SILICA-IMMOBILIZED 8-QUINOLINOL: SYNTHESIS, CHARACTERIZATION, AND APPLICATIONS IN FLOW SYSTEMS

By

MONTE ALTON MARSHALL

Bachelor of Science
Southeastern Oklahoma State University
Durant, Oklahoma
1978

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
July, 1984

SILICA-IMMOBILIZED 8-QUINOLINOL: SYNTHESIS,
CHARACTERIZATION, AND APPLICATIONS
IN FLOW SYSTEMS

Thesis Approved:

Horacio A Mottola
Thesis Adviser

Linda B. McGown

E J Eisenbraun

J P Chandler

Norman N Durham
Dean of the Graduate College

PREFACE

This thesis is divided into four parts for convenience and clarity. The first section (Chapter II), being historical in nature, covers the pertinent literature in the area of silica-immobilized chelating agents. The three following chapters (Chapters III, IV, and V) are concerned with synthesis, characterization, and analytical applications of silica-immobilized 8-quinolinol, respectively. Chapter I is simply an introduction while Chapter VI draws some conclusions. Each chapter dealing with experimental work contains its own experimental section. This makes the thesis more fluid and results in easily located experimental detail for those reading the thesis.

I should mention that during the first year and a half of this work, a number of synthetic approaches were examined, none of which were successful. I was somewhat discouraged at that point when, Dr. Mottola, my adviser, told me something I will always remember when research is not going so well. He said, "Research is failure, failure, failure, failure,..., success,..., failure, failure, failure, failure,...success." Whenever I was having problems with an experiment, I always thought about the success that would come after only a few more failures and that gave me the inspiration to continue.

I wish to express my appreciation to all of those who assisted me during this work. I am particularly grateful to my adviser, Dr. Mottola, not only for the inspirational quotation attributed to him above, but also for his patience, understanding, and especially for his guidance

and knowledge in the field of analytical chemistry. I am also indebted to the other members of my committee, both past and present, Dr. E. J. Eisenbraun, Dr. J. P. Chandler, Dr. L. B. McGown, and Dr. H. L. Gearhart.

Financial support of the Oklahoma State University Water Research Institute and the Chemistry Department is acknowledged as well as summer scholarships from Conoco, Inc. in 1982 and 1983.

Thanks also go to all of the past and present members of the research group who provided friendship, companionship, solace, comfort, intellectual stimulation, and innovative ideas. I am extremely grateful and I will remember each of you always. Design and construction of equipment by Mr. Heinz Hall is appreciated as well.

I would like to thank Dr. J. L. Robinson for allowing me to become acquainted with, and inspiring me in, the field of analytical chemistry through a summer job (that turned into a year) at Southeastern Oklahoma State University in Durant. Finally, I want to express my gratitude to my mother, Christine, without whom I probably would have never even made it through college and to my late father, Clarence, who placed such a high value on an education. I am forever indebted.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The attachment of functional groups to the surface of solid supports in order to enhance interaction between the immobile (stationary) phase and a solute in the solution (mobile) phase is the basis of a number of analytical techniques. For example, such common techniques as reverse-phase chromatography, immobilized enzyme reactors, and ion-exchange processes rely on the interaction between solutes and immobilized functional groups. Immobilized chelating agents, capable of interacting with many metal ions in solution, have become of particular interest in the past ten years. Much of the technology developed for the attachment of hydrocarbonaceous and other types of stationary phases in reverse-phase chromatography has proven applicable to the immobilization of chelating agents on similar supports (1). As expected, the major application of these immobilized chelating agents lies in the area of preconcentration and/or separation of ionic species in solution, particularly metal ions. However, other applications are possible as will be shown later.

Immobilized chelating agents, which are known as chelating ion exchangers (although an actual exchange of ions may not take place), offer several attractive advantages over conventional ion exchange materials in the above applications. The most important advantage is the fact that chelating ion exchangers exhibit much greater selectivity than conventional, non-chelating ion exchangers. The greater selectivity of chelating ion exchangers can be explained on the basis of the different processes involved in the exchange and chelation processes. The exchange process occurring on conventional ion exchangers is based on a purely electrostatic, diffusion-controlled exchange of ions. The ion that is favored by the ion exchanger depends primarily on the relative charges and sizes of the competing ions (2). On the other hand, chelating ion exchange, which also is usually a diffusion controlled process, differs in that chelation involves a chemical reaction which leads to the formation of covalent bonds. The ion that is favored by a chelating ion exchanger depends primarily on the relative values of the overall formation constants (β_n):

Overall formation constant (β_n)

$$\beta_n = \prod_{i=1}^{n} K_{f_i} \tag{1}$$

Stepwise formation constant (K_f)

$$K_{f_n} = \frac{[ML_n]}{[ML_{n-1}][L]} \tag{2}$$

The value of the overall formation constant in turn is affected by the electronic configuration of the metal ion and by the Lewis base character and polarizability of the ligand (3). Formation constants can vary over a wide range. For example, the overall formation constants of 8-quinolinol metal complexes vary from about 10^2 for Ba^{2+} to about 10^{38} for Fe^{3+} (4). Therefore, it is observed that conventional ion exchangers exhibit little selectivity whereas chelating ion exchangers can show considerable selectivity depending on the relative values of the formation constants of the complexes formed.

The enhanced selectivity observed for chelating ion exchangers is a very important characteristic of these materials. This is particularly true in the area of preconcentration of trace metal ions in high ionic strength aqueous solutions which is the most common application of these materials. For example, the preconcentration of trace metal ions in seawater is essentially impossible on conventional cation exchangers because the relatively large amounts of alkali and alkaline earth metal ions quickly saturate the exchange sites. On the other hand, chelating ion exchangers, which usually show little affinity for alkali and alkaline earth metal ions, are well suited for this application and, generally, preconcentration is readily achieved (5). It should be pointed out that the chelation reaction involved is normally also strongly influenced by pH.

For the reaction:

$$M + L \rightleftharpoons ML \ , \tag{3}$$

$$\beta_1 = K_{f_1} = \frac{[ML]}{[M][L]} \tag{4}$$

But, in general, L can also interact with protons since L is normally a Bronsted base so that:

$$H + L \rightleftharpoons HL \tag{5}$$

and therefore,

$$C_L = [HL] + [L] \tag{6}$$

We define:

$$\alpha_1 = \frac{[L]}{C_L} = \frac{Ka_1}{[H] + Ka_1} \qquad (7)$$

which upon rearrangement becomes:

$$[L] = \alpha_1 C_L \qquad (8)$$

Therefore, we have:

$$K_{f_1} = \frac{[ML]}{[M]\ \alpha_1\ C_L} \qquad (9)$$

and,

$$\alpha_1 K_{f_1} = \frac{[ML]}{[M]\ C_L} = K'_{f_1} = \text{Conditional Formation Constant} \qquad (10)$$

As pH decreases, $[H^+]$ increases, α_1 decreases, and therefore, K'_f decreases which indicates that fewer ligands are available for interaction with metal ions. Therefore, although a chelating ion exchanger may form complexes at high pH with some metal ion present as a major component, preconcentration of metal ions present at lower concentration (but with larger formation constants) may still be achieved. For example, alkaline earth metal ions present at relatively high concentration can usually be excluded from the chelating ion exchanger since the conditional formation constants of the complexes formed are often comparatively small (4). Preconcentration of trace metal ions present at ppb concentrations or less in water samples is necessary in order to successfully employ such common instrumental techniques as X-ray fluorescence spectroscopy (XRF), neutron activation analysis (NAA), or atomic absorption spectroscopy (AAS). These techniques are not always directly applicable to ppb or sub-ppb concentrations of metal ions and preconcentration is required in order to increase the concentration of metal ions above the limit of detection of the technique.

Although the use of chelating ion exchangers under chromatographic conditions has not been extensively explored, the possibility of using these materials for cation separations is obvious. Selectivity in conventional cation exchange chromatography of metal ions is generally achieved by adding a complexing agent to the mobile phase (6). However, for chelating ion exchangers, the differences in formation constants (which result in increased selectivity) are often large enough to allow separation by a pH gradient alone. Complexing agents can be added to the mobile phase, however, in order to further enhance the selectivity (5).

Most chelating ion exchangers in the past have been attached to the surface of, or incorporated into, some type of organic polymeric support. Of these, the most important have been based on styrene-divinylbenzene (S-DVB) copolymers. However, other common organic polymer supports include phenol-formaldehyde condensation resins, cellulose, cellulose derivatives, and polymers prepared by polycondensation of monomeric ligands (7). There are several disadvantages of polymer-based chelating ion exchangers that should be considered.[1]

---

[1] A recent review covers the subject of organic polymer-based chelating agents (7).

1. These materials are not easily prepared by a novice. Unlike conventional ion exchange resins, few chelating ion exchange resins are commercially available. Synthetic procedures are generally tedious, time-consuming, and irreproducible.

2. Many organic polymer-based chelating resins suffer from a slow rate of metal ion uptake. Batch equilibration requires on the order of 30 minutes to several hours (8, 9). This severely restricts flow rates in preconcentration procedures. Flow rates in small columns are generally restricted to 3-5 mL/min for quantitative recovery. These flow rates are not practical when it is necessary to pass several liters of solution through the column. The use of macroreticular resins such as the XAD resins has improved the metal ion exchange kinetics of these materials. However, the immobilization procedures are still rather difficult (10).

3. Swelling and shrinking of organic polymers as the composition of the solution changes can affect flow rate and pressure drop adversely in both preconcentration and separation applications. Swelling is particularly troublesome as it can increase the pressure drop beyond the pressure limit of the pump used.

4. The lack of mechanical stability at high pressures precludes the use of most polymer-based chelating agents in chromatographic systems where high pressures are often required. Many organic polymers are not rigid enough to withstand the high pressures encountered.

5. Cellulose and cellulose derivatives have the additional problem of degradation due to microbial action.

It is apparent that an alternative to organic polymer supports is desirable in order to alleviate as many of the above problems as possible.

Silica[2] has been the most widely studied alternative support for the immobilization of chelating agents. The reasons for the choice of silica as an immobilization support include:

1. The availability of abundant data concerning the properties of silica due to the popularity of silica as a stationary phase in adsorption chromatography.

2. The commercial availability of silica in a wide variety of particle sizes, surface areas, and porosities.

3. The surface chemistry of silica is relatively well understood and reactions occurring at the silica surface have been studied extensively due to the explosive growth of bonded phase chromatography. In particular, techniques developed for covalent modification of the silica surface in reverse phase high performance liquid chromatography (HPLC) have been adapted for the immobilization of chelating agents.

4. Immobilization procedures are relatively simple. Basic laboratory equipment is sufficient and an extensive knowledge of organic synthetic procedures is usually unnecessary.

5. Silica has good mechanical stability even at the high pressures often encountered when small diameter particles are used in HPLC. Silica is also less susceptible than organic polymers to shrinking and swelling associated with solution composition changes although silica is limited to use at $pH < 10$ due to hydrolysis of the silica framework in basic media.

---

[2] "Silica" is used as a short, convenient designation for "silicon dioxide" in all its crystalline, amorphous, and hydrated or hydroxylated forms (11).

Silica, then, offers a viable alternative to organic polymer-based chelating agents that eliminates many of the difficulties associated with the preparation and use of the latter materials. One of the primary disadvantages of silica has been the relatively low exchange capacity of many silica-based chelating agents compared to organic supports. This generally limits their use, at present, to trace levels of metal ions although some of the silica-based chelating agents have capacities approaching those of conventional ion exchangers and organic-based chelating exchangers. New and optimized synthetic procedures, however, can increase the exchange capacity considerably.

Most commercial silica is available as silica gel which is "a coherent, rigid, three dimensional network of contiguous particles of colloidal silica" (11). Silica gel is usually formed by coagulation and drying of colloidal silica which is formed by the polymerization of silicic acid. Porous glass, another popular support, is essentially a special form of silica gel. It is formed by phase separation of a homogeneous borosilicate glass followed by dissolution of the boron-rich glass phase by strong acid leaving a silica-rich highly porous glass.

The initial chemical reaction involved in the immobilization of functional groups on silica by covalent attachment relies on the reaction of surface silanol groups (Figure 1). The fully hydroxylated silica surface contains about 8 μmoles of silanol groups per m^2 of surface. Only about 4.5 μmoles/m^2 of the silanol groups can be reacted, however, due to steric considerations (12). The remainder of the silanol groups are shielded by the reacted groups.

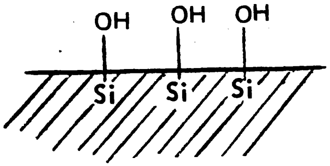


Figure 1. Surface Silanol Groups

Of the reactions used to attach functional groups to the silica surface, the most useful are based on the silylation reaction. Reaction of organo-functional chloro or alkoxy silanes with surface silanol groups results in covalent attachment to silica via siloxane bond formation (i.e., Si-OSi). Silylation reactions are generally superior to other possible surface reactions because of the much greater hydrolytic stability of the Si-OSi linkage relative to Si-OR, Si-C, or Si-N bonds. Silylated surfaces are hydrolytically stable in the pH range of about 1 to 10 and are therefore suitable for use under pH gradient conditions within this range.

Two types of approaches have been taken for the immobilization of chelating agents on silica. The first, and simplest, is the incorporation of a functional group capable of chelation into the silane used for the silylation reaction itself. The most common type of chelating ligand immobilized in this manner is ethylenediamine. The other more common approach to chelating group attachment on silica is the heterogeneous modification of some suitable immobilized silane by using known organic synthetic transformations. Most chelating agents immobilized on silica are prepared in this manner.

The material presented up to this point is primarily meant as a short introduction to the basic concepts and rationale behind the preparation and use of silica-immobilized chelating agents. The work described in this thesis describes the preparation of silica-immobilized 8-quinolinol, also known as 8-hydroxyquinoline or oxine (HOx), and its use as a preconcentrating medium for trace transition metal ions. The aims of this work include the development of a new, time saving synthetic route to silica-immobilized HOx, characterization of the material in order to systematically choose the correct analytical conditions for a given application, and the use of the material in unsegmented continuous flow analysis (commonly called flow injection analysis (FIA)) for on-line preconcentration and sample handling using atomic absorption spectroscopy (AAS) as the detection device. The studies undertaken can be grouped as follows:

1. Synthetic routes to silica-immobilized HOx, including the evaluation of several routes to silica-immobilized HOx in an effort to devise an improved synthetic procedure with the aim of improving capacities and saving time (Chapter III).

2. Carrier optimization in which the properties of the support such as pore size and specific surface area were shown to have a marked effect on capacity. This work is included as a part of Chapter IV which discusses characterization.

3. Studies in which the properties of silica-immobilized HOx were evaluated. Characteristics such as stability, metal ion extraction behavior, and acid-base characteristics were determined and comparisons to free 8-quinolinol in solution were made (Chapter IV).

4. The use of silica-immobilized HOx under flow conditions, particularly its use in unsegmented continuous flow analysis combined with preconcentration and atomic absorption spectroscopy for the determination of trace amounts of metals ions in water (Chpater V).

A review of synthetic aspects, characterization studies, and analytical applications of silica-immobilized chelating agents is presented in Chapter II.

CHAPTER II

A LITERATURE REVIEW OF ANALYTICAL AND SYNTHETIC ASPECTS OF SILICA-IMMOBILIZED CHELATING AGENTS

This chapter is concerned with the more important contributions made in the preparation, characterization, and use of silica-immobilized chelating agents up to the present time. As this review is restricted to chelating agents, no discussion of the use of monodentate ligands will be presented although there are numerous papers devoted to this subject. Therefore, only ligands capable of forming two or more bonds per molecule will be considered. Also, due to the stability enhancement afforded by covalent attachment, the review will be restricted to those chelating agents attached to the silica surface through actual covalent bond formation.

Although the number of chelating agents attached to silica matrices is limited, some of the more common chelating ligands such as the dithiocarbamates and ethylenediamine have been studied extensively and have found a host of applications. Many of the reported silica-immobilized chelating agents, however, have not been investigated in detail except with regard to the probable structure of the immobilized species, and, in some cases, the observed exchange capacities. The most common, and therefore most widely studied materials will be reviewed first, followed by a section on miscellaneous silica-bound chelating agents which have been less widely studied and used.

Ethylenediamine

Preparation

As mentioned previously, ethylenediamine is one of the few chelating agents that has been attached to the silica surface in one step by incorporating the diamine function into the silylating agent. The preparation and utilization of silica-bound ethylenediamine has largely been due to the work of Leyden and coworkers (13-20). However, the attachment of this functional group to the silica surface was first reported by Dow-Corning Corporation which first marketed the silane, N-2-aminoethyl-3-aminopropyltrimethoxysilane (AEAPTS) under the code Z-6020 (21). The silylation reaction leading to the immobilized diamine is depicted in Figure 2.

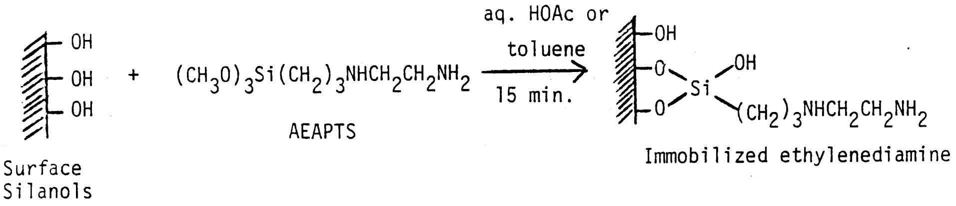


Figure 2. Synthesis of Silica-Immobilized Ethylenediamine

The reaction can be carried out in either dilute aqueous acid solution (usually acetic acid) or in nonaqueous solvents such as toluene or benzene (13). In aqueous solution, the alkoxy groups are rapidly hydrolyzed to silanol groups which then condense with the silanol groups on the silica surface. It is interesting to note that, unlike most alkoxysilanes, the aminoalkoxysilanes are generally very stable in aqueous solution and polymerization is normally slow in acidic media (22). Therefore, aminoalkoxysilanes deposited onto silica gel from dilute aqueous acid solution usually give approximately monolayer coverage (23). This is in contrast to most simple alkoxysilanes which polymerize rapidly to form a siloxane polymer on the silica surface which results in multilayer coverage (22). The stability of aminoalkoxysilanes also makes them easy and convenient to handle. Reaction in non-aqueous solution is thought to proceed via hydrolysis of the alkoxy groups by surface adsorbed water followed by condensation with surface silanol groups. The non-aqueous medium helps to keep polymer formation to a minimum and aids in the dissolution of sparingly soluble silanes.

As indicated in Figure 2, it has been shown that silylating reagents containing di- or tri-alkoxy or chloro groups can be attached to the silica surface only through two of these groups (24, 25) due to steric considerations. The remaining silanol group is usually condensed with a neighboring silanol group by heating. This process, which is called "curing", results in a more hydrolytically stable surface structure than the uncured product. The proposed curing process is shown in Figure 3.

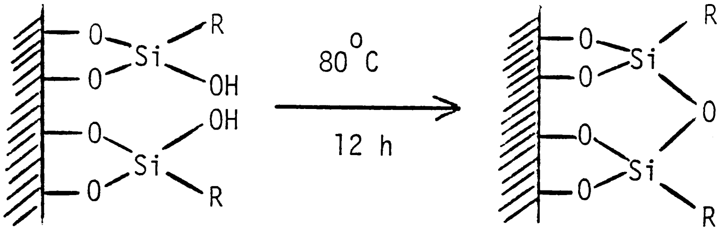


Figure 3. Curing Process

The optimal loading of AEAPTS onto the silica surface has been reported to occur when 10 mL of a 10% (v/v) aqueous solution of AEAPTS is added to 5g of silica gel (13). This corresponds to 0.2 mL of silane per gram of silica gel at a concentration of 10% by volume. The effect of pH on the loading, however, was not mentioned in this paper although in another publication that year, the same workers indicate that the silylating solution should be prepared in dilute aqueous acid (14). As mentioned previously, acidic media causes rapid hydrolysis of the alkoxy groups while keeping polymerization to a minimum. Non-aqueous silylation has also been performed in benzene (26, 27) and toluene (28, 29). The primary advantage of using organic solvents is the greater stability of the silylating solution. This, however, is not very important in the case of AEAPTS as long as the silylating solution is used within a few hours since AEAPTS, as well as most aminosilanes, is relatively stable (22). The bound ethylenediamine is also a convenient intermediate in the preparation of immobilized dithiocarbamates as well as other silica-bound species as will be shown later.

Characterization

The characterization of silica-bound ethylenediamine as well as other chelating ligands involves several parameters.

Capacity. Of initial importance after completing the synthesis of an immobilized chelating agent is the amount of chelating agent that has actually been attached to the silica substrate. An indirect measure of this quantity is available by measuring the capacity of the solid material. The capacity is the maximum quantity of metal ion (usually in μmoles or mmoles) which can be extracted from solution per gram of dry chelating ion exchanger.

Ideally, the capacity should be independent of the particular metal ion used. This is not always the case, however, since different metal ions may exhibit different stoichiometries with the chelating agent. Also, differing sizes of ions may cause a sieving effect in which the larger ions are excluded from the smaller pores. Another important factor to consider is the magnitude of the conditional formation constant which, of course, is pH dependent. The conditional formation constant should be as large as possible so that a convenient concentration of metal ion can be used at a relatively low pH where the problems of precipitation of the metal ion, as hydroxide, and extraction of metal ion by residual silanol groups are avoided. A word of caution should be interjected here in that, although it is tempting, extrapolation of knowledge about the chelating agent in homogeneous solution should be kept to a minimum. It is observed that while the order of formation constants usually remains the same (although magnitudes may change), the stoichiometry of the metal ion-chelating agent reaction is often very different for heterogeneous systems than that observed in homogeneous solution (9). This will be discussed in more detail later.

A quantity which is related to capacity and is used quite often in the literature is the surface coverage. The surface coverage is related to the capacity through the following equation:[3]

$$\text{Surface Coverage } (\mu\text{moles/m}^2) = \frac{\text{Capacity } (\mu\text{moles/g})}{\text{Specific Surface Area } (\text{m}^2/\text{g})} \quad (11)$$

The capacity of a chelating ion exchanger is usually determined by one of six possible methods.

1. Metal ion uptake in which the change in concentration of a standard metal ion solution (with the metal in excess) is measured after equilibration with a known weight of chelating ion exchanger.[4]

2. Metal ion elution in which the solid chelating ion exchanger is rinsed with a strong mineral acid solution in order to remove any extracted metal ion after equilibration with an excess standard metal ion solution.

3. pH titration in which the number of hydronium ions or hydroxide ions required to neutralize the ion exchanger is measured.

---

[3] Actually, surface coverage refers to the ligand whereas capacity refers to the metal ion. Therefore this equation, as written, is applicable only for 1:1 complexes which are most common in immobilized systems. If the metal to ligand ratio is not 1:1, but is known, this equation becomes: Surface Coverage = Capacity/(Specific Surface Area x Metal:Ligand Ratio).

[4] The so-called "column breakthrough method" is essentially a metal ion uptake method which is carried out in a column under dynamic rather than static conditions. The amount of metal ion extracted by the ion exchanger is measured at the point at which the metal ion is first detected in the column effluent.

4. Elemental analysis in which the percent of carbon, nitrogen, or some other element is used to calculate the number of immobilized ligands on the support. This gives an indirect measure of capacity.

5. Ion-pair formation in which the uptake of an anion by the chelating ion exchanger in a protonated form is measured.

6. Weight increase in which the increase in mass upon immobilization is measured.

Some of the capacity values obtained for silica-immobilized ethylenediamine by various workers are presented in Table I. It is apparent that the capacity values (and related surface coverages) vary considerably depending on a number of variables such as the specific surface area of the support, the reaction conditions, the silylating solvent, the probe used, and the method of calculating the capacity. Since there is no universally accepted procedure used to determine capacities, comparison between various values is difficult. However, a few points should be made about the data of Table I.

1. Since the exchange capacity refers to the amount of metal ion which can be extracted per gram of dry exchanger, methods employing metal ions are generally preferable to other methods. If the amount of bound chelating agents is of interest, elemental analysis can be used. This is particularly useful when trying to determine the metal to ligand ratio of the immobilized complex. Elemental analyses, however, yield values which reflect the total amount of bound organic matter. This can include bound, non-chelating, organic species as well as chelating species which cannot complex the metal due to steric considerations. Therefore, elemental analysis often yields higher capacity values than those obtained using other methods such as metal ion exchange or ion-pair

TABLE I

SUMMARY OF CAPACITY STUDIES FOR SILICA IMMOBILIZED ETHYLENEDIAMINE

Row	Mean Specific[a] Surface Area (m^2/g)	Mean Pore Diameter (Å)	Solvent for Silylation	Capacity (μmoles/g)	Apparent[b] Surface Coverage ($\mu moles/m^2$)	Capacity Method	Probe	Metal Determination Method	Ref.
1	550^c	60	aq.	520 470	0.95 0.85	2 2	Zn^{2+} Cu^{2+}	EDTA Titn.	13
2	550^c	60	aq.	540 570	0.98 1.04	5 5	MoO_4^{2-} CrO_4^{2-}	Visible Spect.	15
3	292	114	toluene	642 701^d	2.2 2.4	5 5	Cl^-	$AgNO_3$ Titn.	30
4	292	144	aq.	1080 1110^d	3.7 3.8	5 5	Cl^-	$AgNO_3$ Titn.	30
5	100^e	240	aq.	798^g	7.98^f	4	----	----	23
6	167	180	$benzene^h$	558^d 468^g	3.34 2.80	4 4	----	----	26,27
7	204	153	$benzene^h$	702^d 553^g	3.44 2.71	4 4	----	----	26,27
8	400	85	$benzene^h$	1300^d 932^g	3.25 2.33	4 4	----	----	26,27

TABLE I (Continued)

Row	Mean Specific[a] Surface Area (m^2/g)	Mean Pore Diameter (Å)	Solvent for Silylation	Capacity (μmoles/g)	Apparent[b] Surface Coverage (μmoles/m^2)	Capacity Method	Probe	Metal Determination Method	Ref.
9	490	70	benzene[h]	1330[d] 1018[g]	2.71 2.08	4 4	----	----	26,27
10	305	--	toluene	320 700[d]	1.05 2.30	2 2	Cu^{2+}	Atomic Absorp.	18
11	305	--	toluene	510 1030[d]	1.67 3.40	2 2	Cu^{2+}	Atomic Absorp.	18
12	305	--	toluene	270 650[d]	0.89 2.10	2 2	Cu^{2+}	Atomic Absorp.	18
13	256	--	toluene	410 1100[d]	1.60 4.30	2 2	Cu^{2+}	Atomic Absorp.	18
14	117	--	toluene	310 730[d]	2.65 6.20	2 2	Cu^{2+}	Atomic Absorp.	18

---

[a] Before silylation (silylation causes a decrease in specific surface area).

[b] Assuming a 1:1 metal to ligand ratio although this may not be the case.

[c] Silica gel G (contains gypsum binder for TLC).

[d] Based on % carbon

TABLE I (Continued)

Row	Mean Specific[a] Surface Area (m^2/g)	Mean Pore Diameter (Å)	Solvent for Silylation	Capacity (μmoles/g)	Apparent Surface Coverage (μmoles/m^2)	Capacity Method	Probe	Metal Determination Method	Ref.

[e] Controlled Pore Glass.

[f] Average of six values on six different materials (std. dev. = 0.50).

[g] Based on % nitrogen.

[h] 5% AEAPTS in benzene which gave the largest capacities.

formation as can be seen in Table I. This is true even if a 1:1 metal to ligand ratio is observed. If ion-pair formation is of interest, anions can be employed as probes in order to obtain the capacity (e.g., Cl^-, MoO_4^{2-}, CrO_4^{2-}).

2. Surface coverage values calculated in Rows 1 and 2 of Table I are considerably smaller than surface coverage values obtained by other workers. This could be due to the presence of gypsum (calcium sulfate hemihydrate) binder which, being unreactive with AEAPTS, causes a decrease in the capacity that would be obtained in the absence of gypsum.

3. As shown in the work of Okamoto and coworkers (Rows 6-9), as specific surface area increases (pore diameter decreases), the capacity generally increases. However, the surface coverage remains approximately constant until the pore size decreases to 70-85 Å at which point the surface coverage drops off. This is probably due to steric considerations and hindered diffusion of AEAPTS into the pores of the silica gel.

Stability. The stability of immobilized chelating agents is, of course, of major importance. Three types of stability are most often considered:

1. Storage stability which is simply a measure of shelf life of a given material.

2. Temperature stability, usually measured by thermogravimetric techniques which show a percent weight loss as a function of temperature.

3. Hydrolytic stability which generally involves the stability of a material as a function of pH.

Storage and hydrolytic stability are most important since immobilized chelating ligands are normally used under ambient temperature conditions so that stability at elevated temperatures is not required. Usually, a decrease in stability is measured by comparing capacities determined before and after some particular treatment. For example, storage stability is determined by storing the dry silica material for a period of time after which the change in capacity is measured. From a practical point of view, hydrolytic stability is the most important type of stability if the storage stability is good. As mentioned previously, none of the silica bound chelating agents can be used above a pH of about 10 due to hydrolysis of the silica matrix itself.

Stability studies of silica-immobilized ethylenediamine are scarce. Leyden and Luttrell (13) indicate that no appreciable hydrolysis occurs between pH 1.5 and 10.5 for time periods up to several hours. They base their observations on the lack of colored species in the filtrate of colored complexes. No capacity studies were performed to confirm this observation. They also report that amino-functional groups are thermally stable up to 150°C. At temperatures above 150°C for periods of 1 hour or more the amine materials became yellow which is claimed to be an indication of oxidation. Again, however, no capacity studies were performed and no attempt was made to identify the yellow product(s). Oxidation problems were also observed in solutions containing significant amounts of very strong oxidizing agents.

The results obtained by Gimpel and Unger (30) are quite different in that a significant decrease in Cu^{2+} capacity was found when a 0.1 M acetate buffer solution (pH 5) which contained Cu^{2+} was used under dynamic conditions. They attribute this effect to 1) a loss of functional groups due to washing of the column, and 2) the competition of the buffer solution for Cu^{2+} ion which would cause an apparent loss of capacity when using on-line monitoring of Cu^{2+}. No discussion of mass transfer rates or possible oxidation of the amino groups to nitro groups was mentioned. It was indicated that the decomposition of silica-immobilized ethylenediamine followed a pseudo-first-order rate law. Little information is available concerning the storage stability or shelf life of silica-immobilized ethylenediamine. Leyden et al., however, indicate that the immobilized ethylenediamine is air stable and may be stored at room temperature (20). No capacity studies were presented to confirm this.

Rate Studies. As mentioned earlier, one of the problems of polymer-based ion exchangers is the slow rate of equilibration with metal ions. For this reason, the rate of metal ion uptake for many silica-bound ligands has been studied in order to assess the ability of these materials to undergo rapid ion exchange. Fast exchange rates are essential in chromatographic applications in order to avoid undue band broadening and in preconcentration applications in order to allow larger flow rates when passing large volumes of sample (time savings).

