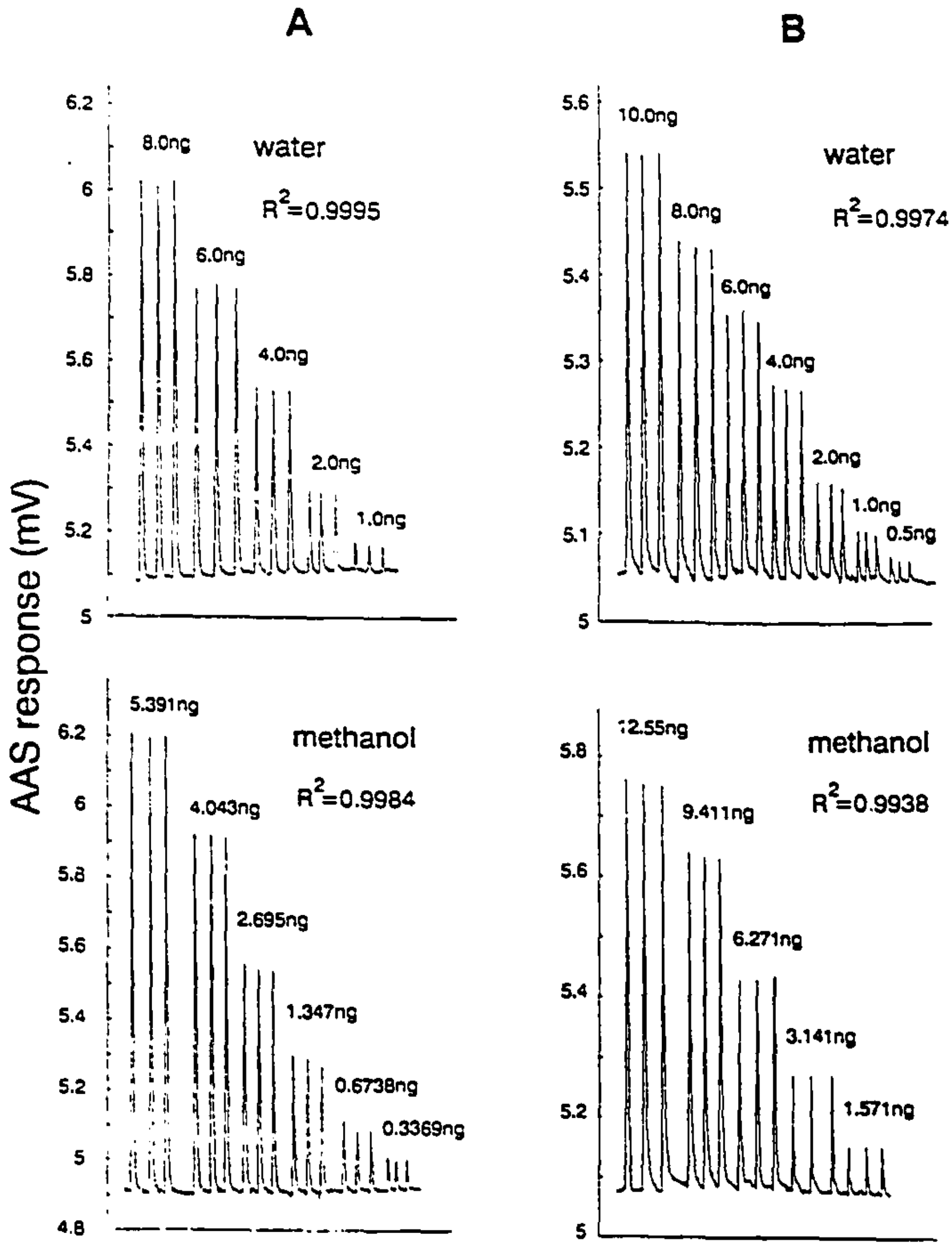


Figure 3-6. Typical AAS responses for the flow injection of various quantities (as metal analyte) of Pb (A.) or Mn (B.) standard into a methanolic, or an aqueous mobile phase.

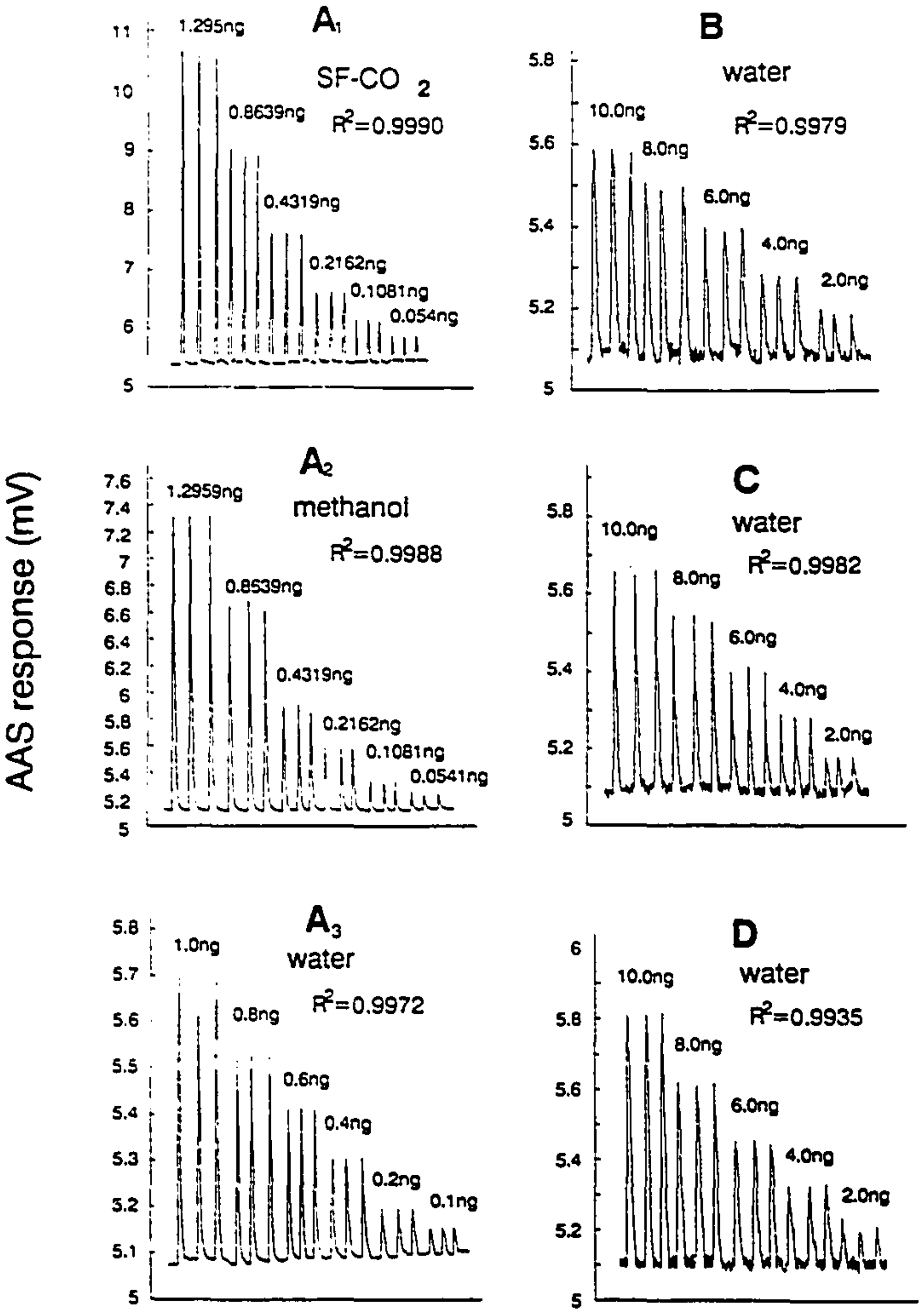


Figure 3-7. Typical AAS responses for the flow injection of various quantities (as metal analyte) of Cd (A_1, A_2 or A_3), Se (IV) (**B.**), Se (VI) (**C.**), or As (III) (**D.**) standard into a SC-CO_2, methanolic, or an aqueous mobile phase.

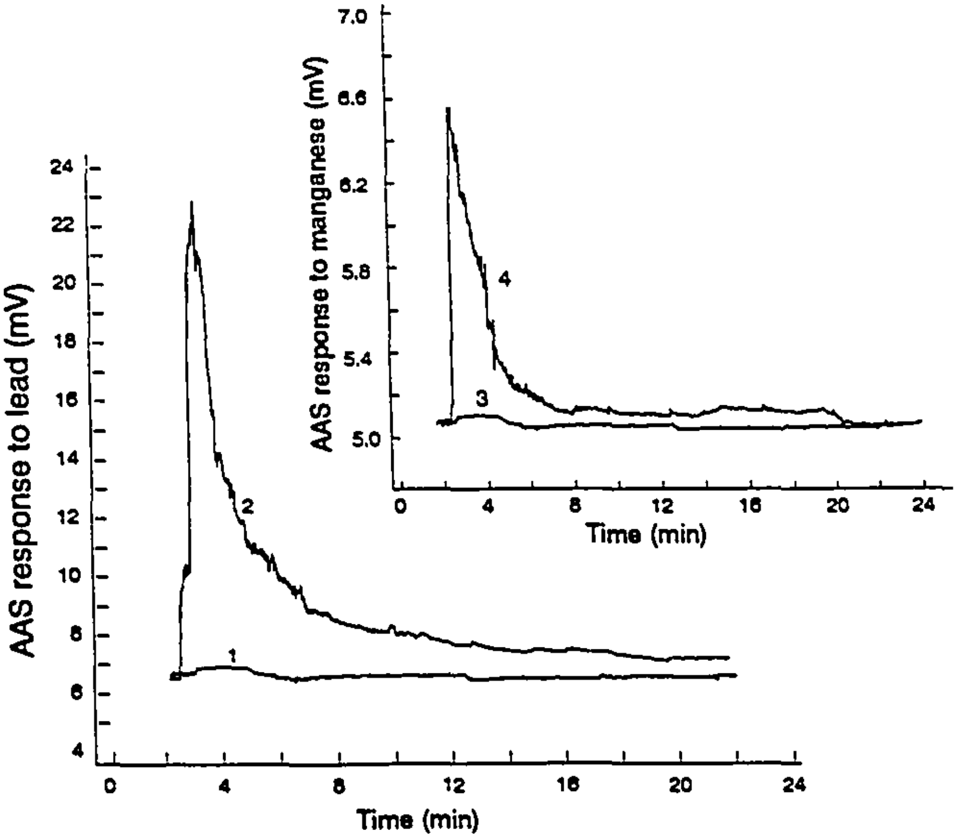


Figure 3-8. AAS response curves for the complexometric extraction into SC-CO_2 of 3 mL aqueous solution containing 30 µg (as metal analyte) of either $Pb(NO_3)_2$ or $Mn(NO_3)_2$. Extractions were performed at 28.86 MPa and 50 °C without pre-saturation of the mobile phase with complexing reagent. Curves 1 and 3 represent AAS signal in the absence of complexing ligand.

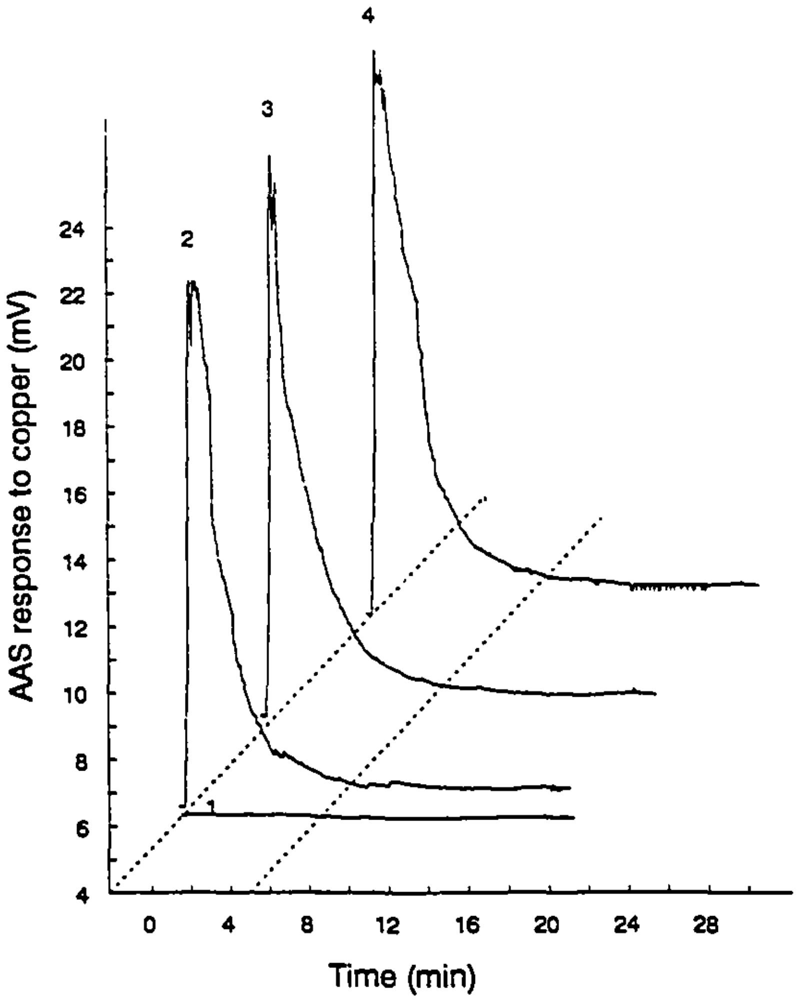


Figure 3-9. AAS response curves for the three replicate complexometric extractions into SC-CO_2 of 3 mL aqueous solution containing 10 µg/mL (as metal cation) of $Cu(NO_3)_2$. Extractions were performed at 28.86 MPa and 50 °C without pre-saturation of the mobile phase with complexing reagent. Curve 1 represent AAS signal in the absence of complexing ligand.

the complexing reagent was performed - rather the analyte was purged from the sample using mobile phase that had dissolved complexing agent as it passed through the saturation vessel. These extractions were performed for 40 min at 27.48 (Cu) or 28.86 MPa (Mn, Pb) and 50 °C. At the termination of each trial, the residual quantity of analyte metal in the aqueous aliquot was determined by FIA with quantitation by standard additions and by the method of external standards. The results were identical using both calibration techniques. As recorded in Table 3-2, the extractions were both very efficient for all three metals (>92% removal) and highly repeatable (~1% RSD). The cumulative area under the AAS response curve for 40 min of extraction however was appreciably more variable (±12.1, ±15.8 and ±14.2% RSD for Cu, Mn and Pb respectively). A portion of this variation was apparently caused by longer term instrumental drift in that the variability in the AAS signal (in terms of cumulative area) increased modestly with longer extraction times. Additionally, the AAS signal typically did not return to values for mobile phase in the absence of pairing ion. For this reason, the mathematical models (section 3.3.5) that were used in these studies included a term (Y_o) to compensate for the contribution of complexing reagent in the mobile phase to the AAS response. Variability was further explored by extracting aqueous solutions containing 0.1-3.0 μg of Cd as $Cd(NO_3)_2$ (Figure 3-10). The extractor and interface operating conditions and the resulting efficiencies of extraction are summarized in Table 3-3. Despite a modest change in the shape of the AAS response as well as a modest decrease in the efficiency of the process with increasing metal loading in the aqueous sample, the cumulative area under the AAS signal was highly correlated with the concentrations of Cd in the sample solutions (for extraction performed with the same interface operating parameters). As reported in Table 3-3, R^2 for the linear regression of the optimized model of

Table 3-2. Figures of merit for the dynamic complexometric extraction into SC-CO_2 of copper, manganese or lead from aqueous solution containing 5 or 10 μg/mL of analyte metal cation.

Analyte	**Conc. (μg/mL)**	**% RSD for the cumulative peak area**			**% Removal**[a]
		10 min	**20 min**	**40 min**	
Cu	5	± 10.2	± 11.3	± 12.1	92.0± 0.008
Mn	10	± 10.9	± 12.2	± 15.8	93.6± 0.01
Pb	10	± 10.1	± 11.5	± 14.2	94.9± 0.01

[a] Percent removal was calculated from three replicate determinations of the residual levels of analyte in the aqueous sample as determined by FI-AAS.

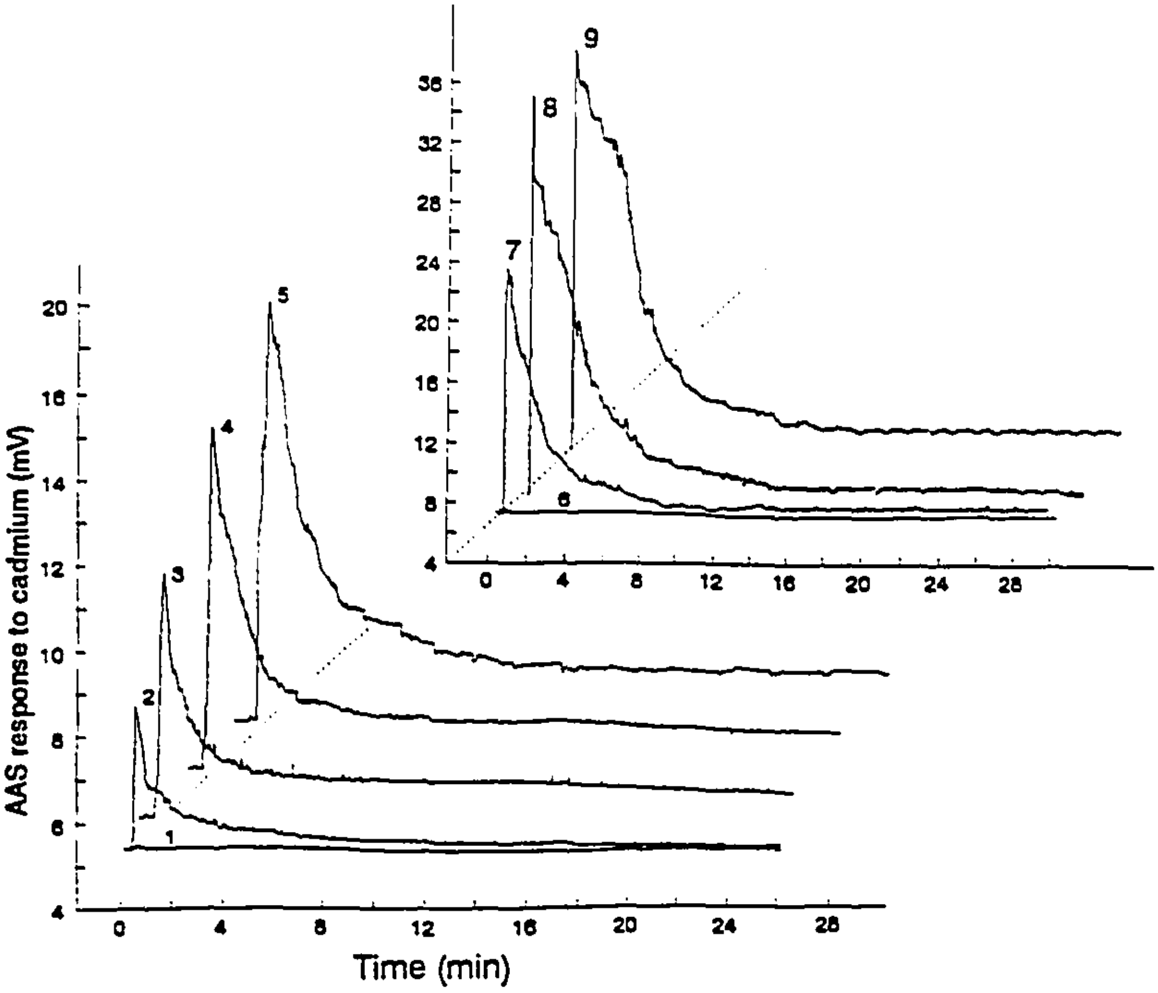


Figure 3-10. Variation in the AAS profile with $Cd(NO_3)_2$ loading in the aqueous sample. Extractions of 3 mL aliquots were performed at 27.48 (curves 1-5) or 25.42 MPa (curves 6-9) and 50 °C with gas flow rates to the detector of 450 and 1250 (curves 1-5) or 220 and 987 mL/min (curves 6-9) of O_2 and H_2 respectively.

Table 3-3. Purging of cadmium cation from aqueous solution into SC-CO_2 by complexometric extraction with TBADBDTC.

Solution number[a]	[Cd^{2+}] (µg/mL)	Extraction conditions[b] and duration (psi/°C/min.)	AAS support gases (mL/min)		% Analyte removal[c]	R^2 [d]
			O_2	H_2		
1	0.10	3,500/50/25	525	700	98.9	
2	0.20	3,500/50/25	525	700	97.1	
3	0.30	3,500/50/25	525	700	95.3	
4	0.40	3,500/50/25	525	700	94.4	0.9803
5	1.0	3,700/50/30	120	987	93.1	
6	2.0	3,700/50/30	120	987	92.8	
7	3.0	3,700/50/30	120	987	92.1	0.9893

[a] Solution numbers are identical to the AAS response curves of Fig. 3-10.

[b] Solutions were extracted dynamically without prior pre-saturation of the mobile phase with the complexing agent.

[c] Percent removal was calculated from the residual levels of Cd^{2+} in the aqueous sample as determined by FI-AAS.

[d] For the linear regression of the area under the modeled AAS response curve $[(Y-Y_0) = A_1 e^{(-x/t1)} + A_2 e^{(-x/t2)}]$ on $[Cd^2]$.

the AAS response on [Cd^{2+}] for four or for three standard solutions extracted under identical operating conditions was 0.9801 and 0.9893, respectively.

3.3.4 Further investigation of Metal Carbonate(s) with SFE-AAS System

It is possible that a side-reaction can take place during the extraction process in which the analyte metal is transposed to its corresponding carbonate or bicarbonate salt. The sparingly soluble salt might be precipitated, in part, under the operating conditions of the extraction. The precipitated portion of the analyte could be transposed back to the non-polar dithiocarbamate complex but at a rate which would be controlled by the rate of dissolution of solid phase analyte. These reactions can be summarized by following scheme:

$$CO_2 + H_2O \rightleftharpoons H_2CO_{3(aq)}$$

$$H_2CO_{3\,(aq)} \rightleftharpoons HCO_3^- + H^+$$

$$HCO_3^- \rightleftharpoons CO_3^{2-} + H^+$$

$$M^{2+} + CO_3^{2-} \rightleftharpoons MCO_{3\,(s)}$$

$$MCO_{3\,(s)} \rightleftharpoons M^{2+} + CO_3^{2-} \rightleftharpoons M(DTC)_2$$

From *off line* SFE experiments (Chapter 2), it was postulated that the bi-phasic response might have been the result of some of the metal being converted to its corresponding carbonate. However, this suggestion was not corroborated with *off line* experiments. To determine whether any appreciable proportion of the M^{2+} in distilled deionized water was converted to the corresponding carbonate during the extraction process, the *on-line* SFE-AAS system was used. One possible way to test this hypothesis was to pre-saturate the aqueous sample with CO_2 for a time longer than the dynamic extraction. After equilibration, the system was to be vented to atmospheric, then the sample was to be subjected to a complexemetric extraction. The kinetics of the dynamic extraction were then directly compared with the kinetics of extraction for a second fresh aliquot of the sample which had not been pre-equilibrated with CO_2.

There was no evidence for any perceptible formation of insoluble Cu product(s) over the course of either extraction. By contrast, relative to the 30 min. complexometric extraction of a 3 mL aliquot of a Zn^{2+} solution (20 μg/mL) without pre-treatment, the pre-equilibration of a separate aliquot of the same solution with SC-CO_2 for 40 min appreciably modified the form of the AAS response curves (Figure 3-11) and of the cumulative recovery curve observed for the subsequent 30 min complexometric extraction of this sample (Figure 3-12). This behaviour was consistent with an extensive transposition of Zn^{2+} to a product (presumably $ZnCO_3$) which was mobilised from the sample at an appreciably slower rate.

Equilibrium models of the Zn-species distributions for $Zn(NO_3)_2$ solutions (1.0-100 x 10^{-4} mol/L) maintained at 50 °C under CO_2 atmospheres between 10.35-44.87 MPa, predicted the absence of any Zn-precipitate(s). The principal Zn-species in solution were predicted to be Zn^{2+} (83% decreasing to 71 %) and $ZnHCO_3^+$ which collectively account for greater than 99% of the total metal. The saturation index for $ZnCO_3 \bullet H_2O(s)$ and for Smithsonite actually decreased modestly ($\approx$ 20%) with increasing pressure over this range. This behaviour is not surprising given the predicted changes in dissolved CO_2 species with increasing pressure. As presented in Table 3-4, in the absence of metal ion(s), the pH and $pHCO_3^-$ of an aqueous medium is predicted to decrease whereas the $[H_2CO_3]$ is increased more than 10-fold and $[CO_3^{2-}]$ remains essentially invariant over this pressure range. Thus, the postulated transposition of soluble Zn species to an insoluble carbonate during equilibration with SC-CO_2 under conventional extractor operating conditions is not supported by this equilibrium model. Interestingly, analogous models for the variations in species

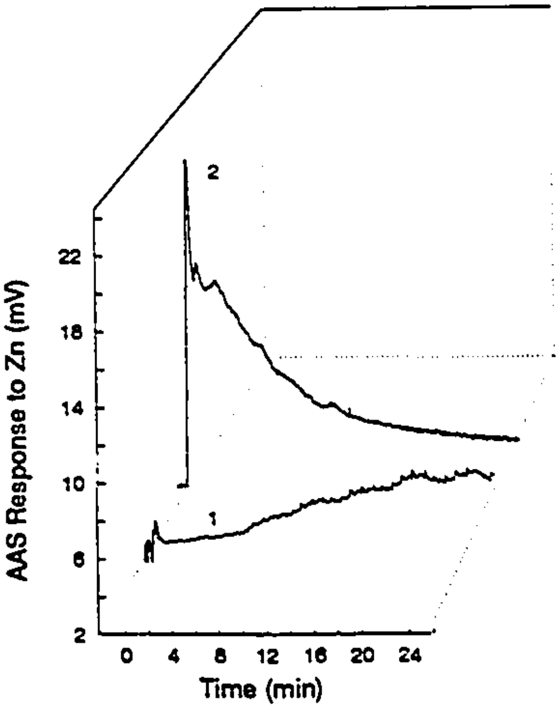


Figure 3-11. AAS response curves for the extraction of zinc from separate aliquots of an aqueous solution (1.) which had been pre-saturated with SC-CO_2 for 40 min, then complexometrically for 30 min or (2.) extracted complexometrically for 30 min without pre-equilibration. Extraction were performed at 24.45 MPa and 50 °C.

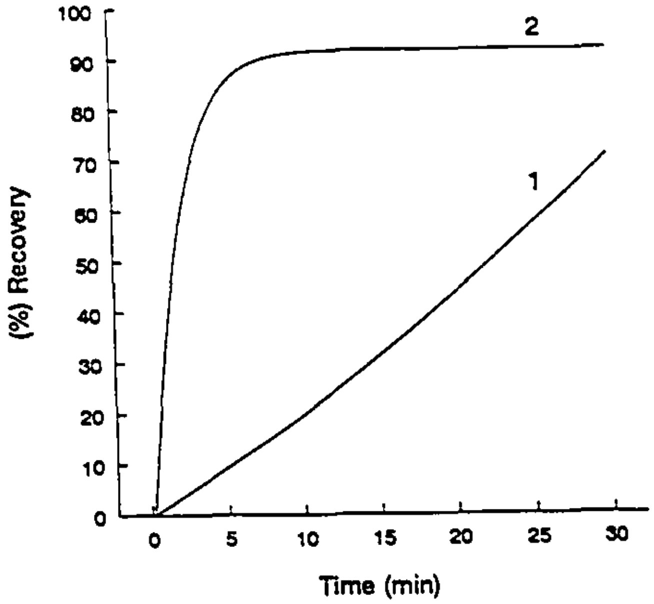


Figure 3-12. Cumulative recovery of Zn from separate aliquots of the Zn-solution (1.) which had been pre-saturated with SC-CO_2 for 40 min, then extracted complexometrically for 30 min or (2.) subjected to a complexometric extraction 30 min without any pre-equilibration. These cumulative recovery curves were generated by integrating the corresponding AAS response curves of Figure 3-11.

Table 3-4. Predicted[a] species concentrations for CO_2, H_2O and copper or zinc nitrates at 50 °C and various extractor operating pressures.

Pressure (psi)	**pK_{CO_2} [b]**	**pH**	**$pHCO_3^-$**	**pCO_3^{2-}**	**pOH**	**$[H_2CO_3]$**
Distilled Water						
6,500	15.53	2.69	2.69	10.12	10.55	11.30
5,500	15.60	2.72	2.72	10.12	10.52	9.63
4,500	15.69	2.76	2.77	10.14	10.48	7.83
3,500	15.80	2.82	2.82	10.12	10.42	6.08
2,500	15.95	2.90	2.90	10.12	10.34	4.30
1,500	16.62	3.23	3.24	10.13	10.02	0.92

Pressure (psi)	**pK_{CO_2} [b]**	**pH**	**$pHCO_3^-$**	**pM^{2+}**	**$pMHCO_3^+$**	**$pMCO_{3\,(aq)}$**
		$Zn(NO_3)_2$, 1.5×10^{-4} M, 50 °C				
3,500	15.80	2.82	2.83	3.93	4.48	8.76
		$Cu(NO_3)_2$, 2.4×10^{-3} M, 50 °C				
3,500	15.80	3.79	4.22	2.64	3.98	6.47

[a] Calculations were performed using the data base contained in MINEQL$^+$ (Ver 3.0).

[b] $pK_{CO_2} = -\log (K_H \times K_1 \times K_2 \times P_{CO_2})$, estimated using a Henry's Law constant = $10^{-1.5}$, $K_{2H_2CO_3} = 10^{-10.33}$, $K_{1H_2CO_3} = 10^{-6.35}$, partial pressure CO_2 (atm).

distributions of $Cu(NO_3)_2$ solutions (4.8 μmol/L) over the same pCO_2 range predicted the absence of any Cu-precipitate(s).

To corroborate the hypothesis that precipitation was involved, a separate Zn solution (150 μg/mL) was pre-equilibrated with SC-CO_2 for 40 min (24.12 MPa and 50 °C) then decompressed to atmospheric pressure after which the sample possessed a milky white turbidity. If the extraction vessel was resealed and the turbid sample re-equilibrated with mobile phase for a further 24 h (under the same operating conditions) the sample clarified and provided extraction kinetics which were indistinguishable from the kinetics for an aliquot of the same sample which had not been pre-equilibrated. Since there was no provision for stirring the aqueous sample during the extraction, it was not possible to study the rate of the CO_2 equilibration process in depth. It seems probable that, with sufficient stirring, the precipitation phenomenon would be minimized. Interestingly, the metal analyte remaining in the residual solution (40 min static equilibration followed by complexometric extraction of 30 min) only gave the FI-AAS response when it was acidified with nitric acid. This is a further indication that the residual zinc was present as $ZnCO_3$. For Cu, the kinetics of extraction suggested only a single mobilization mechanism, and no apparent difference between the mobilization kinetics of Cu from fresh or pre-equilibrated sample.

3.3.5 Kinetic Analysis for the Simultaneous Mobilization of Different Forms of the Analyte Metal by SFE-AAS

3.3.5.1 Theoretical Considerations

It was postulated that the variations, with time, of the AAS detector response to analyte metal in the SC-CO_2 extractor eluate might furnish additional information concerning the interactions (if any) of that metal with the sample matrix. The avidity of the interaction(s) of the analyte with the sample matrix might tend to make the metal less available to the complexing ligand and thereby decrease its net rate of mobilization/purging from the extractor. Conventional approaches to the determination of the separate physical and chemical forms of an analyte element (a process termed chemical speciation) within a complicated natural matrix has been to separate and isolate each of these "forms" prior to their determination. An alternate approach to speciation is based on the premise that the individual components of a mixture of analyte metal species which cannot be mobilized into SC-CO_2 directly, might be fractionated based on the relative rates at which separate components are transposed to a form which can react with the complexing ligand. Thus, this approach attempts to distinguish species on the basis of differences in their rates of mobilization into SC-CO_2. The objectives of this study were to investigate the kinetics for the dynamic extraction of analyte metal(s) into SC-CO_2 and to develop a kinetic model for this process. The extraction rates were to be estimated from the rates at which the AAS response curve declined with time.

