PRECONCENTRATION OF TRACE METAL IONS WITH $TPPS_4$

PRECONCENTRATION OF TRACE METAL IONS FROM NATURAL WATERS: COMPLEXATION WITH MESO-TETRA(p-SULFONATOPHENYL)PORPHINE AND ADSORPTION ONTO XAD RESINS

By

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ABSTRACT

A new preconcentration method had been developed for the determination of some trace-metal ions in lakewater. This method is based on the formation of their $TPPS_4$ complexes at elevated temperatures in solution and adsorption of the complexes onto a column of the macroporous acrylic ester resin, XAD-7. The complexes are eluted with a methanolic solution and the metal concentration determined by graphite-furnace atomic absorption spectroscopy. Optimal conditions were established for the group preconcentration of Cd, Cu, Ni and Pb. The concentration of these trace metals in several lakewater samples were determined successfully, with a precision comparable to that of the accepted Chelex-100 method. Application of the method to natural seawater samples failed, largely because of aggregation in the highly saline medium.

Adsorption isotherms of $TPPS_4$ and its complexes on XAD resins were obtained. Adsorption onto XAD-2 and XAD-4 resins likely involves π-π interactions while adsorption onto XAD-7 is probably due to weak Van der Waals forces. The adsorption at high $TPPS_4$ concentrations is complicated by aggregation.

An attempt to create a simple chelating ion-exchange

resin by adsorption of $TPPS_4$ onto XAD resin failed. Similarly, the development of a rapid room-temperature preconcentration method specifically for Pb and Cd in natural waters was not successful.

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CHAPTER 1. INTRODUCTION

1.1 General

The exponential growth of population and the corresponding expansion of industry have led to increasing pollution of the environment and the detrimental effects have been noticed with growing awareness by the public. There is particular concern for the contamination of inland waters, not only because of the effects on aquatic life therein but also because inland waters provide a source of water for human consumption and recreation. This concern is reflected in the increased amount of research by scientists from many different areas.

An important group of pollutants are "heavy metals"*. They are toxic and do not evaporate or biodegrade. These metals, such as Cd, Co, Cu, Cr, Fe, Pb, Mn, Hg, Ni, Sn, and Zn (1-5), are found in trace (mg L^{-1} or ppm) to ultra trace (µg L^{-1} or ppb) quantities in natural waters (e.g., Table 1). They are introduced into the water system by natural processes such as weathering of rocks, soil drainage

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* The term "heavy metal" is deliberately ambiguous and is defined in this thesis according to Burrell (1) (see Figure 1).

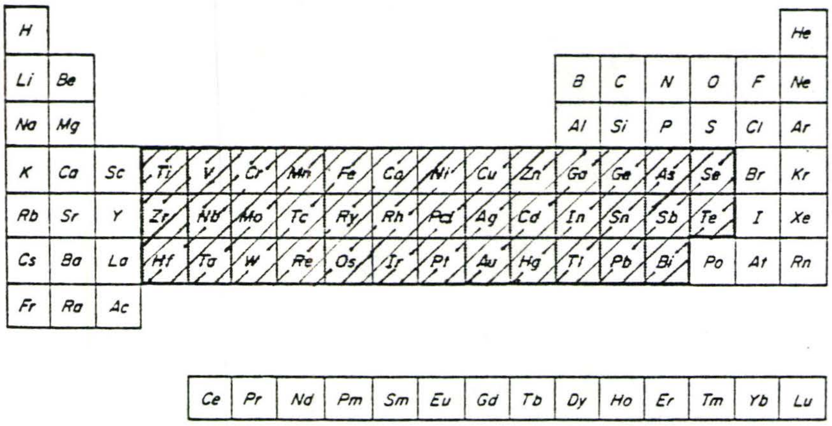

Figure 1. Heavy metals as defined by Burrell (1)

Table 1. Concentrations in Two Natural Water Samples

Element	Concentration ($\mu g\ L^{-1}$) Niagara River (6)	 Atlantic Ocean, Open (7)
Cd	0.052	0.030
Co	0.21	0.003
Cu	3.5	0.12
Fe	86.7	0.20
Mn	2.84	0.018
Ni	1.56	0.27
Pb	0.59	0.095
Zn	1.42	0.28

and volcanic activity, but man's activities have accelerated the flux of metals into the environment. This has been done by direct dumping of industrial, agricultural and domestic effluents, or indirectly by atmospheric fallout of burned fossil fuels and pesticides, or increased exposure of fresh rock from mining and construction operations. Furthermore, acid rain may convert an inactive metal species to an active form by lowering the pH of the water body. Metals such as Cd, Hg, and Pb are very toxic in low concentration and they tend to bioconcentrate in fish and man (8). Other elements such as Co, Cr, Cu, Mn, Ni and Zn are essential for life but are toxic at higher concentration; for some, there exists a narrow concentration range between essential and toxic levels (8). The assessment of toxicity is difficult because it not only depends on the particular heavy metal, but also on its physico-chemical form and its ability to react with biological membranes (9). For example, the organic forms of Hg (specifically alkylmercurials) are known to be more toxic than the inorganic forms (10).

1.2 Determination of Trace Metals in Natural Waters

Necessity of Preconcentration

Environmental concern has led to increased demands for high quality analytical data of heavy metal content in natural waters. The data are used to create accurate toxicity standards for drinking water quality, to establish dumping regulations and to monitor heavy metal pollution. The analysis of natural waters is plagued by problems of analyte contamination from a number of sources such as reagents, containers and the atmospheric environment (11-16), and analyte loss by adsorption onto vessel walls (17-22). Low analyte concentration and interference from the matrix further complicate the analysis. Therefore, the determination of heavy metals in natural waters requires careful sampling, storage and handling procedures in addition to special analysis techniques.

Direct analysis of natural water is ideal since it decreases the probability of contamination and loss of the analyte and is less time consuming. Direct analysis, however, is not always feasible due to problems of low heavy metal levels and/or matrix interferences. Comparison of the limits of determination and detection of commonly used instruments for heavy metals in aqueous samples is shown in Tables 2 and 3. Neutron activation and inverse (stripping) voltammetry have been used to perform direct analysis of

Table 2. Limits of Determination for Various Analytical Methods ($\mu g\ L^{-1}$) (23)

Element	Neutron Activation	Inverse (Stripping) Voltammetry	XRF	ICPAES	AAS (Flame)	GFAAS
Cd	30	0.01	700	5	50	0.1
Co	30	0.1	100	10	200	4
Cu	5	0.02	1000	5	200	3
Fe	140,000	--	1000	20	200	2
Mn	0.1	--	300	2	100	0.8
Ni	100	20	500	20	200	10
Pb	25,000	0.02	2000	20	600	2
Zn	50	0.03	1000	5	20	0.06

NOTE: "Limit of determination" is defined as 10 * $|s_b|$, where $|s_b|$ is the standard deviation of the blank (23-25). The factor of k=10 is felt to be more practical when the capabilities of analytical methods at very low levels are compared and when non-Gaussian distribution prevails. The compiled data refer to the determination of elements in pure aqueous solutions which contain only the element in question.

Table 3. Limits of Detection for Recently Modified ICPAES Techniques (µg L^{-1})

Element	Electrothermal Atomization - ICPAES (26)	Ultrasonic Nebulizer - ICPAES (27)
Cd	3.0	0.091
Co	--	0.55
Cu	0.2	0.91
Fe	2.0	0.35
Mn	0.1	0.42
Ni	--	0.46
Pb	10.0	0.94
Zn	0.2	0.12

NOTE: "Limit of detection" (c_l), is the lowest concentration which can be detected by the analytical procedure. "c_l" is a function of "x_l", which is defined as $x_l = \bar{x}_b + k * |s_b|$. "$x_l$" is the smallest acceptable measurable signal, "$\bar{x}_b$" is the mean value of the blank, "k" is a constant which, by convention, equals three. This expression was originally defined by Kaiser (28) and accepted by IUPAC (29).

natural waters but there are disadvantages to both techniques. Neutron activation analysis is possible only if the appropriate facilities are available. Also, analysis can be lengthy (long irradiation and decay times) and the matrix, especially that of seawater, may create spectral interference. Inverse voltammetry requires few reagents, simple instrumentation and permits limited measurement of speciated forms but it is not very selective. Other instruments frequently used are X-ray spectrometry (XRS), inductively coupled plasma atomic emission spectrometry (ICPAES) and atomic absorption spectrometry (AAS). For most water samples, the analyte concentration is below the detection limit of these instruments. XRS is best suited to solid samples where the metal ions may be electrochemically plated out on thin electrodes or retained on resin or filter paper. The solid support media, however, often causes some interference problems. ICPAES and AAS suffer from matrix interferences (30). Interference effects are more severe in flameless or graphite furnace AAS (GFAAS) than in ICPAES although this problem has been some what alleviated by methods developed to reduce these interferences (31-37). In particular, non-specific absorption from volatilization of salts, loss of analyte as volatile chlorides and analyte co-volatilization with other volatile chlorides (38) are special problems in GFAAS. GFAAS also has lower precision but its advantages are lower detection limits, increased sensitivity, _in situ_ sample treatment and small sample volumes for measurement. ICPAES, on the other hand, suffers more from spectral line interference although it has simultaneous multi-element capability. In general, the instrumental methods available do not have the necessary selectivity, sensitivity, large linear dynamic range, and freedom from matrix interferences. Therefore, there is a need to interface an appropriate preconcentration* method with the instrument chosen for the analysis.

Methods of Preconcentration

Various preconcentration methods have been applied to trace element determinations in aqueous samples. Leyden and Wegscheider (39), and Bachmann (40) provide extensive reviews. Techniques include solvent evaporation (41,42), coprecipitation (43-51), electrodeposition (52-57), solvent extraction, conventional and chelating ion-exchange, and surface adsorption. The latter four techniques are popular.

Solvent or liquid-liquid extraction is a widely used preconcentration method. Usually, a group of metal ions are complexed in the aqueous medium by a chelating agent and then extracted into a small volume of a water-immiscible solvent. There have been many applications of trace metal preconcentration from natural waters by solvent extraction. Two

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* The term "preconcentration" includes both the process of analyte concentration and separation from the matrix.

widely used chelating agents are ammonium pyrrolidinedithiocarbamate (APDC) (58-61) and diethyldithiocarbamate (DDTC) (60, 62-65). Although solvent extraction is fast and simple, concentration factors over 20 or 30 are not often achieved due to a decrease in the percent extraction with increasing aqueous-to-organic phase volume ratio. Also, there may be problems obtaining a clean separation of a small volume of the organic phase from the bulk aqueous phase, particularly when an emulsion forms.

Conventional ion-exchange methods are based on a reversible exchange of ions between a liquid phase and a solid phase with ionic exchange sites. Cation and anion exchange resins essentially operate on an ion association basis. In the past, these resins have found some use in trace metal analysis (i.e., 66-68). Recently, however, Amberlite XAD-7 has been found to retain metal ions and has been used to preconcentrate trace metals from lake and seawater samples (69-71). It is thought that carboxylic acid impurity sites on XAD-7 causes it to act as a weak acid ion-exchange resin (72). Amberlite XAD-2 and -4 also act as cation-exchange resins but of even lower capacity than XAD-7 (73,74). A macro porous cation-exchange resin, AG MP-50, is thought to be useful for the HPLC separation of metal ions (75-77) and preconcentration of metals from seawater on a single anion-exchange bead has been performed (78).

In chelating ion-exchange, chelating ligands are fixed on various supports such as resins, activated charcoal, glass and silica gel. These ligands are immobilized either by adsorption or by chemical bonding to the support material. One commercially available chelating ion-exchange resin that has proven very useful is Chelex-100 (61, 79-83), a resin with chemically bound iminodiacetate groups. The resin has a high distribution ratio for a wide range of metals such as Cd, Co, Cu, Fe, Mn, Ni, Pb, and Zn (84). The disadvantage of this resin is that exchange sites are occupied by alkali and alkaline-earth metals which co-elute with the trace metals. These ions in high concentration interfere in GFAAS measurement. Differential elution of these ions has been attempted (84) but this procedure is thought to bring about some loss of trace metals (85). In column operation, there is often difficulty in swelling and shrinking when the resin is converted from one form to another. The large preconcentration factors anticipated have not been achieved due to the large volume of eluant (e.g., 25 mL of 2 M HNO_3) required to strip the metals quantitatively (79). Use of a larger sample volume (if available) gives rise to a greater concentration factor but the concentration of acid used still creates a high reagent blank.

Many other resins with chemically bound chelating ligands have been synthesized and characterized. Muzzarelli and Rocchetti modified chitosan, a natural polymer, with a glucosamine functional group (86-88). Fritz and co-workers have modified XAD-4 resin to bind propylenediaminetetraacetic acid groups (89), n-butylamide groups (90), hexylthioglycolate groups (91), thioglycoloxymethyl groups (92), and N-substituted and N-unsubstituted hydroxamic acid groups (93). Vernon and Eccles have synthesized resins with N-substituted hydroxylamine (94) and 8-hydroxyquinoline (95). Leyden et. al. have immobilized chelating functional groups prepared by reacting silica gel with various silylating reagents (96-98). Sugii et. al. synthesized resins with oxime and diethylamino functional groups (99), and a resin with a pyridine moiety (100). Nakayama et. al. have synthesized a chelating resin with mercapto and azo groups (101), and Colella et. al. made a poly(acrylamidoxime) resin (102). 8-Hydroxyquinoline has been chemically fixed onto silica gel (7) and various polymer substrates (103) by Sturgeon et. al. and onto controlled pore glass by Sugawara et. al. (104). More recently, silica-bound diphenylcarbazone (105) and nitrobenzamide (106) has been synthesized. Also calcein has been immobilized onto cellulose (107) and an affinity gel has been coupled to carboxymethyl(imino)-bis(ethylenenitrilo)tetraacetic acid (108). Macroreticular resins have been modified to contain mercapto (109) and triazolethiol (110) functional groups. These are a few examples of the many chelating resins with chemically bound ligands that have been synthesized. Exchange capacities of up to 4 meq g^{-1} are typical. Although these resins have little affinity for the alkali and alkaline-earth metals, they usually require a fairly high concentration of acid for elution. Also, preparation of these resins involve long and difficult synthesis procedures.

By comparison, fewer ion-exchange resins containing ligands immobilized by adsorption have been investigated but have gained popularity in recent years. To cite a few examples, dithiazone has been incorporated into gel particles (111), dithiazone and DDTC have been immobilized on polyurethane foam (112). Silica gel has been coated with 1-nitroso-2-naphthol (113), a hydrazone (114) and a mixture of a liquid anion-exchanger (Aliquat 336) and Eriochrome Black T (115). A naphthalene disulfonic acid (SPADNS) and a benzene sulfonic acid (Orange II) was supported on a conventional anion-exchange resin (116). 2-Mercaptobenzothiazole was loaded onto glass beads (117) and 1-(2-pyridylazo)-2-naphthol (118) and a ferroin chromogen (119) were adsorbed on Amberlite XAD-4 and XAD-2 respectively. 8-Hydroxyquinoline adsorbed onto XAD-2 and -4 resin was unsuccessful in use because of severe ligand bleeding (120). While resins with ligands immobilized by adsorption are more easily made than chemically bound ones, generally they suffer from bleeding problems. They also have lower capacities (μeq g^{-1} range) but this is adequate for several applications in trace metal preconcentration.

Trace metals have been preconcentrated by adsorption onto a solid substrate. For example, metal ions have been directly adsorbed onto substrates such as activated charcoal (121-123), tungsten wire (124), polyether foam (125) and cellulose (126). Also, metal complexes are formed in solution and then adsorbed onto various solids. 8-Hydroxyquinoline metal complexes have been adsorbed onto activated charcoal (127) and onto C_{18} chemically bonded silica gel (128). Copper N-(dithiocarboxy)sarcosine (129) and chromium diphenylcarbazone (130) has been adsorbed onto an XAD-2 resin. Metal complexes of N-methyl flurohydroxamic acid (131), bis(2-hydroxylethyl)dithiocarbamate (132) and N-(dithiocarboxyl)sarcosine (133) have been adsorbed onto XAD-4. Also, ferroin metal chelates are adsorbed onto activated carbon (134) and APDC chelates onto C_{18}-bonded silica gel (135).

A novel approach to preconcentration of metal ions are flow injection systems, which permit a large number of samples to be processed rapidly in an automated fashion. These systems have been developed for anodic stripping voltammetry (136), coprecipitation-flotation (137) and solvent extraction/AAS (138,139). The most popular flow injection systems are the column-AAS or ICPAES systems. The columns are either packed with ion-exchange material (140-142) or chelating resins such as immobilized 8-quinolinol (143,144) or Chelex-100 (145). The development of flow injection systems is recent but they are gaining acceptance.

The desirable characteristics of a good preconcentration method are as follows: it should provide a large analyte preconcentration factor and matrix isolation simultaneously; it should require few reagents and steps in the procedure so as to minimize contamination; it should provide multi-element enrichment for heavy metals and it should be simple and inexpensive. Although several methods may possess some or even most of these advantages, there is still a need to develop better methods of preconcentration.

The Natural Water Sample

For metal determination in natural waters, the sample is normally passed through a 0.45 µm membrane filter. By convention, the filtrate is known as the "soluble" or "dissolved" portion, even though this includes colloidal particles. Metal ions assume various forms such as simple hydrated metals, inorganic and organic complexes, and metals adsorbed onto inorganic and organic colloids. The bioavailability, and hence toxicity, of metal ions is highly dependent on chemical form. Therefore, experimental determination of the distribution of these various forms has become an important but challenging endeavour. Several attempts to develop speciation schemes have been made. These and other studies have been recently reviewed by Florence and Batley (9,146,147). Although more meaningful information on metal bioavailability is derived from speciation studies, this thesis is concerned with total (soluble) metal-ion determination.

One final point must be emphasized about preconcentration with regard to natural complexing agents present in natural waters. Because of the several bound forms of metal ion, there is no assumption that the preconcentration method yields quantitative separation. The standard additions method (SAM) is often used to compensate for the effects of the complexing matrix. Analyte separation is dependent on the thermodynamic stabilities and labilities of the various natural complexes relative to those formed by the preconcentrating agent. Given suitable labilities, a sufficiently powerful preconcentration method can completely remove the analyte element from its matrix so that the application of SAM is not necessary. If quantitative separation of the analyte is not possible or proven, SAM should be applied and complete equilibration of the spike should be ensured prior to the preconcentration procedure and instrumental measurement (148).

1.3 Meso-Tetra(p-sulfonatophenyl)porphine, $TPPS_4$

Background

Porphyrins are conjugated tetrapyrrole macrocyclic molecules. They are frequently found in nature (for example, heme and chlorophyll), but many porphyrins have been synthesized. A metalloporphyrin is the metal chelate of a porphyrin that is formed by replacement of the two central hydrogens by a metal ion. Metalloporphyrins have been made with almost every metallic and semi-metallic element (149). There is an exhaustive amount of literature related to porphyrins and metalloporphyrins (i.e., 150,151). Sulfonated derivatives of tetraphenylporphine (TPP) are especially interesting. Because of their water solubility, one derivative extensively studied is meso-tetra(p-sulfonatophenyl)porphine ($TPPS_4$), shown in Figure 2.

The porphyrin ring is basically planar as shown by X-ray studies (152-154), with a ring diameter of about 8.5 Å and cavity diameter about 4.2 Å. Although $TPPS_4$ has a "ruffled" structure as a crystal (152), in solution the phenyl and porphyrin planes are thought to reach a near-planar conformation so that a resonance interaction can occur between the π systems (155-157). The metal in the metalloporphyrin may be four, five, six or eight co-ordinate. Most are four co-ordinate with square planar geometry with the metal-ion in the plane of the pyrrole nitrogens, or five

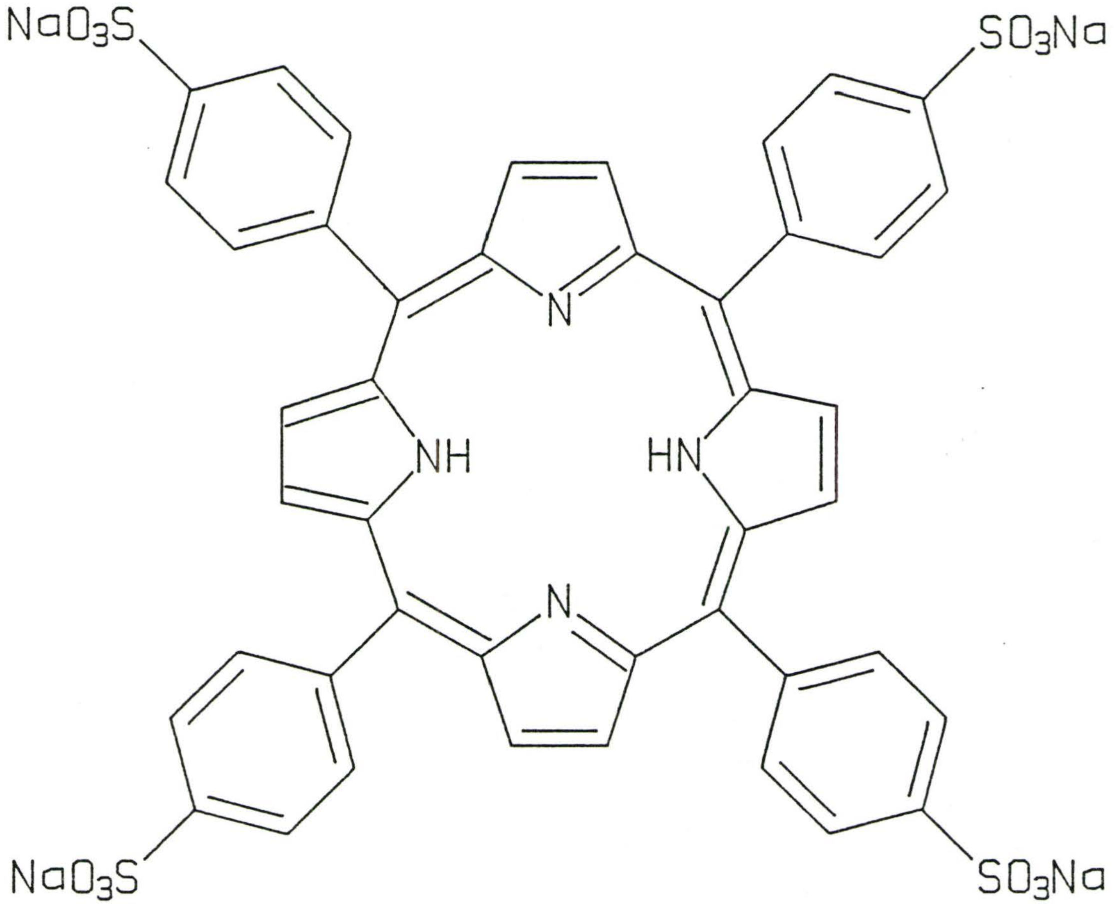

Figure 2. Structure of $TPPS_4$

co-ordinate with square-pyramidal geometry due to binding with an axial ligand (149,152).

In general, porphyrins have a distinctive 5-banded absorption spectrum from 350-650 nm (158). The most intense band is called the Soret band. Its maximum is between 350-450 nm, with a molar extinction coefficient about 10^5 cm^{-1} M^{-1}. The order of the other four bands depends on the type of porphyrin. The spectrum of $TPPS_4$ is "etio" type where the bands increase in intensity with energy. Protonation or metallation of the porphyrin leads to changes in the absorption spectrum due to symmetry changes in the molecule. Figure 3 shows the absorption spectrum of $TPPS_4$ and its protonated form. The dissociation scheme for the porphyrin (P), is as follows:

$$PH_4^{+2} \overset{K_4}{\rightleftharpoons} PH_3^{+} \overset{K_3}{\rightleftharpoons} PH_2 \overset{K_2}{\rightleftharpoons} PH^{-} \overset{K_1}{\rightleftharpoons} P^{-2} \qquad (1)$$

where K_1, K_2, K_3, K_4 are dissociation constants in ascending magnitude. The pH range of dissociation for PH_4^{+2} and PH_3^{+} overlap severely and the average pK_4 and pK_3 value is reported to be about 5 (159-161). The species PH^{-} and P^{-2} are so basic that K_2 and K_1 cannot be determined in an aqueous solution.

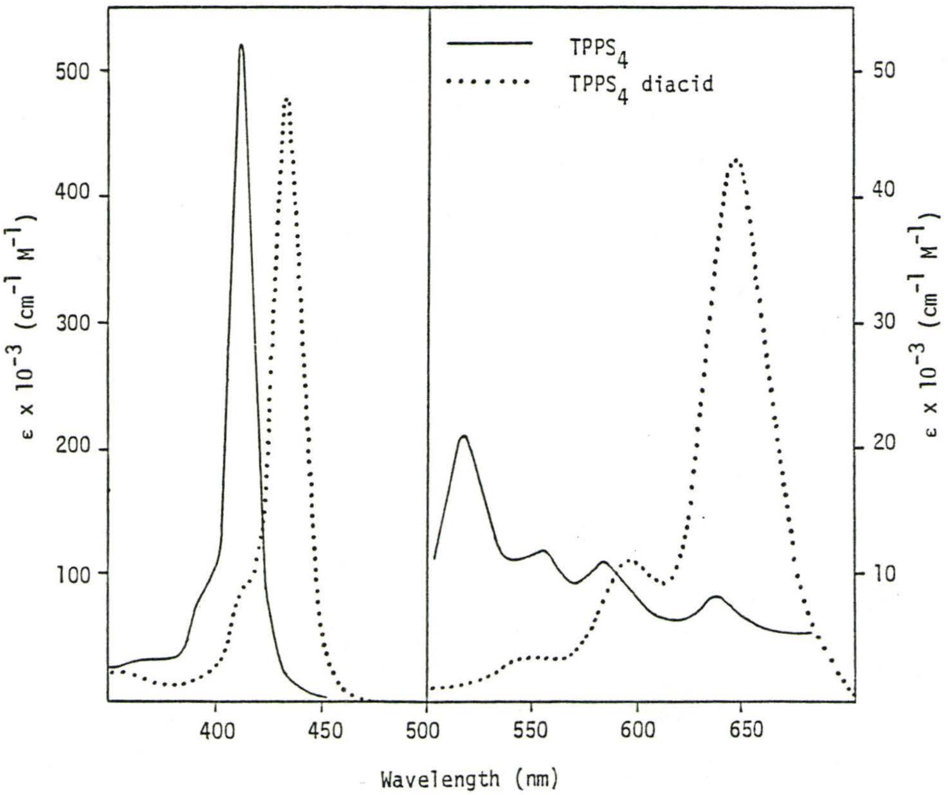

Figure 3. Electronic absorption spectrum of monomeric $TPPS_4$ and its diacid (both 1 X 10^{-6} M) (162)

Previous Work

A considerable amount of work has been reported on $TPPS_4$ and derived metalloporphyrins. Herrmann et. al. developed a novel method for metal insertion into the porphyrin by heterogeneous reaction with the metal reactant present in the insoluble form (either as the metallic element or metallic oxides) (163).

Herrmann and Corsini (164) also studied the aggregation of $TPPS_4$. In the neutral form (PH_2), the model that best accounts for both absorption and NMR spectral changes with increasing concentration involves two main equilibria:

$$2\ \text{MONOMER} \overset{K_D}{\rightleftharpoons} \text{DIMER} \qquad (2)$$

$$2\ \text{DIMER} \overset{K_{TT}}{\rightleftharpoons} \text{TETRAMER} \qquad (3)$$

In neutral to basic solution*, the monomer form exists below 3×10^{-6} M. In the concentration range 10^{-6} M to 10^{-4} M, the monomer-dimer equilibrium is dominant, with K_D(ave) = 1.51×10^4 M^{-1}. At high concentrations of $TPPS_4$ (>10^{-4} M), the dimer-tetramer equilibrium is important. The K_{TT} value (4-15 depending on the buffer used), is not a true equilibrium

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* The studies were done in phosphate buffer (pH 7) and sodium acetate media (pH 9), 0.1 M ionic strength, 25°C.

constant because the ionic strength could not be maintained constant at the high concentration levels of $TPPS_4$. It was also discovered that increasing the salt concentration increased aggregation while increasing the temperature decreased aggregation.

The protonated $TPPS_4$ behaves quite differently. Above the concentration of about 3×10^{-6} M*, changes in the absorption and NMR spectra pointed to the formation of micelles, a collection of about 10-100 monomer units which align so that their hydrophobic and hydrophillic portions meet. The variation in the critical micelle concentration (CMC) in different ionic media suggests that the porphyrin molecules are stacked with their planes parallel for maximum interaction between the π systems, and with adjacent molecules displaced horizontally relative to each other such that the sulfonate group of one porphyrin molecule interacts with the protonated centre of the next. At very high concentration of $TPPS_4$, precipitation was noticed.

Metalloporphyrins also aggregate but are pH insensitive over a wider range of acidities due to substitution of the protons for a metal ion. $Cu-TPPS_4$ and $Zn-TPPS_4$ were found to aggregate at concentrations above 5×10^{-7} M and 2×10^{-4} M, respectively**.

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* The study was done in formate buffer, pH 3.55, 0.1 M ionic strength, 25°C.

** These studies were done in phosphate buffer, pH 7, 0.1 M ionic strength, 25°C.

The applicability of $TPPS_4$ for metal-ion determination was briefly investigated by Herrmann and Corsini (162). In acidic solution, the reagent was found to show good selectivity for Cu(II), with Zn(II) and Ag(I) as the major interfering ions. Cu was quantitated by monitoring the absorbance of the Cu-$TPPS_4$ Soret band. Protonation of the free ligand shifts the Soret band so that excess ligand does not interfere with the Cu determination.

DiFruscia et. al. (165) showed that in neutral to basic solution, $TPPS_4$ was found to react with 12 metal ions at elevated temperatures, within an hour. The reactivities are shown in Table 4 and absorption spectra of several metalloporphyrins are shown in Figures 4 to 9. Note that the Cd(II), Hg(II) and Pb(II) metalloporphyrin spectra are a combination of the porphyrin and metalloporphyrin spectra since the complexes dissociate extensively in solution. Also, although Mn(II) is the reactive species, the higher (III) oxidation state is stabilized.

The different reactivities of the metal ions ultimately led to a procedure for the simultaneous preconcentration of Cd(II), Co(II), Cu(II), Ni(II) and Pb(II) from seawater (165). In this method, the $TPPS_4$ complexes were extracted by ion-association into methylisobutylketone (MIBK) with tricaprylmethylammonium chloride (TCMA), followed by subsequent determination by GFAAS. In comparison to the frequently used APDC system, $TPPS_4$ and its complexes are

Table 4. Reactivity of $TPPS_4$ with Metal Ions; Aqueous Solution, pH 5 - 9 (165)

Observed Reaction (1 hour)		No Observed Reaction (3 days, steam bath)			
Mn^{+2}	Pd^{+2}	Be^{+2}	Cr^{+3}	Gd^{+3}	Th^{+4}
Fe^{+2}	Ag^{+}	Mg^{+2}	Fe^{+3}	Ho^{+3}	UO_2^{+2}
Co^{+2}	Cd^{+2}	Ca^{+2}	La^{+2}	Er^{+3}	VO^{+2}
Ni^{+2}	Hg^{+2}	Sr^{+2}	Ce^{+3}	In^{+3}	
Cu^{+2}	Sn^{+2}	Ba^{+2}	Nd^{+3}	Sc^{+3}	
Zn^{+2}	Pb^{+2}	Al^{+3}	Eu^{+3}	Y^{+3}	
12		21			

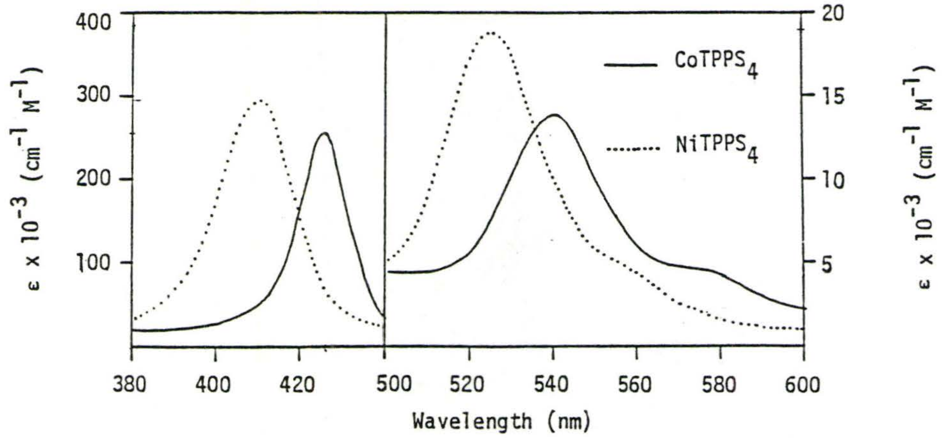


Figure 4. Absorption spectra of Co-TPPS$_4$ and Ni-TPPS$_4$ (both 1 X 10^{-6} M, 1 cm cells for λ < 500 nm; 2 X 10^{-5} M, 1 cm cells for λ > 500 nm) (162)

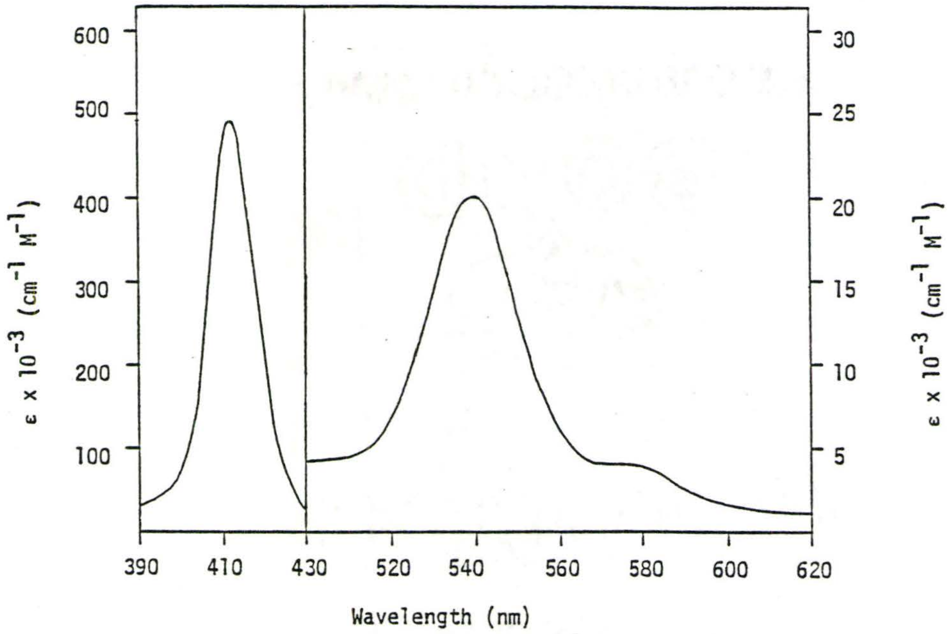


Figure 5. Absorption spectrum of Cu-TPPS$_4$ (conditions same as in Figure 4) (162)

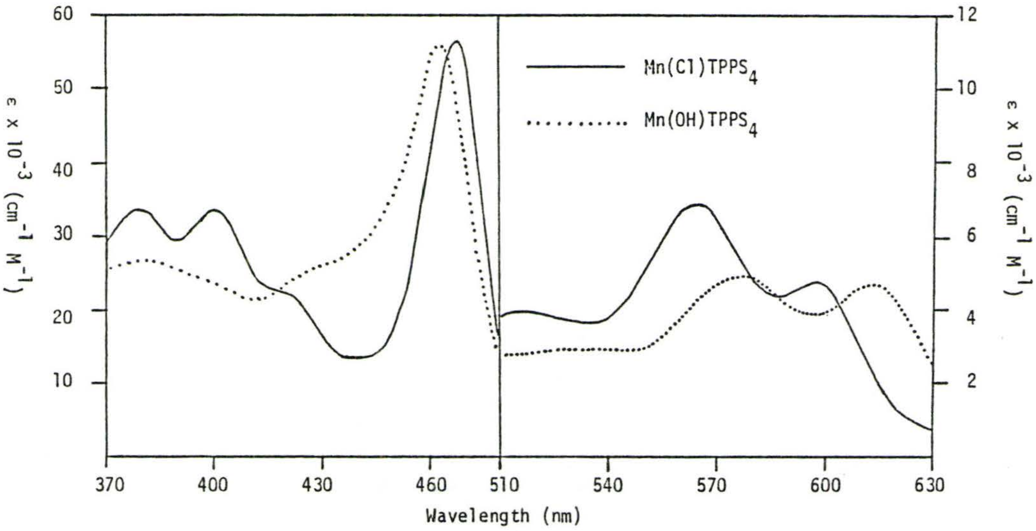


Figure 6. Absorption spectra of Mn(III)-TPPS$_4$ (1 X 10^{-5} M for $\lambda < 500$ nm; 1 X 10^{-4} M for $\lambda > 500$ nm; 1 cm cells in both cases) in 0.25 M HCl and in 0.25 M NaOH (162)