Leyden and coworkers (13-15) have performed rate studies with a variety of immobilized ligands, metal ions, and anions. The time required for 50% and 90% extraction of some selected metal ions at pH 7 is presented in Table II. These metal ions were chosen for study because they exhibit a wide range of affinities for ethylenediamine. Both copper(II) and mercury(II), which exhibit large formation constants with ethylenediamine (31), are extracted very quickly from dilute aqueous solution. On the other hand, silver(I) which tends to form binuclear ethylenediamine complexes rather than chelates, manganese(II) whose ethylenediamine complexes have very small formation constants, and

TABLE II

TIME REQUIRED FOR 50% AND 90% EXTRACTION OF SEVERAL METAL IONS AT pH 7.0 BY SILICA-IMMOBILIZED ETHYLENEDIAMINE

Cation*	Time Required (Min.)	
	50%	90%
Hg^{2+}	< 0.5	< 0.5
Ag^{+}	1	17
Cr^{3+}	5	22
Mn^{2+}	7	22
Cu^{2+}	< 0.5	3

*All metals determined by x-ray fluorescence spectroscopy.

chromium(III) which is notorious for slow exchange, all exhibit slower exchange rates. Polymer-based ion exchangers, however, may require several hours in order to achieve 50% extraction and therefore the values obtained for silica-bound ethylenediamine are considered to be very good. It should be pointed out that pH 7 is not the optimal pH for each metal ion and rates of exchange would be faster if the pH was optimized for each individual metal ion.

The rate of metal oxyanion extraction under acidic conditions has also been investigated for SeO_4^{2-}, MnO_4^-, AsO_4^{3-}, and $Cr_2O_7^{2-}$ on silica-ethylenediamine (15). These anions form particularly strong ion pairs with the protonated diamine. An unusual decrease in extraction efficiency of permanganate as the pH increases is observed which is thought to be due to the strong oxidizing nature of MnO_4^- coupled with the small formation constant of the Mn(II)-ethylenediamine complex. Oxidation of the diamine by MnO_4^- is believed to occur followed by complexation of the resulting Mn(II) with either unoxidized diamine or the oxidized form of the diamine. The Mn(II) complex thus formed then dissociates due to the relatively low pH (pH = 3.5 ± 0.2) and the small formation constant of the Mn(II) complex. Dichromate and selenate are also strong oxidizing agents. However, they do not display the same behavior as Mn(II). This is probably due to larger formation constants of the metal complexes formed from the reduced forms of the metal ion. In the case of dichromate and permanganate, ESCA studies have indicated the presence of Cr(IV) or Cr(V) and Mn(II). No Cr(VI), Cr(III), or Mn(VII) was detected which indicates that at least partial reduction has occurred. Although AsO_4^{3-}, which also exhibits a decrease in extraction efficiency with increasing pH, is only a moderately strong oxidizing agent, it may be able to oxidize the diamine and therefore behave similarly to MnO_4^-.

pH Dependence. The formation of metal complexes is, of course, dependent on pH among other things. The effect of pH on the extraction of metal ions is usually measured in a batch experiment in which the distribution of a particular metal ion probe between the solution phase and the solid phase is measured as a function of pH. Two quantities are most often used to express this distribution:

The Distribution ratio (D) which is defined as follows:

$$D = \frac{\text{Amount of Metal Probe Extracted / g Ion Exchanger}}{\text{Amount of Metal Probe in Solution / mL of Solution}} \qquad (12)$$

and Percent extracted (%E) which is given by the following equation:

$$\%E = \frac{\text{Amount of Metal Probe Extracted}}{\text{Amount of Metal Probe Added}} \times 100 \qquad (13)$$

These two quantities are related through the equation:

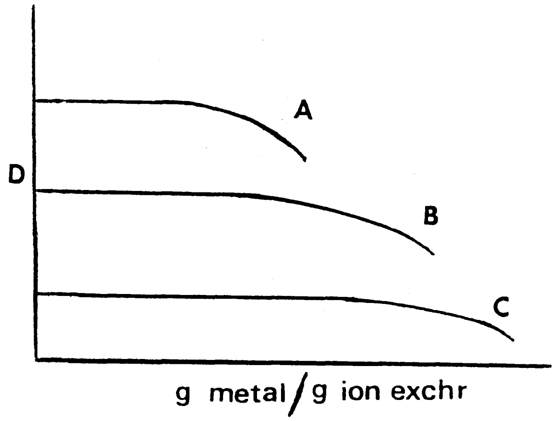
$$D = \frac{\%E}{100-\%E} \times \frac{\text{mL of Solution}}{\text{g Ion Exchanger}} \quad . \qquad (14)$$

The measurement of D (or %E) requires that the ratio of the amount of metal ion probe / amount of ion exchanger be small enough so that the linear capacity of the ion exchanger is not exceeded. This is in contrast to the practice in capacity determinations where an excess of metal ion is used. The effect of the metal / ion exchanger ratio on the apparent distribution ratio value (D) for various pH values is shown in Figure 4. The flat regions of these curves correspond to the linear capacity regions. Capacity measurements are generally done under higher pH conditions (curve A for example) at large metal loading values so that the linear capacity is exceeded. This is done to ensure that all of the exchange sites are saturated. It should be mentioned that D can be measured under dynamic conditions in which case:

$$D = \frac{V_R - V_M}{W_S} \tag{15}$$

where:

V_R = Retention volume of the probe (mL).
V_M = Void volume of the system (mL).
W_S = Weight of the stationary phase (g).



pH A >pH B > pH C

Figure 4. Effect of Loading on the Distribution Ratio (D)

The extraction of various metal ions onto immobilized ethylenediamine as a function of pH has been determined by Leyden and coworkers (13, 14). They indicate that the extraction pattern observed is that which would be expected based on the formation constants of ethylenediamine complexes in solution. In fact, they claim that pH values at 50% extraction agree very well with the literature values of log β_2 for the ethylenediamine-metal complexes in solution which would indicate a 1:2 metal to ligand ratio for the immobilized complex. However, a plot of $pH_{1/2}$ versus log β_2 is actually not very linear having a correlation coefficient of approximately 0.90. These workers also point out that some of the $pH_{1/2}$ values are sufficiently different to allow for useful batch or column separations. Gimpel and Unger (30) have also reported the percent extraction of copper(II) as a function of pH for the immobilized diamine and both of the above groups have investigated the extraction of copper(II) by silica gel itself at pH>5. In addition, Leyden and Luttrell have studied the extraction of other metal ions by silica gel as a function of pH. However, selectivity of the silanol groups is poor. For example, the extraction of copper(II) and zinc(II) as a function of pH was shown to be almost identical, thus preventing separation of these metal ions (13).

As ion-pair formation is also pH dependent, the extraction of certain anions (AsO_4^{3-}, MnO_4^-, $Cr_2O_7^{2-}$, SeO_4^{2-}, WO_4^{2-}, and VO_4^{3-}) as a function of pH has also been performed (15). The anions generally exhibit a maximum (or plateau) in the %E vs. pH curve. At low pH, the diamine is diprotonated and therefore allows for the possibility of ion-pair formation between the anion and the immobilized diamine. The competition of H^+ for the anion, however, can cause the anions of weaker acids (e.g. MoO_4^{2-} and $HAsO_4^{2-}$) to exhibit a decrease in %E as pH decreases. As pH increases, eventually the diamine loses its protons and ion-pair formation ability is lost. A similar behavior has been observed for

$UO_2(CO_3)_3^{4-}$ and MoO_4^{2-} (19). Extraction of such common anions as I^-, Br^-, IO_3^-, IO_4^-, or BrO_3^- was not observed (15). It was postulated that the central atom in extractable anions must be metallic in nature resulting in more basic anions.

Miscellaneous Studies. Probably the most important studies of immobilized ethylenediamine that have not been discussed up to this point are structural studies of the immobilized material. One of the most important questions to be answered is "What is the metal to ligand ratio in the immobilized complex formed?" The answer to this question can be tentatively provided by carrying out capacity studies using different capacity determination methods and probes. For example, immobilized ethylenediamine was shown to apparently form a 1:2 metal to ligand complex with copper(II) by comparing capacities determined by copper(II) elution, molybdate elution, and carbon determination (18). The results are given in Table III. The results obtained for molybdate elution and carbon determination agree very well whereas the copper(II) value is about half of these values. This is strong evidence that a 1:2 metal to ligand complex is formed. A similar result was obtained by Jezorek et al. (48) using copper(II) uptake and carbon determination. Later work using photoacoustic spectroscopy (PAS), however, indicates that, actually, a mixture of mono (1:1) and bis (1:2) complexes exist on the surface even at low surface coverage (32). The formation of mono or bis complexes depends on the proximity of one immobilized diamine to another immobilized diamine. When possible, the bis complex is favored due to its greater stability. Therefore, at very low copper concentrations, formation of the bis complex occurs first, followed by formation of the mono complex. The two complexes can be distinguished by differences in their absorption maxima using PAS. An approximately 2.5:1 ratio of mono to bis sites was found. This clearly points out the difficulties encountered when attempting to extrapolate knowledge of the ligand in solution to the solid phase.

TABLE III

EXAMPLE OF STOICHIOMETRY DETERMINATION FOR THE SILICA-IMMOBILIZED Cu(II)-ETHYLENEDIAMINE COMPLEX

Probe	Capacity (μmoles/g)
Cu(II)	510
MoO_4^{2-}	1050
%C	1030

Kvitek and coworkers (23, 33) have used potentiometric and thermometric titrimetry to study the interaction between copper(II) and the immobilized ethylenediamine. They propose a model based upon their data in which copper(II) initially reacts with the primary amino group from each of four bound ethylenediamine ligands. This step is then followed by reaction of another copper(II) with the bound $Cu(en)_4$ complex resulting in two $Cu(en)_2$ complexes. This, of course, is a very different mechanism than that in solution in which copper(II) sequentially reacts with both nitrogens of two ethylenediamine molecules. It should be mentioned that the above results were obtained for relatively high surface coverages of the bound diamine. At lower surface coverages, formation of the $Cu(en)_4$ complex may not be possible. In fact, the PAS work of Leyden et al. previously mentioned (32) indicates that the predominant species is probably Cu(en) with smaller amounts of $Cu(en)_2$ at low surface coverages.

Applications

Silica-immobilized ethylenediamine has proven to be useful for a variety of analytical purposes. Many of these applications, however, are not dependent on the complex-forming ability of ethylenediamine. For example, one of the most useful and novel applications of silica-bound ethylenediamine to date is the determination of orthophosphate at the ppb level by x-ray fluorescence (XRF) determination of Mo. This procedure utilizes the reaction of PO_4^{3-} with MoO_4^{2-} to yield the heteropoly acid, 12-molybdophosphoric acid (12-MPA) (20). Usually this complex is reduced to the heteropoly blue complex for spectrophotometric determination. The oxidized form, however, is easily extracted, free of excess molybdate, into organic solvents. This procedure involves extraction of 12-MPA into ethylacetate followed by the ion-pair extraction of 12-MPA (as a molybdate-like anion) onto silica-bound ethylenediamine at pH 2.8. Molybdenum is then determined by XRF. Results obtained using this procedure were shown to agree very well with those obtained using an EPA-approved continuous flow procedure.

Ion-pair extraction has also been used for the determination of uranium in mining samples by extraction of uranium, as $UO_2(CO_3)_3^{4-}$ or $UO_2[(CO_3)_2 \cdot 2H_2O]^{2-}$, from aqueous carbonate solutions at pH 5 to 7 (34). The extracted uranium was then determined by XRF. High concentrations of carbonate, however, reduce extraction efficiency through competition.

Results of a colorimetric procedure agree fairly well with those obtained using the XRF procedure (slope = 0.90, correlation coefficient = 0.9999) with the x-ray method giving slightly lower results.

Other examples of the usefulness of silica-immobilized ethylenediamine as an ion-pair forming material can be found (15, 18, 19). These papers deal primarily with the preconcentration or separation of oxyanions of Mo, W, U, As, and Se in synthetic samples. Real samples have not been used.

Another non-chelating application of silica-immobilized ethylenediamine is its use as a polar bonded stationary phase for liquid chromatography. Okamoto and coworkers have separated isomeric aromatic amines on this material (26) as well as the nucleotides, AMP and ADP (27). However, reverse-phase packings still provide better efficiency. The amine separations described are also very slow.

There are two major applications of silica-immobilized ethylenediamine in which the chelating ability of this material has been exploited. The first is preconcentration of trace metal ions prior to determination by some instrumental technique such as XRF. Silica-bound ethylenediamine, however, displays some bleeding of metal ions from the column and is less selective than many other chelating agents. Therefore, little work has been done in this area. Leyden and coworkers, however, have demonstrated the preconcentrating ability of this material by preconcentrating Zn(II), Co(II), and Cu(II) from a synthetic test mixture (16). Again, no real samples were analyzed.

The second technique in which the chelating ability of the bound ethylenediamine is useful is in the area of ligand exchange chromatography, a technique by which ligands can be separated by interaction with metal ions which have been complexed by an immobilized ligand. Generally, the bound metal ion is not coordinatively saturated and therefore can interact with a ligand, added as the sample, through the vacant (or solvated) coordination sites. Usually the mobile phase contains a ligand which competes with the sample ligands for these vacant coordination sites. The major problem encountered in ligand exchange chromatography is bleeding of metal ion from the column. Therefore, the common practice is to add small amounts of the metal ion to the mobile phase. However, chelating ion exchangers show less metal ion bleed than do conventional ion exchangers.

Masters and Leyden (35) have used copper(II)-loaded ethylenediamine on controlled pore glass for the separation of certain amino hexoses and amino acids using ammonia as the eluting ligand. Copper bleeding was compensated by 10^{-4} M copper in the mobile phase. Column lifetime was limited to approximately 50h under continuous operation at pH 9 in 0.1M ammonia due to hydrolysis of the silica matrix. It was also reported that copper was irreversibly lost at pHs 3 and 4 which would indicate that the functional groups were being removed from the column. This is surprising in light of previous reports of hydrolytic stability down to pH 1.5 (13). Three amino sugars and eighteen amino acids were investigated. At best, only certain pairs of amino acids could be separated using this approach. The poor efficiency is probably due to the large particle size which results in a relatively large contribution to plate height due to eddy diffusion and mobile phase contributions. Also, ligands are typically slow to desorb from the metal which results in an increased contribution to plate height due to mass transfer in the stationary phase. Optimization of the support could improve separation. However, the study was done with a low pressure system consisting of peristaltic pump, septum-type on-line injector, glass column, and photometric detector (maximum pressure was about 15 psi) for which the properties of porous glass (i.e., large pore diameter and relatively large particle size) are well suited.

Cooke et al. (36) have also used the ligand exchange technique with Cd(II)-loaded ethylenediamine on Nucleosil (5 and 10 μm) spherical, porous silica. $CdSO_4$ was added to the mobile phase to compensate bleeding. They apply this technique to the separation of some selected sulfa drugs and dipeptides. It was shown that those sulfa drugs having pK_a values higher than the pH of the mobile phase (pH 6.8) exhibit low partition ratio (k') values that remain approximately the same both in the presence and absence of added metal (Note: $k' = (V_R - V_M)/V_M$). However, the order of elution does not strictly follow the order of pK_a values as sulfapyridine ($pK_a = 8.4$) elutes after sulfacetamide ($pK_a = 5.4$). This is probably due to stronger interaction between Cd(II) and the heterocyclic nitrogen of sulfapyridine than between Cd(II) and the oxygens of sulfacetamide. Useful separation of the selected sulfa drugs and dipeptides were demonstrated using 5 μm Nucleosil-ethylenediamine-Cd(II) stationary phase using an aqueous acetonitrile mobile phase. A summary of applications of silica-bound ethylenediamine is shown in Table IV.

Silica-immobilized ethylenediamine is one of the most widely used silica-bound chelating agents. It is extremely easy to prepare and yields good capacities approaching those found in conventional ion exchange resins. It also appears to be hydrolytically stable in the pH range of 2 to 9. However, it suffers from instability at extreme pH and

TABLE IV

SUMMARY OF APPLICATIONS OF SILICA-IMMOBILIZED ETHYLENEDIAMINE

Application	Species Determined	Determination Technique	Comments	Ref.
P(A)	Mo,W	XRF	1 liter synthetic sample in 35% NaCl (18 ppb Mo, 32 ppb W)	18
S(A)	U,Mo	AA(Mo)	Synthetic sample, $(NH_4)_2CO_3$ mobile phase	19
P(A)	PO_4^{3-}	XRF(Mo)	Formation of 12-MPA followed by extraction into ethylacetate	20
P(A)	As,Se	XRF	2 liter synthetic sample, 100% recovery	15
P(A)	U	XRF	20 mL mining samples, carbonate solution, batch extraction, (120 ppb U)	34
P(C)*	Zn,Co,Cu	XRF	Synthetic sample, 10 ppm of each metal	16
S(C)*	Fe,Cu,Ni,Zn	NA[a]	Poor resolution (only 1 metal could be separated from the other 3)	28
S(O)	aromatic[b] amines	S	25:1 n-hexane/ethanol mobile phase. Slow (35-80 min)	26
S(O)	AMP,ADP[c]	S	0.5M KH_2PO_4 mobile phase (<10 min)	27
S(LE)*	amino sugars amino acids	S	Cu(II)-ethylenediamine complex on controlled pore glass. Poor efficiency.	35

TABLE IV (Continued)

Application	Species Determined	Determination Technique	Comments	Ref.
S(LE)*	sulfa drugs, dipeptides	S	Cd(II)-ethylenediamine complex on Nucleosil. Good efficiency.	36

*Involves chelation.

[a] TLC separation (non-quantitative).

[b] Selected isomeric aminotoluenes and nitroanilines.

[c] AMP = adenosine monophosphate   ADP = adenosine diphosphate.

Application

P = Preconcentration
S = Separation
A = Anions
C = Cations
LE = Ligand exchange
O = Organics

Determination Technique

XRF = X-ray fluorescence
AA = Atomic absorption
S = Spectrophotometric

is relatively easily oxidized over a period of time. Also, other chelating agents can be immobilized which exhibit larger formation constants with most metal ions (which aids in preconcentration) and some chelating agents are more selective so that they may be better suited for a given application. It is surprising that its use under column chromatographic conditions for metal ion separations has not been investigated.

Dithiocarbamates and Related Structures

Preparation

As mentioned previously, the preparation of most silica-bound dithiocarbamates has been carried out after immobilization of ethylenediamine as described earlier. Immobilized dithiocarbamates, however, can also be prepared from other bound amines. The general structure of silica-immobilized dithiocarbamates is shown in Figure 5 below.

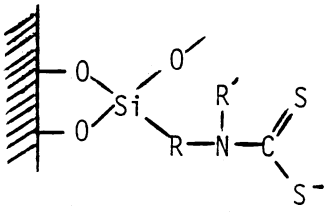


Figure 5. General Structure of a Silica-Immobilized Dithiocarbamate

A related species that has also been described is a silyl xanthate (Figure 6) which has the structure shown below and is prepared from silica gel itself (13).

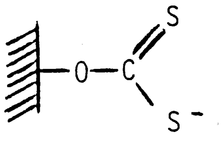


Figure 6. Silyl Xanthate

The synthesis of immobilized dithiocarbamates is performed by reacting an immobilized amine (e.g., ethylenediamine) with carbon disulfide in the presence of a base as shown in Figure 7 (13).

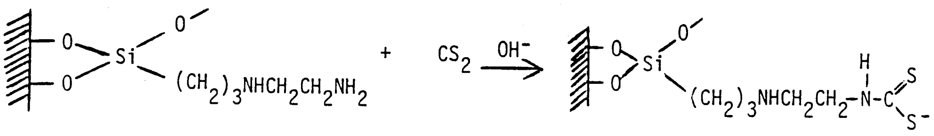


Figure 7. Synthesis of Silica-Immobilized Dithiocarbamates

The reaction conditions (i.e., solvent, base, etc.) can be modified in order to obtain a bis-dithiocarbamate from immobilized ethylenediamine (13). The structure of the bis-dithiocarbamate formed from immobiized ethylenediamine is shown in Figure 8. Leyden and Luttrell (13) have described the preparation of several dithiocarbamates as well as a silyl xanthate. In addition to the silyl xanthate (Figure 6) and dithiocarbamates (Figures 7 and 8), they have also prepared the immobilized dithiocarbamate from N-methyl-3-aminopropyltriethoxysilane (Figure 9).

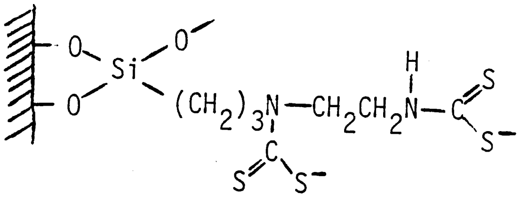


Figure 8. The Bis-dithiocarbamate From Silica-Immobilized Ethylenediamine

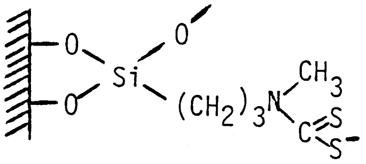


Figure 9. The Dithiocarbamate From Silica-Immobilized N-methyl-3-aminopropyltriethoxysilane

It appears that reaction of the immobilized amine with CS_2 in a non-aqueous solution of tetramethylammonium hydroxide for 15 min. yields the mono-dithiocarbamate. Reaction of diamines (e.g., ethylenediamine) in aqueous NaOH for 15 min., however, yields the bis-dithiocarbamate. No explanation was proposed for this observation. The silyl xanthate was prepared from silica gel itself under the conditions described for preparation of the mono-dithiocarbamates. Essentially all subsequent work using silica-immobilized dithiocarbamates use the above procedures.

Characterization

Capacity.

Some of the capacity values determined by Leyden and Luttrell (13) for various silica-bound dithiocarbamates and silyl xanthate are summarized in Table V. As expected the bis-dithiocarbamate (Figure 8) exhibits a capacity that is about twice that observed for the mono-dithiocarbamates (Figures 7 and 9) due to the two reactive sites per bound silane for the bis-dithiocarbamate. It is also observed that the mono-dithiocarbamates have about the same capacity as their ethylenediamine precursors which indicates a good synthetic efficiency. This also indicates that the metal to ligand ratio should be the same for the bound mono-dithiocarbamates and the bound ethylenediamine precursor, although this was not mentioned by Leyden and Luttrell. The capacity of the silyl xanthate determined from pH titration is higher than the mono-dithiocarbamates probably because it can be prepared in one step, and therefore is not dependent on the presence of an immobilized precursor such as ethylenediamine.

Stability.

The stability of immobilized dithiocarbamates is, at best, poor. They are unstable under a variety of experimental condi-

TABLE V

SUMMARY OF CAPACITY STUDIES FOR SILICA-IMMOBILIZED DITHIOCARBAMATES AND SILYL XANTHATE

Structure (Figure No.)	Mean Specific[a] Surface Area (m^2/g)	Mean Pore Diameter (Å)	Capacity[b] (μmoles/g)	Apparent[c] Surface Coverage (μmoles/m^2)	Capacity Method	Probe	Metal Determination Method
9	550	60	500	0.91	2	Zn^{2+}	EDTA Tit'n
			530	0.96	2	Cu^{2+}	EDTA Tit'n
7	550	60	490	0.89	2	Zn^{2+}	EDTA Tit'n
8	550	60	940	1.71	2	Zn^{2+}	EDTA Tit'n
			970	1.76	2	Cu^{2+}	EDTA Tit'n
6	550	60	1000	1.82	3	----	----

[a] Silica gel G (contains gypsum binder for TLC)/all values are before silylation.

[b] All from reference 13.

[c] Assumes 1:1 metal to ligand ratio although this may not be the case.

tions, particularly in acidic solution. Leyden and Luttrell (13) report that solutions in contact with the immobilized dithiocarbamates became cloudy after about 15 minutes at pH values below 2.5. They also indicate that the cloudiness observed was more apparent for the dithiocarbamates prepared from immobilized ethylenediamine (both bis- and mono-). Again, however, no capacity studies were presented to obtain a quantitative measure of the amount of degradation. The cloudiness was attributed to acid catalyzed hydrolysis of the nitrogen-carbon bond as shown in Figure 10.

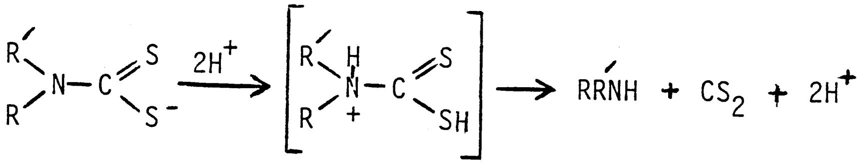


Figure 10. Acid Catalyzed Hydrolysis of Dithiocarbamates

The immobilized dithiocarbamates, like their solution counterparts, are also easily oxidized by air and a variety of other oxidizing agents. Leyden and Luttrell, however, report no evidence of oxidation by air but the mono- and bis-dithiocarbamates prepared from bound ethylenediamine may be oxidized by copper(II) (13).

In a later publication (37), Leyden and coworkers report that the immobilized bis-dithiocarbamate, once prepared, was stored under refrigeration for a maximum of 10 days which indicates the limited storage stability of these materials. A maximum storage time of 3 days in a refrigerator has also been reported for the bisdithiocarbamate of ethylenediamine bound to filter paper which contains silica gel (38). No capacity studies were reported in either case.

Rate Studies. Metal ion extraction onto silica-bound dithiocarbamates is generally very fast. It was observed that, with the exception of Cr(III), all of the ions studied were over 90% extracted in less than 4 min. onto the N,N-dialkyldithiocarbamate (Figure 9). This is much faster than the silica-bound ethylenediamine mentioned previously and is obviously an improvement over chelating resins. As mentioned previously, Cr(III) has notoriously slow exchange rates and, in fact, Cr(III) is more slowly extracted onto the silica-dithiocarbamate than onto the silica-ethylenediamine. Again, pH values were not optimized and therefore extraction rates could be higher at optimal pH for each metal. Leyden and coworkers (15) have also investigated the rate of extraction of $Cr_2O_7^{2-}$ as an illustrative oxyanion on a silica-immobilized N-alkyldithiocarbamate. About 90% of the $Cr_2O_7^{2-}$ was found to be extracted at pH 3.5 within about 2 min. which was the fastest extraction rate of all of the immobilized species studied (i.e. bound ethylenediamine, various dithiocarbamates, silyl xanthate). The percent extracted, however, decreases after reaching the maximum (only about 70% extracted after 1 hr.) probably due to oxidation of the dithiocarbamate or possibly due to acid catalyzed hydrolysis at pH 3.5.

pH Dependence. Leyden and Luttrell (13) have also investigated the pH dependence of the extraction process for the silyl xanthate (Figure 6) and the N,N-dialkyldithiocarbamate (Figure 9). Work on the silyl xanthate was limited, although it chelates very well, because of the vulnerability of the silyl xanthate to acid hydrolysis. Only Cu(II), Zn(II), and Ni(II) were studied on the silyl xanthate. For preconcentration of cations, however, the N,N-dialkyldithiocarbamate was shown to be very useful. Due to the large formation constants of most dithiocarbamate complexes, most metal ions can be extracted efficiently even at low pH. However, acid hydrolysis prevents working at low pH. Common practice involves use at pH 6-8 where hydrolysis is minimized but extraction efficiency is good. One problem encountered, however, is in the subsequent elution of the extracted metal. Elution is very difficult due to large formation constants even at low pH where degradation may occur. For this reason, preconcentration using dithiocarbamates is normally restricted to determination techniques in which the extracted species can be determined directly on the solid chelating material (e.g., XRF, ESCA, etc.). Unlike the silica-immobilized ethylenediamine, the pH values at 50% extraction are not very well separated which indicates that the bound dithiocarbamate is not well suited for column separations under chromatographic conditions due to the lower selectivity.

Applications

The large formation constants observed for dithiocarbamate complexes as well as the lack of selectivity and the instability at low pH severely restricts the applications of these materials. Their primary use, as mentioned, lies in the preconcentration of trace metals prior to determination by some instrumental technique which can be used directly on the solid phase.

Probably the first use of a silica-bound dithiocarbamate was reported by Hercules et al. (39). Fiberglass disks were used to support an immobilized dithiocarbamate prepared from bound ethylenediamine (Figure 7). The modified disks were then used to preconcentrate Pb(II), Ca(II), Hg(II), and Tl(I) from 100 mL of 10^{-6}M synthetic samples. A minimum detectable quantity (MDQ) of about 1.0 μg was observed for each metal ion. No real samples were analyzed nor were possible interferences investigated. Also of interest is the fact that conventional ion exchange resins are not amenable to use in ESCA which is, essentially, a surface technique, due to diffusion of metal ions into the bulk of the exchanger whereas silica-bound materials comprise a sort of "two dimensional" ion exchanger in which the ions remain on the surface.

Leyden et al. (16) have demonstrated the use of the silica-immobilized N,N-dialkyldithiocarbamate (Figure 9) for the preconcentration of trace levels of various metal ions in lake water and in water for pharmaceutical preparations prior to determination using XRF. Samples of 1L volume were taken and the heavy metal ions preconcentrated on the CPG-bound dithiocarbamate. In the case of the lake water, the CPG material was then analyzed for Zn(II), Cu(II), Ni(II), Mn(II), Hg(II), Pb(II), As(III), and Cr(III). Only Zn(II), Cu(II), Ni(II), and Mn(II) were detected. The other metals, if present, were below the minimum detectable quantity which was reported to be about 0.5 μg for all the elements determined. Larger volumes of sample, however, might be used to preconcentrate the other metal ions so that they are present at quantities greater than the MDQ. In the analysis of water used for pharmaceutical formulations, Pb(II), Cu(II), and Fe(III) were determined in the low ppb range. Again, other metals could be determined by increasing the sample size.

The silica-immobilized bis-dithiocarbamate of ethylenediamine (Figure 7) has also been used for preconcentration of trace levels of certain metal ions on a special type of filter paper (which contains silica gel) prior to XRF determination of the metal ions (38). The method was applied to distilled water samples and to some organic samples (i.e., dyes, excipients, etc.). Acidified samples of 10 mL to 150 mL were adjusted to pH 5.3 using acetate buffer and ammonia and then passed through a filter at a surface-specific flow rate of 10 mL/(cm^2-min). The sample solution was recycled through the filter 10 times, followed by drying, and mounting on a Teflon disk for x-ray measurement. Metal ions determined included Cu(II), Zn(II), Pb(II), Ni(II), and Fe(III). MDQ in a mixture of these metal ions ranged from 2 μg for Pb(II) to 11 μg for Zn(II). Results obtained from distilled water using this method agree very well with those obtained by evaporation followed by coprecipitation with sodium dibenzyldithiocarbamate. Extreme care must be taken to avoid contamination when using the latter technique, however. Preconcentration of trace metals from some organic samples after digestion with H_2SO_4/HNO_3 was also reported. However, no data was presented.

Leyden et al. (37) have also critically compared several preconcentration methods for trace metal ion determination by XRF including one method involving the use of CPG-immobilized bis-dithiocarbamate from ethylenediamine (Figure 7). Other preconcentration techniques investigated include ion-exchange impregnated filter paper, sodium diethyldithiocarbamate precipitation, ammonium pyrrolidinedithiocarbamate precipitation, and the metal oxinate complexes adsorbed on activated carbon. This paper is one of the few papers which carefully studied interferences due to the presence of elements other than the element of interest. Only Cu(II) and Ag(I) determinations are uninfluenced by the presence of other metal ions. Differences between energy and wavelength dispersive XRF were also discussed. The metal ions of interest included Cr(III), Mn(II), Fe(II), Co(II), Zn(II), Hg(II), As(III), Pb(II), and Ag(I). The MDQ values using the silica-bound dithiocarbamate range from 2.0 μg for Pb(II) using energy dispersive XRF to 100 μg for Cr(III) using wavelength dispersive XRF. The authors apply this technique to some environmental water samples (wastewater, lake water, river water, and tap water). The CPG-dithiocarbamate method gives results which agree fairly well with those obtained using activated carbon adsorption of metal oxinates and precipitation with ammonium pyrrolidinedithiocarbamate. The ion-exchange impregnated filter, which is based on a sulfonic acid ion exchanger, gives consistently lower results which is probably due to saturation of the exchange sites by Ca(II) and Fe(III) in the water samples. The sodium diethyldithiocarbamate precipitation method also gives lower results. However, no explanation was given for this behavior. Leyden (9) also reports the use of a mixed silica-immobilized ethylenediamine plus the silica-immobilized dithiocarbamate (Figure 9) for the preconcentration of metal ions prior to XRF determination. Nitrogen pressure was used to force the sample solution through a packed Teflon column at a flow rate of 30-40 mL/min. The silica material was then dried and mounted between thin Mylar sheets for the XRF measurement.