In exploratory studies, SC-CO_2 mobile phase was saturated with dibutyldithiocarbamate complexing agent in the form of a non-polar ion pair (tetrabutylammonium DBDTC) prior to passage through the sample dissolved/suspended in an aqueous medium. The head space above the sample was continuously purged with fresh mobile phase and the effluent from the extractor was monitored *on line* by atomic absorption spectrometry (AAS). For aqueous standards containing Zn, Cd, Cu, Pb, or Mn at low to sub-µg/ml concentrations complexometric extractions into SC-CO_2 had been demonstrated to be rapid and very efficient. These studies also demonstrated that under the extractor operating conditions (*i.*) mobile phase SC-CO_2 rapidly became saturated with the ion pair reagent and (*ii.*) a sufficiently large excess of the reagent (relative to the concentration of analyte metal) was present throughout the extraction to permit the assumption that the concentration of complexing agent remained constant. As a working model for the process (Figure 3-13), it was assumed that analyte metal (M) present in aqueous solution or slurry in various physical and/or chemical forms (M_i) are in equilibrium with free metal cation (M^{n+}) or an equivalent form which is available for reaction with the complexing ligand. Free metal cation is in equilibrium with non-polar complex $\{M(DBDTC]_{n(aq)}\}$ in the aqueous phase which, in turn is in equilibrium with complex in the SC-CO_2 phase $\{M(DBDTC)_n\ sc\text{-}co_2\}$. In this Figure, k_i and k_{-i} represent rate constants (in the forward and reverse directions respectively) associated with the equilibrium K_i. Provided that there is an appreciable excess of complexing ligand, and provided that the rates of M^{n+} complexation, transfer of the resulting complex to the SC-CO_2 phase and exchange of the head space gas are also fast, then the rate determining step(s) will be the rate(s) of conversion from M_i to a form (M^{n+}) which can react with the complexing agent. Provided that these assumptions are valid, a pseudo first-order model is predicted to adequately describe the kinetics of the extraction process. It might then be possible to discriminate between different chemical species M_i of analyte metal M based on their relative rates of mobilization/purging from the sample. In short, this approach attempts to describe the kinetics of the extraction in terms of a ligand competition model and would only be potentially applicable to those systems in which the rate(s) of conversion from M_i to $M(DBDTC)_n$ is the rate limiting step of the process.

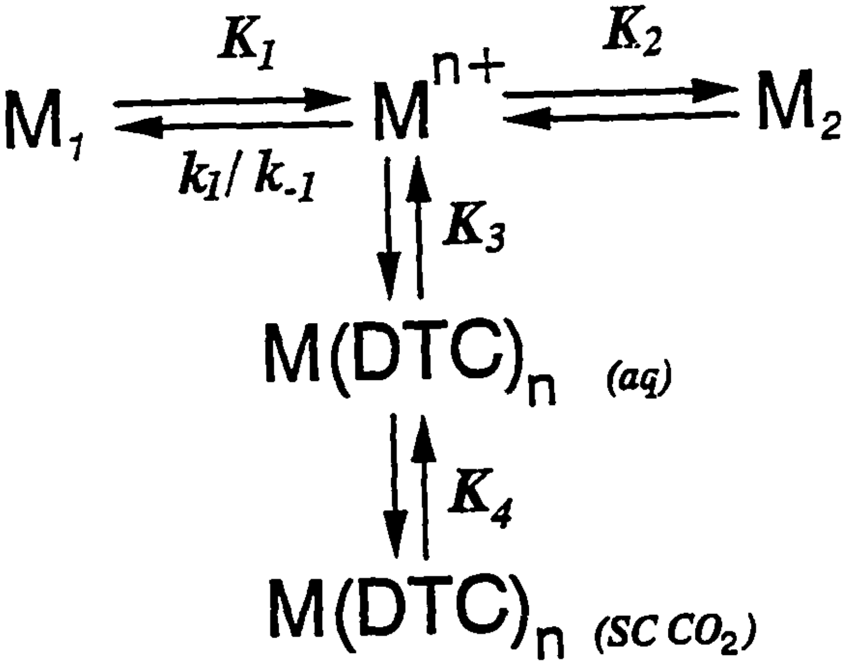


Figure 3-13. Model for the *in situ* sequestering of the different chemical species (M_i) of analyte metal M in aqueous medium by dithiocarbamate (DTC) complexing ligand followed by transfer of the product complex $M(DTC)_n$ to the SC-CO$_2$ phase and purging from the extractor by rapid exchange of the head space above the reaction medium.

3.3.5.2 Kinetic Analysis for Aqueous Solution

Aqueous solutions containing zinc, copper or cadmium were extracted to demonstrate the feasibility of using SFE-AAS (prototype 2) to characterize samples with respect to their metal burdens. Following 20 min of static equilibration at 24.05 MPa, 3 mL of aqueous solution [20 µg of Cd/mL as $Cd(NO_3)_2$] contained in the 4.6 mL capacity EV was extracted for 20 min with a $SC\text{-}CO_2$ saturated with TBADBDTC (decompressed flow rate, 800 mL/min). The change in AAS response with time is presented in Figure 3-14. Earlier results had demonstrated that, under the extractor operating conditions, (*i.*) the mobile phase rapidly became saturated with the complexing reagent and (*ii.*) a large excess of this reagent (relative to the quantity of analyte metal) was present in the mobile phase during the entire extraction.

The decay portion of the AAS response with time was modeled using a single-exponential decay or the sums of either two- or three-exponential decay functions. Using χ^2 as a parameter of the goodness of fit of the model to the data, the sum of two exponential decay functions provided an appreciably better fit to the data than either the single or the combination of three decay functions (Table 3-5). However, there was no significant lack of fit for any of the models to the data. Flow injection analysis of the levels of residual Cd in the extracted sample indicated greater than 94% removal after 30 min extraction (Table 3-5). These results corroborated earlier observations that the extraction process can be made to be very efficient. Analogous results (Table 3-5) were obtained when 3 mL of $Zn(NO_3)_2$ or $Cu(NO_3)_2$ in distilled water (12 µg of Zn or 5 µg of Cu/mL) was extracted at 50 °C and 24.05 or 27.48 MPa, respectively. Detection of Cu in the extractor eluate was performed using a modified interface (prototype 3) to nebulize/atomize this analyte.

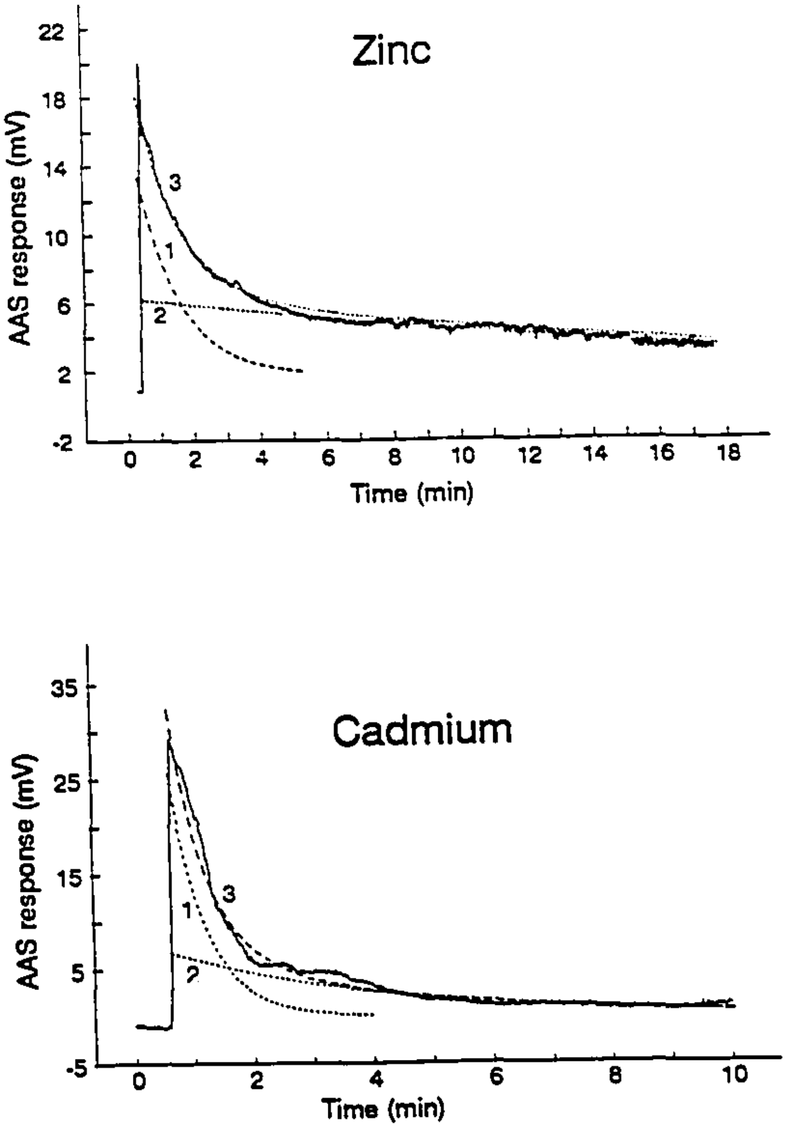


Figure 3-14. AAS response curves for the SC-CO_2 complexometric extraction of 12 µg/mL $Zn(NO_3)_2$ or 20 µg/mL $Cd(NO_3)_2$ performed at 24.05 MPa and 50 °C for 25 min. The modeled declination portion of the response curve (3) was decomposed mathematically into two component exponential decay curves (1 and 2).

Table 3-5. Kinetic models, apparent percent composition and efficiency for the SC-CO_2 complexometric extraction of cadmium, copper or zinc from aqueous solution.

Analyte	Model[a]	t_1	t_2	t_3	χ^2	% Components[b]			% Removal[c]
						1	2	3	
Cd[d]	one	0.63			3.018	100			
	sum of two	0.04	2.55		0.644	2.5	97.5		94.4
	sum of three	0.51	3.05	10.9	1.631	23.5	52.0	24.5	
Cu[d]	one	4.49			1.349	100			
	sum of two	0.49	2.18		0.039	4.7	95.3		91.9
	sum of three	1.29	2.80	55.7	0.050	30.5	48.6	20.9	
Zn[d]	one	1.92			0.201	100			
	sum of two	1.26	27.3		0.057	15.0	85.0		92.1
	sum of three	1.05	2.20	15.2	0.064	15.9	10.7	73.4	

[a] Responses were modeled as one, or the sum of two or the sum of three exponential decay curves.

[b] The apparent fractional composition was estimated by extrapolating the declination portion of the AAS response curve back to a maximum and assumed an identical response for equivalent quantities of different analyte "chemical species".

[c] Efficiency was determined from the residual levels of analyte in the aqueous sample after 30 min extraction.

[d] Whereas the detection of Cd or Zn was performed with the prototype 2 interface the detection of Cu was performed with the prototype 3 interface .

The apparent rate constants that result from this fitting process can be interpreted to represent operationally dependent baseline values and can serve as a basis of comparison with slower extractions of other analyte metal species. It is to be recognized that the ability to successfully distinguish between kinetically distinct processes (to mathematically decompose an overall detector response curve into its components) will depend on the differences in the magnitude of the rate constants for the processes and on the proportion of the total analyte that undergoes each process. That 97.5 and 95.3% of the purged Cd and Cu in these extractions were best fitted to a single kinetic process is gratifying. The zinc results are somewhat more difficult to rationalize in that only 85% of the substrate analyte was fitted by the dominant process. None the less, after 4 half-lives of the faster process 3.5 min, when the contribution zinc mobilization from this faster process will be less than 1% (6.25% x 15%), the molar fraction of analyte remaining in the aqueous sample (m/m_o) is predicted to be 0.75 for the slower process. A maximum of less than 40% will have been mobilized by this time. Interestingly, a somewhat analogous mathematical expression (the sum of exponential decay curves) results from a model (hot ball model, Bartle *et al.*, 1992) for SF extraction which predicts the changes in rate of solute recovery in terms of the size of the host matrix particles and the diffusivity of the solute or solvent through the host particles (unspecified process which can be approximated by a diffusion controlled process). For a single solute, this model predicts that a plot of $\ln (m/m_o)$ vs time will be curvilinear initially, becoming linear during the latter stages of the extraction. It is postulated that the slower of the two kinetically distinct mobilization/purging processes reflects the mobilization of zinc from zinc carbonate.

3.4 *On-line* Coupling SFE to Flame-AAS

3.4.1 Flame Nebulizer Assembly Modification

Earlier in this Chapter (Section 3.3) an all quartz T-tube interface was described for the detection of heavy metal analyte in SC-CO_2 eluate. Despite sub-ng to low pg limits of detection for As, Cd, Cu, Mn, Pb, Se and Zn, the prospects of extending the range of analytes to other elements did not seem promising with this somewhat energy limited device. Although this device was compatible with conventional flow rates of analytical-scale SC-CO_2 extractions, scale up to larger flow rates associated with bench scale operation was also anticipated to be difficult. An alternate approach to *on line* detection would be to attempt to modify the spray chamber of a conventional flame-AAS instrument to provide efficient nebulization/volatilization of the extractor eluate. An exploded view of the modified nebulizer assembly of the Zeiss FDM-3 AAS is presented in Figure 3-15. For these studies background correction was not employed. The nebulizer assembly - spray chamber was modified to accept the 50 μm i.d. silica capillary restrictor. The demountable glass impact bead of the nebulizer chamber was removed and the nebulizing jet of the spray nozzle was replaced with a 20 cm length of hollow needle (0.15 mm o.d. X 0.10 mm i.d., Figure 3-15), which extended some 10 cm into the all Teflon spray chamber. This stainless steel guide tube served to position the exit tip of silica restrictor within the nebulizer. Extractor eluate, within the capillary restrictor, was superheated by positioning the exposed portion of the silica restrictor tube - guide tube assembly inside a 20-turn heating coil (20-guage Kantal-A high resistance heating wire coiled around a 4 cm x 0.8 cm o.d. silica tube). The coil was jacketed with insulating Fibrefax and encased within two sections of shaped firebrick which were held together with a screw clamp. Air, which was transferred from the gas control module of the instrument to the nebulizing jet assembly (Figure 3-15) and separately to the spray

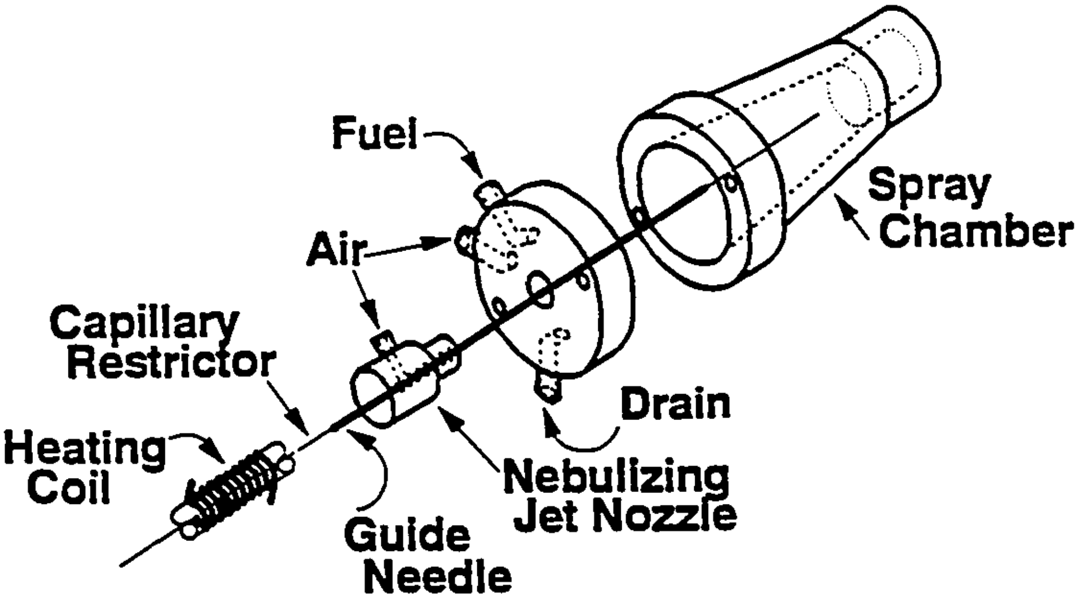


Figure 3-15. Exploded view of the modified AAS nebulizing assembly in which a 20 cm x 0.1 mm i.d. stainless steel hollow needle served to position the silica capillary restrictor within the spray chamber . An insulated heating coil superheated the mobile phase within the restrictor causing efficient volatilization by thermospray effect, into the spray chamber. Air flows to both the nebulizing nozzle and directly to the spray chamber were heated to 200 °C (an identical approach was described by Larsen and Blais, 1993).

chamber via 6.4 mm o.d. x 3.2 mm i.d. high temperature flexible silicone tubing (rated to 250 °C, Cole-Parmer Instrument Co., Niles, IL, USA), was pre-heated be passing the separate lines of silicone tubing through radiant heaters (CRFC-756/60 vacuum formed ceramic fiber radiant heater 15.24 cm x 7.62 cm o.d. x 0.63 i.d., OMEGA Technologies Company, Que., Canada). All three heaters were separately energized by direct currents from variable transformers (Variac). Studies of a heated restrictor tip proved to be the basis for the successful detection by flame-AAS of Cu-pyrollidine dithiocarbamate which was flow injected into a SC-CO_2 carrier stream (Bysouth and Tyson, 1992). Supplemental heating of the air entering the nebulizer assembly coupled with carrier phase volatilization, by thermospray effect, provided a low ng LODs to Cd, Cu or Pb in an aqueous carrier (Larsen and Blais, 1993). The AAS responses to analyte metal in the mobile phase was recorded with a personal computer and a chromatographic capture and data reduction program (Turbochrome 3, Perkin Elmer Corp., Wilton, CT).

3.4.2 Interfacing with Supercritical CO_2 Flowing Streams

By venting the extractor eluate to atmospheric pressure, the SC-CO_2 spontaneously reverts to a gas which appreciably facilitates detector interfacing. However, the attendant adiabatic cooling can cause problems including reduced flow and/or intermittent plugging of the capillary restrictor especially if the mobile phase contains appreciable quantities of water. These problems have been circumvented by heating the restrictor or by maintaining the collection solvent at a constant temperature.

3.4.3 Calibration and Limits of Detection (LODs)

For the current flame-AAS interface design, SC-CO_2 extractor eluate was nebulized, by thermospray effect, into a mixture of fuel and oxidant gases within the spray chamber of the nebulizing assembly. As anticipated, preliminary experiments indicated that additional inputs of thermal energy by heating the air streams to both the nebulizing nozzle and to the spray chamber increased the AAS signal to analyte complexes without perceptibly changing the background noise. de Galan (1981) has demonstrated that when sufficient energy is supplied to the nebulizing assembly to assure complete analyte vaporization, there is no requirement for an aerosol to physically transport the analyte to the detector. The gas flow configuration of the instrument permitted the total flows of acetylene and air to be varied as well as the proportion of air directed to either the nebulizing nozzle or the spray chamber. Somewhat arbitrarily, the voltages to the ceramic heaters were adjusted (≈ 35 V) to provide air flows at 200 °C. Subsequently, the system response (peak height or peak area) to analyte element entrained by flow injection into an aqueous (0.6 mL/min), methanolic (0.6 mL/min), or SC-CO_2 carrier phase (631 mL/min, measured as decompressed gas flow) was maximized by optimizing the flow rates of air and acetylene to the nebulizing assembly. Whereas metal-dibutyldithiocarbamate (M-$DBDTC_2$) complex were used for either the methanolic or SC-CO_2 mobile phase, aqueous cation was used for the aqueous carrier phase. Optimized interface operating parameters and the estimated limits of detection (LODs) for some analyte elements are presented in Table 3-6. For volatile elements such as Cu or Cd, the LOD for flow injected analyte was in the low ng range.

For the SC-CO_2 mobile phase, maximum responses to the flow injected analyte metals were obtained at lower thermospray operating temperatures (270-350 °C) than for either the methanolic (650-770 °C) or the water mobile phase (950-1,000 °C). At optimal settings, typical responses for the FI of analyte metal, entrained in SC-CO_2, are presented in Figure 3-16, 3-17 and 3-18. These results indicated

Table 3-6. Estimated Limits of detection[a] (LODs) for the flame-AAS detection of analyte element which had been injected into SC-CO_2, methanolic or an aqueous carrier phase.

Analyte[b]	Wavelength (nm)	Air 1[c] (L/min)	Air 2[d] (L/min)	Thermospray T (°C)	Estimated LOD (ng)
		SC-CO_2[e] mobile phase			
Cu	324.7	6.0	6.5	270	2.2
Zn	213.9	6.1	6.4	280	5.5
Cd	228.8	5.5	6.1	275	1.0
Mn	279.5	6.5	7.1	320	17.8
Fe	248.3	7.2	7.4	350	22.6
		MeOH mobile phase			
Cu	324.7	6.2	6.9	667	1.7
Zn	213.9	5.8	6.3	740	4.6
Cd	228.8	5.4	6.8	650	1.1
Mn	279.5	6.9	7.5	750	10.4
Cr	357.9	7.2	7.9	770	28.5
		H_2O mobile phase			
Cu	324.7	6.0	7.1	1000	2.5
Zn	213.9	6.6	7.3	980	3.8
Cd	228.8	6.3	7.0	950	1.6
Mn	279.5	6.6	7.9	1000	24.5
Cr	357.9	7.5	8.1	1000	32.1

[a] Limit of detection for FIA-AAS were estimated as three (peak to peak baseline noise)/slope of the calibration plot for standards.

[b] Analytes were AAS standards diluted in H_2O mobile phase or M-$DBDTC_2$ standard in methanol (methanolic or SC-CO_2 mobile phase).

[c] Air stream directed to the nebulizer nozzle.

[d] Air stream directed to the spray chamber.

[e] Operating conditions were 50 °C, 24.12 MPa and a decompressed CO_2 flow rate of 631 mL/min.

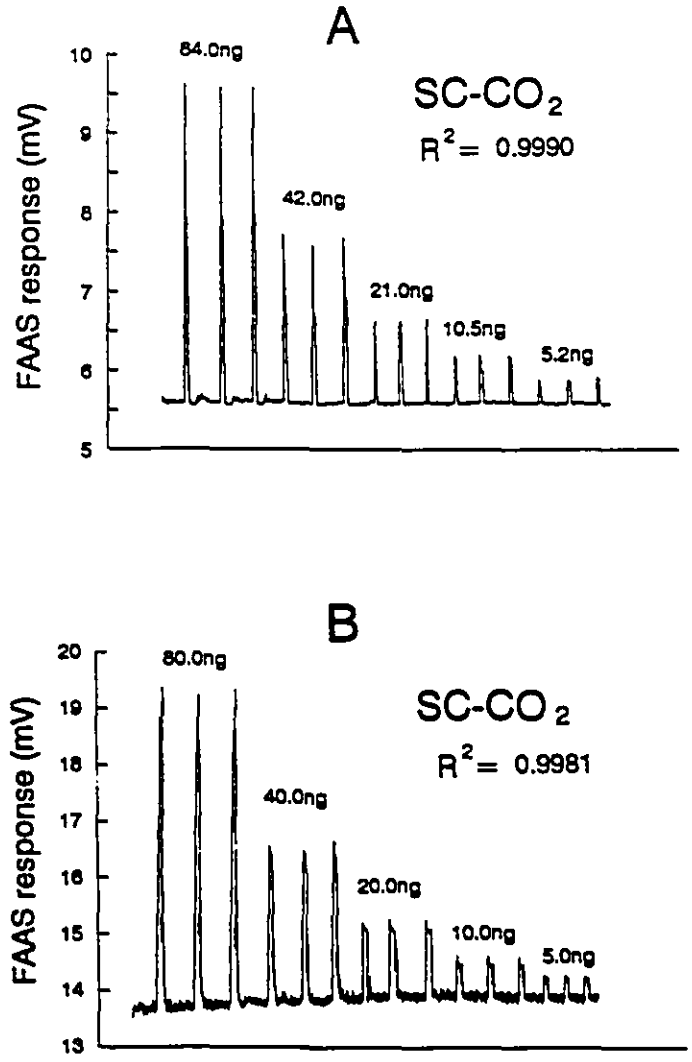


Figure 3-16. Typical Flame-AAS responses to methanolic solutions of (**A.**) Cu- or (**B.**) Zn-dibutyldithiocarbamate complex which had been flow injected into SC-CO_2.

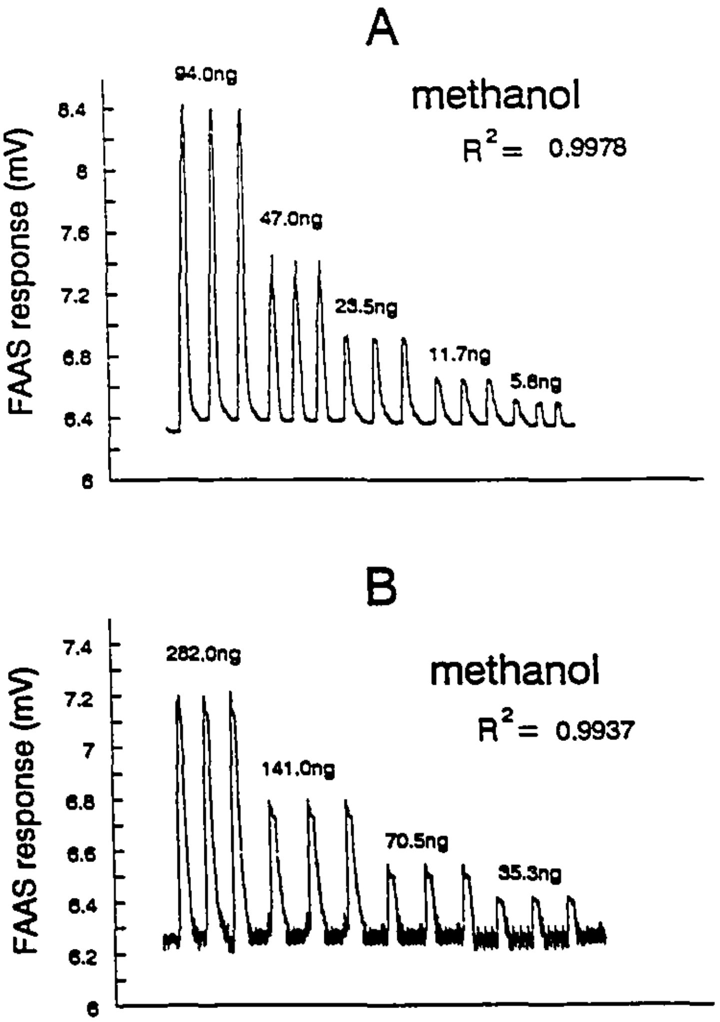


Figure 3-17. Typical Flame-AAS responses to methanolic solutions of (**A.**) Cu- or (**B.**) Cr-dibutyldithiocarbamate complex which had been flow injected into a methanolic mobile phase.

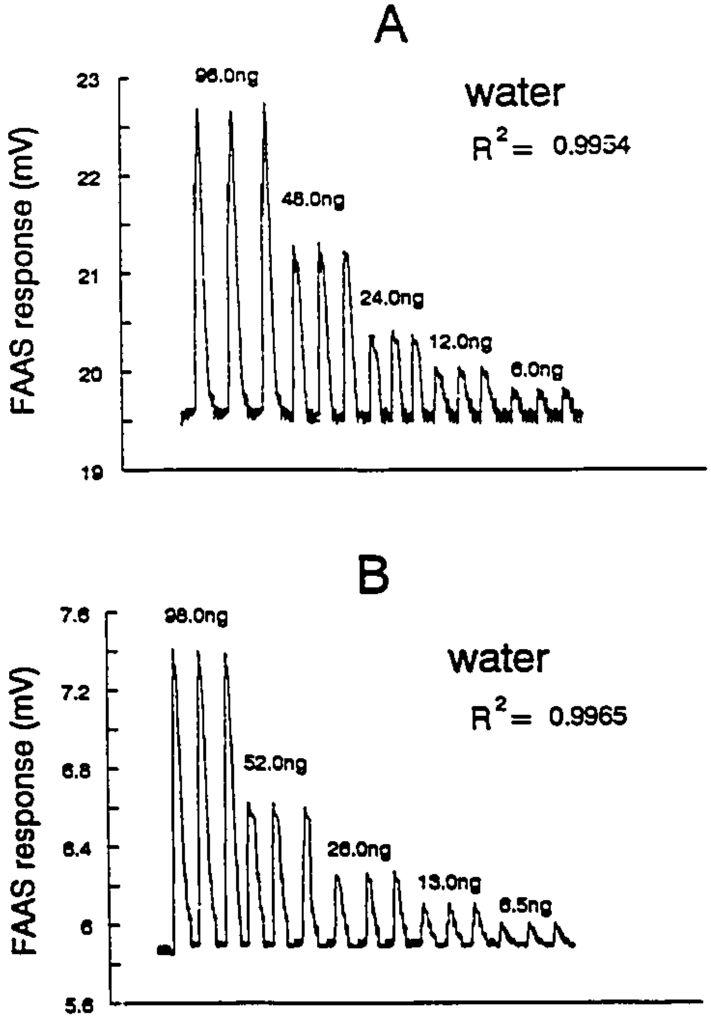


Figure 3-18. Typical Flame-AAS responses to aqueous solution of (**A.**) Zn(II) or (**B.**) Cd(II) standard which had been flow injected into a water mobile phase.

approximately a 10-fold improvement in response over the conventional sample aspiration mode of operation.

In summary, the all silica T-tube interface for coupling flowing streams with detection by AAS provided sensitive responses to seven analyte metals in SC-CO_2, methanol or aqueous mobile phases. In addition, this *on line* detection device permitted the declination of the AAS response with time to be mathematically modeled so as to obtain quantitative estimates of the rate(s) of mobilization/purging of component chemical species of the analyte metal. However, for practical purposes, this T-tube device was limited to the detection of the seven relatively volatile analyte elements and to flow rates of decompressed SC-CO_2 corresponding to ≤ 1 L/min. An alternate approach to interfacing involved the modification of the nebulizer assembly of a conventional flame-AAS. A combination of efficient analyte volatilization, by thermospray effect, and supplemental heating of the air oxidant gas to the nebulizing assembly resulted not only in low nanogram limits of detection for volatile elements but also provided sensitive responses to other analyte elements including chromium and ion. In subsequent chapters, the applications of these devices as dedicated detectors for supercritical extractions are described.

Chapter 4

The Influence of Natural Complexants on the Mobilization/Purging of Copper from Aqueous Media into Supercritical CO_2

4.1 Application SFE-AAS to "real world" samples

Increasing environmental concerns require that novel characterization/remediation technologies be developed/optimized for the characterization and efficient removal of heavy metal toxicants from processing wastes and contaminated environmental media. To date, there are relatively few remediation procedures which can be optimized for natural matrices (soils, sediments, or waste waters) which have been contaminated with both organic and heavy metal toxicants. Rather, conventional remediation strategies have traditionally been directed to either organic or metallic toxicants. For organic pollutants, a number of techniques can be considered for the remediation of a particular site, including thermal treatment, steam and air stripping, bioremediation, chemical treatment and various other extraction/concentration processes. By contrast, fewer treatment techniques are available for the remediation of heavy metal laden environmental media. Particularly troublesome are metallic species which can be volatilized during conventional processes involving heat treatment. Conventional chemical stabilization tends to mobilize arsenic and chromium(VI) can be leached from conventionally stabilized waste (Daley, 1989). One promising technology which can be made to be efficient for mobilizing heavy metals, is the use of supercritical fluid extraction. The envisaged process would remove/concentrate non-polar materials in an initial extract and subsequently, volatile complexing agent(s) would be added to the supercritical fluid to sequester and mobilize heavy metal toxicants. The principal advantages of extractions with SC-CO_2 are the following: (*i.*) by controlling the density of the mobile phase, contaminants can be selectively solubilized/mobilized from the extraction vessel then recovered in a receiving vessel; (*ii.*) the natural organic matter of soils, sediments and natural waters are not removed by this process, so that the decontaminated medium remains biologically productive; (*iii.*) the most troublesome metallic (inorganic) pollutants for stabilized wastes or for incineration processes (e.g. As, Cd, Cr, Cu, Hg, Pb or Se) might be readily handled by the proposed process and (*iv.*) the composition of the sample matrix can vary over wide ranges without appreciably influencing the efficiency of the extraction process.

Interest has also increased in the use of supercritical fluid extraction as a means of quantifying the avidity of the interaction(s) of these metal analytes with other components of the sample matrix. For extractor operating conditions under which the rate(s) of transposition of the different forms of analyte metal to a chemical form which is available to the complexing reagent becomes rate limiting, different species of the analyte metal will be mobilized at different rates. Appreciable interaction of a component species with the matrix will limit its availability to the complexing reagent and decrease its rate of mobilization/purging from the extractor.

The feasibility of *on line* monitoring of the extractor eluate by atomic absorption spectrometry (AAS) led to the suggestion that the heavy metal burden in complex biological/environmental samples might be characterized on the basis of the relative rate(s) of mobilization of the different physical and/or chemical forms of the analyte metal. This approach attempts to fractionate the total analyte metal burden into different groups or classes based on their relative rates of mobilization from the matrix. The objectives of the following studies were to develop methods for the efficient mobilization of metal analytes from environmental matrices by supercritical extraction into carbon dioxide.