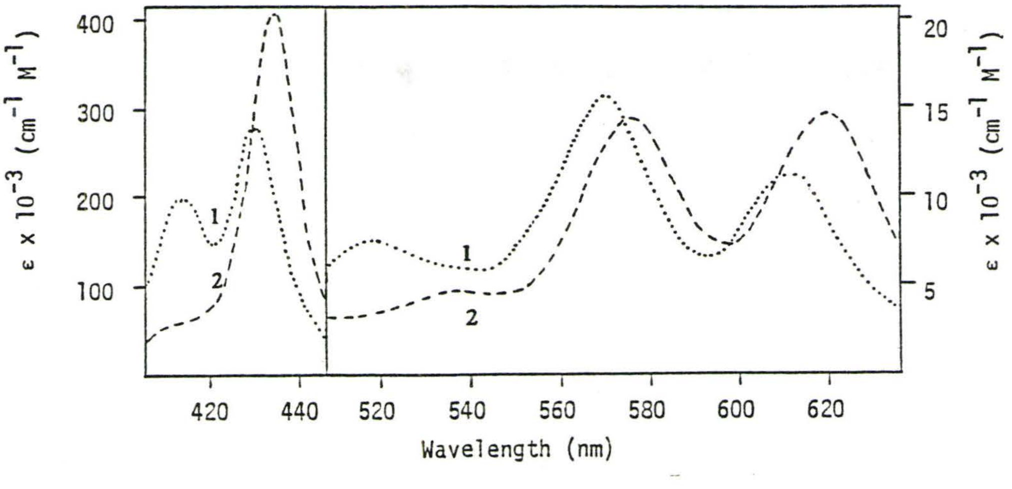


Figure 7. Absorption spectrum of Cd-TPPS$_4$ (1, neutral solution; 2, strongly alkaline solution; both 2 X 10^{-4} M, 0.10 mm cells for $\lambda < 500$ nm, 1.0 mm cells for $\lambda > 500$ nm) (162)

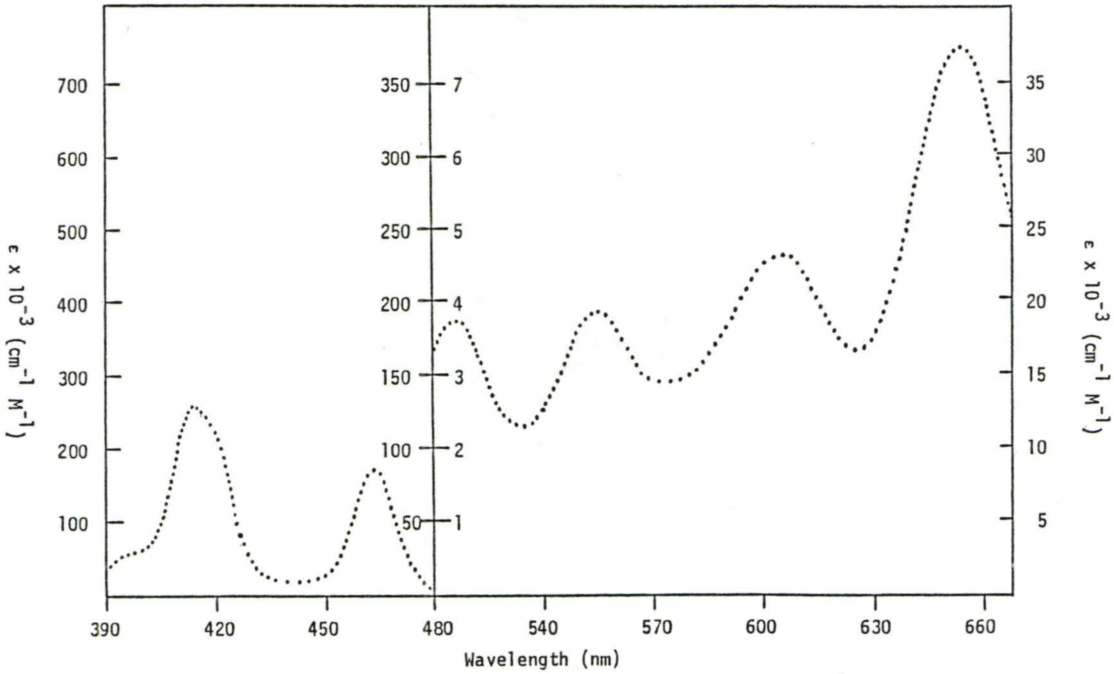


Figure 8. Absorption spectrum of Pb-TPPS$_4$ (2 X 10^{-4} M, 0.10 mm cells for λ < 500 nm, 1 cm cells for λ >500 nm) (162)

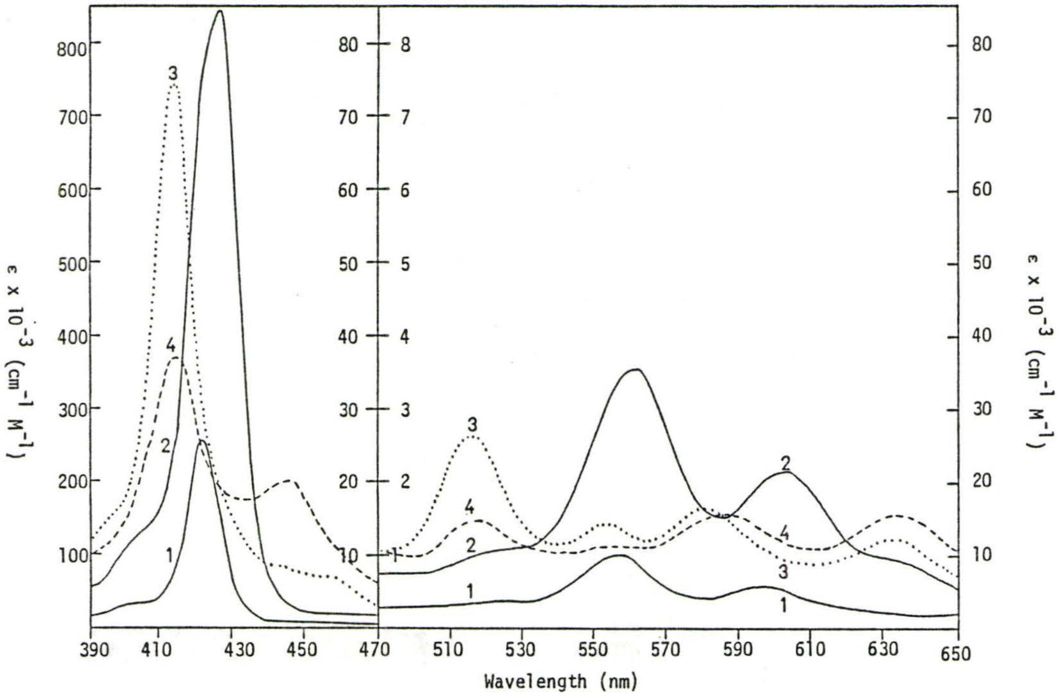


Figure 9. Absorption spectra of Hg-TPPS$_4$ (162)

a) Complex prepared for Hg metal (1 X 10^{-5} M; 1 mm cells for $\lambda < 500$ nm, 1 cm cells for $\lambda > 500$ nm; 1, neutral solution; 2, strongly alkaline solution

b) Complex prepared from HgO (1 X 10^{-4} M; 1 mm cells for $\lambda < 500$ nm, 1 cm cells for $\lambda > 500$ nm; 3, neutral solution; 4, strongly alkaline solution

NB: Outer scales refer to spectra 1 and 2, inner scales refer to 3 and 4.

generally more stable and more extractable than APDC and its complexes, but the formation of the $TPPS_4$ metal complexes is more difficult.

Because of the intense absorption of the Soret band, porphyrins have become popular reagents for sensitive spectrophotometric determinations of metal ions at trace and ultra trace levels in aqueous solutions. For example, emulsified TPP has been used to determine Cu(II) (166). Tetrakis(4-carboxyphenyl)porphine [T(4-CP)P] has been used to determine trace amounts of Cu(II) (167) and Cd(II) (168); tetraphenylporphine trisulfonic acid (TPPS) to determine Cu(II) (169), Pb(II) (170), Cd(II) (171) and Pd(II) (172); and $TPPS_4$ for determination of Pd(II) (173) and Cd(II) (168). Several other porphyrins have also been used; e.g., tetra(3-N-methylpyridyl)porphine (174,175), tetrakis(1-methyl-3-pyridyl)porphine [T(3-MPy)P] (176-179), tetrakis(1-methyl-4-pyridyl)porphine [T(4-MPy)P] (176,179,180), tetrakis(5-sulfothienyl)porphine (181) and tetrakis(4-trimethylammoniophenyl)porphine (182,183). Some of these procedures have been applied to natural water samples (174,176,178, 181). Although the sensitive spectrophotometric determination often eliminates the need for preconcentration, it lacks selectivity and, consequently, various metal ions and salts interfere. Attempts to eliminate the interferences by addition of masking agents (171) or by separation of the analyte from the interfering ions by extraction (172,178) have been made. Problems also arise if the Soret band of the free ligand (usually added in excess), overlaps with that of the metal complex. If the metal complex is stable in acid, the solution can be either acidified to shift the Soret band of the free ligand (166,174), or the excess free ligand can be converted to another metal complex having a different wavelength (178,184). If the complex is labile, the remaining ligand can be converted to a stable metal complex and the labile complex decomposed by acidification and the absorbance of the liberated ligand monitored (177). Spectral interference caused by the overlap of the Soret band of the free ligand with that of the metal complex can also be eliminated by an extraction process. For instance, Cu(II) has been complexed with T(3-MPy)P and extracted into 2-nitropropane from an acidic solution with perchlorate (185). Another example is the extraction of Cu(II)-T(4-MPy)P with dodecylbenzenesulfonate (186).

Fluorometric techniques have also been developed to measure metalloporphyrin concentrations; for example, Cu(II)-TPPS (187), Mg(II)-T(4-MPy)P (188), and Zn(II)-TPPS (189). In some cases, the fluorescence of some porphines and their complexes has been advantageously used.

High pressure liquid chromatography (HPLC) has been used for the separation, identification and quantitation of metalloporphyrins. The disadvantage of this method is that only the absorbance at one wavelength is monitored and hence the method is limited to a few metal ions; e.g., Cu(II), Zn(II) and Pd(II) determined as T(4-MPy)P complexes (190).

Several groups (191,192) have shown that the relative incorporation rates in aqueous solution depend on the metal-ion ($Cu^{2+} > Zn^{2+} > Co^{2+}, Fe^{2+}, Mn^{2+} > Mg^{2+}, Ni^{2+} >> Al^{3+}, Fe^{3+}, Cr^{3+}$). This rate order approximately parallels the rates of water exchange by aquo ions (191). Generally, the rate of aqua metal-ion incorporation into porphyrins is very slow compared to non-cyclic chelating agents because more than one water molecule at a time must be removed for metal-ion incorporation to occur (193). The reaction mixture has to be heated to force the reaction to reach completion but once formed, the complexes are quite stable (149). Hg(II), Cd(II) and Pb(II) complexes, however, form readily even at room temperature, and are more labile. These complexes have been advantageously used to accelerate formation of other metalloporphyrins. Electrophilic substitution of one metal ion for another which is coordinated to a porphyrin, has been previously studied with TPP (194), $TPPS_4$ (195) and TMPyP (196) and the following room-temperature stability sequence was found: (Cu, Zn) > (Hg > Pb) > (Li > Na > K). More recently, Cd(II), Hg(II), and Pb(II) in anionic porphyrins such as $TPPS_4$ and [T(4-CP)P] have been shown to cause substitution almost instantly by Cu(II), Zn(II), Co(II) and Mn(II) at room temperature, in aqueous alkaline solution (197). Small amounts of Mn(II) and Co(II) have been spectrophotometrically determined by metal substitution of Cd-[T(4-CP)P] (198) and Cd-$TPPS_4$ (199), respectively. Although this is a faster method of trace metal-ion determination, there are still problems with interference from other anions and cations. Recently, a method for Zn(II) determination in the presence of Cd(II) was reported based on the different dissociation rates of their complexes with $TPPS_4$ (200).

A kinetic method has been developed for the determination of trace amounts of Hg(II), Cd(II) or Pb(II). The method is based on the catalytic effect of these metal ions on the complexation of other metal ions such as Mn(II). The net decrease in absorbance of the free-porphyrin Soret band at a fixed time after the initiation of the Mn(II) insertion is proportional to the concentration of Hg(II), Cd(II) or Pb(II). Thus, Hg(II) has been determined in nanogram amounts through its catalytic effect on the complex formation reaction of Mn(II)-TPPS (201). Similarly, the determination of Cd(II) by its catalytic effect on Mn(II)-$TPPS_4$ formation has been reported (202,203). Obviously, the determination of one of the three metal ions mentioned is interfered with by the other two. But these interferences can be eliminated; for instance, in the Hg(II) determination, metallic mercury can be separated by distillation at room temperature (201) and in the Cd(II) determination, Hg(II) was reduced by addition of hydroxylamine while Pb(II) was removed by coprecipitation with MnO_2 (202).

Although there have been many studies on metal-ion incorporation into the porphyrin, the mechanisms of such reactions are by no means clear. The overall scheme of metallation involves the metal ion losing much of its solvation sphere and the porphyrin releasing two protons. Most of the proposed mechanisms involve a metal ion-porphyrin intermediate with its protons intact. The following sequence of reactions best fits the current published data (204-206):

deformation:

$$H_2P \rightleftharpoons H_2P(\text{deformed}) \tag{4}$$

outer-sphere complexation:

$$ML_6 + H_2P(\text{deformed}) \rightleftharpoons [L_6M,\ H_2P(\text{deformed})]^{2+} \tag{5}$$

ligand dissociation and first bond formation:

$$[ML_6,\ H_2P(\text{deformed})]^{2+} \rightleftharpoons L_5M\text{-}H_2P^{2+} + L \tag{6}$$

second bond formation (SAT):

$$L_5M\text{-}H_2P^{2+} \rightleftharpoons L_nM{=}H_2P^{2+} + (5-n)L \tag{7}$$

metalloporphyrin formation:

$$L_nM{=}H_2P \rightleftharpoons MP + L_n + 2\ H^+ \tag{8}$$

Several fast pre-equilibrium steps are thought to occur: deformation of the porphyrin followed by outer-sphere complexation with the metal-ion-containing species; ligand dissociation of the metal-ion species with first bond formation with the porphyrin (H_2P); second bond formation to bring about the rate-determining "sitting-atop" or "SAT" intermediate (the metal-ion species sits above the plane of the porphyrin coordinated to central nitrogen atoms which still retains the two central protons); the final step is the concerted loss of the two hydrogen atoms and insertion of the metal ion into the porphyrin.

It was Fleischer and Wang who first proposed the SAT model in 1959 (207). Although the SAT complexes could not be isolated and the original SAT species was later shown to be similar to ion pairs (208), examples have been presented to lend strong support to the existence of SAT complexes as a common intermediate (209-212).

Tabata and Tanaka have proposed a metal-ion assisted mechanism of metalloporphyrin formation based on their kinetic method for the determination of trace amounts of Hg(II), Cd(II) and Pb(II) (203,213,214). The presence of Pb(II), Cd(II) or Hg(II) greatly accelerates the rate of metal insertion. Hg(II), Cd(II) and Pb(II) are larger ions* that cannot fit into the porphyrin cavity but form out-of-plane, weakly bonded metalloporphyrins. The formation of this mononuclear activated complex is rapid. The activated complex is readily irreversibly substituted by medium-sized ions such as Mn(II), Co(II) and Ni(II) to form stronger metal-nitrogen bonds. Using Hg(II), Mn(II) and $TPPS_4$ as examples, the following sequence is postulated:

$$\mathrm{Hg(II)} + \mathrm{H_2TPPS_4} \rightleftharpoons \mathrm{Hg(II)\text{-}TPPS_4} + 2\ \mathrm{H^+} \qquad (9)$$

$$\mathrm{Hg(II)\text{-}TPPS_4} + \mathrm{Mn(II)} \rightleftharpoons \mathrm{Mn\text{-}TPPS_4} + \mathrm{Hg(II)} \qquad (10)$$

---

* The crystal ionic radii of some elements are: Ni(II), 0.69 Å; Cu(II), 0.72 Å; Mn(II), 0.80 Å, Cd(II),0.97 Å; Hg(II), 1.10 Å; Pb(II), 1.20 Å (215).

The Hg(II)-TPPS$_4$ complex forms rapidly, with Hg(II) sitting on the nucleus. This appears to be a favorable configuration for attack by Mn(II) from the back to form a heterodinuclear activated complex with Hg(II) and Mn(II). Reaction 9 is a rapid equilibrium step and reaction 10 is the rate-determining step. Hg(II) is released in reaction 10 and used again in reaction 9 as a catalyst in Mn-TPPS$_4$ formation. Therefore, the mechanism of metalloporphyrin formation is dependent on the effective radius of the catalytic metal ion (i.e., Hg(II) > Cd(II) > Pb(II)), pH, and the concentration of the metal ions involved.

1.4 Adsorption of Metal Complexes on XAD Resins

Adsorption Isotherm, Langmuir Equation

Adsorption is the result of non-specific forces that cause retention of a gas, liquid, solute or colloid onto the surface of a solid. Freundlich and Langmuir isotherms are used most frequently to represent data on adsorption of solutes from solution. A convex isotherm is described by the Freundlich equation:

$$\Gamma = k\ [M]^{1/n} \quad (11)$$

where k and 1/n are characteristic constants, [M] is the equilibrium concentration of adsorbate and Γ is the amount adsorbed per gram of adsorbent. Although the equation is easy to use it does not account for common experimental observations, i.e., it does not show a linear proportionality of [M] to Γ at low concentrations and a limiting plateau value of [M] at high concentrations (216).

The Langmuir equation, originally developed for adsorption of gas molecules onto solids, also describes a convex isotherm:

$$\Gamma = \frac{\Gamma_{max}\ [M]}{(1/K) + [M]} \qquad (12)$$

where Γ_{max} is the limiting adsorption value and K is the heterogeneous equilibrium constant. At low values of [M], (i.e., [M] << 1/K), Γ approaches a linear function of [M]; at high values, Γ approaches a limiting value, Γ_{max}. In this model, Langmuir assumed that only one gas molecule per active site is adsorbed (monolayer adsorption), that the sites are identical in affinity, and that the occupation of a site did not affect the properties of other neighboring sites. This equation can be applied to dilute solutions but deviation from the theoretical model may be evident for several reasons (217):

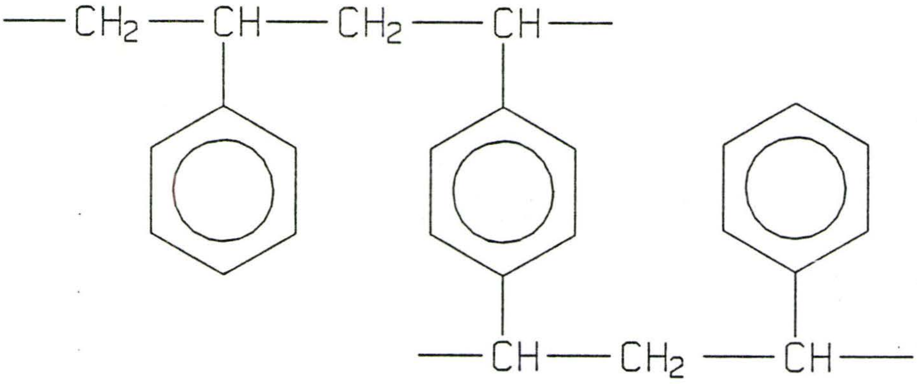
1. the adsorption process is more complex, involving solvent-solute, solvent-adsorbent, and solute-adsorbent interactions. Other factors such as pH, ionic strength and other possible adsorbates increase the complexity;
2. at the molecular level, solid surfaces are more often not homogeneous;
3. adsorption frequently proceeds beyond the monolayer capacity.

Adsorption isotherms of $TPPS_4$ and their metalloporphyrins will be interpreted with these limitations in mind.

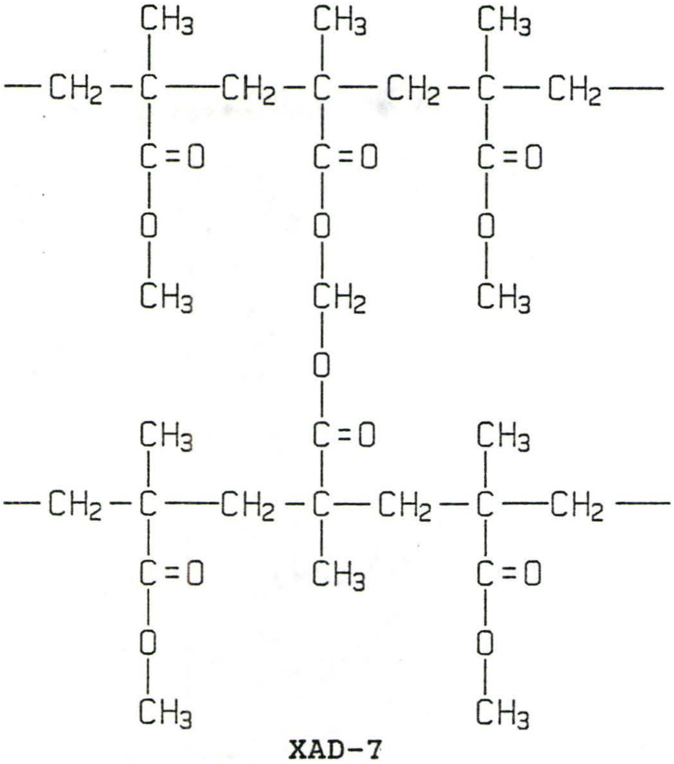
Properties of XAD Resins and Some Applications

The process of adsorption is the basis of adsorption chromatography and of some preconcentration methods. In adsorption chromatography, samples are introduced on a column with a flowing mobile phase and separation of individual compounds is achieved based on differences in partition coefficients. Preconcentration methods differ from adsorption chromatography in that sample retention can be by either batch equilibration or column percolation and species are often eluted simultaneously. Adsorbents frequently used are silica, alumina, diatomaceous earth and activated carbon (218). Factors which determine adsorbent capabilities include surface area, pore size, and type and arrangement of the functional group on the surface. Recently, macroreticular XAD resins have become popular solid supports, the frequently used types being XAD-2, -4 and -7. XAD-2 and -4 are styrene-divinylbenzene copolymers of differing pore size and surface area, and XAD-7 is an acrylate ester copolymer. The structures of these resins are shown in Figure 13, and some physical and chemical properties are listed in Table 5. The resins have a rigid, porous structure which give them good physical durability and flow characteristics (i.e., readily solvated with minimal swelling and contraction (219)). XAD-7 is thermally stable up to 150°C and XAD-2 and -4 to 250°C (220). The resins are stable over the entire pH range (221) and to solvents of all polarities (219). These characteristics make the resins attractive analytical column materials.

XAD resins retain weak organic acids and bases, and ampholytes. They have been extensively applied to the separation and preconcentration of organic molecules. For example, they have been used in the analysis of air samples (e.g., glass capillary GC separation of polynuclear aromatic



XAD-2, XAD-4 (see Table 5 for difference in properties)



XAD-7

Figure 10. Structures of Some XAD Resins (220)

Table 5. Some Chemical and Physical Properties of XAD Resins (219)

Resin	Chemical Nature	Polarity	Dipole Moment (debye)	Ave Pore Diameter (Å)*	Surface Area (m^2/g)*
XAD-2	polystyrene divinylbenzene	hydrophobic	0.3	90	330
XAD-4	"	"	0.3	50	750
XAD-7	methyl-methacrylate	hydrophillic	1.8	80	450

* These properties refer to the dry resin. Values for the solvated beads are different due to swelling of the gel phase.

hydrocarbons (PAH's) adsorbed on XAD-2 (222)); water samples (e.g., reverse-phase HPLC separation of organic acids on XAD-2, -4 and -7 (223,224); drugs (e.g., LC separation of analgesics using XAD-7 (225)); and mineral oil (e.g., LC separation of PAH on XAD-2 (226). XAD resins have also been used to preconcentrate compounds prior to identification. Polychlorobiphenyls (PCB's) and low molecular weight compounds (i.e., benzene, acetone, heptane, butyl acids) have been concentrated from air using XAD-2,-4 and -7 (227,228). Also, PAH's, halogenated hydrocarbons, trace organic contaminants, and humic and fulvic compounds have been concentrated from water samples on XAD resins (229-234). XAD-2 and -4 have also been used to remove chlorophenols from chlorine-treated water (235), chlorinated pesticides from industrial waste streams (236), colored impurities in sugar (237), and colour and odour contaminants in water (238).

There have been a limited number of reports on the use of XAD resins to preconcentrate trace metals. As referred to previously, metal ions have been retained directly on XAD-7 (69-72) and on XAD-2 and -4 (73,74). Also metal ions have been indirectly retained as metal complexes adsorbed onto XAD-2 (119,129,130) and XAD-4 (118,120,131-133).

1.5 Aim of Research and Summary

Previous work in this laboratory showed that $TPPS_4$ is apparently strongly adsorbed on XAD-4 and is not completely eluted by a number of solvents (239). This observation became the basis for a simple approach to the creation of a chelating resin which avoided the lengthy procedures that would be necessary for the synthesis of a resin with a chemically-bonded chelating moiety. In the course of the present work, however, it became clear that a preconcentration column procedure based on this chelating ion-exchange resin was not practical. For metal-ion complexation, it was necessary to heat the column (jacketed) to about 98°C. This heating caused the resin to "float" to the top of the column. Furthermore, excessive bleeding of the ligand occurred from the resin, presumably due to increased solubility of $TPPS_4$ at elevated temperatures. Even at room temperature, some bleeding of the ligand occurred upon percolation of buffer solution (about pH 10) or eluting agent (1% HNO_3). Because of these difficulties, the development of a chelating column based on $TPPS_4$ was not pursued further.

The aim of the research became to develop a preconcentration method for several trace metals in natural waters based on the formation of their $TPPS_4$ metal complexes in solution, subsequent adsorption of the complexes onto an appropriate XAD resin, elution from the resin and finally

GFAAS measurement. In this work, a general method was developed which enables determination of Cd(II), Cu(II), Ni(II), and Pb(II) by simultaneous metal complexation with $TPPS_4$ at elevated temperatures (about 90-98°C); adsorption of the metal complexes onto XAD-7 resin by column percolation; elution of the complexes with a 80% methanol/water mixture; and measurement by GFAAS. SAM was used for calibration.

A method specific for Cd(II) and Pb(II) was also investigated. Preliminary evidence indicated that in the above group of metal ions, Cd(II) and Pb(II) seemed to react quantitatively at room temperature with $TPPS_4$. This observation implied that a very simple and rapid batch method could be developed for the preconcentration of these two ions as complexes adsorbed on XAD resin. As described later, however, more detailed studies showed that the room-temperature reaction is not complete at low metal ion concentration. Although incomplete reaction could be compensated by the use of SAM, the precision, particularly for Pb, is unsatisfactory.

CHAPTER 2. EXPERIMENTAL SECTION

2.1 General

Laboratory Apparatus

Adsorption losses to vessel walls are severe at low metal-ion concentrations and at high pH. In accordance with recommendations from studies to minimize these losses (17-22), polypropylene or polyethylene vessels were used as much as possible and water samples were stored at pH 1.6 - 1.7.

Borosilicate glass columns (Bio-Rad Laboratories, Mississauga, Ontario) were used for loading the resins. These columns were 20 cm X 1.0 cm i.d. or 10 cm X 1.0 cm i.d. with a 35-µm pore bed support interfaced to a nylon three-way value and disposable pipette tip.

The delivery of small volumes was made with Eppendorf pipettes (fixed-volume and continuously variable digital types). The accuracy of the pipettes ranged from 1.2% at 5 µL to 0.5% at 1000 µL; the precision ranged from 0.5% at 5 µL to 0.15% at 1000 µL. Both accuracy and precision were adequate for the work of this thesis. The pipettes were calibrated periodically according to the manufacturer's instructions.

Reagents

Most of the chemicals were of reagent-grade quality or better and were used without further purification. Aqueous solutions were prepared with distilled deionized water (DDW); i.e., distilled water passed through a Barnstead Ultrapure Cartridge (Sybron Corporation, Boston, Massachusetts).

Electrolyte stock solutions (1.0 M NH_4OAc and 1.0 M NH_4Cl) and saline solutions (0.77 M NaCl) were passed through a column of Chelex-100 (Bio-Rad, 200-400 mesh) before use to remove trace metals. Buffer solutions were prepared from the purified electrolyte solutions and appropriate amounts of Baker Instra-Analysed grade concentrated HNO_3, HOAc or NH_4OH (Baker Chemical Company, Phillipsburg, New Jersey).

Tricaprylmethylammonium chloride or TCMA (Aliquat 336, General Mills) was washed with 2 M HCl and 2 M NH_4OH to remove trace metal contaminants.

Solutions of metal ions were prepared by dilution of 1000 ppm standard solutions (1% accuracy) of Cd(II), Co(II), Cu(II), Mn(II), Ni(II) and Pb(II) (Fisher Scientific Co., Toronto, Ontario).

Humic substances (sodium salt) were obtained from the Aldrich Chemical Company (Milwaukee, Wisconsin). A stock solution of 10,000 mg L^{-1} was prepared by dissolution of the appropriate amount of humic substances in dilute aqueous ammonia (approximately pH 11). Upon standing, undissolved amounts settled out and this residue was removed by filtration (0.45 µm membrane filter). Working solutions were obtained by appropriate dilution and adjustment of pH.

The reagent, $TPPS_4$, in the sodium-salt form was synthesized and purified in the laboratory by the procedure of Herrmann et. al. (162). Further purification was performed as per DiFruscia et. al. (165) for use in trace-metal analysis.

Test Solutions

Freshwater and seawater samples were used to demonstrate method application. A Sandy Cove coastal seawater sample was obtained from the Atlantic Regional Laboratory (Halifax, Nova Scotia). This sample had been previously filtered through a white-sand filter and acidified to about pH 1.7 prior to storage. Three Lake Ontario samples were supplied by Canada Centre for Inland Waters (Burlington, Ontario): one was a nearshore sample (Station 41); another an offshore sample (Station 302); and the other was taken at lat. 43° 36' 30", long. 78° 00' 0" (1 meter depth). The samples were centrifuged to remove suspended particulates and acidified to about pH 1.7 for storage.

Resins and Resin Preparation

The Amberlite resins XAD-2, XAD-4 and XAD-7 (Rohm and Haas) were supplied by BDH Chemicals. The particle size of the purchased resins is 20-50 mesh and smaller particle sizes were obtained by manual grinding with a pestle and mortar or by mechanical grinding with the SPEX mixer/mill. Sorting was achieved by dry-sieving through a set of C.E. Tyler standard sieves to give the particle size ranges shown in Table 6. The fines of the resin fractions were removed by decanting from a water suspension. The resin was then dried at 100°C for 24 hours.

These resins are contaminated with organic and inorganic impurities and must be cleaned prior to use. A modified clean-up procedure by Junk et. al. and James et. al. was used to remove the organic contaminants (231,240). The resins (about 5-10 g) were washed batchwise under vacuum with methanol (100-200 mL), acetonitrile (100-200 mL) and diethyl ether (100-200 mL) in turn, and rinsed thoroughly with DDW. The inorganic contaminants were removed by washing with 5% (v/v) nitric acid (100-200 mL) followed by copious rinsing with DDW. For subsequent batch experiments, the resins were either used directly after cleaning or dried in an oven at 100°C and stored for later use. After use, the resins were regenerated for future use by the above purification procedure. The use of resins for batch equilibration studies is discussed in Section 2.5.

For columns work, the resins were slurry-loaded in methanol. After loading, the bed was rinsed with DDW, washed with about 200 mL of 5% HNO_3, and then with dilute ammonia until the effluent was pH 8-9 and finally rinsed with DDW

Table 6. Resin Particle Size

Mesh Size Range	Particle Diameter Range (µm)
< 60	> 250
60 - 80	250 - 180
80 - 100	180 - 150
100 - 150	150 - 106
> 150	< 106

until the effluent was neutral. The column was backwashed to ensure uniform packing and then conditioned with about 50 mL of the appropriate buffer solution. After use, the XAD-2 and XAD-4 resins were discarded since $TPPS_4$ could not be removed from these resins (see Section 3.1). After use, the XAD-7 column was regenerated by rinsing with HNO_3, dilute ammonia, and DDW and backwashed before storage for the next experiment. Applications of the columns are described in Sections 2.7 and 2.8.

The Chelex-100 resin (sodium-ion form, 100-200 mesh) was obtained from Bio-Rad Laboratories. The operation of this column followed the procedure described by Kingston (84) and Sturgeon (61). Inorganic contaminants were removed from the resin by washing batchwise with 5M HNO_3, 4M HCl and DDW. This was followed by washing with 1 M ammonia to convert the resin to the ammonium ion form, and rinsing with DDW. The Chelex-100 was then slurry-loaded into a borosilicate column to a bed size of 1 X 6 cm. Two 5-mL portions of 5 M HNO_3, two 5-mL portions of 4M HCl, 5 mL of DDW and 5 mL of 2 M ammonia was passed through the column. The column was backwashed and rinsed with DDW until the effluent pH was 8-9. Before use, the column was conditioned with 50 mL of the appropriate buffer. After an experiment, the column was rinsed with dilute ammonia and DDW and stored. The use of Chelex-100 is described in Section 2.8.

2.2 Instrumentation

General

A horizontal laminar-flow work station, bench-top model (Canadian Cabinets, Ottawa, Ont.) was used for the preparation of solutions, filtration, and the loading and stripping of columns. The unit contains a high efficiency particulate air filter (HEPA filter) which removes 99.97% of particles of 0.3 microns or larger.

For vacuum filtration, a Fisher Filtrator, which is designed to filter and collect liquid directly into a beaker, was used. The filters were of the Millipore HA type, 0.45 µm (Millipore Corporation, Bedford, Mass.).

Measurements of pH were make with a Fisher Accumet 520 Digital pH meter equipped with an Orion 91-05 combination electrode. The pH meter was standardized with the appropriate Fisher (NBS) buffers prior to use and in the manner recommended by the manufacturer.

In batch experiments, the resin-solution mixture was agitated with a Burrell Wrist-Action Shaker, Model 75 (Pittsburgh, Penn.), on the continuous mode and stopped manually at measured times.

A Colora Ultra-Thermostat water bath was used to heat solution mixtures when required.

Atomic Absorption

Metal-ion determinations were made with a Perkin-Elmer Model 373 double beam spectrophotometer equipped with a D_2-arc background corrector and a HGA 2200 graphite-furnace atomizer/controller unit. Perkin-Elmer hollow cathode lamps and pyrolytic graphite tubes were used. Purified argon was used as purge gas. Sample injections were made manually with 5, 10 or 20 µL Eppendorf micropipettes. In Table 7, the conditions are listed for measurement of each metal in acidic media. Both the room-temperature method and the Chelex-100 method yield final solutions that are acidic. In Table 8, conditions are given for measurement of metal ions in a methanol-water mixture (80% v/v) for the general method. Drying times were selected according to the volume of sample injected; i.e., 10 µL required 12 sec, 20 µL required 22 sec. Charring times of 20 sec and atomization times of 5 sec were appropriate.

UV-Visible Spectrophotometer

The concentrations of $TPPS_4$ and metal-$TPPS_4$ complexes were measured with a GCA McPherson double-beam spectrophotometer, Model EU-700. Kinetic studies on metalloporphyrin formation were done on the Hewlett Packard UV-visible diode array spectrophotometer, Model HP 8451. Canlab quartz cells (1 cm) and Hellma Spectrosil quartz cells (0.1, 5, 10 cm) were used.

Table 7. Analytical Conditions for GFAAS Measurement of Trace Metals in Acidic Media

Element	Analytical Wavelength, nm	Temperature Settings, °C		
		Drying	Charring	Atomization
Cd	228.8	100	250	2100
Co	240.7	100	1000	2700
Cu	324.7	100	900	2700
Mn	279.5	100	1100	2700
Ni	232.0	100	1000	2700
Pb	283.3	100	700	2300

Table 8. Analytical Conditions for GFAAS Measurement of Trace Metals in Methanol-Water Mixture (80% v/v)

Element	Analytical Wavelength, nm	Temperature Settings, °C		
		Drying	Charring	Atomization
Cd	228.8	100	300	1700
Co	240.7	100	600	2400
Cu	324.7	100	900	2700
Mn	279.5	100	600	2700
Ni	232.0	100	600	2700
Pb	283.3	100	500	2100

2.3 Studies on XAD/$TPPS_4$ as a Chelating Resin

As noted in the Introduction, the initial purpose of this work was to test XAD resins on which $TPPS_4$ was adsorbed, as possible chelating resins for preconcentration of trace metals ions. Accordingly, preliminary qualitative experiments were done with the main purpose being to determine the resistance of the adsorbed $TPPS_4$ to a variety of chemical and physical conditions, some of which would prevail in the loading and stripping of metal ions onto and from the chelating resin.

XAD-4 (3.3 g, 20-50 mesh) was moistened with methanol, placed in 100 mL of 2 X 10^{-5} M $TPPS_4$ in DDW and shaken at room temperature for about two days. At the end of this period, the surface of the resin had assumed a purple coloration from the adsorbed $TPPS_4$. This resin was filtered and washed with about 200 mL of DDW, then 0.3 g portions were placed in 50 mL of the following media: phosphate buffer (0.01 M, pH 7.0), 0.1 M NaOH, 1 M NaOH, 1 M HCl, 6 M HCl, naphthalene sulfonic acid, ethanol, benzene, cyclohexane and DMSO. The supernatants were examined visually for coloration after the mixtures had been agitated and left to sit in each media for 24 hours at room temperature. Portions of the XAD-4/$TPPS_4$ resin (0.3 g), were also agitated for 2 hours in 0.1 M and 1 M NaOH, 1 M and 6 M HCl at 60-70°C and in ethanol, benzene, cyclohexane and DMSO at 40°C.

Ligand bleeding from a chelating resin is important since loss of chelating agent means a reduced capacity as well as possible loss of chelated metal ions during sample passage. Therefore, a study was done to investigate the extent of bleeding of $TPPS_4$ from columns packed with XAD-2/$TPPS_4$ and XAD-4/$TPPS_4$ resins, under pH conditions required for metal-ion complexation (pH 10.2 (165)), and for stripping of metal ions from the chelating resins (1% v/v HNO_3 in DDW).