In one of the few applications other than preconcentration of trace metals, Chow and Grushka (40) have used a Cu(II)-loaded N-alkyldithiocarbamate prepared from silica-bound propylamine for the ligand exchange chromatographic separation of aromatic amines. The differences in retention (as reflected in the capacity factors, k') for the solutes were observed to increase for nearly all solutes when Cu(II) is bonded to the bound dithiocarbamate. Only those solutes which do not have an amine group eluted faster on the column containing Cu(II) in some cases. Also, it was observed that the less basic aniline isomers (e.g., o-chloroaniline) exhibited a smaller increase in the capacity factor than the more basic isomers. This is, of course, due to less affinity of weakly basic amines for the Cu(II). Another interesting observation was the increased plate heights observed for solutes on the Cu(II)-loaded column relative to the column without Cu(II). This is in spite of the fact that greater selectivity is observed on the Cu(II)-loaded column. This decrease in efficiency was attributed to slow dissociation of the solute-Cu(II) complex as was observed previously for a Cd(II)-diamine complex (36). A separation of eight substituted anilines in 8 min. on the Cu(II)-dithiocarbamate column was shown as an example. The applications of silica-immobilized dithiocarbamates are summarized in Table VI.

Unlike the previously discussed silica-bound ethylenediamine, it appears that the major use of silica-bound dithiocarbamates lies in the preconcentration of trace levels of metal ions prior to determination by some instrumental technique that can be used directly on the solid. The primary advantage of silica-immobilized dithiocarbamates is the large formation constants of their metal complexes which allows quantitative extraction of many metal ions even at relatively low pH (although low pH cannot be used due to hydrolysis). However, the large formation constants also prevent elution once extraction has taken place so that surface analytical techniques must be used for determination of the metal ions. Additionally, the poor selectivity and considerable instability

TABLE VI

SUMMARY OF APPLICATIONS OF SILICA-IMMOBILIZED DITHIOCARBAMATES

Structure (Figure No.)	Application*	Species Determined	Determination Technique	Comments	Ref.
7	P(C)	Pb,Ca,Hg, Tl	ESCA	100 mL synthetic samples. Fiberglass support. (MDQ = 1.0 μg for all metals)	39
9	P(C)	Zn,Cu,Ni, Mn,Hg,Pb As,Cr	XRF	Lake water and water for pharmaceuticals. 1 L samples. (MDQ = 0.5 μg for all metals).	16
8	P(C)	Cu,Zn,Pb, Ni,Fe	XRF	Silica-containing filter paper support. 10-150 mL samples of distilled water or digested organic samples. (MDQ = 2-11 μg)	38
8	P(C)	Cr,Mn,Fe, Co,Cu,Zn, Hg,As,Pb, Ag	XRF	Various environmental water samples (100 mL). Interferences studied. Compared to other preconcentration techniques. (MDQ = 2-100 μg)	37
9	S(LE)	aromatic amines	S	Cu(II)-loaded dithiocarbamate compared to Cu(II)-loaded β-diketone.	40

*All applications involve chelation.

Application

P = Preconcentration
S = Separation
C = Cations
LE = Ligand exchange

Determination Technique

ESCA = Electron spectroscopy for chemical analysis
XRF = X-ray fluorescence
S = Spectrophotometric

of the bound dithiocarbamates indicate little promise for their use under chromatographic conditions. Their poor storage stability also means that fresh material should be prepared often which can become time consuming in routine use.

8-Quinolinol

Preparation

One of the most promising of the silica-immobilized chelating agents that have been reported to date is based on the immobilization of 8-quinolinol which is also sometimes called 8-hydroxyquinoline or oxine (HOx). The immobilization of HOx on silica supports generally involves several steps which is in contrast to the preparation of silica-bound ethylenediamine which requires only one step and the preparation of the silica-bound dithiocarbamates which requires two steps. Actually, the immobilization procedure that has been used almost exclusively for bonding of HOx to silica has been adopted from the work of Weetall (41, 42) which describes the covalent attachment of enzymes to silica substrates. Hill (43) and Sugawara et al. (44, 45) first described the preparation of silica-immobilized HOx.

Both immobilization procedures involve the following five steps:

1. Silylation using aminopropyltriethoxysilane (or other trialkoxy- or trihalo-aminosilane).

2. Amidization of the resulting aminosilane with p-nitrobenzoyl-chloride.

3. Reduction of the resulting aromatic nitro group to an amine with sodium dithionite.

4. Diazonium salt formation with nitrous acid.

5. Diazo coupling to HOx.

The synthetic pathway described above is illustrated in Figure 11.

Diazo coupling is a convenient method for immobilization. The azo bond, once formed, can be cleaved by using a reducing agent (such as sodium dithionite) and the resulting aromatic amine can be rediazotized and coupled to the same, or a different, ligand without repeating the entire immobilization procedure (41, 44). Also, the diazo coupling reaction results in the formation of an azo dye which is brightly colored. This gives an immediate qualitative indication of the success, or failure, of the immobilization procedure since light yellow or light orange products give low capacities and further evaluation in such cases is unnecessary. It should be pointed out, however, that only certain chelating agents can be immobilized via diazo coupling since the weakly electrophilic diazonium salt requires the presence of a strongly electron releasing group on the aromatic ring at which coupling takes place. This generally restricts use to aromatic amines and phenols.

Although the reactions employed by Hill (43) and Sugawara et al. (44, 45) are essentially the same (Figure 11), many of the reaction conditions are different. For example, Hill uses a 4 hour reaction of a 4% solution of aminopropyltriethoxysilane in acetone for the initial step whereas Sugawara et al. use the overnight reaction of a 10% solution of the same silane in refluxing toluene. There are also other differences in reaction times, solvents, and conditions used for many of the other steps. For this reason, Fulcher et al. (46) have carried out an extensive study and have attempted to optimize many of the synthetic aspects related to HOx immobilization on silica substrates. Table VII summarizes the synthetic modifications which have been investigated and

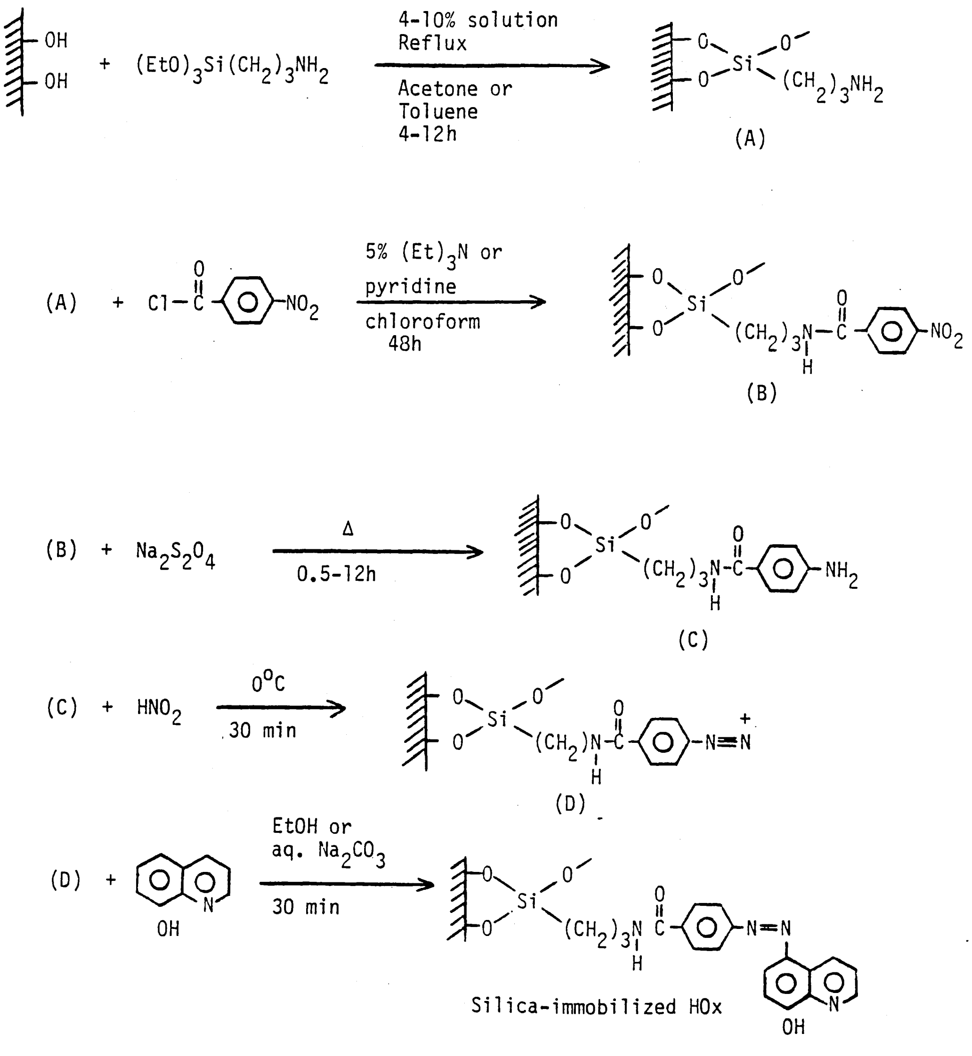


Figure 11. Synthesis of Silica-Immobilized 8-Quinolinol

TABLE VII

EFFECT OF SYNTHETIC VARIATIONS OF SILICA-IMMOBILIZED HOx (SUMMARIZED FROM REFERENCE 46)

Step[a]	Variation	Effect
1	Bound ethylenediamine vs. bound monoamine	Bound ethylenediamine yields slightly larger capacities
1	Refluxing toluene solvent vs. 45°C acetone solvent (both for 4h)	Unclear[b]
1	Vacuum degassing prior to reflux	Unclear[b]
1-2	Curing after silylation	Unclear[c]
2	Pyridine vs. triethylamine to remove liberated acid	Triethylamine yields larger capacities
2	15-fold excess of acid chloride vs. 2-3 fold excess	2-3 fold excess gives larger capacities
3	Boiling dithionite (30-60 min) vs. 45°C dithionite (overnight)	Boiling dithionite gives 50% higher capacities
4	$NaNO_2$ in HCl vs. $NaNO_2$ in $HC_2H_3O_2$	No difference
4	150 min. reaction vs. 30 min. reaction	No difference, therefore shorter reaction time is preferred
5	30 min. to 150 min. reaction time	No effect, therefore shorter reaction time is preferred

TABLE VII (Continued)

Step[a]	Variation	Effect
5	aq. Na_2CO_3 at 0-6°C for 24h vs. EtOH at room temperature for 30 min.	EtOH preferred due to quick reaction

[a] Step 1 = silylation; Step 2 = amidization; Step 3 = $-NO_2$ reduction; Step 4 = diazonium salt formation; Step 5 = coupling.

[b] Degassing was also performed with toluene but never with acetone. Therefore, increased surface coverages observed with toluene may be due to solvent, temperature, or degassing or a combination.

[c] Curing was normally performed although the authors claim that it was unnecessary. However, they do not present stability data to confirm this claim.

the results of these modifications. Many other aspects of the reactions such as pH, solvents, different reagents, etc., have still not been studied in detail.

It should be mentioned that, although Fulcher et al. (46) have attempted to optimize the existing synthesis, little work to date has been directed towards providing a more direct route to silica-bound HOx (see Chapter III).

Characterization

Capacity. Capacities (as well as surface coverages) obtained for silica-immobilized HOx are generally lower than capacities obtained for silica-immobilized ethylenediamine or silica-immobilized dithiocarbamates as can be seen in Table VIII. In some cases, capacities are lower by one order of magnitude or more. There are several plausible explanations for this observation. One probable contributing factor to the lower capacities observed is the longer synthetic route involved in most HOx immobilizations. A low yield at any one of the five steps could cause decreased capacities and surface coverages. The bulkiness of the HOx group could also contribute to lower capacities and surface coverages due to steric hindrance of neighboring reactive sites. This latter point has been made by Fulcher et al. (46). Pore size restrictions may also contribute to decreased capacities due to steric hindrance of bulky or lengthy groups attached to the surface due to limited pore size as pointed out previously (46). However, one would expect to see much lower surface coverages for high surface area/small pore diameter silica supports than for the lower surface area/large pore diameter silica supports. This has not been clearly indicated by the limited amount of data presented

TABLE VIII

SUMMARY OF CAPACITY STUDIES FOR SILICA-IMMOBILIZED 8-QUINOLINOL

Row	Mean Specific[a] Surface Area (m^2/g)	Mean Pore Diameter (Å)	Capacity (μmoles/g)	Apparent[b] Surface Coverage $(\mu moles/m^2)$	Capacity Method	Probe	Metal Determination Method	Ref.
1	70	550	18	0.26	1	Cu^{2+}	AA	47
2	70	550	22	0.31	2	Cu^{2+}	EDTA Titn	48
3	70	550	17	0.24	2[c]	Cu^{2+}	Visible Spec.[d]	45
4	80	550	57	0.71	1	Fe^{3+}	Visible Spec.[e]	44
5	57	544	39	0.68	1	Cu^{2+}	AA	47
6	200	150	54	0.27	2	Cu^{2+}	EDTA Titn	48
7	200	150	61	0.30	2	Cu^{2+}	AA	49
8	550	60	60	0.11	2	Cu^{2+}	EDTA or AA	46
9	550	60	140	0.25	2	Cu^{2+}	EDTA or AA	50
10	480	70	178	0.37	2	Cu^{2+}	EDTA or AA	50
11[f]	550	60	138	0.35	2	Cu^{2+}	EDTA or AA	46

[a] Before silylation and subsequent ractions.

[b] Assuming 1:1 metal to ligand ratio although this may not be the case.

TABLE VIII (Continued)

Row	Mean Specific[a] Surface Area (m^2/g)	Mean Pore Diameter (Å)	Capacity (μmoles/g)	Apparent[b] Surface Coverage (μmoles/m^2)	Capacity Method	Probe	Metal Determination Method	Ref.

[c] Column breakthrough (dynamic).

[d] Bathocuproine procedure.

[e] 1,10-phenanthroline procedure.

[f] Based on bound ethylenediamine instead of bound propylamine.

in Table VIII. Perhaps smaller pore diameters than those studied thus far are necessary in order to observe a significant steric effect due to pore size.

Stability. Hill has reported that the silica-bound HOx is stable between pH 0 and 9 for periods of at least two months (43). Absorption spectra of solutions at pH>10 indicate strong absorption at 500 nm (red) within 24h of mixing. This red color indicates that the azo bond remained intact and therefore the decomposition was probably due to hydrolysis of the silica support. After one month, solutions at pH 9 and in 10M HCl absorbed only slightly at 500 nm. It was also reported that the silica-immobilized HOx was stable in chloroform, benzene, acetic acid, and acetone for at least two months. No capacity studies were undertaken to indicate the amount of decomposition that occurred. Sugawara et al. (44, 45) also indicate good hydrolytic stability in neutral and acidic media with slight degradation indicated by a brownish tint in ammoniacal medium. Only visual inspection was performed. No quantitative results were presented.

Fulcher et al. have presented thermogravimetric evidence of the thermal stability of these materials at temperatures up to 250^{o}C. Actually, some weight loss is observed at temperatures of 25^{o}C up to about 150^{o}C but this has been attributed to loss of adsorbed water and is also observed for silica gel itself. Although little information is available, it appears that the shelf life is good.

Rate Studies. An extremely limited amount of data is available concerning the rate of extraction of metal ions from solution onto silica-immobilized HOx. Sugawara et al. (44) indicate that after only

2 min. of shaking, 95% of Fe(III) was extracted at pH 5.0 (84% after 1 min.) onto a CPG-bound HOx. Other metal ions with smaller formation constants with HOx were not studied. However, in the patent issued to Schucker, Sugawara, and Weetall (45), they indicate an extremely slow rate of extraction for Ni(II) onto CPG-bound HOx at pH 8.0 and pH 8.5 in aqueous ammonia. Apparently, the ammonia ligand is only very slowly displaced by the bound HOx and 60 hours are required for quantitative extraction of the Ni(II). Ni(II), however, is known to undergo slow ligand exchange as pointed out by Jezorek and Freiser (48) and therefore, it is probably the exception rather than the rule. Sturgeon et al. (49) have reported the use of 0.60g portions of silica-immobilized HOx in columns at flow rates up to 80 mL/min with quantitative recovery of all metal ions studied except Mn(II) which was only 55% recovered. The metal ions studied included Cd(II), Pb(II), Zn(II), Cu(II), Fe(III), Mn(II), Ni(II), and Co(II). This was shown to be a significant improvement for trace metal preconcentration over the conventionally used Chelex-100, an iminodiacetate chelating resin which is generally limited to flow rates of 1-2 mL/min. and requires careful washing to remove Ca(II) and Mg(II).

pH Dependence. The extraction behavior of several metal ions by silica-bound HOx has been investigated by Sugawara et al. (44). Plots of percent extraction as a function of pH under batch conditions, were obtained and the general extraction pattern was similar to that obtained using HOx/chloroform in liquid-liquid extractions. Jezorek and Freiser (48) have also investigated selected metal ion distributions on silica-immobilized HOx. However, these workers determine distribution ratios (rather than percent extracted) under chromatographic as well as batch conditions. They use their data to justify the assumption of a 1:1 metal to ligand ratio for the immobilized metal-HOx complex. The mathematical justification follows.

Let us assume a 1:1 complex is formed:

Therefore:

$$M + Ox_B^- \rightleftharpoons MOx_B \qquad \beta_1 = \frac{[MOx]_B}{[M]_B\,[Ox^-]_B} \tag{16}$$

where the subscript, B, refers to the bound species. Also,

$$HOx_B \rightleftharpoons H^+ + Ox_B^- \qquad K_{a_2} = \frac{[H^+][Ox^-]_B}{[HOx]_B} \tag{17}$$

Solving (17) for $[Ox^-]_B$ and substituting into (16) we have:

$$\beta_1 = \frac{[MOx]_B}{[M]} \times \frac{[H^+]}{K_{a_2}\,[HOx]_B} = D\,\frac{[H^+]}{K_{a_2}\,[HOx]_B} \tag{18}$$

or, rearranging

$$D = \frac{\beta_1 K_{a_2}\,[HOx]_B}{[H^+]} \tag{19}$$

From mass balance, we have:

$$C_{HOx} = [MOx]_B + [H_2Ox^+]_B + [HOx]_B + [Ox^-]_B \tag{20}$$

at neutral pH and low metal ion loading, (2) becomes:

$$C_{HOx} \simeq [HOx]_B \tag{21}$$

and (19) becomes:

$$D = \frac{\beta_1 K_{a_2} C_{HOx}}{[H^+]} \qquad (22)$$

taking common logarithms, we obtain:

$$\log D = \log \beta_1 K_{a_2} C_{HOx} - \log [H^+] = \log \beta_1 K_{a_2} C_{HOx} + pH \qquad (23)$$

or

$$\log D = pH + \log \beta_1 K_{a_2} C_{HOx} \qquad (24)$$

From Equation (24) above, a plot of log D vs. pH should be linear with a slope = 1 at low metal ion loading and at neutral pH if a 1:1 complex is formed. A 1:2 metal to ligand ratio would give a slope = 2. The plots for several metal ions under both batch and column conditions were presented by these workers (48). The slope for the Cd(II) plot under batch conditions is 1.01 ± 0.04. It should be mentioned that the assumptions implicit in this treatment were not pointed out by the authors and these assumptions, in fact, are very questionable under the conditions employed in their experiments (i.e., relatively low pH and relatively high metal loading). The non-linearity of some of the curves is probably attributable to these poor assumptions. These workers also point out differences in apparent D values that can arise between batch and column studies. Generally, metal ions which are known to undergo rapid ligand substitution show little difference between batch and column D values. However, those metal ions which undergo ligand substitution slowly generally exhibit a much lower D value under dynamic column conditions due to non-equilibrium. For example, Cd(II) was shown to give about the same D values by column and batch methods whereas Ni(II), which exhibits slow substitution, gives D values as much as 80% lower by the column method than by the batch method. This paper (48) also was the first to really examine the potential of silica-bound HOx under chromatographic conditions for metal ion separations using a continuous pH gradient.

The extraction onto silica-HOx of certain anions, in particular some selected aliphatic and aromatic sulfonates, as a function of pH was reported by Kuo and Mottola (47) under batch conditions. These anions were strongly extracted as an ion-pair at $pH < 3$ which corresponds to pH values less than the approximate pK_{a_1} of the bound HOx. An approximately linear dependence of D on pH was reported in this region with the aliphatic sulfonates showing considerably larger D values at a given pH. However, individual sulfonates exhibited large enough differences in D values to allow chromatographic separation. No column distribution studies were presented. Sugawara et al. (44) also studied the extraction of two anions, molybdate and tungstate, as ion-pairs onto silica-bound HOx. The extraction behavior observed was similar to that observed for these same anions onto silica-immobilized ethylenediamine with essentially 100% extraction at low pH with a decreasing percent extraction observed as pH increases above pH 4.

Miscellaneous Studies. Infrared characterization (46) and acid-base studies (48, 51) have been reported. The infrared characterization is of little use due to the strong affinity of water vapor for the silica gel support, the small amount of organic material bound to the surface, and light scattering problems particularly with larger particles.

Essentially the only information that could be obtained was about the reduction of the aromatic -NO_2 species to the amine. The -NO_2 stretching vibrations are strong enough to be readily observed and are in a region of the spectrum unobscured by matrix absorption.

Of course, HOx is amphoteric, containing both a weakly basic heterocyclic nitrogen as well as a weakly acidic phenol group. Acid-base titrations have been undertaken in order to characterize these materials as well as to examine the effect of immobilization on acid-base properties. Jezorek and Freiser (48) initially reported $pK_{a_1} = 3.3$ and $pK_{a_2} = 6.2$ using NaOH titrant. This compares to $pK_{a_1} = 4.85$ and $pK_{a_2} = 9.95$ for free HOx. Later work by Jezorek et al. (51) indicates $pK_{a_1} = 7.0 - 7.5$ using HCl as titrant. This discrepancy in apparent pK_{a_1} values is puzzling and no explanation was given for the difference. However, the acidic nature of residual surface silanol groups is neglected in both of these studies although the number of unreacted silanol groups is undoubtedly large. (See Chapter IV.)

Applications

Silica-immobilized HOx, like silica-immobilized ethylenediamine has been used for a variety of purposes. This, of course, is in contrast to silica-bound dithiocarbamates which are relatively restricted in their use. The usefulness of silica-immobilized HOx for preconcentration and separation of trace metal ions was first demonstrated by Hill (43) who investigated the preconcentration of traces of copper(II) from relatively concentrated salt solutions. It was shown that copper(II) could be quantitatively extracted from 1 liter of 10^{-6}M Cu(II) in the presence of 2 M KNO_3, 2 M $Ca(NO_3)_2$, 1.33 M Na_2HPO_4, and 0.75 M $MgSO_4$. This same author also demonstrates the column chromatographic separation of Mn(II), Cu(II), and Zn(II), after preconcentration, by a stepwise pH gradient elution. Mn(II) was completely eluted at pH 3, Zn(II) at pH 2, and Cu(II) at pH 1. This elution order follows the order of increasing formation constants of the metal-HOx complexes in solution as expected. Only synthetic samples were analyzed. No real samples were studied.

Moorehead and Davis (52) used commercially available CPG-immobilized HOx for the cleanup of unwanted trace metal impurities from relatively concentrated solutions (≃1M) which were to be used as supporting electrolytes in the anodic stripping voltammetric determination of gallium. Normally, lower concentrations of supporting electrolyte are used and trace impurities are not a problem. However, in this case more concentrated thiocyanate was required in order to obtain polarographic reversibility of gallium. Purification by controlled potential electrolysis, as is commonly done, was not possible due to anode-generated thiocyanate oxidation products. CPG-bound HOx effectively reduced the background interference due to traces of heavy metal ions.

Preconcentration of trace metal ions in distilled-deionized water was carried out by Sugawara et al. in order to demonstrate the usefulness of CPG-HOx (44). A given volume of distilled/deionized water buffered at pH 5.0 was passed through a column of CPG-HOx at a flow rate of 5 mL/min. The extracted metals were then eluted with 50 mL of 1.0M HCl and the amount of Fe(III) and Cu(II) determined spectrophotometrically using bathophenanthroline and bathocuproine procedures, respectively. Fe(III) was not detected in 4L of distilled/deionized water (< 0.25 ppb) but Cu(II) could be detected at the 8 ppb level using a 1L sample. Guedes da Mota et al. (53) have also employed CPG-HOx in a preconcentration application. Cu(II) in river water was preconcentrated prior to atomic absorption determination. This procedure was compared to one using an anion exchange column for preconcentration of the anionic bromide complex of Cu(II). Higher flow rates could be used with the CPG-HOx column due to less flow resistance. Thus, only 3.5h is required to preconcentrate Cu(II) from a 2L water sample on CPG-HOx whereas the anion exchange column requires 18h. Cu(II) could be determined in the low ppb range using this procedure. More recently, Sturgeon et al. (49) have employed silica-immobilized HOx for preconcentration of a variety of trace metal ions from seawater prior to their determination by graphite furnace atomic absorption spectroscopy. Metals determined included Cd, Pb, Zn, Cu, Fe, Mn, Ni, and Co. Seawater aliquots (500 mL for coastal samples and 900 mL for open ocean samples) were preconcentrated at pH 8.0 using a column containing 0.6g of silica-HOx. The extracted metals were then eluted using 10 mL of a 1.0 M HCl/0.1 M HNO_3 solution with which quantitative recovery of the extracted metals was obtained. A standard addition technique was used to reduce matrix effects. The above elements could be determined at sub-ppb levels even in seawater of 35% salinity. Results obtained using the silica-HOx/graphite furnace atomic absorption technique were shown to agree extremely well with results obtained using an iminodiacetate chelating resin preconcentration followed by determination using graphite furnace atomic absorption spectroscopy, isotope dilution spark source mass spectrometry, and inductively coupled argon plasma spectroscopy.

In one of the more novel applications, Jezorek and Freiser (48) have used silica-bound HOx for the separation of several metal ions under continuous pH gradient conditions. A commercially available, dual-pump chromatograph was used. Their work showed that efficient isocratic elution is limited to a narrow range of D values between about 1 and 4. At $D < 1$ the metal ion elutes approximately in the void volume and if $D > 4$, peaks are too diffuse to be observed. However, a relatively large particle size material (Porasil B, 37-74 μm diameter) was used and the effect of particle size on efficiency was not explored. Smaller particle size could increase the efficiency and extend the applicable range of D values to higher values. However, the pressure drop observed with the larger diameter material is small and this allows a low pressure system to be used. Also, the low capacity of the silica-HOx used may contribute to the relatively poor efficiency (plate heights 2.5 - 5.0 mm). This points out the need for more efficient synthetic procedures in order to increase the capacity. Another factor that contributes to the low efficiency is the post column reactor used in the detection step of the metal ions. The reactor adds considerable dead volume to the system which results in increased band broadening. Detection was based on the reaction of the separated metal ions with 4-(2-pyridylazo)resorcinol (PAR) at high pH to form a red metal-PAR complex that possesses a large molar absorptivity ($\simeq 10^4$) at 500 nm (54). Detection of the alkaline earth metals and others which do not react with PAR directly depends on the displacement of Zn(II) from a Zn-EDTA complex which is added to the PAR solution. The displaced Zn(II) then reacts with PAR to form the red Zn-PAR complex. The reaction takes place in two mixing coils of 1 m length each (0.8 mm i.d. tubing) which corresponds to approximately 1.0 mL volume. No attempt was made to optimize the size and configuration of the post-column reactor. Despite the poor efficiency, some separations can be achieved.

The separation of some aromatic sulfonates by ion-pair chromatography on CPG-HOx has been reported by Kuo and Mottola (47). Actually, the separation of aliphatic sulfonates is also possible. However, on-line detection using UV monitoring is difficult for the aliphatic species. As an example, benzenesulfonate, p-toluenesulfonate, 2-mesitylenesulfonate, and 1-napthalenesulfonate in a synthetic mixture were separated within an hour on a 99 x 0.20 cm column packed with CPG-HOx (37-74 μm) at pH 2.8 using perchlorate as the eluent. Again, the relatively large particle size and low capacities are deleterious to efficiency as plate heights are 3.0 - 3.5 mm for the 37 - 74 μm material.

More recently, Shahwan and Jezorek (50) have demonstrated the use of the silica-immobilized Fe(III)-HOx complex for the ligand exchange chromatographic separation of phenols. Unlike the previously discussed applications of ligand exchange chromatography which required the presence of metal ion in the mobile phase (35, 36, 40), no such measure is required in the case of the silica-bound Fe(III)-HOx complex. The reason for this is believed to be due to the very large formation constant for the Fe(III)-HOx complex as is observed in solution. Also, Fe(III) has great affinity for oxygen and therefore is able to readily interact with the phenol group of the sample species. An acetonitrile/acetate buffer solution (pH = 4.0) mobile phase was used. In some cases, gradient elution was applied. Also, a small particle size silica gel (10 μm) with larger capacities than normally encountered was used. Application of this technique to the separation of 11 phenolic compounds found on EPA's priority pollutant list was demonstrated with reasonably good efficiency and resolution. However, as of yet, ligand exchange has not been able to compete with good reverse-phase columns in terms of efficiency. A summary of applications of silica-bound HOx is presented in Table IX.

It appears that silica-bound HOx is an extremely useful and versatile chelating agent. Soluble HOx reacts with more than 60 metal ions to form complexes having a wide range of aqueous phase formation constants. This points out the general applicability to a variety of metal ions on the one hand and the considerable selectivity on the other hand. For this reason, silica-immobilized HOx appears to be one of the best all-around silica-bound chelating agents available. The primary problem with silica-bound HOx is the low capacities observed and lengthy synthetic route normally required.

It should be mentioned that more selectivity, if required, might be obtained by simply immobilizing one of the more selective derivatives of HOx such as 8-mercaptoquinoline or 2-methyl-8-quinolinol. Both of these reagents have been well characterized in solution although neither has been immobilized to date.

β-Diketones

Preparation

Although not as widely used as the previously discussed bound chelating agents, the so-called β-diketones have found some applications and also have served a very important role in the development of spectroscopic techniques for the study of silica-immobilized species. Actually, many of the so-called β-diketones are β-keto-amides or β-keto-esters. However, throughout this section all of these species will be referred to as "β-diketones" as they are in the literature.