4.2 Materials and Methods

4.2.1 Instrumentation

The supercritical fluid extractor was a commercial unit (Figure 4-1, CSC Instrument Systems, Model 3100, Avondale, PA, USA) which had been fitted with a separate 50 mL capacity saturation vessel (SV) and a 10 mL or 5 mL capacity extraction vessel (EV). Each vessel was mounted vertically within a separate compartment of the extractor and jacketed with a thermostatted heater to provide a constant temperature over the course of the extraction. The EV was charged with solid complexing reagent, tetrabutylammonium dibutyldithiocarbamate (TBADBDTC), contained in a snugly fitting paper thimble. An accurately measured aliquot of aqueous sample, approximately 6 mL, was transferred to the EV which was connected in series with the SV. Pneumatically actuated two-way ball valves positioned before and after the SV and EV permitted several modes of operation. Mobile phase could be (*i.*) pre-equilibrated with the complexing reagent during a static phase (no net flow) of the process, (*ii.*) pre-equilibrated with the aqueous sample during a separate static stage of the process (*iii.*) once charged with complexing reagent, mobile phase could be percolated through the aqueous sample during a dynamic stage of the process, or (*iv.*) transferred directly to the EV so as to mobilize/purge any solute which was soluble directly into SC-CO_2. The extractor was fitted with a heated variable restrictor to facilitate the collection of eluate.

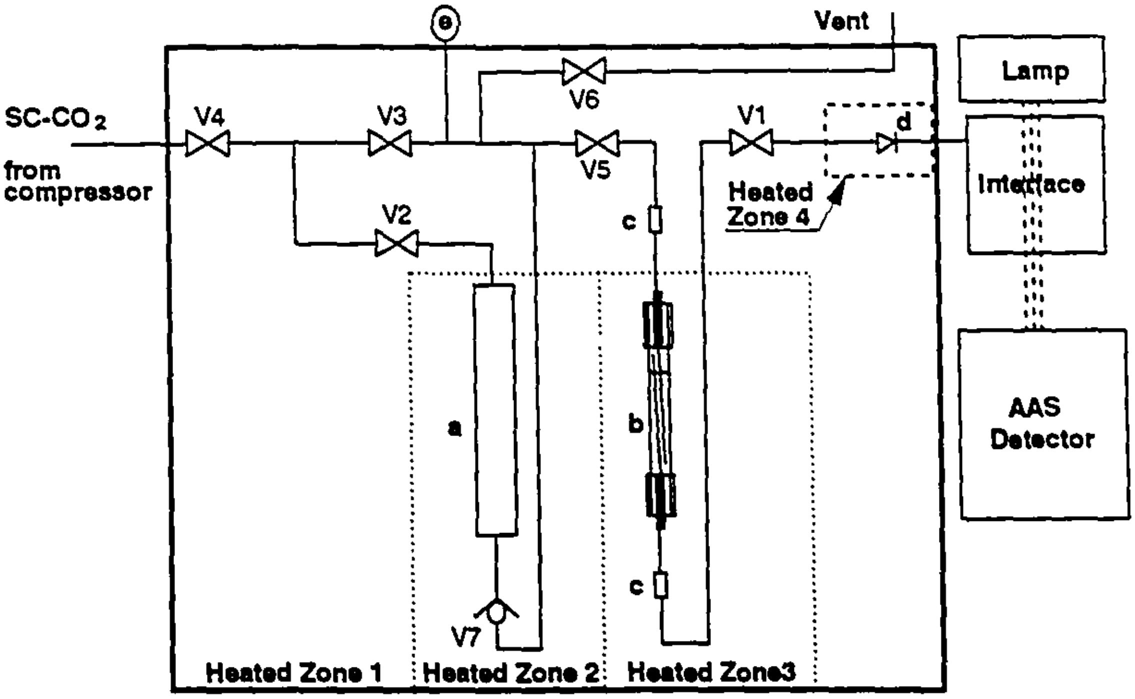


Figure 4-1. Supercritical fluid extractor (Series 3100, CCS Instrument Systems) consisting of (**a.**) a saturation vessel (**b.**) a demountable extraction vessel (**c.**) reducing unions (**d.**) variable restrictor (**e.**) pressure transducer and seven valves (valves 1-6: solenoid valve, valve 7: check valve). Extractor eluate was channeled, via a 23 cm x 0.05 mm i.d. silica transfer line, to the silica T-tube interface mounted within the optical beam of the AA spectrometer.

Extractions were performed for 30-60 min. with or without presaturation of the $SC\text{-}CO_2$ mobile phase with complexing/pairing reagent. Typically, mobile phase was not pre-saturated but solubilized excess reagent as it traversed the SV prior to entering the EV. Preliminary trials had indicated that omitting the static equilibration step had no measurable effect on the efficiency of the extraction.

On line monitoring of analyte metal in the extractor eluate was performed. A jacketed polymeric PEEK transfer line (1.6 mm o.d.), heated to approximately 50 °C, directed $SC\text{-}CO_2$ eluate from the outlet of the extractor to an all-silica T-tube interface mounted within the optical beam of a Philips Model PU 9100 atomic absorption spectrometer. Alternatively, for higher analyte metal burdens, the extractor eluate was transferred via a capillary restrictor to a Zeiss FMD-3 AAS operated in the conventional air/acetylene flame mode. The impact bead had been removed and nebulizer assembly had been modified as described in section 3.4. A silica capillary restrictor [capillary silica tube 0.050 mm (i.d.) x 23 cm SGE, Austin, TX] located at the exit of the transfer line served to control the mobile phase flow rate within the extractor and to provide a rapid depressurization of mobile phase inside the interface. For these studies background correction was not employed. In operation, extractor eluate was thermosprayed directly into the base of the Teflon spray chamber and mixed with fuel and with air which had been heated to approximately 200 °C. The AAS signal was captured with a chromatographic reduction software (Turbochrome 3, Perkin Elmer Corp., Montreal, QC). Curve fitting was performed with a commercial software (Microcal Origin, V 3.5, Microcal Software Inc., Northampton, MA, USA) using an unweighted least squares interative routine.

4.2.2 Chemical Reagents and Sample Preparation

All chemicals used were of ACS reagent grade or better. Working solutions of Cu(II) was prepared from 1,000 μg/mL analytical reagent grade stock solution (in dilute nitric acid) purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Acid washed sand (60-100 mesh) was purchased from BDH Co. (Montreal, Quebec, Canada). The fulvic acid was isolated from the Bh horizon of an imperfectly drained podzol sandy loam soil from Armadale (Prince Edward Island, Canada) and kindly donated by Dr. D. Gamble (Agriculture Canada). This material has been under continued study for many years. Tetrabutylammonium dibutyldithiocarbamate (TBADBDTC), was synthesized as described in Chapter 2. Tetraalkylammonium halide (R_4NX) was used as received (Aldrich Chemical Co.). Sand suspensions were analyzed for residual Cu content after digestion in 8 M HNO_3 for 4 h at 90 °C. The digestion solution was filtered, washed, then diluted to volume and assayed by flow injection - AAS using the method of standard additions. Extraction efficiency was expressed relative to the Cu level determined for a separate aliquot of sample which had been digested but not extracted.

4.3 Results and Discussions

4.3.1 Factors Controlling SF Extraction of Heavy Metals from Environmental Matrices

The mobilization of heavy metals by complexometric extraction into supercritical carbon dioxide has become an area of considerable interest. The lipophilic product metal complexes are partitioned into the SC-CO_2 mobile phase and purged from the extraction vessel by replacing the head space above the sample with fresh mobile phase. Thus, in theory at least, supercritical fluid extraction offers the possibility of the intentional remediation of polluted matrices which are burdened with both lipophilic organic contaminants and heavy metals pollutants.

The development of supercritical fluid extraction methods for the recovery of pollutants from environmental samples basically requires three steps. First, the analyte must be efficiently or rapidly partitioned from the sample matrix into the bulk supercritical fluid. Second, the analyte must be swept from the sample extraction vessel. Finally, the analyte must be efficiently collected in a form that is compatible with the analytical method used to determine the analyte content of the extract (Hawthorne *et al.*, 1993b). The partitioning step might be controlled by analyte solubility in the extraction fluid, kinetic limitations, and/or the ability of the extraction fluid to disrupt matrix-analyte interactions. Whereas the first step is mainly controlled by the chemistry of the system, steps two and three are essentially controlled by the physical characteristics of the matrix including the dimensions of the sample size, as well as the flow-rate of the supercritical fluid, and the efficiency of the collection device. However, the physicochemical processes that control SF extraction efficiencies are not well understood. It is becoming increasingly clear that (*i.*) high solubility of a particular species in the supercritical fluid is not a sufficient condition to yield high extraction efficiencies, and (*ii.*) the ability of the supercritical fluid to overcome matrix-analyte interactions is often more important than high solute solubility for achieving rapid quantitative recoveries. The extraction rate does not depend appreciably on the fluid flow-rate, and the total volume of extraction fluid which has passed through a sample apparently has little relevance to extraction efficiency. The contact time of the sample and the fluid is more important than the amount of extraction fluid that is used. The extraction is carried out by placing the sample in contact with a static supercritical fluid in a static mode or by percolating a supercritical fluid through the sample in a dynamic mode. The static mode allows a more efficient penetration of the matrix by the fluid, but the latter method permits saturation of the extraction fluid to be avoided, resulting to better recoveries and shorter extraction times. Thus, in most instances, extractions combine these two modes of operation - a short period of time is allowed for static equilibration, before the sample is dynamically extracted.

4.3.2 Influence of Natural Complexants on the Mobilization of Cu

Metals are know to interact strongly with both the humic and fulvic acid fractions of natural organic matter in the environment. Fulvic acid (FA) is an operationally defined fraction of the organic matter in natural waters, soils and/or sediments (Stevenson *et al.*, 1994). It is considered to be that fraction of the humic material which is soluble under all pH conditions. As such, FA is a complex highly heterogeneous mixture of polymeric ions (a macromolecular polydisperse) which is characterized by (*i.*) a multiplicity of functional groups capable interacting with both cationic and anionic species, (*ii.*) a range of apparent molecular weights and (*iii.*) local and seasonal variations in composition. Several observations have spurred a continuing interest in the interactions of heavy metals with components of natural organic matter. Transition metal ions such as Cu(II) or Cd(II) are known to be less toxic to aquatic organisms if they are complexed to FA or to other components of natural organic matter (Florence *et al.*, 1983). Aquatic FA as well as other fractions of adsorbed or dissolved organic matter can: (*i.*) alter the geochemical mobility of metal ions, (*ii.*) mediate changes in the oxidation state of soluble metal ions (Wittbrodt and Palmer, 1995), and (*iii.*) solubilize inorganic materials which would otherwise be considered water insoluble (Waite *et al.*, 1988). Finally, it is becoming increasingly recognized that particles within most aquatic systems are coated with a layer of organic matter which exerts a major influence on the adsorption equilibria of trace metals (Shindler, 1990). The sorption of metals onto FA is an important mechanism for retarding the migration of metals in environmental matrices. The objectives of current studies were (*i.*) to attempt to deal with increasingly concentrated solutions/suspensions of Cu analyte and (*ii.*) to evaluate the influence of increasingly complicated sample matrices which would provide progressively more realistic models of natural aquatic systems.

Fulvic acid is known to bind up to six equivalents (Gamble and Underdown, 1980) of Cu although the avidity and binding capacity of representative FAs for this cation and for other HMs is somewhat influenced by temperature, pH and metal:ligand ratio as well as the ionic strength of the aqueous medium. Decreasing pH decreases the apparent metal-FA complex stability presumably by increasing the proton competition with metal ions for weakly acidic binding sites on the FA. Increasing ionic strength of the medium decreases the overall complex stability by shielding macro-ionic charge and decreasing the complexing metal ion activity in the domain of the macromolecule. Increasing metal:ligand ratio decreases the overall complex stability since metal ions bind to progressively weaker sites on heterogeneous poly-ligand macromolecules as the metal loading is increased. The complexation of Cu by representative FAs have generally been described as rapid with equilibrium being achieved within a few minutes (Dalang *et al.*, 1984). Cross linking of separate macromolecules of FA by complexation with a divalent cation (which serves as a bridging atom) has been suggested as the probable explanation for the increased aggregation observed with increasing HM loading on this material. The process of aggregation (Underdown *et al.*, 1981), which is also pH dependent, is quite distinct from the flocculation/precipitation which is observed in complexometric titrations of humic materials with HM cations.

Based principally on laboratory studies of the interactions between dissolved metals and solid mineral surfaces, these processes have been described as reversible coordination reactions between dissolved metals and hydroxyl groups of the particle surface. The avidity of the interactions with mineral surfaces are considered to be weaker than interactions with humic substances and studies with "natural" particles have indicated that desorption rate(s) are appreciably smaller than those for adsorption. The complexation of metal ions by natural ligands can alter their adsorption by mineral surfaces. Typically, metal fulvate complexes are not adsorbed by the surfaces of silica, manganese oxides, calcite or aluminosilicate minerals (Davis and Kent, 1990). In these cases, the mineral surface site and dissolved ligands compete thermodynamically for coordination of metal ions and the net adsorption, in theory at least, can be modeled as the sum of two independent processes. FA adsorbed to the surface of these materials can also adsorb HMs. Modeling of the interactions of HMs with natural complexants has been attempted successfully by many groups. The vast majority of these studies have been concerned with thermodynamic calculations of these processes at equilibrium and have taken either of two forms. The binding sites on the matrix have been modeled either as a series of discrete ligands (Dzombak *et al.*, 1986) or as a continuous distribution (Grimm *et al.*, 1991). Appreciably fewer studies have focused on the kinetics of these interactions (Chakrabarti *et al.*, 1994). The favored approach to monitoring the kinetics of desorption of HM from humic materials has been to choose experimental conditions which favor first-order kinetics (Shuman *et al.*, 1983). Despite the complexities of the interactions of metals with organic matter and/or mineral surfaces it was anticipated that studies of the kinetics of extraction of well defined/characterized sample matrices would provide further insight into the mechanistic processes which occur during the SC-CO_2 extraction.

4.3.3. The Efficiency of Extraction of Cu from Cu-FA Complexes

The fulvic acid (FA) used for these studies had been well characterized and was known to strongly adsorb up to 3 μmoles of Cu^{2+} per mg (which corresponded to 1.05 μmole assuming an average MW=951). It was of interest to evaluate the efficiency of complexometric extraction of Cu from Cu-FA solutions using tetrabutylammonium dibutyldithiocarbamate (TBADBDTC) as the source of the dithiocarbamate complexing ligand. Previous studies had indicated that Cu^{2+} (12-20 μg/mL) was rapidly and efficiently purged from distilled deionized water. The extraction strategy was to pre-charge the supercritical CO_2 mobile phase with the solid TBADBDTC complexing reagent contained in a separate saturation vessel and to percolate the reagent-charged mobile phase through the aqueous sample. The Cu analyte was sequestered *in situ* and the resulting Cu-DBDTC complex was partitioned into the SC-CO_2 mobile phase and purged from the extraction vessel by replacing the head space above the aqueous sample with fresh solvent. The course of the extraction was to be monitored by atomic absorption spectrometry with the view to modeling the kinetics of the process. Provided that the concentration of complexing DBDTC ligand in the aqueous phase was sufficiently large to be considered constant and that the rates of Cu complexation, transfer of the Cu-complex to the SC-CO_2 phase and exchange of the head space were sufficiently large, then pseudo first-order kinetics would be anticipated to provide an adequate description of the purging process. Thus

$$\frac{[\mathrm{Cu-DTC}]_t}{[\mathrm{Cu-DTC}]_0} = Y_0 + Ae^{-(t-t_0)/\lambda} \tag{4.1}$$

where: $[\text{Cu-DTC}]_t$= the concentration of Cu in the extractor eluate at time t min.

$[\text{Cu-DTC}]_0$= the concentration of Cu in the extractor eluate at time t_0.

λ = a time constant characteristic of the overall extraction process.

Y_0= a fitting parameter to account for baseline displacement resulting from pairing reagent in the effluent.

If more than one physical or chemical form (chemical species) of the Cu analyte was present in the aqueous sample, appreciable differences in the relative rates of their conversion to Cu^{2+} (or some other chemical form which is available to the complexing ligand) would be reflected by an appreciably better fit of the decay in the AAS-Cu signal with time to a model comprising the sum of i = 2 or more exponential decay curves.

$$\frac{[\text{Cu - DTC}]_t}{[\text{Cu - DTC}]_0} = Y_0 + \sum A_i e^{-(t-t_0)\lambda_i} \tag{4.2}$$

Since the AAS detection process is a relative technique some means of calibrating the Cu-response was required. Two approaches were considered; (*i.*) the addition of a methanolic spike of $Cu(DBDTC)_2$ standard directly to the extractor eluate via a manual rotary injection valve interposed between the exit of the extractor and the silica T-tube interface of the AAS detector or (*ii.*) using a measure of the residual Cu content of the sample after extraction (and the known initial copper content) to determine the ratio of the absorbance at time *t* to the absorbance at time zero (the time corresponding to the maximum AAS signal). Somewhat arbitrarily, the latter approach was chosen. Previous studies has demonstrated a high level of correlation for the linear regression of the AAS Cu response (peak area) and the quantity of $Cu(DBDTC)_2$ standard added, as a methanolic solution, to $SC\text{-}CO_2$ mobile phase in a flow injection mode.

The configuration of the extractor did not permit mobile phase (containing TBADBDTC) from the exit of the saturation vessel to be transferred directly to the detector, an approach which would have facilitated the optimization of the detector response to Cu in the extractor effluent. Instead, the valve controlling flow from the saturation vessel was opened permitting mobile phase containing the complexing reagent to flow through the extraction vessel to the detector via the transfer line which ended with a silica capillary restrictor. The restrictor had to be positioned within the entry of the interface. The positioning of the exit tip of the restrictor within the interface influenced the magnitude of the AAS response. In addition, there was a short time delay while the extraction vessel was pressurized and the aqueous sample became saturated with the complexing reagent. Time zero was arbitrarily chosen to be the onset of the rapid increase in the AAS signal.

The declination portion of the AAS-Cu signal with time for the extraction of 2.4 μmol Cu/mL from aqueous solution (Figure 4-2, curves 1-3) at 30.85, 27.57 and at 24.12 MPa were well fit by a single exponential decay model and were characterized by a half-life, $t_{1/2}$ (= λ_i) corresponding to 3.1-4.6 min. (Table 4-1) indicating a moderate pressure dependence for this variable. Increasing density of the mobile phase [as estimated by extrapolation from the measured flow rates (Table 4-2) of decompressed gas exiting the extractor], resulted in a corresponding decrease in the number of exchanges of headspace fluid per half-life of the analyte in the aqueous sample. The overall rate of purging (in this pressure range) was not limited by rates of complexation, or transfer of the product into the SC-CO_2 phase; Presumably, a more rapid rate of purging would have been achieved by increasing the flow rate of the mobile phase. Whereas the time from the maximum AAS response to the onset of a clearly exponential decay was quite short for the 2.4 μmol Cu/mL loading (Fig. 4-2, curves 1-3), the 4.8 μmol Cu/mL loading caused this time lag to become appreciable

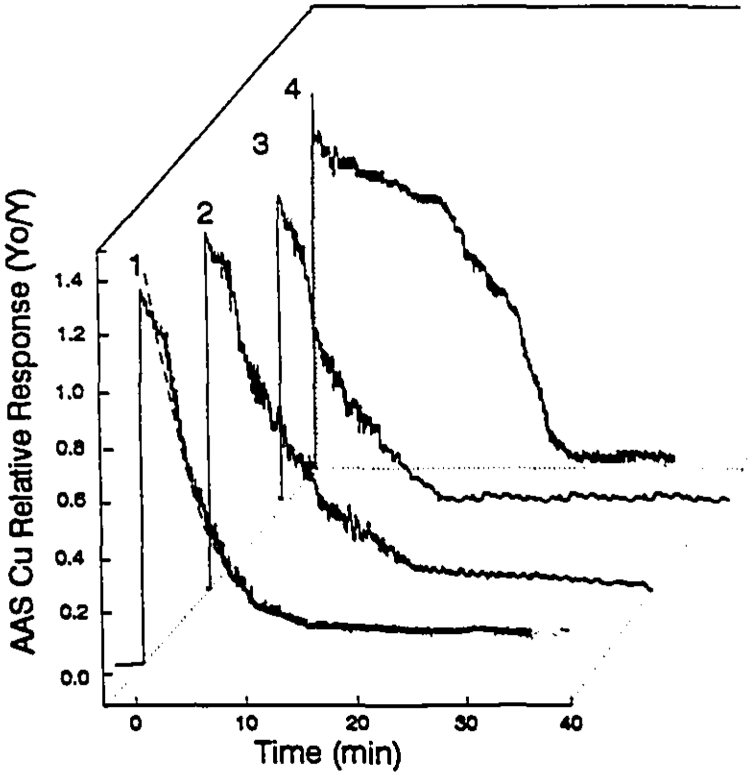


Figure 4-2. AAS response curves for the complexometric extractions, at 50 °C, of a 3 mL aqueous solutions containing 2.4 (curves 1-3, 24.12, 27.57 and 30.85 MPa respectively) or 4.8 μmol Cu/mL (24.12 Mpa). The optimized single exponential decay model (dashed line), has been added to the declination portion of curve 1.

Table 4-1. Parameters of the optimized exponential decay models for the extraction of samples containing 0.078, 2.4 or 4.8 μmol $Cu(NO_3)_2$/mL solution.

Cu Loading (μmol/mL)	Extractor operating conditions	χ^2	$t_{1/2}$ (min)	Decompressed flow rate (mL/min)	Exchange vols./$t_{1/2}$ [a]	% Mobilization[b]
0.078[c]	20 min static + 30 min dynamic 24.12 MPa & 50°C	0.39	3.1			97.2 ± 2
2.4[d]	40 min dynamic 31.01MPa & 50 °C	0.46	3.1	952	1.74	98.5 ± 1
2.4[d]	40 min dynamic 27.50MPa & 50 °C	0.21	4.6	901	2.51	98.1 ± 2
2.4[d]	40 min dynamic 24.12MPa & 50 °C	0.28	3.2	845	3.12	97.5 ± 1
4.8[d]	40 min dynamic 24.12MPa & 50 °C	4.76	3.8	852	3.68	95.2± 2

[a] Number of exchange volumes of head space per $t_{1/2}$.

[b] Mean ± RSD (based on three replicate determinations of residual Cu in the sample after extraction).

[c] Detection was performed with the silica T-tube interface AAS.

[d] Detection was performed with the flame-AAS.

Table 4-2. Measured flow rates of decompressed gas exiting the extractor.

T (°C)	P (MPa)	Length of restrictor (cm)	Inner diameter of restrictor (mm)	Interface	CO_2 flow rate (mL/min)
50	24.12	30	0.05	SFE-FAAS	631
50	27.57	30	0.05	SFE-FAAS	656
50	30.85	30	0.05	SFE-FAAS	682
50	24.12	24	0.05	SFE-QTAAS	845
50	27.57	24	0.05	SFE-QTAAS	901
50	30.85	24	0.05	SFE-QTAAS	952

(curve 4). Nonetheless, the trailing portion of this curve (from ≈17-45 min) was well fit with a single exponential decay function (Table 4-1). In all cases, the % mobilization of analyte (as determined by the residual quantities of Cu in the aqueous medium after extraction), was virtually quantitative.

Addition of 1 mg/mL of FA to the aqueous sample (followed by equilibration in the dark for 20 h at room temperature) appreciably retarded the rate of metal mobilization/purging from the matrix (Figure 4-3, curve 2). Estimates of the $t_{1/2}$ corresponding to a single exponential decay ranged between 17 and 19 min (Table 4-3). The goodness of fit for a single or the sum of either two or three exponential decay processes (as measured by chi-square, χ^2) were excellent. Moreover, each of the models predicted Cu residues in the extracted sample which were in close agreement with the observed levels. The inclusion of the constant (Y_0) term in the single exponential decay model improved the goodness of fit of the model to the data somewhat. One possible interpretation for this behaviour is that some 7% of the Cu analyte was either not available to the complexing ligand or that it was mobilized so slowly as to be kinetically indistinguishable from the inert Cu fraction. Alternatively, the goodness of fit would be expected to be improved with the inclusion of a second fitting parameter in the model. Without knowing the form of the residual Cu it does not seem possible to decide which of these models best fits the data. The inclusion of a second and a third decay term in the model did not improve the goodness of fit for this extraction. Control extractions performed under identical operating conditions in the absence of complexing reagent (TBADBDTC) failed to mobilize detectable quantities of Cu.

Increasing the concentration of Cu in the FA solution to either 2.4 or 4.8 μmol/mL appreciably affected the AAS response curve for the extraction although the overall

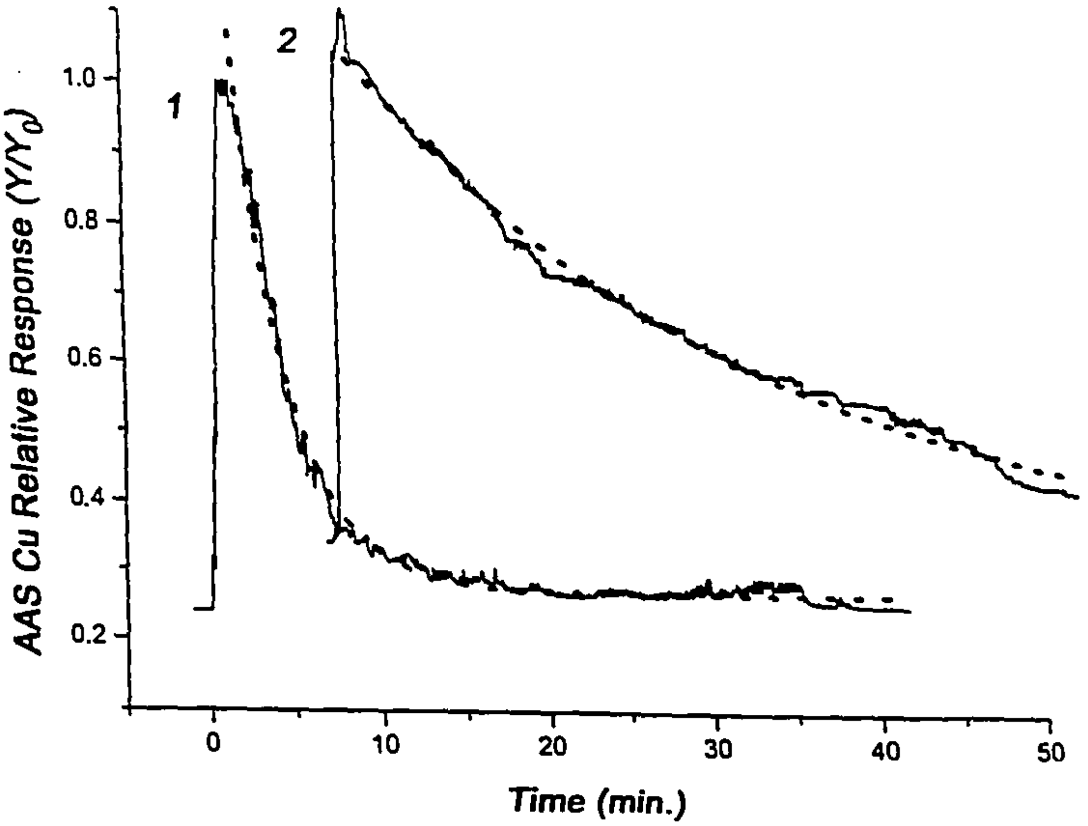


Figure 4-3 Fit of the optimized single exponential decay model to the declination portion of the AAS response to Cu for the complexometric extraction (24.12 MPa and 50 °C) of 1, an aqueous suspension containing 10 μg Cu plus 0.17g sea sand/mL or 2, 1.2 μmol Cu plus 1.05 μmol FA/mL.

Table 4-3. Exponential decay models for the declination portion of the AAS response to copper in the SC-CO_2 extract from aqueous solutions containing 1.2, 2.4 or 4.8 µmol Cu /mg fulvic acid. Sample were extracted dynamically, at 24.06 MPa and 50 °C, for 60 min.

χ^2 ($\times 10^3$)	Model[a]	$t_{1/2}$ (± 1 RSE)[b]	Y_0	Coefficient (A_i)	% residue@ 60 min.	
					Predicted	Observed[c]
Cu, 1.2 µmol /mg FA						11.5 ± 2
0.89	single	18.7 ± 1.1	0	1.0	10.8	
0.33	single +Y_0	17.0 ± 0.8	0.071	0.93	14.9	
0.30	sum of two	12.3 ± 14	0	0.52	14.3	
		32.1 ± 25		0.46		
0.30		11.8 ± L	0	0.60		
	sum of three	43.2 ± L	0	0.33	15.7	
		25.8 ± L	0	0.06		
Cu, 2.4 µmol /mg FA						20.8 ± 2
1.0	single + Y_0	4.04 ± 0.6	0.387	0.63	38.7	
0.5	sum of two	2.92 ± 0.9	0	0.57	30.6	
		92.4 ± 1.9		0.49		
0.7	sum of three	2.92 ± 4.2	0	0.57		
		88.5 ± L	0	0.32	30.9	
		100.4 ± L	0	0.17		
Cu, 4.8 µmol /mg FA						41.6 ± 3
0.3	single +Y_0	3.88 ± 0.7	0.468	0.51	46.8	
0.2	sum of two	2.48 ± 0.7	0	0.54		
		184.1± 1.4	0	0.54	42.7	
0.15	sum of three	2.29± 2.0	0	0.55		
		97.9 ± L	0	0.35	42.3	
		VL	0	0.19		

[a] Fraction of Cu remaining: $=Yo + \Sigma A_i e^{-(x-xo)\lambda}$ where: Yo is a constant, x is time (min) and λ is the time constant ($\lambda = 0.693/ t_{1/2}$).

[b] RSE = relative standard error of estimate.

[c] Observed residues are the average of three replicate determinations ± 1 RSD.

shape was not changed drastically (Figure 4-4). Exponential decay models indicated that an increasing proportion of the Cu was either not accessible to the complexing ligand or was immobilized either during the equilibration or during the extraction. There was no visible change in the solution during the 20 h pre-extraction equilibration of any of the samples. Yet the portion of the Cu which was mobilized, was mobilized more rapidly than had been observed for the 1.2 μmol/mL loading. For the 2.4 μmol Cu/mg FA/mL solution, it was not possible to fit a single exponential decay model to the data without the addition of a constant to the model (Y_0 of Table 4-3). Each of the exponential decay models overestimated the Cu residues in the sample after extraction (mean = 33% predicted *vs.* 21 % observed). Each of the models predicted that approximately 60% of the initial Cu burden (mean 59 ± 3%) was mobilized very rapidly (mean $t_{1/2}$ = 3.3 min. ± 20 %) and the remainder (0.96 μmol) was immobilized (Y_0 = 39 %) or purged from the extractor at a rate(s) which was slow relative to the 60 min extraction. Apparently, during the extraction a portion of the bound Cu was released and the remainder was encapsulated within the flocculated/coagulated FA. Upon prolonged standing in the dark, a sealed unextracted aliquot of the same solution developed a visible dark brown flocculant.

The results for the exponential decay models of the 4.8 μmol/mg FA/mL solution were qualitatively similar. As was the case for the 2.4 μmol Cu/mg FA/mL solution, it was not possible to obtain an acceptable fit of a single decay model to the data without the addition of a constant term (Y_0 of Table 4-3). However, for this substrate there was close agreement between the observed Cu residue (41.6 ± 3 %, 2 μmol) and the residue predicted by the three decay models (mean 43.9 ± 6 %). Each of the three models predicted a rapid purging of approximately one half (mean 53 ± 4 %) of the Cu with $t_{1/2}$ = 2.88 min. ± 30 %. The remaining Cu was either immobilised (Y_0) or purged at a rate which was slow relative to the 60 min duration of the extraction. By

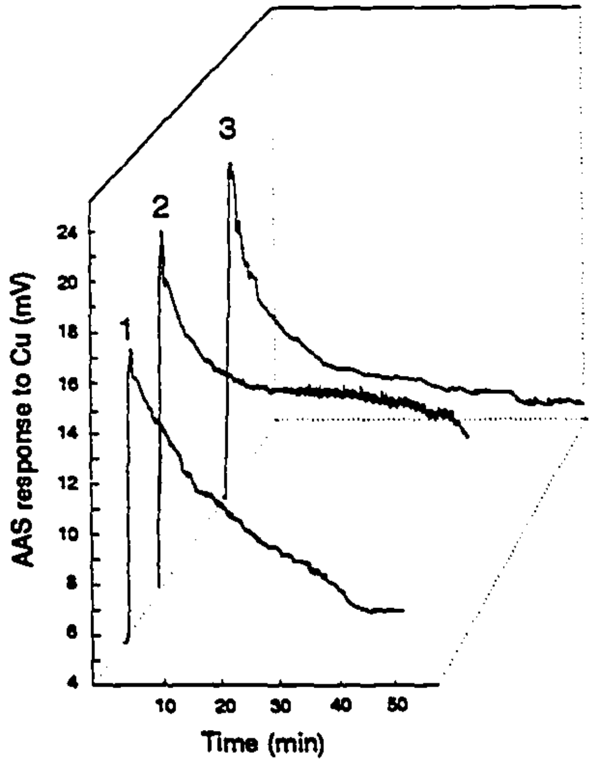


Figure 4-4. AAS copper response profiles for the complexometric extraction, into SC-CO_2, of fulvic acid (FA) solution, which contained (1.) 1.2 μM Cu^{2+} and 1.0 mg FA/mL or (2.) 2.4 μM Cu^{2+} and 1.0 mg FA/mL or (3.) 4.8 μM Cu^{2+} and 1.0 mg FA/mL. Extractions were performed for 60 min at 20.04 MPa and 50 °C after the reactants had been equilibrated at room temperature and atmospheric pressure in the dark for 20 h.

contrast, if the Cu load was decreased 10-fold (0.48 μmol Cu plus 0.1 mg FA/mL), no visible coagulant was evident even after prolonged storage in the dark and recoveries of Cu were virtually quantitative.