About 0.5 g of XAD-2 and XAD-4 resin (80-100 mesh) was moistened with 2 mL methanol and each resin type was shaken with 25 mL of 1×10^{-3} M $TPPS_4$ until the absorbance of $TPPS_4$ at 414 nm ceased to decrease with time. This indicated that equilibrium was reached with the attainment of maximum adsorption of $TPPS_4$ (48 hours). From the decrease in absorbance, the amount of $TPPS_4$ adsorbed on each resin could be calculated. The resin was then washed well with pH 10.2 buffer and packed into a column and back-washed with the buffer. Each of the columns were eluted with a series of 1% HNO_3 washings (100 mL collected in 10 mL aliquots) and pH 10.2 buffer washings (50 mL collected in 25 mL aliquots) at the rate of 1 mL min^{-1}. The amount of $TPPS_4$ stripped off the column was determined spectrophotometrically and the concentrations were calculated from calibration curves of absorbance at 414 nm versus $TPPS_4$ concentration in pH 10.2 buffer and calibration curves of absorbance at 436 nm versus $TPPS_4$ concentration in 1% HNO_3 (Figures 35-38, Appendix).

The accumulated amount of stripped $TPPS_4$ was calculated as a percentage of the original quantity adsorbed on the resin.

A similar study was done in which 100 mL of pH 10.2 buffer was passed through a jacketed column heated at 98°C and collected in 10 mL aliquots.

2.4 Studies on Optimal Experimental Conditions

As stated in the Introduction, the attempt to create a chelating ion-exchange resin was unsuccessful and led to the ultimate development of the general method for the simultaneous preconcentration of several metal ions. This method entails metal-ion complexation followed by adsorption of the complexes. Therefore, preliminary studies were conducted to optimize the pH for complexation and particle size for complex adsorption.

pH and Buffer Type

An investigation was carried out to confirm the optimal pH for metal-ion complexation by following the formation of Hg, Cd, Pb and Ni metalloporphyrins over a pH range of 8 to 12. For these metal ions, the Soret band of the corresponding metalloporphyrins do not seriously interfere with that of the free ligand and so the extent of metal insertion could be monitored spectrophotometrically. The study was carried out in saline solution to simulate seawater media.

Aliquots of 100.0 mL (0.1 M NH_4OAc, 0.77 M NaCl) were adjusted from pH 8.0 to 10.6 with appropriate amounts of NH_4OH and from pH 11 to 12 with appropriate amounts of NaOH solution. The aliquots were spiked with a particular metal ion and $TPPS_4$. The $TPPS_4$ concentration was about 10^{-3} M, with the metal ions concentration in excess of 100 times (10 ppm). For the Ni solutions, 400 µL of hydrazine was added to aid in complex formation (165).

The solutions were heated at 90-98°C for 1 hour. Since Cd-$TPPS_4$ is thought to be light sensitive* (165), all flasks in this experiment (and columns in subsequent studies) had to be covered with aluminum foil to prevent decomposition of the complex. The absorbance values of the Soret bands were measured at 443, 443, 465 and 403 nm for the Hg, Cd, Pb and Ni complexes, respectively.

Particle Size

Various mesh sizes of XAD-7 were used to determine the particle size with the greatest capacity for $TPPS_4$.

Portions (0.5 g) of dry XAD-7 (20-60, 60-80 and 80-100 mesh) was moistened with 2 mL of methanol. The resin was cleaned batchwise as stated in Section 2.1. The cleaned resin was agitated with 20 mL of approximately 2×10^{-4} M $TPPS_4$ at pH 10.2 until adsorption equilibrium was reached. The amount adsorbed was calculated from the difference between the initial and final concentration of $TPPS_4$. The concentrations were calculated from calibration curves of absorbance at 414 nm versus $TPPS_4$ concentration in pH 10.2 buffer (Figures 35-36, Appendix).

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* That only the Cd complex is light sensitive seems curious. No attempt was made in this study to verify the light sensitivity of the Cd complex but the appropriate precautions were taken in the event that it was.

2.5 Batch Adsorption of $TPPS_4$ and Complexes on XAD Resins

Adsorption Isotherms

The adsorption of $TPPS_4$ and its complexes on XAD resins was studied. Adsorption isotherms for $TPPS_4$ on XAD-2, XAD-4 and XAD-7 and for Cu-$TPPS_4$ (chosen as a representative metalloporphyrin) on XAD-7, were obtained.

Solutions of Cu-$TPPS_4$ (10^{-3} M) were prepared by reacting $TPPS_4$ and Cu(II) in a 1:1 molar ratio at pH 10.2 for one hour at 90-98°C. Appropriate dilutions were made to obtain the concentration range required in the study.

For each type of resin, 0.5 g amounts of dried resin (80-100 mesh) were moistened with 2 mL of methanol and then agitated with 25-mL aliquots (pH 10.2) of various concentrations of $TPPS_4$ and Cu-$TPPS_4$ until adsorption equilibrium was attained. Equilibrium was assumed when the absorbance of small aliquots of supernatant (removed periodically) remained constant. The quantity adsorbed was calculated from the difference between the initial and equilibrium concentrations. Cu-$TPPS_4$ and $TPPS_4$ were monitored at 412 and 414 nm, respectively.

2.6 Column Operation Studies

Breakthrough Curves

Cu-$TPPS_4$ was selected as a representative metalloporphyrin to illustrate the column capacity of XAD-7 for the general method.

XAD-7 (80-100 mesh) was cleaned and dried and 0.5 g was packed in the column (1 X 3-cm resin bed) and conditioned as mentioned previously (Section 2.1). The Cu-$TPPS_4$ solution was prepared as described in Section 2.5. A solution of 1 ppm of Cu-$TPPS_4$ (1.6×10^{-5} M) was passed through the XAD-7 column at a rate of 1 mL min^{-1}. The concentration of Cu-$TPPS_4$ in the effluent was monitored by collecting 0.50 mL aliquots of effluent periodically and analyzing the aliquots spectrophotometrically at 412 nm. The procedure was repeated for 100 ppb solutions (1.6×10^{-6} M).

Elution Profiles

The preconcentration factor is determined by the ratio of sample volume and eluent volume required to quantitatively strip the column of analyte. The optimal methanol-water mixture for elution of Cu-$TPPS_4$ was sought.

An XAD-7 column (1 X 3-cm resin bed) was prepared in the usual manner (Section 2.1). A solution (100.0 mL, pH 10.2) with 4 ppb (6.3×10^{-8} M) of Cu(II) and 2×10^{-6} M of $TPPS_4$, was reacted at 90-98° C for an hour. This solution was percolated through the conditioned XAD-7 column at a rate of 1 mL min^{-1}. The analyte was eluted with a range of methanol-water mixtures from 25 to 95% v/v, collected in 0.50 mL aliquots and analyzed by GFAAS to yield elution profiles for Cu-$TPPS_4$.

2.7 Applications

General Method

The method was tested with synthetic samples before application to lakewater samples.

The synthetic lakewater samples (four 100.0-mL aliquots at pH 10.2) contained 10 mg L^{-1} humic acid and the following metal-ion concentrations: Cd(II), 0.08 ppb; Co (II), Mn(II), Pb(II) each 1.0 ppb; Cu(II), Ni(II) each 2 ppb. A combined metal-ion spike was added (0, 0.5, 1 and 1.5 times the concentration of each ion above), as well as 200 µL of 95% N_2H_4 and $TPPS_4$ (final concentration, 2 X 10^{-6} M). A blank solution with all the reagents except the metal ions was also prepared. The solutions were placed in screw cap polyethylene bottles, wrapped in aluminum foil and heated for 1.5 hours in a water bath at 90-98°C. The solutions were cooled and percolated through foil-wrapped XAD-7 columns (1 X 3-cm resin bed, 80-100 mesh), at a rate of 1 mL min^{-1}. The columns were rinsed with 10 mL of pH 10.2 buffer and eluted with a 80% methanol-water mixture into 5-mL volumetric flasks. The eluted metal complexes were measured by GFAAS.

Actual lakewater samples, previously acidified to pH 1.7, were filtered (0.45 µm membrane filter) and equilibrated with a combined metal-ion spike for 48 hours. The samples (200 mL) were adjusted to pH 10.2 with NH_4OH buffer, the appropriate amount of N_2H_4 and $TPPS_4$ added, and then processed in the same manner as the synthetic samples. The blank was prepared as for the synthetic samples.

Chelex-100

The accepted Chelex-100 method was applied to lakewater samples in order to compare results with the $TPPS_4$ general method.

A clean 1 X 6 cm column (Section 2.1) was conditioned with 50 mL of 0.010 M $NH_4OAc/HOAC$ buffer (pH 5.4). Aliquots of 200 mL of both synthetic and filtered real lakewater samples were adjusted to pH 5.4 in the same manner. They were passed through the Chelex-100 columns at 1 mL min^{-1} and then rinsed with 50 mL of the $NH_4OAC/HOAc$ buffer to remove alkali and alkaline-earth metal ions. The metal ions were eluted with 14% HNO_3 into 25-mL volumetric flasks. The concentrations of metal ions were determined by GFAAS using external calibration curves.

2.8 Studies on the Room-Temperature Method

Room-Temperature Complexation of Metal Ions

According to the literature, certain metal ions such as Cd, Pb and Hg complex with $TPPS_4$ faster than other metal ions. Accordingly, the extent of complexation at room temperature and at an elevated temperature were compared for these ions. If the extent of reaction is about the same, a simpler method not requiring heating could be developed.

Two sets of solutions containing each metal ion (1 X 10^{-4} M) in 100-fold excess of $TPPS_4$ (1 X 10^{-6} M) were prepared at pH 10.2. In ppm, the concentrations of metal ions were 10, 20 and 20 for Cd, Pb and Hg, respectively. One set was kept at room temperature for one hour and the other set was heated at 98°C, each for an hour, and then cooled. The Cd-$TPPS_4$, Pb-$TPPS_4$ and Hg-$TPPS_4$ concentrations were monitored spectrophotometrically at 433 nm, 464 nm, and 434 nm, respectively.

Resin Type

The capacity of XAD-2 and XAD-4 resins for $TPPS_4$ were compared to determine which was best suited for this method.

About 0.5 g of clean resin (dry, 80-100 mesh) was moistened with 2 mL of methanol and 25 mL of aqueous 10^{-3} M $TPPS_4$ at pH 10.2 was added. The mixtures were agitated until adsorption equilibrium occurred. The $TPPS_4$ concentration was monitored at 414 nm. The concentrations were calculated from calibration curves of absorbance at 414 nm versus $TPPS_4$ concentration (see Figures 35 and 36 in the Appendix). The amount of $TPPS_4$ adsorbed on each resin was calculated from the difference between the initial and final concentration of $TPPS_4$ in the supernatant. As described later (Results and Discussion) the capacity of XAD-4 for $TPPS_4$ exceeded that of XAD-2. The effect of particle size of XAD-4 on adsorption was determined next.

Particle Size

Various mesh sizes of XAD-4 were used to determine the particle size with the greatest capacity for $TPPS_4$.

Portions (0.5 g) of clean dry XAD-4 of 20-60, 60-80 and 80-100 mesh, were wet with 2 mL of methanol and treated with $TPPS_4$ solutions as above. The amount of $TPPS_4$ adsorbed was calculated as above.

Time and Extent of Complexation

The minimum time for maximum reaction of Pb(II) and Cd(II) with $TPPS_4$ at room temperature and pH 10.2 was determined.

Pb(II) (20 ppb or 9.6×10^{-8} M) and $TPPS_4$ (1×10^{-6} M) were mixed together to give a solution of 100.0 mL. The solution was agitated and aliquots were taken periodically for spectrophotometric measurement of free TPPS (414 nm) until negligible change in absorbance occurred. The free $TPPS_4$ concentration was monitored rather than the concentration of Pb-$TPPS_4$ because the Pb-$TPPS_4$ Soret band is overshadowed by a shoulder of the $TPPS_4$ band. The percent Pb complexed, and hence the extent of reaction, was calculated from the amount of $TPPS_4$ reacted and the original concentration of Pb(II).

The procedure for the formation of the Cd-$TPPS_4$ complex was the same except that 10 ppb Cd (8.9 X 10^{-8} M) was used, and repeated with 100 ppb Cd(II) (8.9 X 10^{-7} M) and 2 X 10^{-6} M $TPPS_4$.

To investigate the time and extent of reaction for lower concentrations of Cd and Pb (as found in seawater), the extraction procedure developed by DiFruscia et. al. (165) was used as follows. Two 100-mL aliquots of 0.77 M NaCl solutions containing 0.4 ppb Pb(II) (3.9 X 10^{-10} M), 0.05 ppb Cd(II) (4.9 X 10^{-11} M) and 1 X 10^{-6} M $TPPS_4$ were buffered to pH 10.2. Both solutions were placed in 100-mL polypropylene volumetric flasks wrapped in aluminum foil. One solution was agitated at room temperature for one hour and the other heated at 90-98°C for 1 hour and cooled to room temperature. They were both saturated with MIBK then extracted with 5 mL of 10% w/w TCMA/MIBK. The organic phase was analyzed by GFAAS for Pb and Cd extracted as the ion-associated complex, $(TCMA)_4$-metal-$TPPS_4$.

Effect of Auxiliary Ligands on Reaction

The literature suggests that hydroxylamine and hydrazine serve as chelating catalysts (e.g., 165,202). The possibility that they might hasten the room-temperature reaction of Cd and Pb with $TPPS_4$ was investigated.

Four solutions of 100 mL were prepared at pH 10.2, each with 0.05 ppb Cd (4.9×10^{-11} M), 0.4 ppb Pb (3.9×10^{-10} M), 1×10^{-6} M $TPPS_4$ and 0.77 M NaCl. They were placed in 100-mL polypropylene volumetric flasks wrapped in aluminum foil. One solution was agitated for one hour at room temperature, a second heated at 90-98°C for 1 hour, a third was agitated at room temperature for one hour after addition of 200 µL of $(NH_2)_2$, and a fourth, containing 9.4×10^{-3} M NH_2OH was agitated at room temperature for one hour. The solutions were saturated with MIBK, extracted with 5 mL of 10% w/w TCMA/MIBK, and the extract analyzed by GFAAS.

Time of Adsorption

The minimum amount of time for quantitative batchwise adsorption of the Pb and Cd complexes of $TPPS_4$ on XAD-4 resin was determined.

Solutions (100.0 mL) of 4 ppb Pb(II) (2×10^{-8} M), 2 ppb Cd(II) (1.8×10^{-8} M), and $TPPS_4$ (1×10^{-6} M) were agitated for an hour at room temperature to ensure maximum reaction. Then, 0.5 g of clean XAD-4 (80-100 mesh) was added and the mixture agitated. Shaking times were 30, 15, 10, 5 and 1 minute. The resin was poured into a column and eluted with 10 ml of 1% HNO_3. Measurement of metal concentrations by GFAAS followed.

Adsorption Isotherm

The adsorption of Pb-$TPPS_4$ on XAD-4 was investigated. Because of the light sensitivity of Cd-$TPPS_4$, its adsorption isotherm was not determined but presumably should be similar to that for the Pb complex.

The Pb-$TPPS_4$ complex was synthesized by room-temperature reaction of $TPPS_4$ (1×10^{-3} M) with Pb(II) (2.5×10^{-3} M) at pH 10.2. Appropriate dilutions were made to obtain the concentration range for Pb-$TPPS_4$ required.

Batch amounts of XAD-4 (0.5 g, 80-100 mesh) were moistened with 2 mL of methanol and agitated with 25-mL aliquots of Pb-$TPPS_4$ solutions of various concentrations, until adsorption equilibrium was attained. The amount of Pb-$TPPS_4$ adsorbed was calculated from the difference between the initial and final concentrations, determined spectrophotometrically at 464 nm.

Breakthrough Curves

The breakthrough curve for the XAD-4 column (1 X 3-cm resin bed, 80-100 mesh) was determined using Pb-$TPPS_4$. The column was prepared as previously described. A solution containing 1 ppm of Pb-$TPPS_4$ (4.8×10^{-6} M) was passed through the column at a rate of 1 mL min^{-1}. The concentration of Pb-$TPPS_4$ in the effluent was monitored by periodic collection of 0.5-mL aliquots of effluent and spectrophotometric measurement at 464 nm. The procedure was repeated for a 100-ppb solution of Pb-$TPPS_4$ (4.8×10^{-7} M).

Elution Profiles

Pb-$TPPS_4$ and Cd-$TPPS_4$ were adsorbed onto the XAD-4 column and the elution profiles with 1% v/v HNO_3 were determined as follows. A solution (100.0 mL, pH 10.2) containing 4 ppb Pb(II), 2 ppb Cd(II) and 1×10^{-6} M $TPPS_4$ was agitated for one hour at room temperature then percolated through the conditioned XAD-4 column. Pb(II) and Cd(II) were eluted with 1% HNO_3 which was collected in 0.5 mL increments and analyzed by GFAAS.

Applications

The method was tested with synthetic freshwater and salt-water samples before application to natural samples.

Synthetic freshwater samples contained humic acid (10 mg L^{-1}), $TPPS_4$ (1×10^{-6} M), Cd(II) (2 ppb) and Pb(II) (4 ppb) at pH 10.2. Calibration was by SAM, with spikes of 0, 0.5, 1.0 and 1.5 times the concentration of each metal ion. The blank was prepared with all reagents present except the trace metal ions. The spiked samples were agitated for one hour at room temperature in foil-covered screw cap polyethylene bottles and then percolated through foil-covered XAD-4 columns at a rate of 1 mL min^{-1}. The columns were rinsed with 10 mL of pH 10.2 buffer and then eluted with 5 mL of 1% HNO_3. The metal-ion concentrations were determined by GFAAS.

Synthetic salt-water samples and corresponding blanks containing 0.77 M NaCl were processed in the same manner.

Natural lakewater samples (100 mL), previously acidified to pH 1.7 and filtered were equilibrated with a combined metal-ion spike for 48 hours. After the pH was adjusted to 10.2, the samples and corresponding blanks were processed as above.

CHAPTER 3. RESULTS AND DISCUSSION

3.1 Studies on XAD/$TPPS_4$ as a Chelating Resin

The preliminary studies to determine the resistance of $TPPS_4$ to stripping from XAD-4 by various reagents showed that in general, $TPPS_4$ is quite firmly adsorbed. These experiments were merely qualitative in nature in that a visual estimate of the intensity of coloration of the supernatant solution or solvent was made; the more intense the coloration, the greater the extent of stripping. In strongly acidic media, $TPPS_4$ exists as the green dication, and in neutral and basic solution, as the purple neutral form. In most organic solvents, $TPPS_4$ is purple. A relative ordering of the reagents in terms of stripping ability was not made because of the different sensitivity of the eye to these colours. However, it could be readily concluded from the faint coloration of all the supernatants that only a small fraction of the $TPPS_4$ was stripped. To the eye, maximum stripping appeared to occur with naphthalene sulfonic acid at room temperature, and 1 M and 6 M HCl at 60-70°C. On the whole, $TPPS_4$ is held strongly onto XAD-4, likely by chemisorption involving a rather strong interaction between the π-electrons of the XAD-4 phenyl rings and the extensive π-system of $TPPS_4$ (73).

An essential characteristic of a resin used in ion-exchange columns is that it be stable to experimental conditions so that it can be repeatedly used and regenerated. With this idea in mind, $TPPS_4$ was adsorbed batchwise onto 80-100 mesh XAD-2 and XAD-4* and the two columns were packed with the resulting products. For XAD-2, the initial amount of $TPPS_4$ adsorbed was 5.9 µmoles on 0.5 g dry resin; for XAD-4, 9.6 µmoles on 0.5 g dry resin. Solutions of 1% HNO_3 and pH 10.2 buffer were alternately percolated at room temperature, thorough each column. The pH 10.2 buffer was selected because this was found to be the optimal pH for complexation (165). The 1% HNO_3 solution was selected in anticipation of this being a suitable eluting agent for the metal ions retained on the column as their $TPPS_4$ complexes. As seen from Figures 11 and 12, the average rate of bleeding of $TPPS_4$ from XAD-2 and XAD-4 resin on passage of 1% HNO_3 is about the same (i.e., 0.05% mL^{-1} and 0.04% mL^{-1}, respectively). With the pH 10.2 buffer, the amount of $TPPS_4$ stripped decreases upon elution of more buffer. For the XAD-2 resin, the amount of bleeding per 50-mL aliquot ranges from 4.6% to 1.0 % (Figure 11). For the XAD-4 resin, the amount of bleeding per 50-mL aliquot, ranges from 3.7% to 0.2 % (Figure 12). It is

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* Recall that these resins are similar except for pore size and surface area (see Table 5).

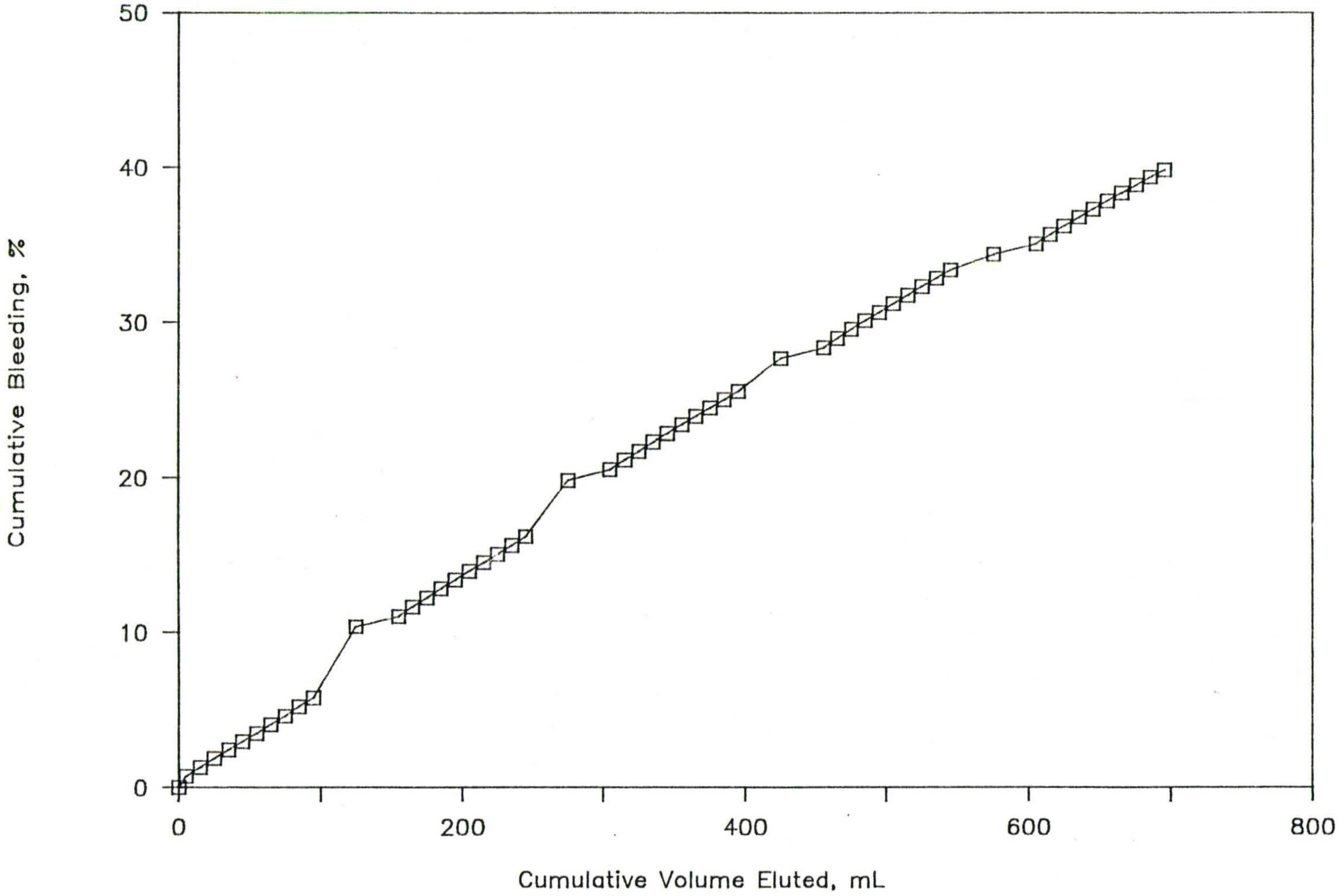

Figure 11. Bleeding of $TPPS_4$ adsorbed on XAD-2 column (0.5 g) upon alternate passage of 1% HNO_3 and pH 10.2 buffer solutions at room temperature. First 100 mL, 1% HNO_3; second 50 mL, buffer; third 100 mL, 1% HNO_3; fourth 50 mL, buffer; etc.

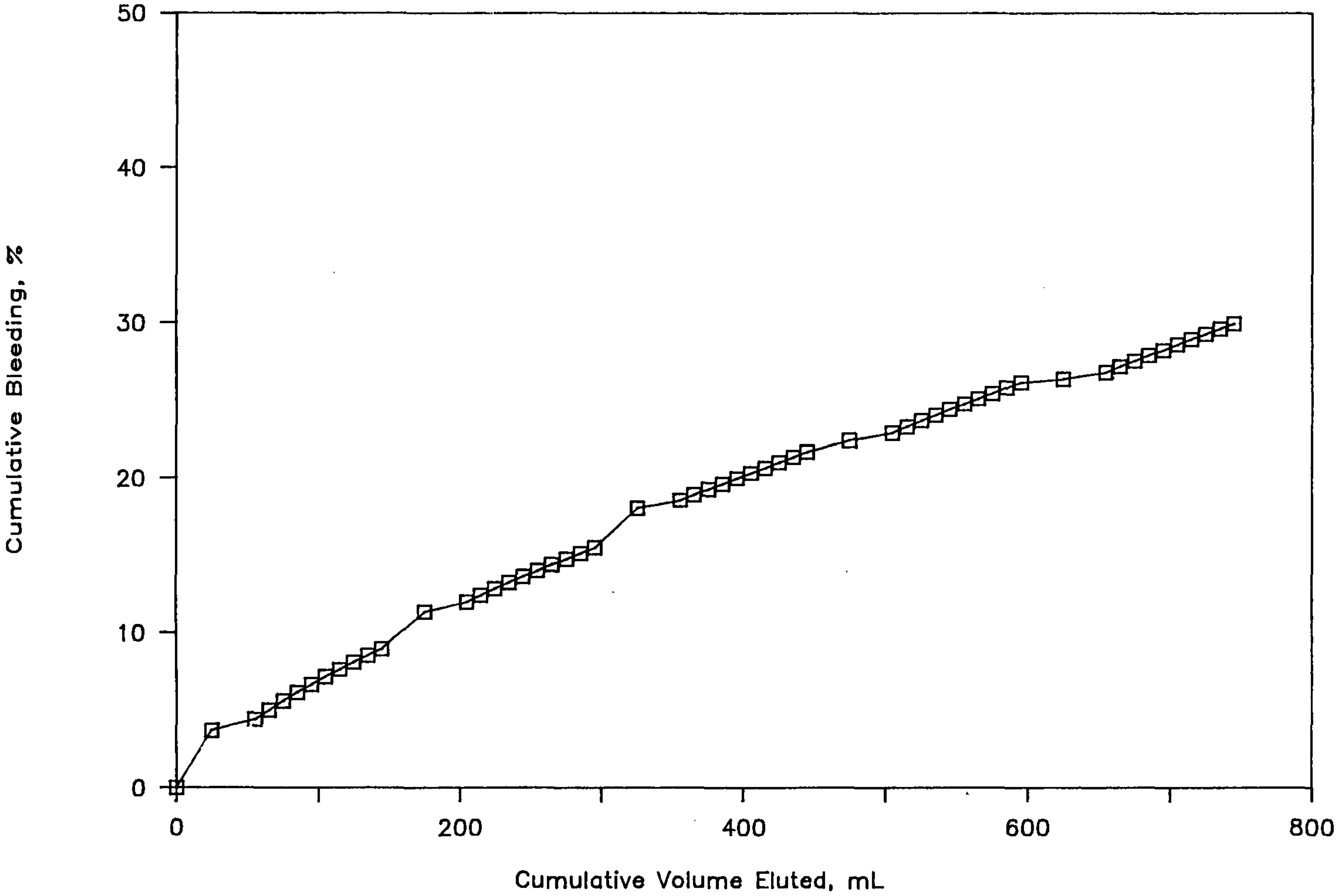

Figure 12. Bleeding of $TPPS_4$ adsorbed on XAD-4 column (0.5 g) upon alternate passage of pH 10.2 buffer and 1% HNO_3 solutions at room temperature. First 50 mL, buffer; second 100 mL, 1% HNO_3; third 50 mL, buffer; fourth 100 mL, 1% HNO_3; etc.

clear that repeated use of the $TPPS_4$/XAD resins will cause loss in their capacity upon passage of both buffer and HNO_3 solutions.

A test at 90-98°C was done because elevated temperatures were found necessary to facilitate metal-ion insertion into $TPPS_4$ (165). When the buffer was percolated through the resin at 90-98°C, there was more bleeding of $TPPS_4$ compared to percolation at room temperature. This is presumably due to the increase in solubility of $TPPS_4$ at elevated temperatures. A large amount of bleeding during sample percolation reduces column capacity and possible loss of complexed metal ions. A quantitative study on $TPPS_4$ bleeding at elevated temperatures was not done since the $TPPS_4$/XAD resin floated to the surface of the solution in a heated column and proper percolation was impossible.

In summary, this study shows that the use of $TPPS_4$/XAD chelating resins is not feasible under the conditions needed for complexation and elution of metal ions. Therefore, the focus of this thesis became the preconcentration of trace metals by prior formation of their $TPPS_4$ complexes in solution, followed by adsorption and stripping of the complexes from an appropriate XAD resin. This study led to the development of a method general for several trace metal ions (discussed in Sections 3.2 - 3.5), and a more simple and rapid method specifically for Pb and Cd (discussed in Section 3.6).

3.2 Studies on Optimal Experimental Conditions

pH and Buffer Type

A previous study (165) on the stability of pre-formed metalloporphyrins over the pH range 2-14 showed that the Cu, Co and Mn complexes are stable over the entire range. Below pH 6-7, the Pb and Cd complexes decompose completely and the Hg complex is stable only in the pH range 11-13. The recommended pH for the simultaneous formation of several $TPPS_4$ complexes was 10.2.

In the present work, an investigation was carried out to confirm the optimal pH for formation of the Hg, Cd, Pb and Ni complexes of $TPPS_4$. The optimal pH for metalloporphyrin formation was different for each metal ion; pH 11.6-11.8 for Cd (Figure 13), pH 11.0-11.3 for Hg (Figure 14), pH 10.2-10.4 for Ni (Figure 15), pH 9.8-10.0 for Pb (Figure 16). Obviously, the optimal pH could not be chosen for each metal ion, and a compromise value of pH 10.2 as suggested by DiFruscia (165) was selected.

Mechanistic considerations of metal-ion insertion support experimental findings that complexation is facilitated in basic media. The near-planar conformation of $TPPS_4$ in the neutral H_2P form, is flexible (152,205). The protons on the pyrrole nitrogens exchange rapidly, which correlates with out-of-plane vibrations of the porphyrin. This motion allows stepwise substitution of the ligands of the metal-ion

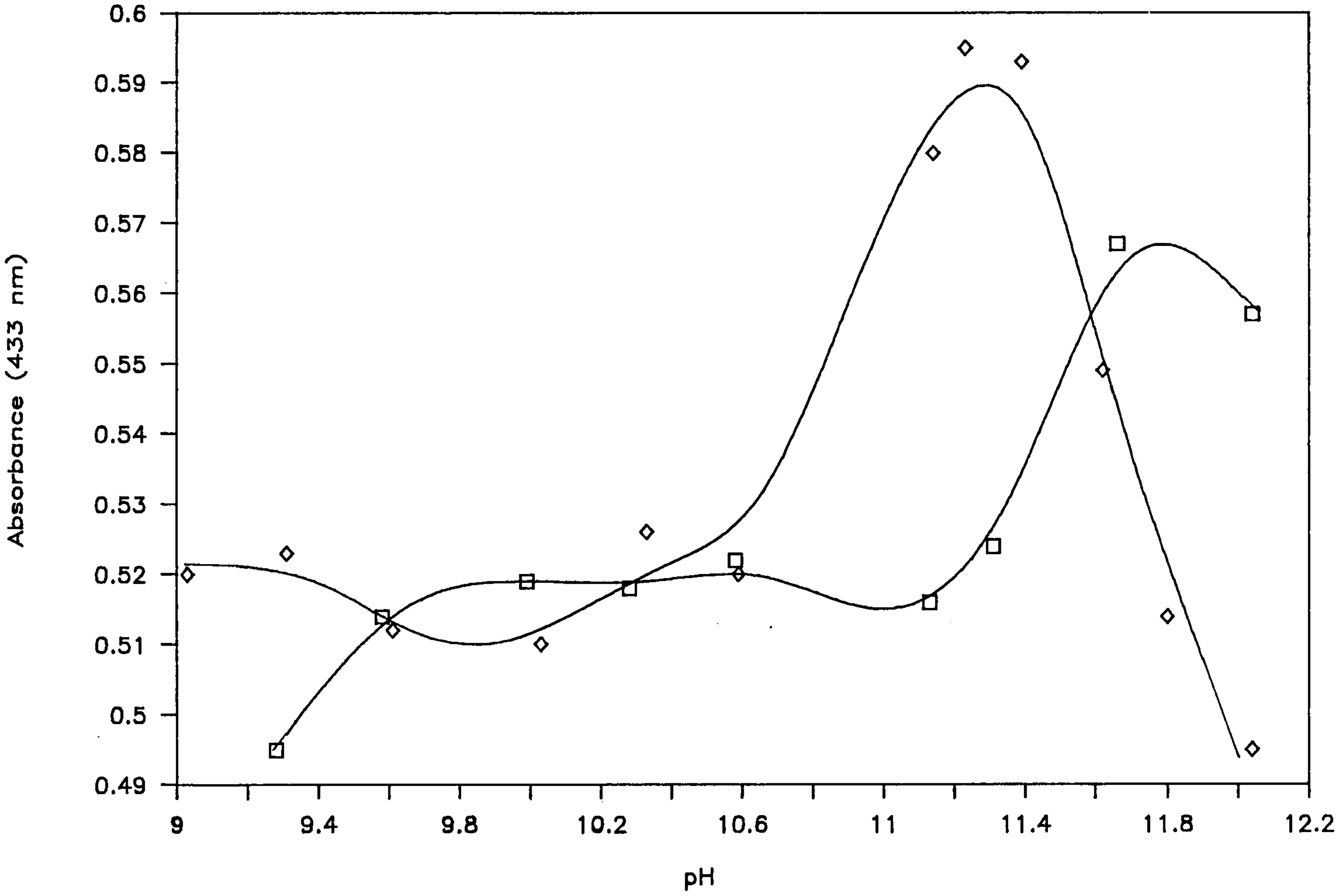

Figure 13. Cd–TPPS$_4$ formation as a function of pH (10 ppm Cd(II), 10^{-3} M TPPS$_4$). □ = set 1, ◇ = set 2.

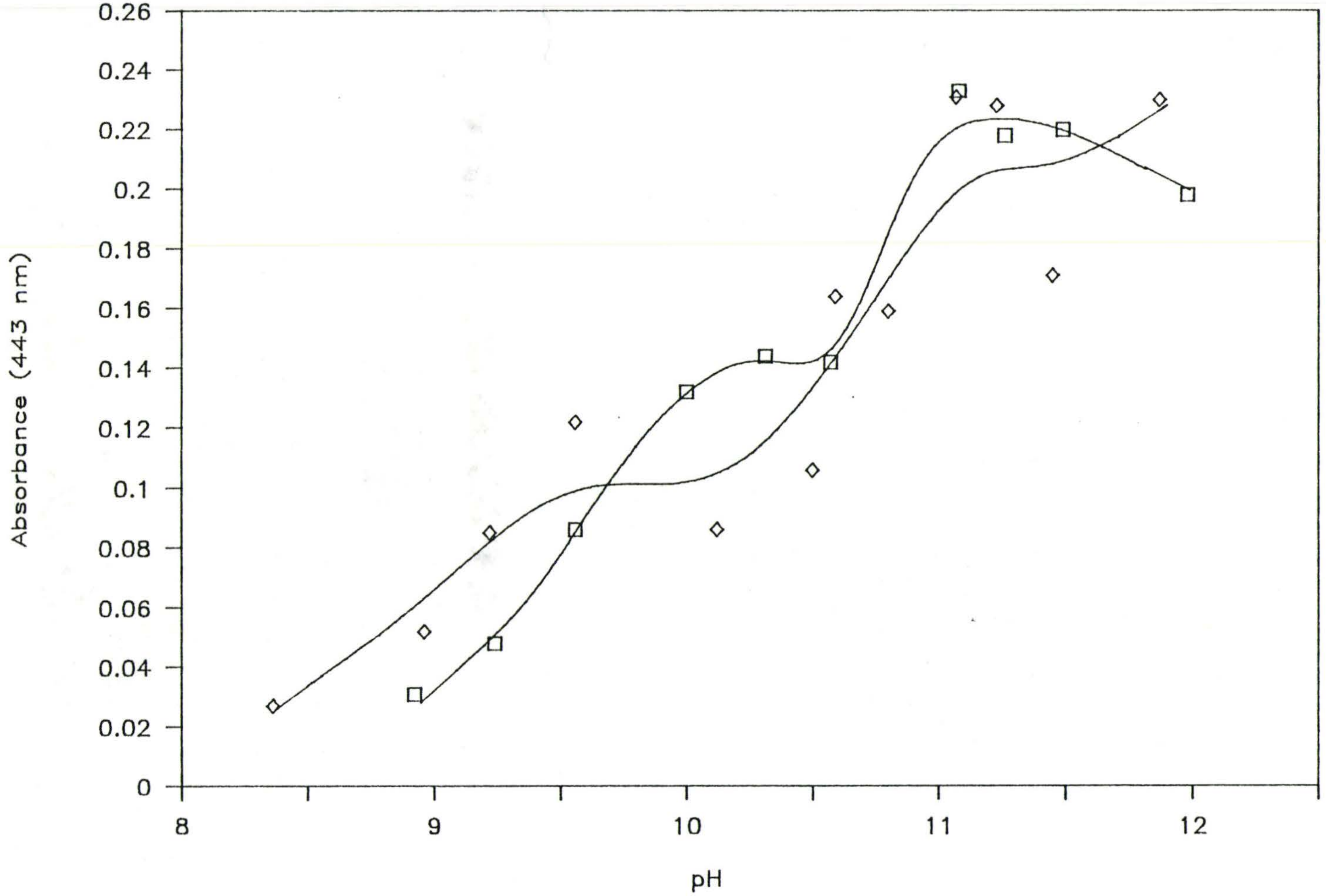

Figure 14. Hg-TPPS$_4$ formation as a function of pH (10 ppm Hg(II), 10^{-3} M TPPS$_4$). □ = set 1, ◇ = set 2.