The preparation of silica-bound β-diketones generally involves reaction of an immobilized primary amine (i.e., bound propylamine or bound ethylenediamine) with diketene or ethylacetoacetate as shown in Figure

TABLE IX

SUMMARY OF APPLICATIONS OF SILICA-IMMOBILIZED 8-QUINOLINOL

Application	Species Determined	Determination Technique	Comments	Ref.
S(C)*	Mn,Cu,Zn	NA[a]	Synthetic sample (2 x 15 cm column). Stepwise pH gradient elution.	43
P(C)*	Cu	S	Synthetic sample (1 L) containing conc. alkali and alkaline earth metal salts.	43
P(C)*	Pb,Cu,Cd[b] Zn	ASV	Actually used for cleanup of NaSCN used as supporting electrolyte for ASV.	52
P(C)*	Fe,Cu	S	Distilled/deionized water samples (1 L) (Samples in low ppb range).	44
P(C)*	Cu	AA	River water samples, compared to anion-exchange preconcentration. (Low ppb range for samples). Sample size not given.	52
P(C)*	Cd,Pb,Zn Cu,Fe,Mn Ni,Co	GFAAS	Seawater samples (500-900 mL). Sub-ppb levels for all metal ions. Compared to other accepted methods.	49
S(C)*	Various transition metal ions and lanthanides	S	Synthetic samples, pH continuous gradient elution. Post column detection.	48
S(A)	Selected aromatic sulfonates	S	Synthetic samples, manual pH gradient elution, (0.2 x 99 cm column). Low pressure system.	47

TABLE IX (Continued)

Application	Species Determined	Determination Technique	Comments	Ref.
S(LE)	Selected phenols	S	Bound Fe(III)-HOx complex. No need to add metal ion to mobile phase. Application to EPA priority pollutants. High pressure system.	50

[*] Involves chelation.

[a] Determination technique not specified.

[b] Detected but not determined.

Application

P = Preconcentration
S = Separation
C = Cations
A = Anions
LE = Ligand Exchange

Determination Technique

ASV = Anodic stripping voltammetry
S = Spectrophotometry
AA = Atomic absorption spectrometry
GFASS = Graphite furnace atomic absorption spectrometry

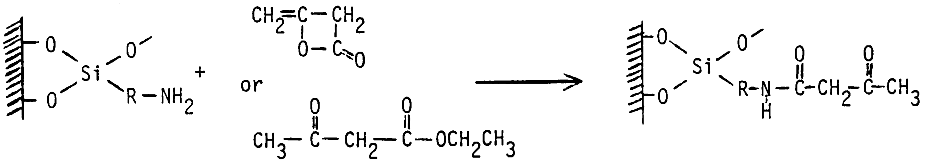


Figure 12. Synthesis of Silica-Immobilized β-Diketones

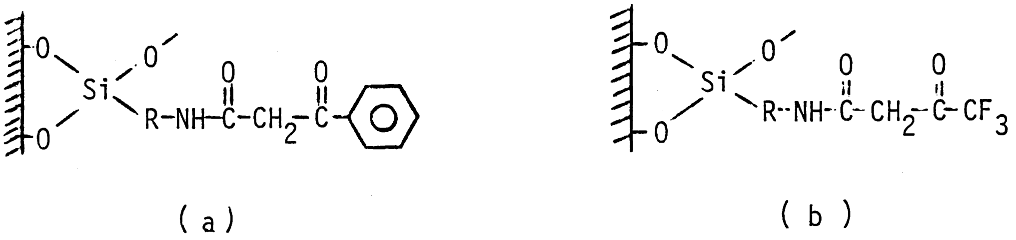


Figure 13. Benzene-Substituted β-Diketone (a) and Trifluoromethyl-substituted β-Diketone (b)

12 (28, 55-57). Diketene is preferred since ethylacetoacetate gives an unwanted Schiff base by-product. Ethylbenzoylacetate has also been used to give the benzene substituted β-diketone (Figure 13(a)) (28, 40) and 4,4,4-trifluoroacetoacetate has been used to give the trifluoromethyl-substituted β-diketone (Figure 13(b)) (28, 29). Both of the reactions leading to these products also suffer from the presence of the Schiff base by-products. Another β-diketone (Figure 14) has been prepared by the reaction of acetylacetone (2,4-pentanedione) with an immobilized benzylchloride (58-60). This reaction does not suffer from the unwanted

side reactions. Also, although not reported, this reaction should be applicable to β-diketones other than acetylacetone.

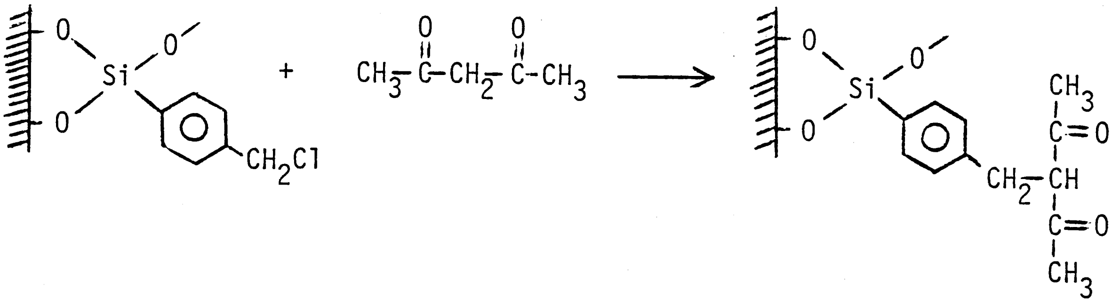


Figure 14. Alternative Synthesis of Silica-Immobilized β-Diketones

Characterization

Capacity. Very little systematic study of capacities of different silica-bound β-diketones has been performed. Table X summarizes the values reported. Although a comparison is difficult due to incomplete information about the support and capacity determination methods, some information can be extracted. Seshadri and Kettrup (55) indicated that the immobilized β-diketone (Figure 12) showed affinity only for Cu(II) and Fe(III) in neutral and acidic solutions. No significant affinity for Co(II), Mn(II), Ni(II), Zn(II), Mg(II), or La(III) was observed. Leyden et al. (57), however, have suggested that other bound metal-β-diketone complexes may form at higher pH where the enol form of the ligand is predominant. In fact, they showed spectroscopically that the

TABLE X

SUMMARY OF CAPACITY STUDIES FOR SILICA-IMMOBILIZED β-DIKETONES

Structure (Figure No.)	Mean Specific[a] Surface Area (m^2/g)	Mean Pore Diameter (Å)	Capacity (μmoles/g)	Apparent[b] Surface Coverage ($\mu moles/m^2$)	Capacity Method	Probe	Metal Determination Method	Ref.
12[c]	----	---	230	----	1	Fe^{3+}	Visible	55
	----	---	140	----	1	Cu^{2+}	Spectro.	
	----	---	196	----	1	UO_2^{2+}		
12[c]	300	---	200	0.67	---	Fe^{3+}	---	57
	----	---	520[e]	1.73	---	Cu^{2+}	---	
12[d]	300	---	100	0.33	---	Fe^{3+}	---	57
	----	---	430	1.43	---	Cu^{2+}	---	

[a] Before silylation and subsequent reactions.

[b] Assuming 1:1 metal to ligand ratio although this may not be the case.

[c] From bound ethylenediamine.

[d] From bound propylamine.

[e] Determined at pH 11 (aqueous NH_3).

Fe(III) and UO_2(II) complexes observed by Seshadri and Kettrup (55) were due to keto complexes rather than the more common enol complexes which are normally found in solution. Cu(II), however, appeared to form enol complexes only. This may provide the explanation for the large differences in capacities obtained by the two groups at different pH values when using Cu(II) as the metal probe. At the relatively low pH used in the studies of Seshadri and Kettrup (55), the amount of enol tautomer is less than the amount of keto tautomer present and therefore the amount of Cu(II) extracted does not correspond to the total amount of ligand on the surface. However, at the high pH used by Leyden et al. (57), the ligand is probably almost completely in the enol form and therefore the amount of Cu(II) extracted increases. No such effect was observed for Fe(III) which seems to form both keto and enol complexes. Seshadri et al. also reported a capacity of 1450 μmoles/g for silica-bound β-diketone (Figure 12) using carbon determinations (56). This seems to indicate a 1:2 metal to ligand ratio. However, as shown previously, several different ratios of metal to ligand can exist on the same material dedepending on the proximity of neighboring ligands and carbon determinations also suffer from errors due to the presence of non-chelating organic material.

Stability. Seshadri and Kettrup (55) reported hydrolytic stability of silica-immobilized β-diketone (Figure 12) at pH values between 0 and 8.5 although no data was presented. Presumably, the instability at pH > 8.5 was due to hydrolysis of the silica support. These same workers report thermal stability at temperatures of 100 - 105°C for periods of 2h or more. No mention of storage stability was found.

Rate Studies. The rate of extraction of uranyl cation onto silica-bound β-diketone (Figure 12) at pH 5 was determined (55). It was reported that 78% was extracted after 1 min., 96% after 2 min., and 98.4% after 5 min. Again the superiority of silica-bound chelating ligands over chelating resins in terms of rates of extraction is apparent. It should be pointed out that, unlike the rate studies previously mentioned, in this case pH was optimized for UO_2^{2+}.

pH Dependence. Again, little information is available, and as mentioned, silica-immobilized β-diketones react with only a few metal ions in neutral and acidic solution. The distribution ratios of Fe(III), UO_2(II), and Cu(II), however, have been determined under batch conditions as a function of pH in 0.5 M NaCl (55). It was shown, as expected, that an increase in pH results in an increase in D. However, D for UO_2(II) increased much more rapidly than for Fe(III) or Cu(II) up to pH 6. This indicates a high selectivity for UO_2(II) at this pH which, in turn, suggests the potential application of this material for UO_2(II) preconcentration and separation.

Miscellaneous Studies. As mentioned, silica-bound β-diketones have played an important role as model systems for the study of the applicability of spectroscopic techniques to bound surface species. Leyden and his coworkers have been largely responsible for these studies (57, 59, 60). The three most useful techniques which have been used to study surface-bound species are (1) carbon-13 cross polarization with magic angle spinning nuclear magnetic resonance spectroscopy (^{13}C-CPMAS-NMR), (2) Fourier transform infrared spectroscopy (FTIR), and (3) ultraviolet and visible photoacoustic spectroscopy (UV-VIS PAS). When these techniques are used along with a soluble model system, the structure of the bound species can, in some cases, be determined.

The reaction of acetylacetone with bound benzylchloride as shown in Figure 14 has been followed by using ^{13}C-CPMAS-NMR (59). Upon reaction of the benzylchloride, a peak observed at 50 ppm (relative to TMS), which was assigned to the methylene carbon of the benzyl chloride was observed to disappear and a peak at 31 ppm appeared. However, no carbon resonances due to the diketone were observed. This point remains unexplained. Loss of chlorine followed by XRF, as well as PAS and FTIR spectra of the bound species, indicated that a reaction had occurred. Other silanes (non-chelating) were also investigated and ^{13}C-CPMAS-NMR was generally able to confirm the expected structure which demonstrated the usefulness of the technique. The bound β-diketone (Figure 14) has also been studied by FTIR and UV-VIS PAS (60). Comparison to the model compound, 3-benzyl-2,4-pentanedione, was also performed to confirm the structure of the bound species. It was shown that the immobilized β-diketone was largely in the keto form and that the small fraction of the enol tautomer is hydrogen bonded to the surface. A similar study was carried out for the bound β-diketone in Figure 12.

Applications

The separation of UO_2(II) from lanthanides and thorium at pH 5 using the silica-immobilized β-diketone in Figure 12 was demonstrated in the preliminary work of Seshadri and Kettrup (55). This separation was deemed important since uranium occurs in nature in conjunction with these metals. Uranium was retained strongly on the column and was subsequently eluted with 1 M HCl. Sweet and coworkers have also studied the separation of metal ions (28, 29). However, in these studies, a thin-layer chromatographic technique was employed using the bound trifluoromethyl-substituted β-diketone (Figure 13b). Excellent separation of Fe(III), Cu(II), Ni(II), and Zn(II) was achieved using a trifluoroacetylacetone in acetone mobile phase. R_f values were 0.00, 0.81, 0.12, and 0.41 for the above metals, respectively. A variety of other silica-bound β-diketones were investigated with less than satisfactory results.

The use of silica-immobilized β-diketone (Figure 12) as a stationary phase in HPLC has also been studied (56). Application to the separation of o-, m-, and p-nitroanilines, aromatic carboxylic acids, urea herbicides, and o-, m-, and p-aminophenols was demonstrated. The effect of the presence of metal ions in the mobile phase or complexed to the bound ligand was not examined and only synthetic samples were used. It was shown that separation was due to the immobilized β-diketone and not due to the bound amine precursor.

Chow and Grushka (40) have also investigated the use of a silica-bound β-diketone in HPLC. They used the silica-immobilized β-diketone (Figure 13b) complexed with Cu(II) for the ligand exchange chromatographic separation of some aromatic compounds, mostly amines. These workers also investigated the use of a silica-bound Cu(II)-dithiocarbamate complex as discussed previously and most of the same observations are valid for the Cu(II)-diketone complex as well. However, the diketone column seems to be superior to the dithiocarbamate column due to the fact that smaller plate heights were obtained for the Cu(II)-diketone column than for the Cu(II)-dithiocarbamate column. Also, as mentioned, dithiocarbamates are relatively unstable. The Cu(II)-diketone column was shown to be useful for the separation of some fluorenes and fluorenones. Reverse-phase systems, however, can still provide better performance. A summary of applications of silica-immobilized β-diketones is presented in Table XI.

Although less information is available concerning silica-immobilized β-diketones relative to the other silica-bound ligands discussed thus far, it is apparent that these β-diketones may be useful for certain specific applications. Silica-bound β-diketones exhibit acceptable capacities, and, unlike the dithiocarbamates, are relatively stable. The major advantage, and disadvantage, to the use of silica-bound β-diketones for metal ion preconcentration/separation lies in the selectivity of the material. In acidic and neutral media, only UO_2(II), Cu(II), and Fe(III) appear to react to any extent. This makes preconcentration/separation of these metals in the presence of other metals possible which is an advantage. However, if other metal ions are of interest, or if a general-purpose chelating ion exchanger is desired, silica-immobilized β-diketones are probably not the best choice. The side reactions that occur in the preparation of some of the silica-immobilized β-diketones is also an undesirable aspect of these materials.

Crown Ethers

The macrocyclic polyethers, commonly called crown ethers, comprise a group of chelating agents which differ from previously discussed ligands in their potential applications. The previously discussed bound chelating agents generally have little, if any, affinity for alkali metals. However, the applications of the crown ethers discussed in this section will deal primarily with the separation of alkali metals with a common anion. The most stable and important complexes of the alkali metal ions are those with crown ethers and cryptates (which are bicyclic species similar to the crown ethers but containing nitrogen). The selectivity of the crown ethers is strongly dependent on how well the ion fits

TABLE XI

SUMMARY OF APPLICATIONS OF SILICA-IMMOBILIZED β-DIKETONES

Structure (Figure No.)	Application	Species Determined	Determination Technique	Comments	Ref.
12	S(C)* P(C)*	U	S	Poor selectivity for metals other than Cu, Fe, and U.	55
13b	S(C)*	Fe,Cu,Ni,Zn	---[a]	TLC separation. Good separation using a β-diketone in the mobile phase.	28,29
12	S(O)	Nitroanilines urea herbicides aromatic acids amino phenols	S	Good separation but inferior to reverse-phase HPLC.	56
13b	S(LE)	Aromatic amines	S	Bound Cu(II)-β-diketone complex. Superior to Cu(II)-dithiocarbamate. Reverse-phase HPLC is still superior.	40

*Involves chelation.

[a] TLC procedure (non-quantitative).

Application

S = Separation
C = Cations
O = Organics
LE = Ligand Exchange

Determination Technique

S = Spectrophotometric

into the cavity of the crown ether. Other factors affecting selectivity include the counterion, solvent, and proximity of other crown ethers in heterogeneous systems.

Preparation

Several different immobilization procedures as well as several different structures have been described for silica-bound crown ethers. The simplest of these are the silica-bound monomeric crown ethers (61 - 64). Three different structures have been described for these materials.

The most direct attachment of a crown ether (benzo-15-crown-5) to silica was reported by Blausius et al. (61, 62) (Figure 15). It was prepared by reaction of 4-hydroxymethylbenzo-15-crown-5 with chlorinated silica gel. However, the Si-O-C linkage is known to be highly susceptible to hydrolysis and therefore, this structure, although simple, is not very useful in aqueous solutions.

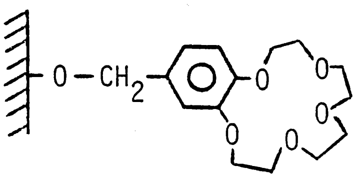


Figure 15. Directly Attached Benzo-15-Crown-5

Waddell and Leyden (63) have reported the preparation of silica-bound dibenzo-18-crown-6 by treating a bound benzyl chloride with dibenzo-18-crown-6 and aluminum chloride in carbon disulfide in a typical Friedel-Crafts alkylation reaction. The structure of the resulting product is shown in Figure 16. This is a very convenient method for immobilizing benzene ring-containing crown ethers on silica surfaces since it requires no modification of the commercially available crown ether prior to immobilization.

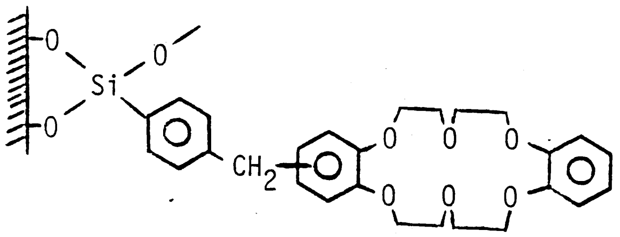


Figure 16. Silica-Immobilized Dibenzo-18-Crown-6

Benzo-15-crown-5 has been bound to silica by condensation of the benzoyl chloride derivative of benzo-15-crown-5 with 3-aminopropyltriethoxysilane in chloroform followed by reaction of the resulting silane with silica gel (Figure 17) (64). This material proved useful for the separation of some alkali metals. However, silica-bound bis- and polycrown ethers have been shown to be even more useful (64, 65). Preparation of the bis(benzo-15-crown-5) has been performed by condensing the benzoyl chloride derivative of the crown ether with silica-immobilized ethylenediamine (65). The silica-immobilized bis(benzo-15-crown-5) structure is shown in Figure 18. The synthetic pathway leading to the silica-immobilized poly(benzo-15-crown-5) is shown in Figure 19 (65, 66). The synthesis used is, of course, applicable to other crown ethers as well.

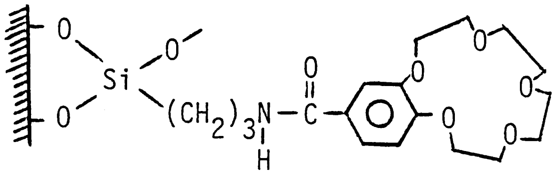


Figure 17. Silica-Immobilized Benzo-15-Crown-5

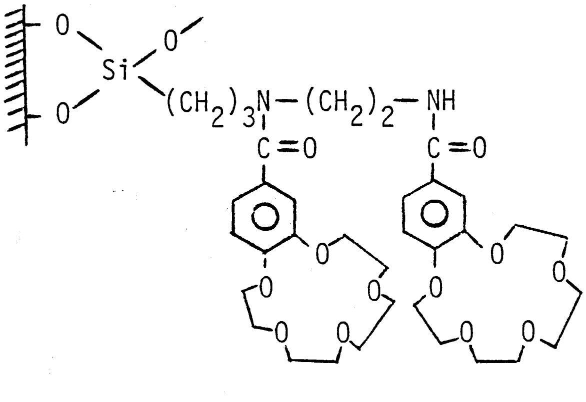
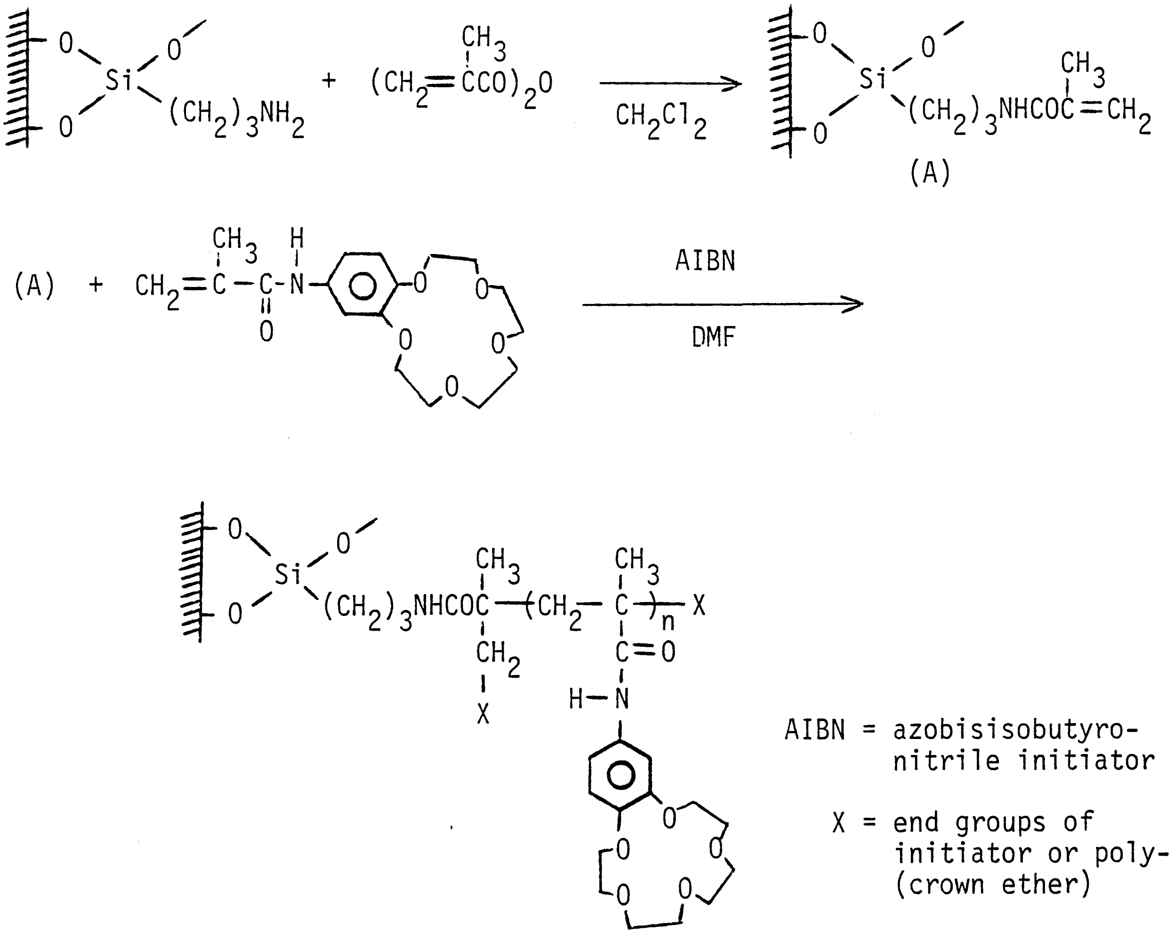


Figure 18. Silica-Immobilized Bis(Benzo-15-Crown-5)



AIBN = azobisisobutyronitrile initiator

X = end groups of initiator or poly(crown ether)

Figure 19. Synthesis of Silica-Immobilized Poly(Benzo-15-Crown-5)

Characterization

Capacity. The capacities obtained for most of the silica-bound crown ethers mentioned above are presented in Table XII. Comparisons are difficult to make due to the structural differences as well as the lack of information about the supports. However, the capacities appear

TABLE XII

SUMMARY OF CAPACITY STUDIES FOR SILICA-IMMOBILIZED CROWN ETHERS

Structure (Figure No.)	Mean Specific[a] Surface Area (m^2/g)	Mean Pore Diameter (Å)	Capacity (μmoles/g)	Apparent[b] Surface Coverage (μmoles/m^2)	Capacity Method	Probe	Metal Determination Method	Ref.
16	550[c]	60	340	0.62	4	---	---	62
17	----	--	520-580	----	4/6	---	---	63
18	----	--	390	----	6	---	---	64
			370	----	4	---	---	
19	----	--	200-220	----	4/6	---	---	63
19	----	--	920	----	6	---	---	64
			900	----	4	---	---	

[a] Before silylation and subsequent reactions.

[b] Assuming 1:1 metal to ligand ratio although this may not be the case.

[c] Silica gel G (contains gypsum binder for TLC).

to be good for silica-based chelating ion exchangers (even though metal ion exchange capacities were not determined). One puzzling aspect of the above values is the large difference in capacity observed for the bound poly(benzo-15-crown-5) (Figure 19). The explanation for this difference was not given.

Stability. No detailed stability studies of these materials have been performed. The only mention of stability was made by Blausius and Janzen (63) who reported the instability of the silica-bound benzo-15-crown-5 (Figure 15) due to the Si-O-C link as mentioned already.

Applications

Blausius et al. (61, 62) and Kimura et al. (64, 65) have both demonstrated the applicability of silica-immobilized crown ethers for alkali metal separations. However, the size of the hydrated ion relative to the size of the crown ether ring, as mentioned earlier, strongly affects the selectivity. For this reason, the monomeric crown ethers cannot be applied to the separation of all the alkali metal ions. For example, small cations such as Li^+ or Na^+ can fit into the crown ether cavity of benzo-15-crown-5 and therefore can be separated whereas the larger alkali metal cations (K^+, Rb^+, Cs^+) are excluded and thus cannot be separated. They then elute from a column in the void volume. Actually, monomeric crown ethers are usually most selective for the alkali metal cations of intermediate size since the small cations are strongly hydrated (and desolvation requires considerable energy) and the larger cations are unable to attract and organize the ligand as well as the smaller cations due to the more diffuse charge on the cation. For this reason, the bound bis- and poly(crown ethers) have been used to increase the selectivity. The bis- and poly(crown ethers) have been shown to form 2:1 crown ether ring to metal cation complexes which can be described as "sandwich-type complexes" (64, 65). This allows for extraction and relatively high affinity for those alkali metal ions which are larger than the cavity of the crown ether. For example, silica-bound poly(benzo-15-crown-5) shows high extractability of K^+ and Rb^+ and therefore they are retained on the column strongly. Cs^+ may also form 2:1 crown ether ring to cation complexes with the poly(benzo-15-crown-5), however, Cs^+ is too large to fit into the "sandwich" well and therefore elutes before Rb^+ and K^+. On the other hand, Na^+ forms 1:1 complexes with the bound benzo-15-crown-5 group (as is expected from solution behavior). It appears that 1:1 complexes are not as strong as the "sandwich" complexes and therefore Na^+ elutes before Cs^+. Lastly, Li^+, being strongly solvated tends to remain solvated and therefore elutes first. Thus, the elution sequence is $Li^+ < Na^+ < Cs^+ < Rb^+ < K^+$ for both bis- and poly(benzo-15-crown-5) immobilized on silica. Anions with a common cation can also be separated with the largest anion being the most strongly retained. Some of the alkaline earth metals were also separated.

Silica-immobilized crown ethers occupy a special niche in the area of silica-bound chelating agents because of the high selectivity of these materials for alkali metal ions (as well as alkaline earth metal ions to a lesser extent). Although little work has been done on these materials and no real samples have been analyzed, the development and use of the bis- and poly(crown ether) sationary phases stands out as the major contribution to this particular area.

Miscellaneous Chelating Agents

The most widely studied chelating agents of analytical importance have been discussed in the previous sections. However, there are several silica-immobilized chelating agents reported briefly in the literature which are of some interest. The most important of these are summarized in Table XIII.

Probably the most important for trace metal preconcentration/separation is the ACDA-silica derivative in Row 1. It is very similar to the dithiocarbamates in its behavior which suggests its use for preconcentration of trace metal ions. However, unlike the dithiocarbamates, it appears to be stable in acidic solution.

The applications of the 3,6-dioxaoctanedioic acid derivatives (Rows 2 and 3) are similar to those of the crown ethers previously described. The bis- and poly(crown ethers) show much better selectively and are more easily prepared, however, which makes them preferable.

The iminodiacetate and ethylenediamine triacetate ligands (Rows 4 and 5) are useful for preconcentrating a large number of metal ions. The primary problem with their use is their lack of selectivity. Alkaline earth metals can interfere in preconcentration/separation procedures.

The 3-glycidoxy derivative has been shown to be very useful for separation of tryptophan and tyrosine racemates using ligand exchange chromatography. One of its advantages is the fact that the 3-glycidoxysilane is commercially available.

Several other bound chelating agents have been reported by Waddell, Leyden, and Hercules in a chapter of a monograph (58). However, little information is presented. Essentially, only the synthesis of each material is described and, as pointed out by the authors themselves, the structures have not been confirmed.

TABLE XIII

MISCELLANEOUS SILICA-BOUND CHELATING AGENTS

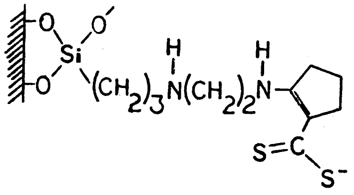
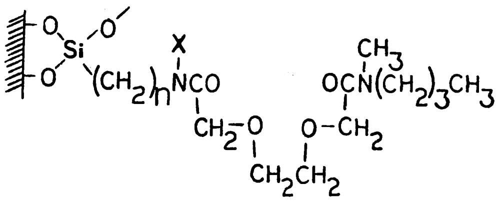
1.	 2-amino-1-cyclopentene-1-dithiocarboxylic acid derivative (ACDA) - Ref. 66	Similar to the dithiocarbamates, however more stable. Capacity ≃ 1000 μmoles/g using Ag(I), Hg(II), or Pb(II). Fast exchange rate. Support = silica gel (50 m^2/g SA, 60 Å pores).
2.	 3,6-Dioxaoctanedioic acid derivative (I) Ref. 67	Some selectivity for alkali and alkaline earth cations. Separation efficiency is poor although an HPLC support (Lichrosorb -NH_2) was used.

TABLE XIII (Continued)

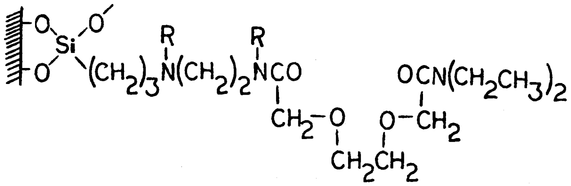
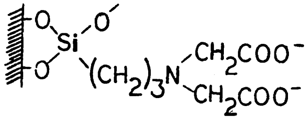
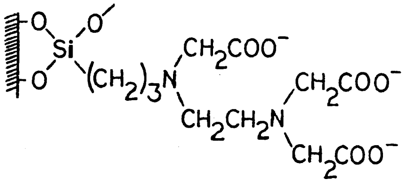
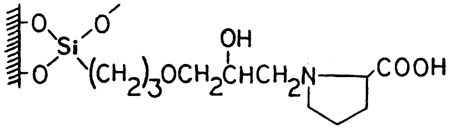
3.  3,6-Dioxaoctanedioic acid derivative (II) Ref. 68	Similar to 2 above. Two supports used: 1. Porous glass (200-400 mesh, 200 Å pores). 2. Lichrospher (10 μm diam., 250 m^2/g SA, 100 Å pores). Poor efficiency for metal ion separations.
4.  Iminodiacetate derivative Refs. 30, 69	Used for preconcentration of inorganic Hg(II) and studied as possible support for Cu(II) in ligand exchange chromatographic separation of carboxylic acids, amino acids, and triphenylphosphonium salts.

TABLE XIII (Continued)

5.	 Ethylenediaminetriacetate and Derivatives Refs. 30, 70	EDTA analog. Complexes of Cd(II), Zn(II), Cu(II), and Pb(II) and effect of pH studied. Application to ligand exchange chromatography with complexed Cu(II) for carboxylic and amino acid separations.
6.	 3-Glycidoxy Derivative Ref. 71	Made from commercially available silane. Used for separation of tryptophan and tyrosine racemates by ligand exchange chromatography with complexed Cu(II). Good resolution.

Schucker et al. (45) also report the immobilization of such common chelating agents as dithizone, neocuproine, 1,10-phenanthroline, and salicylaldoxime using a diazo coupling procedure identical to that used for the immobilization of 8-quinolinol on silica. However, no further information has been reported on the use or characterization of these materials.