4.3.4 Extraction of Cu from Suspensions Containing either Sea Sand, Sea Sand plus FA or Sea Sand, FA and Oxalic Acid

The extraction of Cu from suspensions containing either sea sand, sea sand plus FA or sea sand, FA and oxalic acid were also performed. The mobilization of Mn, Zn or Pb from sea sand solutions into SC-CO_2 by complexometric extraction with TBADBDTC was presented in Table 4-4. These results indicated a surprising similarity in the efficiencies of mobilization of different metal analytes from the same matrix. The shapes for the profiles of the AAS Cu signal with time were somewhat different from the previous curves. The time required to reach a maximum AAS signal was increased appreciably and typically consisted of two parts; an initial very rapid increase reminiscent of the earlier AAS response curves followed by a level response or slight decrease prior to the onset of a rapid decay (Figure 4-5). Although it was possible to fit an exponential decay model to the declination portion of this response curves (Figure 4-5), an appreciable portion (up to 30 %) of the total area under the curve fell outside this region. The estimated $t_{1/2}$ for Cu extraction from the sand suspension was 2.22 min. For curve 1 of Figure 4-3, 82 % of the total area of the AAS response curve fell within the boundaries of the exponential decay model. It was deemed appropriate to attempt to model as much of the AAS response curve as possible. Both Lorenzian and Gaussian distribution models were considered. The latter models generally provided a better fit (smaller χ^2) to the data. For most of the sample matrices (Table 4-5), it was necessary to use a model comprising the sum of two Gaussian distributions. Within the fitting criteria used and with the exception of the 2.4 μmol Cu loading, it was not possible to obtain an acceptable fit to the AAS

Table 4-4. Purging of Metals from sea sand suspensions into SC-CO_2 by complexometric extraction with TBADBDTC.

Sample composition (mL)	**SFE operating conditions**				**(%) recovery**
	T (°C)	**P (MPa)**	**T (min)**		
			Static	**Dynamic**	
10μg $Mn(NO_3)_2$ + 0.17 g AWS[a]	50	24.05	5	50	78 ± 3
10μg $Zn(NO_3)_2$ + 0.17 g AWS	50	24.05	5	50	80 ± 2
10μg $Pb(NO_3)_2$ + 0.17 g AWS	50	24.05	20	50	83 ± 2

[a] AWS, acid washed sea sand.

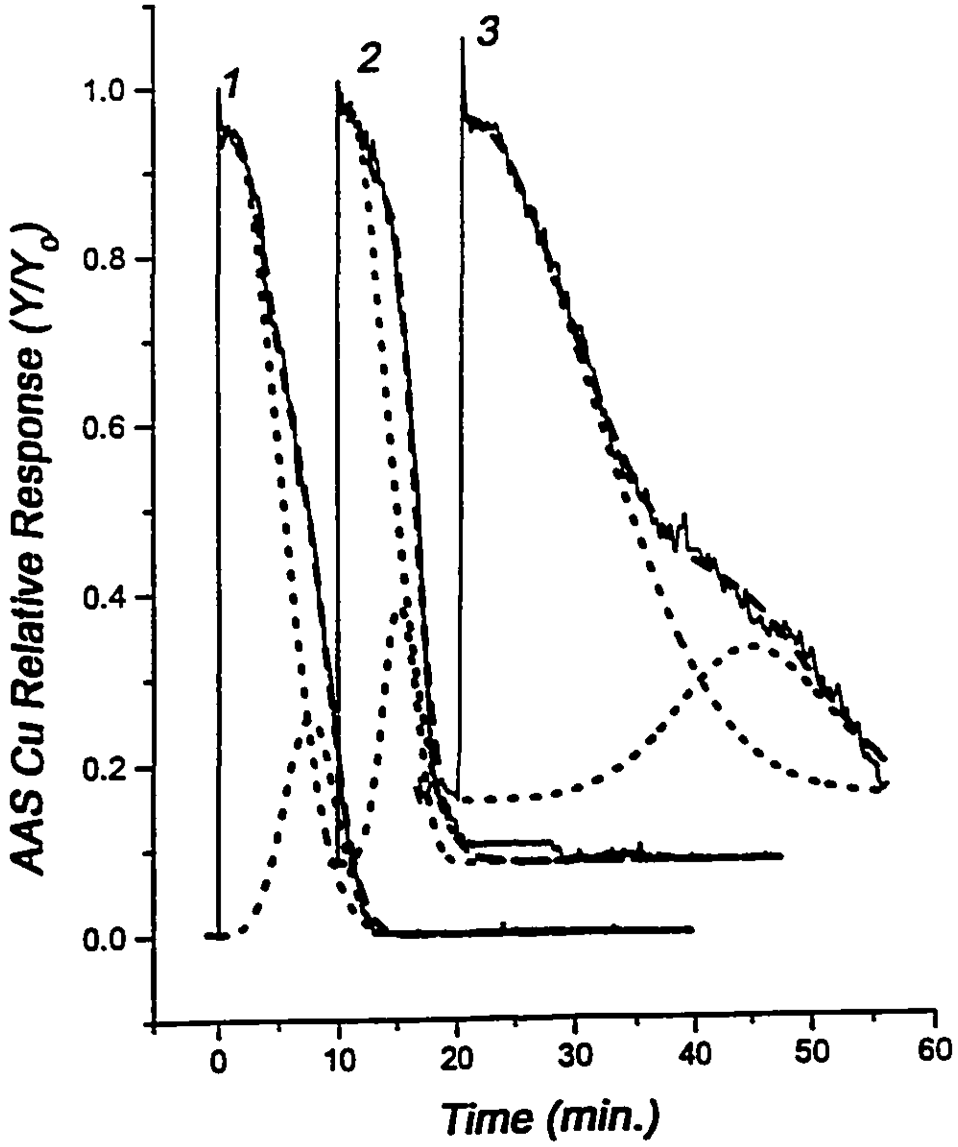


Figure 4-5. Fit of the optimized sum of two Gaussian distributions to the AAS response to Cu for the complexometric extraction (at 24.12 MPa and 50 °C) of 1, 2.4 µmol Cu in 0.1 % (w/w) oxalic acid; 2, 0.48 µmol Cu plus 0.1 µmol FA/mL or 3, an aqueous suspension containing 2.4 µmol Cu plus 0.17 g sea sand plus 0.1 % oxalic acid/mL suspension.

Table 4-5. Gaussian distribution models (single or sum of two) for the AAS response to the eluate from the SC-CO_2 dynamic extraction of aqueous suspensions of sea sand which had been pre-equilibrated with copper and fulvic acid.

Sample[a]	χ^2	Y_0[b]	$X^c_{1\,max}$ (min.)	W_1[d]	A_1[e]	$X^c_{2\,max}$ (min.)	W_2[d]	A_2[e]	% Recovery[f]
Cu^{2+} 10μ g/g AWS[g]/mL	0.07	0	2.5	8.3	0.89	21.4	21.8	0.11	82 ± 3
Cu^{2+} 0.48 μ mol + 1mgFA/mL	0.001	0	0.70	7.1	0.88	5.3	2.9	0.12	93 ± 2
Cu^{2+} 1.2 μ mol+1mg FA+167mg AWS/mL[h]	0.21	0.67	2.2	6.6	0.91	17.6	14.3	0.09	70 ± 5
Cu^{2+} 2.4μ mol+1mg FA+167mg AWS/mL	0.02	0	1.0	4.7	1				33 ± 2
Cu^{2+} 4.8μ mol+1mg FA+167mgAWS /mL	0.28	0	0.74	4.4	0.87	21.6	41.0	0.13	28 ± 3
Cu^{2+} 2.4μ mol+1mg FA+167mgAWS/mL 1% (w/w) OX[j]	0.11	0	0.73	20.6	0.88	28.2	12.9	0.12	43 ± 3
Cu^{2+} 2.4μmol in 1% (w/w) OX	0.001	0	1.0	8.4	0.91	7.7	4.0	0.09	95.3 ± 2

[a] Solutions of Cu and FA were equilibrated during three weeks then mixed with sea sand and re-equilibrated for one week.

[b] Baseline offset.

[c] Peak maxima were measured relative to the time corresponding to the initial rapid rise in the AAS response profile.

[d] Peak width at half height.

[e] Peak area relative to the total area under the modeled curve.

[f] Mean recovery based on three replicate determinations of the residual Cu in the sample after extraction.

[g] Acid washed sea sand.

[h] Single distribution model.

[j] Oxalic acid.

response profiles with a single Gaussian or Lorenzian distribution function. Table 4-5 summarizes the baseline offset (Y_0), peak maximum, width at half height and relative peak area for each of the 2 components of the best fit distributions. Given the large number of data points (1,500-2,800), and the 6 or 7 variables optimized by the fitting process, it is not surprising that the "lack of fit" parameter, χ^2, was very low. One possible advantage of this model is that it should be independent of the concentration of Cu provided that the distribution of binding avidities remains identical.

The mean recoveries of Cu (30 μg in sample) from the sand suspension [10 μg Cu/mL suspension containing 0.17g sand] or from the sand-FA mixture (1.2 μmol Cu/mL suspension containing 1 mg FA plus 0.17g sand] were acceptably high (Figure 4-6, curves 2-3, 82 and 70 % respectively), but were lower than the recoveries from distilled water (97.2 %). Higher loading of Cu in the FA-sand suspensions caused a further decrease in recoveries (Table 4-5). An attempt to improve the recovery by adding oxalic acid to the sample matrix (Table 4-5) was only moderately successful. Oxalic acid is known to mediate the reductive dissolution of certain solid phase metal oxyhydroxides (with the formation of CO_2) and to complex strongly with Cu. If appreciable coagulation of the dissolved fraction of the matrix had occurred during the equilibration or extraction then the presence of this reagent might have increased the fraction of Cu analyte in the solution phase. In general, the pH is believed to play an important role in the fixation of heavy metals on complex matrices. First, the pH is important in determining the solubility and the state of hydrolysis of the metal cations and therefore their ability to complex with fulvic acids. Second, the conformation(s) and complexing avidities of the humic matter are also pH dependent. At very low pH values, fulvic acid molecules for instance are aggregated into colloidal particles with very little or no charge. If the pH is increased the negative charge on the molecule increases as dissociation occurs. At

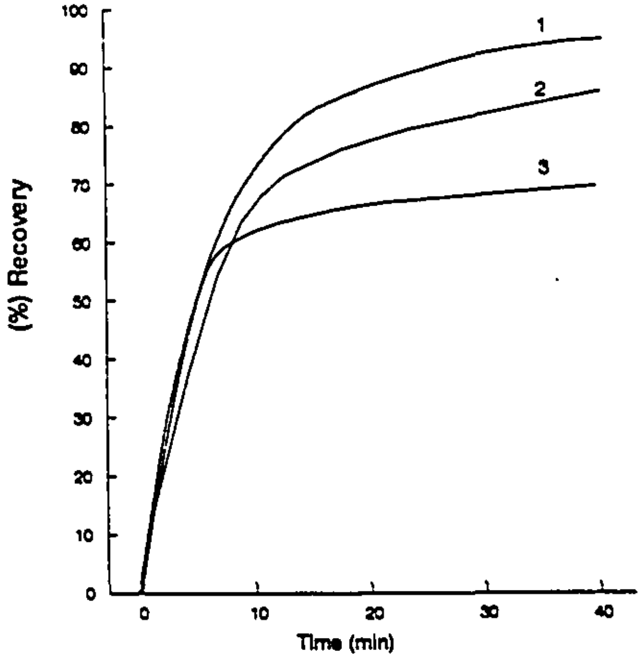


Figure 4-6. Cumulative recovery curves for the of complexometric extraction of Cu from (1.) 1% (w/w) oxalic acid (2.4µmol Cu^{2+}/mL), (2.) sand suspension (10µg Cu^{2+} + 0.17g sand)/mL or (3.) the sand-fulvic acid (FA) mixture (1.2.µmol Cu^{2+} + 1 mg FA + 0.17g sand)/mL. Extractions were performed at 24.12 MPa and 50 °C.

the same time the aggregates disintegrate, and as the molecules become more hydrated, they become completely dissolved in solution. However, it was not known whether the relativly low pH of the extraction and/or the saturation of the sample with CO_2 during the extraction would influence the course of the dissolution/complexation. The presence of the oxalic acid increased the Cu recovery moderately (Table 4-5, 43 *vs.* 33 %) but apparently appreciably retarded the purging rate of the analyte for the extraction of 2.4 μmol Cu loading in the presence (Figure 4-7, 1.) or absence (Figure 4-7, 2.) of oxalic acid. By contrast, the extraction of a solution containing 0.48 μmol Cu + 0.1 mg FA/mL in which there was no visible coagulation/precipitation even on prolonged storage, w_i was determined to be 7.1 min. Increased Cu loading (>1.2 μmol Cu + 1 mg FA/mL) caused a modest decrease in peak width of the optimized models. For an array of binding sites which are known to be heterogeneous with respect to the avidity of interaction with Cu, the strongest sites would be preferentially occupied during the equilibration. Higher loading would be anticipated to result in a greater proportion of weaker sites being occupied which might, in turn, be displaced more readily and possibly more rapidly as well. For companion control extractions (at 24.12 MPa and 50 °C), the recoveries of Cu (2.4 μmol/mL) from 1% (w/w) oxalic acid was quantitative (Figure 4-6, curve 1, 95.3 ± 2 %).

To evaluate the efficiency of mobilization of heavy metal from these matrices several factors that control the extent of metal release from fulvic acid should be considered. The most important factors are considered to be (*i.*) the magnitude of the stability constants for the different metal-fulvic acid complexes, and (*ii.*) the pH which provides a measure of the competition from carbonate and hydroxyl ligands. The ionic strength of the medium containing metal-fulvic acid complexes has been observed to exert a marked effect on stability constants. With increasing

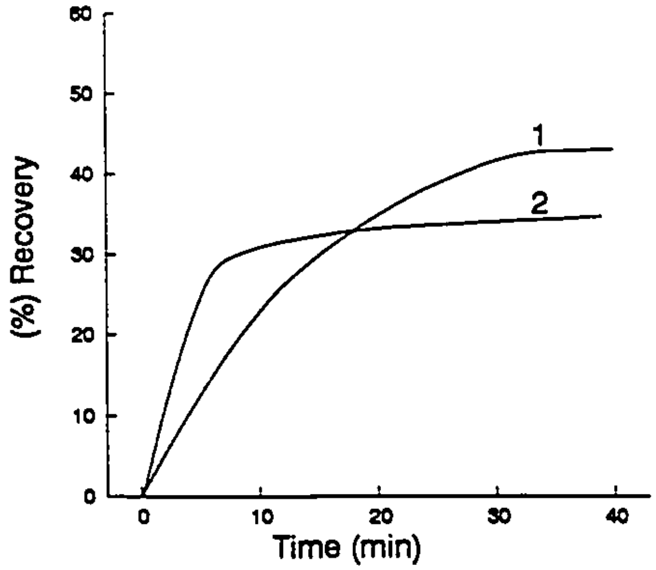


Figure 4-7. Cumulative recovery curves for the complexometric extraction of copper (2.4 µmol Cu^{2+}/mL) from aqueous solution in the presence (**1.**) or absence (**2.**) of 0.1 % (v/v) oxalic acid. Extractions were performed at 24.12 MPa and 50 °C.

ionic strength, the stability constants decrease. This is probably the result of increased competition between the different ions in solution for complexing sites on the organic matter. Humic compounds of different origin differ in their metal binding behavior because of differences in their functional group distributions and contents. The lower molecular weight fractions apparently have a greater metal holding capacity than the higher molecular fractions because of a relatively higher content of metal binding functional groups.

These results of the current studies indicate that recoveries from Cu-FA solutions or Cu-FA-sand suspensions are dependent on both the Cu-loading and the level of FA in the sample. Whereas pseudo first-order kinetics provided an adequate model for the extraction kinetics for solution containing 1.2 μmol Cu plus 1 mg FA/mL, higher Cu loading caused an apparent coagulation of the FA which was accompanied by the release of a portion of the bound Cu but the immobilization of the remainder. A kinetic analysis of the changes in rates of analyte purging with time can provide additional information on the composition of the sample matrix. Thus, providing sufficient data points can be recorded, not only will an estimate of the level of metal analyte be available, but also parameters of the avidity of interaction of the analyte with component(s) of the matrix can be obtained.

Chapter 5

Metal Anion Mobilization from Aqueous Media and Characterization of Zinc and Copper in Biological Materials

5.1 Synopsis

The supercritical fluid extraction of cationic metal species has been described in previous Chapters. A variety of reagent additives (Lin and Wai, 1994) to supercritical carbon dioxide, added either directly to the sample matrix or the SC mobile phase, have been applied to the successful mobilization of metallic cations from aqueous media and semi-permeable solids. With the exception of the crown ethers (Wang and Wai, 1995), these ligands characteristically behave as univalent, bidendate anionic ligands and are capable of rapidly forming a non-polar complex(es) with a wide variety of cationic analytes. The mobilization of nascent anionic species into SC-CO_2 has not been explored in detail. Hawthorne and co-workers (1991) described the quantitative purging of linear alkylbenzenesulfonates from soil, sediment and anaerobic digested sludge into methanol amended SC-CO_2 and Laintz *et al.* (1992c) have reported the complexometric extraction, with bis(trifluoroethyl)dithiocarbamate, of As(III) and Sb(III) (as well as As(V) and Sb(V) after pre-reduction) from natural waters. However, dithiocarbamates or xanthates, are not predicted to react with most anionic species. Selenite (SeO_3^{2-}) but not selenate (SeO_4^{2-}) (Hutchins *et al.*, 1982) and arsenite (AsO_3^{2-}) (Suzuki *et al.*, 1986) but not arsenate (AsO_4^{3-}) react with dithiocarbamate, via a redox reaction, to form a non-polar selenobis(dithiocarbamate) [$R_2NC(S)SSeSC(S)NR_2$] and arsenobis(dithiocarbamate) respectively. Alkylxanthate ligands [$RONC(S)S^-$]

apparently undergo analogous redox reactions with these analytes (Singh *et al.*, 1986).

That other anions might be susceptible to extraction is suggested by liquid phase partitioning studies. Liquid membranes (Li, 1971) are water-immiscible emulsions composed of surfactants and various additives, typically in a hydrocarbon solvent which serves to encapsulate droplets of an aqueous solution of an ion carrier (ion pairing or ion associating reagent) capable of stripping target ions from the bulk aqueous feedstock. Emulsions containing a variety of "ion carriers" including tetraalkylammonium ions, tertiary, secondary or primary amines, oximes, and phosphinic, thiophosphinic or phosphonic acids have been used successfully to partition a variety of metallic cations and/or anions from aqueous media including NH_4^+, Cr(VI), Hg^{2+}, Cd^{2+} and Cu^{2+} (Kitagawa *et al.*, 1977), Cr(VI) and Hg^{2+} (Moore, 1972), V(V) (Palet *et al.*, 1995) and U(VI) (Chiarizia *et al.*, 1990). When coupled with stripping processes to immobilize the target ion(s) while regenerating the ion carrier, efficient and rapid processes for remediating aqueous streams have been developed.

The objective of the current studies were to extend the applicability and selectivity of the extraction process with supercritical fluids to nascent metallic/organometallic anions including anions of chromium and selenium. If promising, the approach was then to be applied to a processing stream containing ionic metals. Inorganic wood preservative chemicals such as chromated copper arsenate (CCA) have become increasingly used because of problems and restrictions associated with the organic wood preserving agents such as pentachlorophenol, creosote and coal tars. Formulations of CCA (American Wood Preservers Association, 1978) contain 47.7 % Cr as CrO_3, 18.5 % Cu as

CuO and 34.0 % As as As_2O_5 and are applied to different species of wood by heat/pressure methods following recommended procedures which typically involve the recycling of metal-containing condensates.

5.2 Materials and Methods

5.2.1 Instruments

The supercritical fluid extractor (Figure 4-1, CSC Instrument Systems, Model 3100, Avondale, PA, USA) had been described previously in Chapter 4. Extractions were performed for 30-50 min. with or without pre-saturation of the SC-CO_2 mobile phase with complexing/pairing reagent. Typically, mobile phase was not pre-saturated but solubilized excess reagent as it traversed the SV prior to entering the EV. Eluate from the extractor was transferred to the AAS detector via a heated ($\approx$ 50 °C), jacketed 1.6 mm outer diameter (o.d.) x 0.1 mm inner diameter (i.d.) PEEK HPLC grade tubing which ended in a 23 cm x 50 μm i.d. silica capillary restrictor (SGE, Austin, TX). The capillary restrictor served to control the mobile phase flow rate within the extractor and to provide a rapid depressurization of mobile phase inside the interface. Detection was performed by thermospray induced volatilization of the complexed analyte(s), within a silica T-tube interface interposed within the optical beam of an atomic absorption spectrometer (AAS) or by flame AAS as described in Chapter 3.

5.2.2 Reagents and Chemicals

All chemicals used were of ACS reagent grade or better. Working solutions of Cr(VI), Se(IV), or Se(VI) were prepared from 1,000 μg/mL analytical reagent grade stock solution in dilute nitric acid purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Tetrabutylammonium dibutyldithiocarbamate

(TBADBDTC) and metal-DBDTC complexes were synthesized as described in Chapter 2. Tetrahexylammonium bromide (THAB) and tetraheptylammonium bromide (Aldrich Chemical ([illegible].) was used as received. A condensate of CCA wood preserving suspension was supplied by a local treatment facility. Freeze dried bovine liver (standard reference material 1557a) purchased from the National Institute Standard and Technology (NIST, Beltsville, ME, USA) or fresh bovine liver purchased from a local supermarket was solubilized (*i.*) in 10% (w/v) KOH in 20% (v/v) methanol solution or (*ii.*) by homogenizing [polytron homogenizer (30,000 rpm)] 5g of chopped wet tissue with 50 mL of distilled water followed by sonicating (Model W-185D, Ultrasonics Inc., Plainview, NY) three successive times (each time for 5 min) with external cooling in an ice bath.

5.2.3 Statistical Analyses

Curve fitting was performed with a commercial software (Microcal Origin, V 3.5, MicroCal Software Inc., Northampton, MA) using an unweighted least squares iterative routine.

5.3 Results and Discussions

5.3.1 Cr(VI) Anion Mobilization

Chromium occurs in oxidation states ranging from -2 to +6. The widespread use of chromium in industrial applications and the attendant emissions from metallurgical and processing industries, as well as the toxicity of its hexavalent forms have caused well publicized concerns. The forms of chromium in aqueous solution are dependent on the pH of the medium. In general, H_2CrO_4 is the principle species below pH 0.6 in relatively concentrated solutions. In the pH range 1-6 the $HCrO_4^-$ is the primary species, and CrO_4^{2-} is the predominant species at pH greater than 6 (Nriagu and Nieboer, 1988). Because the pH is decreased to approximately 3.5 for CO_2 saturated water at normal SF extraction conditions (Hendrick and Taylor, 1992), it seemed probable that Cr(VI) exists as its monoprotic form, $HCrO_4^-$, during the SC-CO_2 extraction process. To achieve efficient mobilization from aqueous media, initial efforts were directed to identifying an ion pairing reagent which (*i.*) would possess appreciable solubility in SC-CO_2 and (*ii.*) would rapidly form a non-polar ion associate with $HCrO_4^-$, which would also be appreciable soluble in SC-CO_2. A preliminary series of liquid phase extractions suggested that tetrahexylammonium ion partitioned chromate into chloroform more efficiently than either tetraheptylammonium or tetrapropylammonium ion. Subsequent trials tested tetrahexylammonium and tetraheptylammonium bromide (R_4NBr) or tetrabutylammonium dibutyldithiocarbamate as SC-CO_2 additives for the mobilization of this analyte. The strategy was to saturate the mobile phase with a suitable ion pair source capable of sequestering Cr (VI) *in situ* and purge the resulting non-polar ion associate/ion pair from the extractor by replacing the headspace fluid with fresh SC-CO_2 mobile phase. The SC-CO_2 was pre-equilibrated with the test ion pairing reagent and sample solution for 10 min, followed by 30 min of dynamic extraction. Residual Cr in the aqueous samples after extraction was determined by flow injection FAAS and by graphite furnace AAS. There were no significant differences in residual Cr levels as determined using either quantitation technique. The percentage of Cr mobilized (as determined by the differences between the initial and residual levels of analyte in the sample) are presented in Table 5-1. Apparently, the tetrahexylammonium bromide ion pairing reagent at the higher extractor operating pressure (30.85 MPa) resulted in the most efficient analyte purging (85% recovery). By contrast, tetraheptylammonium bromide provided the lowest recovery (25%) among these trials. For liquid phase extractions, Cr(VI) is

Table 5-1. Mobilization/purging of Cr(VI) from aqueous media by ion pair extraction into SC-CO_2.

Pairing reagent	**Extractor operating conditions**				**(%) Recovery**[a]
	T (°C)	**P (MPa)**	**T (min)**		
			Static	**Dynamic**	
THAB[b]	50	24.51	10	30	75 ± 3
THAB +10 %MeOH	50	24.51	10	30	80 ± 2
THAB	50	30.85	10	30	85 ± 3
THPAB[c]	50	24.51	10	30	25 ± 2
TBADBDTC[d]	50	24.51	10	30	68 ± 2

[a] Mean recovery (± 1 relative standard deviation) based on three replicate determinations.

[b] Tetrahexylammonium bromide.

[c] Tetraheptylammonium bromide.

[d] Tetrabutylammonium dibutyldithiocarbamate.

known to form a rather polar ion associate with alkylammonium ions (Chiarizia, 1991). Indeed switching from an aliphatic hydrocarbon (dodecane) to an aromatic diluent in a liquid membrane appreciably increased the Cr(VI) permeation across that membrane (Pearson, 1983). That the polarity of the product ion associate might have been the dominant factor limiting the recovery is corroborated by the increased analyte recovery in the presence of polar modifier or at the higher extractor operating pressure. A subsequent trial was performed at 27.58 MPa and 50 °C without pre-saturation of the mobile phase with ion pairing reagent or pre-equilibration of the sample with this reagent. Figure 5-1 (1.) presents the declination of the flame-AAS response to Cr with time for the extraction (with tetrahexylammonium bromide) of an aqueous sample containing of 400 μg Cr(VI). Mathematical modeling using a single exponential decay function or the sum of two or three exponential decay functions indicated an appreciably better fit [minimal chi^2 (χ^2)] for a model with two decay functions (Table 5-2.). Nonetheless, 92 % of the purged analyte is predicted to have had a mean half-life ($t_{1/2}$) in the sample of 19.5 min which is almost six times greater than had been observed previously for the mobilization of several metal cations (including Cd, Cu and Pb) under comparable extractor operating conditions. Further, the model predicts a residual Cr content of 24 % in reasonable agreement with the observed 21 %.

5.3.2 Se(IV) or Se(VI) Anion Mobilization

The ion pairing/ion association approach was also evaluated for selenate (SeO_4^{2-}) and for selenite (SeO_3^{2-}). For these trials, (Table 5-3.) no static pre-equilibrations were performed. For selenite, the tetrabutylammonium dibutyldithiocarbamate additive to the mobile phase resulted in a moderately more efficient mobilization (90 vs. 73 % purging) relative to the tetrahexylammonium ion pairing reagent.

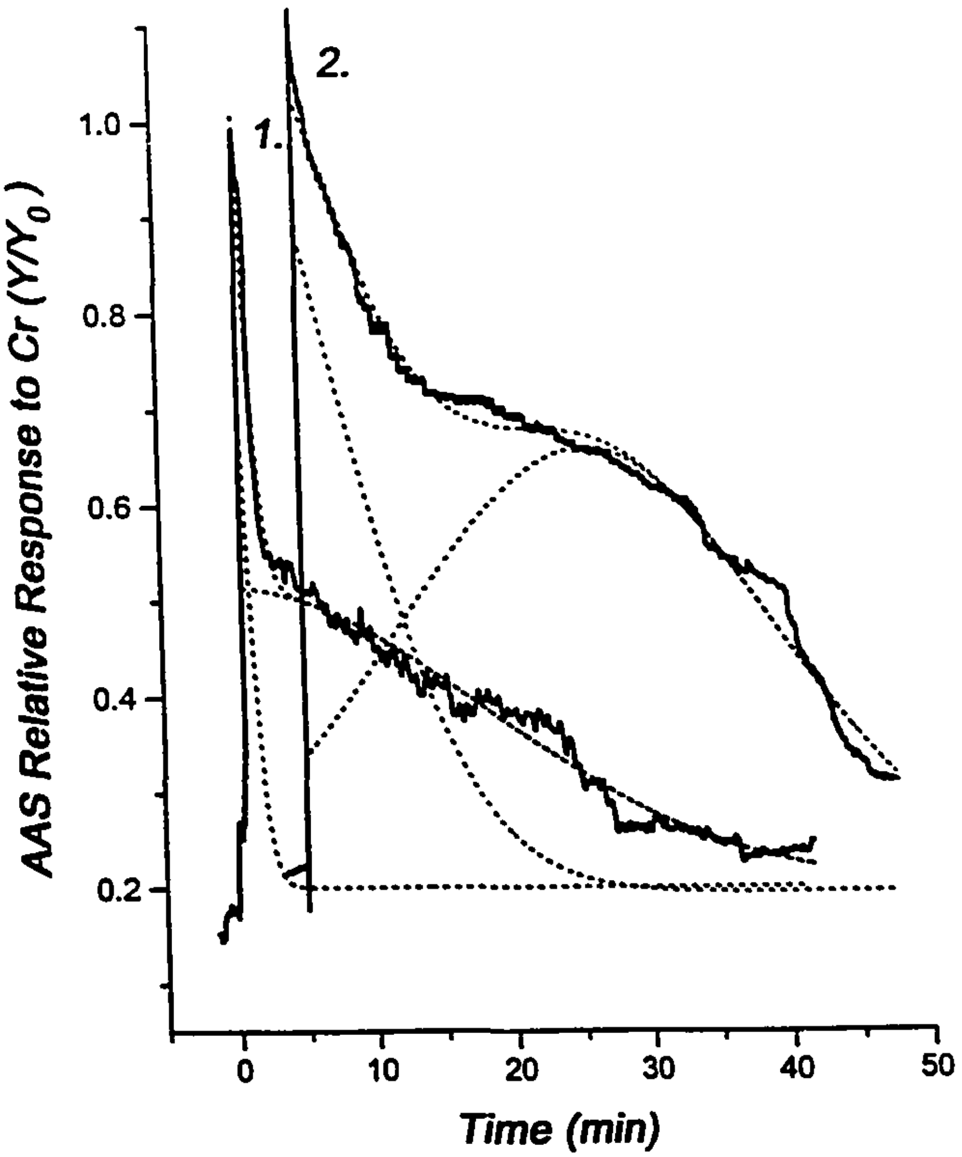


Figure 5-1. Changes, with time, in the relative Cr-FAAS response (solid line) for the ion pairing extraction of (1.) aqueous standard Cr(VI) containing 400 µg/6 mL analyte or (2.) aqueous suspension containing chromated copper arsenate (CCA). Dotted curves present the two modeled component Gaussian distributions (and their sum) corresponding to the optimized fit to the declination portion of the AAS response. Arbitrarily, time zero was set to the initial rapid increase in the AAS response.

Table 5-2. Optimized exponential decay models for the declination portion of the chromium, selenium or the Cu-AAS response to eluate from the ion associate or complexometric extraction into SC-CO_2.

Analyte	Optimized Model[a]	Y_0	t_1[b] (min)	t_2[b] (min)	χ^2 (×1,000)	% Components		%Removal[c]
Cr(VI)	sum of two	0	0.4	19.5	0.43	8	92	65 ± 2
Se(VI)	one	0.34	3.6		0.53	100		69 ± 4
Cu(II)	one	0	3.3		0.70	100		96 ± 3
Cu(II)[d]	one	0	7.8		3.3	100		98 ± 3

[a] Responses were modeled as one, the sum of two or the sum of three exponential decay curves.

[b] Half-life as estimated from the optimized exponential decay model.

[c] Mean % removal ± 1 RSD, after 40-min (Cr), 30-min (Se or Cu) or 45-min (Cu[d]) at 24.1 MPa and 50 °C as determined by residual analyte metal in the sample.

[d] Cu analyte was present in a suspension of CCA wood preservative.

Table 5-3. Mobilization/purging of Se(IV) or Se(VI) from aqueous media by ion pair extraction into SC-CO_2.

Analyte	**Pairing reagent**	**SFE operating conditions**			**(%) recovery**[a]
		T (°C)	**P (MPa)**	**T (min)**	
Se(IV)	THAB[b]	50	24.12	40	73 ± 2
Se(IV)	TBADBDTC[c]	50	27.57	40	90 ± 1
Se(VI)	THAB	80	30.57	40	93 ± 2
Se(VI)	THAB	50	24.12	40	66 ± 2
Se(VI)	TBADBDTC	50	24.12	40	62 ± 1

[a] Mean recovery (± 1 relative standard deviation) based on three replicate determinations.