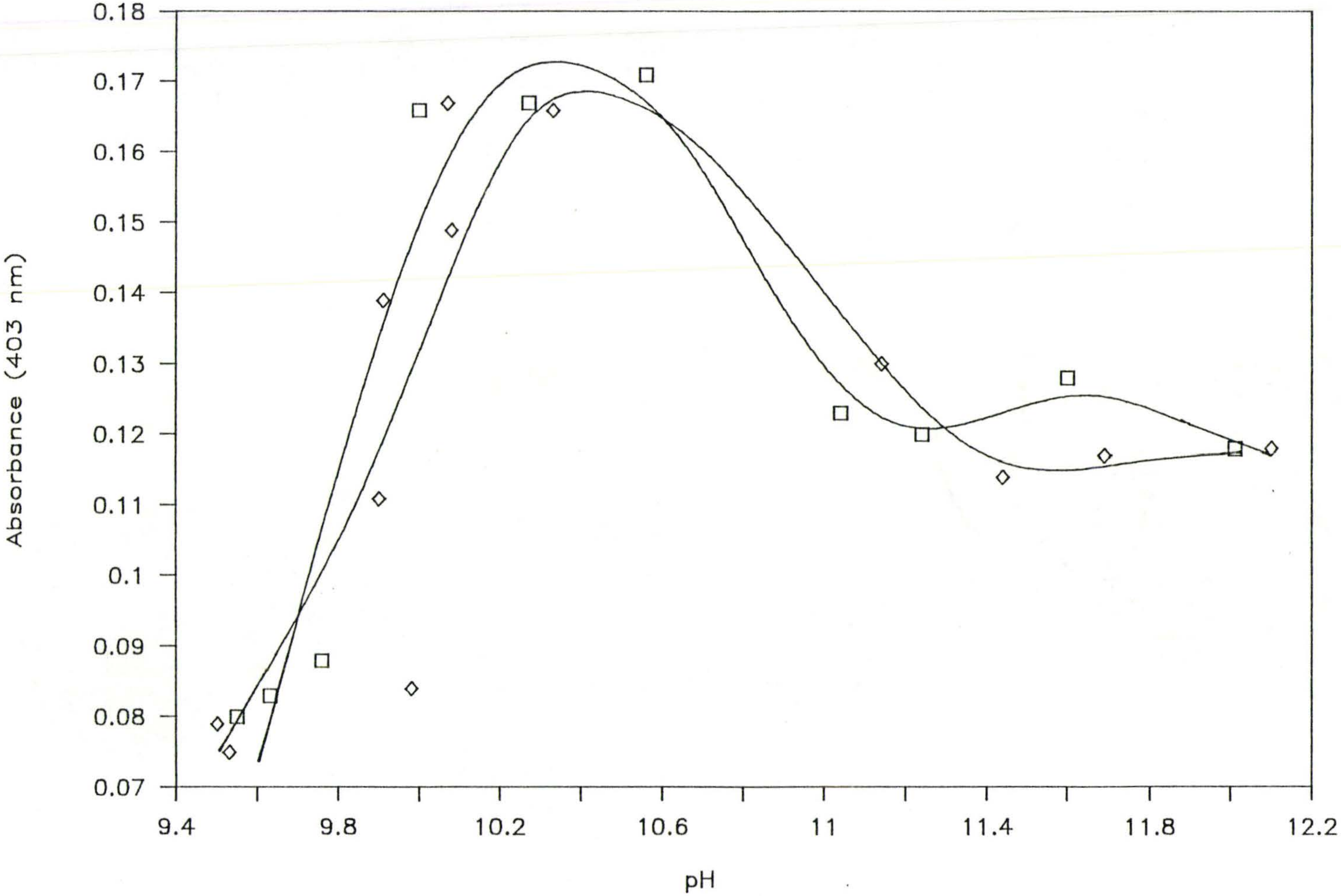

Figure 15. Ni–TPPS$_4$ formation as a function of pH (10 ppm Ni(II), 10^{-3} M TPPS$_4$). □ = set 1, ◇ = set 2.

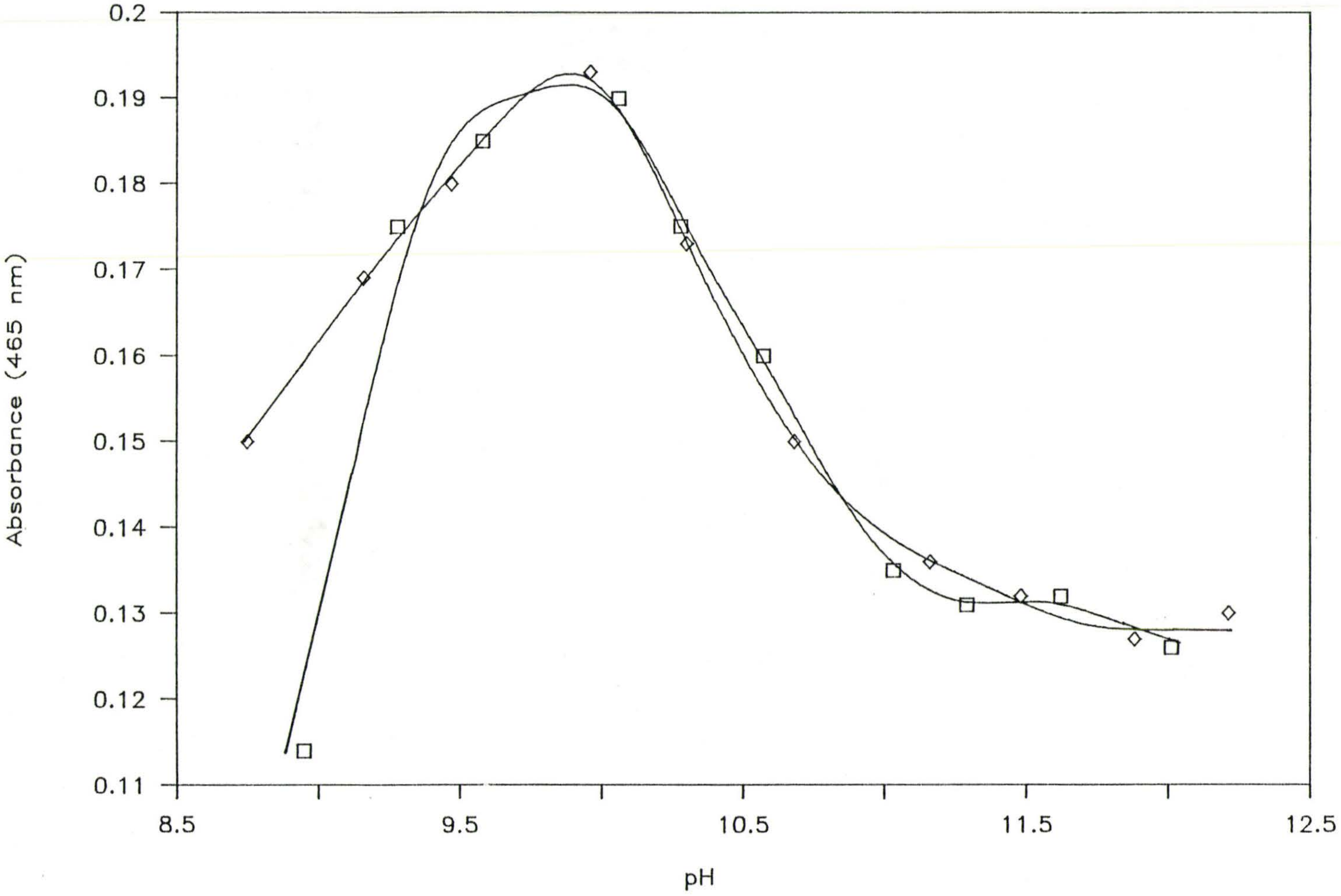

Figure 16. Pb−TPPS$_4$ formation as a function of pH (10 ppm Pb(II), 10^{-3} M TPPS$_4$). □ = set 1, ◇ = set 2.

by the porphyrin nitrogens. In the diacid form (H_4P^{2+}), the porphyrin assumes a frozen non-planar conformation making it very difficult to form metalloporphyrins. Electrostatic repulsion also makes insertion of metal ions into the H_3P^+ and H_4P^{2+} forms difficult (204).

Salt effects have been studied but are not well understood. Present data show that specific effects are more important than general salt effects. The rate of metal-ion insertion depends more on the associated ligand than the medium used (205). For the present work, either an NH_3/NH_4Cl or NH_3/NH_4OAc buffer could be chosen for pH 10.2. Although the NH_3/NH_4Cl buffer would be useful since NaCl is already present in natural waters, an NH_3/NH_4OAc buffer was chosen since OAc^- generally forms weaker complexes with the metal ions of concern (241). Also, OAc^- keeps ions such as Pb(II) from precipitating as its chloride and possibly helps increase the rate of formation of the $TPPS_4$ complex. Therefore, in subsequent studies, all solutions were buffered to pH 10.2 with a NH_3/NH_4OAc (0.01 M NH_4OAc) buffer.

Particle Size

For a given weight of resin, the smaller the particle size, the larger the total external surface area. The overall surface area remains virtually constant since the increase in external surface area is small relative to the internal surface area. Therefore, if adsorption occurs on both the external and internal surfaces the effect of decreasing the particle size should not greatly affect the amount of $TPPS_4$ adsorbed. The results in Table 9 suggest, although not clearly, that external surface adsorption is an important component of the overall adsorption process (although this does not preclude some internal adsorption). The values for the 60-80 and 80-100 mesh seem out of line and, unfortunately, were not repeated. Ideally, smaller divisions in mesh size (i.e., smaller ranges of particle sizes), should have been used in this study in order to draw a firmer conclusion.

In subsequent studies, 80-100 mesh was chosen because in addition to allowing adequate adsorption, it allows for good column packing at a reasonable flow rate (1 mL min^{-1}). The flow rate for 100-140 mesh is much too slow for practical use (<< 1 mL min^{-1}).

Table 9. Effect of Mesh Size for XAD-7

Mesh Size	Initial $TPPS_4$ Conc. (M)	µmoles $TPPS_4$ Adsorbed per g Dry Resin
20-60	2.14×10^{-4}	2.82
60-80	1.98×10^{-4}	3.51
80-100	2.07×10^{-4}	3.09
100-140	1.75×10^{-4}	4.61

3.3 Batch Adsorption of $TPPS_4$ and Complexes on XAD Resins

Adsorption Isotherms

An understanding of the adsorption process of $TPPS_4$ and its complexes is important in the knowledgeable application to the analytical preconcentration technique. Adsorption isotherms studies were conducted with $TPPS_4$ and Cu-$TPPS_4$, chosen as a model $TPPS_4$ complex, because of its solution and photolytic stability.

The XAD resins are widely used as adsorbents and the retention mechanism has been studied extensively. According to Cantwell and Puon (73), adsorption can be explained in terms of Stern-Guoy-Chapman (SGC) theory. For example diphenylguanidinium cation ($DPGH^+$) is adsorbed onto the XAD-2 surface and forms the "compact" part of the electrical double layer while small inorganic counter-ions (i.e., Cl^-) occupy the "diffuse" part. The adsorbed molecule is thought to lie flat on the resin surface to maximize π-π interactions, with the protonated nitrogen directed toward the solution phase. Here, calculations confirm a closely packed monolayer coverage. This is in agreement with previously studies on the adsorption of phenol on XAD-2 (242) and adsorption of substituted benzene on XAD-4 (243). In the present study, adsorption was examined only in the context of the Langmuir equation, as an initial attempt. A more detailed study should involve the SGC model of adsorption.

XAD-8 is a porous acrylic resin chemically similar to XAD-7. In a study of retention of non-ionic organic solutes on XAD-8, the order of retention was found to be aliphatic solutes > aromatic solutes > alicyclic-carbon solutes (244). The resin also favours solutes in order of decreasing aqueous solubility. The inverse relationship between capacity and solubility is attributed to a hydrophobic effect. The nonpolar organic solutes have an unfavorable entropy of solution due to the ordering of water molecules around the organic solute. This is the driving force for aggregation and subsequent adsorption of nonpolar solutes onto the resin. A similar solubility relationship is shown to hold for XAD-1,2,4 and 7 (244).

In the present study, it was shown that the capacity of the resins for $TPPS_4$ is in the order XAD-4 >> XAD-2 > XAD-7 (Table 10). The adsorption isotherms are shown in Figures 17-19. Although XAD-2 and XAD-4 are both polystyrene divinylbenzene copolymers, they differ in pore size and surface area. The major factor for increased adsorption on XAD-4 is the larger total surface area of XAD-4 compared with XAD-2 (750 versus 330 $m^2 g^{-1}$) (Table 5). In turn, XAD-7 has a lower capacity for $TPPS_4$ than XAD-2 or XAD-4 due to the difference in the chemical nature of the resins (Figure 10). XAD-2 and XAD-4 resins contain benzene rings which are thought to cause a π-π interaction with the porphyrin ring (73). These forces are so strong that none of the solvents

Table 10. Γ_{max} (Resin Capacity) for the Adsorption of $TPPS_4$ and Cu-$TPPS_4$ on XAD Resins, 80-100 Mesh

Resin (Adsorbate)	Γ_{max} (μmole g^{-1})*	K^{-1} (M^{-1})*
XAD-2 ($TPPS_4$)	13.8	1.34×10^{-4}
XAD-4 ($TPPS_4$)	50.0	8.26×10^{-4}
XAD-7 ($TPPS_4$)	8.88	2.71×10^{-4}
XAD-7 (Cu-$TPPS_4$)	23.5	6.16×10^{-4}

* Γ_{max} and K^{-1} are calculated from fitting the best line through the data points using the equation, $\Gamma = \Gamma_{max}$ [M]/ (K^{-1} + [M]). The derivation of the equations used for least-squares curve-fitting is given in the Appendix.

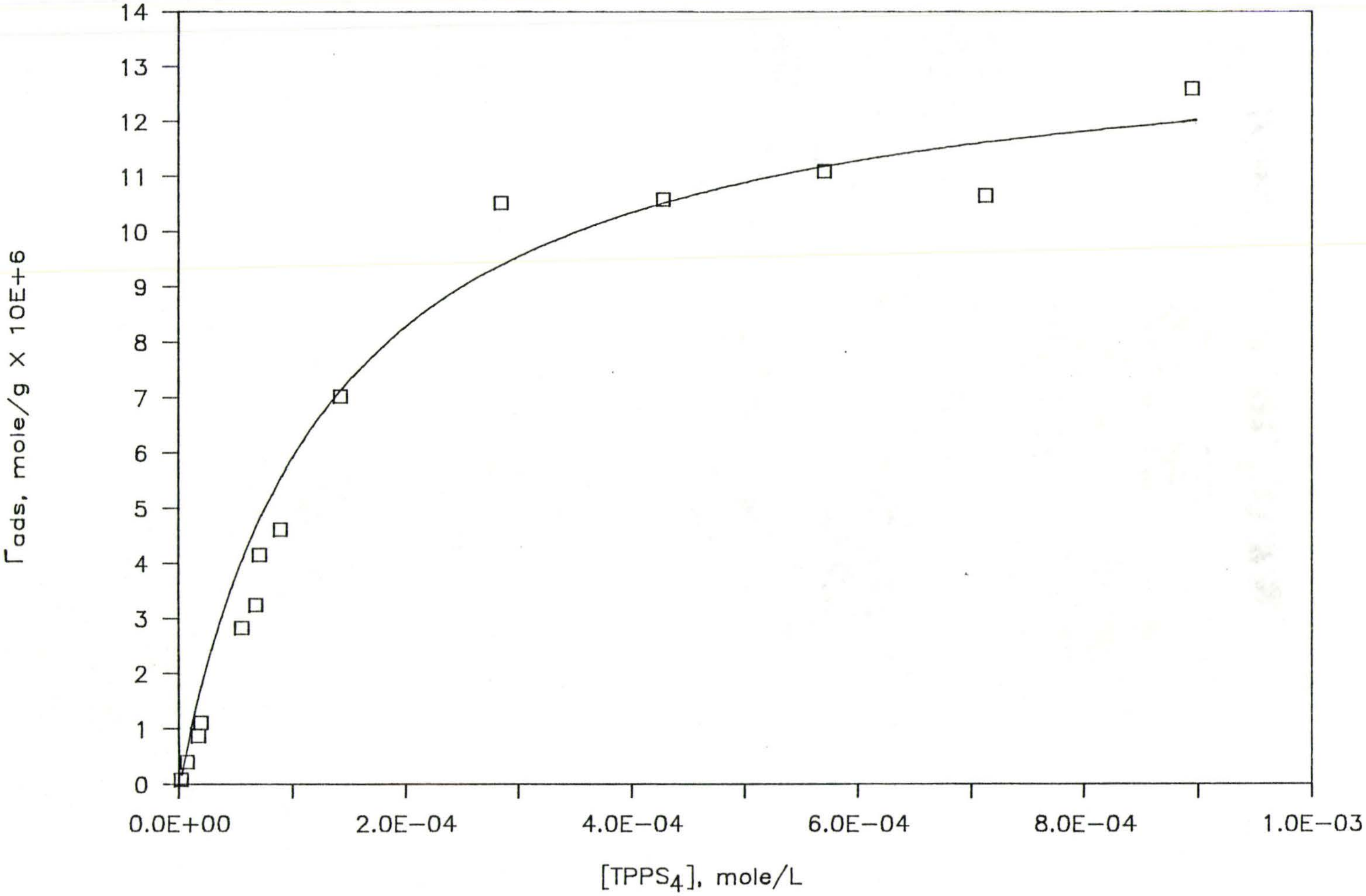

Figure 17. Adsorption isotherm for TPPS$_4$ on XAD-2 (0.5 g, 80-100 mesh) at pH 10.2 and room temperature.

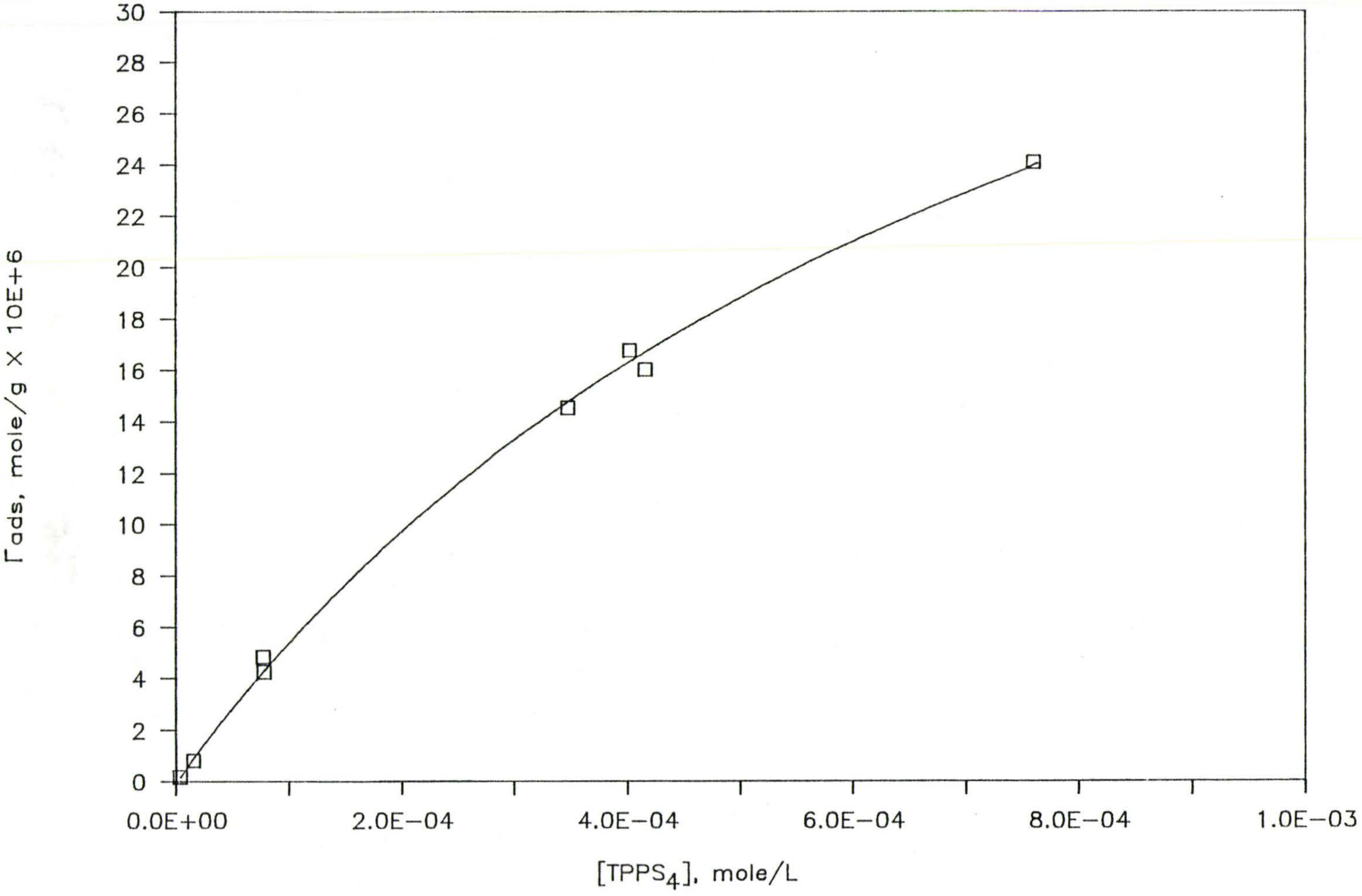

Figure 18. Adsorption isotherm for $TPPS_4$ on XAD-4 (0.5 g, 80-100 mesh) at pH 10.2 and room temperature.

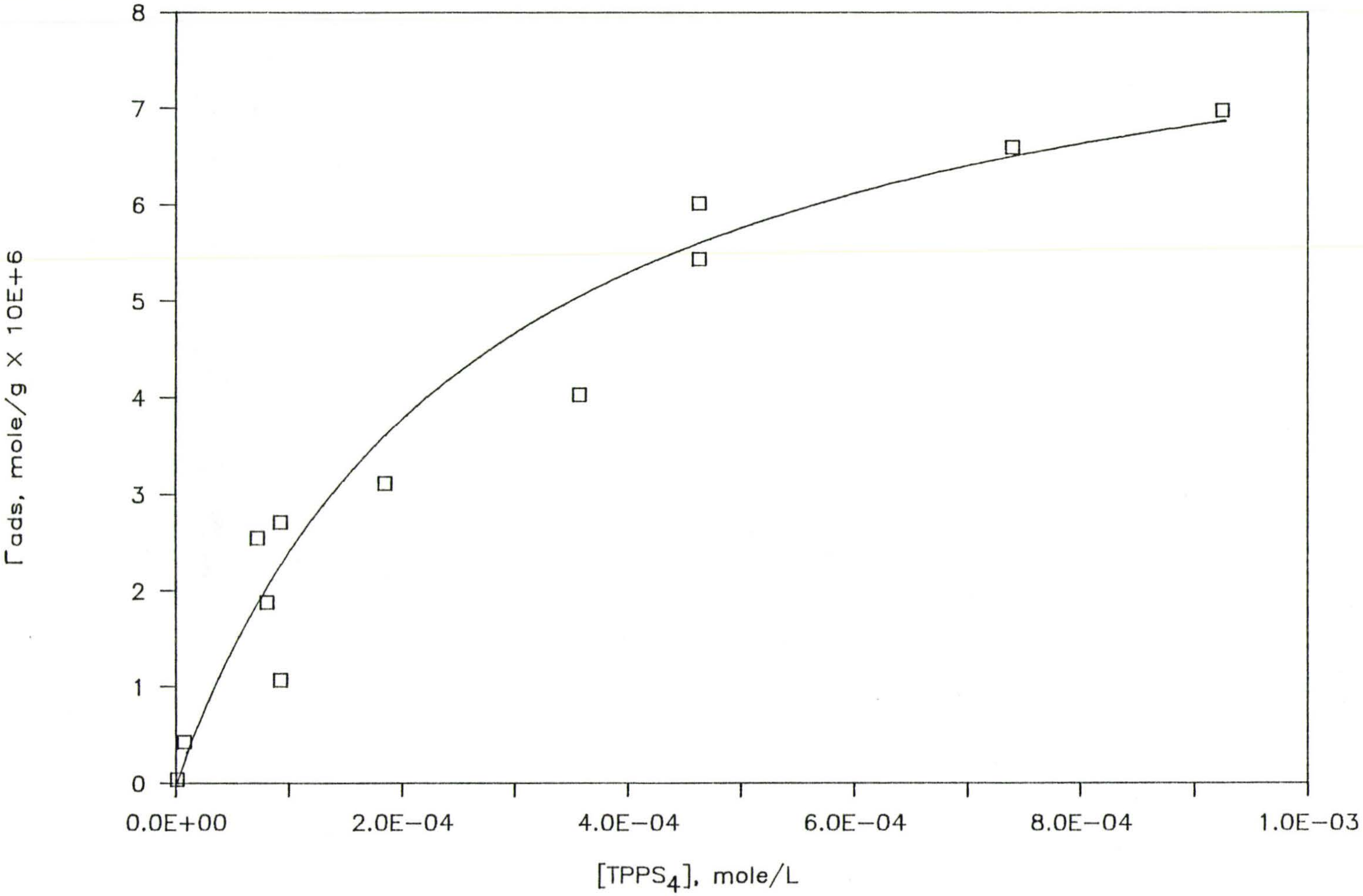

Figure 19. Adsorption isotherm for $TPPS_4$ on XAD-7 (0.5 g, 80–100 mesh) at pH 10.2 and room temperature.

or reagent solutions used in this work quantitatively removed the ligand from the resin (Section 3.1). Adsorption of $TPPS_4$ onto XAD-7 lacks this strong interaction and the porphyrin is perhaps held by weak Van der Waals forces. On present knowledge, there are no cationic sites on XAD-7 which might react with the sulfonate groups of $TPPS_4$. There are a small number of carboxylic acid impurity sites that are unesterified (30 µg mole^{-1} per g dry resin, (72)) but it is difficult to envision their involvement in the binding.

At high $TPPS_4$ concentration, such as those corresponding to Γ_{max}, aggregation probably plays a role in the adsorption process. Studies by Herrmann et. al. (164) demonstrated that as the concentration of $TPPS_4$ increased, aggregation of $TPPS_4$ increased. In neutral or basic media, data best fit the model of monomer-dimer equilibrium from 3 X 10^{-6} M - 3 X 10^{-4} M and a dimer-tetramer equilibrium above 3 X 10^{-4} M. ^1H NMR of $TPPS_4$ in D_2O points to aggregation in which the molecules are stacked vertically in approximately parallel planes. Perhaps this also occurs on $TPPS_4$ adsorption onto the resin at high concentrations, leading to multilayer adsorption.

It is thought that aggregation may adversely affect the extent of metal complexation. Although the adsorption isotherms cover a large range of concentrations, only a low concentration of $TPPS_4$ is needed in the preconcentration methods. Thus for practical applications, $TPPS_4$ is monomeric

(2×10^{-6} M) and its adsorption on the resins only occupies the initial linear portion of the isotherms.

Theoretical calculations of maximum equilibrium adsorption of $TPPS_4$ on the XAD resins were attempted with the objective of providing a reasonable explanation of the experimental results. A good correlation between observed and calculated data does not necessarily reflect physical reality but data-fitting can indicate which model among several will correspond most closely to the experimental observation.

A model based on monolayer coverage of $TPPS_4$ on the resin was used for the calculations. If it is assumed that $TPPS_4$ molecules penetrate the pores of the resin so that adsorption occurs on the total surface area, the calculated values for maximum adsorption are at least an order of magnitude higher than the experimental results (Table 11). If, however, the assumption is made that the monolayer coverage is only on the exterior surface, the calculated values for maximum adsorption are at least two orders of magnitude too low (Table 12). Even after taking into account a multiplication factor of four for the possible presence of $TPPS_4$ tetramer species adsorbed on the resin, the calculated values for maximum adsorption are still too low. At this point, it is hard to envisage which adsorption mechanism would reflect the experimental results. One suggestion is that the pore sizes are not uniform, causing only a part of the interior surface to be covered by restricting entry of the large tetramer molecules.

Table 11. Calculated versus Experimental Data for Maximum Adsorption of $TPPS_4$ on Total Surface of XAD Resins

Resin Type	Total Surface Area of Resin (m^2 g^{-1})	Experimental * Γ_{max} (µmoles g^{-1})	Calculated ** Γ_{max} (µmoles g^{-1})
XAD-2	330	13.8	275
XAD-4	750	50.0	626
XAD-7	450	8.88	375

* For 0.010 M NH_4OAc, pH 10.2, 23 ± 1°C

** Based on the assumption that $TPPS_4$ penetrates the pore of the resins so that adsorption of $TPPS_4$ occurs on the total surface area. Each $TPPS_4$ molecule is assumed to cover a square area with dimensions of 14.1 Å by 14.1 Å providing an area of 200 $Å^2$ or 2×10^{-18} m^2. The calculation of these dimensions is given in the Appendix.

$$\text{i.e., } \Gamma_{max} = \frac{\text{Total surface area of resin } (m^2/g) \times 10^6 \text{ µmoles/mole}}{\text{Area of } TPPS_4 \ (m^2/\text{molecule}) \times \text{Avogadro's no.(molecules/mole)}}$$

Table 12. Calculated versus Experimental Data for Maximum Adsorption of $TPPS_4$ on Exterior Surface of XAD Resins

Resin Type	Resin Density ($m^3\ g^{-1}$)	Approx. External Surface Area* ($m^2\ g^{-1}$)	Experimental Γ_{max}** (μmoles g^{-1})	Calculated Γ_{max}*** (μmoles g^{-1})
XAD-2	1.49×10^{-6}	0.054	13.8	0.045
XAD-4	1.61×10^{-6}	0.058	50.0	0.048
XAD-7	1.54×10^{-6}	0.056	8.88	0.047

* To calculate the total exterior surface area, solve for the number of particles in 1 g of resin, n, assuming each particle is perfectly spherical.

i.e., volume/g resin $= 4/3\ \pi r^3\ n$
$= 1/\text{density}$

e.g., for XAD-2 (80-100 mesh), the particle diameter = 150-180 μm and the approximate mean particle radius = 82.5 μm.

$$n = \frac{3 \times (1.54 \times 10^{-6}\ m^3)}{4\pi \times (0.825 \times 10^{-4}\ m)^3}$$

$$= 6.55 \times 10^5$$

Total exterior surface area $= 4\ \pi r^2\ n$
$= 4\ \pi\ (0.825 \times 10^{-4}\ m)^2 \times (6.55 \times 10^5)$
$= 0.054\ m^2/g$ resin

** For 0.010 M NH_4OAc, pH 10.2, 23 ± 1°C

*** Based on the assumption that $TPPS_4$ is adsorbed only on the external surface of the resins. Proceeding as per Table 11,

$$\Gamma_{max} = \frac{\text{Exterior surface area of resin } (m^2/g) \times 10^6 \text{ μmoles/mole}}{\text{Area of } TPP_S4\ (m^2/\text{molecule}) \times \text{Avogadro's no.(molecules/mole)}}$$

The adsorption isotherm of Cu-$TPPS_4$ is shown in Figure 20 and the Γ_{max} value is given in Table 10. Cu-$TPPS_4$ is known to form aggregates at much lower concentration than $TPPS_4$ (164). A monomer-dimer equilibrium is thought to exist above 5 X 10^{-7} M. In natural water samples, Cu(II) concentrations are usually lower than this (less than 10 ppb or 1.57 X 10^{-7} M) and thus, the Cu-$TPPS_4$ formed in solution should be present in the monomeric form.

The time required to reach equilibrium adsorption is concentration dependent (Table 13). Also, Γ_{max} and the time required to reach equilibrium are dependent on resin capacity. Figures 21 to 24 illustrate the rate of $TPPS_4$ and Cu-$TPPS_4$ adsorption at different concentrations on XAD-7. Other adsorption rate curves for $TPPS_4$ on XAD-2 and XAD-4 are shown in the Appendix (Figures 39-42).

Adsorption of $TPPS_4$ and its complexes is complicated by the competitive adsorption of water and various other organic and inorganic species onto the resin. Since the fundamental assumptions of the Langmuir equation are not valid, the parameters (i.e., Γ_{max} and K) obtained from the equation are empirical constants for the conditions as described.

Because the choice of pH is dictated by complexation reaction conditions, the adsorption isotherms were not repeated at different pH values. Also, high ionic strength induces extensive $TPPS_4$ aggregation and therefore adsorption isotherms were not measured at high ionic strength.

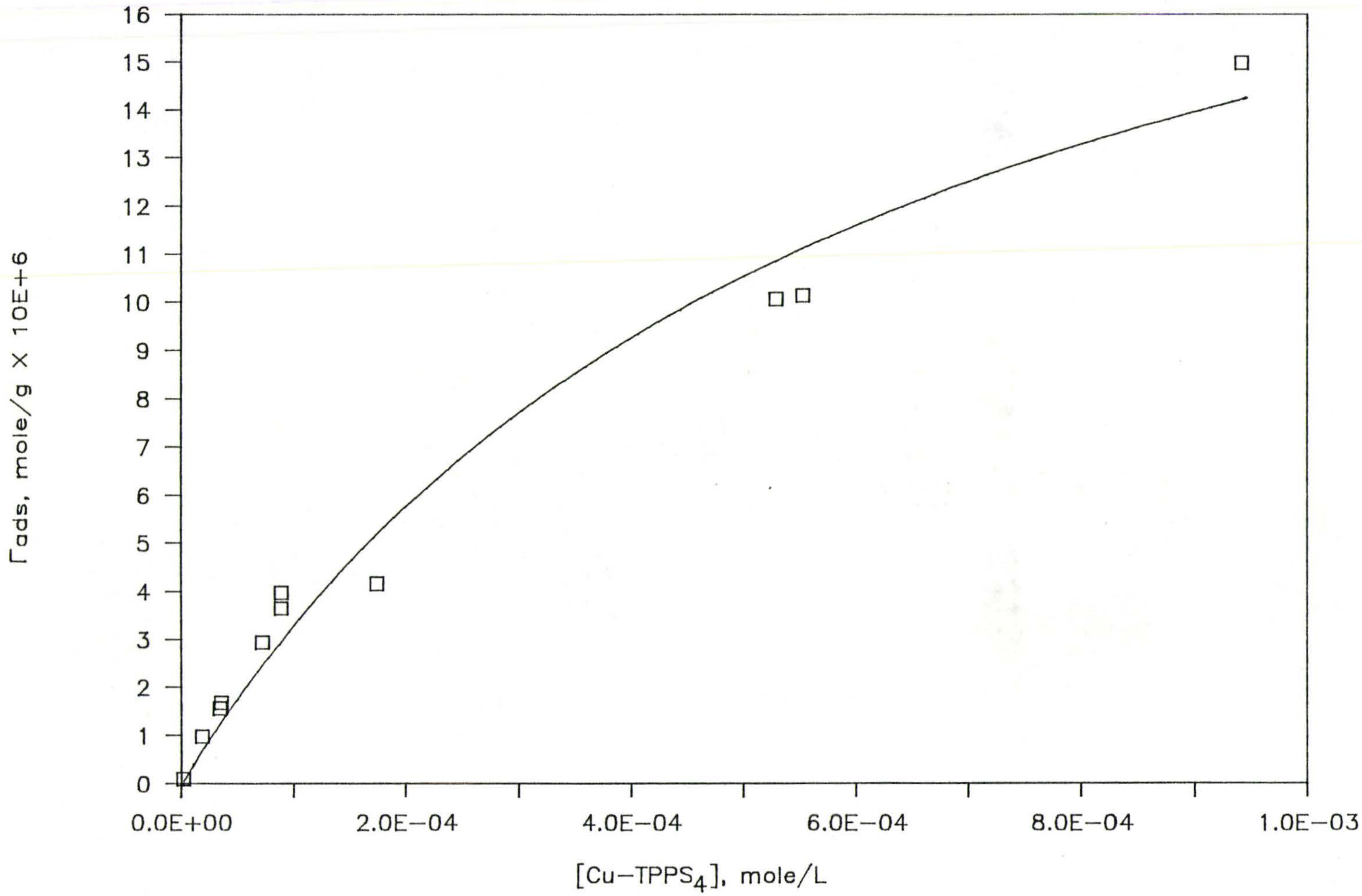

Figure 20. Adsorption isotherm for Cu-TPPS$_4$ on XAD-7 (0.5 g, 80-100 mesh) at pH 10.2 and room temperature.