Comparison of Reviewed Species

Of the five major silica-immobilized chelating agents in use today, the most popular seem to be ethylenediamine, the dithiocarbamates, and 8-quinolinol. The two most generally useful of the silica-immobilized chelating ligands are ethylenediamine and 8-quinolinol. Both of these ligands have good selectivity for metal ions although 8-quinolinol appears to be somewhat more selective. Both also have acceptable to excellent stability with bound 8-quinolinol appearing to be somewhat more stable. The silica-bound ethylenediamine, however, is much more easily prepared than the silica-immobilized 8-quinolinol (particularly when using the conventional procedure for preparing the silica-8-quinolinol). The silica-bound ethylenediamine also has higher capacities.

The major use of silica-bound ethylenediamine to date, however, has been the preconcentration of anions using ion pair extraction. On the other hand, silica-bound 8-quinolinol has found widest applicability for trace metal ion preconcentration from high ionic strength aqueous samples.

The silica-immobilized dithiocarbamates are more restricted in their use due to poor selectivity and poor stability. However, large formation constants of metal dithiocarbamates and high capacities allow for successful metal ion preconcentration with the restriction that determination of those metal ions must be made in situ on the preconcentrating medium itself.

The silica-immobilized crown ethers are a special case in that they exhibit great selectivity for alkali and alkaline earth metal cations. Many are easily prepared with fair capacities. The silica-immobilized bis- and poly(crown ethers) are the most useful due to the great selectivity enhancement.

The silica-immobilized β-diketones appear to be of limited value for metal ion preconcentration or separation. The keto-enol tautomerism complicates complexation reactions and some of the synthetic routes suffer from unwanted side reactions. However, the bound β-diketones hold some promise for ligand exchange chromatography and their usefulness in the development of some instrumental techniques for structural studies cannot be overlooked. A comparison of the most widely used silica-bound chelating ligands is presented in Table XIV.

The field of silica-immobilized chelating ligands is still in its infancy. Innumerable other chelating agents hold the possibility of being ideally suited for a given application. New synthetic procedures should increase the efficiency and decrease the preparation time of many of the ligands. Also, relatively unexplored areas exist particularly in the area of ligand exchange chromatography and metal ion separations under continuous gradient elution.

TABLE XIV

COMPARISON OF IMPORTANT SILICA-IMMOBILIZED CHELATING AGENTS

Bound Species	Ease of Preparation	Capacity	Stability	Metal Ion Selectivity	Major Applications	Comments
Ethylenediamine	++	++	0	+	P(A),S(O)	Most widely used. Very easy to prepare. Precursor to many other bound species.
Dithiocarbamates	+	++	--	--	P(C)	Generally restricted to heterogeneous determinations. Very unstable.
8-Quinolinol	--	--	++	++	P(C),S(C)	Long synthetis generally. Low capacities limit aplications to trace levels.
β-Diketones	-	0	+	--	S(LE)	Unwanted by-products often present. Keto-enol tautomerism complicates reactions.
Crown Ethers	+	+	NA^a	++^{b}	S(C),S(A)	Selective for alkali and alkaline earths. "Sandwich" complexes afford good separation.

[a] not available.

[b] Strongly dependent on the crown ether and the configuration (ie. bis-, poly-, etc.).

KEY

++	Excellent
+	Good
0	Acceptable
-	Poor
--	Very Poor

Applications

P = Preconcentration
S = Separation
C = Cations
A = Anions
O = Organics
LE = Ligand Exchange

CHAPTER III

SYNTHETIC ROUTES TO SILICA-IMMOBILIZED 8-QUINOLINOL

Previously, silica-immobilized 8-quinolinol (HOx) has been prepared utilizing one of two similar procedures as mentioned in Chapter II (43, 44). These immobilization procedures involve a rather lengthy synthesis (3 to 4 days) and the products obtained generally have rather low capacities. The traditional diazo coupling method has also proven to be rather irreproducible, with capacities varying widely from batch to batch. This chapter describes several possible synthetic routes to silica-immobilized 8-quinolinol, one of which has proven to be a valuable alternative to the traditional method (72).

Schiff-Base Coupling

The immobilization of enzymes on silica supports using a dialdehyde such as glutaraldehyde was first reported by Robinson et al. (73). This immobilization procedure involves silylation of the silica surface with an aliphatic aminoalkoxysilane such as aminopropyltriethoxysilane, reaction of the immobilized amine with glutaraldehyde, and then coupling of the free aldehyde group of the immobilized glutaraldelyde with the enzyme. The nature of this reaction is not fully understood, however, it is believed to proceed through a Schiff base (imine) intermediate. The reaction proceeds quickly under mild conditions and therefore seemed to be a likely candidate to provide an improved synthetic route to silica-immobilized HOx. The results of an investigation into this immobilization approach are presented in this section.

Experimental

Reagents. All reagents were analytical grade and used as received unless otherwise indicated. All water was deionized by reverse osmosis followed by distillation in a borosilicate glass still equipped with a quartz immersion heater (Corning Model AG-la, Corning, NY). Aminopropyl-controlled pore glass (AMP 00500A, Electro-Nucleonics, Fairfield, NJ) was used as the immobilization support. This is simply controlled pore glass which has been previously derivatized with an aminopropyl group. Glutaraldehyde (Kodak Laboratory Chemicals, Rochester, NY) was obtained as a 50% solution in water. 5-amino-8-quinolinol was prepared by low pressure catalytic hydrogenation of 5-nitro-8-quinolinol (Aldrich Chemical Co., Milwaukee, WS) and isolated as the dihydrochloride salt.

Apparatus. A custom-made shaker based on the use of a vacuum driven windshield wiper motor was used for batch equilibrations. Atomic absorption measurements were performed with a Perkin-Elmer Model 290B (Norwalk, CT) atomic absorption spectrophotomer. UV-VIS Spectrophotometry was carried out on a Bausch and Lomb Spectronic 505 Spectrophotometer, Rochester, NY).

Procedure. The dihydrochloride salt of 5-amino-8-quinolinol was synthesized by dissolving or suspending 5.0 g of 5-nitro-8-quinolinol in 100 mL of methyl cellosolve (ethylene glycol monomethylether) plus 50 mL of N,N-dimethylformamide, adding 0.5 g of 5% Pd/C, and hydrogenating at 30 psig and room temperature until the hydrogen uptake was complete (1 - 1.5 hours). The solid was then quickly filtered through Dicalite after which 10 mL of concentrated HCl was added to the filtrate. The orange crystals were filtered, dissolved in a small amount of 0.01 M HCl, treated with charcoal, and diluted with an equal volume of concentrated HCl while hot. The solution was then boiled down to a small volume and the orange/brown crystals were dried in vacuo over KOH pellets. The yield was approximately 65% of theoretical. Mass spectral analysis of the product showed it to be pure. 46.9% C, 4.3% H, 12.1% N, 7.1% O and 30.4% Cl was obtained experimentally versus 46.4% C, 4.3% H, 12.0% N, 6.9% O, and 30.4% Cl as the theoretical values.

Immobilization of 8-quinolinol was performed via a procedure originally described for enzyme immobilizations (73). A 2.0 g portion of aminopropyl-controlled pore glass (AMP-CPG) was added to 20 mL of a 1% aqueous glutaraldehyde solution at $0^{o}C$. The reaction was allowed to proceed for 30 min. with constant shaking. The derivatized AMP-CPG was then rinsed with water and suspended in 10 mL of 0.05 M phosphate buffer (pH 7.5). To this suspension was added 70 mg of 5-amino-8-quinolinol dihydrochloride. At this point nitrogen gas was bubbled through the solution for agitation and to inhibit decomposition of the unstable 5-amino-8-quinolinol. The reaction was carried out for 2 hours after which the buffer was decanted and the derivatized glass was rinsed with water until the rinsings were clear. The solid was then allowed to air dry and stored in a desiccator. In some cases $NaBH_4$ in ethanol was used to reduce the imine bonds after immobilization.

Capacities were determined by adding a known excess of copper(II) to a given amount of solid. Typically, 20.00 mL of a 150 μg/mL standard stock solution of copper(II) (prepared from $CuSO_4 \cdot 5H_2O$) in 0.2 M acetate buffer (pH 5.0) was added to approximately 0.2g of the solid in a 30 mL screw cap vial. This mixture was allowed to equilibrate for 30 min. with constant shaking after which an aliquot of the liquid was removed and the capacity determined from the change in copper concentration observed. The capacity of a silica blank was also determined in order to confirm that the silica support was not responsible for any significant portion of the observed capacity.

All copper determinations were carried out at the 3247Å line of copper with an Intensitron No. 2252 hollow cathode lamp (Perkin-Elmer). Calibrations were performed by using a series of standards prepared from a 993 µg/mL copper atomic absorption standard stock solution (Aldrich).

Results and Discussion

The immobilization reactions leading to the Schiff base-coupled 8-quinolinol are shown in Figure 20. The reaction is easily performed with very few problems except for the instability of the 5-amino-HOx. A very dark decomposition product appeared in the reaction vessel as the reaction proceeded.

A total of nine batches of Schiff base coupled material were prepared. The mean capacity of these materials was 12±2 µmol/g which is a rather low value for chelating ion-exchangers. Commercially available silica-immobilized HOx on a similar support is reported to have a capacity of 30 µmol/g. Capacity determinations using Fe(III) as the probe gave larger capacity values (27±9 µmol/g). However, the silica blank also indicated a capacity of 22±3 µmol/g which indicates extraction of Fe(III) by silanol groups at pH 5. In fact, the differences in capacities of the immobilized HOx and the silica blank agree very well with those obtained using Cu(II). The blank value using Cu(II) at pH 5 was 0±2 μmol/g.

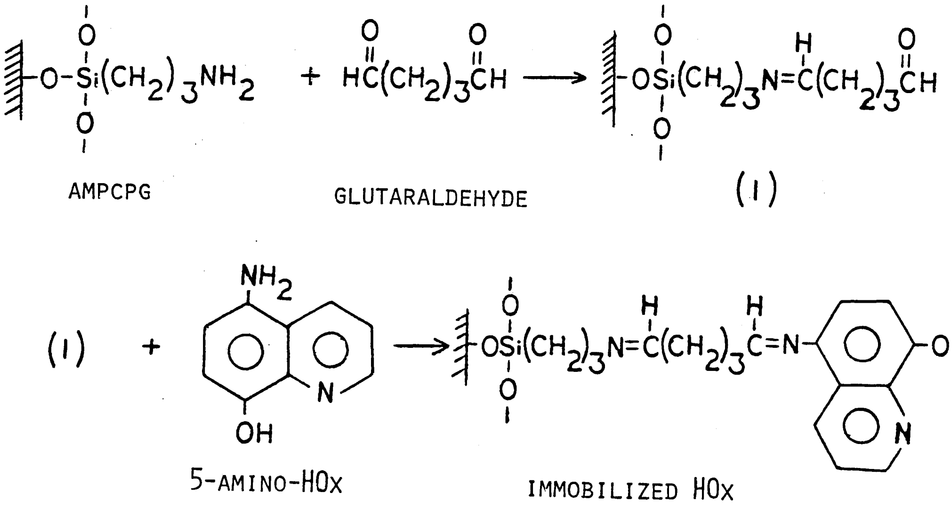


Figure 20. Schiff-Base Coupling of 8-Quinolinol to Silica

A more serious disadvantage, however, arises due to the poor hydrolytic stability of these materials. Upon treatment of the Schiff base-coupled HOx with 0.1M HCl, hydrolysis of the imine bonds was observed. This was indicated by the presence of a dark color to the rinsings. In an effort to improve the hydrolytic stability, the imine bonds were reduced by 0.35M $NaBH_4$ in ethanol. This treatment, however, resulted in a decrease in capacity to 8 μmol/g. The results of UV-VIS spectrophotometric spectral analysis of various rinse solutions (0.2g solid + 25 mL 0.1 M HCl) are shown in Table XV. It appears that the reduction of the

TABLE XV

UV-VIS SPECTROPHOTOMETRIC RESULTS FOR HYDROLYTIC STABILITY

Solution	λ_{max} (nm)	Absorbance
5-amino-HOx in 0.1M HCl	202	----
	251	----
0.1M HCl rinsings of solid (0.2g, untreated)	204	0.43
	263	0.45
0.1M HCl rinsings of solid (0.2g, $NaBH_4$ treated)	202	0.28
	256	0.14

imine bonds improves the stability but this additional step adds time to the synthesis and the capacities, as indicated, are disappointingly low.

Diazo Coupling

Immobilization of 8-quinolinol on silica surfaces has traditionally been done via diazo coupling (41-51). A review of this area has already been presented in Chapter II. As indicated in that review, however, the traditional (or conventional) method suffers from several problems. The most serious of these problems are the time-consuming synthetic procedure which takes 3 to 4 days and the low, irreproducible capacities of the product. Diazo coupling, however, has many attractive features which were also pointed out in Chapter II. The azo bond can be cleaved with reducing agents allowing coupling to another ligand without repeating the entire immobilization procedure (41, 44). Also, the final product, being an azo dye, is brightly colored which yields qualitative information about the success of the synthesis.

A new, less circuitous, route to silica-immobilized HOx is described here which retains the desirable features of diazo coupling reactions while reducing the preparation time and yielding products having relatively high capacities. The results presented here have been published in a recent paper (72).

Experimental

Reagents. Reagents were analytical grade and, unless expressly indicated, were identical to those listed for the Schiff base coupling procedure. (Aminophenyl)trimethoxysilane (Petrarch Systems, Inc., Bristol, PA) was obtained as mixed isomers and stored under refrigeration. Organic solvents were stored over molecular sieves.

Silica gel (Woelm TLC grade, ICN Pharmaceuticals, Cleveland, OH), Porasil C (Waters Associates, Milford, MA), and Controlled Pore Glass (CPG 00500, Electro-Nucleonics, Fairfield, NJ) were dried at 120°C for 12 hours before use and were stored in an oven at 120°C.

Apparatus. The same apparatus was used as described previously for the Schiff base coupling.

Procedure. A 10% solution of (aminophenyl)trimethoxysilane in dry toluene (20 mL) was added to the dry silica support (2.0g). A vacuum was then applied in some cases to remove any trapped air. The reaction mixture was refluxed for 4 hours, filtered through a filter funnel, rinsed sequentially with toluene and acetone, and dried overnight at 80°C in an oven. This results in the formation of an arylamine silica.

This arylamine silica was then diazotized and coupled in essentially the same manner as previously described (46). First, 100 mL of 2% $NaNO_2$ in 2M HCl was added to the arylamine silica at 0°C and reaction was allowed to occur for 30 min. This reaction results in the formation of a diazonium salt-silica. Degassing was performed in some cases. The diazonium salt-silica was quickly filtered, washed with three 25 mL-portions of cold water, and added to 100 mL of a 2% solution of HOx in ethanol. Degassing was also performed at this point in some cases. Appearance of a deep red color indicates the formation of the diazo-coupled HOx. This coupling reaction was carried out for 30 min., after which the silica-immobilized HOx was filtered and washed sequentially with ethanol, 0.1M hydrochloric acid, and water. This material was then air dried and stored in a desiccator. Capacities were determined as described previously.

Results and Discussion

The procedure described above for the immobilization of HOx on silica via diazo coupling is shown in Figure 21.

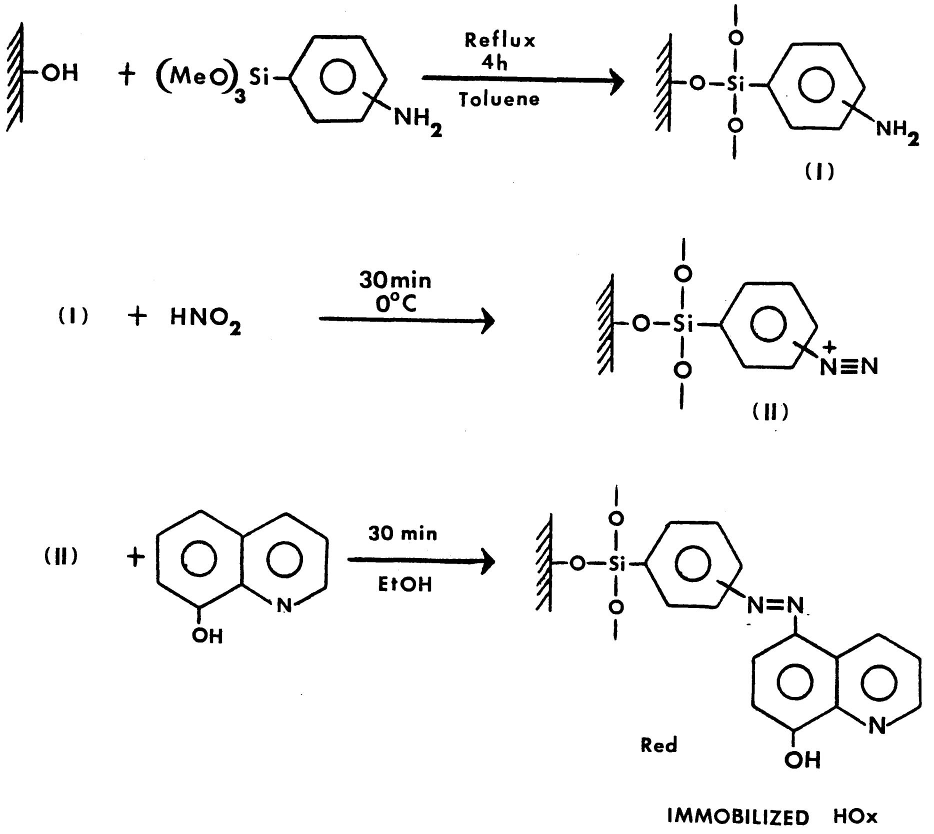


Figure 21. Diazo Coupling of 8-Quinolinol to Silica

This procedure involves, initially, the formation of an immobilized aromatic amine in one step. In diazo coupling immobilization, the presence of an aromatic amine is required in order to form a stable diazonium salt (i.e. stable relative to aliphatic diazonium salts) that, being a weak electrophile, can couple to an electron-rich aromatic species such as an aromatic amine or phenol. The previous route to silica-immobilized HOx requires a 2 to 3 day process to obtain the immobilized aromatic amine (Chapter II). The method described here is obviously an improvement in terms of the time involved. It has other advantages, as well, as will be discussed later. Although we have not found it to be a necessity, curing was normally performed as a precautionary measure.

Degassing prior to refluxing with the silane did not result in a larger capacity for the final product. In fact, it appears that slightly higher capacities are obtained without degassing as shown in Table XVI. This observation seems to indicate that the increased surface coverage of the silane (i.e. (aminopropyl)triethoxysilane) observed by Fulcher et al. (46) when performing the silylation in degassed, refluxing toluene instead of the milder, nondegassed acetone, is probably due to the more vigorous refluxing conditions rather than degassing. Another possible explanation, however, is that the surface coverage of the silane is increased by degassing but has no effect on the final capacity due to steric problems during the diazonium salt formation or coupling reactions. In fact, this could provide an explanation for the lower capacities observed with degassing since a higher surface coverage of the silane could result in more steric problems in subsequent reactions.

The capacities of silica-immobilized HOx prepared by using the procedure described here are comparable to (if not slightly larger than) the largest capacities reported to date for silica-immobilized HOx prepared via the conventional route. Table XVII lists some pertinent physical characteristics of some of the supports used in this study and the

TABLE XVI

EFFECT OF DEGASSING ON CAPACITY

Treatment	Capacity (μmol/g)[a]	
No Degassing	260	230
Degassing (silylation step only)	217	216
Degassing (silylation step and diazonium salt formation step)	214	184
Degassing (all steps)	199	191

[a] Carrier: Woelm TLC Grade Silica Gel. Two batches.

TABLE XVII

PHYSICAL PROPERTIES OF SOME SILICA SUPPORTS USED AND SUMMARY OF CAPACITIES OBTAINED

	CPG-00500 (Controlled Pore Glass)	Porasil C (Porous Silica)	Silica Gel (TLC Grade)
Particle Size (μm)	37-74	75-125	5-15
Mean Pore Diameter (Å)	515	300	60
Mean Specific Surface Area (m^2/g)	41.6	50	550
Average Capacity[a] (μmoles/g)	58	104	216 ± 20[b]
Average Surface Coverage (μmoles/m^2)	1.4	2.1	0.39
Previously Reported Capacities[a,c] (μmoles/g)	18[d] 39[e] 22[f] 17[g]	54[h] 61[i]	60[j] 70[k] 140[l] 178[m]

[a] Based on Cu(II) as the metal probe.

[b] 12 samples (range = 184 to 260 μmoles/g).

[c] Particle sizes, specific surface areas, and mean pore diameters may vary slightly.

[d] Reference 47 (70 m^2/g SA, 550Å pores).

[e] Reference 47 (57 m^2/g SA, 544Å pores).

[f] Reference 54 (70 m^2/g SA, 550Å pores).

TABLE XVII (Continued)

	CPG-00500 (Controlled Pore Glass)	Porasil C (Porous Silica)	Silica Gel (TLC Grade)

[g] Reference 45 (70 m^2/g SA, 550Å pores).

[h] Reference 54 (Porasil B, 140-230 m^2/g SA, 150Å pores).

[i] Reference 49 (Porasil B).

[j] Reference 46 (550 m^2/g SA, 60Å pores).

[k] Reference 43.

[l] Reference 50 (550 m^2/g SA, 60Å pores).

[m] Reference 50 (480 m^2/g SA, 70Å pores).

corresponding capacities and surface coverages obtained. Also presented in this table is a comparison to capacities obtained by using the conventional immobilization on similar supports. The somewhat larger capacities obtained by using the procedure described may be due to several factors. First, the synthetic route used, involving only three steps, is expected to be more efficient than the five-step conventional procedure since the chemical reactions involved are similar. It is also observed, using molecular models that the immobilized (aminopropyl)triethoxysilane, being more flexible due to the aliphatic spacer may tend to lie down on the surface becoming less available for reaction. Another factor that may contribute to the increased capacities observed is the shorter length of the group attached by the procedure described here. The shorter length of the new material may result in slightly less obstruction of the pores as the pore diameter decreases. The relationship of pore diameter to capacity and length of the immobilized group will be discussed in more detail in Chapter IV.

Miscellaneous Routes

In addition to the two synthetic routes discussed in detail above, a number of unsuccessful attempts were made to immobilize HOx on silica. These attempts are summarized in Table XVIII. In all of these cases, the capacities obtained were essentially 0 μmol/g.

Conclusions

Of the several possible synthetic procedures leading to silica-immobilized HOx that have been presented in this chapter, the new diazo-coupling procedure is the most useful. The stability of the diazo-coupled

TABLE XVIII

UNSUCCESSFUL SYNTHETIC APPROACHES TO SILICA-IMMOBILIZED 8-QUINOLINOL

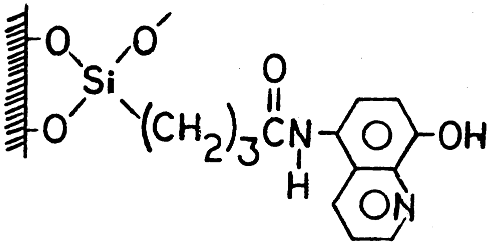
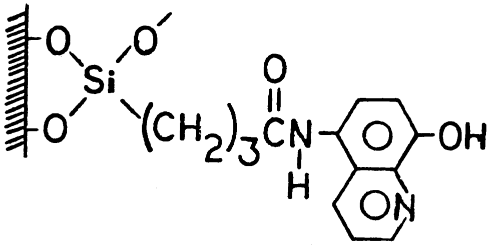
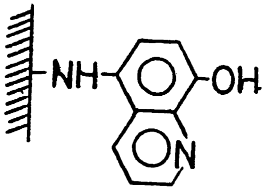
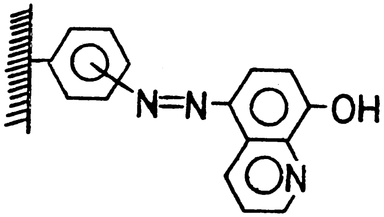
Immobilization Reaction	Support	Anticipated Structure[a]
Amidization with 5-amino-HOx	Carboxyl-CPG	
Amidization after conversion of support to acid chloride	Carboxyl-CPG	
Tosylation of silica followed by nucleophilic displacement with 5-amino-HOx	CPG	

TABLE XVIII (Continued)

Immobilization Reaction	Support	Anticipated Structure[a]
Phenyl Grignard, nitration, reduction, diazo-coupling	CPG	

[a] All capacities are 0 μmol/g which indicates no reaction.

product is very good (see Chapter IV), the capacities are relatively high, and the new procedure can be quickly and reproducibly performed. This method offers a significant improvement over the conventional method, particularly in terms of time savings and reproducibility. The remaining chapters in this dissertation will focus on the characterization of the new diazo-coupled silica-immobilized HOx, particularly with regards to its use for metal ion preconcentration, and its analytical utility in preconcentration/flow injection analysis.

CHAPTER IV

CHARACTERIZATION OF SILICA-IMMOBILIZED 8-QUINOLINOL

Once a chelating agent has been immobilized on a particular support, it is important to characterize the material so that information can be obtained which will guide the analyst in systematically choosing conditions which can be applied in a given situation. Many properties such as stability are essential for use of the material in any situation. Other parameters such as the effect of the physical characteristics of the support on the capacity are very important in order to achieve the highest possible capacity. Other variables can also affect the capacity such as the presence of various isomers of the silane, some of which are more sterically hindered than others. Properties such as metal ion extraction behavior as a function of pH, rate of extraction of metal ions, and acid-base characteristics are essential in understanding what particular conditions should be used in applying the silica-immobilized material to preconcentration, or even separation, of metal ions. These properties can also give insight to, or provide explanations for, differences between the immobilized species and the same species in solution. This chapter will deal with the characterization of silica-immobilized HOx with an emphasis on those properties which affect the use of the material for metal ion preconcentration. All of the silica-immobilized HOx has been prepared using the new diazo coupling procedure described in Chapter III.

Capacity Studies

The success, or failure, of a particular synthetic route to silica-immobilized HOx is determined by measuring the amount of chelating agent that has been immobilized on a given amount of carrier or support. This is determined by measuring the capacity of the material. In Chapter III some results of capacity studies using uptake of copper(II) as a measure of capacity were reported. This method of determining the capacity, however, is only one of several methods. Ideally all methods would result in the same capacity. This is not always the case, however, and useful information about stoichiometry, for example, can be obtained by comparing different capacity determination methods. Also, various properties of the silylating reagent and the support can have a marked effect on the capacity. The various factors involved in capacity determinations are presented in this section along with a comparison of some capacity determination methods.

Choice of Metal Ion Probe and pH

Probably the most straightforward method of determining the capacity involves measurement of the maximum amount of metal ion extracted onto a known mass of solid chelating ion-exchanger. The capacity obtained, ideally, should be independent of the metal ion used. This, however, is not always true. The metal ion probe used for capacity studies should have a large formation constant with the chelating agent and should not be extracted by bound species other than the chelating agent of interest (e.g., surface silanol groups). The pH should also be chosen so that conditional formation constants are large. This is generally true at high pH. However, at high pH problems of metal ion solubility and extraction by surface silanol groups dictate a somewhat lower pH value at which solubility is still high, extraction by silanol groups is absent, and conditional formation constants are high.

In this work, copper(II) was chosen as the metal ion probe at pH 5.0. The formation constant for the soluble Cu(II)-HOx complex is large (74) which means that only a small excess of Cu(II) is required for saturation of all the immobilized HOx sites. Also, pH 5.0 is optimal since copper(II) is extracted at higher pH by unreacted surface silanol groups (75). In fact Fe(III), which has also been used as a probe and whose HOx complex exhibits a larger formation constant than Cu(II), gives capacities on controlled pore glass (44) that are somewhat higher than those observed when using Cu(II) on similar supports (48). This effect is probably due to the extraction of Fe(III) by silanol groups which have the ability to extract Fe(III) down to pH 1.0 (75). A similar effect was mentioned in Chapter III with regard to capacity determination of Schiff base-coupled HOx. No evidence of extraction of Cu(II) onto a silica blank was observed at pH 5.0 in this work.

Experimental Procedures

Several different methods were used to determine capacities. The first method was the copper uptake method already described in Chapter III. Alternatively, the equilibrated silica-immobilized material was sometimes filtered and rinsed with water and the extracted copper(II) eluted with 100 mL of 0.1M hydrochloric acid followed by 15 mL of 1.0M hydrochloric acid in some cases. A third capacity determination method involved carbon determinations with a Perkin-Elmer Model 240 elemental analyzer. This gave a direct measure of the total amount of immobilized organic material.

Isomers of (aminophenyl)trimethoxysilane were preparatively separated, when necessary, by vacuum distillation. Analytical separation of the isomers was performed by capillary gas chromatography on an OV101 column (length = 39m, inside diameter = 0.5 mm, injection port temperature = 200^{o}C, column temperature = 210^{o}C, detector temperature = 250^{o}C). A Hewlett-Packard Model 5880 Level Four gas chromatograph (Palo Alto, CA) was used with flame ionization detection. 'H-NMR spectroscopy was performed on a Varian (Palo Alto, CA) Model XL-300 FT-NMR. Various silica supports used for the pore size/surface area studies included: Bead Gel spherical silica gel (Davison Chemical, Baltimore, MD), silica gel (Davison Grade 12, Supelco, Bellefonte, PA), Woelm TLC-grade silica gel (ICN Pharmaceuticals, Cleveland, OH), silica gel for column chromatography (J. T. Baker, Phillipsburg, NJ), Porasil F and Porasil C (Waters Associates, Milford, MA), and four controlled-pore glasses (CPG-00075, CPG-00500, and CPG-02000 from Electro-Nucleonics, Fairfield, NJ; CPG-550 from Corning Glass, Corning, NY).

Rate studies were performed by quickly adding 10.00 mL of 150 μg/mL Cu(II) (pH 5.0) to 0.100 g of silica-HOx, allowing it to react with constant shaking for a given time interval, and filtering quickly. The filtrate was then analyzed for Cu(II). Time intervals of 30 sec. were chosen as shorter time intervals were impossible using the manual procedure described.

Rate of Extraction of Copper(II)

When performing capacity studies, it is essential that sufficient equilibration time is allowed so that the maximum amount of copper(II) can be extracted. It was believed that silica-immobilized chelating agents should exhibit fast mass transfer relative to polymer-based materials. The bound HOx, being at the surface of the silica carrier, should be readily available for complexation. Figure 22 shows the rate of extraction of Cu(II) onto silica-immobilized HOx at pH 5.0. A silica carrier with a pore size as small as 60Å was chosen since a restricted pore size, it was believed, could hinder mass transfer. However, as shown, the rate is really rather fast, the maximum capacity being obtained within approximately 3 to 4 minutes. Since batch equilibrations were normally performed for 30 minutes, all capacities obtained were assumed to be the maximum capacities.

The rate of extraction reported here compares to values of 6.5 to 140 minutes required for 50 percent extraction on an HOx chelating resin based on crosslinked polystyrene (76). The wide range reported was due to differences in the water regain and swelling properties of the polymer. Incorporation of sulfonate groups into the resin caused a reduction of the 50 percent extraction time to about 2.5 minutes. Later work on various supports containing HOx indicated a further reduction to less than 2 minutes in some cases (10). However, use of silica-immobilized HOx, as shown, results in a 50 percent extraction within about 30 seconds.