[b] Tetrahexylammonium bromide.

[c] Tetrabutylammonium dibutyldithiocarbamate.

Recoveries from these two trials are not strictly comparable because of the higher operating pressure of the latter (27.6 vs. 24.1 MPa). It is interesting to note that the ion pairing mechanism probably contributed appreciably to the mobilization of this analyte. For selenate, where the reductive complexation mechanism can not contribute to mobilization, tetrabutylammonium and tetrahexylammonium pairing ion resulted in similar recoveries (62 vs. 66 %) suggesting little difference in polarities of the product ion pairs (if indeed solubility in the mobile phase is rate limiting). By operating at higher temperature (80 vs. 50 °C) and pressure (30.6 vs. 24.1 MPa) the recovery of Se(VI) was increased to 93 %. The declination curve, which resulted from *on-line* monitoring (Figure 5-2., quartz T-tube AAS) of the mobilization of selenate (50 °C, 24.1 MPa) with TBADBDTC, was also modeled. The parameters of the optimized fit for exponential decay models to this declination curve is included in Table 5-2. It was not possible to obtain an acceptable fit to a single decay model without the inclusion of a constant term (Y_0). Higher order models, which did not improve the fit to the experimental data significantly, inevitably included a decay curve(s) with a rate constant corresponding to very long $t_{1/2}$s such that an appreciable fraction ($\approx$ 35 %) of the analyte was predicted not to be mobilized over the course of the extraction. Although the reason for this behavior [rapid mobilization ($t_{1/2}$ = 3.6 min) of approximately 2/3 of the analyte burden yet immobilization of the remainder] remains unknown, again there was good agreement between predicted and observed residual levels of Se(VI).

5.3.3 Mobilization of Metal Analyte(s) from Waste Wood Preservative Suspension

To evaluate the proposed approach to anion mobilization with an environmental sample, a turbid suspension of condensate from a CCA wood treatment facility

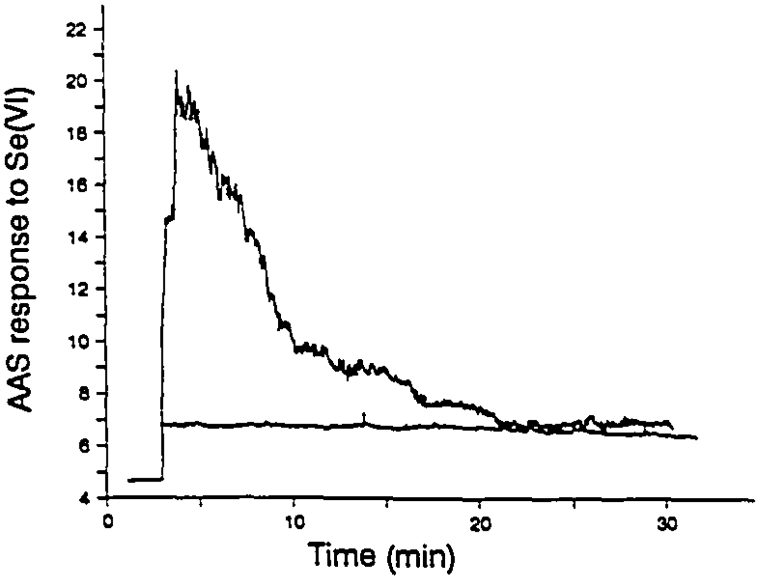


Figure 5-2. AAS response to the complexometric extraction (with tetrahexylammonium bromide) of Se(VI) from aqueous solution. Extraction, performed at 24.12 MPa and 50 °C, was monitored by QTI-AAS. The lower curve represents the AAS response to SC-CO_2 eluate containing THAB but no Se.

served as the substrate. In addition to Cu, Cr and As, the sample was known to contain waxes and other undisclosed additives. For these trials, a 1:1 (m/m) mixture of a complexing reagent, TBADBDTC, and an ion pairing reagent, THAB, was used in an effort to simultaneously mobilize both cations and anions in a single step extraction. With a larger mobile phase flow rate [2.2L/min of decompressed CO_2, obtained by doubling the inner diameter of the capillary restrictor to 100 μm i.d.)] and 45 min of dynamic extraction, 89 ± 2 % of Cr and 98 ± 3 % of the Cu were removed from the suspension. The mean percent removal of Cr was slightly higher from the wood preservative suspension than from aqueous media (Table 5-1.) presumably because of the higher flow rate of mobile phase. However colored components of the sample were also efficiently mobilized resulting in a colorless substrate after treatment. Figure 5-1, curve 2 represents the declination in the FAAS response to Cr with time for the extraction of the wood preservative suspension. Curve 2 could not be satisfactorily modeled with an exponential decay model but was successfully modeled as the sum of two Gaussian distributions. The relevant curve fitting parameters are contained in Table 5-4. Apparently there was an appreciable interaction of the analyte with other components of the sample as judged by the decreased rate of mobilization despite the increased flow rate of mobile phase. The peak width at half-height (w_1) for the fitted model (Table 5-4.) was increased approximately 6-fold and the peak width for the second Gaussian curve (w_2) was reduced modestly. The FAAS declination curve for the extraction of Cu from wood preservative suspension is presented in Figure 5-3, curve 2. Here again, the rate of mobilization of Cu from the suspension is slower than from aqueous solution (Figure 5-4, curve 2, larger slop). The fitting parameters for the optimized fit of exponential decay models to these curves are recorded in Table 5-2 and for the optimized fit of the sum of two Gaussian distributions in Table 5-4. The predicted half-life ($t_{1/2}$) for Cu was

Table 5-4. Parameters for the optimized Gaussian distribution models (sum of two) for the FAAS response to the eluate from the SC-CO_2 dynamic extraction of aqueous solutions/suspensions containing Cr and/or Cu.

Sample[a]	χ^2 (×1,000)	Y_0[b]	X^c_{1max} (min)	W_1[d]	A_1[e]	X^c_{2max} (min)	W_2[d]	A_2[e]	% Recovery[f]
Cr(VI) standard, 66.7µg/mL	0.36	0.20	-1.7	3.2	0.28	-0.52	36.7	0.72	82 ± 3
Cr(VI) in suspension, containing CCA	0.42	0.03	-7.1	19.0	0.58	20.6	26.9	0.42	89 ± 2
Cu^{2+} standard, 30 µg/mL	0.15	0	0.09	4.7	0.77	5.7	7.0	0.23	96 ± 3
Cu^{2+} in suspension, containing CCA	0.43	0	-4.1	23.3	0.84	14.2	5.6	0.16	82±3

[a] Aqueous solutions/suspensions were extracted at 24.5 MPa and 50 °C without pre-equilibration with pairing complexing reagent.

[b] Baseline offset.

[c] Peak maxima were measured relative to the time corresponding to the initial rapid rise in the AAS response profile.

[d] W = peak width at half height.

[e] Peak area relative to the total area under the modeled curve.

[f] Mean recovery based on three replicate determinations (± 1RSD) of the residual Cr or Cu in the sample after extraction.

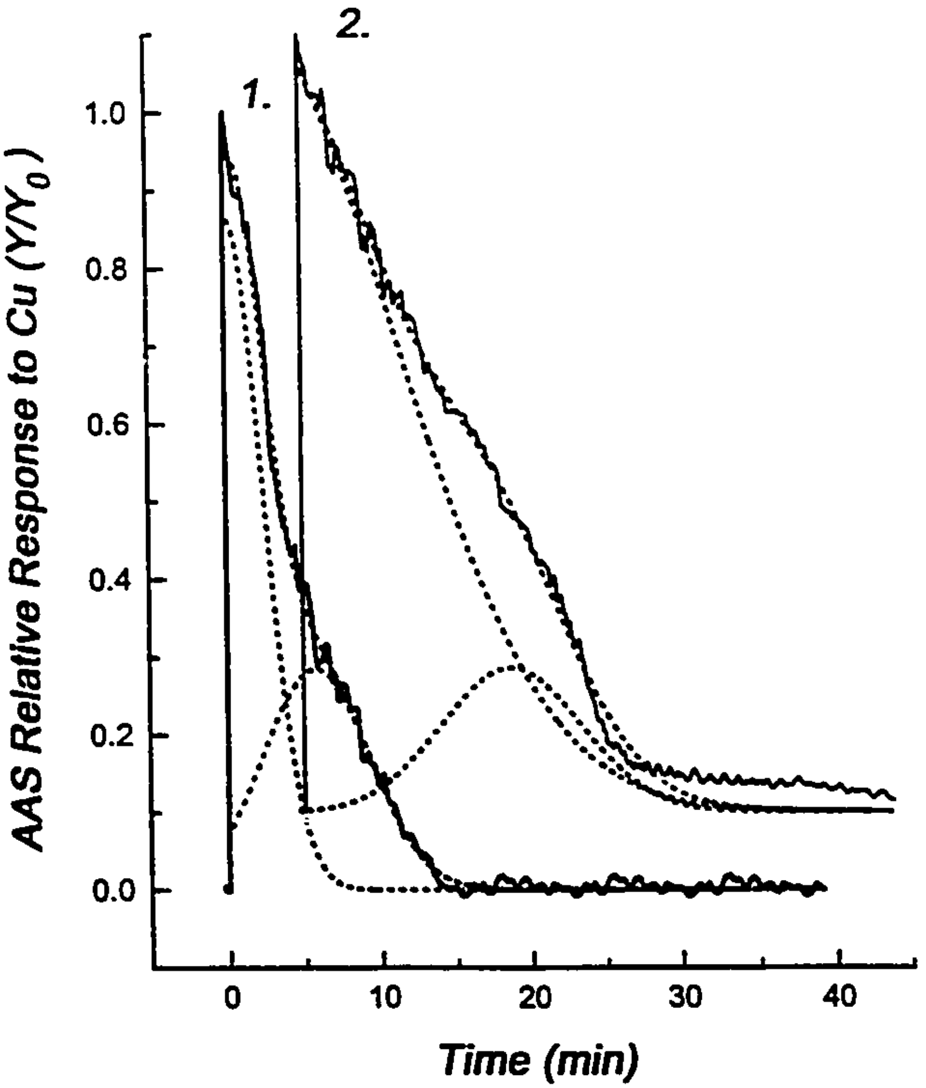


Figure 5-3. Changes, with time, in the relative Cu-FAAS response (solid line) for the ion pairing extraction of (1.) aqueous standard Cu containing 100 μg analyte or (2.) aqueous suspension containing CCA. Dotted curves present the two modeled component Gaussian distributions (and their sum) corresponding to the optimized fit to the declination portion of the AAS response. Arbitrarily, time zero was set to the initial rapid increase in the AAS response.

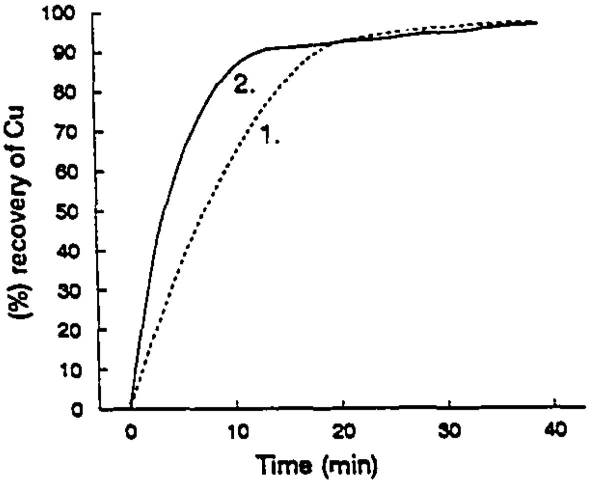


Figure 5-4. Cumulative recovery curves for the complexometric extraction of Cu from: (1.) the wood preservative waste suspension or (2.) aqueous solution. Extraction were performed at 24.12 MPa and 50 °C.

increased from 3.3 to 7.8 min (Table 5-2) and w_1 was increased approximately 5-fold whereas w_2 was decreased by only 0.8 (Table 5-4). However, the differences in the increases in w_1 (6-fold vs. 5-fold) might not be sufficiently different to conclude with certainty that the Cr(VI) and the Cu(II) mobilizations were affected differently by the sample matrix. These trials were all performed at 24.12 MPa and 50 °C.

In total, these results demonstrate that metal analyte(s) both cations and anions, can be rapidly and efficiently mobilized/purged from aqueous media/waste suspension of wood preservative by *in-situ* ion association or chelation into SC-CO_2. Apparently, the rate limiting step in the process for Cr(VI) mobilization is the solubility of the product ion pair (ion associate) in the SC-CO_2 mobile phase. Previous studies had demonstrated that, for identical extractor operating conditions (24.1 MPa, 50 °C, 630 mL CO_2/min) approximately 400 μg Cu were purged with a predicted $t_{1/2} < 4$ min. This observation suggests that excess pairing ion was probably present in the Cr extraction as well. For Se(IV) mobilization, (predicted $t_{1/2} = 3.6$ min) (*i.*) the rate of mobilization was not appreciably different from the rates of mobilization of cationic analytes and (*ii.*) the ion paring mechanism apparently predominated over the dithiocarbamate mediated reduction to Se(-II) followed by formation of selenobis(dithiocarbamate). No evidence was observed for any difference between the polarities of the Se(VI) and the Se(IV) ion pairs. It is also feasible to mix a complexing reagent with an ion pairing reagent to effect the mobilization of both cations and anions.

Although process optimization was not the objective of these feasibility studies, the efficiencies of mobilization were increased appreciable by changing the parameters of extraction. It seems likely that rates of mobilization could be increased further by: (*i.*) increasing the density of the mobile phase (higher extractor operating pressures), (*ii.*) changing the temperature to disrupt the interactions between analyte(s) and other components of the matrix and /or (*iii.*) reducing the head space volume above the sample solution. A kinetic analysis of the changes in rates of analyte purging with time can provide additional information on the interactions of analyte ion with the sample matrix. These results also suggested that mobilization of toxic metal ions (anion/cation) from aqueous waste suspension is a promising approach to environmental remediation.

5.3.4 Characterizing Analyte Metals in Biological Samples

To demonstrate the feasibility of working with biological materials, a standard reference material (NIST Standard Reference Material 1557a) of freeze dried bovine liver was slurried dissolved in methanolic KOH and subjected to SC-CO_2 extraction at 20.03 MPa and 50 °C for 20 min. The resulting AAS Zn traces are presented in Figure 5-5. Whereas the extraction of aliquots of the slurry solution with SC-CO_2 alone did not result in a perceptible AAS Zn response (Figure 5-5, curve 1), saturation of the mobile phase with tetrabutylammonium dibutyldthiocarbamate (TBADBDTC) resulted in an AAS response curve (Figure 5-5, curve 2) that was similar but not identical to the AAS response curve for the extraction of 12 µg of $Zn(NO_3)_2$ contained in 3 mL of distilled water (Figure 5-5, curve 3). Surprisingly, when fresh bovine liver, purchased from a local supermarket, was homogenized and then extracted under identical conditions, a more rapid rate of mobilization of the Zn content was evident (Figure 5-5, curve 5) and approximately 86% of the Zn burden was recovered. When a separate aliquot of the same slurry of the fresh liver was extracted with SC-CO_2 in the absence of complexing ligand, a portion (5.6%) of the zinc was mobilized from this matrix (Figure 5-5, curve 4). The chemical identity of the non-polar fraction of the total

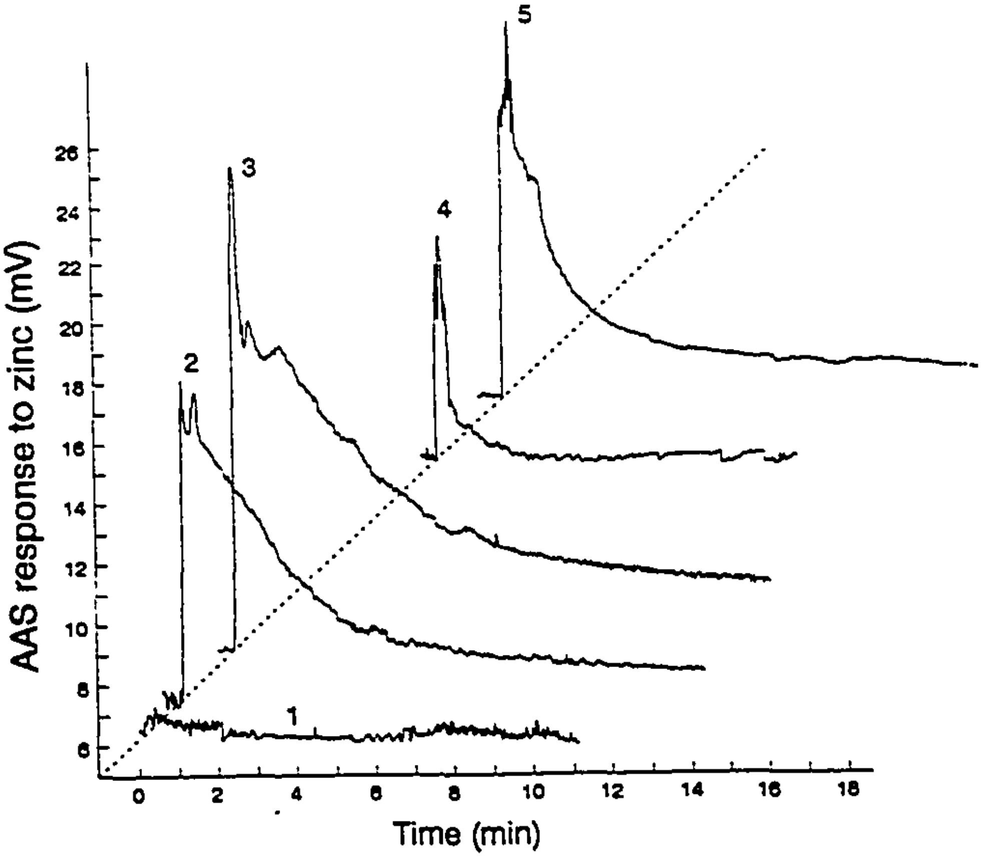


Figure 5-5. AAS zinc response profiles for the extraction, into SC-CO_2, of freeze dried bovine liver standard in the absence (1.) or presence (2.) of complexing agent or (3), an aqueous solution containing 23 µg Zn in the presence of complexing agent. The response curves 4 and 5 resulted from the SC-CO_2 extraction of fresh bovine liver homogenate in the absence (4) or presence (5.) of TBADBDTC complexing reagent. Extractions were performed at 20.04 MPa and 50 °C with a static equilibration of the mobile phase with the complexing agent for 20 min prior to dynamic mobilization of analyte Zn.

Zn content remains to be determined. Apparently, the preparation and/or slurrying of the freeze-dried material in KOH had appreciably altered the interactions of the Zn with this matrix. The efficiency of extraction, as determined by the residual zinc content in the aliquots of samples after extraction, are recorded in Table 5-5. Figure 5-6 presents cumulative recovery curves for the extraction of Zn from (*i.*) fresh bovine liver homogenate in the absence of complexing agent (*ii.*) fresh bovine liver homogenate in the presence of complexing agent (*iii.*) freeze dried bovine liver standard in the presence of complexing agent and (*iv.*) an aqueous Zn solution.

There is a requirement for a rapid technique which will permit the characterization of natural matrices not only with respect to metal burdens but also with respect to the avidity of the interaction between the metal and the matrix. It is to be anticipated that binding sites (which for most natural matrices are undersaturated with respect to metals loading) will influence/control the analyte metal concentration which is available to the complexing reagent. The effect of these sites will be to attenuate the rates of metal removal from the matrix. Sites of differing avidity will affect the recovery curves to different extents. Thus, the shape of the declination curve for the AAS signal with time for the extraction of analyte metal can provide a wealth of information concerning the form(s) of the analyte metal in biological samples.

Figure 5-7 present a plot of the AAS response *vs.* time for the complexometric extraction of zinc from a suspension of freeze dried bovine liver. The dashed line presents the single exponential decay which provided the better fit with the experimental data than summation models of either two or three exponential decays. The result suggested a single group of species might predominate in the

Table 5-5. Mathematical models for the declination portion of the zinc or cadmium AAS response to eluate from $SC\text{-}CO_2$ complexometric extraction of fresh and defatted freeze-dried bovine liver.

Matrix	Model[a]	t_1	t_2	t_3	χ^2	% Components			%Removal[b]
						1	2	3	
SRM-Freeze dried live	sum of two	2.50	22.3		0.025	92.0	8.0		90.1
Fresh liver hgt[c] **$SC\text{-}CO_2$+L[d]**	sum of three	1.03	4.51	78.8	0.036	17.5	17.3	65.2	85.6
$SC\text{-}CO_2$	one	0.55			0.208	100			5.6
$Zn(NO_3)_2$ in distilled water	sum of two	1.26	27.3		0.057	15.0	85.0		92.1

[a] Responses were modeled as one, or the sum of two or the sum of three exponential decay curves.

[b] After 30 min extraction at 50 °C and 24.5 KPa as determined by residual level of analyte metal in the sample.

[c] hgt = homogenate.

[d] L = DBDTC ligand.

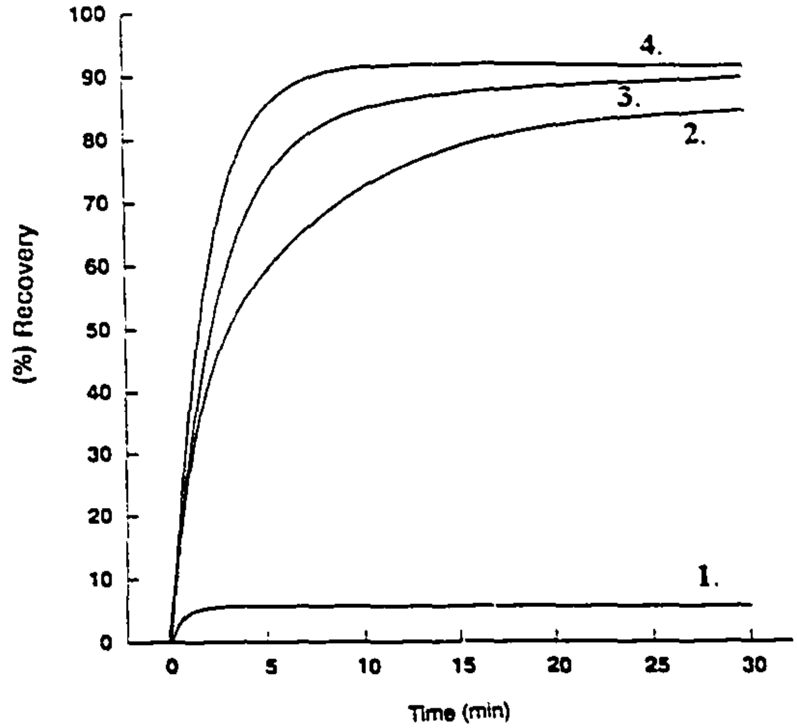


Figure 5-6. Cumulative recovery curves of Zn for extraction from (1.) fresh bovine liver homogenate in the absence of complexing agent (2.) fresh bovine liver homogenate in the presence of complexing agent (3.) freeze dried bovine liver standard in the presence of complexing agent (4.) an aqueous solution. Extractions were performed at 20.04 MPa and 50 °C.

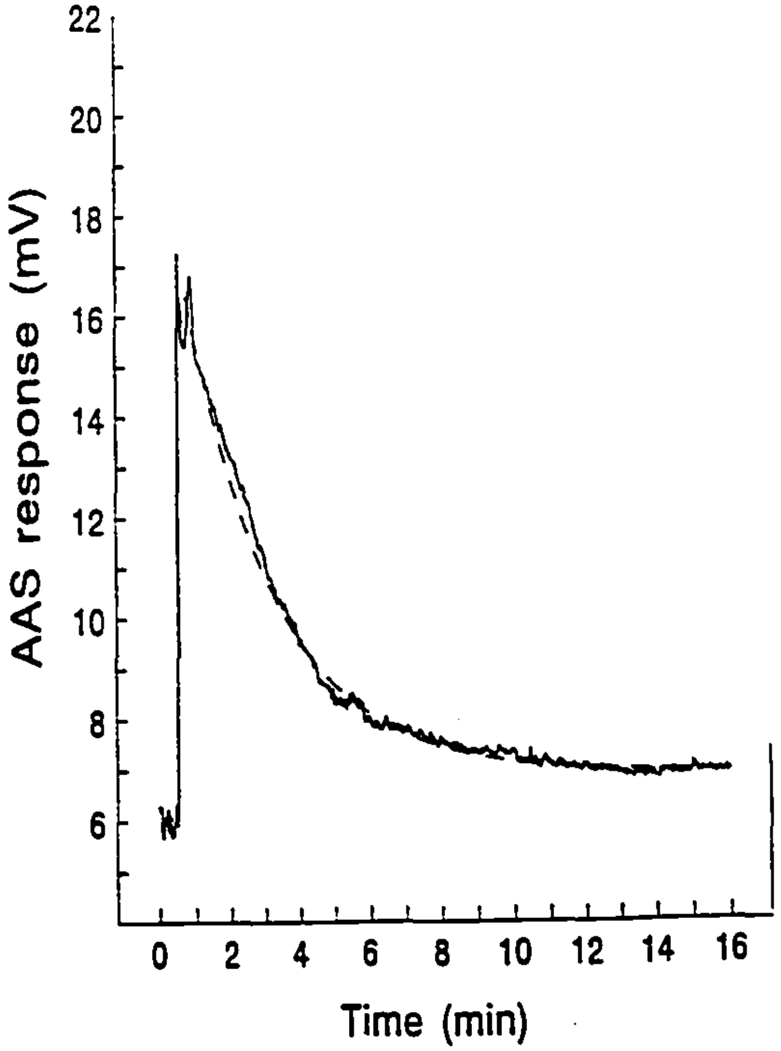
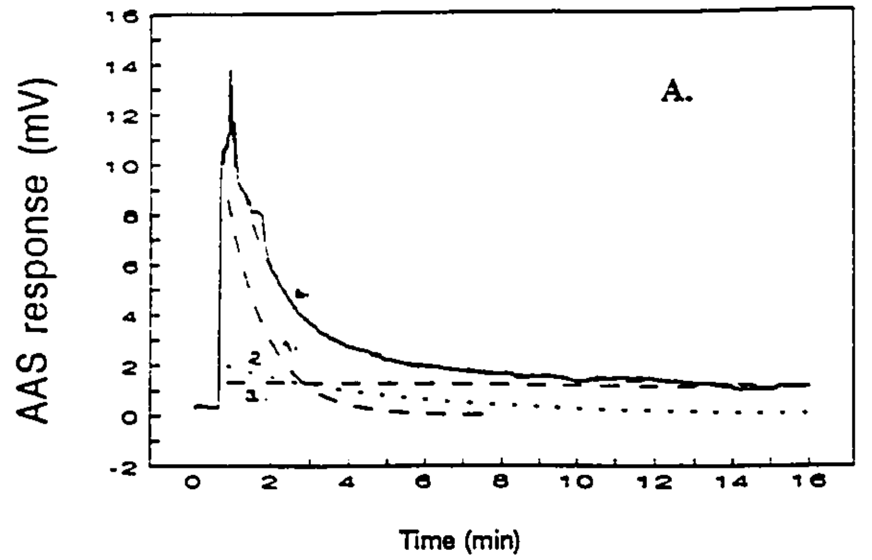


Figure 5-7. AAS response curves for the SC-CO_2 extraction of freeze-dried bovine live standard performed at 20.04 MPa and 50 °C for 30 min. The dashed line represent modeled declination response curve.

extraction process. Because KOH/MeOH was used to suspend/solubilize the sample, presumably, the proteins were denatured and the zinc analyte was released from the binding sites of the biological ligand(s). Figure 5-8 presents the plots of the AAS response *vs.* time for the extraction of zinc from crude bovine liver homogenated. The trace metal(s) content of a biological system can be classified into one of three groups based on interactions with the matrix: (*i.*) free metal ion (*ii.*) metal which is weakly bound to protein, amino acid, or organic phosphates and (*iii.*) metal which is tightly bound to protein, or other biological ligands. During the dynamic complexometric extraction process free metal ions are anticipated to be complexed directly, then mobilized into SC-CO_2. The weakly bound fractions are transposed to the more stable dibutyl dithiocarbamate complexes. It is presumed that this transposition is quiet rapid because the tetraalkylammonium dialkyldithiocarbamate is present in large excess and can rapidly form the metal complexes (stability constant log $K_F \geq 15$), which are more stable than most biological metal complexes (stability constant logK 2-4 for Zn bound to ATP, ADP or AMP. 3-6 for Cu) (Helmut, Volume 1, 1974). A small portion of the metal which is tightly bound to biological ligands (e.g. Zn bound to lysine or tyrosine logK around 24-25) (Helmut, Volume 2, 1974) might be directly mobilized into SC-CO_2 as a biological complex if it is sufficiently non-polar. For this reason, a larger AAS response was observed in Figure 5-8 (A) compared with Figure 5-8 (B). These results suggest that, at least in theory, all these "groups" of analyte metal are included in a extraction process. In the absence of complexing reagent in SC-CO_2, presumably, only the metals which are tightly bound to protein/or other biological ligands in the form of an apolar complex(es) can be purged from the sample. Therefore, the smaller AAS response is detected compared with complexometric extraction. All bovine liver samples were homogenated and diluted to approximately the same concentration.



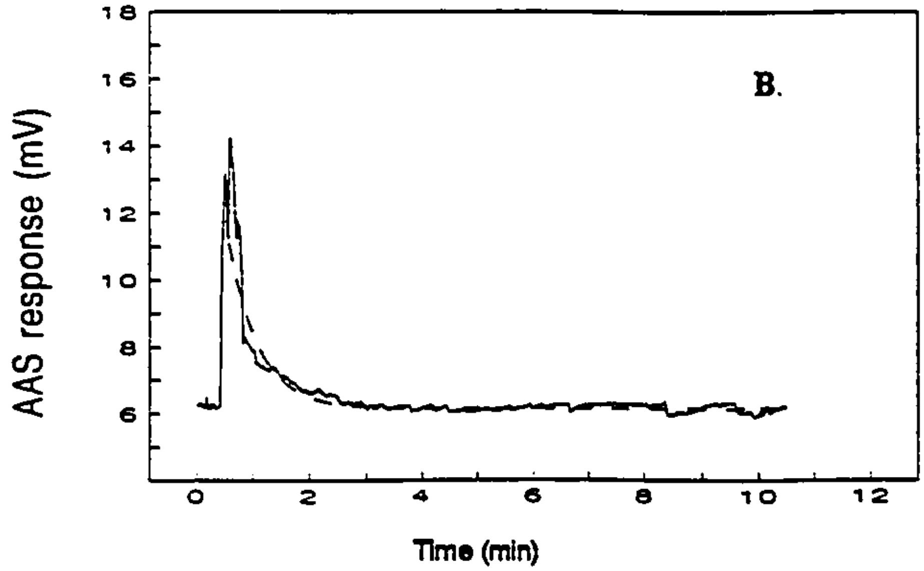


Figure 5-8. AAS response curves for the SC-CO_2 extraction of fresh bovine live in the presence (A.) or absence (B.) of tetrabutylammonium dibutyldithiocarbamate complexing reagent performed at 20.04 MPa and 50 °C for 30 min. The modeled declination portion of the response curve (4) was decomposed mathematically into three component exponential decay curves (1, 2 and 3).

Modeling of the AAS response curve for the complexometric extraction of zinc from crude homogenate bovine liver was performed (Figure 5-8, A. dashed line). The sum of three exponential decays provided a better fitting with the experimental data when compared with either a single or the sum of two exponential decay which suggested that three groups of species might predominate in the extraction process. The dashed line (Figure 5-8, B.) presents the kinetic model for the extraction of zinc from crude homogenate bovine liver in the absence of complexing reagent. A single exponential decay provided the better fit to the experimental data than did either sum of two or three exponential decays. This might suggest that only a single group of species has been mobilized into SC-CO_2. Theoretically, only a fraction which is tightly bound to a biological ligand(s) can be mobilized into SC-CO_2 in the form of a non-polar complex. The resulting models (Table, 5-5), in every case, provided no significant lack of fit at the 99% level of confidence. These initial studies have demonstrated that the experimental approach of *on-line* detection with AAS can be used to investigate the processes associated with supercritical fluid extraction of heavy metals. When compared with the labor intensive steps of conventional liquid-liquid, or solid-liquid extraction, the *on-line* SFE-AAS approach offers some appreciable advantages for investigating trace metals in biological samples. It also reduces manipulative preparation steps, provides an efficient analyte preconcentration and minimizes sample contamination and losses. All these factors can be important sources of error when analyzing biological samples. This experimental technique can also be used to characterize the various "forms" of the analyte metal within a biological sample.