Table 13. Rate of Adsorption of $TPPS_4$ and Cu-$TPPS_4$ onto XAD Resins, 80-100 Mesh

Resin (Ligand)	Initial $TPPS_4$ Conc. (M)	Time Required to Reach Equilibrium
XAD-2 ($TPPS_4$)	$1.18 X 10^{-6}$	< 5 min.
	$1.18 X 10^{-5}$	2.5 hours
	$1.18 X 10^{-4}$	29 hours
	$2.80 X 10^{-3}$	29 hours
XAD-4 ($TPPS_4$)	$3.06 X 10^{-6}$	15 hours
	$1.49 X 10^{-5}$	2 days
	$0.77 X 10^{-4}$	3 days
	$0.75 X 10^{-3}$	3 days
XAD-7 ($TPPS_4$)	$0.94 X 10^{-6}$	9 min.
	$0.94 X 10^{-5}$	30 min.
	$0.94 x 10^{-4}$	1 hour
	$0.82 X 10^{-3}$	1 hour
XAD-7 (Cu-$TPPS_4$)	$0.76 X 10^{-6}$	50 min.
	$2.19 X 10^{-5}$	1 hour
	$0.78 X 10^{-4}$	1 hour
	$0.75 X 10^{-3}$	1 hour

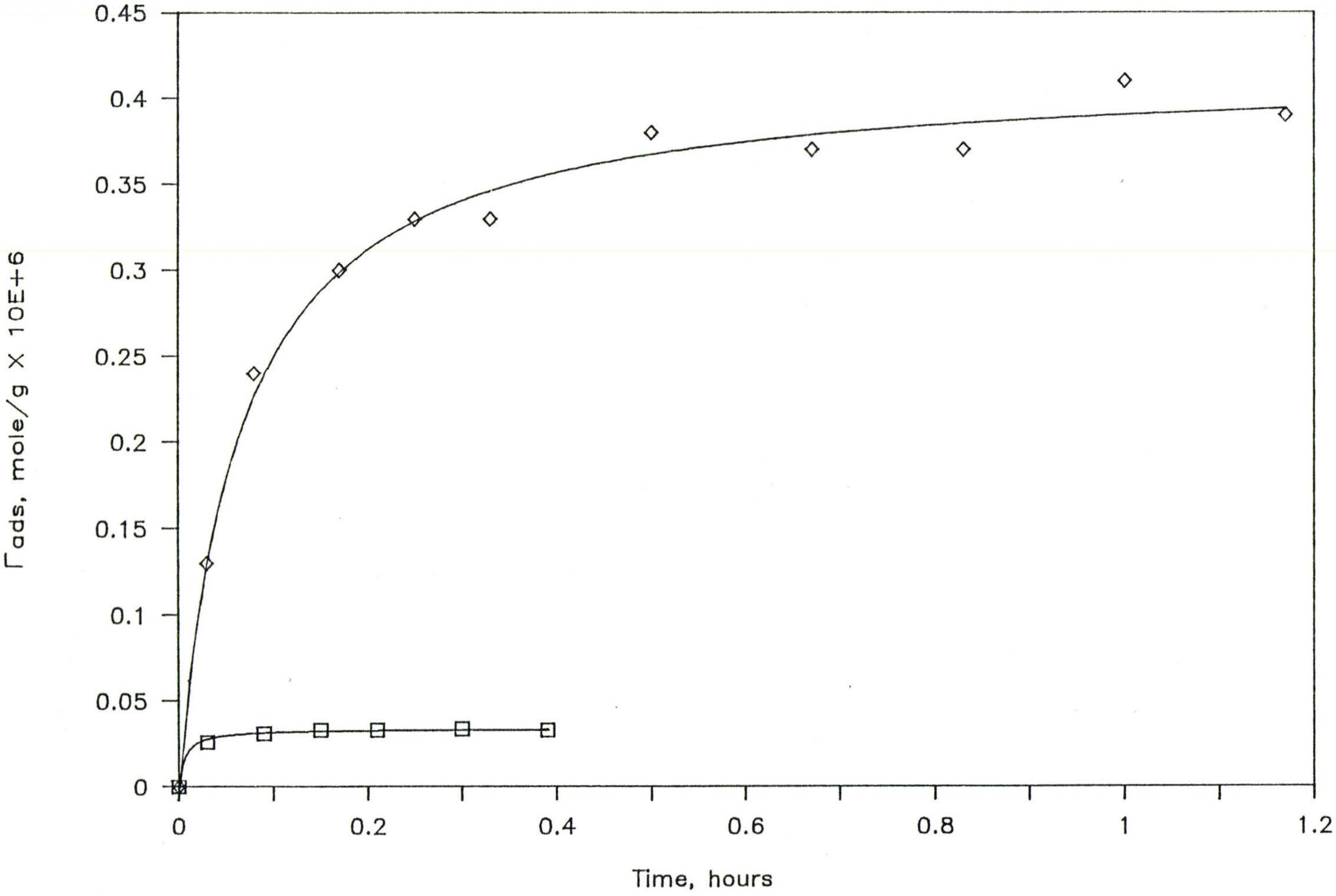

Figure 21. Rate of adsorption of TPPS$_4$ (□ = 1.01 X 10^{-6} M, ◇ = 1.01 X 10^{-5} M) on XAD-7 (0.5 g, 80–100 mesh) at pH 10.2 and room temperature.

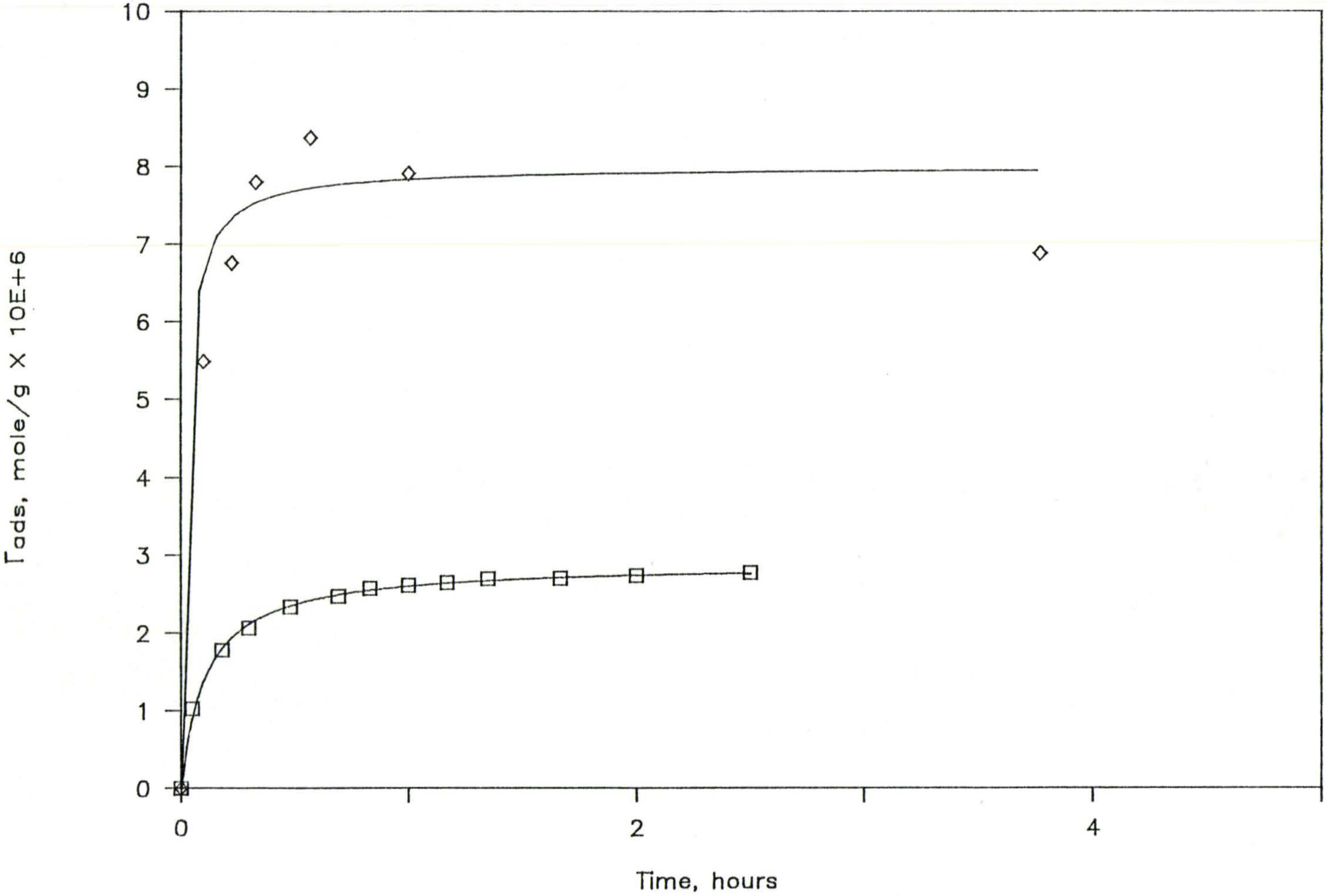

Figure 22. Rate of adsorption of $TPPS_4$ (□ = 0.96 X 10^{-4} M, ◇ = 8.17 X 10^{-4} M) on XAD-7 (0.5 g, 80-100 mesh) at pH 10.2 and room temperature.

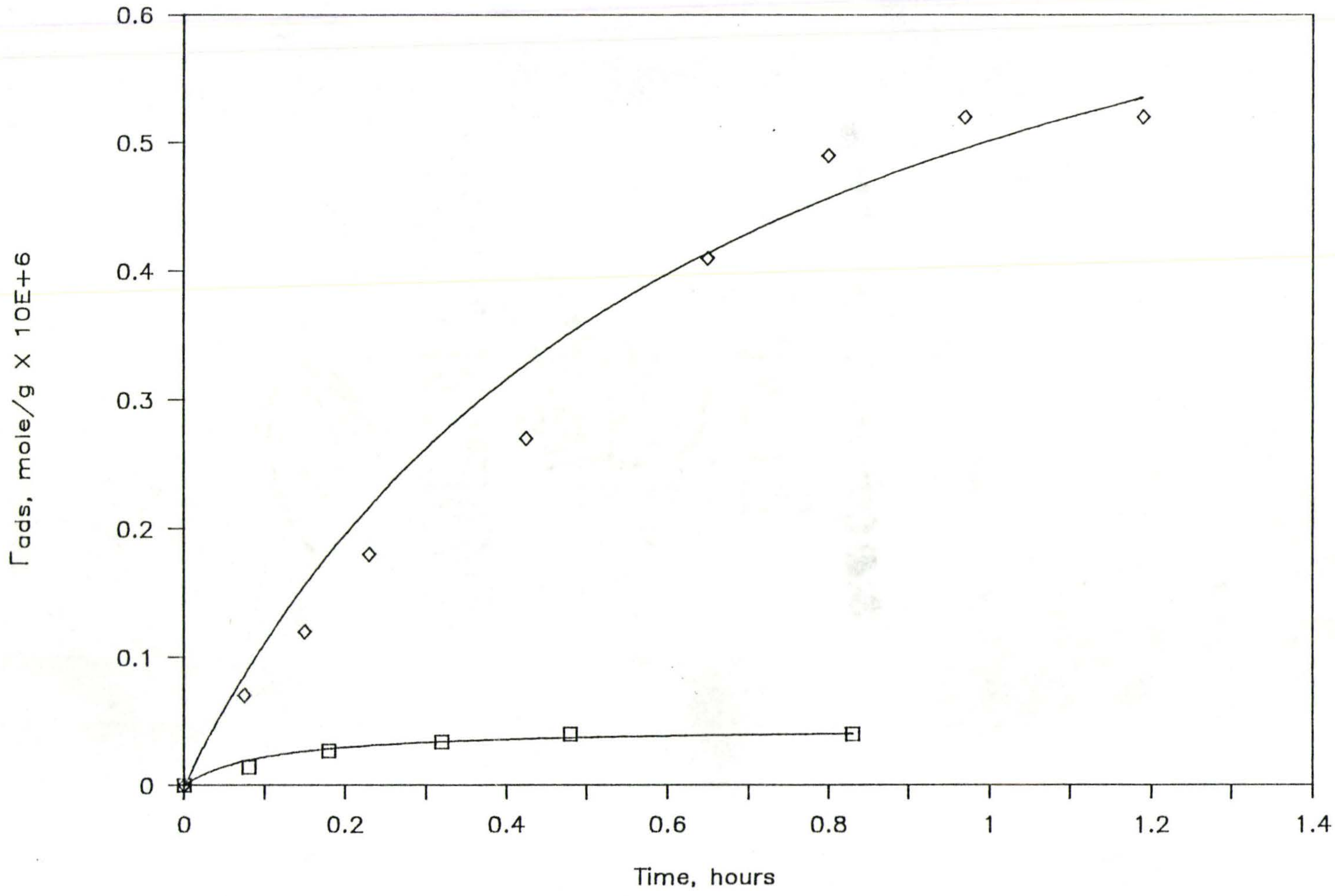

Figure 23. Rate of adsorption of Cu-TPPS$_4$ (□ = 8.85 X 10^{-7} M, ◇ = 2.03 X 10^{-5} M) on XAD-7 (0.5 g, 80-100 mesh) at pH 10.2 and room temperature.

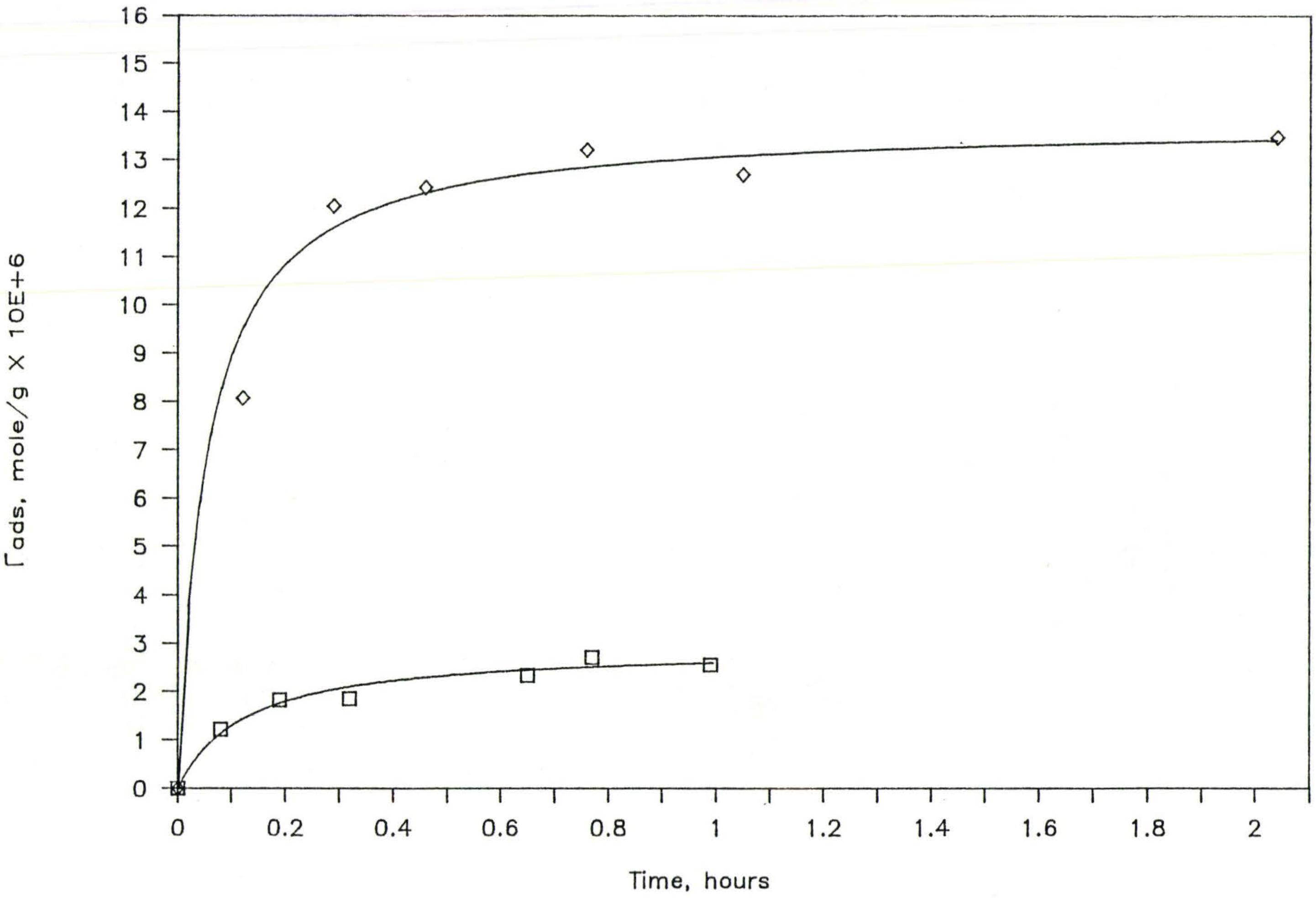

Figure 24. Rate of adsorption of Cu-TPPS$_4$ (□= 7.93 X 10^{-5} M, ◇= 8.19 X 10^{-4} M) on XAD-7 (0.5 g, 80-100 mesh) at pH 10.2 and room temperature.

3.4 Column Operation Studies

Breakthrough Curves

Whereas adsorption isotherm studies give the batch capacity of the resin, breakthrough curves give the capacity of the resin in the column configuration. In the breakthrough studies, a higher concentration of metalloporphyrin was used than would be formed on application of the preconcentration procedure to natural water samples; otherwise, passage of too large a solution volume would be required to obtain breakthrough.

In practice, the preconcentration method would require 100-500 mL of natural water sample with a total concentration of trace metals of up to 10 ppb. As seen from Table 14, the concentrations and the breakthrough volumes for Cu-TPPS$_4$, used as a representative metalloporphyrin, far exceed that expected for natural waters. Thus, the capacity of an XAD-7 column (with a 1 X 3-cm resin bed) is sufficient for the preconcentration method proposed. The breakthrough curves for Cu-TPPS$_4$ are shown in Figures 25 and 26.

Elution Profiles

TPPS$_4$ and its complexes are not strongly held on XAD-7, compared to XAD-2 and XAD-4, and can readily be eluted with methanol and methanol-water mixtures.

Since a 1 X 3-cm column of 80-100 mesh XAD-7 has

Table 14. Breakthrough Volumes for Cu-$TPPS_4$ on 1 X 3-cm XAD-7 Column (80-100 mesh)

Conc. of Cu-$TPPS_4$ (ppm)	Breakthrough volume* (mL)
0.1	490
1.0	15
2.0	5

---

* The breakthrough capacity (concentration of Cu-$TPPS_4$ X volume of Cu-$TPPS_4$ solution used), are not identical because the column conditions do not allow for equilibrium of the solution and resin phase as in batch studies. A lower flow rate would bring the breakthrough capacities into closer agreement with each other.

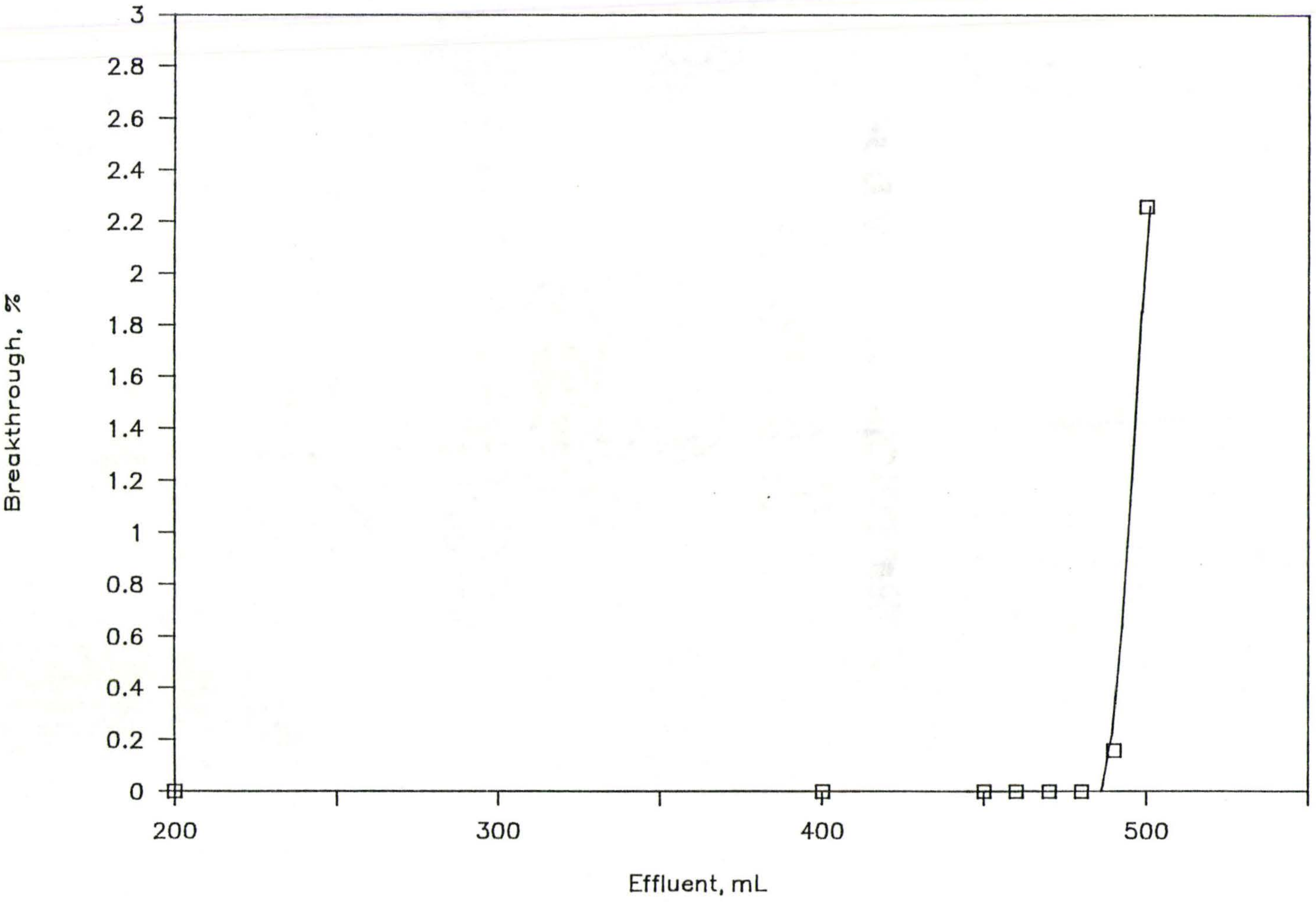

Figure 25. Breakthrough curve for Cu-TPPS$_4$ (100 ppb = 1.57 X 10^{-6} M) on 1 X 3-cm XAD-7 column (80-100 mesh) at pH 10.2. Flow rate = 1 mL/min.

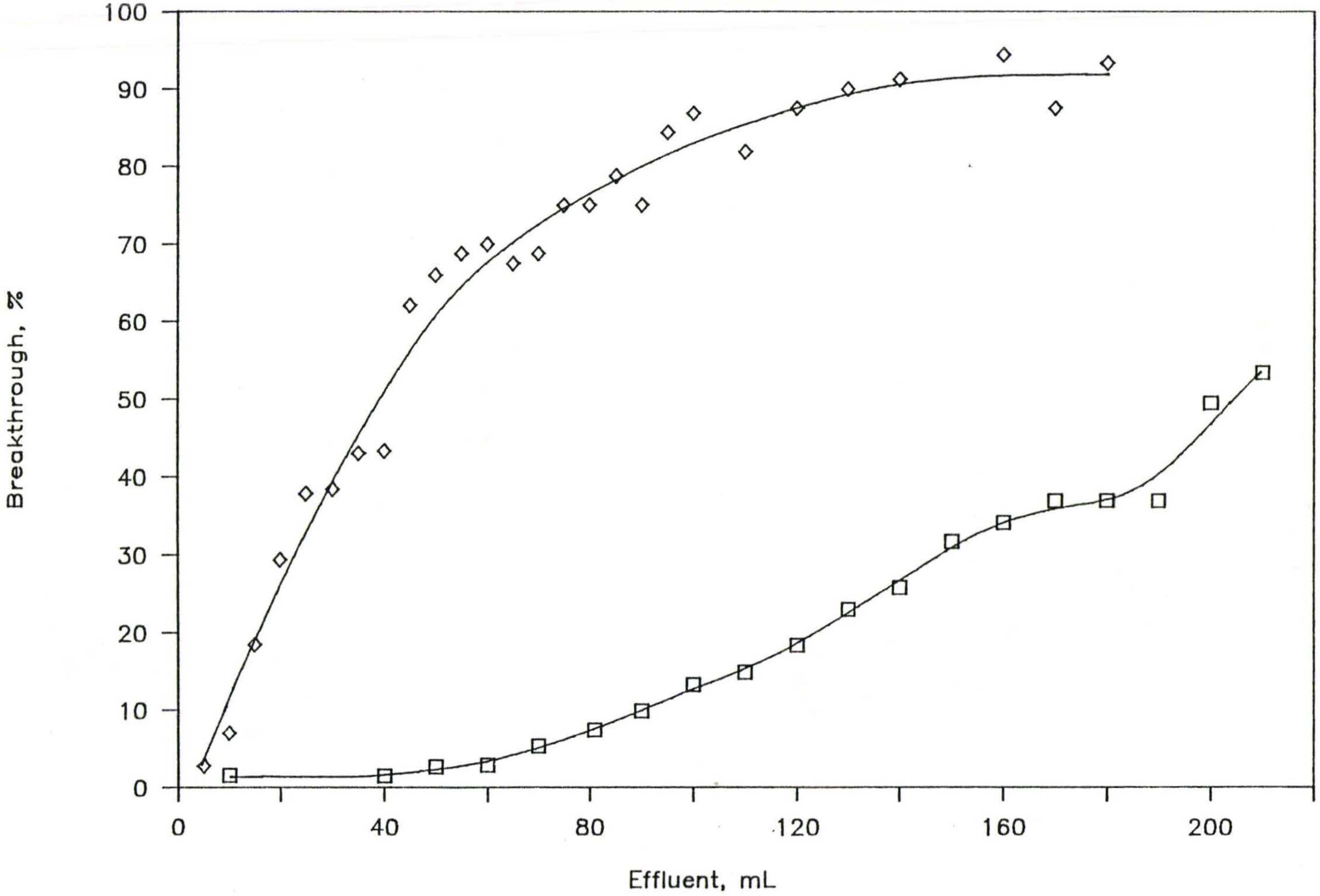

Figure 26. Breakthrough curves for Cu-TPPS$_4$ on 1 X 3-cm XAD-7 column (80-100 mesh) at pH 10.2 and room temperature. Cu-TPPS$_4$ concentrations of 1 ppm or 1.57 X 10^{-5} M (□), and 2 ppm or 3.14 X 10^{-5} M (◇) were used. Flow rate = 1 mL/min.

adequate capacity, it was decided in the elution study to keep these column parameters constant but vary the methanol-water ratio of the eluent. The optimum methanol-water ratio would provide quantitative elution of the metal complexes within a 5-mL preconcentration volume but with a maximum water content so as to decrease solvent volatility and make volume injection into the GFAAS easier and more reproducible.

The volume required for quantitative elution with various methanol-water mixtures are given in Table 15. Elution profiles of 95%, 80% and 30% mixtures are shown in Figures 27-29. The remaining elution profiles are found in the Appendix as Figures 43-49. As the ratio of water to methanol increases, the elution profile becomes broader and a larger volume is required to elute the $TPPS_4$ complexes quantitatively. To obtain quantitative elution with minimum eluent volatility, the 80% methanolic mixture was selected.

Table 15. Elution Volumes for Cu-$TPPS_4$ from 1 X 3-cm XAD-7 Column (80-100 mesh)

Ratio of Methanol:Water	Volume Required for Complete Elution of Cu-$TPPS_4$ (mL)
95:5	4.5
90:10	4.0
85:15	4.0
80:20	5.0
75:25	5.0
70:30	6.0
60:40	6.5
50:50	7.5
40:60	7.5
30:70	9.0

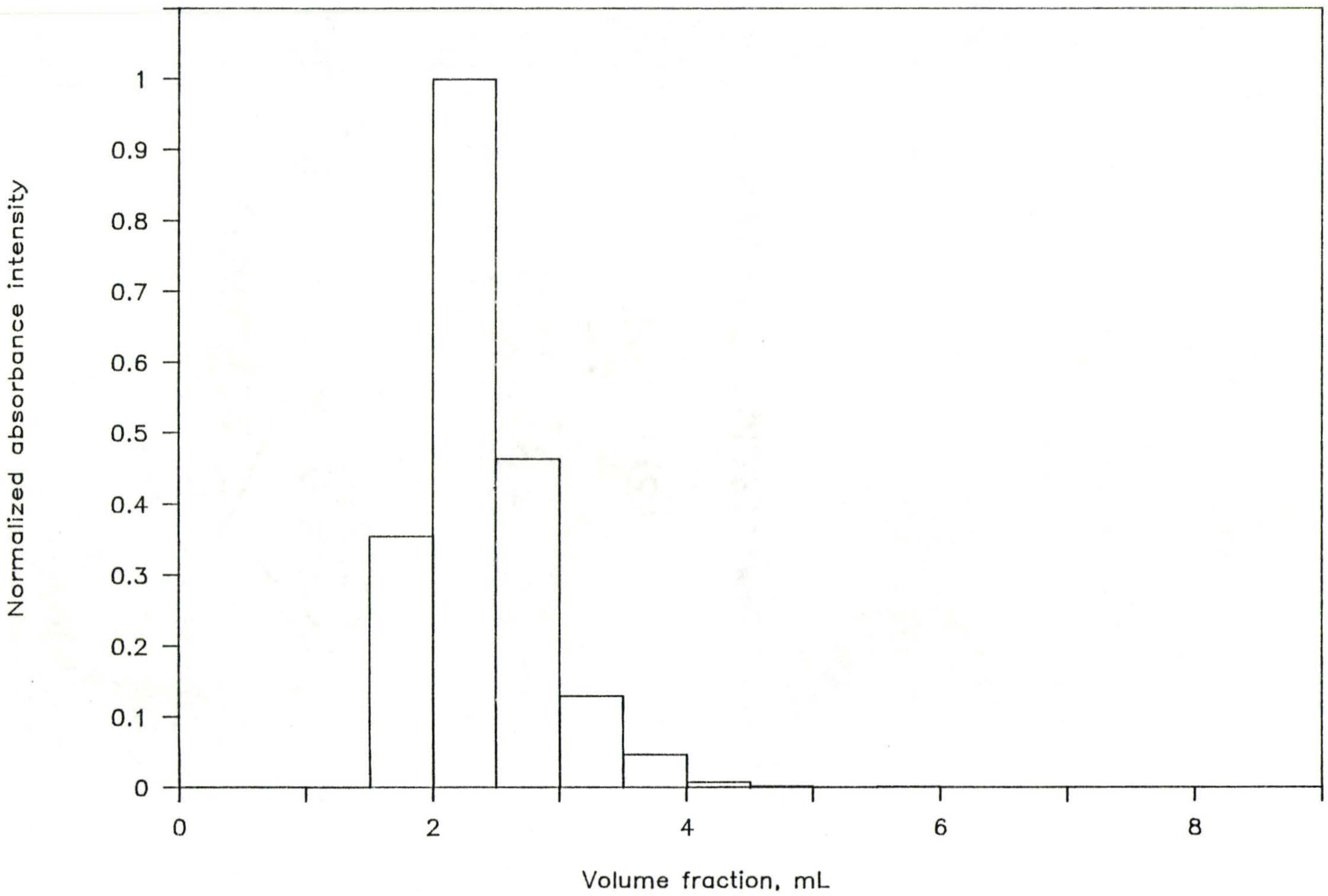

Figure 27. Elution of Cu-$TPPS_4$ from 1 X 3-cm XAD-7 column (80-100 mesh) by 95% v/v methanol. Mass of Cu(II) on column = 0.4 μg and flow rate = 1 mL/min.

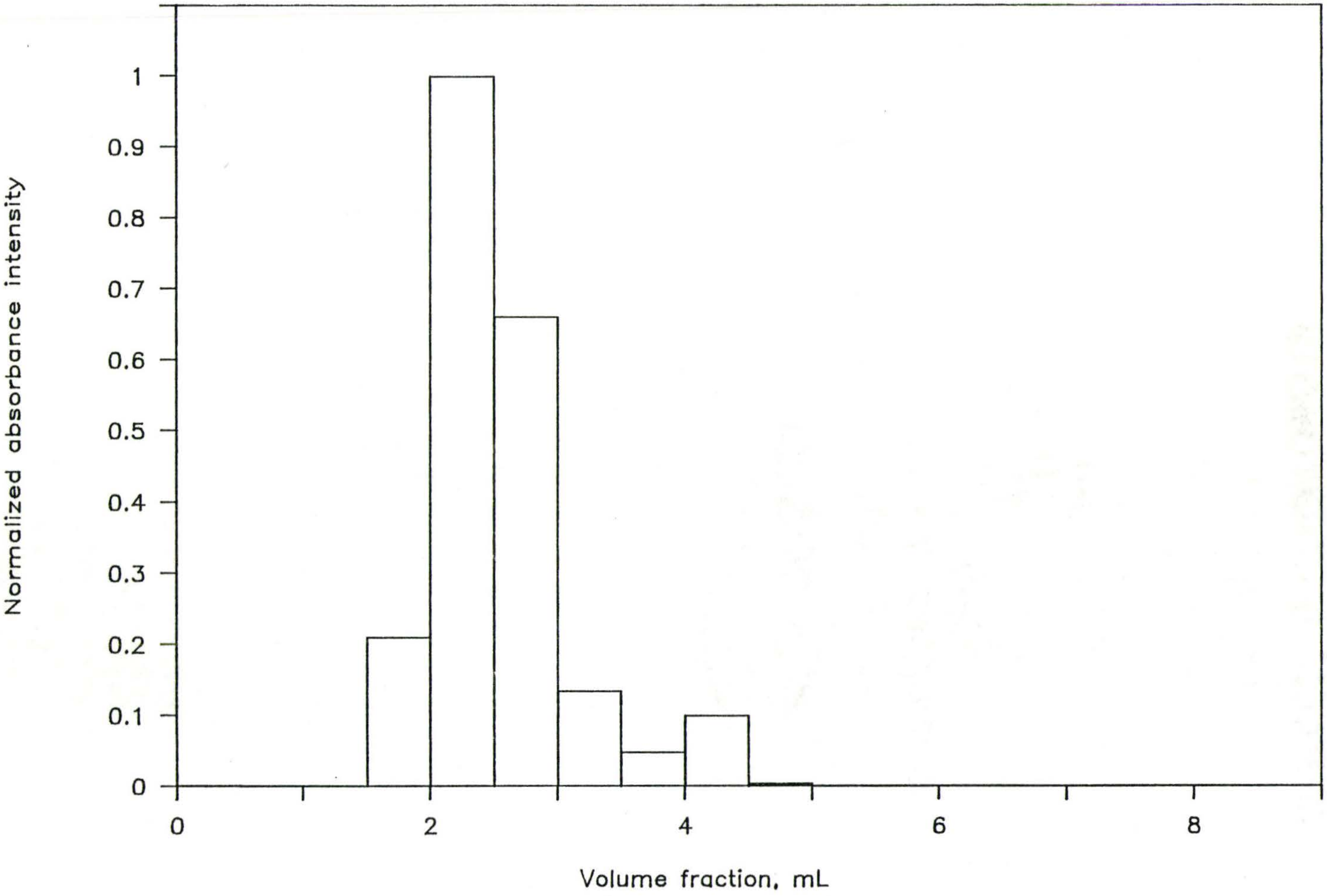

Figure 28. Elution of Cu–$TPPS_4$ from 1 X 3-cm XAD-7 column (80–100 mesh) by 80% v/v methanol. Mass of Cu(II) on column = 0.4 μg and flow rate = 1 mL/min.