Comparison of Various Capacity Determination Methods

Three different capacity determination methods were performed in order to obtain several different measures of capacity. The results are presented in Table XIX. The values for Cu(II) elution were obtained by eluting the extracted Cu(II) with 0.1 M HCl. Only 75-85% of the capacity

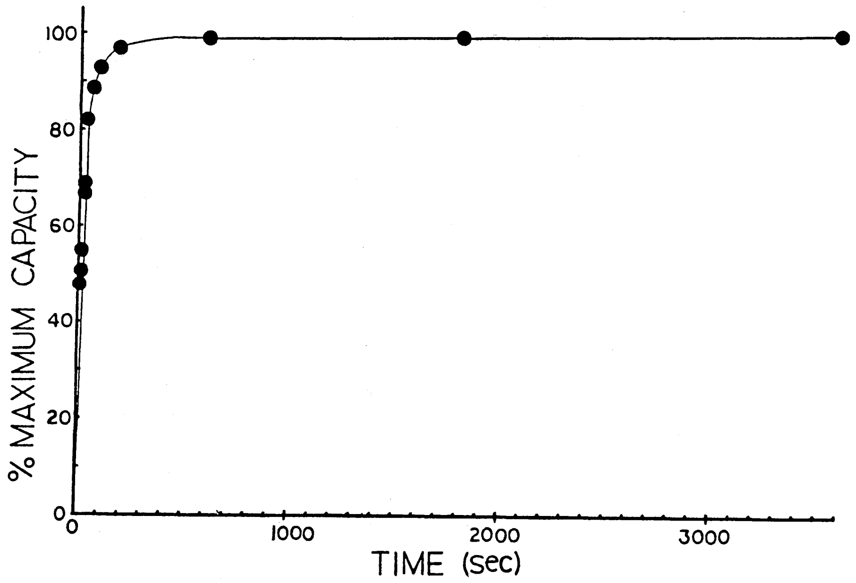


Figure 22. Rate of Extraction of Copper(II). Carrier = Woelm TLC Grade Silica Gel (60Å Pore Size, 550 m^2/g Surface Area). $[Cu^{2+}]$ = 150 μg/mL, pH = 5.0 (Acetate). Maximum Batch Capacity = 206 μmol/g

TABLE XIX

CAPACITY RESULTS OBTAINED BY DIFFERENT DETERMINATION APPROACHES

	Capacity Determination Technique[a]		
Material	Cu(II) Uptake	Cu(II) Elution[b]	Carbon Determination
CPG-00500-HOx	61	46	100
Porasil C-HOx	107	89	194
TLC-SG-HOx #1	227	188	467
TLC-SG-HOx #2	225	173	517

[a] Units are μmol/g.

[b] Elution with 100 mL of 0.1M HCl. Further elution with 15 mL of 1M HCl results in agreement with Cu(II) uptake values (see text).

determined by Cu(II) uptake was obtained when eluting, even when using as much as 100 mL of 0.1 M HCl. Because of the large formation constant of the Cu-HOx complex (74), a more concentrated acid solution was required for quantitative recovery of Cu(II). It was found that 100 mL of 0.1 M HCl followed by 15 mL of 1M HCl was sufficient to account for all of the Cu(II) present. Further elution with 15 mL portions of 1M HCl/0.1M HNO_3, 1M HNO_3, and concentrated HCl did not result in elution of additional Cu(II).

Carbon determinations yielded values that are higher than those obtained by Cu(II) uptake or Cu(II) elution. At first glance, it was believed that this was evidence of the formation of a 2:1 HOx to Cu(II) complex since the capacities obtained using carbon as the probe are approximately twice those values obtained using Cu(II) as the probe. However, if it is assumed that the bound HOx is uniformly distributed over the entire surface area of the support, calculations show that the bound groups are too far apart for formation of the 2:1 complex. A more likely explanation is that a 1:1 complex is formed, but some of the initially bound silane is not coupled to HOx due to steric hindrance. This steric problem is probably of two types: (1) the bulkiness of the HOx molecule probably results in coverage of more than one of the bound silanes which carry the reactive aromatic amine and (2) the ortho isomer of the silane is probably not readily coupled due to the fact that the amino group is pointed toward the silica surface, thus blocking further reaction (both of these points will be addressed in the next section). Previous work on the conventionally coupled silica-immobilized HOx also indicates a 1:1 complex (54).

Isomer Effect

In an effort to provide an explanation for the larger than expected capacities obtained from carbon determinations, the effect of various isomers of the silane was studied. The silane ((aminophenyl)trimethoxysilane) is commercially available as an isomeric mixture. Figure 23 shows the chromatogram obtained from capillary gas chromatography of the commercially available material. The three major peaks (A, B, and C) correspond to 29.2%, 45.6%, and 22.6% of the mixture respectively. Isomer A appears to be the ortho isomer which is easily separated from the other two isomers on a preparative scale by vacuum distillation. This ease of separation is due to the ability of isomer A to intramolecularly hydrogen bond whereas isomers B and C can only form intermolecular hydrogen bonds. Positive identification of the lower boiling point isomer as the ortho isomer was performed by 'H-NMR spectroscopy. Two doublets and two triplets observed in the aromatic ring region of the spectrum was indicative of the ortho isomer.

After separation of the ortho isomer from the commercially available material of mixed isomers, synthesis of silica-immobilized HOx from the various fractions gave the results shown in Table XX. As expected, the ortho isomer gave much lower capacities than the meta and para isomers or the entire mixture of isomers. This is probably due to steric hindrance of the reactive amine function due to the close proximity of the silica surface. The bulky HOx molecule cannot couple due to these steric problems.

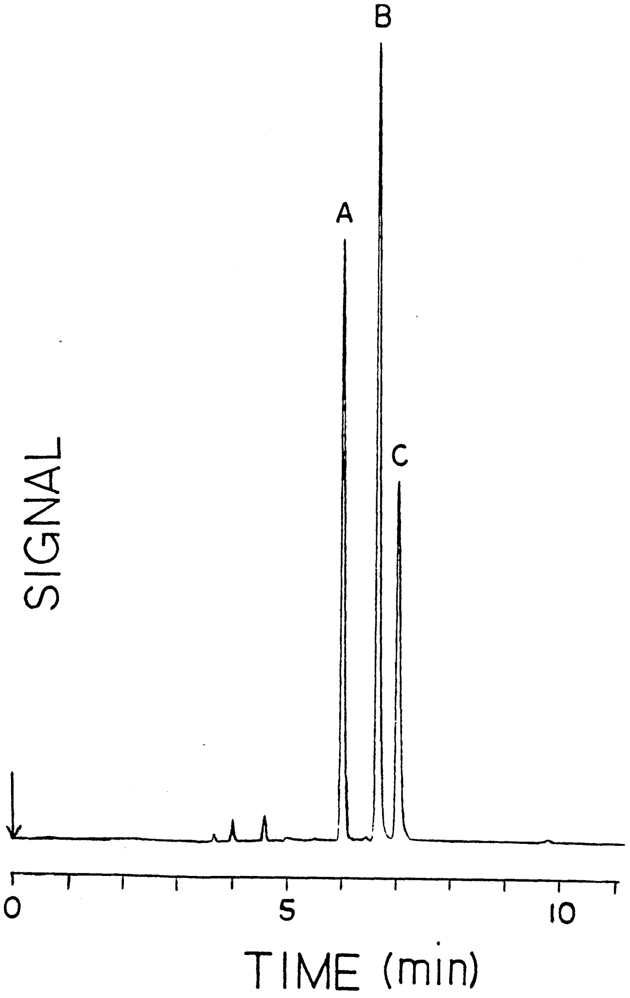


Figure 23. Capillary Gas Chromatogram of Commercially Available (Aminophenyl)trimethoxy Silane (Column Temp. = 210°C, Isothermal, OV101 Column, 39 m x 0.5 mm i.d.) . See text

TABLE XX

EFFECT OF ISOMERS OF (AMINOPHENYL)TRIMETHOXYSILANE ON CAPACITY

Isomer	Capacity (μmol/g)
ORTHO (90%)	41
META+PARA (97%)	237
ALL (97%)	220 ± 20

Somewhat disappointing, however, was the relatively small increase in capacity observed when performing the synthesis with a fraction containing primarily meta and para isomers instead of the entire mixture of isomers. This indicates that probably a large amount of the silane is attached to the silica surface, but the bulky HOx molecule "covers up" more than one of the bound aromatic amine groups. Therefore, it appears that the limit to capacity occurs due to the bulky nature of the HOx molecule. The presence of unreacted silane, however, provides an explanation for the larger capacities obtained from carbon determination versus metal ion (Cu(II)) studies since a large amount of organic matter is attached to the silica surface but is not subsequently coupled to HOx.

Effect of Pore Size/Surface Area

Up to this point in this dissertation, the factors affecting capacity have been related to some aspect of the synthetic procedure (i.e.

various synthetic routes, solvent degassing, isomer effect, etc.). Another approach to improving (increasing) capacity, however, involves changing the support, or optimizing and/or modifying its properties. Changing the support is an unattractive option because of the swelling and diffusional problems encountered in the use of most organic polymer supports (Chapter I) and the ease with which the silica surface undergoes reaction. Therefore, optimization or modification of any property of the support which could result in a larger capacity is of interest. The pore size and related specific surface area of the support can have a dramatic effect on the capacity; this effect has been alluded to previously (51, 72) and the results of the work included here have been published (77). Again, the synthetic route used was the new diazo coupling procedure described in Chapter III.

The relationship between capacity and mean pore diameter is shown in Figure 24. The capacity increases gradually at first, then rapidly as the mean pore diameter decreases to about 50 - 60Å; for smaller diameters, the capacity drops off sharply. This behavior is thought to be the result of two different effects. With large pore diameters, the specific surface area is relatively small which means that, for a given amount of silica support, the number of surface silanol groups available for silylation is also small. A decrease in pore size (i.e. increase in surface area) increases the number of surface silanol groups, which results in increased capacities. However, as the pore size becomes even smaller, a second effect (steric hindrance) predominates over the effect of increased surface area. The size of the immobilized functional groups begins to approach the size of the pores. A similar effect has been observed for enzyme immobilizations in which enzyme activity drops off

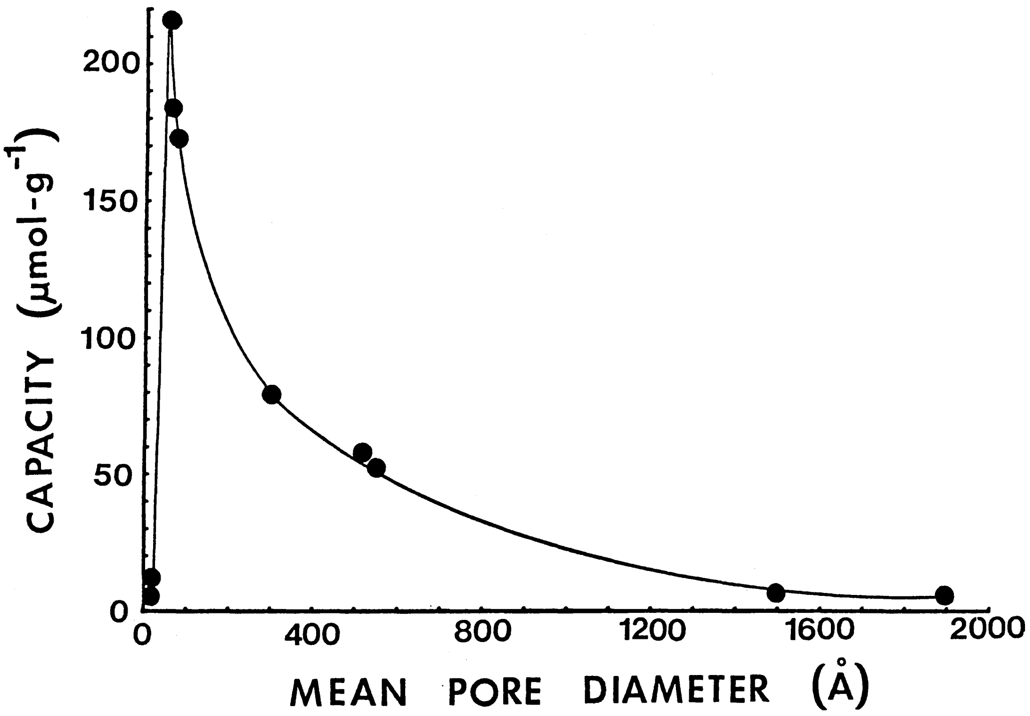


Figure 24. Effect of Mean Pore Diameter on Capacity

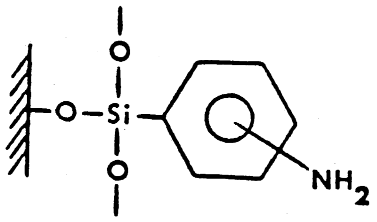
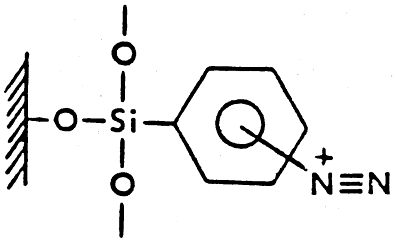
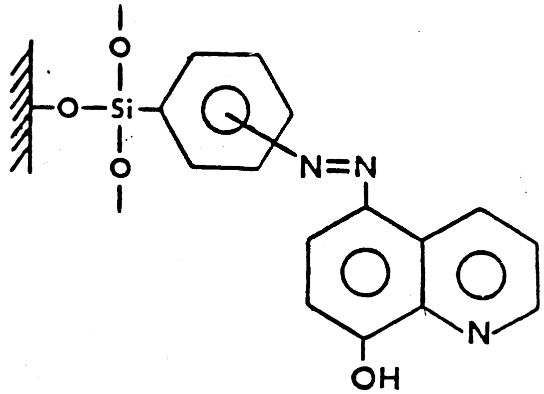
sharply below a certain cut-off pore diameter (78). The relationship between pore size and length of the immobilized functional group has also been shown to have a marked effect on chromatographic efficiency in high performance liquid chromatography (79).

It has been proposed (46) that, once the silylation reaction or one of the subsequent reactions has been completed, the pores become even smaller and therefore may block reaction of the active functional group (i.e., 8-quinolinol) at reaction sites within the pores. Another possibility is that the functional group becomes attached, but metal ions (particularly those that are large or highly hydrated) are prevented from diffusing into the pores and therefore cannot react with the chelating agent attached within the pores (46). Our observations seem to indicate that, in the case of silica-immobilized 8-quinolinol, the first explanation is more likely. On addition of an ethanolic solution of 8-quinolinol to the diazonium salt silica support, almost no red color (due to azo dye formation) was observed when performing the reaction with small pore diameter silica supports. Some 8-quinolinol, of course, is coupled at reaction sites on the exterior surface of the silica.

In an effort to rationalize this behavior, molecular models of the silica-immobilized species were assembled and the length of the attached group was estimated for each step of the immobilization reaction. The results are shown in Table XXI. It is apparent that formation of two bound 8-quinolinol moieties near the opening of a pore could block an entrance of roughly 24-28 Å. This corresponds fairly well to the observed cut-off pore diameter. Also, a diameter of 60Å, for example, should accommodate two opposing immobilized 8-quinolinol moieties (24-28 Å) and still allow for eady passage of other reactant species and

TABLE XXI

ESTIMATED LENGTH OF VARIOUS SILICA-IMMOBILIZED SPECIES

Step	Structure	Estimated Length (Å)
1		7-8 (para) 6-7 (meta and ortho)
2		8-9 (para) 7-8 (meta) 6-7 (ortho)
3		12-14 (para) 6-12 (meta) _____ (ortho)[a]

---

[a] Cannot form.

copper(II). This is in agreement with the high capacities observed for silica gels having pore diameters in this range. Therefore, the optimum pore diameter in terms of increased capacities appear to be in the range 40-60 Å. This optimum could be determined more accurately if silica supports with a wider variety of pore diameters and specific surface areas were available.

Stability Studies

The problem of hydrolytic instability of Schiff-base coupled HOx led to the investigation of stability of diazo-coupled HOx. In addition to hydrolytic stability, however, the investigation of storage stability (shelf life) and thermal stability were also performed.

Experimental Procedures

Hydrolytic stability studies were performed by mixing approximately 0.15 - 0.20g of silica-immobilized HOx with 20 mL of appropriate solutions adjusted to integral pH values between approximately -1 and 12. Buffer solutions were used when possible. Hydrochloric acid (pH-1 and 0), 0.1M potassium chloride (pH 1 and 2), 0.1M phthalate buffer (pH 3 and 4), 0.1M acetate buffer (pH 5 and 6), 0.1M phosphate buffer (pH 7, 8, and 9), 0.1M carbonate buffer (pH 10 and 11), and 0.1M sodium carbonate (pH 12) were used. After equilibration for a period of time (24 h or 72 h), the solid was removed by filtration, rinsed with water, and dried. The capacity of the dried material was then determined by copper(II) uptake as previously described. Storage stability was checked by measuring the capacity after various times of dry storage in a desiccator. The thermal stability was determined by thermogravimetry on a

DuPont Model 951 Thermogravimetric Analyzer at a scan rate of 10^{o}C/minute. In all cases Woelm TLC grade silica gel was used as the support for the immobilization of HOx.

Hydrolytic Stability

The hydrolytic stability of the silica-immobilized HOx is excellent in the pH 0-10 range. Table XXII indicates the percentage of the original capacity remaining after equilibration with solutions of various integer pH values. The material is very stable even in strongly acidic solutions. At pH 11 and 12, rapid hydrolysis of the silica framework was observed which was indicated by the dark red color of the solution after only a few minutes of contact. The red color seems to indicate that the azo bond remained intact. It is also observed that once the red solution was removed from the solid at pH 11 and 12, a gelationous red precipitate formed which was probably due to repolymerization of some of the dissolved species formed from the hydrolysis of the silica matrix. The solution slowly loses some of its red color when this occurs, finally becoming amber in color. Also, a drop in pH was observed at pH 11 and 12 which indicates the presence of monomeric silicic acid or higher soluble polymers which are products of the hydrolysis. These species may be responsible for the repolymerization observed.

Storage Stability

The storage stability or shelf life of silica-immobilized HOx is also excellent. No change in capacity (within experimental error) was observed after 1 year of dry storage. The initial capacity was 225 μmol/g and after 1 year was unchanged.

TABLE XXII

HYDROLYTIC STABILITY OF SILICA-IMMOBILIZED HOx

pH	24 h	72 h
-1[a]	104	95
0	102	100
1	101	100
2	100	100
3	101	---
4	99	98
5	99	---
6	101	100
7	102	---
8	103	99
9	102	100
10	102	105
11	91	91
12	62	62
Original Capacity (μmoles/g)	216	212

[a] Approximate pH (concentrated hydrochloric acid).

Thermal Stability

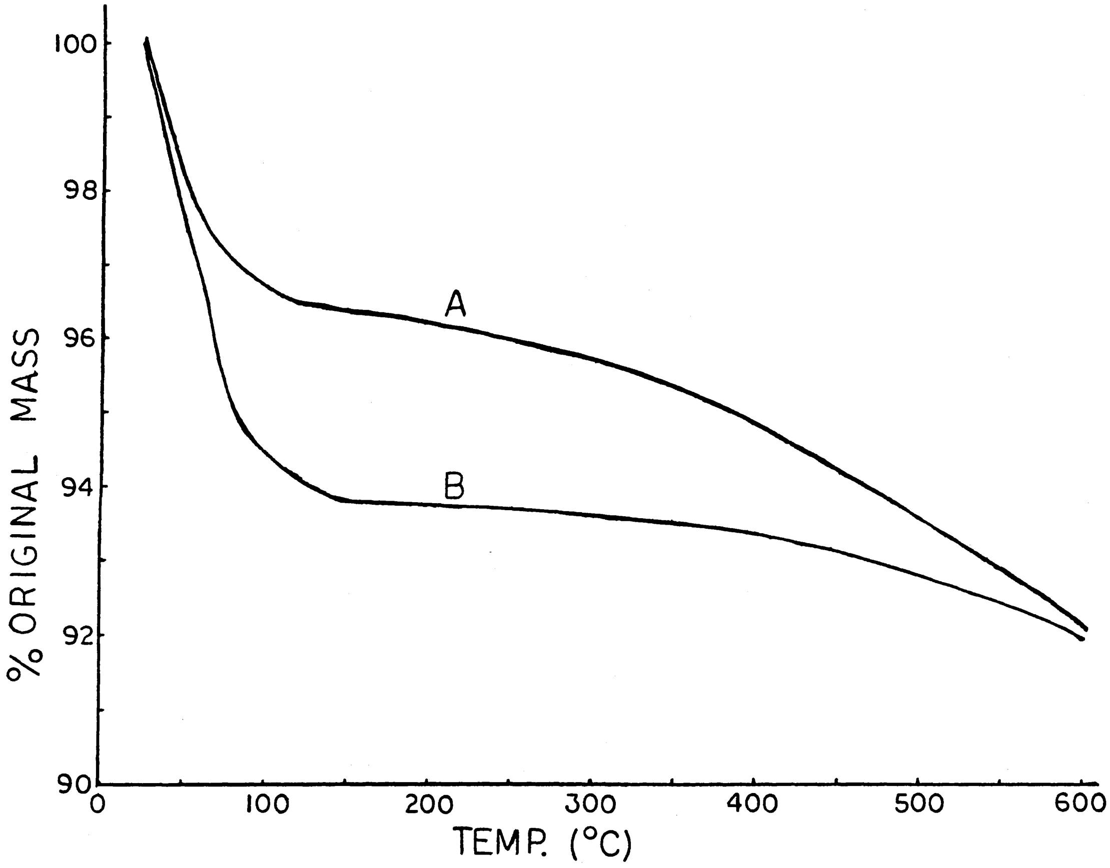
From a practical point of view, thermal stability is not as important as hydrolytic stability or storage stability. However, it may be useful to use silica-immobilized HOx under elevated temperature conditions in certain applications. Thermogravimetric analysis of silica-immobilized HOx indicated good thermal stability. Most of the weight loss appears to be due to surface-adsorbed water. The immobilized group makes up only a small fraction of the total mass present. Typical thermogravimetric curves are shown in Figure 25. The weight loss due to silica gel itself is greater than for the silica-immobilized HOx. This may be due to displacement of some surface-adsorbed water by immobilized HOx which reduces the moisture weight loss. As temperature increases above 250^{0}C, however, the rate of weight loss for silica-immobilized HOx begins to surpass that of silica gel itself which is an indication of loss of the immobilized functional group. The capacity after treatment at 600^{0}C drops to zero which indicates loss of the immobilized HOx. It appears that the upper limit for use of silica-immobilized HOx is probably about 200-250^{0}C.

Metal-Ion Distribution Studies

The use of silica-immobilized 8-quinolinol for metal ion preconcentration/separation was of interest. In order to choose preconcentration or separation conditions, it was necessary to characterize the material with regard to metal-ion distribution as a function of pH.

Experimental Procedures

Metal-ion solutions of varying pH were prepared from standard stock



A = Silica-Immobilized HOx; B = Silica Gel

Figure 25. Thermogravimetric Curves

metal-ion solutions and buffer solutions when possible. Typically, 20.00 mL of 100 μg/ml metal ion solution was added to a 100 mL volumetric flask and diluted to the mark with a 0.125M buffer solution. Buffers used included potassium chloride (pH 1-3), formate (pH 3-4), acetate (pH 4-6), phosphate (pH 6-8), and borate (pH 8-9). Usually 0.2g, of silica-immobilized HOx was equilibrated with 10 mL of standard stock metal ion solution for 1 h after which the solid was filtered and the remaining metal ion concentration in the filtrate was determined. In all cases, care was taken to ensure that the bound HOx groups were present in at least an eight fold excess. Actual excesses ranged from 8- to 14-fold. Copper(II) was determined by atomic absorption as previously described, iron(III) by the 1,10-phenanthroline/reducing agent method, cobalt by the thiocyanate method, nickel by the dimethylglyoxime/oxidizing agent method, and calcium by the purpurate (murexide) method. All of the colorimetric procedures listed above can be found in a single reference (80).

Percent Extracted Versus pH

The results of metal ion distribution studies for Cu(II), Fe(III), Ni(II), Co(II), and Ca(II) are presented in Figure 26. The selectivity of silica-immobilized HOx for the transition metal ions over the alkaline earth metal ions (i.e. Ca(II)) is readily observed. This allows for the use of these materials in preconcentration of trace heavy metal ions in aqueous samples containing large amounts of alkali and/or alkaline earth metal ions. The use of silica-immobilized HOx for separation of the transition metal ions, however, appears to be a more difficult task. The percent extracted as a function of pH is seen to be very similar for

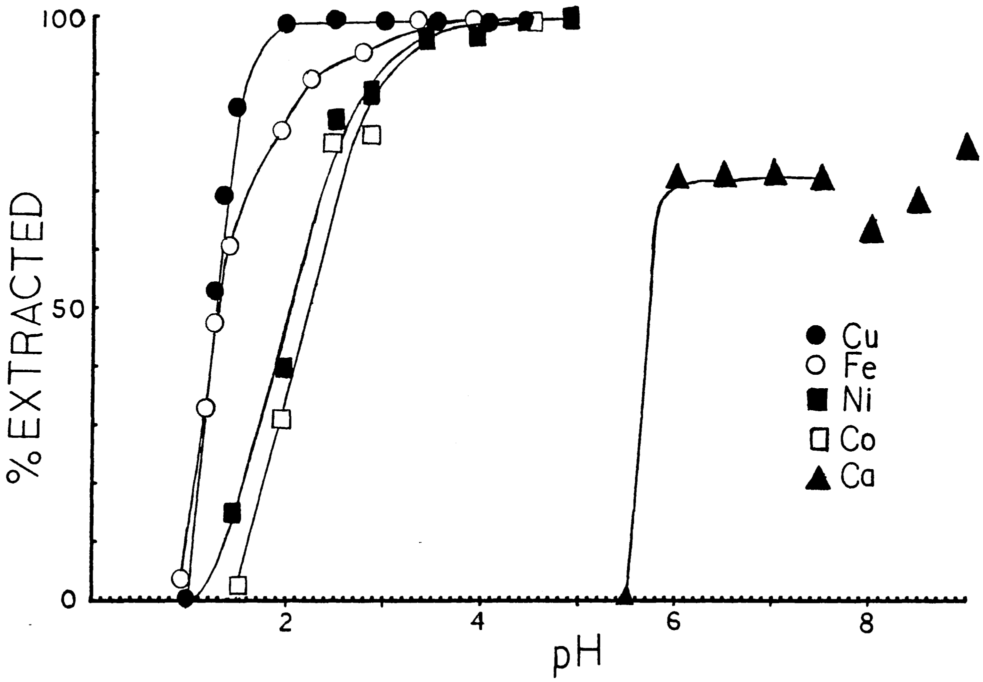


Figure 26. Metal-Ion Distribution Curves

Cu(II) and Fe(III), for example (also Ni(II) and Co(II)). This is somewhat disappointing from the point of view of liquid chromatography of the transition metal ions since this indicates a lack of selectivity between the transition metal ions.

The transition metal ions, it should be noted, are extracted at very low pH. This means that conditional formation constants are large, so that adjustment of the sample pH is really only important for strongly acidic samples. In fact, the percent extracted curves for the silica-immobilized HOx are shifted to lower pH values than the percent extracted curves obtained for free 8-quinolinol. Figure 27 shows the percent extracted of Ni(II) versus pH for: (1) new diazo-coupled HOx, (2) conventionally diazo-coupled HOx, and (3) free HOx. The new and conventional diazo-coupling procedures give essentially the same percent extraction behavior whereas free HOx extracts Ni(II) at a higher pH. This indicates that caution is necessary when making comparisons between an immobilized species and that same species in solution. Their properties are, many times, much different and serious errors can be made if one tries to extend knowledge about the species in solution to the immobilized form. The reason for the difference in extraction behavior lies in the acid-base characteristics of the material. This will be discussed in the next section.

Acid-Base Studies

It is apparent that HOx as well as immobilized HOx are amphoteric, containing both a weakly basic heterocyclic nitrogen as well as a weakly acidic phenol group. The protonated form of HOx, the 8-hydroxyquinolinium ion, undergoes stepwise dissociation as shown below:

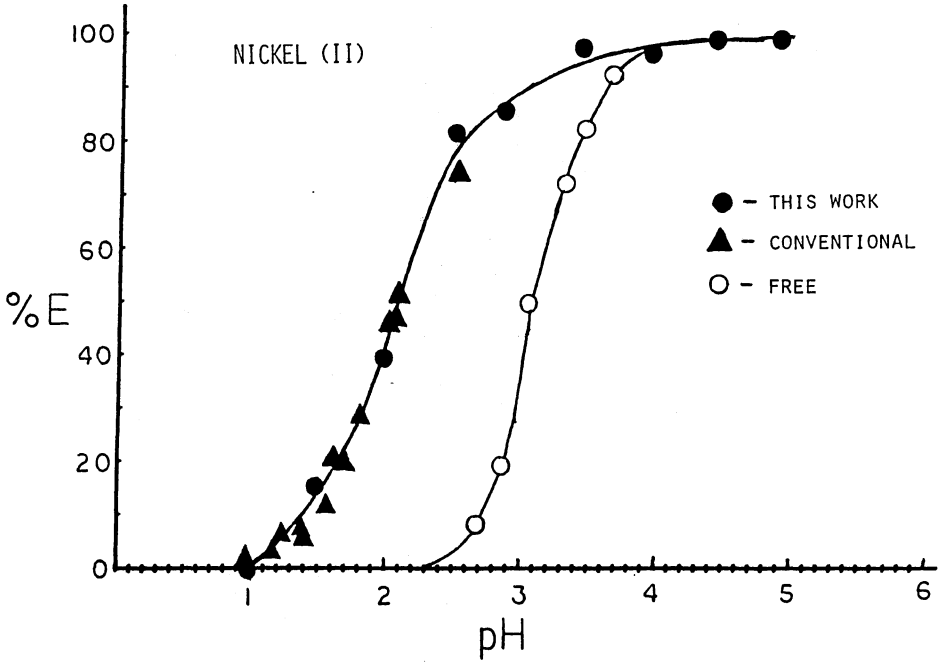


Figure 27. Comparison of Extraction Behavior for Free and Immobilized HOx

$$H_2Ox^+ \rightleftharpoons H^+ + HOx \qquad K_{a_1}$$

$$HOx \rightleftharpoons H^+ + Ox^- \qquad K_{a_2}$$

The difference in the acid-base properties of the free, and bound HOx is based on the effect of substitution of a phenylazo group at the 5-position of the HOx molecule. The effect of this substitution has a large effect on the properties of silica-immobilized HOx; particularly the metal ion extraction behavior as a function of pH.

Experimental Procedures

Potentiometric pH titrations were performed on some of the silica-immobilized HOx prepared. Typically, 0.2g of the solid was added to 25 mL of water. The titration was then performed with 0.01M hydrochloric acid or 0.01M sodium hydroxide. In some cases, the immobilized HOx was rinsed with pH 7 buffer solution or 0.1M hydrochloric acid prior to titration in order to ensure that the material was in the neutral (HOx) form or in the protonated (H_2Ox^+) form, respectively. All pH measurements were made with an Orion Research Model 601A pH meter (Cambridge, MA) equipped with an epoxy-body combination electrode (Sensorex, Westminster, CA). All pKa values were estimated from the pH at the half-equivalence volume.

In some cases, a graphical method similar to one described previously (81) was used to confirm the pKa_1 ibtained from half-neutralization. In this method, it is assumed that at pH $<$ 5, only the first dissociation reaction need be considered. Under these conditions, where

$\bar{X} = [OH^-]_{Total}/[HOx]_{Total}$, a plot of $\bar{X}/(1-\bar{X})$ vs. pH yields an intercept equal to $-pKa_1$.