Chapter 6

Characterization of Metal bound to Metallothionein(s) with Supercritical CO_2

6.1 Metallothioneins

More than three decades have been passed since the first publication on metallothionein appeared (Kagi and Vallee, 1960). Numerous biochemical studies have characterized metallothionein as a series of low molecular weight metal-binding proteins which are rich in cysteinyl residues and heavy metals. Spectroscopic features are characteristic of tetrahedral metal-thiolate(-mercaptide) complexes and the behavior of this protein is dominated a strong avidity for Ag^{1+}, Cu^{1+}, Cd^{2+}, Hg^{2+} and Zn^{2+}. This class of metal binding polypeptide is widely distributed throughout the animal kingdom. The most conserved features among the four structural sub groups of this class of protein are the twenty odd cysteine (cys) residues which collectively bind seven divalent metal ions in two separate metal thiolate oligonuclear clusters (M_3Cys_9 and M_4Cys_{11}) buried as "mineral cores" in the interior of the two globular domains formed by the carboxyl- (α-domain) and amino-terminal (β-domain) halves of the polypeptide chain. A simplified model depicting the chain folding of the two domains of rat liver-MT-2 and the Cd-thiolate clusters, determined by nuclear magnetic resonance (NMR) measurements, is presented in Figure 6-1 (A). (Schultze *et al.*, 1988). The disposition of the polypeptide chain and the metal-thiolate clusters of the crystal structure obtained by X-ray diffraction analysis indicate a very close similarity to the NMR structure of Cd-MT-2 (Figure 6-1 B, Robbins, 1991). The structures reveal that the clusters are located as "mineral cores" in the interior of the approximately equal size globular domains each formed by two large helical turns

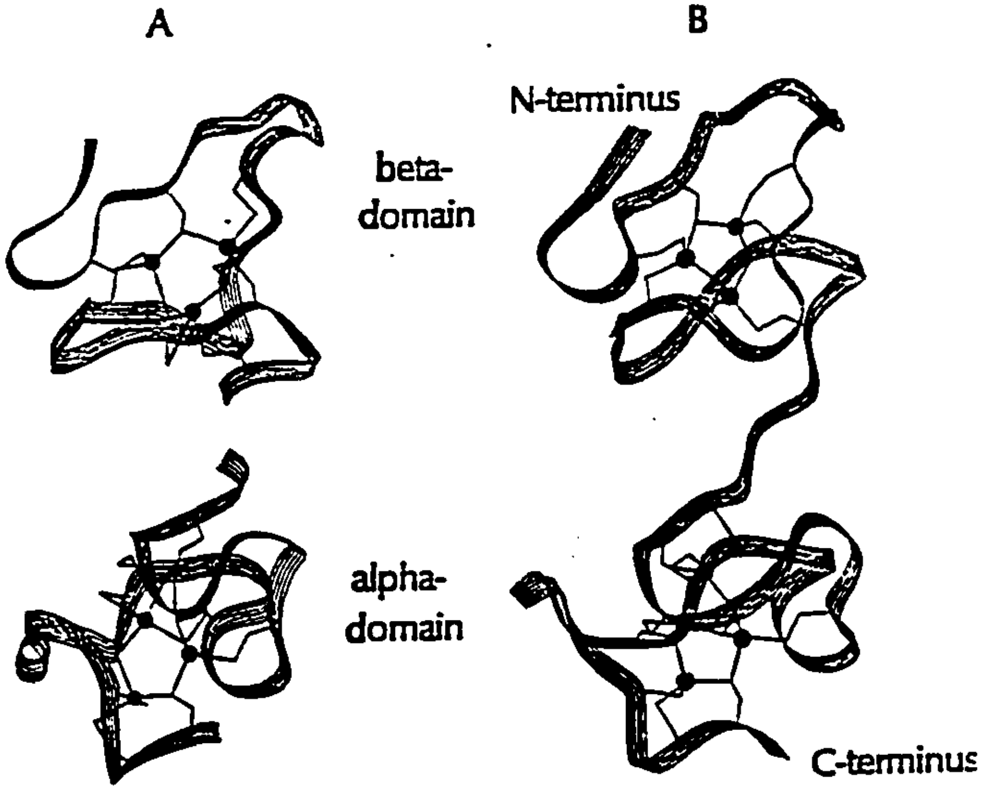


Figure 6-1. A simplified model depicting the folding of the two domains of rat liver-MT-2 and the Cd-thiolate clusters, as determined from NMR measurements (A, Schultze *et. al.*, 1988); The disposition of the polypeptide chain and the metal-thiolate clusters crystal structure (**B**, Robbins, 1991) determined by x-ray diffraction analysis.

of the respective halves of the polypeptide chain. In the amino-terminal beta-domain the chainfold is right handed yet, in the carboxyl-terminal alpha-domain it is left-handed. Despite the fact that zinc is bound more tightly within the α-domain than within the β-domain, zinc is lost from the α-domain to a competing ligand, nitrilotriacetate (NTA) appreciably more rapidly than from the β-domain (Suzuki *et al.*, 1992). Nitrilotriacetate displayed biphasic kinetics of Zn removal from Zn-MT under pseudo first-order reaction conditions and the kinetics of Zn loss from $Ag_6{}^{\beta}Zn_4{}^{\alpha}$-MT were very similar to the more rapid rate of loss in the biphasic rate process.

The objective of the current study was to explore the possibility of applying kinetic speciation with SC-CO_2 to a relatively homogeneous metallothionein sample from a commercial source with a view to gaining insight into the behavior of this molecule during the extraction process.

6.2 Materials and Methods

6.2.1 Chemical Reagents

Metallothionein (catalog # 7641, 5398, Sigma Chemical Co., St Louis, MO, USA) and tris(hydroxymethyl)methylamine (TRIS) were used as received. Tetrabutylammonium dibutyldithiocarbamate was prepared as described (Chapter 2) previously. Solvents were HPLC grade and all other chemicals were ACS Reagent grade or better.

6.2.2 Size Exclusion Chromatography

Extractor eluate, trapped in distilled water, was evaporated to dryness at room temperature under N_2, redissolved in chromatographic mobile phase and applied to the head of a 7.5 mm x 30 cm column Progel-TSD 3000 PW and eluted at 0.7ml/min with 0.15 mmol/ml tris(hydroxymethyl)methylamine buffer (pH 7.5) containing 15% (v/v) methanol. Chromatographic eluate was monitored by AAS for Cd, Cu or Zn content. Occasionally, 10 μl of 50 mmol/L β-mercaptoethanol was injected after 4 min to detect any non protein-bound analyte metal in the sample or the chromatographic column. Cd-metallothionein or Cd-β-mercaptoethanol complex were eluted from the column after 7.6 and 18.4 min respectively.

6.2.3 SF Extractor

The laboratory assembled , analytical scale supercritical fluid extractor (Chapter 2.) was interfaced with a Philips Model PU 9100 AA Spectrometer. In operation, a compressor delivered CO_2 from a syphon-equipped K-type cylinder to extraction vessel. For complexometric extractions, the resulting SC-CO_2 mobile phase was then equilibrated with solid tetrabutylammonium dibutyldithiocarbamate (TBADBDTC) in a saturation vessel (SV) during a 20 min static stage of the process. Analyte(s), (3 mL aqueous solution) contained in a extraction vessel (EV, 4.6 mL capacity) were then sequestered *in situ* and the product dithiocarbamate complexes were partitioned into the SC-CO_2 phase and purged from the aqueous sample during the subsequent dynamic stage of the process. Alternately, the aqueous sample was pre-equilibrated with SC-CO_2 in the absence of TBADBDTC for 20 min then extracted dynamically. Whereas the pressure within the extractor was maintained by the compressor, the flow rate of mobile phase was controlled by a linear restrictor (12-20 cm x 0.050 mm i.d. silica capillary tubing) which was attached to the exit end of the stainless steel transfer line [0.5 mm (o.d.) x 0.16 mm (i.d.)] from the extractor. Eluate was either transferred to an all silica T-tube mounted within the optical beam of the spectrometer or was trapped in 12 mL aqueous solution. Details of the extractor and AAS interface have been provided in Chapters 2 and 3 respectively.

6.2.4 Calibration of HPLC/QTI-AAS System

Calibration of the AAS detector response to analyte metal in HPLC column eluate was performed with a commercial metallothionein standard. Typical quartz tube interface (QTI)-AAS responses to Zn, Cu, or Cd for the flow injection of MT are presented in Figures 6-2 and 6-3. Linear regressions of the detector response to Cd and Zn bound to the metallothionein substrate in the range of 1-10 ng metal (corresponding to 13-104 ng and 0.16-1.2 μg protein respectively) were highly correlated ($R^2 > 0.996$). The results of peak area regressions on concentration are summarized in Table 6-1. Moreover, the slopes of these calibration plots were not significantly different from slopes of calibration curves generated by using the mobile phase to transfer aqueous standards of Cd or $Zn(NO_3)_2$ directly to the detector in a flow injection mode. Thus the presence of protein in the column eluate had no perceptible effect on the detector response to the analyte metal. The detector response was highly repeatable as indicated by the relative standard deviation of 0.56% for three replicate injection of 160 ng protein substrate containing 12.3 ng bound Cd as presented in Figure 6-4.

6.3 Reaction Mixtures of Metallothionein with Excess Ligand

6.3.1 Characterization

Spectrophotometric titrations have indicated that all cysteinyl side chains of the protein are involved in the formation of metal thiolate complexes and, hence, that in accordance with the ratio of the cysteinyl residues to the sum of bivalent metal ions bound, each ion is coordinated to at least three thiolate ligands. This result

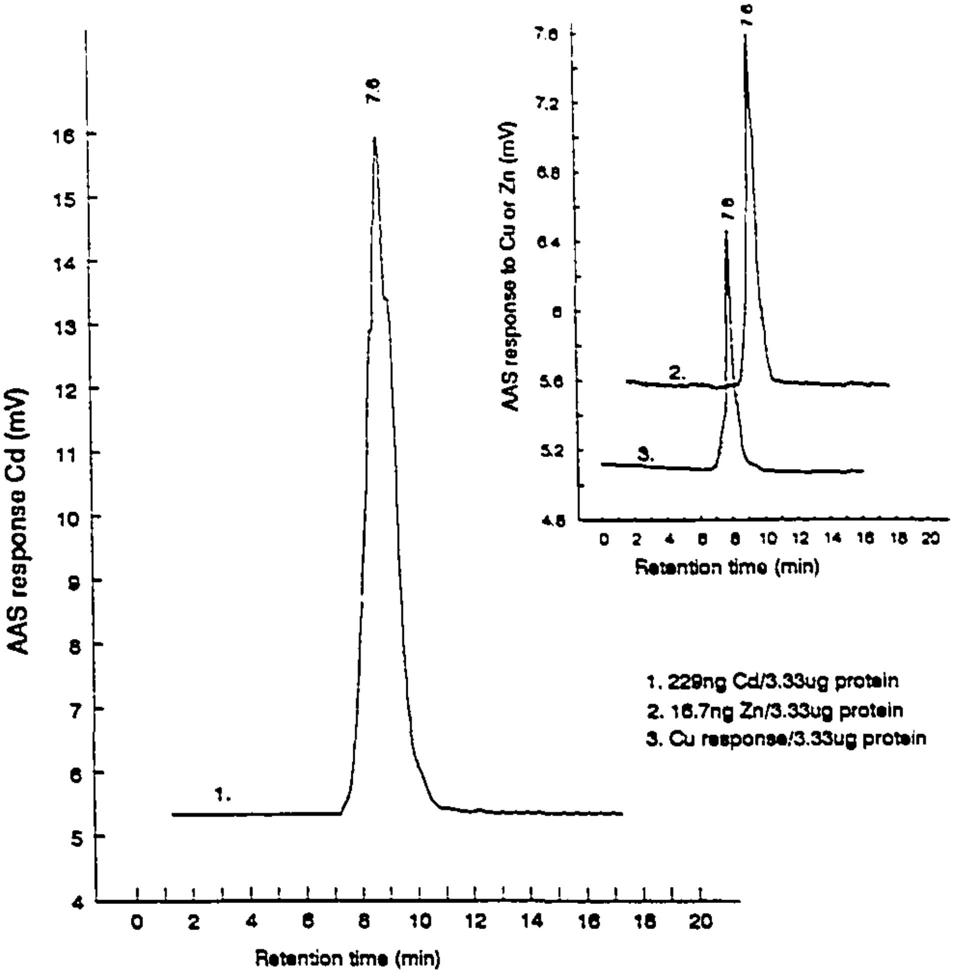


Figure 6-2. QTI-AAS responses for the size exclusion chromatography of 3.33 μg methallothionein (MT) standard which had been eluted with 15 mM tris (hydroxymethyl)methylamine buffer, pH 7.5 containing 15% (v/v) methanol and monitored by AAS for Cd, Zn or Cu.

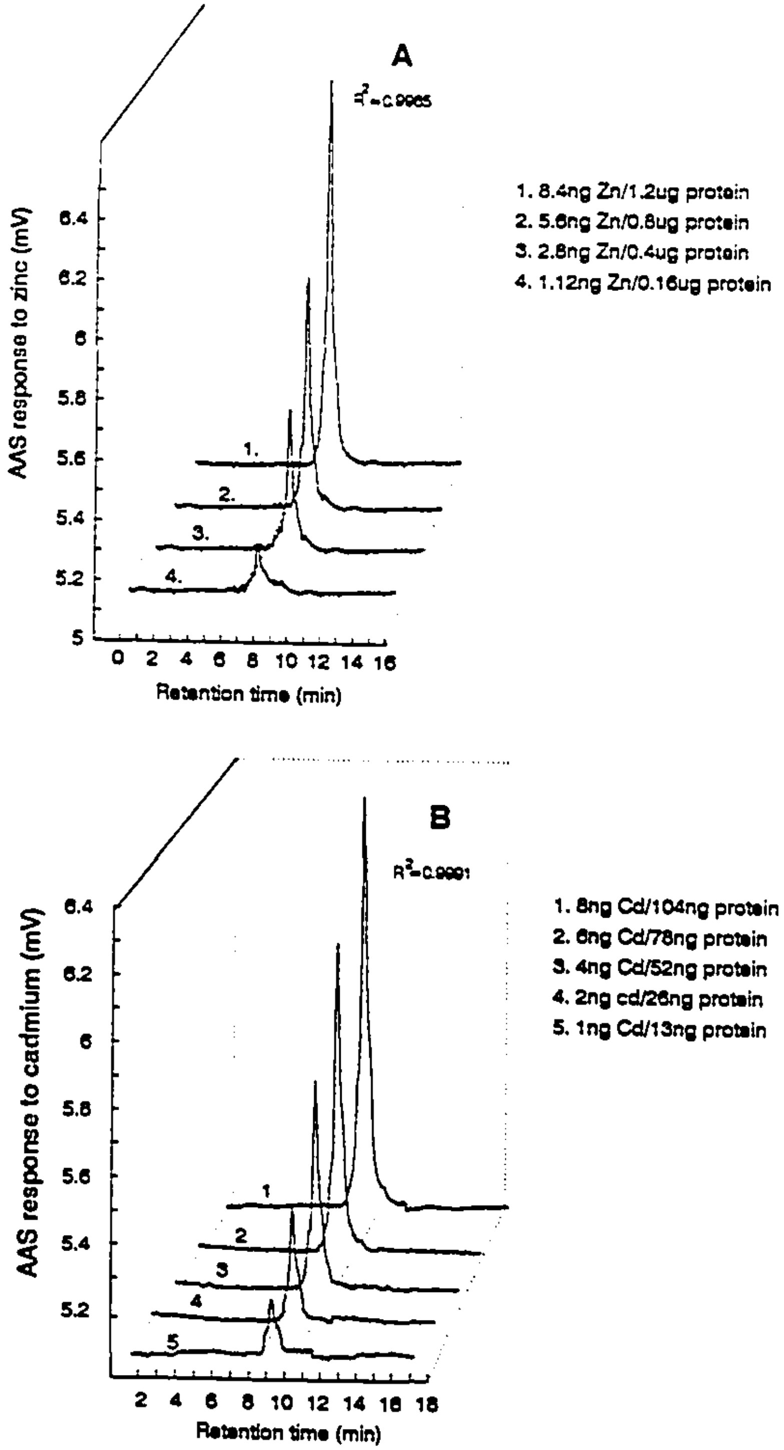


Figure 6-3. Typical QTI-AAS responses for the size exclusion chromatography of various quantities of methallothionein (MT) standard eluted with 15 mM tris (hydroxymethyl)methylamine buffer, pH 7.5 containing 15% (v/v) methanol. Column eluate was monitored by AAS for Zn or Cd.

Table 6-1. Typical AAS responses for the flow injection of various quantities of metallthionein (MT) standard.

Trial	**Analyte**	**Quantity (ng)**	**Peak area (μV sec.)**
1	**Cd**	1.0	5,060
2	**Cd**	2.0	9,939
4	**Cd**	4.0	18,581
4	**Cd**	6.0	28,558
5	**Cd**	8.0	39,026
Linear regression of peak area on quantity of Cd injected			**$R^2 = 0.991$**
$Y = 6.5474 + 4815.6\,[Cd]$			
1	**Zn**	1.1	5,012
2	**Zn**	2.8	13,223
3	**Zn**	5.6	25,451
4	**Zn**	8.4	40,228
Linear regression of peak area on quantity of Zn injected			**$R^2 = 0.991$**
$Y = -412.4 + 4723.97\,[Zn]$			

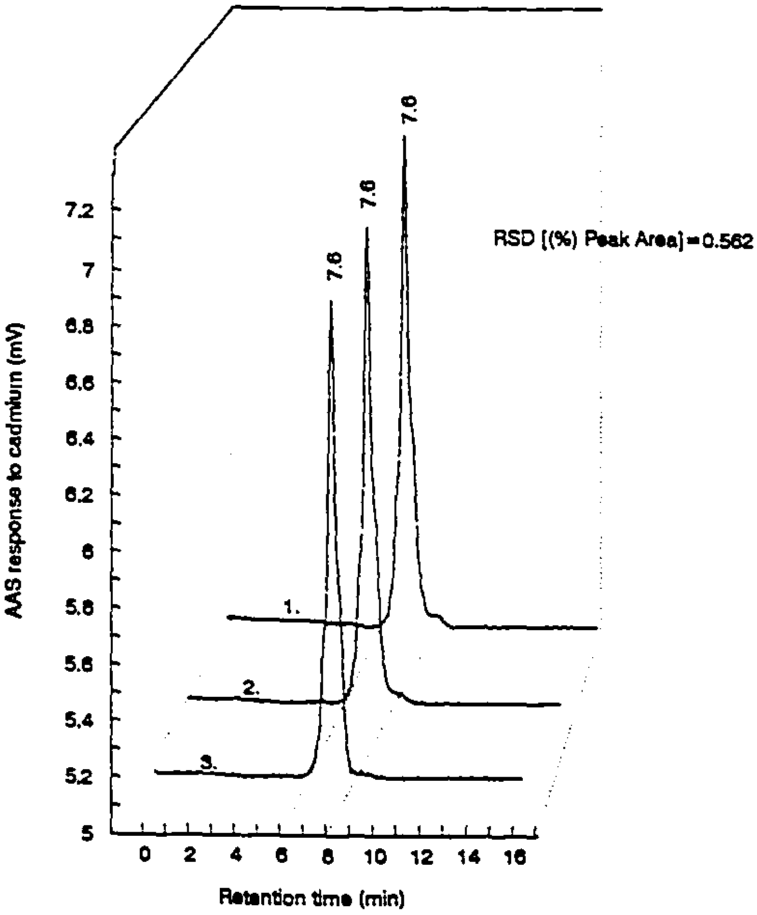


Figure 6-4. QTI-AAS responses for the three replicate chromatograms of 12.3 ng Cu-MT/160 µg MT which were eluted with 15 mM tris (hydroxymethyl)methylamine buffer, pH 7.5 containing 15% (v/v) methanol. Size exclusion chromatographic eluate was monitored by AAS for Cu.

also suggests that certain of the thiolate sites actually bridge between adjacent clusters. The resulting negatively charged trithiolate (trimercaptide) complexes, [Metal^{2+} (Cys$^-$)$_3$]$^-$, are the principal determinants to the overall negative charge of the metal-containing protein. Assuming binding of each metal ion to three cysteinyl residues, dissociation constants of approximately 10^{-25} M and 10^{-21} M were initially calculated for the cadmium and the zinc complexes (Kagi and Vallee, 1961), respectively. At pH 7 the average apparent association (binding) constant for zinc is in the range of 10^{11}-10^{12} /mol, Cd^{II}, Cu^{I} and Ag^{I} are bound more tightly by several orders of magnitude. In copper-metallothionein, the copper became detectable by electron paramagnetic resonance (EPR) measurements only after oxidative treatments. Thus, in accordance with X-ray photoelectron spectrometric measurements, the presence of copper (I)-thiolate units in copper-metallothionein is most probable. Despite binding constants which, at pH 7, are nearly equal to EDTA in avidity and their position within the recesses of the enveloping protein, cluster-bound metal ions are rather mobile in exchange interactions with other metals in solution, with other ligands and with other MT molecules.

Characterization of the metal contained in either the trapped extractor eluate or in the residual sample medium after extraction was performed by size exclusion HPLC interfaced with *on line* detection by AAS. Since the complexing reagent, TBADBDTC, which had been chosen for its solubility in SC-CO$_2$, was not water soluble, a chromatographic mobile phase consisting of 15 % (v/v) methanol Tris-buffer (15 mmol/L pH 7.5) was used to minimize the retention of complexing reagent on the stationary phase. Surprisingly, with identical extractor operating conditions but in the absence of complexing agent, 9.8 % of the Cd was mobilized and recovered in the extractor eluate. Mathematical modeling of the declination portion of the AAS response to levels of Cd in the eluate mobilized by $SC\text{-}CO_2$ in the absence of TBADBDTC (Figure 6-5) indicated an appreciably better fit (chi square, 0.003) of a single exponential decay function relative to models involving the sum of either two or three exponential decay functions. The fitted model to the AAS Cd response curve for this extraction suggested a half-life ($t_{1/2}$) of under 3 min for the Cd in the aqueous sample.

As indicated by the chromatograms of Figure 6-6, approximately 7.7 % of the cadmium present in the sample prior to $SC\text{-}CO_2$ extraction (chromatogram A of Figure 6-6) was purged in the absence of complexing agent and recovered in the trapping solution (chromatogram B of Figure 6-6) in a form which was indistinguishable from the substrate. Specifically, no free metal cation was detected in the chromatograms of extractor eluate when 10 µl β-mercaptoethanol (50 mmol/L in mobile phase) was injected 4 min into the chromatographic run. Similar zinc or copper-specific profiles (in terms of retention time) were obtained (not shown) when size exclusion chromatography of the same solutions was performed with AAS detection at 213.9 or at 324.7 nm respectively.

6.3.2 Ligand Retention

Chromatogram C of Figure 6-6 presents the separation of a synthetic mixture of MT substrate and $Cd(DBDTC)_2$ standard. However, if Zn-MT commercial standard (0.5 nmol) was permitted to react with excess TBADBDTC (0.22 µmol) for 3-120 min, then subjected to size exclusion chromatography (Figure 6-7), the peak area associated with Zn-MT substrate diminished gradually to be partially replaced by traces of higher molecular weight materials [retention time (t_R) 5.7-6.0 min] and more retained fractions at t_R 8.3 and 9.1 min. That the course of this reaction was apparently more complicated than a direct exchange process was

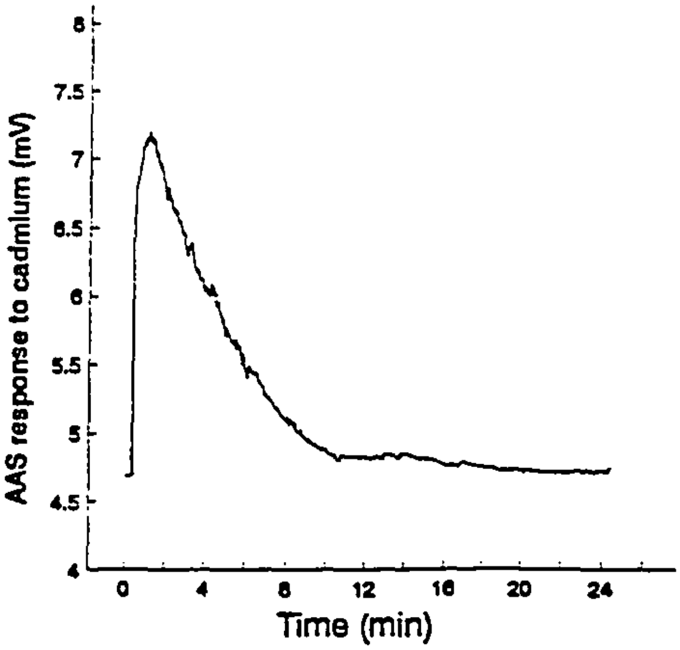


Figure 6-5. *On line* AAS response to Cd in the SC-CO_2 eluate from the extraction of aqueous metallothionein in the absence of dithiocarbamate complexing agent.

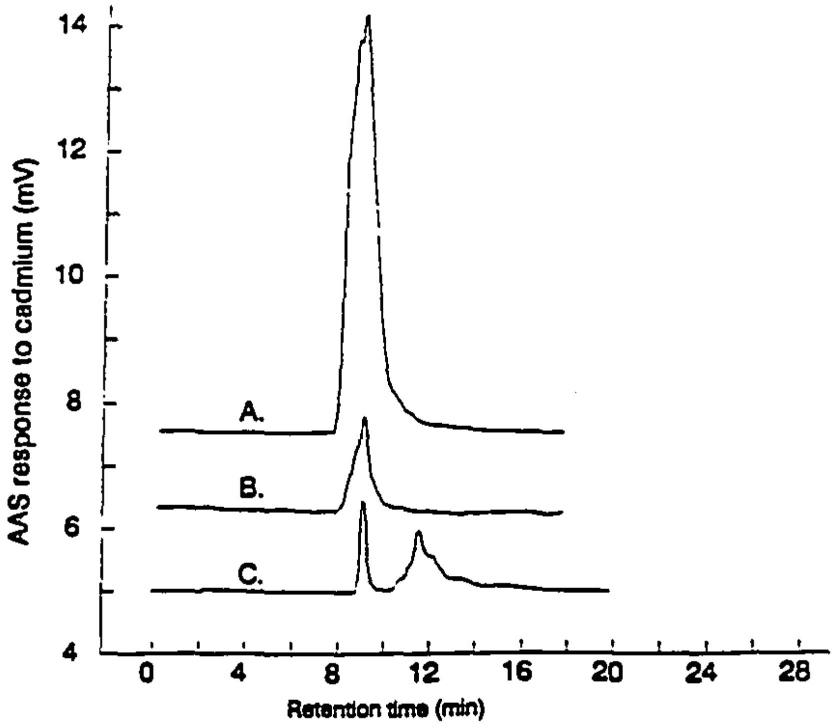


Figure 6-6. AAS Cd-response to column eluate from the size exclusion chromatography of (A.), aqueous metallothionein sample prior to SC-extraction; (B.), trapping solution containing the extractor eluate and (C.) a synthetic mixture of metallothionein and Cd-dibutyldithiocarbamate complex.

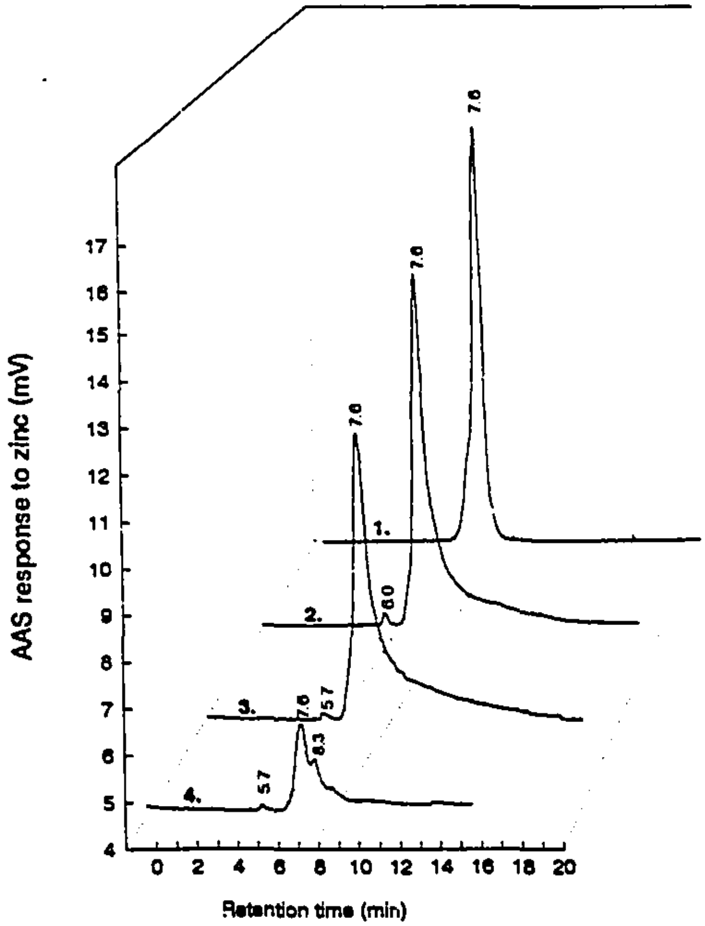


Figure 6-7. AAS Zn size exclusion chromatograms of: (1.) 5 µg metallothionein (MT), or the crude product mixture resulting from the reaction of 5 µg MT with 100 µg TBADBDTC for 3 (2.), 20 (3.) or 120 min (4.)

corroborated by the chromatograms of Figure 6-8 in which MT substrate was mixed with TBADBDTC within the injection loop of the chromatograph then subjected to size exclusion chromatography immediately. The peak areas associate with the various Zn containing fractions are presented in Table 6-2. Interestingly, despite a large excess of complexing ligand, intact Zn-DBDTC was not detected in any of these trails. Apparently, components of the complexing reagent mediate an association among protein monomers (fraction with t_R 6.1 min) and they do not efficiently complex with the Zn over this time frame. Similar results obtained for Cu (Fig. 6-9). Evidence that the TBADBDTC complexing reagent caused appreciable changes in the metallothionein substrate and the SC-CO_2 mobile phase was capable of mobilizing at least a portion of the protein substrate apparently precluded the use of a simplistic kinetic model involving only mobilization via direct exchange of the analyte metal from the protein to the complexing ligand.

6.4 Mobilization of Metallothionein-bound Cu, Zn and Cd

6.4.1 Extractor Operating Parameters

Metallothionein was chosen for study because of the ubiquity of this class of relatively well characterized protein and the avidity of binding of Cu, Cd and Zn to this substrate coupled with their relative ease of exchange. A preliminary complexometric extractions in which SC-CO_2 saturated with tetrabutylammonium dibutyldithiocarbamate was pre-equilibrated during 20 min with 1.85 mg metallothionein, contained in 3 ml distilled water, followed by 60 min of dynamic extraction at 50 °C and 24.05 MPa demonstrated that Cd could be mobilized/purged quantitatively from the protein dissolved in distilled water but that none of the metal could be dislodged directly from the solid substrate using

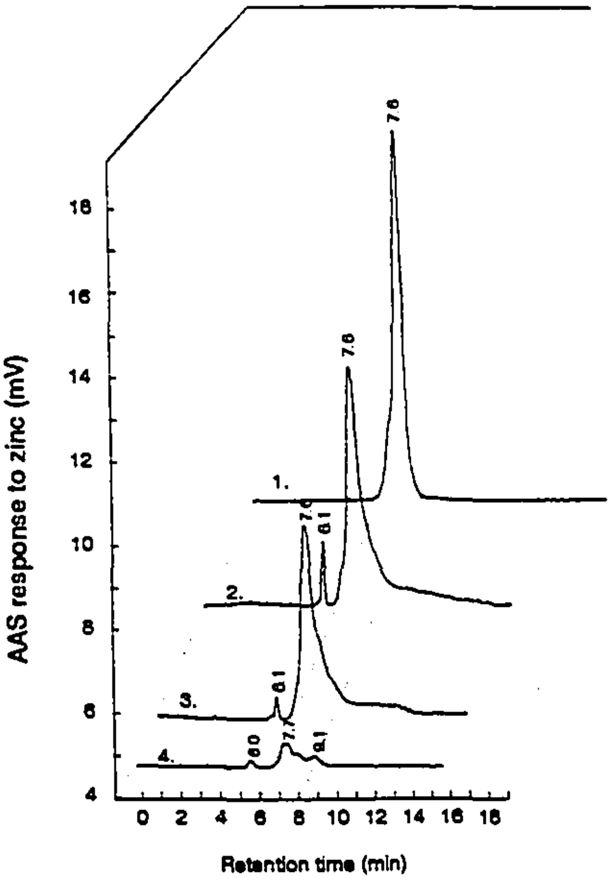


Figure 6-8. Zn-size exclusion chromatograms of the initial reaction mixtures produced by (1.) injecting 10 µg metallothionein (112 ng Zn) alone, or (2. - 4.) by mixing various quantities of this protein with excess TBADBDTC in the injector loop of the chromatograph and injecting immediately. The proportions of reactants and the resulting peak areas are recorded in Table 6-2.

Table 6-2. Peak areas associated with distinct fractions separated by size exclusion HPLC of the reaction mixture of Zn-MT with varying amounts of TBADBDTC.

Chromatogram number	Zn-MT substrate (μg)	TBADBDTC (μg)	Peak area R_t^a (6.1 min)	R_t(7.6 min)	R_t(9.1 min)	Sum
1	10	0		100		100
2	7.3	56	4.1	95.1		99.3
3	6.5	75	1.9	93.7		95.5
4	5.0	500	0.9	6.90	2.5	10.3

[a] Retention time of chromatogram.