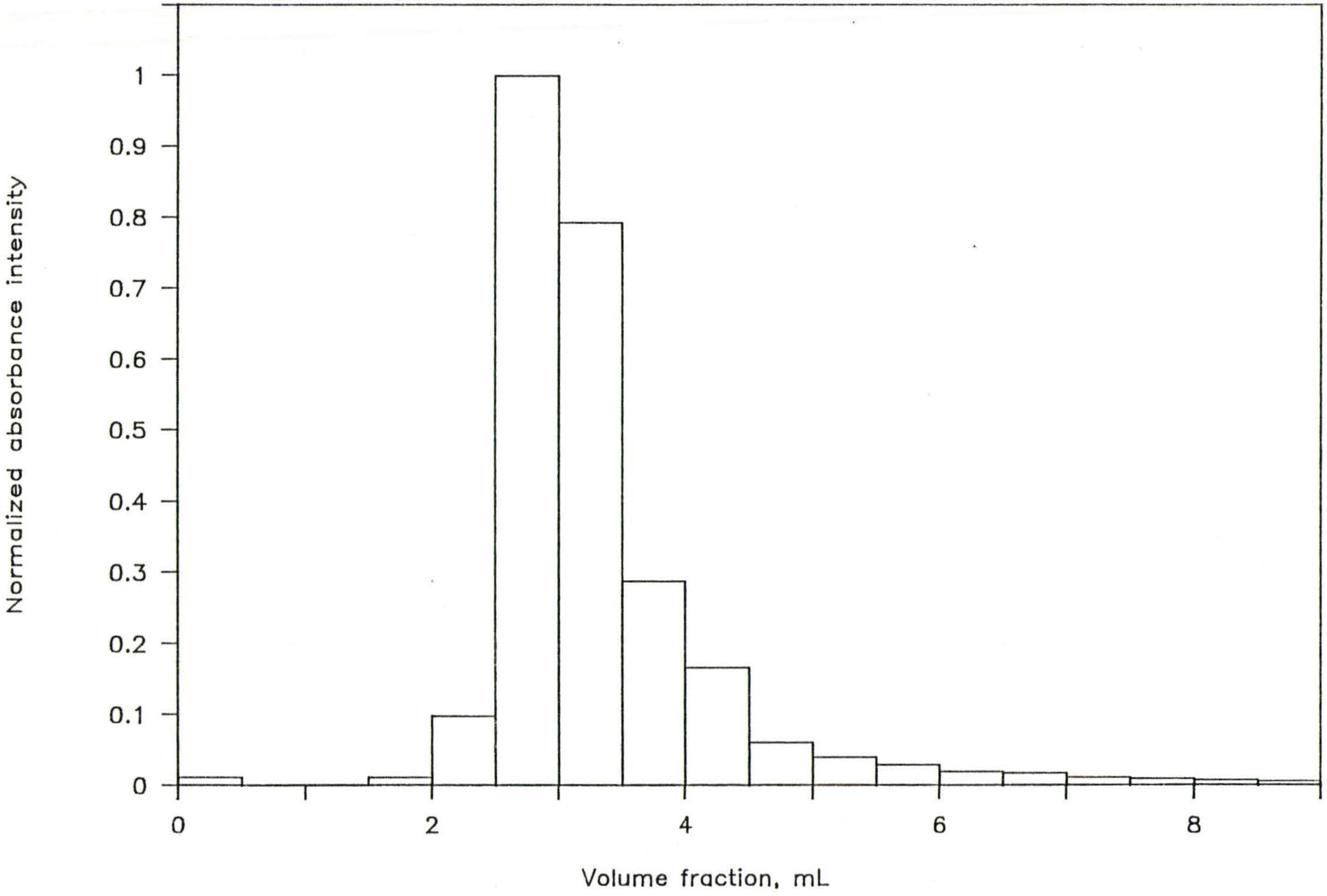

Figure 29. Elution of Cu-TPPS$_4$ from 1 X 3-cm XAD-7 column (80-100 mesh) by 30% v/v methanol. Mass of Cu(II) on column = 0.4 μg and flow rate = 1 mL/min.

3.5 Applications

General Method

The final method for preconcentration of trace metal in lakewater was developed on the basis of the best conditions found in the preceding experiments. A 1 X 3-cm column of 80-100 mesh XAD-7 was selected*. Solutions were adjusted to pH 10.2 with an NH_3/NH_4OAc buffer and reacted with $TPPS_4$ (2×10^{-6} M) and hydrazine at 90-98°C for 1-1/2 hours. The metal complexes were eluted with an 80% methanol-water mixture. Flow rates for adsorption and elution were 1 mL min^{-1}.

It is desirable that alkali-metal and alkali-earth ions not be preconcentrated along with the trace metal since these ions will cause non-specific spectral interference in the GFAAS measurement. It has been previously found (239) that rinsing the column with 10-15 mL of buffer prior to elution was adequate to rid the column of these interfering ions.

Prior to application to natural lakewater samples, the method was applied to synthetic samples (Table 16). The recovery of the trace metals is quite good although the precision on the Co result is unsatisfactory. In practice, Co was not determined in natural lakewater samples because of its very low concentration (< 0.1 µg L^{-1}) and incomplete $TPPS_4$ complex

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* The choice of XAD-7 rather than XAD-4 may seem surprising since XAD-4 not only has a higher capacity but binds the complexes more strongly. However, the complexes and excess ligand, are simply too strongly held on XAD-4 and are too difficult to strip with a small volume of eluent.

Table 16. Analysis of Synthetic Freshwater by $TPPS_4$ Method

Element	Mass Added to 100 mL (ng)	Mass Recovered (ng)
Cd	8	9.4 ± 1.1
Co	100	94.4 ± 46.2
Cu	200	214 ± 11
Mn	100	84.9 ± 5.4
Ni	200	192 ± 12
Pb	100	90.6 ± 10.3

NOTE: The results are based on triplicate determinations, using SAM.

formation (which is likely the reason for the poor precision).

Analysis of three Lake Ontario water samples was performed in which the samples were preconcentrated by a factor of 40 (200 mL to 5 mL). The results were compared to samples preconcentrated using the accepted Chelex-100 method (8-fold preconcentration factor). The data are shown in Tables 17-19. Since there was insufficient sample to do a triplicate analysis on lakewater from CCIW Station 41 using the Chelex-100 method, only a single analysis was done (Table 18). For this particular sample, the results for Pb are in poor agreement, although the Pb value by the $TPPS_4$ method agrees well with Chelex-100 and XAD-7 data acquired by S. Chiang on the same sample (Table 29, Appendix).

In general, there seems to be satisfactory agreement between the $TPPS_4$ general method and the Chelex-100 method with the exception of Mn. Excluding the Mn data, the results from the $TPPS_4$ general method are also in satisfactory agreement with data previously obtained using the XAD-7 two-column method (Tables 28 and 29, Appendix).

The discrepancy in the Mn results using the $TPPS_4$ method and the Chelex-100 method is puzzling. Although, complexation of $TPPS_4$ with Mn is not complete, the determination of Mn should be possible with SAM, which should compensate for this loss. However, the results for Mn using the $TPPS_4$ method are low compared to both the accepted Chelex-100 method and the XAD-7 two-column method using SAM (Tables 28-29, Appendix).

Table 17. Analysis of Lake Ontario Water, CCIW Station 302

	Concentration ($\mu g\ L^{-1}$)	
Element	$TPPS_4$ Method	Chelex-100
Cd	0.057 ± 0.007	0.055 ± 0.014
Cu	1.32 ± 0.17	1.31 ± 0.15
Mn	0.174 ± 0.021	1.25 ± 0.04
Ni	2.02 ± 0.12	1.97 ± 0.11
Pb	0.253 ± 0.024	0.239 ± 0.018

NOTE: The results for the $TPPS_4$ method and Chelex-100 method are based on triplicate determinations using SAM and external calibration method, respectively.

Table 18. Analysis of Lake Ontario Water, CCIW Station 41

Element	Concentration ($\mu g\ L^{-1}$) $TPPS_4$ Method	 Chelex-100
Cd	0.042 ± 0.014	0.031
Cu	8.57 ± 0.41	8.47
Mn	0.212 ± 0.019	1.37
Ni	1.95 ± 0.19	2.11
Pb	0.460 ± 0.113	0.985

NOTE: The results by the $TPPS_4$ method are based on triplicate determinations using SAM. Because of insufficient sample, the Chelex-100 results represent a single determination only, using external calibration method.

Table 19. Analysis of Lake Ontario Water,
(lat. 43° 36' 30", long. 78° 00' 0", 1 meter depth)

Element	Concentration ($\mu g\ L^{-1}$)	
	$TPPS_4$ Method	Chelex-100
Cd	0.045 ± 0.019	0.034 ± 0.005
Cu	1.13 ± 0.14	0.853 ± 0.100
Mn	0.139 ± 0.044	0.504 ± 0.004
Ni	3.09 ± 0.17	2.66 ± 0.28
Pb	0.211 ± 0.045	0.254 ± 0.033

NOTE: The results for the $TPPS_4$ method and Chelex-100 method are based on triplicate determinations using SAM and external calibration method, respectively.

In a separate study, an unidentified form of Mn, thought to be Mn(II) adsorbed on colloidal iron oxides, has been detected in Lake Ontario water (245). Perhaps this unidentified species plays a role in the poor results obtained by the $TPPS_4$ method. Also, the decomposition of the Mn-$TPPS_4$ complex (165) may be involved.

Whereas the general method can be applied to lake-water samples with reasonable success, the outcome of its application to natural seawater samples proved to be disastrous. After heating the sample with the ligand at pH 10.2, a brown-coloured precipitate formed as a layer on top of the XAD-7 column upon sample percolation. The precipitate was found to be $TPPS_4$ and metalloporphyrin aggregates which had formed at the high ionic strength of the solution. $TPPS_4$-metal complexes are known to aggregate under conditions of high concentration and high ionic strength (162). Surprisingly, this behaviour was not observed with synthetic seawater samples, perhaps because the ionic strength was adjusted merely with NaCl and not appropriate Mg and Ca salts. Complexation of $TPPS_4$ with Ca(II) and Mg(II) would greatly increase the concentration of metalloporphyrin in solution, inducing aggregation. Furthermore, for the natural seawater samples, a substantial amount of base was required to raise the pH to 10.2 (as much as 2 mL of concentrated NH_4OH per 100 mL of sample) due to the great buffering capacity of natural water. Thus, the ionic strength was considerably higher in these samples.

Statistical Analysis of the $TPPS_4$ Method

The analytical blank is used in each analysis to correct for contamination incurred during the analytical process. In this study, the blank was prepared by treating distilled deionized water with the same reagents and to the same preconcentration procedure as the real sample. The average blank values for the Chelex-100 and $TPPS_4$ methods obtained over the past year while performing sample analyses, are shown in Table 20. The $TPPS_4$ method has lower blank values than the Chelex-100 method for all of the elements tested except for Co, which is comparable in value. A large contribution to the blank is made by impurities in the chemicals used in the preconcentration method, such as $TPPS_4$ which is particularly difficult to purify. Also, although a laminar-flow work station is used to minimize contamination from the environment, a "Class 100" environment would lower the blank value even further (246,247).

The "limit of detection" (c_l) is defined as the lowest concentration or amount of the element that can be distinguished as statistically different from the analytical blank. According to the IUPAC definition (29),

$$x_l = \bar{x}_b + k * |s_b| \qquad (11)$$

where k is a constant chosen depending on the confidence level desired. By convention, k is equal to 3, which gives a

Table 20. Analytical Blank Values for $TPPS_4$ and Chelex-100 Procedures

	Concentration (µg L^{-1})		
Element	Chelex-100	$TPPS_4$ Method	Detection Limit of $TPPS_4$ Method
Cd	0.11 ± 0.07	0.04 ± 0.02	0.06
Co	0.20 ± 0.11	0.23 ± 0.12	0.36
Cu	1.84 ± 0.90	0.19 ± 0.07	0.21
Mn	0.26 ± 0.04	0.19 ± 0.11	0.33
Ni	1.43 ± 0.95	0.22 ± 0.23	0.69
Pb	0.50 ± 0.29	0.12 ± 0.06	0.18

NOTE: The results of the analytical blank are average values taken during the time period of the experimental work.

confidence level of 99.5% for a normal distribution of blank readings. The average blank value and standard deviation is given by $\bar{x}_b$ and s_b, respectively. The limit of detection is a linear function of x_l,

$$c_l = \frac{(x_l - \bar{x}_b)}{m} \qquad (12)$$

where m is the slope of the analytical calibration curve of signal (x) versus concentration (c). By substituting equation 11 into equation 12, the limit of detection is given by

$$c_l = \frac{k * |s_b|}{m} \qquad (13)$$

The limit of detection for the $TPPS_4$ method is given in Table 20.

The $TPPS_4$ preconcentration method and GFAAS measurement has precision of about 5 to 15% for Cu, Mn, and Ni. The uncertainties are larger for Cd (12-42%) and Pb (9-24%) due at least to some extent, to the large amount of these elements present in airborne particulates from car exhaust and cigarette smoke. Cd is particularly difficult to determine because of its low concentration in water samples and the light sensitivity of its $TPPS_4$ complex. However, the precision is of the same order of magnitude as other trace metal preconcentration methods such as chelating ion-exchange (61,79,85) or solvent extraction (60,61,165).

Assuming normal distribution, the mean value of the metal-ion concentrations determined by the Chelex-100 and the $TPPS_4$ methods can be compared using the Student's t Test (Tables 21-23). The value of t is given by

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{S} * \sqrt{\frac{n_1 n_2}{n_1 + n_2}} \qquad (14)$$

and the value of the pooled S is given by

$$S = \sqrt{\frac{(x_{i,1} - \bar{x}_1)^2 + (x_{i,2} - \bar{x}_2)^2}{n_1 + n_2 - 2}} \qquad (15)$$

where $\bar{x}_1, \bar{x}_2$ and s_1, s_2 and n_1, n_2 are the means, the standard deviations and the number of measurements of the two methods, respectively.

To test if two normal distributions have the same variance, the F Test is used (Tables 21-23). The value of F is given by,

$$F = \frac{{s_1}^2}{{s_2}^2} \qquad (16)$$

With the exception of Mn, there is no significant difference in the means and variances of the Chelex-100 and $TPPS_4$ methods at both the 99% and 95% confidence levels. Since the analysis of CCIW Station 41 lakewater by the Chelex-100 method was only performed once, F could not be calculated for this analysis.

Table 21. Comparison of Analytical Data for Lake Ontario Water (Station 302) by $TPPS_4$ and Chelex-100 Methods: Test for Significant Difference

	Concentration (μg L^{-1})			
Element	$TPPS_4$ Method	Chelex-100	F Calculated	t Calculated
Cd	0.057 ± 0.007	0.055 ± 0.014	3.92	0.19
Cu	1.32 ± 0.17	1.31 ± 0.15	1.27	0.07
Mn	0.174 ± 0.021	1.25 ± 0.04	3.87	39.8
Ni	2.02 ± 0.12	1.97 ± 0.11	1.05	0.56
Pb	0.253 ± 0.024	0.239 ± 0.018	1.70	0.84

NOTE: Values of F with reference to confidence levels of 95% (n_1, n_2 = 3,3): 19.0; 99% (n_1, n_2 = 3,3): 99.0.
Values of t with reference to confidence levels of 95% ($n_1 + n_2 = 6$): 2.78; 99% ($n_1 + n_2 = 6$): 4.60.

Table 22. Comparison of Analytical Data for Lake Ontario Water (Station 41) by $TPPS_4$ and Chelex-100 Methods: Test for Significant Difference

Element	Concentration (µg L^{-1}) $TPPS_4$ Method	Chelex-100	t Calculated
Cd	0.042 ± 0.014	0.031	0.67
Cu	8.57 ± 0.41	8.47	0.21
Mn	0.212 ± 0.019	1.37	52.4
Ni	1.95 ± 0.19	2.11	0.71
Pb	0.460 ± 0.113	0.985	3.97

NOTE: Values of t with reference to confidence levels of 95% ($n_1 + n_2 = 4$): 4.30; 99% ($n_1 + n_2 = 4$): 9.93.

Table 23. Comparison of Analytical Data for Lake Ontario Water (lat. 43° 36' 30", long. 78° 00' 0", 1 meter depth) by $TPPS_4$ and Chelex-100 Methods: Test for Significant Difference

	Concentration ($\mu g\ L^{-1}$)			
Element	$TPPS_4$ Method	Chelex-100	F Calculated	t Calculated
Cd	0.045 ± 0.019	0.034 ± 0.005	13.8	1.02
Cu	1.13 ± 0.14	0.853 ± 0.100	2.04	2.71
Mn	0.139 ± 0.044	0.504 ± 0.004	116	14.4
Ni	3.09 ± 0.17	2.66 ± 0.28	2.75	2.23
Pb	0.211 ± 0.045	0.254 ± 0.033	1.89	1.33

NOTE: Values of F with reference to confidence levels of 95% (n_1, n_2 = 3,3): 19.0; 99% (n_1, n_2 = 3,3): 99.0.
Values of t with reference to confidence levels of 95% ($n_1 + n_2$ = 6): 2.78; 99% ($n_1 + n_2$ = 6): 4.60.

3.6 Studies on the Room-Temperature Method

Preliminary Studies on Room-Temperature Complexation of Metal Ions

Metalloporphyrin formation is much slower than reactions with open chain ligands because the protons of $TPPS_4$ in the H_2P form are effective in preventing bonding of the pyrrole nitrogens to the metal ion; also, the probability of concerted dissociation of water and association of pyrrole nitrogens is small. Thus, high temperatures are necessary to speed up the reaction. However, metal ions such as Pb(II), Hg(II) and Cd(II) are known to form metalloporphyrins rapidly (200-202,212,213). In the present study, it was found that essentially quantitative complexation of Pb(II), Hg(II) and Cd(II) with $TPPS_4$ occurred after shaking the solution at room temperature for one hour; i.e., 97% of the Pb, 98% of the Cd and 105% of the Hg reacted. Therefore, a fast and simple method specifically for the preconcentration of these three metal ions in natural waters seemed possible. The complexes are formed at room temperature and adsorbed quantitatively onto XAD resin and the ions are quantitatively recovered with 1% HNO_3 eluent. Based on these preliminary findings, the development of a room-temperature method seemed promising.

Resin Type

XAD-4 had a greater capacity for $TPPS_4$ than XAD-2 and hence was selected as the resin type for the room-temperature method (Table 24). Although these resins are chemically identical, the greater capacity of XAD-4 is attributed to its larger surface area per gram of resin.

Particle Size

The effect of particle size is shown in Table 24. Since the adsorption capacity does not change significantly with increasing mesh size, adsorption of the $TPPS_4$ in the pores of the XAD-4 resin must be extensive. This behaviour is clearer than that observed previously with XAD-7. Because of good packing properties, the mesh size 80-100 was used in subsequent studies.

Time and Extent of Complexation

From the preliminary study with a 100-fold excess of metal-ion (10^{-4} M) over ligand (10^{-6} M), there was a good indication that quantitative reaction between the metal ions, Cd and Pb, and $TPPS_4$ occurs at room temperature.

This was confirmed by an additional spectrophotometric study in which up to a 10-fold excess of ligand to metal-ion concentration was used. At 10^{-6} M $TPPS_4$ and 10^{-5} M Pb(II), 90-94% conversion to the complex occurred within 25 minutes. With approximately a ten times excess of $TPPS_4$ (10^{-6} M) over Cd(II), 100% Cd-$TPPS_4$ formation occurred within 45 minutes. At a Cd(II) concentration approximately

Table 24. Effect of Resin Type and Mesh Size for the Room-Temperature Method

Resin	Mesh Size	Initial $TPPS_4$ Conc. (M)	µmoles $TPPS_4$ Adsorbed per g Dry Resin
XAD-2	80-100	1.06×10^{-3}	11.4
XAD-4	< 60	1.04×10^{-3}	18.6
XAD-4	60-80	1.04×10^{-3}	21.5
XAD-4	80-100	1.04×10^{-3}	18.4

equivalent to ligand concentration (10^{-6} M), Cd-$TPPS_4$ formation was 96% within 60 minutes. Therefore, an one-hour shaking time was deemed sufficient to ensure complete reaction between the metal ions and the ligand.

However, when a similar study was conducted using the TCMA/MIBK extraction procedure (165) with metal ions at concentrations found in natural water (0.4 ppb Pb, 0.05 ppb Cd), the extent of reaction was found to be less complete. Both metal ions equilibrated with $TPPS_4$ within 45 minutes to one hour but only 84% of the Cd had reacted. The Pb reacted quantitatively to form the complex.

The concentration of Hg was not measured in this study and in subsequent experiments because the GFAAS exhibits a low sensitivity for this element when it is injected directly into the instrument.

Effect of Auxiliary Ligands on Reaction

Because metal complexation of porphyrins is slow, auxiliary complexing agents have been used to accelerate this reaction. Metal-ion incorporation has been catalyzed by pyridine (192,248) and imidazole (168), and by reducing agents such as hydroxylamine (202), ascorbic acid (176) and hydrazine (165).

Hydrazine is known to form coordination compounds with metal ions (249). In general, the number of hydrazine molecules coordinated to the metal ion is half of its usually assigned coordination number. However, it has been shown that the metal-ion hydrazine complexes rarely exist as simple ions but rather as polycationic complexes in which hydrazine serves as a bridging group. Due to the reducing nature of hydrazine, even the number of these complexes are limited.

The addition of hydroxylamine and hydrazine to the solutions did not seem increase the rate of reaction of Cd and Pb at room temperature. It was noted however, that these reagents contributed towards a high blank.

Time of Adsorption

Using concentrations of Cd and Pb ions similar to that found in natural-water samples, the metal ions were complexed with $TPPS_4$ and adsorbed onto XAD-4 resin by batchwise agitation. Adsorption occurred rapidly and it was found that less than five minutes were required for quantitative adsorption of the complexed metal ions onto the resin. Therefore, batchwise adsorption of the metal complexes could provide a fast alternative method to column percolation, especially for large sample volumes.

Adsorption Isotherm

The adsorption isotherm of Pb-$TPPS_4$ is shown in Figure 30; it is less curved than that of Cu-$TPPS_4$. This may be due to a higher amount of metalloporphyrin aggregation, although aggregation of Pb-$TPPS_4$ has not been studied.

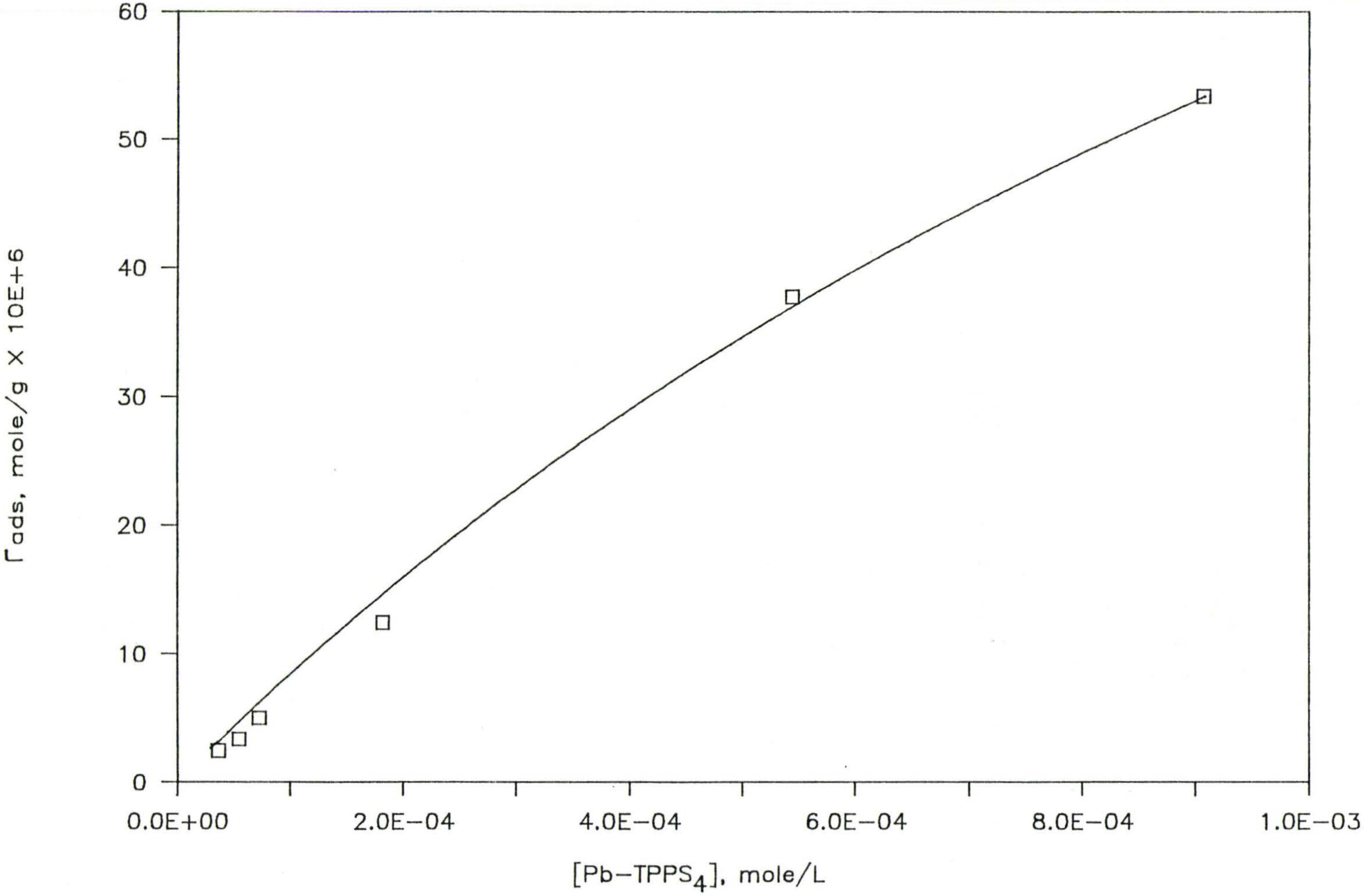


Figure 30. Adsorption isotherm for Pb−$TPPS_4$ on XAD−4 (0.5 g, 80−100 mesh) at pH 10.2 and room temperature.

For Pb-$TPPS_4$, the Γ_{max} value is 156.8 μmoles per gram resin (80-100 mesh) and $K^{-1} = 1.76 \times 10^{-3}\ M^{-1}$. The Γ_{max} of Pb-$TPPS_4$ on XAD-4 is much greater than Γ_{max} of $TPPS_4$ on any of the XAD resins or Γ_{max} of Cu-$TPPS_4$ on XAD-7 (i.e., 9-50 μmoles g^{-1}) (see Table 10). The large Γ_{max} value may also reflect extensive aggregation of Pb-$TPPS_4$ on the resin.

The rate of adsorption is relatively fast considering the large amount of metalloporphyrin found on the resin. For a concentration of 1.30×10^{-3} M Pb-$TPPS_4$, equilibrium is reached in about 20 hours (Figure 31). For the same concentration of $TPPS_4$, equilibrium is attained in about 29 hours for XAD-2 resin, 3 days for XAD-4 resin and 1 hour for XAD-7 resin. For Cu-$TPPS_4$, equilibrium on XAD-7 is reached in an hour (Table 13).

Breakthrough Curves

From Table 25, it is seen that the column capacity far exceeds the amount needed for practical applications, i.e., usually 100-500 mL at a Pb concentration less than 10 ppb (4.8×10^{-8} M) in natural waters. The breakthrough curves for Pb-$TPPS_4$ are shown in Figure 32.

Elution Profiles

The elution profiles for Cd and Pb are shown in Figures 33 and 34. Both Cd and Pb are quantitatively recovered within 5 mL of 1% HNO_3 eluent.

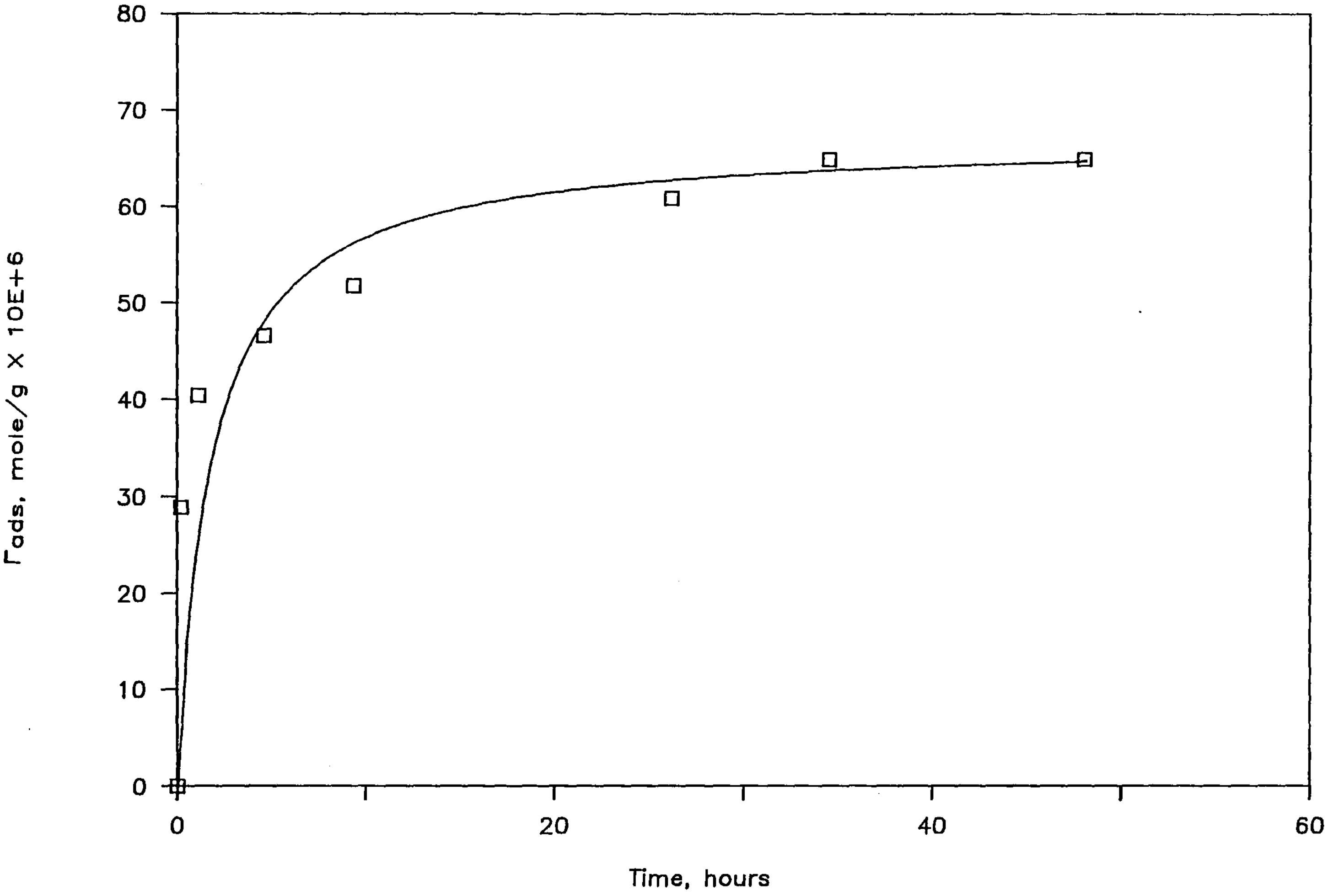


Figure 31. Rate of adsorption of Pb-TPPS$_4$ (1.30 X 10^{-3} M) on XAD-4 (0.5 g, 80-100 mesh) at pH 10.2 and room temperature.

Table 25. Breakthrough Volumes for Pb-$TPPS_4$ on 1 X 3-cm XAD-4 Column (80-100 mesh)

Conc. of Pb-$TPPS_4$ (ppm)	Breakthrough volume (mL)
0.1	430
1.0	5

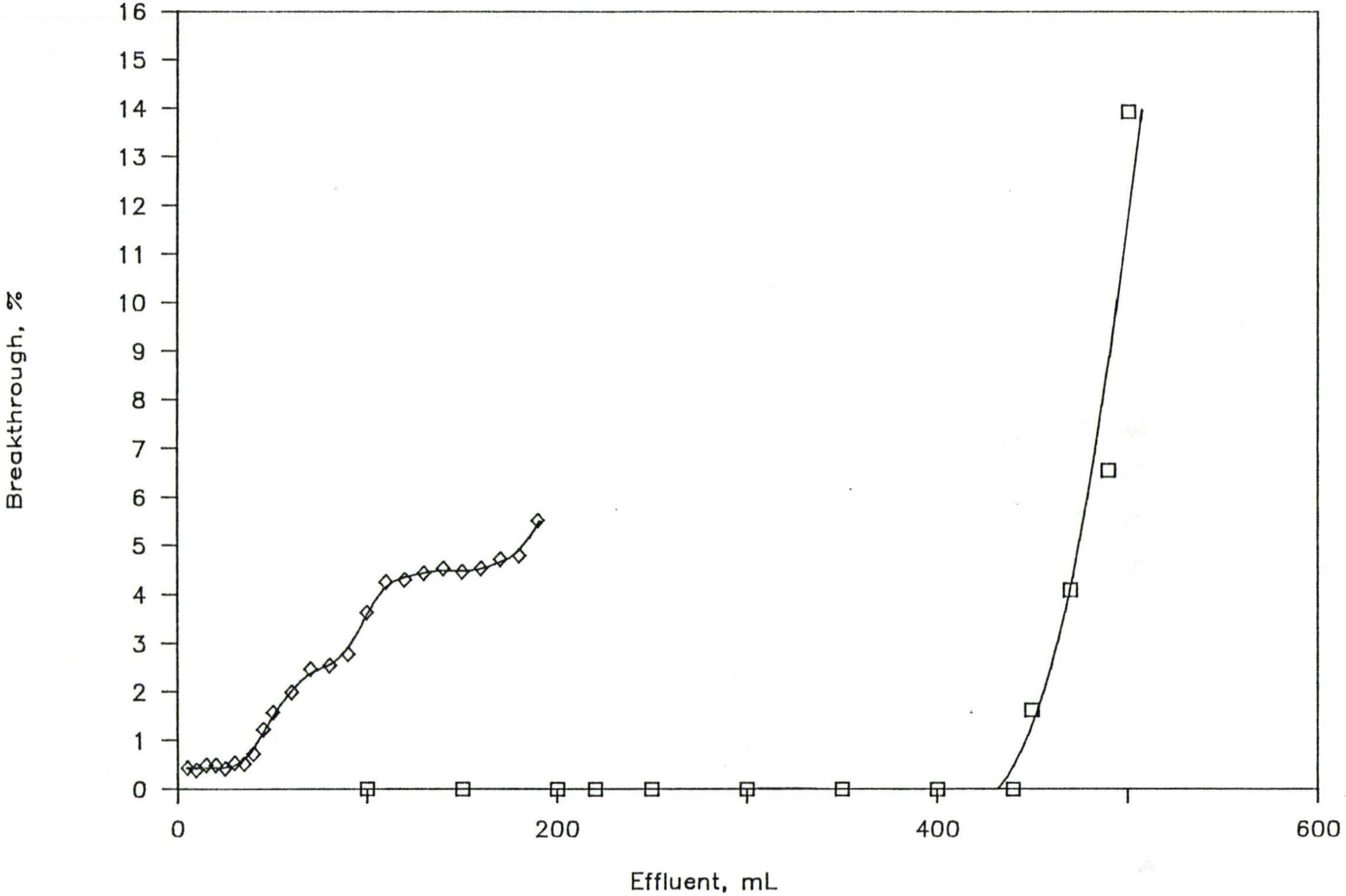


Figure 32. Breakthrough curve for Pb-TPPS$_4$ on 1 X 3-cm XAD-4 column (80-100 mesh) at pH 10.2. Pb-TPPS$_4$ concentrations of 100 ppb or 4.83 X 10^{-7} M (□), and 1 ppm or 4.83 X 10^{-6} M (◇) were used. Flow rate = mL/min.

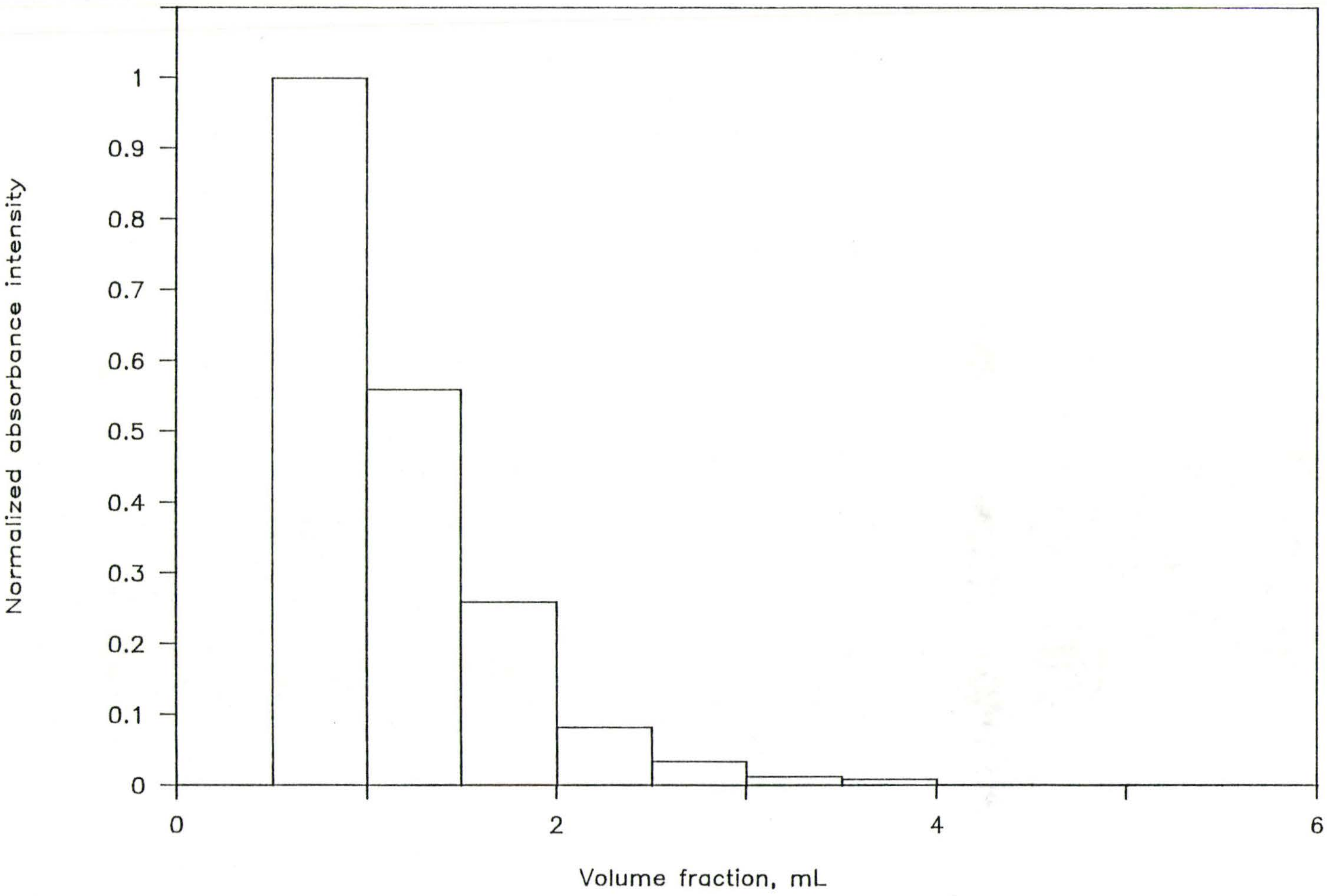


Figure 33. Elution of Cd(II) from 1 X 3-cm XAD-4 column (80-100 mesh) by 1% v/v HNO_3. Mass of Cd(II) on column = 0.2 μg and flow rate = 1 mL/min.