Results of Acid-Base Studies

A comparison of pKa values obtained by various workers for HOx, 5-phenylazo-HOx (the closest free solution analogue to the immobilized material), and immobilized HOx is shown in Table XXIII. An acid-strengthening effect due to substitution at the 5-position is apparent as both pKa_1 and pKa_2 decrease when the phenylazo group is introduced. This gives an explanation for the shift in the metal ion extraction curves for the immobilized HOx. A more acidic phenol group means that the proton is more easily displaced by a metal ion.

The other point to be made about the data of Table XXII is the unusual and inconsistent data observed for silica-immobilized HOx. A conventionally prepared Porasil-immobilized HOx has been reported (48) to exhibit a pKa_1 value that is similar to the pKa_1 of 5-phenylazo-HOx using sodium hydroxide as the titrant. The pKa_2 value, however, is observed to be much different. Later work using hydrochloric acid titrant indicates a very different value for pKa_1 also (51).

In an effort to gain some insight into those apparent discrepancies in reported pKa values, we have performed a large number of potentiometric pH titrations on silica-immobilized HOx and silica gel itself. The results are tabulated in the last three rows of Table XXIII.

The apparent pKa, obtained from the titration of silica-immobilized HOx with hydrochloric acid agrees fairly well with the value reported previously for the conventionally coupled material (51). However, silica gel itself was also observed to yield a similar pKa value which indicates

TABLE XXIII

APPARENT ACID DISSOCIATION CONSTANTS

Material	pKa_1	pKa_2	Titrant	Reference
Free HOx	4.85	9.95	a	82
5-Phenylazo-HOx	3.1	8.9	NaOH[b]	83
Porasil-HOx	3.3	6.2	NaOH	48
Silica Gel-HOx	7.0 - 7.5	----	HCl	51
Silica Gel-HOx	6.3 - 7.1	----	HCl	This Work
Plain Silica Gel	6.1	----	HCl	This Work
Silica Gel-HOx	3.3 - 3.8	7.0 - 7.5	NaOH	This Work

[a] From solvent extraction studies.

[b] In 50% aqueous dioxane.

that the values are possibly due to titration of silicate groups on the silica surface. It is not surprising that hydrochloric acid titration of the weakly basic heterocyclic nitrogen does not yield an inflection point on the titration curve in aqueous solution since the leveling effect of water must be considered.

Titration of silica-immobilized H_2Ox^+ with sodium hydroxide yields two pKa values that agree fairly well with previously reported values for the conventionally coupled material (47). The somewhat higher pKa values reported here may be due to the higher surface coverage of the silica-immobilized HOx used here relative to the surface coverage of the conventionally coupled silica-immobilized HOx materials previously studied. It has been shown that the pKa values of polyelectrolytes (silica-immobilized HOx is a polyelectrolyte) are influenced by the degree of neutralization (84). Higher surface coverage affects the concentration of labile groups and therefore may affect pKa values. In fact, the pKa of surface silanol groups has been shown to vary between about 6.3 and 9.8 depending on the degree of neutralization (84). It is not known to what degree neutralization of HOx moieties can affect pKa values obtained for the remaining immobilized HOx. The magnitude of the effect may be less for immobilized HOx than for silica itself since the titratable groups are farther apart in immobilized HOx.

In order to demonstrate that the observed pKa_1 was not due to residual hydrochloric acid remaining from the pretreatment (which forms H_2Ox^+), a sample of silica gel itself was treated in the same manner and titrated with sodium hydroxide. No evidence of residual hydrochloric acid was present as illustrated in Figure 28. The apparently low pKa_2 relative to 5-phenylazo-HOx is probably due to surface silanol groups.

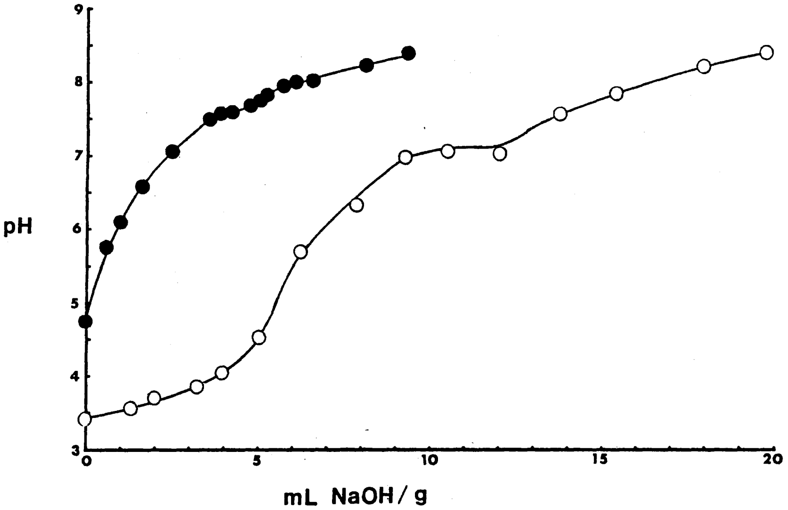


Figure 28. Titration Curves of Silica Gel (●) and Silica Gel Immobilized HOx (O); Titrant, 0.00923 M Sodium Hydroxide

The weakly acidic phenol group may not yield an observable inflection point in aqueous solution since it is such a weak acid. Obviously, it would be desirable to form the oxinate anion (Ox^-) and titrate it with hydrochloric acid to obtain pKa_2. However, the silica matrix cannot withstand the relatively high pH necessary to form the anion.

The pKa_1 values obtained from the sodium hydroxide titration of H_2Ox^+ by using the pH at the half-equivalence volume were confirmed by a graphical method (81). The pKa_1 values obtained graphically ranged from 3.0 to 3.5 which is in good agreement with the pKa_1 values estimated from the half-equivalence volume.

The major conclusions that can be drawn from the titration data presented are: (1) The pKa_1 value of 3.3 - 3.8 is valid and is in good agreement with pKa_1 for 5-phenylazo-HOx in solution; (2) the pKa_2 value is inaccessible by direct titration due to complications arising from the acidic nature of the silica surface and the solubility of silica gel at high pH; and (3) previously reported discrepancies in pKa values of silica-immobilized HOx can be explained when the complicating effect of surface silanol groups is considered.

CHAPTER V

STUDIES AND APPLICATIONS IN FLOW SYSTEMS

Up to this point in this thesis, all experimental work with silica-immobilized HOx has been done under batch, or static, conditions. However, most applications of silica-immobilized HOx (including preconcentration) involve its use under dynamic (i.e. flowing) conditions. Therefore this chapter is devoted to evaluation of the use of silica-immobilized HOx in flow systems, and particularly in flow injection analysis (FIA) in combination with atomic absorption spectroscopy (AAS) for the on-line preconcentration and determination of trace levels of metal ions.

Breakthrough Studies

The total number of immobilized groups per unit mass of solid, commonly called the total capacity has been discussed in Chapter II and is measured, generally, under batch conditions. In column operation another expression is used for column capacity, namely, the breakthrough capacity, which is defined as the amount of ions that can quantitatively be extracted per unit mass of solid under the existing experimental conditions. The breakthrough capacity therefore is always lower than the total capacity and is dependent upon a variety of experimental variables such as particle size, flow rate, temperature, column configuration, pH, etc. (2, 85, 86). Generally any factor which increases the rate of ion exchange increases the breakthrough capacity. In analytical applications such as preconcentration it is essential that the breakthrough capacity not be exceeded in order to have quantitative recovery of the metal ions of interest. Therefore, a study of the effect of variables such as those mentioned above on breakthrough capacity was in order. The effect of particle size on breakthrough capacity was not studied in detail, however, since the use of a low pressure system with a peristaltic pump restricted the choice to relatively large sizes.

Experimental

All breakthrough studies were performed using Cu(II) as the metal ion probe at pH 5. The support, or carrier, used in all breakthrough studies was Baker Silica Gel for Column Chromatography (J. T. Baker, Phillipsburg, NJ, 450 m^2/g surface area, 60Å pore diameter). Several different particle sizes were used other than the 74 - 250 μm range of the commercially available material. These different sizes are noted when applicable. A four channel Gilson minipuls 2 peristalic pump was used for solution delivery. Injection of eluting acid for column regeneration was achieved through the use of a sliding rotary valve injector constructed from plexiglass and Teflon (87). This valve was modified by adding a clamp and ferrules to the injection valve parts so that higher pressures could be tolerated. A 1.0 ml injection loop was used.

Columns were packed by placing a small silanized glass wool plug at one end of a glass column and aspirating a water-slurry of silica-HOx into the column until filled. Another small silanized glass wood plug was then inserted into the other end. All connections were made with silicone rubber tubing, Teflon tubing, and Cheminert fittings.

The experimental arrangement for breakthrough studies is shown in Figure 29. First, with the column removed, Cu(II) was pumped through the system in order to fill all the tubing up to the column. Then, the column was placed in the system and pumping was resumed at a specified flow rate. Fractions were collected in 2 mL vials at 1.0 mL intervals. The Cu(II) content of each vial was then determined by atomic absorption spectroscopy as previously described. Two column dimensions were investigated: Column #1 was 1.43 mm i.d. x 10 cm long and Column #2 was 2.60 mm i.d. x 3.0 cm long. The resulting volumes were both about 160 μL and both columns contained about 0.065g of silica-immobilized HOx. Constant temperatures were maintained using a Brinkmann Instruments Model IC-2 circulator (Westbury, NY) by immersing the column, reagents, and tubing in a thermostatted water bath.

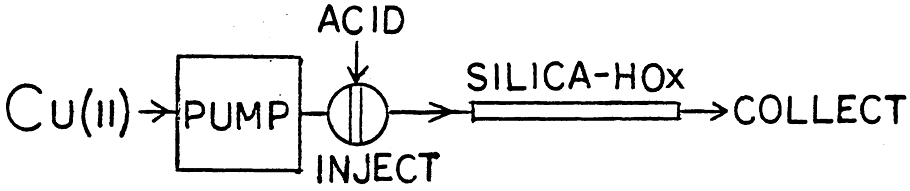
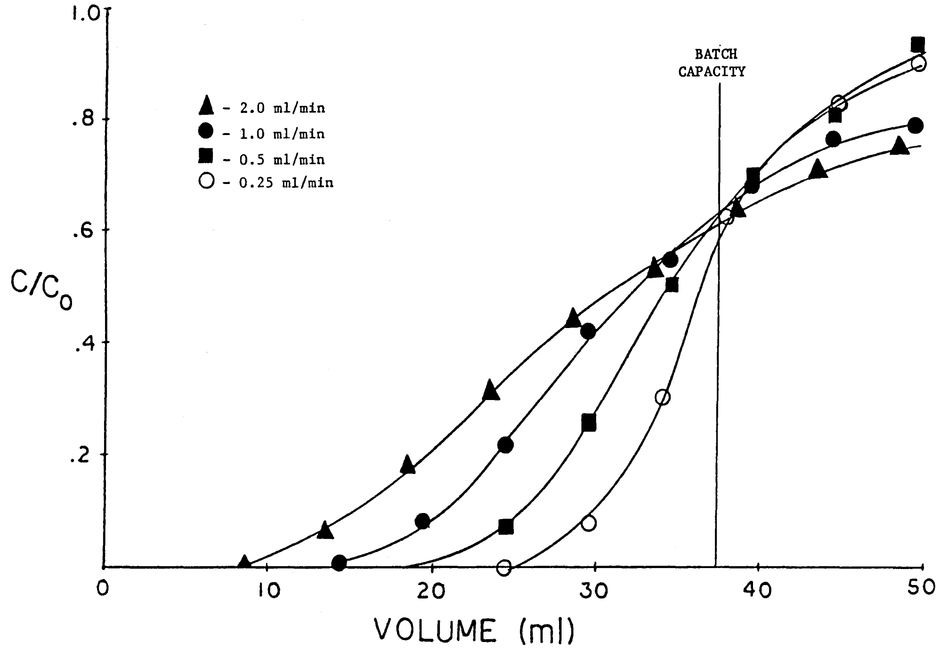


Figure 29. Experimental Arrangement for Breakthrough Studies

Flow Rate Effect

Flow rate was observed to have a very significant impact on the breakthrough capacity. Figure 30 shows a series of breakthrough curves,



C/Co = Effluent Conc/Influent Conc.
Batch Capacity = 185 μmol/g

Figure 30. Breakthrough Curves as a Function of Flow Rate for Column 1

or isoplanes (85, 86), at varying flow rates and the volume corresponding to the batch capacity or total capacity for Column 1. In an isoplane, the ratio of effluent concentration to influent concentration is plotted versus the volume of solution delivered. The curves, then, cross at the volume corresponding to the batch, or total, capacity. The trend of decreasing breakthrough capacities as flow rates increases is apparent. It should be mentioned that breakthrough is defined as the point at which Cu(II) is first detected in the effluent. At a flow rate of 2.0 mL/min, for example, the breakthrough capacity is less than one-fourth of the batch capacity. Therefore it is imperative that the breakthrough capacity of a chelating ion-exchanger be determined under the existing experimental conditions in order to ensure that the capacity of the column is not exceeded in application. Figure 31 depicts the effect of flow rate on capacity for Column 1. At higher flow rates, the breakthrough capacity is observed to decrease accordingly. Column 2 gives similar isoplanes as shown in Figure 32.

Effect of Column Dimensions

As the dimensions of a column change (at constant volume, type of exchanger, and mass of solid) a shift in the isoplanes is also expected (85, 86). In general, long, narrow columns give larger breakthrough capacities than short, wide columns. A convenient way to express the dimensional characteristics of a column is to calculate the aspect ratio which is simply the ratio of length to inside diameter of the column. Therefore, columns with large aspect ratios are generally preferable to those with smaller aspect ratios. Figures 33 and 34 compare two columns,

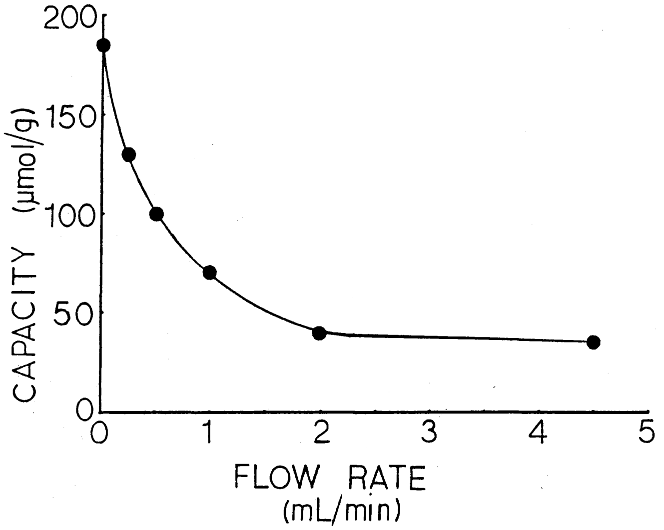
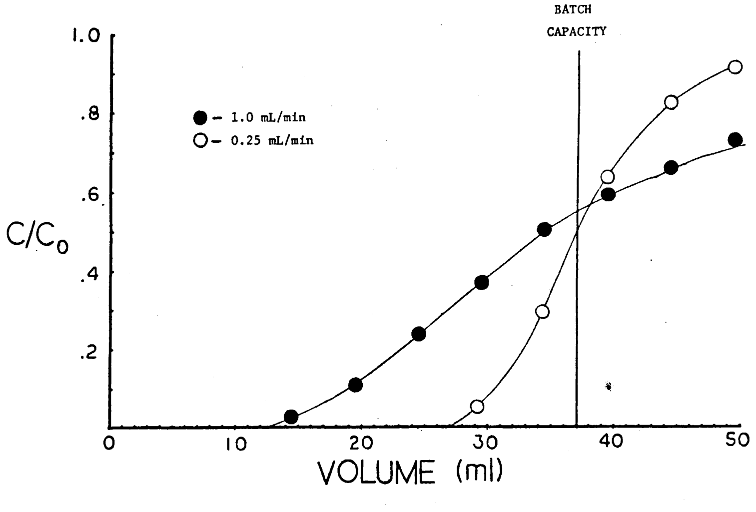


Figure 31. Effect of Flow Rate on Capacity for Column 1



Batch Capacity = 185 μmol/g

Figure 32. Breakthrough Curves as a Function of Flow Rate for Column 2

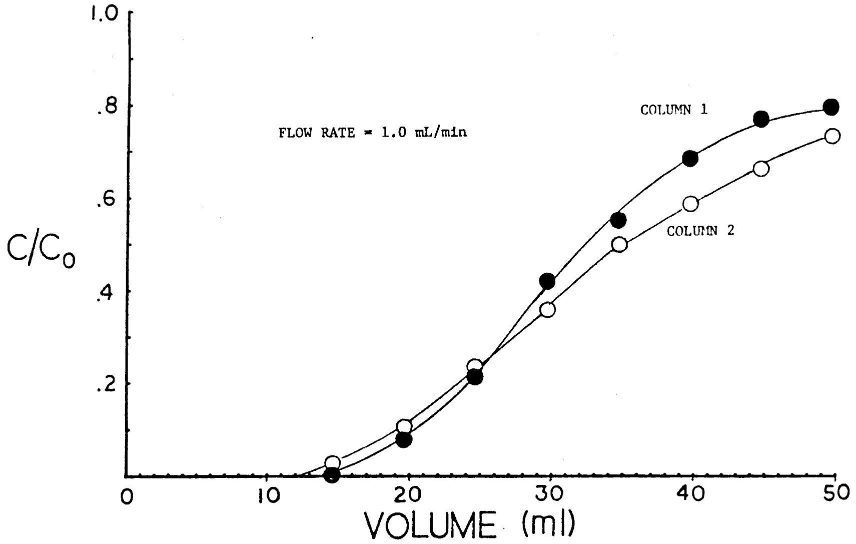


Figure 33. Comparison of Breakthrough Curves for Columns 1 and 2

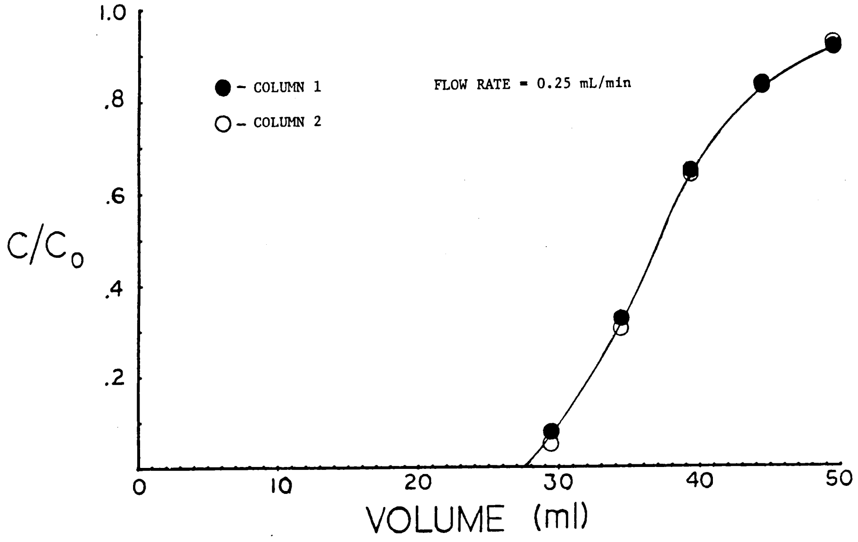


Figure 34. Comparison of Breakthrough Curves for Columns 1 and 2

Column 1 and Column 2, having aspect ratios of 67.9 and 11.6, respectively at two different flow rates. Volumes, type of packing, temperature, etc., are constant. The effect of dimensions (at least within the range studied) on breakthrough capacity was found to be much less than the effect of flow rate. In fact, at low flow rates (Figure 34), the isoplanes are essentially identical. As flow rate increases, however (Figure 33) Column 1 was observed to yield a slightly larger breakthrough capacity as expected. Therefore, in all applications, the column chosen had dimensions similar to that of Column 1.

The effect of column dimensions on the breakthrough capacity appears to be a diffusional effect. At small aspect ratios and relatively high flow rates, it appears that radial diffusion does not occur quickly enough to utilize as many of the exchange sites in the column. However, at lower flow rates or larger aspect ratios, more of the exchange sites are accessible and the breakthrough capacity is increased.

Temperature Effect

An increase in temperature results in an increase in the breakthrough capacity. The effect can be seen in Figure 35. The temperature effect, like the dimension effect, is much less important than the flow rate effect. Only small changes in the isoplanes are observed between 25^oC and 45^oC.

Temperature contributes to larger breakthrough capacities by increasing the rate of ion exchange. There are probably two contributing effects to this increase in ion exchange rate. The first is the increase in the reaction rate (or rate coefficient) and the second effect is the effect of temperature on diffusion coefficients. Depending on the particular system one is using, one, or both factors may contribute to the observed increase in breakthrough capacity. However, since the effect is so small and thermostatting the columns is somewhat cumbersome, use at room temperature is probably preferred.

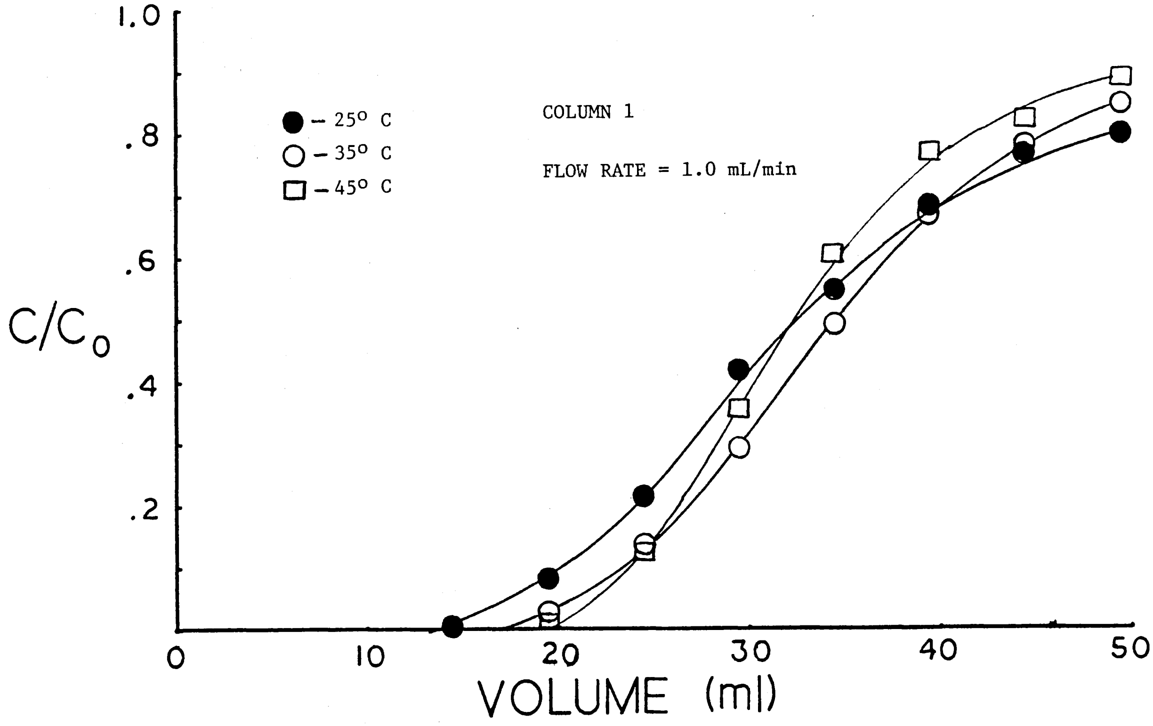


Figure 35. Breakthrough Curves as a Function of Temperature

Applications

The applicability of chelating ion exchangers for preconcentration of metals from dilute aqueous solutions has already been discussed in Chapter II. As mentioned, the impetus for development of chelating ion-exchange materials was to provide a more selective alternative to conventional ion exchange resins so that very small amounts of certain elements of interest could be concentrated, or extracted, in the presence of large amounts of elements of little or no interest, such as the alkali or alkaline earth metals. The extracted elements (usually heavy metals) can then be eluted with acid and determined by a variety of techniques or, alternatively, the extracted elements can sometimes be determined directly on the solid chelating ion exchanger by surface analytical techniques such as ESCA or x-ray fluorescence spectrometry. Direct determination, however, does not allow for reuse of the solid. Therefore, in this respect, techniques such as atomic absorption spectroscopy (AAS) or inductively coupled plasma spectroscopy (ICP) are to be preferred.

Several recent reviews cover the area of trace metal concentration as this area is very important, particularly for the analysis of seawater (88-90).

In the past, preconcentration prior to AAS (or ICP) has been accomplished off-line. That is, preconcentration was carried out on a relatively large volume of sample, the preconcentrated metals were then eluted with aqueous acid into a small volume, and this small volume was then carried to an instrument for determination. Procedures such as the one just described become tedious in practice and are subject to human errors due to the manual manipulations required.

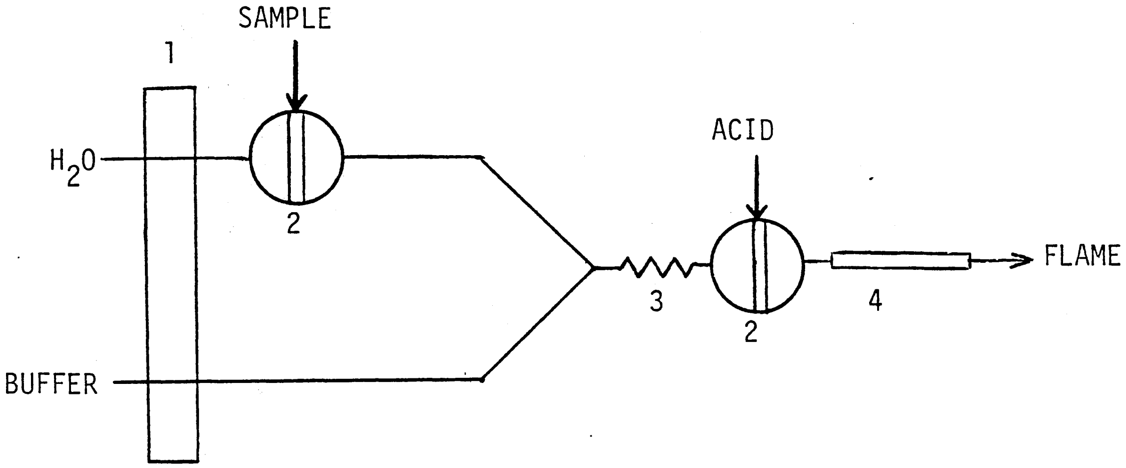
In recent years, however, automated sample processing techniques have been developed which reduce the number of manual manipulations of the sample. One of these sample processing techniques is unsegmented continuous flow sample processing, commonly called flow injection analysis (FIA) (91). Over 300 papers are now in the literature concerning FIA. However, only a very few number of papers have appeared concerning the combination of FIA as a sample processing tool and atomic absorption spectroscopy (AAS) as the detection device. Originally, the basis of FIA was to allow a discrete liquid sample plug to disperse in a nonsegmented continuous liquid carrier stream to an extent controlled by flow rate, tube dimensions, and volume injected, so that a reaction product (or reactant) could be measured by a downstream detector (92). Chemical reactions, however, do not usually play a role in atomic absorption determinations in conjunction with FIA. Therefore, it is better to consider FIA as a means of introducing samples into the spectrometer, albeit that dilutions and the addition of interference-reducing species such as lanthanum can also be performed easily (93-95). A short review of FIA methods used in conuunction with AAS has recently appeared (96). Although chelating ion-exchangers have been used in conjunction with AAS, and FIA has been used with AAS, only one paper has appeared which exploits the full combination of sample processing via FIA, preconcentration with a chelating ion exchanger, and determination by AAS (97). These workers used columns of Chelex-100, an iminodiacetate resin, for preconcentration. However, swelling of the polymeric resin (over 100% swelling has been reported) causes undue backpressure problems and a sophisticated manifold involving switching and backwashing of the column was necessary in order to utilize Chelex-100 in this application. In this chapter, results of an investigation into the use of silica-immobilized HOx for preconcentration in FIA-AAS is reported which eliminates swelling problems, thereby simplifying the necessary manifolds.

Experimental

The experimental arrangement for FIA-Preconcentration-AAS is shown schematically in Figure 36. Distilled/deionized water was used as the carrier at a flow rate of 4.0 mL/min into which an acidified sample could be injected or intercalated. This sample/carrier was then merged with a buffer (0.1M $NH_4C_2H_3O_2$ + HCl, pH 5.0) at a flow rate of 0.5 mL/min to give an overall flow rate of 4.5 mL/min through the delay mixing coil where neutralization of the acidified sample occurred. The sample (now at pH 5.0) then passed onto a silica-HOx column (1.63 mm i.d. x 10 cm long) containing approximately 0.065g of Baker silica-HOx (230 μmol/g capacity, 150-250 μm diameter, 450 m^2/g surface area, 60Å pore size). The metal ions of interest were then concentrated on the column while the alkali and alkaline earth metal ions passed, unextracted, through the column and into the flame of the atomic absorption spectrophotometer. Normally the sample injection volume was 10.0 mL. Therefore, the preconcentration step takes about 2.2 min (10 mL/4.5 $mL\text{-}min^{-1}$) neglecting dispersion. In the interest of ensuring complete passage of the sample (since some dispersion occurs), 4.0 min was normally allowed for the preconcentration step. During this preconcentration step the second injection valve (for acid) was in the "fill" position.

After the preconcentration step the concentrated metal ions were recovered, or eluted, by injecting (intercalating) a 1.0 mL plug of 1.0 M HCl + 1.0 M HNO_3. The eluted metal ions then passed into the flame of the atomic absorption spectrophotometer and the resulting transient signal was recorded.

In some cases the preconcentrating silica-HOx column, the acid injection valve, and the delay mixing coil were removed and samples of



1 - Peristaltic Pump
2 - Injection Valves
3 - Delay Mixing Coil
4 - Silica-Immobilized HOx Column

Figure 36. FIA-Preconcentration-AAS Experimental Arrangement

Cu(II) were injected (intercalated) directly into a water carrier leading into the flame. This was done in order to make a comparison of FIA-AAS to FIA-Preconcentration-AAS.

All instrumentation, with the exception of the recorder (Hewlett-Packard Model 7128A, San Diego, CA), has been previously described. Connecting tubing was Teflon (1.5 mm o.d. x 0.8 mm i.d.). Cheminert fittings were used for all connections except for connections to the column which were made via 1/16 inch barbed connectors and silicone tubing.

Quality control water sample concentrates were obtained from the U.S. Environmental Protection Agency Environmental Monitoring and Support Laboratory (Cincinnati, OH). Standards of Cu(II) were prepared from a 993 μg/mL Cu(II) standard solution for atomic absorption applications (Aldrich Chemical Co., Milwaukee, WI).

Direct Injection FIA-AAS

Direct injection of a sample into a water carrier stream which has been directly connected to the aspiration tube of a flame atomic absorpion spectrophotometer is an extremely simple approach for the determination of metal ions in water samples. In the direct injection mode, the flow injection system serves primarily as a means of sample handling. This sample handling involves simply sample transport and exact timing. Normally, all connecting tubes between the injection valve and the flame are made as short as possible in order to reduce dispersion. This results in larger peak heights (higher sensitivity) and narrower peaks (higher analytical throughput). The flow rate of the carrier in direct FIA-AAS must be at least equal to the aspiration rate of the flame. In this case, this restricted flow rates to values greater than 4.4 mL/min.