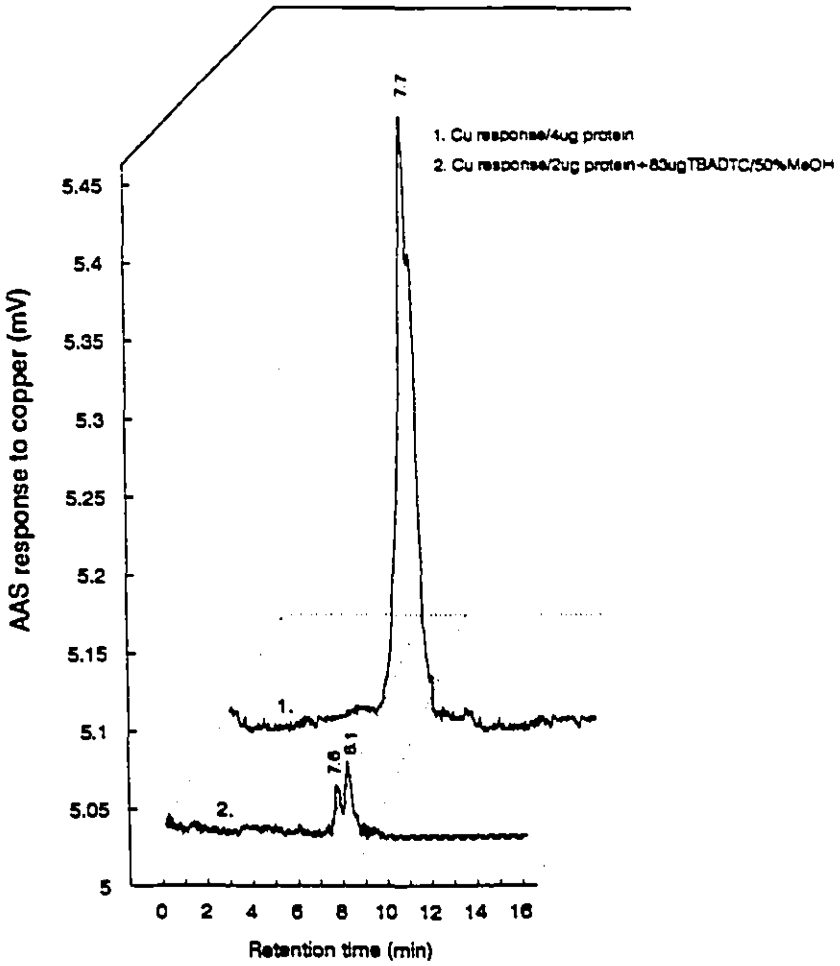


Figure 6-9. AAS Cu-response to column eluate from the size exclusion chromatography (1.) 4 µg MT aqueous protein or (2.) a synthetic mixture of 2 µg MT protein and 83 µg TBADBTC complexing agent. The mobile phase consisted of 15 mM tris(hydroxymethyl)methylamine buffer, pH 7.5, containing 15% (v/v) methanol.

this technique. Quantitation was performed by determining the Cd content of the aqueous sample before and after the extraction. In the absence of complexing reagent, approximately 12, 13 and 15 % of the MT bound Cd, Zn and Cu was mobilized as protein bound analyte from 0.1 mol/ L $NaHCO_3$ solution. However, loss of Zn from the protein was extensive. By changing the extractor operating conditions 19 % of the Cd-MT, 12 % of the Cu-MT and 18 % of the Zn-MT was recovered intact in the extractor eluate and the remaining substrate was apparently unchanged as judged by size exclusion chromatography.

Modest changes in the extractor operating parameters caused appreciable changes in the recoveries of protein bound analyte metal. For these trials (Table 6-3), metallothionein, in 0.1 mol/L sodium bicarbonate, was pre-equilibrated with SC-CO_2, during a static phase of the process then extracted for 60 min during a dynamic phase of the operation. Both the extractor eluate which was trapped quantitatively and the residual aqueous solutions were assayed by size exclusion HPLC with *on line* Cd-, Zn- or Cu- specific AAS detection. The choice of 60 min dynamic extraction was based on the AAS response to Cd in the extractor eluate which indicated that virtually all of this analyte metal was purged within 12 min of extraction.

6.4.2 Factors Relative to Recoveries

All mammalian metallothioneins characterized thus far are negatively charged at neutral pH. The molecular weight range is from 6500 to 7000 depending on the metal composition. On lowering the pH, the metal ions bound to the protein are released yielding apometallothionein (sometimes referred to as thionein). The pH at which 50% of the metal is dissociated depends on the protein concentration and

Table 6-3. Variations in the efficiency of mobilization and the residual content of metallothionein-bound cadmium, zinc and copper as a function of the extractor operating parameters.

Trial	Protein	SFE operating conditions[a]				% Recovery (± RSD[b])			% Residual (± RSD[b])		
	(mg)	Static (min)	Dynamic (min)	P (MPa)	T (°C)	Cd-MT	Zn-MT	Cu-MT	Cd-MT	Zn-MT	Cu-MT
1	2.9	20	60	24.1	50	12.6±0.5	12.9±0.4	13.9±0.7	86.9±1.2	29.1±1.2	65.0±1.2
2	2.2	20	60	20.6	39	6.1±0.3	5.8±0.3	2.3±0.2	93.1±0.9	92.2±1.2	95.1±1.2
3	1.6	0	60	26.1	39	14.4	2.1	4.5	85.6	20.2	89.3
4	1.5[c]	0	60	26.1	39	19.2	2.0	5.7	69.1	95.8	78.6
5	2.15	20	60	26.8	39	19.1[d]	18.1[d]	11.8[d]	79.5	78.7	80.5

[a] Static equilibrations were performed at 20.6 MPa.

[b] Relative standard deviation based on three replicate extractions.

[c] Extraction was performed with 5 % (v/v) methanol added directly to the SC-CO_2 mobile phase.

[d] Cumulative recovery resulting from three sequential 20 min extractions with the pressure vented to atmospheric between successive extractions (see Table 6-2).

on the metal ion concerned. It ranges between pH 3.5 - 4.5 for zinc, pH 2.5 - 3.5 for Cd, and is below pH=1 for Cu (Kagi and Vallee, 1960).

The extractor operating conditions apparently did not affect the total Cd loading on the protein. The sum of the % of Cd bound to MT (Cd-MT) recovered in the extractor eluate plus the % Cd-MT in the residual sample after extraction accounted for virtually all of the Cd (mean for trials 1-5, 97.1 ± 4.9 %) which was initially present in the MT sample. For these trials, a mean 87.7 ± 8.5 % of initial Cu-MT was accounted for and indicates that, on average, some 12 % of the bound copper was lost to the aqueous medium. By contrast, losses of bound Zn to the medium were highly variable. A mean of 71.4 ± 36.5 % of the initially protein-bound Zn remained in this form after the extraction.

Three replicate extraction at 50 °C and 24.05 MPa mobilized a mean of 12.6, 12.9 and 13.9 % of the Cd-MT, Zn-MT and Cu-MT respectively which was initially protein bound. Whereas the residual Cd in the aqueous solution remained protein bound, appreciable portions of the analyte Zn and Cu (57 and 20 % respectively) apparently had been lost to the solvent medium. If loss of these analytes to the aqueous medium was kinetically controlled, it was reasoned that lowering the temperature of the extraction might decrease the rate of loss while at the same time increasing the density (and solvating power) of the mobile phase. It was also unclear whether the 20 min of static pre-equilibration prior to dynamic purging was responsible for the loss of analyte metal from the protein. A subsequent extraction (trial 2) involved a temperature of 39 °C and pressure of 20.61 MPa. Whereas the mean recoveries of protein-bound analyte Cd, Zn and Cu were decreased appreciably (6.1, 5.8 and 2.3 % respectively) relative to the recoveries at the higher operating pressure/temperature, virtually none of the metals were lost from the protein surface (protein bound analyte accounted for 99.2, 98.0 and 97.4% of the Cd, Zn and Cu respectively which was initially bound to the MT substrate). In two other trials (3 and 4), the static pre-equilibration step was omitted and the operating pressure was increased to 26.11 MPa. Relative to trial 2, a direct dynamic extraction (trial 3) improved the recoveries of Cd-MT (14.4 %) and Cu-MT (4.5 %) but decreased the recovery of Zn-MT even further (2.1 %). Moreover, the loss of Zn from the protein was extensive. A more polar mobile phase (SC-CO_2 containing 5 % v/v methanol and saturated with water, (trail 4) further increased the recovery of Cd-MT (19.2 %), did not affect the recoveries of Zn-MT or Cu-MT appreciably, but did decrease the loss of Zn (2.2 %) and Cu (15.7%) from the protein. Collectively these trials suggested that the static pre-equilibration step was not responsible for the loss of metal from the protein, that higher operating pressures increased the mobilization of protein-bound analytes and indicated that the substrate protein could not have been homogeneous with respect to an average metal loading. For operating conditions under which loss of analyte metal from the protein was minimal, changes in operating conditions would have been expected to result in comparable changes in the recoveries of each of the protein bound metals. It is known that the metallothionein fraction from the livers of rabbits which have been challenged with Cd, can be separated into two isoforms by ion exchange chromatography. Even if these two isoforms had appreciably different solubilities in SC-CO_2, it is difficult to rationalize the observed changes in recoveries of the three analytes with changing extractor operating conditions if it is assumed that each of the separate isoforms is characterized by a homogeneous loading of these metals. It seems more probable that appreciable heterogeneity with respect to metal loading exists among the separate isoforms as well.

If the increasing acidity of the sample solution which accompanied its saturation with SC-CO_2 was the dominant cause of the low analyte recoveries, it was reasoned that operation of the extractor in a cycling mode might improve the mobilization of protein-bound metals. An extraction procedure was envisaged in which an initial fraction would be collected, the extractor would be vented to atmospheric pressure then repressurized and a second fraction collected without any static pre-equilibration stage between the collection of fractions. This depressurization-repressurization cycle could be repeated any number of times. To test this hypothesis MT substrate, in 0.1 mol/L $NaHCO_3$, was pre-equilibrated with SC-CO_2 for 20 min then three successive fractions were collected separately with a depressurization step inserted between the collection of fractions. The quantities of protein-bound analytes in each of the fractions (Fig. 6-10) and in the residual solution (after 3 successive 20-min extractions) are recorded in Table 6-4. Appreciable quantities of each analyte was recovered in the second and third fractions and collectively the fraction of mobilized analyte plus the residual protein-bound metal in the aqueous sample accounted for virtually all of the metal initially present in the substrate (98.6 % Cd-MT, 96.8 % Zn-MT and 92.3 % Cu-MT). With the exception of the Cu-MT, losses of analyte metal from the protein were minimal. For comparison, these results have been summarized as Trial 5 in Table 6-3. Collectively, these observations suggest that the decrease in pH results in a change in the protein conformation (possibly with a partial unfolding of the globular domains) which renders the substrate more hydrophilic. This conformational change to a more polar conformation apparently is reversible. When the pressure is reduced to atmospheric, the residual substrate protein resumes a more lipophilic conformation preferred in more alkaline solutions. These pH induced conformational changes occur without appreciable loss of metal analyte from the protein. Finally, it is apparent that the combination of the static

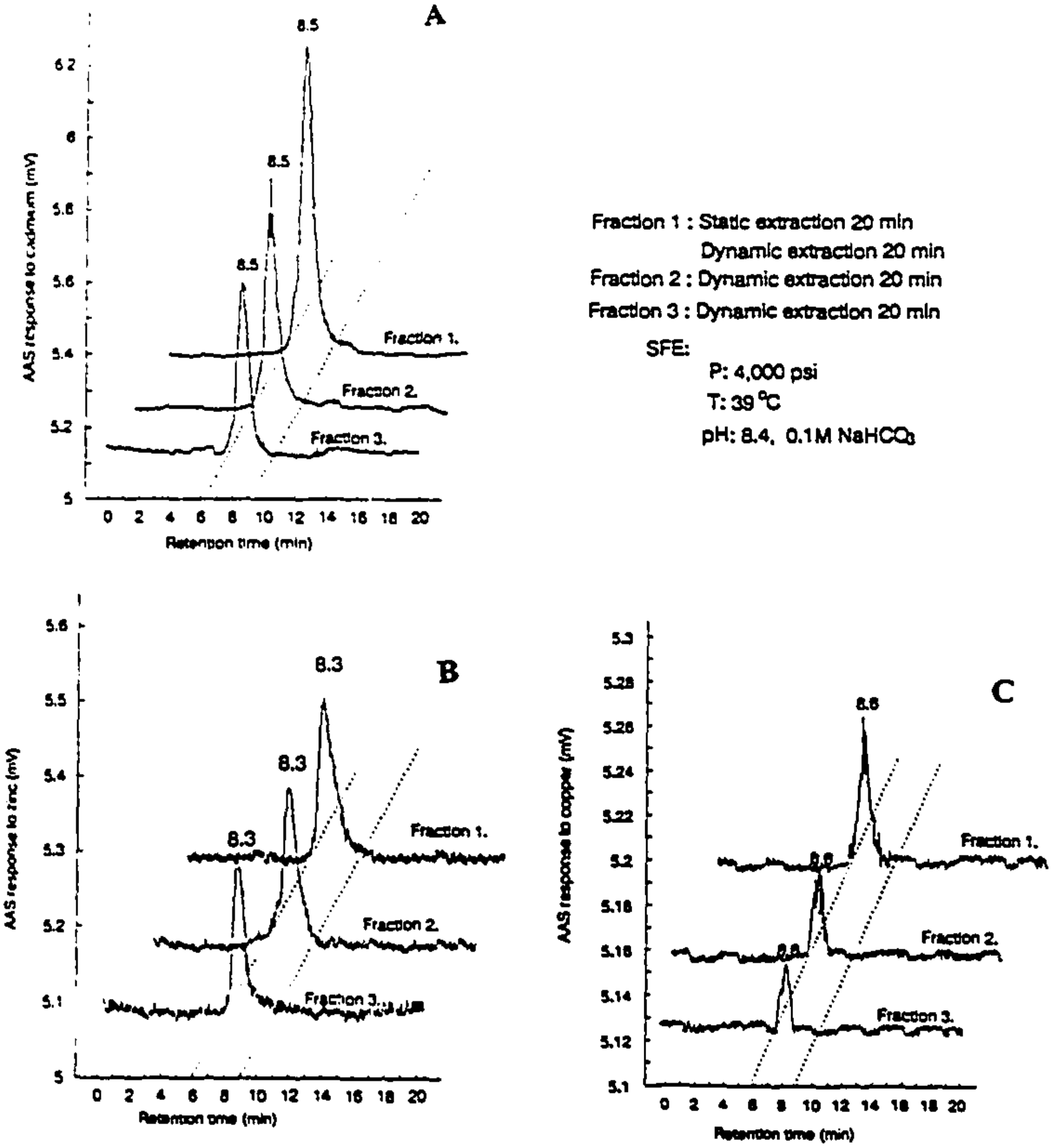


Figure 6-10. The size exclusion chromatograms represent the mobilized fractions collected during three successive extractions (at 26.80 MPa and 39 °C) of commercial metallothionein standard dissolved in 0.1M $NaHCO_3$. A, Cd-MT; B, Zn-MT; C, Cu-MT. The % recovery corresponding to each fraction is recorded in Table 6-4.

Table 6-4. Variations in the recoveries of metallothionein-bound cadmium, zinc and copper from aqueous solution with the number of extractions and levels of these protein-bound analytes in the residual aqueous solution.

Analyte	**(%) Recovery[a]**				**(%) Residual**
	Fraction 1	**Fraction 2**	**Fraction 3**	**Sum 1-3**	
Cd-MT	7.5	6.4	5.2	19.1	79.5
Zn-MT	6.3	6.2	5.6	18.1	78.7
Cu-MT	4.4	3.8	3.6	11.8	80.5

[a] The sample, 2.9 mg protein in 0.1 M $NaHCO_3$ (3mL), was equilibrated (20 min) with SC-CO_3 (20.6 MPa, 39 °C). Then subjected to three successive 20 min dynamic extractions (26.80 MPa, 39 °C). The pressure in the extractor was decompressed to atmospheric between successive extractions.

pre-equilibration step plus the 40 min of the first two dynamic extractions was insufficient to cause complete equilibration of the metals among the two isoforms of the substrate. It also seems unlikely that an efficient recovery can be achieved using SC-CO_2 as the mobile phase. The use of a different supercritical fluid of comparable polarity which would not change the pH of the sample medium appreciably would offer considerable promise for the determination of Cd, Zn and Cu bound to metallothioneins.

Chapter 7

Conclusion and Summary

A novel approach to heavy metal(s) characterization within a complicated sample matrix and the decontamination of that matrix has been developed. These studies indicate that metal analytes, both cations and/or anions, can be rapidly and efficiently removed from aqueous, biological or environmental matrices by *in situ* chelation or ion pairing followed by dynamic mobilization of the resulting product into SC-CO_2. As demonstrated by these studies, the rates of mobilization can be increased appreciably by acidifying the sample matrix prior to the extraction. It also seems likely that rates of mobilization could be further increased by (*i.*) increasing the density of the mobile phase (higher extractor operating pressures), (*ii.*) adding a polar modifier to the mobile phase, and/or (*iii.*) identifying other complexing reagents (ion pairs) which would result in appreciably less polar chelates when reacted with metal analyte(s), and/or (*iv.*) reducing the head space volume above the sample solution.

A series of tetraalkylammonium dialkyldithiocarbamate ion pairs or tetraalkylammonium halide (R_4NX) were synthesized and evaluated for rates of mobilization and efficiency of purging metals from a variety of matrices. A novel all quartz T-tube interface and a modified nebulizer assembly for coupling aqueous, methanolic, or SC-CO_2 solvent streams with *on-line* detection by AAS were developed. For the all silica quartz T-tube interface, the limit of detection for analyte element (Cd, Cu, Zn, Mn, Pb, Se or As), was in the sub-nanogram to low picogram if standard was flow injected into the mobile phase. By modifying the nebulizer assembly of a conventional AAS instrument with supplementary heating of the air (to 200 °C), the number of metal analytes which can be detected in SC-CO_2 extractor eluate can be increased to include Cr and Fe and presumable other elements as well. A device capable of operation with up to 2 L/min of decompressed CO_2 was obtained. For volatile elements such as Cu or Cd, the LOD for flow injected analyte was to low ng which represented a 10-fold improvement over conventional operation. These sensitivities for *on line* detection permitted differences in the rates of mobilization of analyte metal from different matrices to be explored as a technique for probing the interactions of the analyte metal with the matrix. The resulting declination curves with time can be decomposed mathematically to detect and quantify different analyte metal species. Species which differ appreciably in their relative rates of transposition/chelation can be resolved by their different rates of mobilization. The approach of SC-CO_2 extraction coupled with *on-line* detection by AAS provides (*i.*) a novel method for characterizing different physical and/or chemical forms of an analyte trace metal based on differences in their relative rates of mobilization. To the extent that successful mathematical models can be developed for the extraction process, (*ii.*) this approach can be used to estimate analyte metal burdens in samples without having the complete the extraction, and (*iii.*) *on-line* detection will be useful to optimize the extractor operating parameters so as to maximize rates and efficiencies of metal mobilization. These results also suggest that a kinetic analysis of the changes in rates of analyte purging with time can provide additional information on the composition of the sample matrix. Thus, providing sufficient data points can be recorded, not only will an estimate of the level of metal analyte be available but also estimate of the avidity of interaction of the analyte with component(s) of the matrix can be obtained.

To study the influence of natural complexants on the mobilization/purging of copper from aqueous media into supercritical carbon dioxide, the presence of FA appreciably retarded the rate of Cu mobilization from aqueous media but did not eliminate this complexation/purging process entirely. Recoveries from Cu-FA solutions or Cu-FA-sand suspensions were dependent on both the Cu-loading and the level of FA in the sample. For the 1.2 μmol Cu + 1.05 μmol FA, most, if not all of this analyte was mobilized by a process which was adequately described by pseudo first-order kinetics. However for more concentrated Cu-FA solution, an apparent coagulation/precipitation caused a portion of the bound Cu to become more labile, possibly by release from the FA, while fixing the remainder.

For zinc but not for copper, SC-CO_2 extractions under conventional operating conditions caused a portion of the analyte metal to be transposed to a less available form (possibly $ZnCO_3$) which was purged from the extractor at an appreciably slower rate relative to other metal cations such as Cu, Cd, Mn or Pb. Although this apparent precipitation is not supported by species distribution models based on thermodynamic data, solutions of Zn after 40 min (but not after 24 h) incubation at 50 °C and 24.16 MPa, contained a white, acid soluble precipitate which was slowly purged from the extractor under these operating conditions. To my knowledge, this is the first reported demonstration of the extraction of Zn from $ZnCO_3$ suspensions.

Whereas the recoveries of Cu from Cu-sand suspensions, which had been equilibrated in the dark for up to 24 h, were reasonably efficient (≈ 80 %), addition of Cu-FA solutions to sand suspension decreased the recovery in all cases. The presence of sand in the samples caused an appreciable change in the AAS Cu-response curves such that pseudo first-order kinetics no longer provided an acceptable fit to the analytical data. The cause(s) for this more complex behaviour, which was observed for all Cu-FA-sand mixtures, is not known although silica is known to adsorb CO_2 from both the gas phase and from aqueous solutions. A coagulation/precipitation phenomenon was evident in the 2.4 and 4.8 µmol/mg FA loading which apparently caused a portion of the analyte Cu to become physically entrapped within the precipitate. Oxalic acid, added to a separate aliquot of the 2.4 µmol/mg FA/sand incubation mixture caused only a portion of the entrapped Cu to be resolubilized but also appreciably retarded the rate of purging of the analyte from the extractor. The influence of various equilibration times of fulvic acid and sea sand with metal solution on the rates of metal analyte extraction suggests that by monitoring the changes in rates of mobilization/extraction with time it is possible not only to quantify total levels of extractable analyte but also to distinguish between different analyte species based on their rates of transposition to an extractable form.

An alternate strategy employing an ion pairing reagent was developed for the selective mobilization of anions including chromate, selenate and selenite. At 30.6 MPa and 80 °C, SC-CO_2 solubilized sufficient tetrahexylammonium bromide (THAB) to effect the efficient purging (93 %) of selenate (60 µg) from aqueous solution during 40 min extraction. At 24.1 MPa and 50 °C, there was little difference in the recoveries (66 vs. 62 %) using THAB or tetrabutylammonium dibutyldithiocarbamate as pairing reagent. Selenite or chromate was also efficiently mobilized with either reagent. Mathematical models of the declination in AAS response with time suggested that whereas selenite (half-life, $t_{1/2}$ = 3.6 min) was mobilized at a rate which was not appreciably different from cation-dithiocarbamate complexes, chromate was mobilized appreciably more slowly ($t_{1/2}$ = 19.5 min) under identical extractor operating conditions. The Cr and Cu in an aqueous suspension containing CCA wood preservative were simultaneously mobilized using a mixture of THAB and TBADBDTC. Despite appreciable interactions with other components of the sample, 89 and 98 % respectively of the Cr and Cu burdens were purged from the sample during 45 min extraction using a decompressed flow rate of 2.2 L/min. These results suggest that mobilization of toxic metal anions from environmental waste suspensions is a promising technique for remediating polluted media.

In the studies of the supercritical fluid extraction with CO_2 for the characterization of Cd, Cu and Zn bound to metallothioneins, variations in the recoveries of protein-bound analytes under different extractor operating conditions suggested that the low recoveries might be the result of the hydrophilic nature of metallothionein or of pH induced changes in protein conformation that were reversible. A description for this protein which is consistent with the observations from these studies is one of an amazingly malleable molecule which is capable of dynamic changes with respect to the positions of bound metals. Not only do the constituent metals exchange positions via intermolecular transfers, the molecule is able to partially unfold with a concomitant change in overall lipophilic character. This unfolding is quite reversible. It is difficult to explain the extraction efficiencies based on a homogeneous distribution of analyte metals within the various binding sites of the protein. Even within this "standard" material, it seems more likely that different congers are present with appreciably different solubilities in SC-CO_2. Nevertheless, the approach of supercritical fluid extraction (with or without added complexing reagent) coupled with *on-line* detection by atomic absorption spectrometry shows great promise as a speciation technique for protein bound heavy metals.

In summary, the feasibility of extracting heavy metals with supercritical carbon dioxide has demonstrated certain merits. When combined with different extraction modes, sample pretreatments and kinetic modeling, various "forms" of the analyte metal in a biological/environmental sample can be estimated. The target analytes are detected as their dithiocarbamate chelates. Chelation followed by purging of volatile product complexes from the sample matrix can, in theory, provide a degree of selectivity over other potentially interfering metals present in the matrix. With this approach, extensive sample pretreatment and separation are virtually eliminated. In comparison with the *off-line* technique, the absence of sample handling between extraction and analysis is conducive to higher recoveries with less operator intervention. It also eliminates possible analyte loss or contamination. Sample preparation methods normally used by analytical chemists are both time and solvent consuming. Generally, two thirds of the total analysis time are devoted to sample preparation and this step accounts for at least one third of the error generated during the performance of an analytical method. The improvement of SFE sample preparation will therefore lead to a reduction in analysis time and a greater precision of results.

The technique clearly has certain limitations in its present state of development: (*i.*) extraction efficiencies can be variable depending on the specific sample matrix; (*ii.*) the complete range of elements which will be extracted has not yet been determined; and (*iii.*) the extent of chelation/metal extraction has not yet been studied for a wide variety of sample matrices. Further studies should be directed towards (*i.*) expanding the range of elements which can be detected by the SFE-FAAS system (*ii.*) removing excess reagent (especially tetraalkylammonium cation) which is left in the sample after extraction (*iii.*) identifying other more environmentally friendly complexing or ion pairing reagents, and (*iv.*) improving the efficiency of mobilization of heavy metals from environmental matrices such as soils or sediments. In total, these studies have demonstrated that the extraction of heavy metals with supercritical carbon dioxide with *on-line* detection are interesting developments with potentially extensive applications for environmental remediation. There can no doubt that there will be other successful applications of this approach in the future.

Original Contributions to Knowledge

1. The first reported successful coupling of supercritical fluid extraction with *on-line* detection by atomic absorption spectrometry (AAS). Two interface designs proved to be both efficient and robust.

2. A series of tetraalkylammonium dialkyldithiocarbamates provided to requisite volatility and solubility in SC-CO_2 (under conventional extractor operating conditions) to efficiently mobilize both transition metal cations and anions (chromate, selenate and selenite) from aqueous solutions.

3. A novel design of a quartz T-tube interface for coupling AAS with flowing streams of aqueous, methanolic, or SC-CO_2 solvents provided sensitive responses to transition metals.

4. Attempts to were made to characterize the different physical and chemical forms of the analyte metal based on differences in the relative rates of mobilization of these analytes into SC- CO_2.

5. It was demonstrated that Cu could be extracted virtually quantitatively from mixtures of fulvic acid and/or oxalic acid in the solution phase. Recoveries of this analyte that were less than quantitative were the result of a coagulation/precipitation of the fulvic acid which apparently encapsulated a portion of the analyte.

6. A portion of the zinc analyte in aqueous medium was transposed to a less available form (probably $ZnCO_3$) under the conventional extractor operating conditions. Prolonged equilibration of the analyte solution with the solvent resolubilized the precipitate.

7. Although inefficient, the extraction process was able to mobilize mammalian metallothionein without disrupting/dislodging the bound Cu, Cd or Zn. Metallothionein standard from a commercial source proved to be heterogeneous with respect to the metal loading.

REFERENCES

Alexandrou, N.; Lawrence, M.J. and Pawliszyn, J. 1992. Clean Up of complex Organic Mixtures Using Supercritical Fluid and Selective Adsorbents. *Anal. Chem.* 64, 301-311.

American Wood Preservers Association, 1978. Standard P5-78, Washington, DC.

Ashraf-Khorassani, M.; Boyer, D.S. and Levy, J.M. 1991. Optimization of Experimental parameters for the Determination of Polymer Additives Using *On-line* Supercritical Fluid Extraction/Supercritical Fluid Chromatography. *J. chromatogr. Sci.* 29, 517-521.

Ashraf-Khorassani, M.; Memarian, M. and Angaji, M. 1992. Extraction of Biological Markers from Sedimentary Rock Using Supercritical Fluid Carbon Dioxide. *Fresenius J. Anal. Chem.* 344, 492-496.

Ashraf-Khorassani M. and Taylor, L.T. 1989. Qualitative Supercritical Fluid Chromatography/Fourier Transform Infrared Spectroscopy Study of Methylene Chloride and Supercritical Carbon Dioxide Extracts of Double-Base Propellant. *Anal. Chem.* 61, 145.

Ashraf-Khorassani M.; Heligeth, J.W. and Taylor, L.T. 1987. Separation of Metal-containing Compounds by Supercritical Fluid Chromatography. *Anal. Chem.* 59, 2077.

Bartle, K.D.; Beddington, T. and Clifford, A.A. 1992. The Effect of Solubility On the Kinetics of Dynamic Supercritical Fluid Extraction. *J. Supercrit. Fluids.* 5, 207-212.

Berg, B.E.; Hansen, E.M.; Gjoerven, S. and Grelibrokk, T. 1993. *On-line* Enzymatic-Reaction, Extraction, and Chromatography of Fatty-Acids and Triglycerides with Supercritical Carbon Dioxide. *J. High Resolut. Chromatogr.* 16, 358-363.

Bounoshita, M.; Hibi, K. and Nakamura, H. 1993. Determination of Enantiomer ratios of *d, l*-Carvone in Supercritical Fluid Extracts from Caraway Seeds and Spearmint Leaves by High-performance Liquid Chromatography with

Polarimetric and Ultraviolet Spectrophotometric Detection. *Anal. Sci.* 9, 425-428.

Brooks, M.W. and Uden, P.C. **1993.** Extraction and Analysis of Diesel Fuel by Supercritical Fluid Extraction and Microbore Supercritical Fluid Chromatography. *J. Chromatogr.* 637, 175-179.

Buback, M. **1991.** Spectroscopy of Fluid Phases-The Study of Chemical-Reactions and Equilibria Up to high-pressures. *Angew, Chem. Int. Ed.* 30, 641.

Bysouth, S.R. and Tyson, J.F. **1992.** Supercritical carbon dioxide as a carrier for sample introduction in atomic absorption spectrometry. *Anal. Chim. Acta.* 258, 55.

Casabianca, H. **1992.** Use of Supercritical Fluid Extraction in Combination with Gas-Chromatography and Atomic Emission Detection for Analysis of Halogen and Sulfur Based Pesticides. *Analusis.* 20, S9-S11.

Chakrabarti, C.L.; Lu, Y.J.; Gregoire, D.C. and Back, M.H. **1994.** Kinetic-studies of Metal Speciation Using Chelex Cation-exchange Resin- Application to Cadmium, Copper and Lead Speciation in River Water and Snow. *Environ. Sci. Technol.*, 28, 1957-1967.

Chen, S.J.; Randelman, R.E. and Seldomridge, R.L. **1993.** Mass Spectrometer Composition Probe for Bath Cell Studies of Supercritical Fluid Phase Equilibria. *J. Chem. Eng. Data.* 38, 211-216.

Chester, T.L. and Innis, D.P. **1993.** Dynamic Film Formation and the Use of Retention Gaps with Direct Injection in Open-tubular Supercritical Fluid Chromatography. *J. Microcolumn Sep.* 5, 261-273.

Chiarizia, R. Application of Supported Liquid Membranes for Removal of Nitrate Technetium-(VII) and Chromium (VI) from Ground Water. **1991.** *J. Membrane Sci.* 55, 39.

Chiarizia, R.; Horwitz, E.P.; Rickert, P.G. and Hodgson, K.M. Application of Supported Liquid Membranes for Removal of Uranium from Ground Water. **1990.** *Sci. Sep. Technol.* 25.

Chunjie Z.; Smith, J. M. and McCoy, B.J. 1993. Kinetics of Supercritical Fluid Extraction of Coal: Physical and Chemical Processes. *Supercritical Fluid Engineering Science* Chapter. 29, 362.

Cotton, N.J.; Bartle, K.D.; Clifford, A.A. and Dowle, C.J. 1993. Rate and extent of Supercritical Fluid Extraction of additives from Polypropylene: Diffusion, Solubility, and Matrix effects. *J. Appl. Polym. Sci.* 48, 1607-1619.

Daimon, H. and Hirata, Y. 1991. Directly Coupled Supercritical-fluid Extraction/Capillary Supercritical-Fluid Chromatography of Polymer Additives. *Chromatographia.* 32, 549-554.

Dalang, F.; Buffle, J. and Haerdi, W. 1984. Study of the Influence of Fulvic Substances on the Adsorption of Copper(II) Ions at the Kaolinite Surface. *Environ. Sci. Technol.* 18, 135-141.

Daley, P.S. Hazardous-Waste 4. Cleaning Up Sites with On-Site Process Plants. 1989. *Environ. Sci. Technol.* 23, 912.