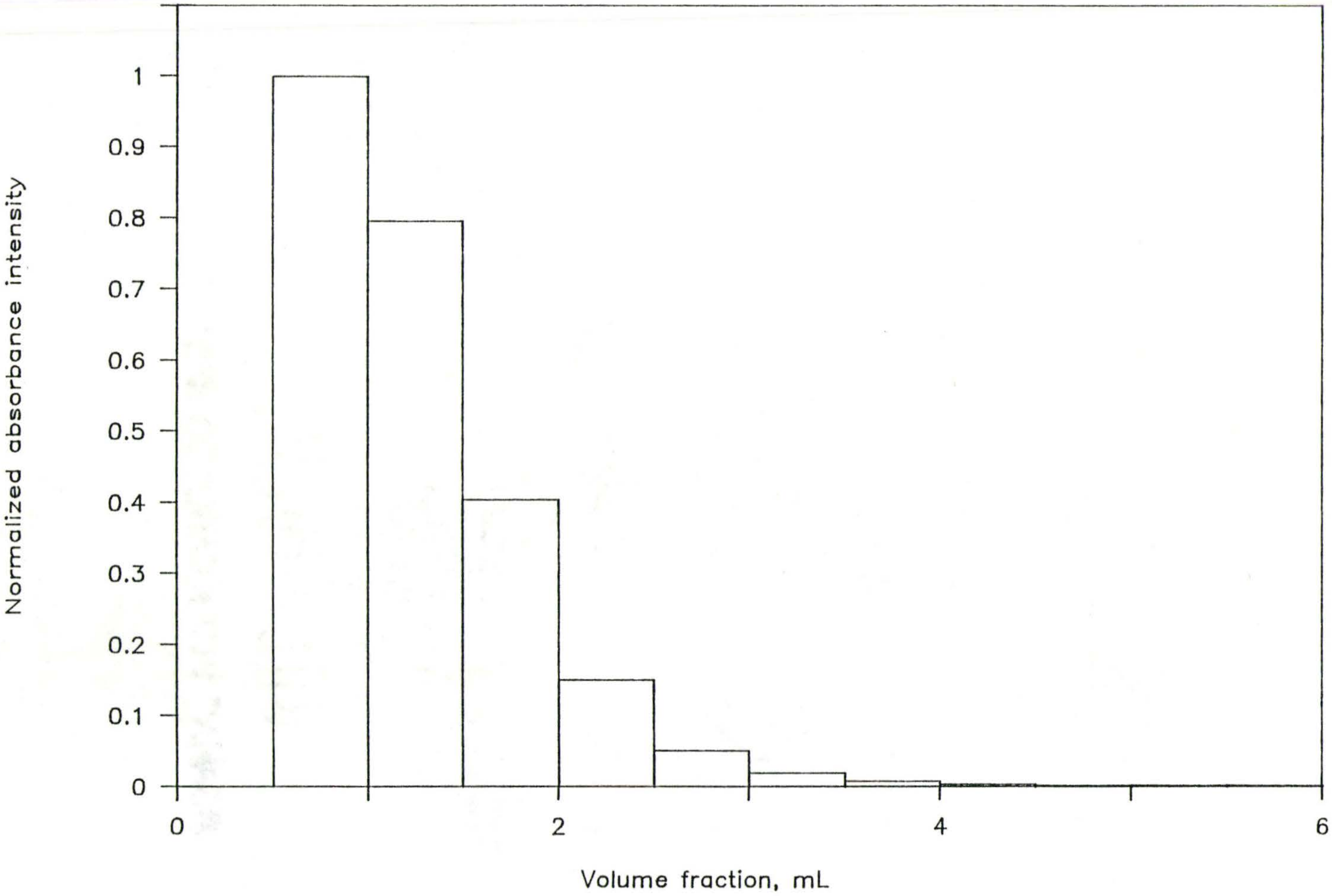


Figure 34. Elution of Pb(II) from 1 X 3-cm XAD-4 column (80-100 mesh) by 1% v/v HNO_3. Mass of Pb(II) on column = 0.4 μg and flow rate = 1 mL/min.

Applications

The conditions arrived at for the room temperature preconcentration were as follows: a 1 X 3-cm column of 80-100 mesh XAD-4 resin; adjustment of pH to 10.2 with a NH_3/NH_4OAc buffer; shaken at room temperature with $TPPS_4$ at 1 X 10^{-6} M for one hour. Ions such as Ca(II) and Mg(II) were washed out of the column with 10-15 mL of buffer, as before. The metal complexes were eluted with 5 mL of 1 % v/v HNO_3. Flow rates for adsorption and elution were 1 mL min^{-1}.

Prior to application to natural-water samples, the method was attempted on synthetic fresh and salt-water samples. The results for the synthetic water are given in Table 26. The average metal-ion recovery varies from good to poor and the standard deviations are high, especially for Pb (21-22% for Cd; 28-88% for Pb).

Analysis of CCIW Station 302 lakewater was performed with a preconcentration factor of 20 (100 mL to 5 mL). The results were compared to samples preconcentrated using the accepted Chelex-100 method. The data and statistical analysis are shown in Table 27. The Cd results for Station 302 are not in agreement with the Chelex method. As for the precision, the standard deviations are high, especially for Pb (4% for Cd; 17% for Pb).

Statistically, there is a significant difference in the variances of the Cd results obtained using the Chelex-100 and room-temperature methods at the 99% confidence level.

Table 26. Analysis of Synthetic Freshwater and Seawater by $TPPS_4$ Specific Method

Sample	Element	Mass Added to 100 mL (ng)	Mass Recovered (ng)
Lakewater	Cd	4	5.4 ± 1.2
	Pb	40	36.5 ± 10.1
Seawater	Cd	5	6.7 ± 1.4
	Pb	40	63.0 ± 55.6

NOTE: The results are based on triplicate determinations using SAM.

Table 27. Analysis and Comparison of Analytical Data for Lake Ontario Water (Station 302) by $TPPS_4$ and Chelex-100 Methods: Test for Significant Difference

	Concentration ($\mu g\ L^{-1}$)			
Element	$TPPS_4$ Method	Chelex-100 *	F Calculated	t Calculated
Cd	0.026 ± 0.001	0.055 ± 0.014	1068	2.77
Pb	0.294 ± 0.050	0.239 ± 0.018	7.22	1.81

* The Chelex-100 method data were taken from Table 17.

NOTE: Values of F with reference to confidence levels of 95% (n_1, n_2 = 3,3) is 19; 99% (n_1, n_2 = 3,3) is 99.0.
Values of t with reference to confidence levels of 95% ($n_1 + n_2 = 6$) is 2.78; 99% ($n_1 + n_2 = 6$) is 4.60.

The mean values for Cd obtained by the two methods are considered the same at the 95% confidence level. For Pb, at both the 95% and 99% confidence levels, the means and variances obtained by the two methods are the same.

3.7 Conclusions

The preconcentration method involving the adsorption of $TPPS_4$ complexes on XAD-7 has been successfully applied to synthetic and some natural lakewater samples. Compared to the Chelex-100 method which is widely used, the $TPPS_4$ adsorption method offers a few advantages: i.e., alkali metal and alkaline earth ions, whose presence is not favorable in GFAAS measurements, are not concomitantly preconcentrated; and the swelling characteristics of XAD-7 are superior to those of Chelex-100. Compared to extraction methods, the $TPPS_4$ adsorption procedure has the potential for larger preconcentration factors (>20-30 times). Also, $TPPS_4$ is a highly water-soluble reagent whereas common extraction reagents like APDC and 8-hydroxyquinoline have only limited aqueous solubility.

The $TPPS_4$ adsorption method, however, has a few serious limitations. For example, a long period of heating at high temperatures is required to bring about complexation. $TPPS_4$ is also difficult to prepare and purify and the Cd complex is light sensitive. The metal-ion concentration must also be determined by SAM, a calibration method which consumes considerable quantities of sample and is more tedious than the external calibration method. In addition, the determination of Co, Mn, Cr or Fe is not possible. Finally, problems with aggregation of the metalloporphyrins at high ionic strength makes seawater analysis impossible and so any application of the method would be restricted to freshwater samples.

3.8 Suggestions for Future Work

1. Assuming $TPPS_4$ to be an interesting and useful reagent for metal-ion complexation, then future research should be directed to fundamental studies which perhaps may ultimately lead to more useful applications. For example, the main disadvantage of the general method is the requirement of heating the solution for an extended period. Since metal-ion insertion into porphyrins can be facilitated by auxiliary ligands, this aspect should be examined with the hope that several metal ions will complex quantitatively, or nearly so, at room temperature. Such a development would overcome the main disadvantage of the procedure and perhaps also permit the use of $TPPS_4$ adsorbed on XAD-4 as an ion-exchange chelating resin.

2. Room-temperature reaction of Cd, Pb and possibly Hg ions with $TPPS_4$, should be investigated further. Perhaps the TCMA/MIBK extraction procedure of DiFruscia et. al. (165) should be interfaced to the room-temperature complexation. If successful, this method would be a fast and specific method of determining key toxic heavy metals in natural waters.

APPENDICES

1. Least-Squares Curve Fitting for the Langmuir Equation

The Langmuir equation is given by, $\Gamma = \Gamma_{max}\,[M]/(K^{-1} + [M])$. If $[M] = x$, $\Gamma = y$, $\Gamma_{max} = a$, and $K^{-1} = b$, then the equation simplifies to $y = ax/(b + x)$. The best-fit curve can be found by minimizing the sum of the squares of the differences between the y-value of each data point (x_i, y_i) and the y-value of an arbitrary curve given by the equation above. That is, the summation given by F(a,b) must be minimized where F(a,b) is given by:

$$F(a,b) = \Sigma\ (y_i - ax_i/(b + x_i))^2$$

Unfortunately, this leads to a set of non-linear equations which become difficult to solve. A simple non-linear least-squares solution can be obtained by multiplying each term in the summation by a factor of $(b + x_i)$ and then minimizing the resultant summation.

$$\begin{aligned} \text{i.e., } F'(a,b) &= \Sigma\ [(y_i - ax_i/(b + x_i))(b + x_i)]^2 \\ &= \Sigma\ (by_i + y_i x_i - ax_i)^2 \\ &= b^2\ \Sigma y_i^2 + \Sigma(y_i^2 x_i^2) + a^2\ \Sigma x_i^2 \\ &\quad + 2b\ \Sigma x_i y_i^2 - 2ab\ \Sigma(x_i y_i) - 2a\ \Sigma(x_i^2 y_i) \end{aligned}$$

To minimize the function, the partial derivatives are taken with respect to a and b and set to zero.

i.e., $\partial F/\partial a = 2a \sum x_i^2 - 2b \sum(x_i y_i) - 2 \sum(x_i^2 y_i) = 0$

$\partial F/\partial b = -2a \sum(x_i y_i) + 2b \sum y_i^2 + 2 \sum(x_i y_i^2) = 0$

The equations can then be solved for the unknown values of a and b which give the best-fit values for Γ_{max} and K^{-1}, respectively.

2. Estimation of $TPPS_4$ Adsorption Area

The diagonal distance between the para carbons of the phenyl rings was assumed to be 15.4 Å based on the average distance for 14 TPP crystal structures found in the Cambridge Crystallographic Database. The C-S bond was estimated to be 1.80 Å and the projection of the S=O bond of the pyramidal SO_3^- group onto the C-S bond was estimated to be 0.47 Å, based on standard bond lengths and angles (215). The area was then calculated assuming square coverage with a diagonal, d, given by:

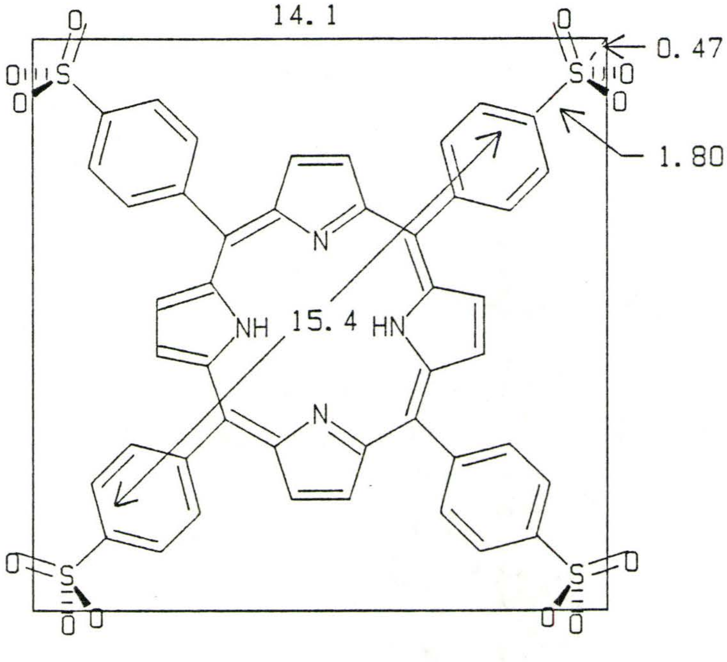
$$d = 15.4 + 2 \times (1.80 + 0.47)$$

$$= 19.9 \text{ Å}.$$

Thus the surface coverage (s.c.) is given by:

$$\text{s.c.} = (19.9 \times \sin 45°)^2$$

$$= 200 \text{ Å}^2$$



3. Other Figures and Tables

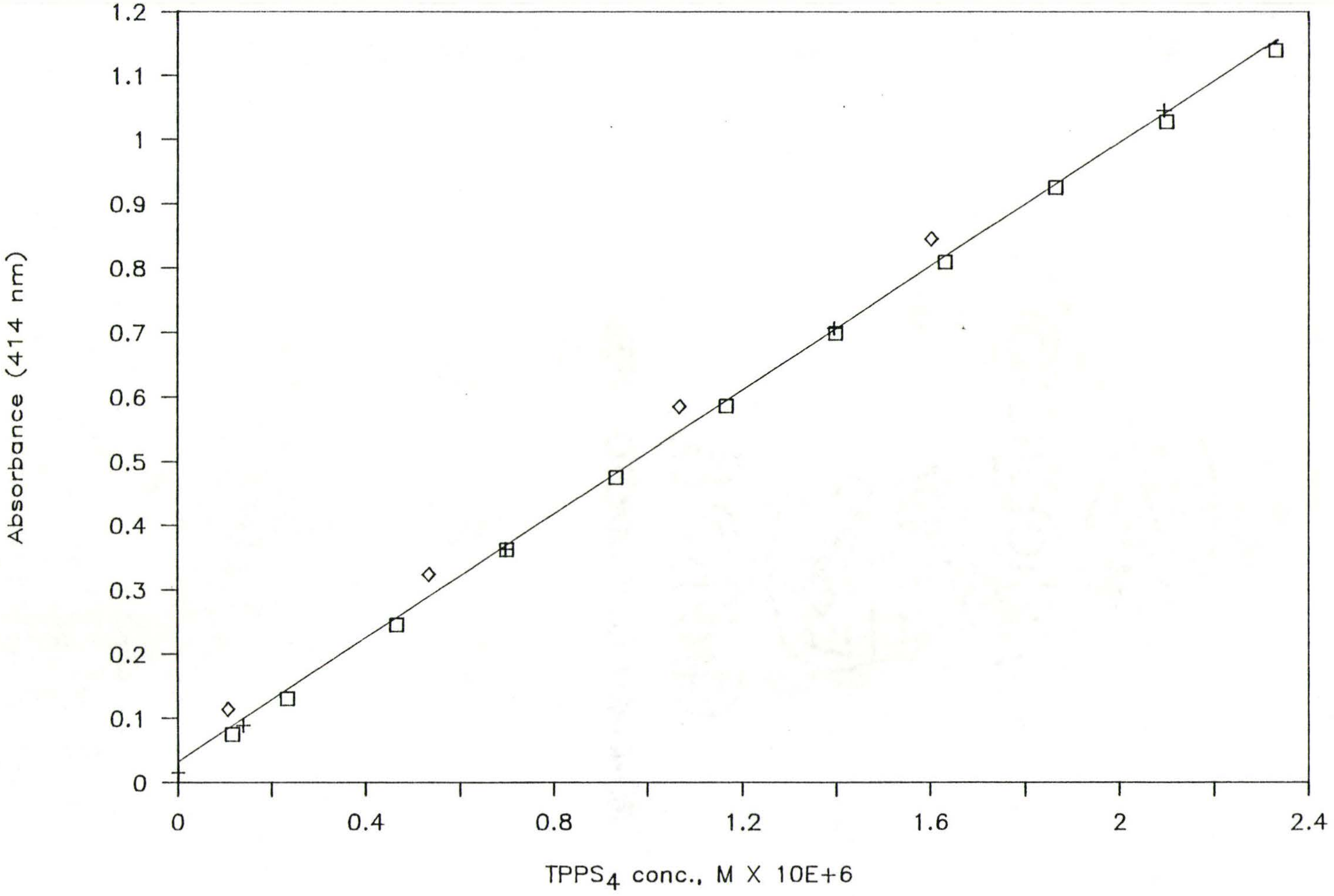


Figure 35. Calibration curve of TPPS$_4$ in pH 10.2 buffer using "new" 1 cm quartz cells. □ = set 1, + = set 2, ◇ = set 3.

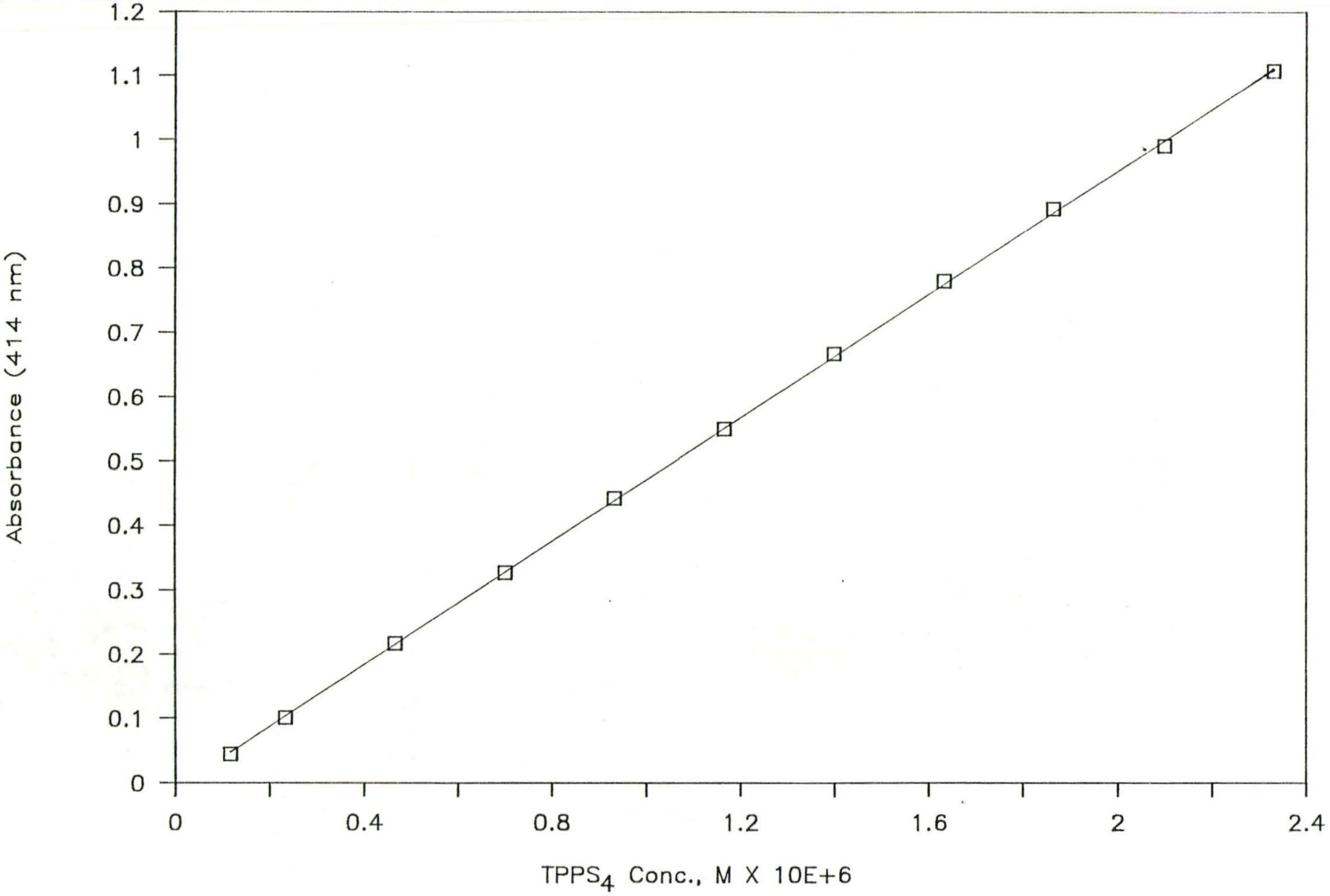


Figure 36. Calibration curve of TPPS$_4$ in pH 10.2 buffer using "old" 1 cm quartz cells.

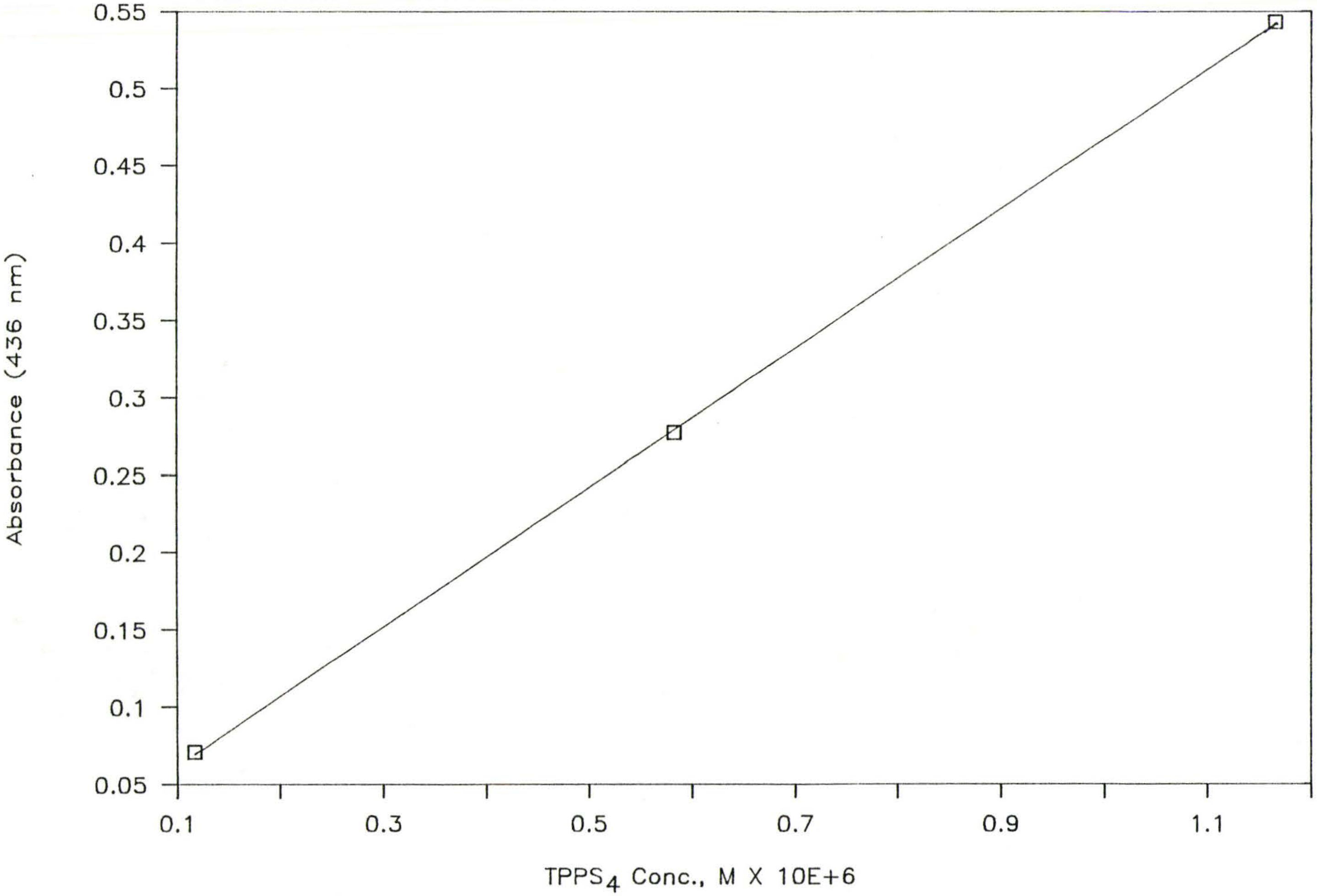


Figure 37. Calibration curve of $TPPS_4$ in 1% v/v HNO_3 using "new" 1 cm quartz cells.

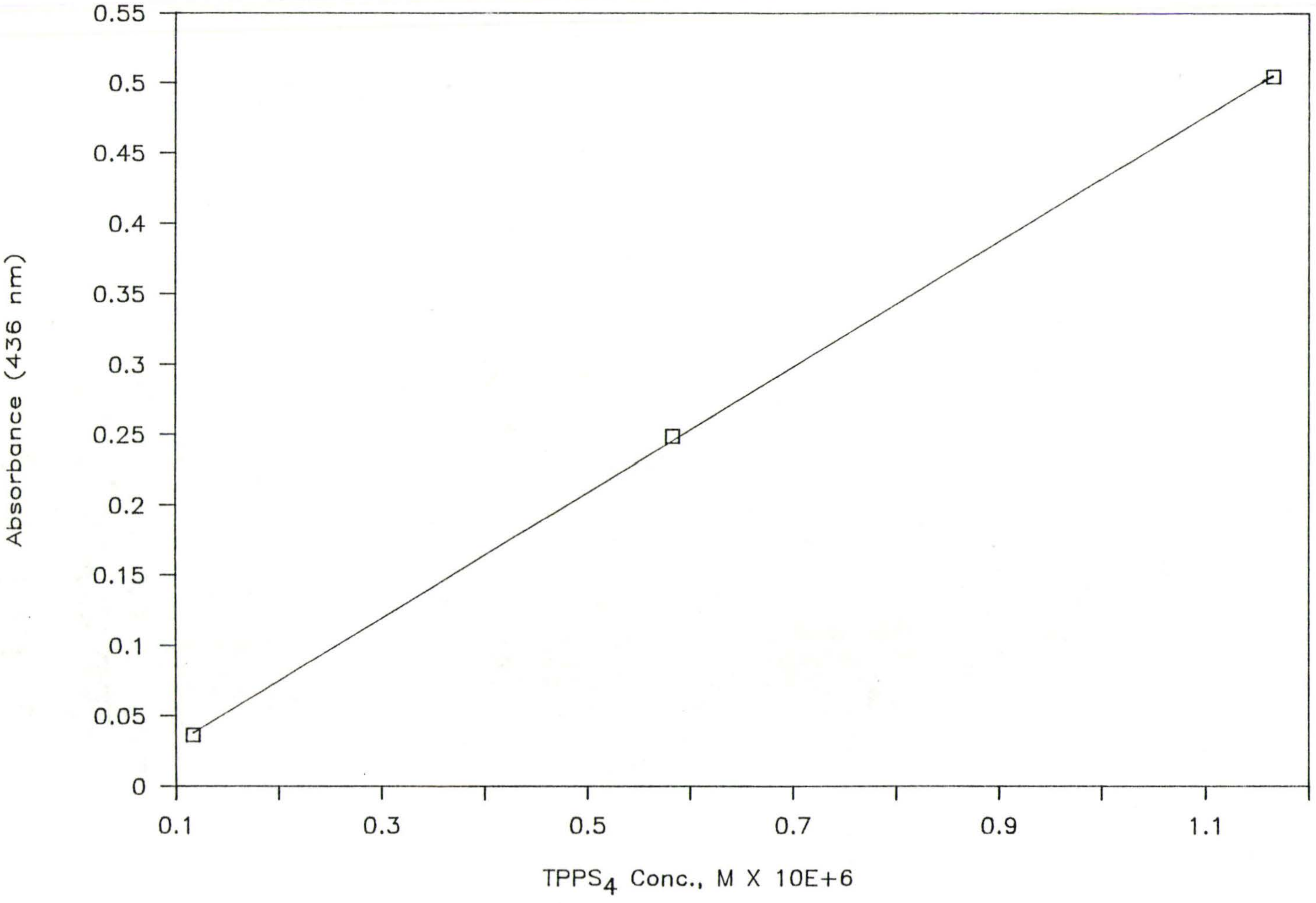


Figure 38. Calibration curve of TPPS$_4$ in 1% v/v HNO$_3$ using "old" 1 cm quartz cells.

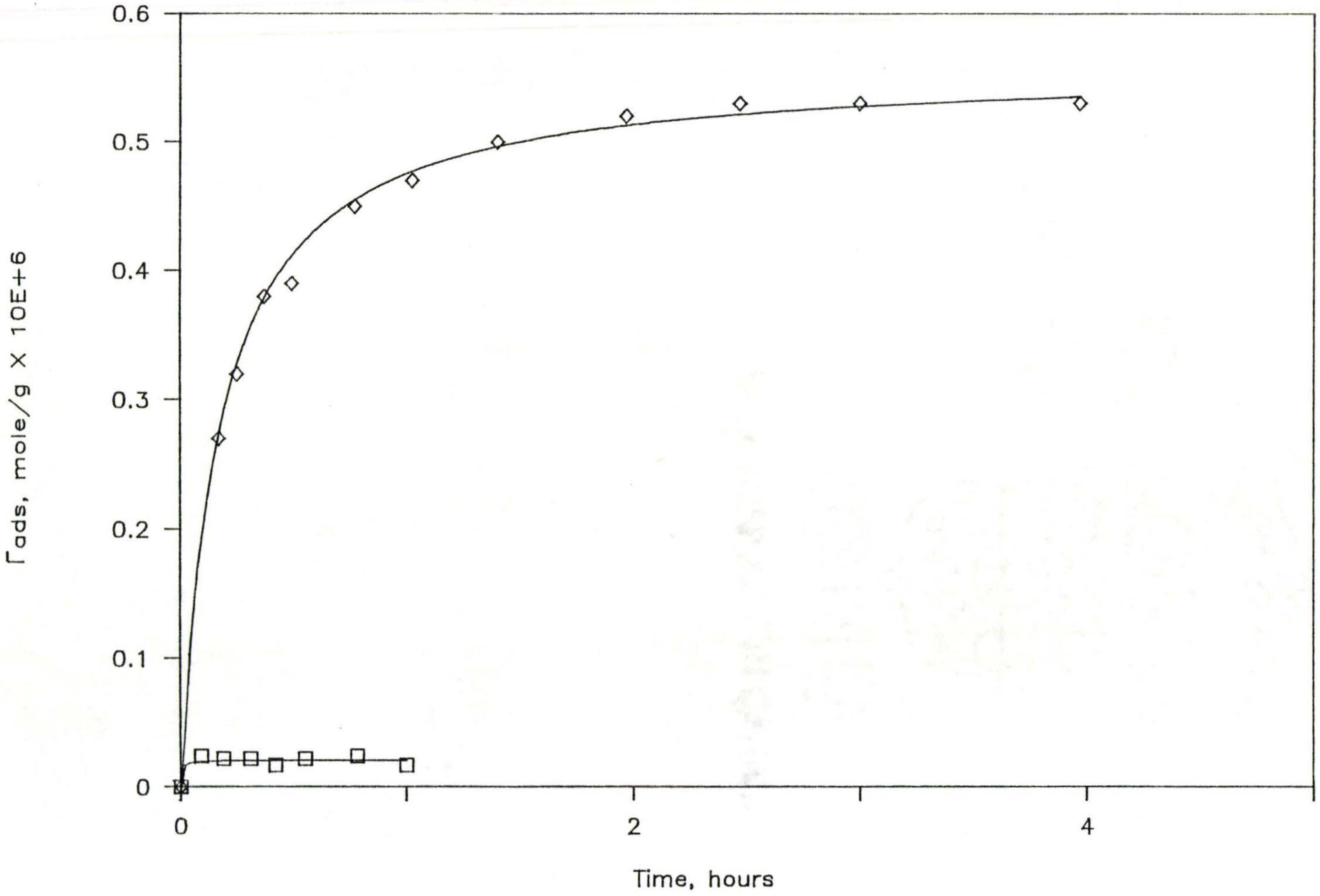


Figure 39. Rate of adsorption of $TPPS_4$ (□ = 1.18 X 10^{-6} M, ◇ = 1.18 X 10^{-5} M) on XAD-2 (0.5 g, 80-100 mesh) at pH 10.2 and room temperature.

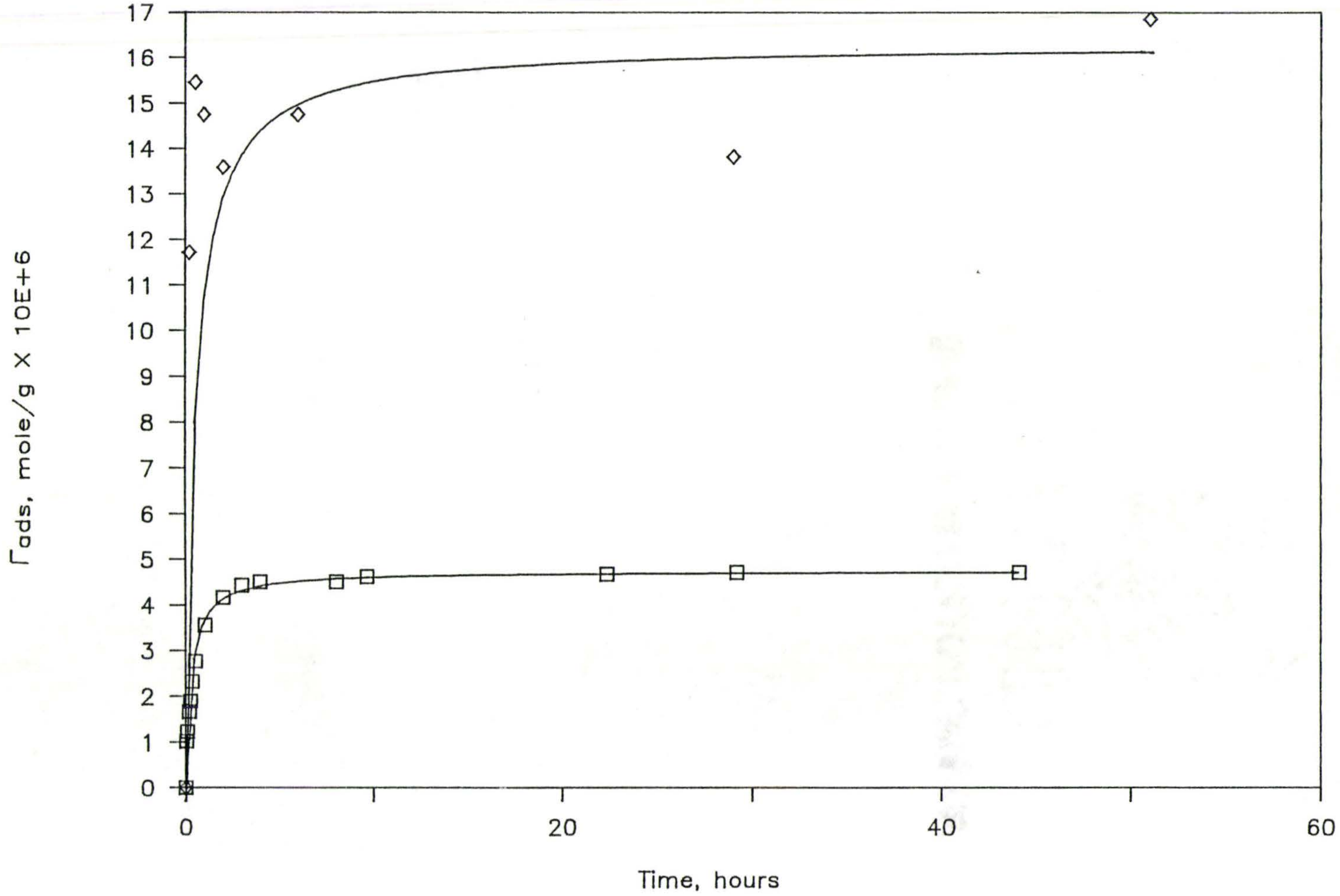


Figure 40. Rate of adsorption of TPPS$_4$ (□ = 1.18 X 10^{-4} M, ◇ = 2.80 X 10^{-3} M) on XAD-2 (0.5 g, 80-100 mesh) at pH 10.2 and room temperature.