A series of standards were injected into a single-line FIA-AAS system and the resulting signal profiles are shown in Figure 37. The sample volume, being 180 μL, does not allow for a steady-state signal to be obtained due to dispersion. The dispersion coefficient $D = C_o/C_{max} = 1.9$ (where C_o = steady-state signal and C_{max} = signal at the peak maximum) indicates limited dispersion of the sample plug (98). Therefore, the bar above the 5 ppm signals represents the height of the steady-state signal that would be obtained from direct, continuous aspiration of 5 ppm copper solution or, alternatively, this signal could also be achieved by increasing the injection volume. In this case D = 1. A steady-state signal was achieved at an injection volume of about 1.0 mL. Of course, the increased peak height (sensitivity) is offset, to some degree, by the larger peak width (lower throughput). A comparison of performance data for the direct injection FIA-AAS and continuous aspiration (steady-state) AAS is presented in Table XXIV. The sensitivities and limits of detection are comparable for both direct injection and steady-state AAS. Direct injection, however, has certain advantages such as higher analytical throughput (Table XXIV) and smaller sample size. Also, use of a pump instead of aspiration reduces the signal dependence due to viscosity changes (96).

The presence of up to 0.2M sodium acetate buffer in the standard had no effect on the signal obtained. It should be mentioned, however, that better limits of detection and sensitivities have been obtained by other workers (97) using different instrumentation. However, the reason for these differences is the more advanced spectrophotometer used in their work; not any fundamental aspect of the flow system.

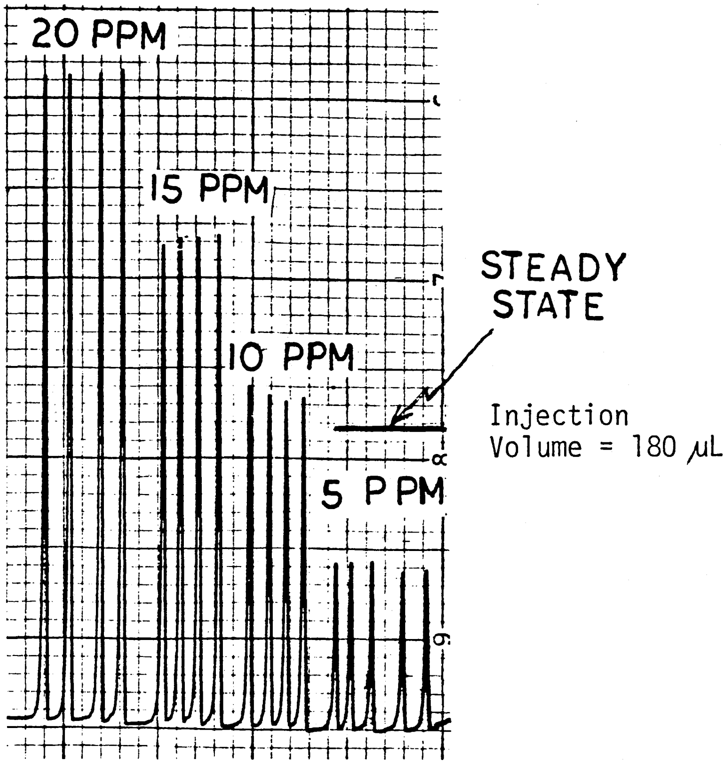


Figure 37. Signal Profiles for Direct Injection FIA-AAS Flow Rate = 4.5 mL/min, Chart Speed = 0.25 in/min

TABLE XXIV

PERFORMANCE OF FIA-AAS VERSUS CONVENTIONAL AAS[a]

	Direct FIA-AAS	Steady-State AAS
Correlation coefficient	0.99995	0.99997
Slope of working curve (cm/ppm)	0.439	0.727
Sensitivity (A.U./ppm)	0.013	0.022[b]
Intercept (cm)	0.010	-0.018
Limit of detection (ppm)[c]	0.19	0.18[d]
Analytical throughput (hr^{-1})	200	100
Sample size (μL)	180	$\geq$1000

[a] Metal = Copper; Wavelength = 324.7 nm.

[b] Perkin-Elmer 290B manual reports 0.029 A.U./ppm for steady-state AAS.

[c] Estimated from the standard deviation of the y-intercept of the working curve (S_i) and defined as the concentration corresponding to $3S_i$.

[d] Perkin-Elmer 290B manual reports 0.015 ppm for steady-state AAS.

FIA-Preconcentration-AAS

In an effort to lower the limit of detection while, at the same time, allowing for good reproducibility and high analytical throughput, an on-line preconcentration system was incorporated into the FIA-AAS system. This system utilized silica-immobilized HOx as the preconcentrating medium.

When combining FIA with AAS, flow rate restrictions, as mentioned, dictate relatively high flow rates (greater than 4.4 mL/min in this case). These large flow rates result in large pressure drops when using packed columns for preconcentration. In fact, the commercially available Baker silica support (particle size 74-250 μm) contains too many small particles resulting in an applicable flow rate range of up to about 2.0 mL/min only. Separation of a 150-250 μm fraction by sieving followed by immobilization of HOx resulted in a silica-immobilized HOx that could be used directly in-line with the aspirator of a flame AAS at flow rates above 4.4 mL/min which was the aspiration rate of the instrument used in this work. Therefore, an overall flow rate of 4.5 mL/min was chosen for operation of the system. The flow system has already been shown in Figure 36.

At relatively high flow rates, of course, the useful capacity of the column (i.e. the breakthrough capacity) is much less than the total (i.e. batch) capacity as demonstrated earlier in this chapter. In fact, on-line determination of the breakthrough capacity at 4.5 mL/min yielded a value of 37 μmol/g compared to a batch capacity of 230 μmol/g. Therefore, at 4.5 mL/min the breakthrough capacity is about 16% of the total capacity. This is in agreement with the breakthrough curves of Figure 30. Under no circumstances can the column be operated after breakthrough since quantitative extraction is not achieved.

A series of standard Cu(II) solutions were subjected to analysis using the FIA-preconcentration-AAS system described. The signal profiles obtained are shown in Figure 38. For comparison, the steady-state signal for a 0.5 ppm copper solution is shown. A considerable enrichment factor was achieved. The enrichment factor obtained when using a 10 mL sample was found to be approximately 35-fold. This corresponds to the ratio of peaks heights obtained after, and prior to, preconcentration. Larger enrichment factors may be obtained by increasing the injection volume. The limit to enrichment, however, is due to the presence of the analyte in the carrier or reagents. The blank signal, in these cases, becomes larger as the injection volume increases.

The working curve obtained from the standards in Figure 37 is shown in Figure 39. A straight line relationship is apparent. (Correlation coefficient = 0.9986, slope = 0.0261 cm/ppb, y-intercept = 0.412 cm). The limit of detection is about 1.5 ppb (0.0015 ppm) with a sensitivity of approximately 0.792 A.U./ppm. Both of these values are about one to two orders of magnitude better than those reported in Table XXIV in the absence of preconcentration. Of course, the sacrifice is in analytical throughput. The analytical throughput, or determination rate, of the preconcentration method with a 10 mL sample volume is about 15 determinations per hour.

The presence of 0.1 M Na^+ in the sample had no effect on the signal obtained and, in fact, sodium was readily observed in the flame (yellow) during the preconcentration cycle which indicates the lack of affinity of silica-HOx for Na^+. Also, no carry over of Cu(II) was observed which indicates efficient elution by 1.0 M HCl + 1.0 M HNO_3.

The design of the manifold for FIA-preconcentration-AAS deserves some mention (see Figure 36). Dispersion in systems such as this one is due primarily to dispersion between the column and the flame. Therefore,

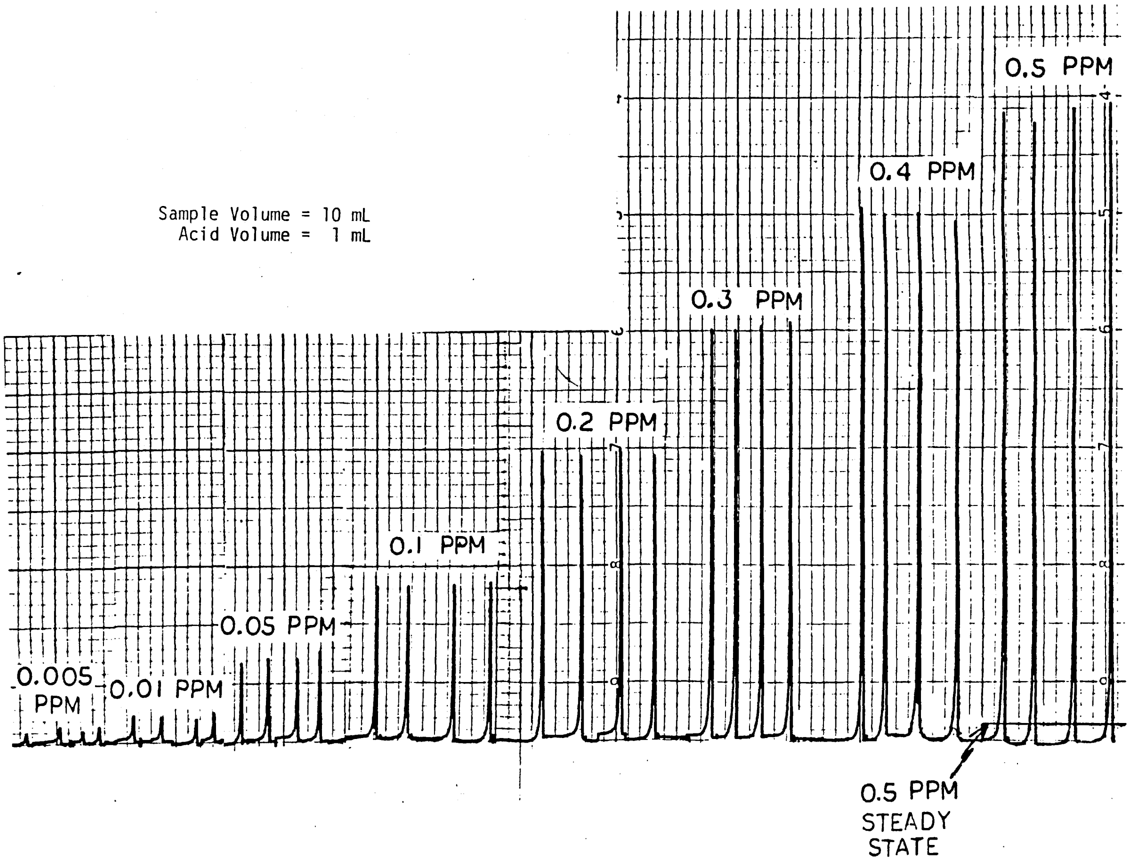


Figure 38. Signal Profiles for On-Line FIA-Preconcentration-AAS Flow Rate = 4.5 mL/Min, Chart Speed = 0.25 in/Min

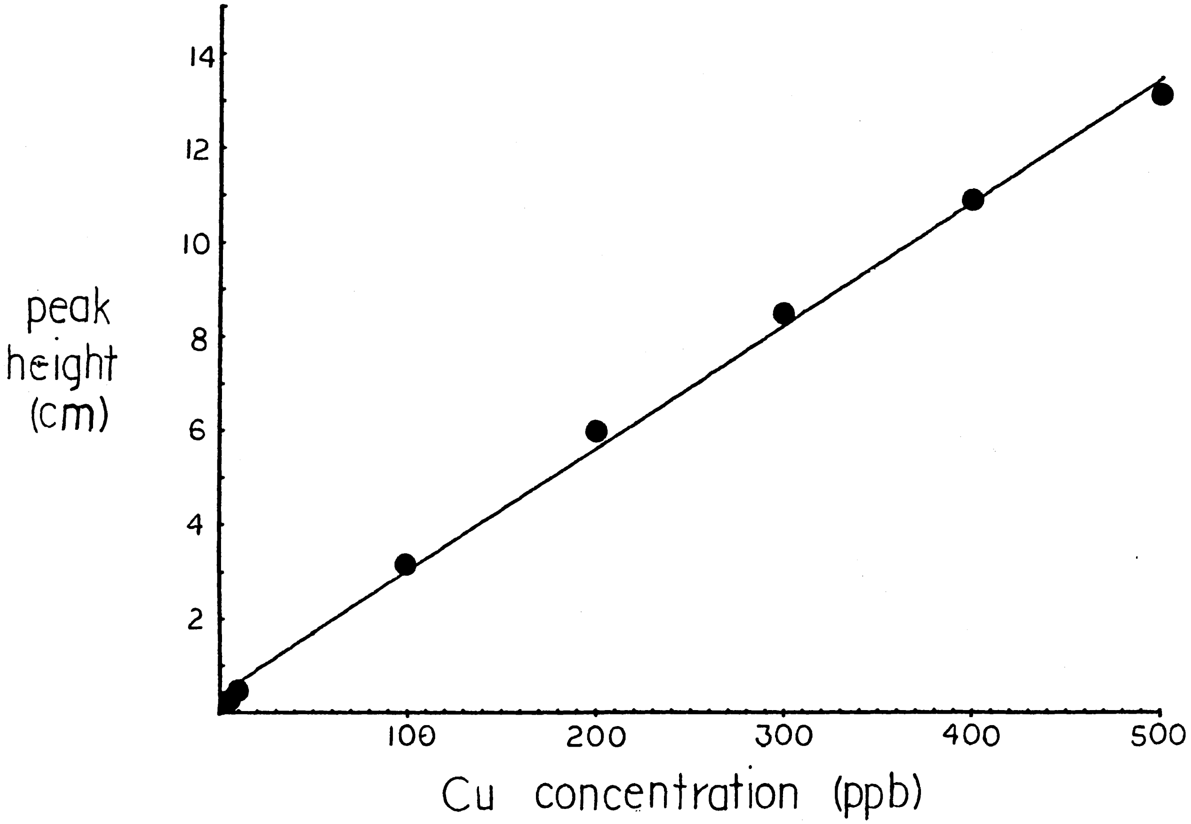


Figure 39. Working Curve for FIA-Preconcentration-AAS

the volume of the tubing upstream from the column is of little consequence. This is very unusual in FIA systems. Also notice that an acid solution (2nd injection valve) is being intercalated into a buffer. However, the acidic solution is relatively concentrated (1M HCl + 1M HNO_3) whereas the buffer is much more dilute (0.1M NH_4OAc). Also, the flow rate is high and connecting tubing is small between the acid injection valve and the flame. The result is that only the extremities of the acid plug are neutralized and dispersion is minimized, thereby allowing for a high local concentration of acid in the center of the plug for efficient elution of the extracted metals.

The system described was applied to the determination of copper in some EPA quality control water samples and in tap water. Typical signal profiles are shown in Figure 40. The "bars" above the EPA samples indicate the peak height that would be expected if the values reported by EPA were the true values. The agreement is very good as can be seen in Table XXV.

TABLE XXV

RESULTS OF COPPER DETERMINATIONS IN REAL SAMPLES

	Copper Concentration (ppb)	
Sample	Determined	Reported
EPA-1	51	50
EPA-2	252	250
Tap H_2O	26	---

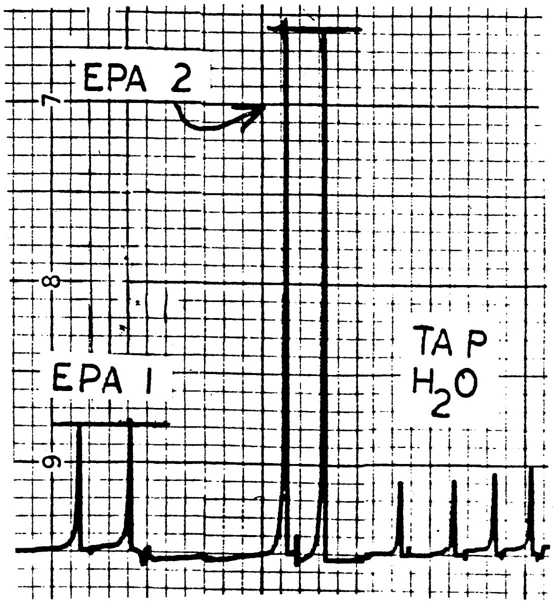


Figure 40. Typical Signal Profiles for Real Samples (Conditions as in Figure 38)

Caution, however, should be exercised when performing this, as well as any, preconcentration procedure. The presence of natural chelating agents in real water samples can plague a determination since some chelated, or bound, metal may escape the preconcentration medium without being extracted. However, as pointed out earlier, HOx exhibits large formation constants with a host of metal ions and silica-immobilized HOx has even larger conditional formation constants than free HOx since it is a stronger acid. Both of these factors point to the usefulness of silica-immobilized HOx as a preconcentrating agent in selective trace metal determinations.

CHAPTER VI

CONCLUSIONS

The work described demonstrates the advantages to be gained by critically evaluating: (1) synthetic aspects, (2) characterization techniques, and (3) analytical applications of silica-immobilized HOx. Synthetic optimization cannot only improve the properties of silica-based reagents but also can save time and be more reliable. Also, by looking at characteristics of silica-bound reagents, we can readily observe (and sometimes explain) differences in behavior between a bound reagent and the same reagent in solution. When applying these materials in practice, it is of utmost importance that an understanding of the behavior of the bound reagent is achieved.

The use of bound chelating agents for preconcentration in unsegmented continuous-flow analysis (FIA) is convenient, reliable, and is useful for determination of trace levels of metals ions in aqueous systems. This is particularly true for water samples containing large amounts of alkali and/or alkaline earth metal ions since silica-immobilized HOx is extremely selective for the transition metal ions. The preconcentrating ability and selectivity of silica-immobilized chelating agents in combination with automated sample processing (FIA) and flame atomic absorption spectroscopy make a very versatile team that can be extremely beneficial in environmental monitoring of toxic metal ions in natural water samples. The possibility of combining FIA and preconcentration with newer detection devices such as inductively coupled plasma spectrometers is also exciting since simultaneous multielement determinations could be performed for extremely low concentrations of metal ions.

BIBLIOGRAPHY

1. Waddell, T. G., D. E. Leyden, D. M. Hercules, in "Silylated Surfaces"; Leyden, D. E., and W. T. Collins, Eds., Gordon and Breach: New York 1980, p. 55-72.

2. Helfferich, F. "Ion Exchange", McGraw Hill: London, 1962.

3. Cotton, F. A., and G. Wilkinson, "Advanced Inorganic Chemistry", 4th edition, Wiley Interscience, New York, 1980.

4. Sillen, L. G., and A. E. Martell, "Stability Constants of Metal Complexes", Supplement 1, The Chemical Society: London, 1971.

5. Minczewski, J., J. Chwastowska, and R. Dybczyński, "Separation and Preconcentration Methods in Inorganic Trace Analysis", Ellis Horwood: Chichester 1982, p. 425.

6. Rothbart, H. L. in "Introduction to Separation Science", Karger, B. L., L. R. Snyder, and C. Horvath, Eds., Wiley Interscience: New York 1973, p. 359.

7. Myasoedova, G. V. and S. B. Savvin, Zh. Anal. Khim. 1982, 37, 499-519; J. Anal. Chem. USSR (Engl. Transl.) 1982, 37, 383-399.

8. Vernon, F. and H. Eccles, Anal. Chim. Acta. 1973, 63, 403-414.

9. Leyden, D. E. in "Silylated Surfaces"; Leyden, D. E. and W. T. Collins, Eds., Gordon and Breach: New York,1980, p. 321.

10. Parrish, J. R., Anal. Chem. 1982, 54, 1890-1892.

11. Iler, R. K., "The Chemistry of Silica", Wiley Interscience: New York, 1979, p. 462.

12. Snyder, L. R. and J. J. Kirkland, "Introduction to Modern Liquid Chromatography", 2nd edition, Wiley Interscience: New York, 1979, p. 272.

13. Leyden, D. E. and G. H. Luttrell, Anal. Chem., 1975, 47, 1612-1617.

14. Leyden, D. E., G. H. Luttrell and T. A. Patterson, Anal. Lett., 1975, 8, 51-56.

15. Leyden, D. E., G. H. Luttrell, W. K. Nonidez, and D. B. Werho, Anal. Chem., 1976, 48, 67-70.

16. Leyden, D. E., G. H. Luttrell, A. E. Sloan and N. J. DeAngelis, Anal. Chim. Acta, 1976, 84, 97-108.

17. Jablonski, B. B. and D. E. Leyden, in "Advances in X-ray Analysis", Barrett, C. S., D. E. Leyden, J. B. Newkirk and C. O. Ruud, Eds., Vol. 21, Plenum Press: New York, 1978, p. 59.

18. Leyden, D. E., M. L. Steele, B. B. Jablonski, and R. B. Somoano, Anal. Chim. Acta, 1978, 100, 545-554.

19. Northcott, S. E. and D. E. Leyden, Anal. Chim. Acta, 1981, 126, 117-124.

20. Leyden, D. E., W. K. Nonidez and P. W. Carr, Anal. Chem., 1975, 47, 1449-1452.

21. Dow-Corning Bulletin No. 03-023, Midland, Michigan.

22. Plueddemann, E. P. in "Silylated Surfaces", Leyden, D. E. and W. T. Collins, Eds., Gordon and Breach: New York, 1980, p. 31.

23. Kvitek, R. J., J. F. Evans, and P. W. Carr, Anal. Chim. Acta, 1982, 144, 93-106.

24. Gilpin, R. K. and M. F. Burke, Anal. Chem., 1973, 45, 1383-1389.

25. Grushka, E. and E. J. Kitka, Anal. Chem. 1977, 49, 1005A-1014A.

26. Okamoto, M. and H. Kishimoto, J. Chromatogr. 1981, 212, 251-260.

27. Okamoto, M. and F. Yamada, J. High Resol. Chromatogr. Chromatogr. Commun. 1982, 5, 443-444.

28. DenBleyker, K. T. and T. R. Sweet, Chromatographia 1980, 13, 114-118.

29. Henry, J. B. and T. R. Sweet, Chromatographia 1983, 17, 79-82.

30. Gimpel, M. and K. Unger, Chromatographia 1982, 16, 117-125.

31. Ringbom, A., "Complexation in Analytical Chemistry", Interscience: New York, 1963, p. 302.

32. Burggraf, L. W., D. S. Kendall, D. E. Leyden, and F. J. Pern, Anal. Chim. Acta 1981, 129, 19-27.

33. Kvitek, R. J., P. W. Carr, and J. F. Evans, Anal. Chim. Acta 1981, 124, 229-231.

34. Jablonski, B. B., and D. E. Leyden, in "Advances in X-ray Analysis", Barrett, C. S., D. E. Leyden, J. B. Newkirk and C. O. Ruud, Eds., Vol. 21, Plenum Press: New York 1978, p. 59-69.

35. Masters, R. G. and D. E. Leyden, Anal. Chim. Acta 1978, 98, 9-15.

36. Cooke, N. H. C., R. L. Viavattene, R. Eksteen, W. S. Wong, G. Davies and B. L. Karger, *J. Chromatogr.* 1978, *149*, 391-415.

37. Leyden, D. E., W. Wegscheider, and W. B. Bodnar, *Intern. J. Envirov. Anal. Chem.* 1979, *7*, 85-108.

38. Gendre, G., W. Haerdi, H. R. Linder, B. Schreiber, and R. W. Frei, *Intern. J. Environ. Anal. Chem.* 1977, *5*, 63-75.

39. Hercules, D. M., L. E. Cox, S. Onisick, G. D. Nichols and J. C. Carver, *Anal. Chem.* 1973, *45*, 1973-1975.

40. Chow, F. K. and E. Grushka, *Anal. Chem.* 1978, 50, 1346-1353.

41. Weetall, H. H., *Biochim. Biophys. Acta* 1970, *212*, 1-7.

42. Weetall, H. H., *Res./Develop.* 1971, 22(12), 18-22.

43. Hill, J. M., *J. Chromatogr.* 1973, *76*, 455-458.

44. Sugawara, K. F., H. H. Weetall and G. D. Schucker, *Anal. Chem.* 1974, *46*, 489-492.

45. Schucker, G. D., K. F. Sugawara and H. H. Weetall, *U.S. Pat.* 1975, 3,886,080.

46. Fulcher, C., M. A. Crowell, R. Bayliss, K. B. Holland and J. R. Jezorek, *Anal. Chim. Acta* 1981, *129*, 29-47.

47. Kuo, M. S. and H. A. Mottola, *Anal. Chim. Acta* 1980, 120, 255-266.

48. Jezorek, J. R. and H. Freiser, *Anal. Chem.* 1979, *51*, 366-373.

49. Sturgeon, R. E., S. S. Berman, S. N. Willie and J. A. H. Desaulniers, *Anal. Chem.* 1981, *53*, 2337-2340.

50. Shahwan, G. J. and J. R. Jezorek, *J. Chromatogr.* 1983, *256*, 39-48.

51. Jezorek, J. R., C. Fulcher, M. A. Crowell, R. Bayliss, B. Greenwood and J. Lyon, *Anal. Chim. Acta* 1981, *131*, 223-231.

52. Moorehead, E. D. and P. H. Davis, *Anal. Chem.* 1974, *46*, 1879-1880.

53. Guedes da Mota, M. M., F. G. Romer and B. Griepink, *Fresenius´ Z. Anal. Chem.* 1977, *287*, 19-22.

54. Jezorek, J. R. and H. Freiser, *Anal. Chem.* 1979, 51, 373-376.

55. Seshadri, T. and A. Kettrup, *Fresenius´ Z. Anal. Chem.* 1979, *296*, 247-252.

56. Seshadri, T., U. Kampschulze and A. Kettrup, *Fresenius´ Z. Anal. Chem.* 1980, *300*, 124-127.

57. Leyden, D. E., D. S. Kendall, L. W. Burggraf, F. J. Pern and M. DeBello, Anal. Chem. 1982, 54, 101-105.

58. Waddell, T. G., D. E. Leyden and D. M. Hercules, in "Silylated Surfaces", Leyden, D. E. and W. T. Collins, Eds., Gordon and Breach: New York 1980, p. 61.

59. Leyden, D. E., D. S. Kendall and T. G. Waddell, Anal. Chim. Acta 1981, 126, 207-212.

60. Kendall, D. S., D. E. Leyden, L. W. Burggraf and F. J. Pern, Appl. Spectrosc. 1982, 36, 436-440.

61. Blausius, E., K.-P. Janzen, W. Klein, H. Klotz, V. B. Nguyen, T. Nguyen-Tien, R. Pfeiffer, G. Scholten, H. Simon, H. Stockemer, and A. Toussaint, J. Chromatogr. 1980, 201, 147-166.

62. Blausius, E. and K. P. Janzen, Pure Appl. Chem. 1982, 54, 2115-2128.

63. Waddell, T. G. and D. E. Leyden, J. Org. Chem. 1981, 46, 2406-2407.

64. Kimura, K., M. Nakajima and T. Shono, Anal. Lett. 1980, 13, 741-750.

65. Nakajima, M., K. Kimura and T. Shono, Anal. Chem. 1983, 55, 463-467.

66. Seshadri, T. and A. Kettrup, Fresenius´ Z. Anal. Chem. 1982, 310, 1-5.

67. Chmielowiec, J. and W. Simon, Chromatographia 1978, 11, 99-101.

68. Grossman, P. and W. Simon, J. Chromatogr. 1982, 235, 351-363.

69. Yao, T., M. Akino and S. Musha, Bunseki Kagaku 1982, 31, 409-412.

70. Guedes da Mota, M. M. and B. Griepink, Fresenius´ Z. Anal. Chem. 1978, 290, 317-318.

71. Gubitz, G., W. Jellenz, G. Löfler and W. Sanfi, J. High Resol. Chromatogr. Chromatogr. Commun. 1979, 2, 145-145.

72. Marshall, M. A. and H. A. Mottola, Anal. Chem. 1983, 55, 2089-2093.

73. Robinson, P. J., P. Dunnill and M. D. Lilly, Biochim. Biophys. Acta 1971, 242, 659-661.

74. Sillén, L. G. and A. E. Martell, "Stability Constants of Metal-Complexes", The Chemical Society: London, 1971, Supplement 1, p. 576.

75. Iler, R. K., "The Chemistry of Silica", Wiley: New York, 1979, p. 672.

76. Parrish, J. R. and R. Stevenson, Anal. Chim. Acta 1974, 70, 189-198.

77. Marshall, M. A. and H. A. Mottola, Anal. Chim. Acta 1984, 158, 369-373.

78. Eaton, D. L. in "Silylated Surfaces", Leyden, D. E. and W. T. Collins, Eds., Gordon and Breach: New York 1980, p. 201.

79. Karch, K., I. Sebestian and I. Halász, J. Chromatogr. 1976, 122, 3-16.

80. Sandell, E. B., "Colorimetric Determination of Traces of Metals", Third edition, Interscience, New York 1959.

81. Hartley, F. R.; C. Burgess and R. M. Alcock, "Solution Equilibria"; Ellis Horwood: Chichester, 1980; p. 179-184.

82. Mottola, H. A. and H. Freiser, Talanta 1966, 13, 55-65.

83. Takamoto, S., Q. Fernando and H. Freiser, Anal. Chem. 1965, 37, 1249-1251.

84. Strazhesko, D. N., V. B. Strelko, V. N. Belyakov and S. C. Rubanik, J. Chromatogr. 1974, 102, 191-195.

85. Samuelson, O., "Ion Exchange Separations in Analytical Chemistry", John Wiley and Sons: New York 1963.

86. Inczédy, J., "Analytical Applications of Ion Exchangers", Pergamon Press: Oxford 1966.

87. Hansen, E. H. and J. Ruzicka, J. Chem. Ed., 1979, 56, 677-680.

88. Van Grieken, R., Anal. Chim. Acta, 1982, 143, 3-34.

89. Murthy, R. S. S., J. Holzbecher and D. E. Ryan, Rev. Anal. Chem. 1982, VI, 113-150.

90. Bächmann, K., CRC Crit. Rev. Anal. Chem., 1981, 12, 1-67.

91. Ruzicka, J. and E. H. Hansen, Anal. Chim. Acta 1975, 78, 145-157.

92. Ruzicka, J. and E. H. Hansen, "Flow Injection Analysis", John Wiley and Sons: New York, 1981.

93. Wolf, W. R. and K. K. Stewart, Anal. Chem. 1979, 51, 1201-1205.

94. Yoza, N., Y. Aoyagi and S. Ohashi, Anal. Chim. Acta, 1979, 111, 163-167.

95. Basson, W. D. and J. F. Van Staden, Fresenius´ Z. Anal. Chem. 1980, 302, 370-374.

96. Tyson, J. F., Anal. Proc., 1983, 20, 488-491.

97. Olsen, S., L. C. R. Pessenda, J. Ruzicka and E. H. Hansen, *Analyst* 1983, *108*, 905-917.

98. Ruzicka, J. and E. H. Hansen, *Anal*. *Chim*. *Acta* 1978, *99*, 37-76.

2

VITA

Monte Alton Marshall

Candidate for the Degree of

Doctor of Philosophy

Thesis: SILICA-IMMOBILIZED 8-QUINOLINOL: SYNTHESIS, CHARACTERIZATION, AND APPLICATIONS IN FLOW SYSTEMS

Major Field: Chemistry

Biographical:

Personal Data: Born in McAlester, Oklahoma, on March 1, 1956, to Dr. and Mrs. Clarence Alton Marshall.

Education: Graduated from McAlester High School, McAlester, Oklahoma, in May, 1974; received Bachelor of Science degree from Southeastern Oklahoma State University, Durant, Oklahoma, in May, 1978; completed the requirements for the Doctor of Philosophy degree from Oklahoma State University in July, 1984.

Professional Experience: Chemical Technician, M.B.S. Program, Physical Sciences Department, Southeastern Oklahoma State University, Durant, Oklahoma, 1978-1979; Graduate Teaching Assistant, Department of Chemistry, Oklahoma State University, Stillwater, Oklahoma, 1979-1981 and 1982-present; Graduate Research Assistant, Department of Chemistry, Oklahoma State University, Stillwater, Oklahoma, 1981-1982.