David, F.; Verschuere, M. and Sandra, P. 1992. *Off-line* Supercritical Fluid Extraction-Capillary GC Applications in Environmental-Analysis. *Fresenius' J. Anal. Chem.* 344, 479-485.

Davis, J.A. and Kent, D.B. 1990. Mineral - Water Interface Geochemistry. *Reviews in Mineralogy.* Vol. 23, Chapter 5, Mineralogical Society of America, 179-260.

De Galan, L. and Ripson, P.A.M. 1981. A Sample Introduction System for an Inductively Coupled Plasma Operating On an Argon Carrier Gas Flow of 0.1 L/min. *Spectrochim. Acta.* 36b, 71.

Dzombak, D.A.; Fish, W. and Morel, F. 1986. Metal Humate Interactions. 1. Discrete Ligand and Continuous Distribution Models. *Environ. Sci. Technol.* 20, 669-675.

Eastoe, J. and Robinson, B.H. 1991. Rotational Dynamics of Aerosol-OT (AOT) Reversed Micelles in Near-Critical and Supercritical Alkanes. *J. Chem. Soc. Faraday Trans.* 87, 1899.

Eckert-Tilotta, S.E.; Hawthorne, S.B. and Miller, D. **1993.** Supercritical Fluid Extraction with Carbon-Dioxide for the Determination of Total Petroleum-Hydrocarbons in Soil. *J.Fuel.* 72, 1015-1023.

Edder, P.; Haerdi, W.; Veuthey, J.L. and Staub, C. **1992.** Quantitative Capillary Supercritical Fluid Chromatography and Supercritical Fluid Extraction of Basic Drugs of Abuse. *Chimia.* 46, 141-148.

Egon S.; Erwin S. and Helmut K.M. **1980.** Extraction of Seed Oils with Liquid and Supercritical Carbon Dioxide. *J. Agric. Food Chem.* 28, 1153-1157.

Engelhardt, H. and Haas, P. **1993.** Possibilities and Limitations of Supercritical Fluid Extraction in the Extraction of Aflatoxin B_1 from Food Matrices. *J. Chromatogr. Sci.* 31, 13-19.

Ezzell, J.L. and Richter, B.E. **1993.** Supercritical Fluid Extraction of Pesticides and Phthalate Esters Following Solid Phase Extraction from Water. *J. Microcolumn Sep.* 4, 319-323.

Fernandez, P.; Alder, A. C. and Giger, W. **1993.** Quantitative Determination of Cationic Surfactants in Sewage Sludges and Sediments by Supercritical Fluid Extraction and HPLC Applying Post-Column Ion-pair. *Natl. Meet. - Am. Chem. Soc., Div. Environ. Chem.* 33, 303-306.

Field, J.A. **1993.** Ion-pair Reactions for Supercritical Fluid Extraction. *Natl. Meet.- Am. Chem. Soc., Div. Environ. Chem.* 33, 300-302.

Florence, T.M.; Lumsden, B.B. and Fardy, J.J. **1983.** Evaluation of Some Physicochemical Techniques for the Determination of the Fraction of Dissolved Copper Toxic to the Marine Diatom Nitzschia-closterium. *Anal. Chim. Acta.* 151, 291-295.

France, J.E. and King, J.W. **1991.** Supercritical Fluid Extraction Enzyme Assay-A Novel Technique to Screen for Pesticide-Residues in Meat-Products. *J. - Assoc. Off. Anal. Chem.* 74, 1013-1016.

Friedrich, J.P.; List, G.R. and Heakin, A.J. **1982.** Petroleum-Free Extraction of Oil from Soybeans with Supercritical Carbon Dioxide. *J. Amer. Oil Chem. Soc.* 59(7): 228.

Froning G.W.; Wehling, R.L. and Cuppett, S.L. **1990.** Extraction of Cholesterol and Other Lipids from Dried Egg Yolk Using Supercritical Carbon Dioxide. *J. Food Sci.* Vol. 55, No. 1.

Furton, K.G.; Jolly, E. and Lin, Q. **1993.** Variables Influencing the Supercritical Fluid Extraction of PCBs and PAHs from Solid Phase Sorbents. *Natl. Meet - Am. Chem. Soc., Div. Environ. Chem.* 33, 307-310.

Gamble, D.S.; Underdown, A.W. and Langford, C.H., **1980.** Copper(II) Titration of Fulvic-acid Ligand Sites with Theoretical, Potentiometric, and Spectrophotometric Analysis. *Anal. Chem.*, 52, 1901-1908.

Gmur, W.; Bosset, J. O. and Plattmer, E. **1987.** Beitrag Zur Direkten Kopplung Fluidextraktion-Kapil-Larfluidchromatographie. *J. Chromatogr.* 388, 143.

Greibrokk, T. **1991.** In Organic Micropblutants in the Aquatic Environment. Proceedings. *The Netherlands.* 112-114.

Griest, W.H.; Ramsey, R.S.; Ho, C. H. and Caldwell, W.M. **1992.** Supercritical Fluid Extraction of Chemical Warfare Agent Simulants from Soil. *J. Chromatogr.* 600, 273-277.

Grimm, D.M.; Azarraga, L.V.; Carreira, L.A. and Susetyo, W. **1991.** Continuous Multiligand Distribution Model Used to Predict the Stability Constant of Cu(II) Metal Complexation with Humic Material from Fluorescence Quenching Data. *Environ. Sci. Technol.* 17, 654-660.

Hammond, D.A. and Karel, M. **1985.** Enzymatic-Reactions in Supercritical Gases" *Appl. Biochem. Biotech.* 11, 393.

Hawthorne, S.B.; Miller, D. J.; Nivens, D. E. and White, D. C. **1992.** Supercritical Fluid Extraction of Polar Analytes Using *in situ* Chemical Derivatization. *Anal. Chem.* 64, 405-412.

Hawthorne, S.B.; Riekkola, M.; Serenius, K.; Holm, Y.; Hiltunen, R. and Hartonen, K. **1993a.** Comparison of Hydrodistillation and Supercritical Fluid Extraction for the Determination of Essential Oils in Aromatic Plants. *J. Chromatogr.* 634, 297-308.

Hawthorne, S.B.; Miller, D.J.; Burford, M.D., and Langenfeld, J.J. **1993b**. Factors Controlling Quantitative Supercritical Fluid Extraction of Environmental Samples. *J. Chromatogr.* 642, 301-317.

Hawthorne, S.B.; Miller, D.J. **1986**. Extraction and Recovery o^f Organic Pollutants from Environmental Solids and Tenax-GC using Supercritical Carbon Dioxide. *J. Chromatogr. Sci.* 24, 258.

Hawthorne, S.B.; Miller, D.J.; Walker, D.D.; Whittington, D.E., and Moore, B.L. **1991**. Quantitative Extraction of Linear Alkylbenzenesulfonates using Supercritical Carbon-Dioxide and a Simple Device for adding Modifiers. *J. Chromatogr.* 642, 301-317.

Hayasi, K.; Sasaki, Y.; Tagashira, S., and Yanagidani, T.J. **1986**. Acid Dissociation Constants of Dialkyldithiocarbamic Acids and O,O'-dialkyl, S-hydrogen dithiophosphates and Formation Constants of their Complexes with Cd(II), Zn(II), and Pb(II) in 1-1 v/v Water DMF Medium. *Bull. Chem. Soc. Jpn* 59, 1255.

Helmut Sigel. **1974**. Metal Ions in Biological Systems. Volume 1, Marcel Dekker, Inc., New York, NY.

Helmut Sigel. **1974**. Metal Ions in Biological Systems. Volume 2, Marcel Dekker, Inc., New York, NY.

Hendrick, J.L., and Taylor, L.T. **1992**. Direct Supercritical Fluid Extraction of Nitrogenous Bases from Aqueous-Solution. *J. High Resolut. Chromatogr. Chromatogr. Commun.* 15, 151.

High, K., Azani, R.; Fazekas, A.F.; Chee, Z.A.; Blais, J.S. **1992**. Theromspray-Microatomizer Interface for the Determination of Trace Cadmium and Cadmium Metallothioneins in Biological Samples with Flow Injection- and High Performance Liquid Chromatography-Atomic Absorption Spectrometry. *Anal. Chem.* 64, 3197.

Hirata, Y., Nakata, F. and Horihata, M. **1988**. Direct Sample Injection in Supercritical Fluid Chromatography with Packed Fused Silica Column. *J. High Resolut. Chromatogr. Chromatogr. Commun.* 11, 81.

Howard, A.L.; Taylor, L.T. **1992**. Quantitative Supercritical Fluid Extraction of Sulfonyl Urea Herbicides from Aqueous Matrices via Solid-Phase Extraction Disks. *J. Chromatogr. Sci.* 30, 374.

Howard, A.L.; Braue, C.: Taylor, L.T. **1993**. Analysis of Thiocarbamate Pesticides in Apples employing SFE and HPLC coupl;ed with Sulfur Chemiluminescence Detection. *Natl. Meet. - Amer. Chem. Soc., Div. Environ. Chem.* 33, 316.

Hunt, T.P.; Dowie, C.J. and Greenway, G. **1993.** Supercritical Fluid Extraction for the Analysis of Liquid Poly(alkyleneglycol) Lubricants and Sorbitan Ester Formulations. *Analyst.* 118, 17-22.

Hutchins, S.R.; Haddad, P.R. and Dilli, S. Reversed-Phase High-Performance Liquid-Chromatography of Diethyldithiocarbamate Complexes Using Radial Compression Columns. **1982.** *J. Chromatogr.* 252, 185.

Jeffrey D. B.; Robert, J. M. and J.W. Hampson **1993.** Membrane Interface for *On-line* Supercritical Fluid Extraction/Flow Injection Analysis. *Anal. Chem.* 65, 2137-2140.

Joe. M. Tingey; Clement R. Yonker and Richard D. Smith **1989.** Spectroscopic Studies of Metal Chelates in Supercritical Fluids. *J. Phys. Chem.* 93, 2140-2143.

Johnston, K.P. and Kim, S. **1989.** Spectroscopic Determination of Solvent Strength and Structure in Supercritical Fluid Mixtures. *Supercritical Fluid Science and Technology* ACS Symposium Series. Vol. 406. Chapter 5, 52.

Kagi, J.H.R. and Vallee, B.L. **1961.** Metallothionein: a Cadmium- and Zinc-containing Protein from Equine Renal Cortex, II Physicochemical properties. *J.Bio. Chem.* 236, 2435-2442.

Kagi, J.H.R. and Vallee, B.L. **1960.** Metallothionein: a Cadmium- and Zinc-Containing Protein from Equine Renal Cortex. *J.Bio. Chem.* 235, 2460-2465.

Kapila, S.; Nam, K.S.; Liu, M.H.; Puri, R.K. and Yanders, A. F. **1992.** Promises and Pitfalls of Supercritical fluid Extraction in Polychlorinated Compound Analyses. *Chemosphere.* 25, 11-16.

King, J.W.; France, J.E. and Snyder, J.M. **1992.** *On-line* Supercritical Fluid Extraction-Supercritical Fluid Reaction-Capillary Gas-Chromatography Analysis of the Fatty-Acid Composition of Oilseeds. *Fresenius' J. Anal. Chem.* 344, 474-478.

King, J.W. **1989.** Fundamentals and Applications of Supercritical Fluid Extraction in Chromatographic Science. *J. Chromatogr. Sci.* 27, 355.

Kitagawa, T.; Hishikawa, Y.; Frankenfeld, J.W. and Li, N.N. Waste Water Treatment by Liquid Membrane Process. 1977. *Environ. Sci. Technol.* 11, 602-605.

Kiyokatsu J. and Muneo S. 1991. Coupling of Supercritical Fluid Extraction with Chromatography. *Analytical Science*, 7, 361-369.

Kueppers, S. 1992. The Use Temperature Variation in Supercritical Fluid Extraction of Polymers for the Selective Extraction of Low Molecular Weight Components from Poly(ethylene terephthalate). *Chromatoggraphia.* 33, 434-440.

Laintz, K.E.; Wai, C.M; Yonker, C.R. and Smith R.K. 1992a. Extraction of Metal Ions from Liquid and Solid Materials by Supercritical Carbon Dioxide. *Anal. Chem.* 64, 2875-2878.

Laintz, K.E.; Yu, J.-J. and Wai, C.M. 1992b. Separation of Metal Ions with Sodium Bis(trifluoroethyl)dithiocarbamate Chelation and Supercritical Fluid Chromatography. *Anal. Chem.* 64, 311-315.

Laintz, K.E.; Shieh, G.M. and Wai, C.M. 1992c. Simultaneous Determination of Arsenic and Antimony Species in Environmental-Sample Using Bis(trifluordoethyl)dithiocarbamate Chelation and Supercritical Fluid Chromatography. *J. Chromatogr. Sci.* 30, 120-123.

Langenfeld, J.J.; Hawthorne, S.B.; Miller, D.J. and Tehranl, J. 1992. Method for Determining the Density of Pure and Modified Supercritical Fluids. *Anal. Chem.* 64, 2263-2266.

Larsen, E.H. and Blais, J-S. 1993. Performance of a Modular Thermospray Interface for Signal Enhancement in Flame Atomic Absorption Spectrometry Coupled On-line to Flow Injection or Liquid Chromatography. *J. Anal. Atom. Spectrom.* 8, 659-664.

Lee, M.; Holtom, G.R. and Hochstrasser, R.M. 1985. Observation of the Kramers Turnover Region in the Isomerism of Trans-stilbene in Fluid Ethane. *Chem. Phys. Lett.* 118, 359.

Leiner, S.; Quirin, K.W. and Gerald, D. 1986. Dense Gases for Extraction and Refining. p 101. *New York, NY.*

Lembke, P. and Engelhardt, H. 1993. Development of A New Supercritical Fluid Extraction Method for Rapid Determination of Total Fat Content Food. *Chromatographia.* 35, 509-516.

Li, N.N. Permeation Through Liquid Surfactant Membranes. 1971. *AICHE J.* 17, 459

Liebman, S.A.; Levy, E.J. and Brame, E.G.Jr. 1992. Supercritical Fluid Technology in the Processing and Analysis of Composite Materials. *Int. SAMPE Electron. Conf.* 6, 759-769.

Lin, Y. and Wai, C.M. Supercritical Fluid Extraction of Lanthanides with Fluorinated β-Diketones and Tributyl Phosphate. 1994. *Anal. Chem.* 66, 1971-1975.

Liu, Y.; Lopez-Avila, V.; Alcaraz, M. and Beckert, W. F. 1993. Determination of Organotin Compounds in Environmental-Samples by Supercritical Fluid Extraction and Gas-Chromatography with Atomic Emission Detection. *J. High Resolut. Chromatogr.* 16, 106-112.

Lixiong Li.; Peishi C. and Earnest F. G. 1993. Kinetic Model for Wet Oxidation of Organic Compounds in Subcritical and Supercritical Water. *Supercritical Fluid Engineering Science.* Chapter 24, 305.

Lopez-Avila, V.; Benedicto, J. and Beckert, W. F. 1993. Supercritical Fluid Extraction of Chlorophenoxy Acid Herbicides from Soil Samples. *Natl. Meet.-Am. Chem. Soc., Div. Environ. Chem.* 33, 320-323.

Lopez-Avila, V. and Beckert, W.F. 1992. Supercritical Fluid Extraction in Environmental Analysis. *Supercritical Fluid Technology.* Chapter 14, *ACS Symp. Ser.* No. 488, 179-205.

Lou, X.; Janssen, H.G. and Cramers, C.A. 1993. Quantitative Aspects of Directly Coupled Supercritical Fluid Extraction Capillary Gas-Chromatography with A Conventional Split Splitless Injector as Interface. *J. High Resolut. Chromatogr.* 16, 425-428.

Marsili, R. and Callahan, D. 1993. Comparison of A Liquid Solvent Extraction Technique and Supercritical Fluid Extraction for the Determination of α- and β-Carotene in Vegetables. *J. Chromatogr. Sci.* 31, 422-428.

Martinez de la, E.; Brandani, V.; Del Re, G. and Ferri, E. 1990. Binary and Ternary Phase Behavior of the System Water-Ethanol-Carbon Dioxide. *Fluid Phase Equil.* 56, 325-340.

Mary, E.P.; McNally and Frank V.B. 1992. Fundamental Studies and Applications of Supercritical Fluids. *Supercritical Fluid Technology.* ACS Symposium Series 488 Chapter 1, 1-15.

Masuda, M.; Koike, S.; Handa, M.; Sagara, K. and Mizutani, T. 1993. Application of Supercritical Fluid Extraction and Chromatography to Assay Fat-Soluble Vitamins in Hydrophobic Ointment. *Anal. Sci.* 9, 29-32.

Maxwell, R.J.; Hampson, J.W. and Cygnarowicz-Provost, M. 1991. Solute Recovery from Equilibrium Solubility Determinations Using A Modified Supercritical Fluid Extraction. *LC-GC Magazing of Separations.* 9, 788-794.

Maxwell, R.J.; Pensabene, J.W. and Fiddler, W. 1993. Multiresidue Recovery at ppb Levels of Nitrosamines from Frankfurters by Supercritical Fluid Extraction. *J. Chromatogr. Sci.* 31, 212-215.

McHugh, M.A. and Krukonis, V.J. editors 1986a. Supercritical Fluid Extraction. *Butterworth publisher.* Chapter 2. 13-23.

McHugh, M.A. and Krukonis, V.J. 1986b. Supercritical Fluid Extraction Principles and Practice. *Boston, MA.*

McNally, M.E. and Wheeler, J.R. 1988. Increasing Extraction Efficiency in Supercritical Fluid Extraction from Complex Matrices. *J. Chromatogr.* 447, 53-63.

McNally, M.E.P.; Deardorff, C.M.; Fahmy, T.M. and Hoffman, D. 1993. SFE: Does It Matter What Soil?. *Natl. Meet. - Am. Chem. Soc., Div. Environ. Chem.* 33, 327-330.

Messer, D.C. and Taylor, L.T. 1992. Development of Analytical Supercritical Fluid Extraction of A Polar Drug from An Animal Food Matrix. *J. High Resolut. Chromatogr.* 15, 238-241.

Mingin W.; Zaiyou L.; Farnsworth, P.B. and Lee, M. L. 1993. Comprehensive Supercritical Fluid Extraction/Gas Chromatographic Analysis of Organic

Compounds in Soil Matrices with an Element-Selective Radiofrequency Detector. *Anal. Chem.* 65, 2185-2188.

Momplaisir, G.-M.; Lei, T. and Marshall, W.D. 1994. Performance of a Novel Silica T-Tube Interface for the AAS Detection of Arsenic and Selenium Compounds in PHLC Column Eluate. *Anal. Chem.* 66, 3533.

Moore, F.L. Solvent-Extraction of Mercury from Brine Solutions with High-Molecular-Weight Amines. 1972. *Environ. Sci. Technol.* 6, 525.

Motley, C.B.; Ashraf-Khorassani, M. and Long, G.L. 1989. Microwave-Induced Plasma as an Elemental Detector for Packed-Column Supercritical Fluid Chromatography. *Appl. Spectrosc.* 43, 737-74.

Murugaverl, V.; Gharaibeh, A. and Voorhees, K.J. 1993. Integrated Supercritical Fluid Flow System for the Analysis of Pesticides in Tissues. *Natl. Meet.- Am. Chem. Soc., Div. Environ. Chem.* 33, 395-397.

Myer, L.J.D.; Damian, J.H.; Liescheski, P.B. and Tehrani, J. 1992. Supercritical Fluid Extraction Versus Soxhlet Sample Preparation: A comparative Study for Extraction and Analysis of Organics in Solid Matrices. *Supercritical Fluid Technology.* Chapter 16, *ACS Symp. Ser.* No. 488, 221-236.

Nair, J. B and Huber, J. W. 1988. *On-line* Supercritical Sample-Preparation Accessory for Chromatography. *LC-GC. Mag. Chromatogr. Sci.* 6, 1071.

Nakamura, K. 1990. Biochemical Reactions in Supercritical Fluids. *Trends Biotechnol,* Oct. 8, 288.

Nakamura, K.; Chi, Y.M.; Yamada, Y. and Yano, T. 1986. Lipase Activity and Stability in Supercritical Carbon-Dioxide. *Chem. Eng. Commun.* 45, 207.

Nriagu, J.O. and Nieboer, E. 1988. Chromium in the Natural and Human Environment . *Advances in Environmental Science and Technology Volume 20,* Chapter 8, 215-229.

Onuska, F.I. and Terry, K.A. 1989. Supercritical Fluid Chromatography of Alkylene Oxide-Fatty Alcohol Condensates: their Quantitation in Water Samples. *J. High Res. Chromatogr. and Chromatogr. Comm.* 12, 357.

Palet, C.; Munoz, M. Hidalgo, M. and Valiente, M. Transport of Vanadium (V) Through a Tricaprylymethylammonium Solid Supported Liquid Membrane. 1995. *J. Membrane Sci.* 98, 241.

Paschke, T.; Hawthorne, S.B.; Miller, D. J. and Wenciawiak, B. 1992. Supercritical Fluid Extraction of Nitrated Polycyclic Aromatic Hydrocarbons and Polycyclic Aromatic Hydrocarbons from Diesel Exhaust Particulate Matter. *J. chromatogr.* 609, 333-340.

Pawliszyn, J. 1993. Kinetic Model of Supercritical Fluid Extraction. *J. Chromatogr. Sci.* 31, 31-37.

Pearson, D. 1983. Supported liquid membranes for metal extraction from dilute solutions in Ion Exchange Membranes. Flett, D.S., Ed., *Ellis Horwood, Chichester.* 55-73.

Perrigo, B. J. and Joynt, B.P. 1992. A Supercritical Fluid Chromatographic Data Base for Analytical Toxicology. *Can. Soc. Forens Sci. J.* 25, 79-89.

Petsche, I.B. and Debenedetti, P.G. 1989. Solute-Solvent Interactions in Infinitely Dilute Supercritical Mixtures: A Molecular Dynamics Investigation. *J. Chem. Phys.* 91, 7075.

Polesello, S.; Lovati, F.; Rizzolo, A. and Rovida, C. 1993. Supercritical-Fluid Extraction As A Preparative Tool for Strawberry Aroma Analysis. *J. High Resolut. Chromatogr.* 16, 555-559.

Ponce-Ramirez, E.; Lira-Galeana, C and Tapia-Medina, C. 1991. Application of the SPHCT Model to the Prediction of Phase Equilibria in CO_2-Hydrocarbon Systems. *Fluid Phase Equil.* 70, 1-18.

Prokopczyk, B.; Hoffmann, D.; Cox, J.E.; Djordjevic, M.V. and Brunnemann, K.D. 1992. Supercritical Fluid Extraction in the Determination of Tobacco-Specific N-Nitrosamines in Smokeless Tobacco. *Chem. Res. Toxicol.* 5, 336-340.

Pyle, S.M. and Setty, M.M. 1991. Supercritical Fluid Extraction of High Sulfur Soils, with Use of A Copper Scavenger. *Talanta.* 38, 1125-1128.

Ramchandran, B.; Riggs, J. B. and Heichelheim, H.R. **1992**. Dynamic Modeling and Control of Supercritical Fluid Extraction Process. *ISA, Adv. Instrum. control*. 763-776.

Randolph, T.W.; Blanch, H.W.; Prausnitz, J.M. and Wilke, C.R. **1985**. Enzymatic Catalysis in a Supercritical Fluid. *Biotech. Letters*. 7, 325.

Raynor, M.W.; Davies, L.; Clifford, A.A.; Williams, A.; chalmers, J.W. and Cook, B.W. **1988**. Supercritical Fluid Extraction/Capillary Supercritical Fluid Chromatography/Fourier Transform Infrared Microspectrometry of Polycyclic Aromatic Compounds in A coal Tar Pitch. *J. High Resolut. Chromatogr. Chromatogr. Commun.* 11, 766.

Raynor, M. W. and Bartle, K.D. **1993**. Supercritical-Fluid Chromatography and Extraction in Surface-Coatings Analysis. *J. Supercrit. Fluids*. 6, 39-49.

Reverchon, E. **1992**. Fractional Separation of SCF Extracts from Marjoram Leaves-Mass-Transfer and Optimization. *J. Supercrit. Fluids*. 5, 256-261.

Robbins, A.H. and Stout, C.D. **1991**. X-ray Structure of Metallothionein. *Methods in Enzymology*. 205, 485-502.

Rocca, J.L.; Sauvage, E. and Toussaint, G. **1991**. Extraction and Analysis of An Active Ingredient by Combined Supercritical Fluid Extraction and Supercritical Fluid Chromatography. *Analusis*. 19, M50-M51.

Rochette, E.A.; Harsh, J.B. and Hill, H.H. **1993**. Supercritical Fluid Extraction of 2, 4- D from Soils Using Derivatization and Ionic Modifiers. *Talanta*. 40, 147-155.

Russell, A.J.; Beckman, E.J. and Chaudhary, A.K. **1994**. Studying Enzyme Activity in Supercritical Fluids. *CHEMTECH*. 33,

Saito, N.; Ikushima, Y. and Goto, T. **1990**. Liquid-Solid Extraction of Acetylacetone Chelates with Supercritical Carbon Dioxide. *Bull. chem. Soc. Jpn*. 63, 1532-1534.

Schuitze, P.; Worgotte, E.; Braun, W.; Wagner, G.; Vasak, M. and Wuthrch, K. **1988**. Conformation of (CD7)-Metallothionein-2 from Rat-Liver in Aqueous-Solution Determined by Nuclear Magnetic-Resonance Spectroscopy. *J. Mol. Biol*. 203, 251-268.

Shindler, P.W. 1990. Mineral - Water Interface Geochemistry. *Reviews in Mineralogy*. Vol. 23, Hochella, M.F.; White, A.F., Eds., Mineralogical Society of America, Washington, D.C., 281-307.

Shuman, M.S.; Collins, B.; Fitzgerald, P.J. and Olsen, D.L. 1983. Distribution of Stability Constants and Dissociation Rate Constants Among Binding Sites on Estuarine Copper-organic Complexes. *Aquatic and Terrestrial Humic Materials*. Christman, R.F. and Gjessing, E.T., Eds., Ann Arbor Science, Ann Arbor, MI, 349-370.

Singh, N. and Garg, A.K. Spectrophotometric Determination of Selenium (IV) with Potassium Butyl Xanthate. 1986. *Analyst*. 111, 247.

Smith, R.M. and Burford, M.D. 1992a. Optimization of Supercritical Fluid Extraction of Volatile Constituents from A Model Plant Matrix. *J. Chromatogr.*, 600, 175-181.

Smith, R.M. and Burford, M.D. 1992b. Supercritical Fluid Extraction and Gas-Chromatographic Determination of the Sesquiterpene Lactone Parthenolide in the Medicinal Herb Feverfew (Tanacetum Parthenium). *J. Chromatogr.* 627, 255-261.

Smith, R.D.; Fulton, J.L. and Blitz, J.P. 1990. Reverse Micelles and Microemulsions in Near-Critical and Supercritical Fluids. *J. Phys. Chem.* 94, 781.

Soave, G. 1991. Correlation of the Solubility of Ill-Characterized Solid Compounds in Supercritical Fluids by A Cubic Equation of State. *Fluid Phase Equil.* 64, 165-171.

Stalling, D. L.; Saim, S.; Kuo, K. C. and Stunkel. J. J. 1992. Integrated Sample Processing by *On-line* Supercritical Fluid Extraction Gel-Permeation Chromatography. *J. Chromatogr. Sci.*, 30, 486-490.

Stevenson, F.J., 1994. Stability Constants of Metal Complexes with Humic Substances. in Humus Chemistry. Genesis, Composition, Reactions. Second edition, John Wiley and Sons Inc., New York. Chapter 17. 405-428.

Sugiyama, K.; Saito, M.; Hordo, T. and Senda, M. 1985. Directly Coupled Laboratory-Scale Supercritical Fluid Extraction-Supercritical Fluid

Chromatography, Monitored with a Multiwavelength Ultraviolet Detector. *J. Chromatogr.* 332, 107.

Suzuki, K.T.; Imura, N. and Kimura, M. Birkhauser. 1992. Metallothionein III-Biological Roles and Medical Implications. *Proceedings of the third international conference on metallothionein.* Tsukuba, Japan, 29-55, 57-74.

Suzuki, N.; Satoh, K. Shoji, H. and Imura, H. Liquid-Liquid- Extraction Behavior of Arsenic (III), Arsenic (V), Methylarsinate and Dimethylarsinate in Various Systems. 1986. *Anal. Chim. Acta.* 185, 239.

Tan, C.S.; Yarn, S.J. and Hsu, J.H. 1991. Vapor-Liquid Equilibria for the Systems Carbon Dioxide-Ethylbenzene and Carbon Dioxide-Styrene. *J. Chem. Eng. Data.* 36, 23-25.

Tan, Y.; Momplaisir, G-M.; Wang, J. and Marshall, W.D. 1994. Performance of a Silica T-tube Interface for the Determination of Cadmium, Copper, Lead, Zinc and Mercury in Flowing Liquid Streams by Atomic Absorption Spectrometry. *J. Anal. Atom. Spectrom.* 9, 1153-1159.

Underdown, A.W.; Langford, C.H. and Gamble, D.S. 1981. Light Scattering of Polydisperse Fulvic Acid. *Anal. Chem.* 53, 2139-2140.

Unger, K.K. and Roumeliotis, P. 1983. *On-line* High-pressure Extraction-high-performance Liquid Chromatography. *J. Chromatogr.* 282, 519.

Vannoort, R.W.; Chervet, J-P, Lingerman, H; DeJong, G.J. and Brinkman, U.T. 1990. Coupling of Supercritical Fluid Extraction with Chromatographic Techniques. *J. Chromatography.* 505, 45-77.

Venema, A.; Van de Ven, H.J.F.M.; David, F. and Sandra, P. 1993. Supercritical-Fluid Extraction of Nylon-6-An Investigation into the Factors Affecting the Efficiency of Extraction of Caprolactam and Its Oligomers. *J. High Resolut. Chromatogr.* 16, 522-524.

Waite, T.D.; Wrigley, I.C. and Szymczac, R. 1988. Photoassisted Dissolution of a Colloidal Manganese Oxide in the Presence of Fulvic-acid. *Environ. Sci. Technol.* 22, 778-785.

Wang, S. Eishani, S. and Wai, C.M. Selective Extraction of Mercury with Ionizable Crown Ethers in Supercritical Carbon Dioxide. 1995. *Anal. Chem.* 67, 919.

Wells, P.A.; Chaplin, R.P. and Foster, N.R. 1990. Solubility of Phenylacetic Acid and Vanillan in Supercritical Carbon Dioxide. *J. Supercrit. Fluids.* 3, 8-14.

Wenzel, T.J.; Townsend, K.J.; Frederique, D.E. and Baker, A.G. 1993. Supercritical Fluid Extraction of Metal-Containing Selective Sorbents. *J. Chromatogr.* 637, 187-194.

Wigfield, Y.Y. and Lanouette, M. 1993. Supercritical Fluid Extraction of the Fortified Residues of Fluozifop-p-butyl (Fusilade II) and Its Major Metabolite, Fluazifop-p, In Onions. *J. Agric. Food Chem.* 41, 84-88.

Wilson, T.C; Harris, R.K.; Winters, T.; Greaves, J.G. and Haggerty, W.J. 1993. Supercritical Fluid Extraction of Polar Materials from Animal Feeds and Plant Materials. *Natl. Meet-Am Chem, Soc., Div. Environ. Chem.* 33, 391-394.

Wittbrodt, P.R. and Palmer, C.D. 1995. Reduction of Cr(VI) in the Presence of Excess Soil Fulvic Acid. *Environ. Sci. Technol.* 29, 255-263.

Wolfe, M.F.; Seiber, J.N. and Hinton, D.E. 1993. Aqueous Sample Preparation for Bioassay Using Supercritical Fluid Extraction. *Natl. Meet - Am. Chem. Soc., Div. Environ. Chem.* 33, 385-387.

Wong and Johnston. 1986. Solubilization of Biomolecules in Carbon Dioxide Based Supercritical Fluids. *Biotechnology Progress 2* 1, 30.

Yonker, C.R. and Smith, R.D. 1988. Solvatochromism: A Dielectric Continuum Model Applied to Supercritical Fluids. *J.Phys, Chem.* 92, 235.

Young, J.C. and Games, D.E. 1993. Supercritical Fluid Extraction and Supercritical Fluid Chromatography of the Fungal Metabolite Ergosterol. *J. Agric. Food Chem.* 41, 577-581.

Zagrobelny and Bright, F.V. 1992. *In Situ* Studies of Protein Conformation in Supercritical Fluid: Trypsin in Carbon Dioxide. *Biotechnol. Prog.* 8, 421-423.

Zerda, T.W.; Song, X. and Jonas. **1986**. Raman-Study of Intermolecular Interactions in Supercritical Solutions of Naphthalene in Carbon Dioxide. *J. Appl. Spectrosc.* 40, 1194.

Zou, M.; Yu, Z.R.; Kashulines, P.; Rizvi, S.S.H. and Zollweg, J.A. **1990**. Fluid-Liquid Phase Equilibria of Fatty Acids and Fatty Acid Methyl Esters in Supercritical Carbon Dioxide. *J. Supercrit. Fluids*. 3, 23-28.