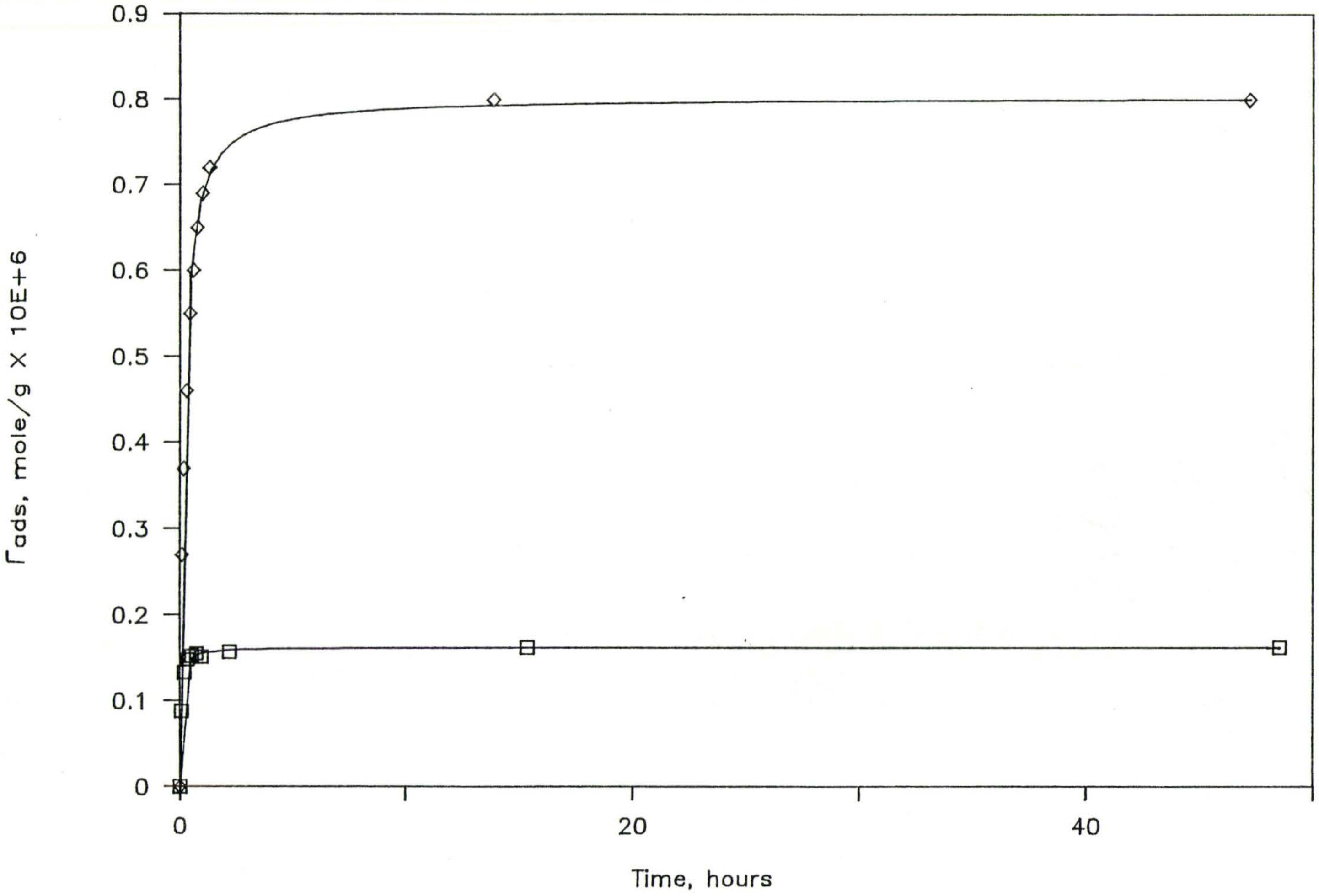


Figure 41. Rate of adsorption of TPPS$_4$ (□ = 3.06 X 10^{-6} M, ◇ = 1.49 X 10^{-5} M) on XAD-4 (0.5 g, 80-100 mesh) at pH 10.2 and room temperature.

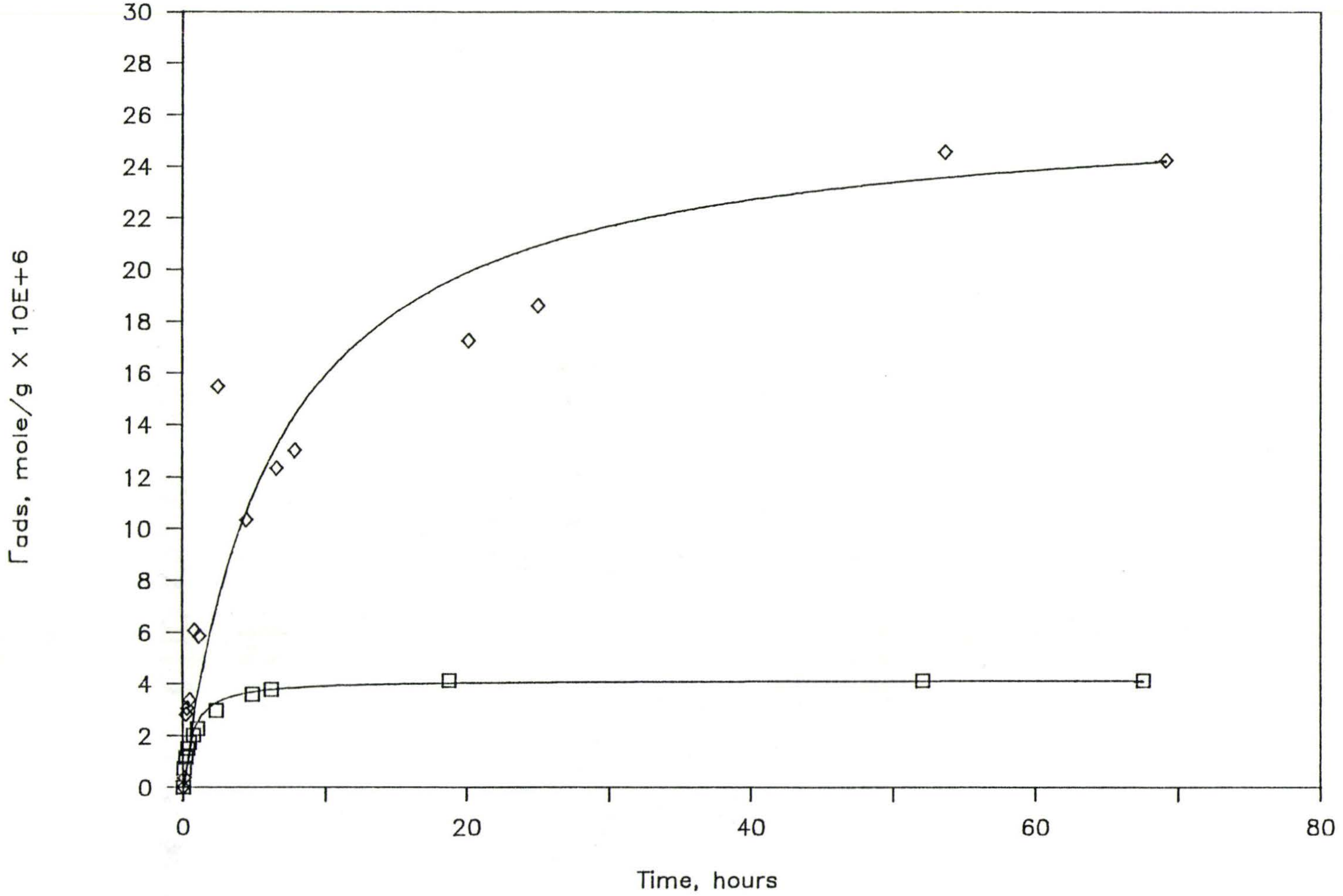


Figure 42. Rate of adsorption of $TPPS_4$ (□ = 7.68×10^{-5} M, ◇ = 7.46×10^{-4} M) on XAD-4 (0.5 g, 80-100 mesh) at pH 10.2 and room temperature.

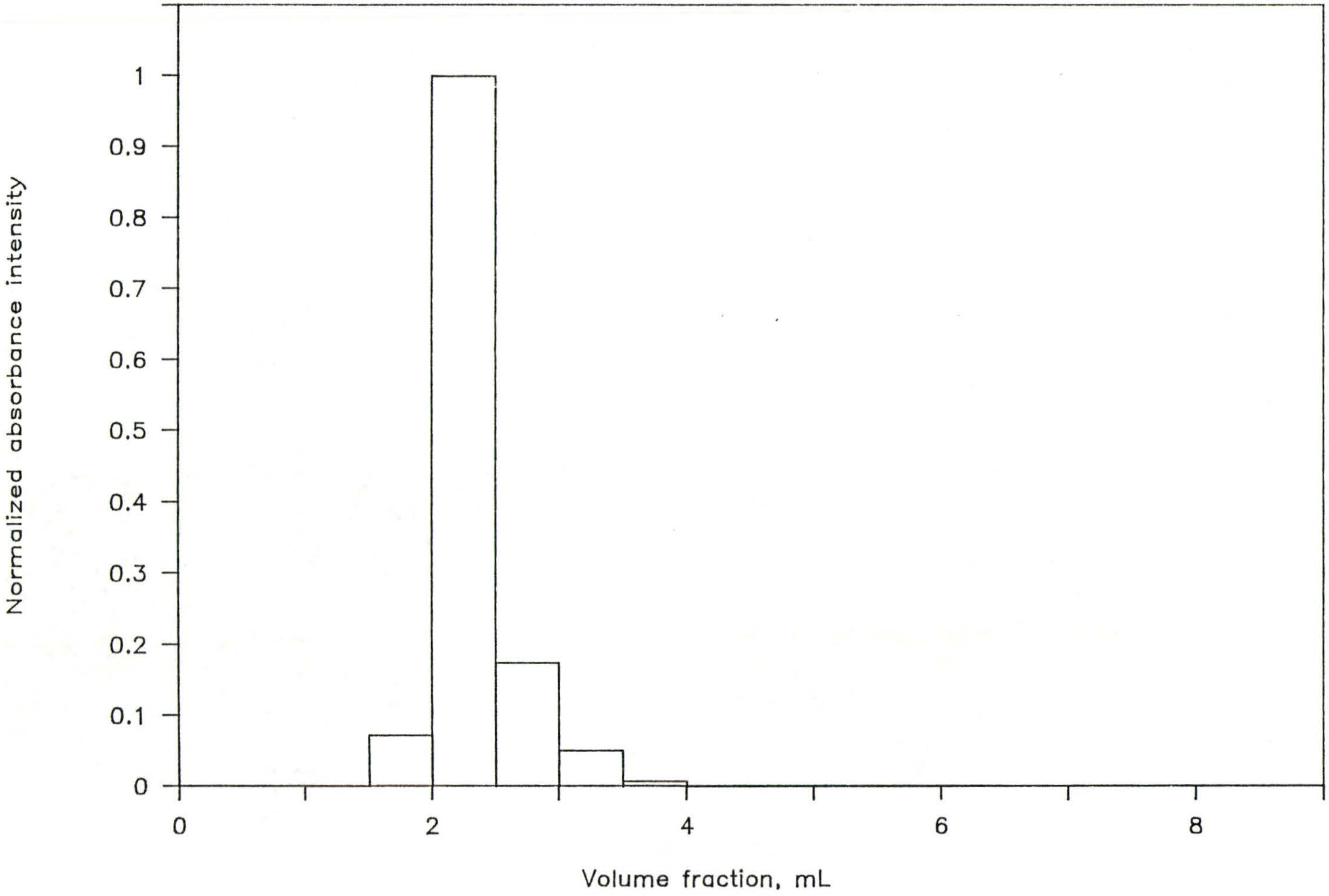


Figure 43. Elution of Cu-TPPS$_4$ from 1 X 3-cm XAD-7 column (80-100 mesh) by 90% v/v methanol. Mass of Cu(II) on column = 0.4 μg and flow rate = 1 mL/min.

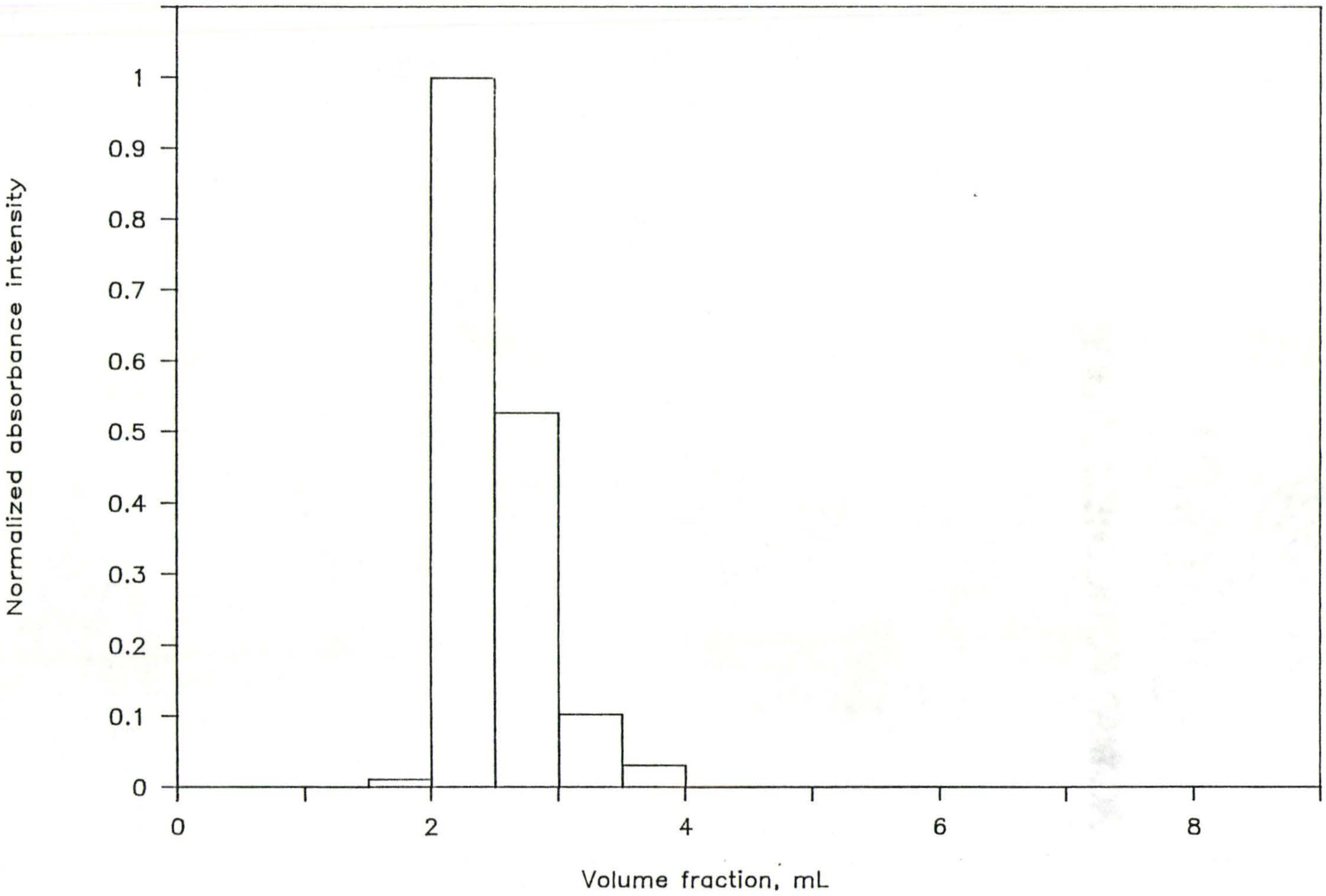


Figure 44. Elution of Cu-TPPS$_4$ from 1 X 3-cm XAD-7 column (80-100 mesh) by 85% v/v methanol. Mass of Cu(II) on column = 0.4 μg and flow rate = 1 mL/min.

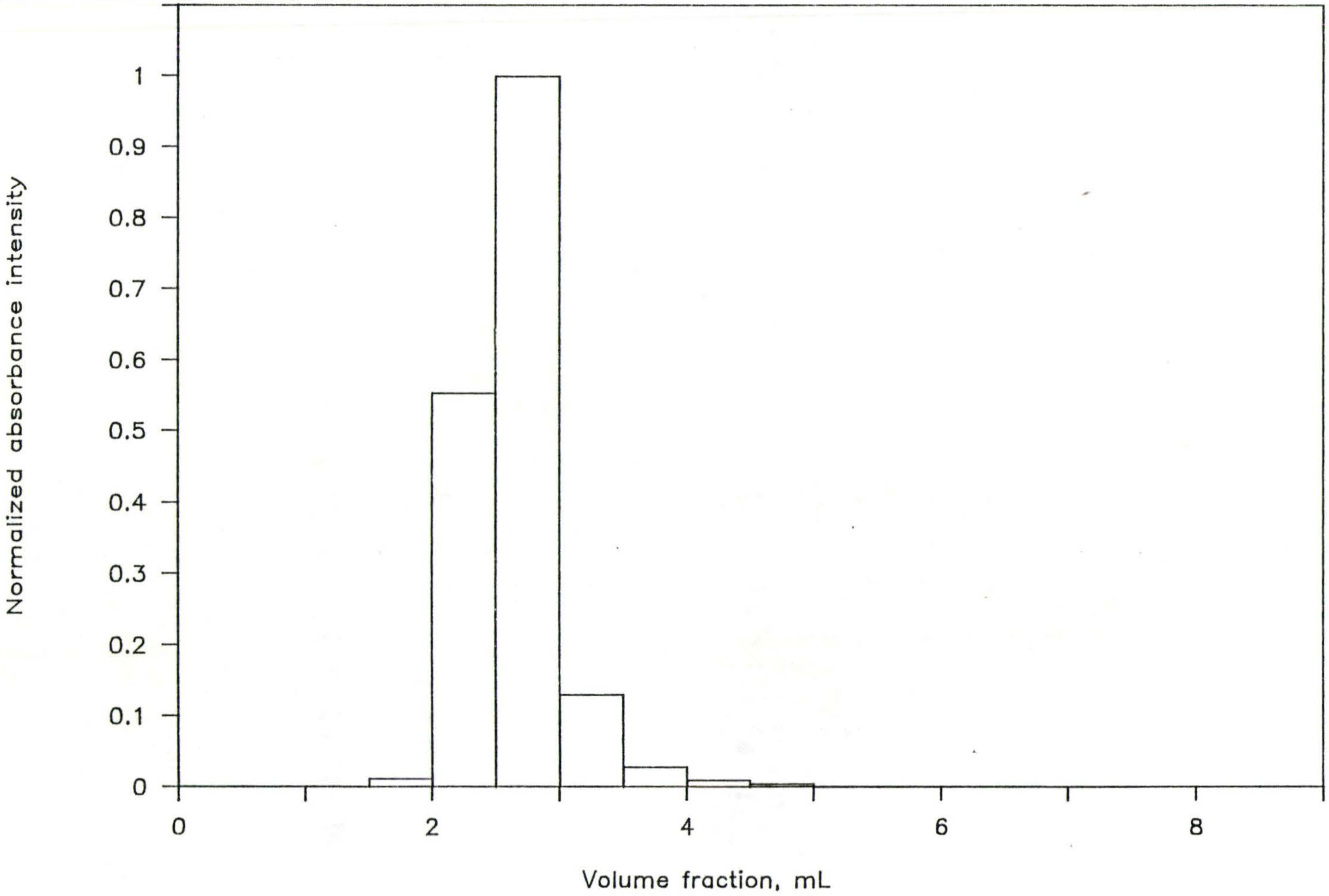


Figure 45. Elution of Cu-$TPPS_4$ from 1 X 3-cm XAD-7 column (80-100 mesh) by 75% v/v methanol. Mass of Cu(II) on column = 0.4 μg and flow rate = 1 mL/min.

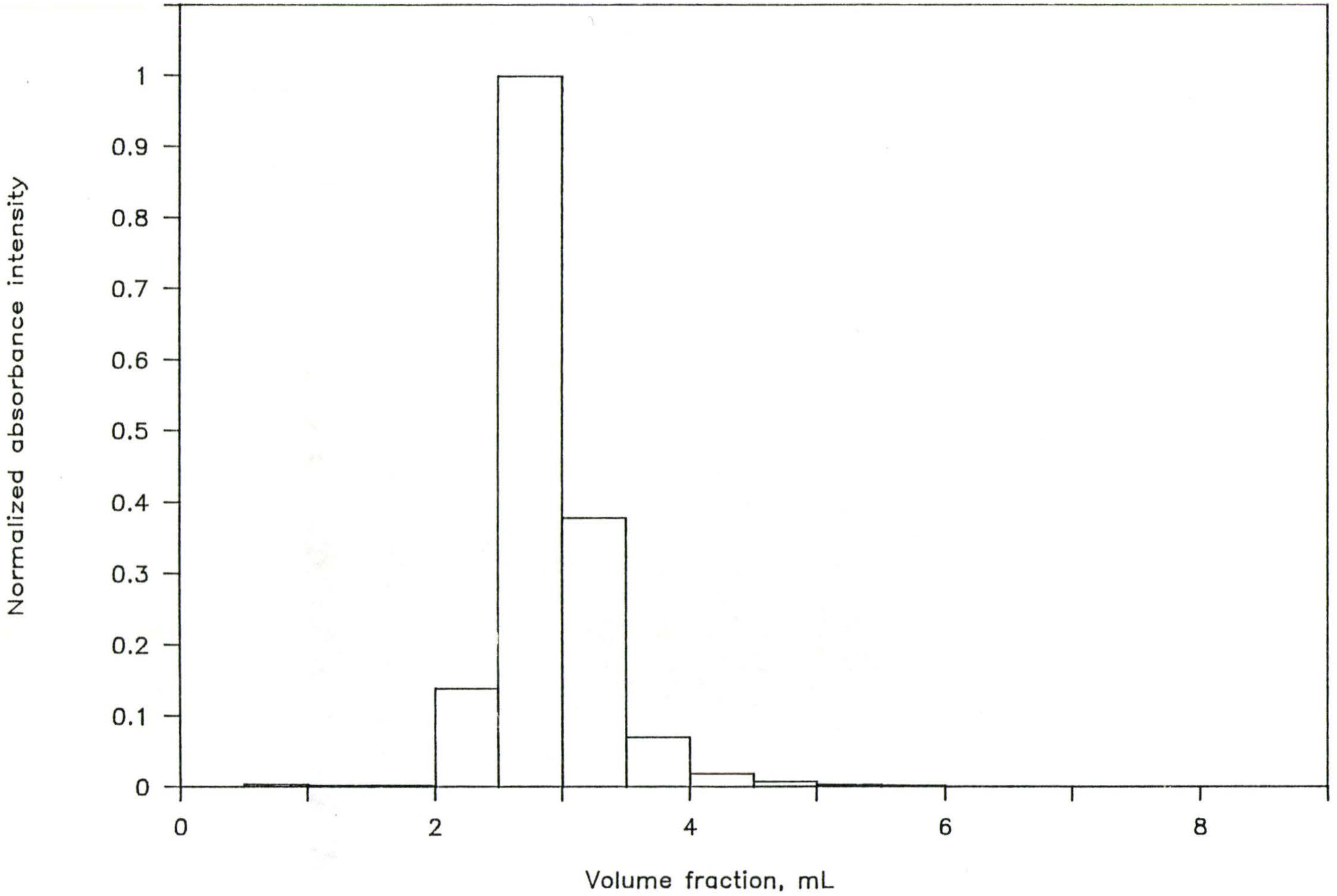


Figure 46. Elution of Cu-$TPPS_4$ from 1 X 3-cm XAD-7 column (80-100 mesh) by 70% v/v methanol. Mass of Cu(II) on column = 0.4 μg and flow rate = 1 mL/min.

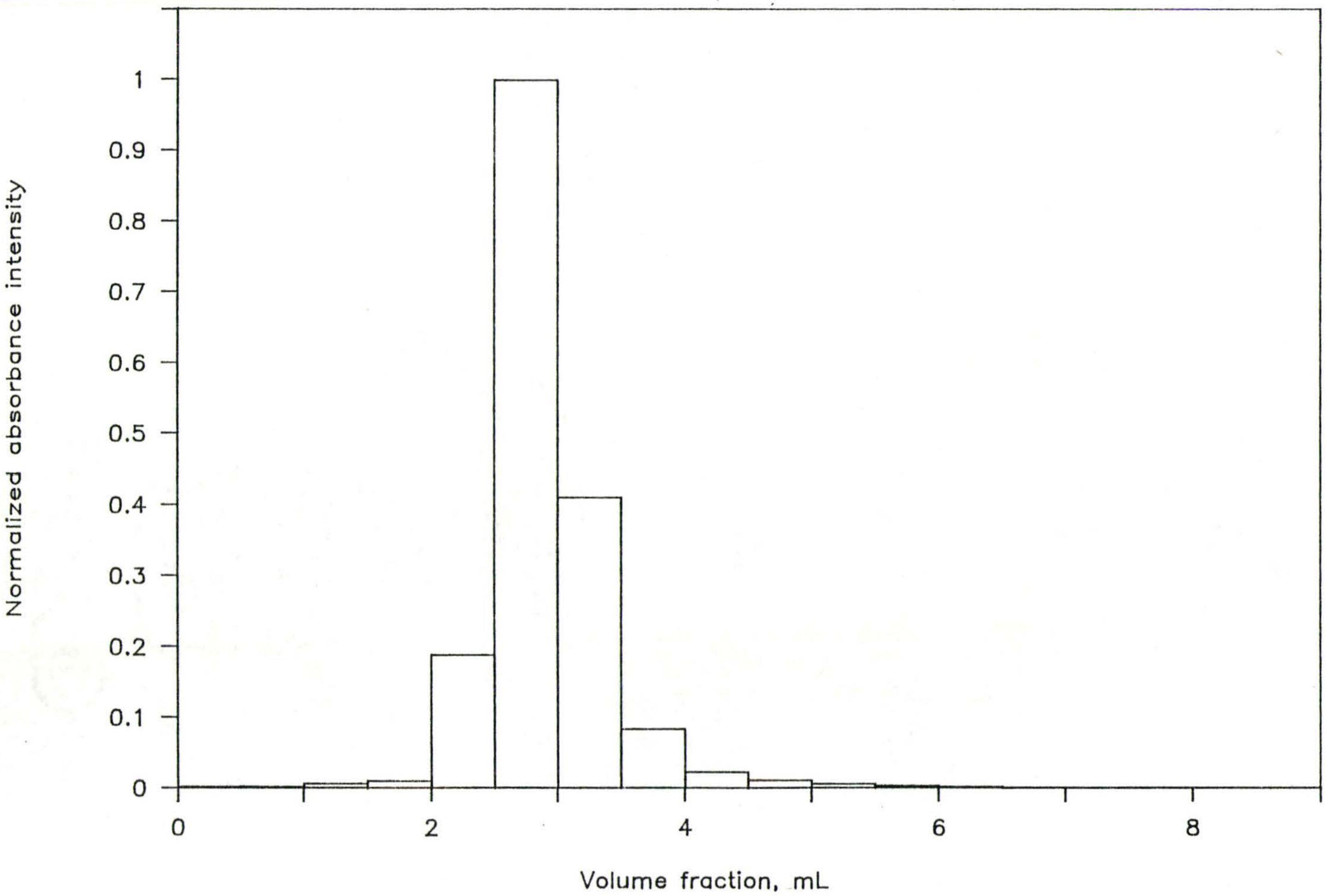


Figure 47. Elution of Cu-$TPPS_4$ from 1 X 3-cm XAD-7 column (80-100 mesh) by 60% v/v methanol. Mass of Cu(II) on column = 0.4 μg and flow rate = 1 mL/min.

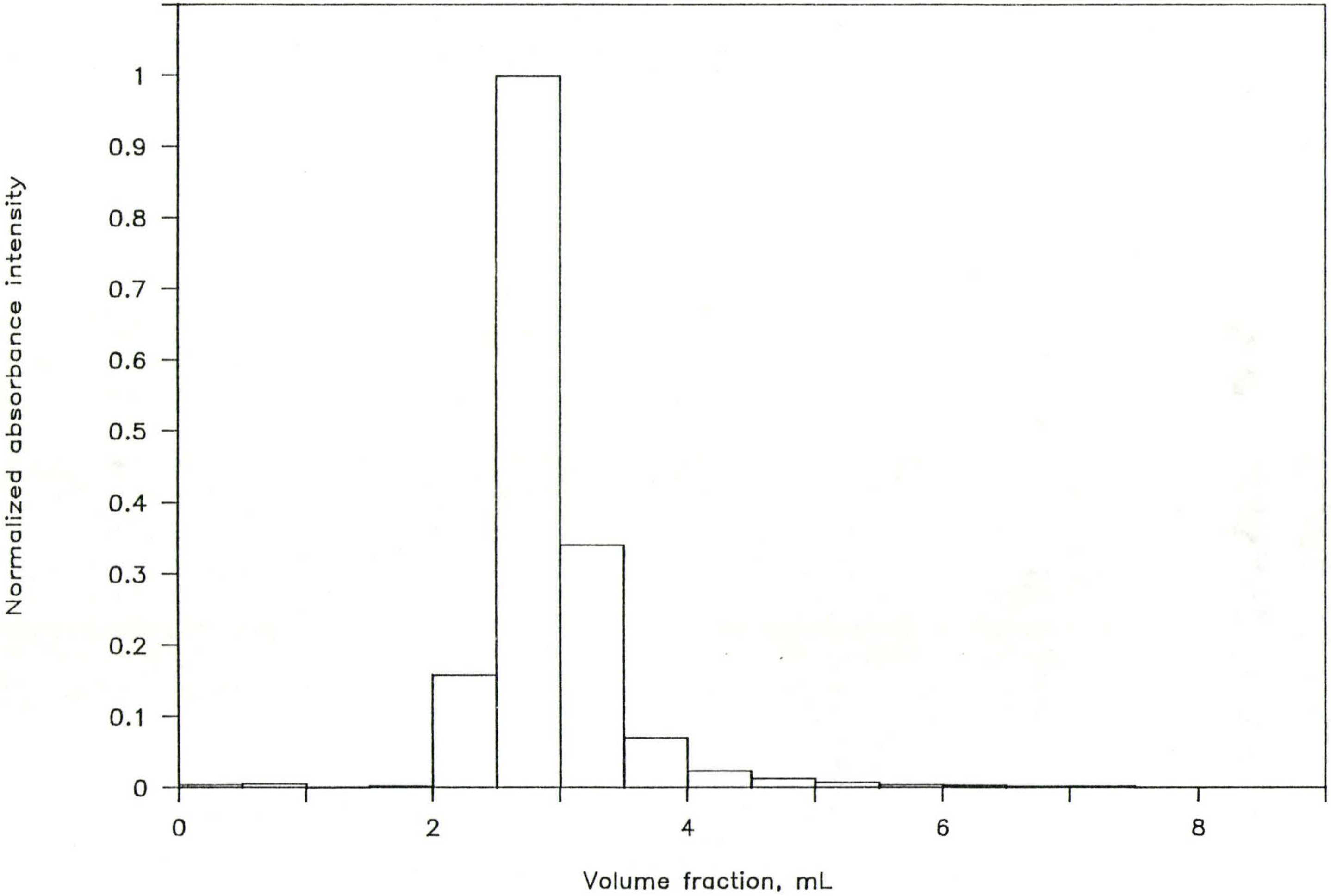


Figure 48. Elution of Cu-$TPPS_4$ from 1 X 3-cm XAD-7 column (80-100 mesh) by 50% v/v methanol. Mass of Cu(II) on column = 0.4 μg and flow rate = 1 mL/min.

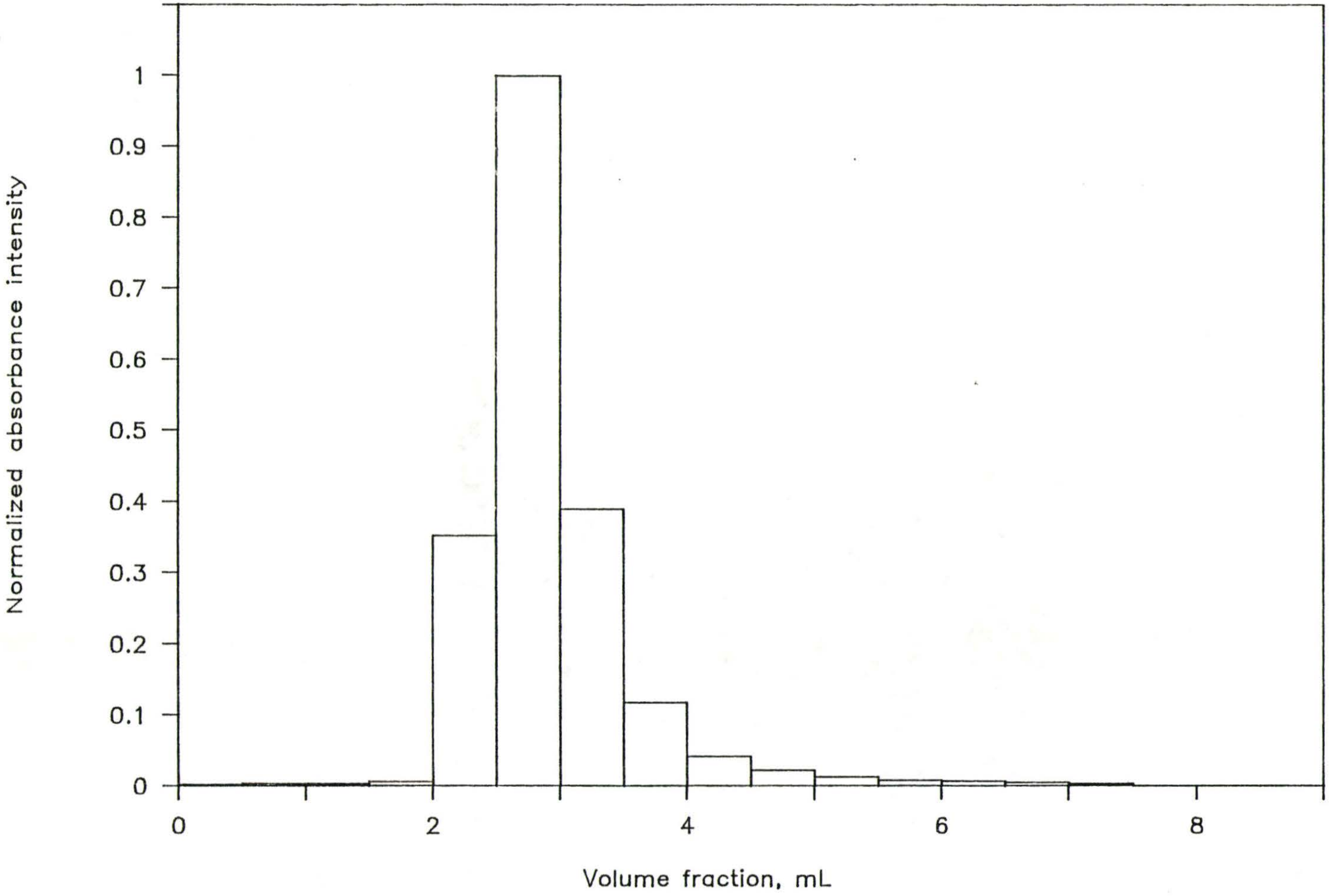


Figure 49. Elution of Cu-$TPPS_4$ from 1 X 3-cm XAD-7 column (80-100 mesh) by 40% v/v methanol. Mass of Cu(II) on column = 0.4 μg and flow rate = 1 mL/min.

Table 28. Analysis of Lake Ontario Water, CCIW Station 302 (72)

	Concentration ($\mu g\ L^{-1}$)*		
		XAD-7 Two-Column Method***	
Element	Chelex-100**	Calibration Curve	SAM
Cd	0.031 ± 0.002	0.038 ± 0.004	0.047 ± 0.010
Cu	2.17 ± 0.41	1.95 ± 0.07	1.41 ± 0.22
Mn	0.72 ± 0.04	0.125 ± 0.011	0.80 ± 0.02
Ni	1.38 ± 0.11	1.47 ± 0.26	1.71 ± 0.06
Pb	0.41 ± 0.04	0.64 ± 0.18	0.78 ± 0.09

* Means and standard deviations are based on triplicate determinations.

** A 1 X 6 cm (80-100 mesh, NH_4^+ form) Chelex-100 column and 200 mL of sample were used. 0.01 M NH_4OAc at pH 5.4 was used as the buffer electrolyte. Metal ions were eluted with 25.0 mL of 14% v/v HNO_3 (preconcentration factor 8.0). Flow rates = 1 mL min^{-1}.

*** The precolumn and analytical columns were 1 X 3 cm (80-100 mesh) and 200 mL of sample were used. 0.01 M NH_4Cl at pH 8.0 was used as the buffer electrolyte. Metal ions were eluted with 5.0 mL 1% v/v HNO_3 (preconcentration factor 40.0). Flow rates = 1 mL min^{-1}.

Table 29. Analysis of Lake Ontario Water, CCIW Station 41 (72)

	Concentration ($\mu g\ L^{-1}$)*		
		XAD-7 Two-Column Method***	
Element	Chelex-100**	Calibration Curve	SAM
Cd	0.026 ± 0.002	0.033 ± 0.011	0.032 ± 0.017
Cu	6.67 ± 0.66	6.75 ± 0.18	6.39 ± 0.23
Mn	1.13 ± 0.03	0.148 ± 0.028	1.07 ± 0.02
Ni	2.32 ± 0.32	2.30 ± 0.18	1.83 ± 0.22
Pb	0.28 ± 0.04	0.38 ± 0.06	0.34 ± 0.01

* Means and standard deviations are based on triplicate determinations.

** A 1 X 6 cm (80-100 mesh, NH_4^+ form) Chelex-100 column and 200 mL of sample were used. 0.01 M NH_4OAc at pH 5.4 was used as the buffer electrolyte. Metal ions were eluted with 25.0 mL of 14% v/v HNO_3 (preconcentration factor 8.0). Flow rates = 1 mL min^{-1}.

*** The precolumn and analytical columns were 1 X 3 cm (80-100 mesh) and 200 mL of sample were used. 0.01 M NH_4Cl at pH 8.0 was used as the buffer electrolyte. Metal ions were eluted with 5.0 mL 1% v/v HNO_3 (preconcentration factor 40.0). Flow rates = 1 mL min^{-1}.

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