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A Column for the High Pressure Liquid Chromatography of Some Potentially Hazardous Metal Ions

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A COLUMN FOR THE HIGH PRESSURE LIQUID CHROMATOGRAPHY OF SOME POTENTIALLY HAZARDOUS METAL IONS

by

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Stephen R. Priebe

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INTRODUCTION

Background

Improved methods for the determination of trace metals continue to be a major focus of modern analytical chemistry. One application of these methods which is receiving widespread attention is the determination of potentially hazardous metals in consumer products, notably foods.[1] Trace levels of a number of metals are known to be essential to human physiological function.[2] However, at higher levels they can exhibit a deleterious effect. This type of behavior is seen with cobalt, which is closely associated with vitamin B_{12}. Deficiency of vitamin B_{12} is a factor in pernicious anemia and various mental and behavioral disorders.[3] A recent example of the severe toxic effects of cobalt at higher levels was demonstrated by deaths resulting from the ingestion of beer to which cobalt had been added to preserve the foam layer.[4] Other metals, notably lead and mercury, have no known bodily function and are toxic at quite low levels.

A large arsenal of techniques for trace level analysis of metals is available to the analyst and include photometry,[5] atomic spectroscopy,[6] and electrochemical analysis.[7] Often difficulties with the sample measurement process are minimal, while a major portion of the analyst's efforts may be directed toward reduction of, or compensation for, interferences.

An interference may be broadly defined as any substance that, through manifestation of its chemical properties, causes a significant reduction in the accuracy and/or precision of an analysis. A large number of techniques for minimizing interferences in trace metal determinations have been suggested, but the most commonly practiced are: masking, precipitation, liquid-liquid extraction, oxidation-reduction, and chromatographic separation of the analyte from the interferant. Of these techniques, chromatography would appear to be the most generally applicable and the least demanding experimentally. Yet, chromatographic sample preparation has been the least widely applied technique of those listed above, perhaps due to a lack of sufficiently selective materials as well as rapid methodology.

Chromatography, by definition, consists of a mobile phase and a stationary phase. Gaseous mobile phases have not been particularly applicable to trace metal analyses as a result of the limited volatility of most metallic compounds. The separation and determination of organometallic compounds[8] and certain metal chelates[9] are important exceptions to this general statement.

Liquid-solid adsorption chromatography was among the first types of chromatography to be studied in terms of its metal ion separation capabilities. A book reviewing the application of liquid-solid chromatography to the separation of metal ions and metal complexes has been written by Michal.[10] The resolution achieved is generally not great and decreases with increasing

sample size and complexity. This mode of chromatography has been applied, for the most part, to the separation of different coordination compounds of a specific metal.[11] The chelating groups tend to decrease interaction of the metal with the adsorbant. Irreversible adsorption is a commonly encountered difficulty. As a result of these problems, a general procedure for the formation and separation of chelates for the analysis of complex mixtures has not appeared.

Liquid-liquid, also known as partition or extraction, chromatography has been successfully applied to the separation of a wide variety of metal ions, notably the rare earths.[12] In this type of chromatography a bulky coordinating compound is dissolved in an organic solvent, slurried with a solid support, and the coated support packed into a column for use with aqueous mobile phases. Separation is effected by exploiting differences between the metal ions' ability to form certain coordination compounds and the preferential solubility of these compounds, or lack thereof, in the organic phase. The subject has been comprehensively reviewed by Braun and Ghersini.[13] The major disadvantage of this type of chromatography is the gradual loss of the organic phase with time and the resulting loss of resolution and reproducibility.[14] This limitation becomes even more severe when higher flow rates are used to speed up the separation.[15]

Paper chromatography, a variant of liquid-liquid chromatography, has also been applied to inorganic separations.[10] Its major

limitations are difficulties in the quantitation and the long periods of time required for the separation of complex mixtures of metal ions. These limitations also apply to thin layer chromatography.

The ion exchange mode of liquid-solid chromatography is currently the most widely used chromatographic method for the separation of metal ions.[16] This type of chromatography is inherently limited by its lack of selectivity, i.e., metal ions of the same charge and similar size are difficult to separate. This limitation may be overcome for specific applications by the addition of one or more auxiliary complexing agents to the mobile phase in order to alter the charge or size of a particular species and thus effect its elution from the column. In order to accomodate a variety of sample types, a great deal of manipulation of elution conditions has been found to be necessary.[16] The addition of strong acids or complexing agents is a drawback if a collected fraction is to be quantitated by another method.[17] In addition, the corrosive solutions often employed necessitate the use of nonmetallic components of the chromatograph which are not readily available for high pressure work.

To increase selectivity, chelating resins have been developed. The most widely used resins of this type incorporate the iminodiacetic acid functional group and are commercially available from several sources. These resins offer somewhat greater selectivity than conventional strong ion exchange resins, however auxiliary

coordinating agents are still necessary for complex mixtures. Optimization of elution conditions when using resins of this type often involves extensive calculations.

Sporadic attempts have been made to incorporate more selective chelating groups into resins. Notable among these studies are the incorporation of pyridine-2,6-dicarboxylic acid,[18] salicylic acid,[19] diethyldithiocarbamate,[20] 4-(2'-pyridylazo)-resorcinol,[21] and 8-hydroxyquinoline.[22] For the most part, good stability and selectivity were reported in the above studies.

Resins, in general, have characteristics which limit their use in high performance liquid chromatography. Even those resins which possess a relatively high degree of crosslinking exhibit a certain amount of nonrigidity. Chromatographic resins tend to shrink or swell with changes in ionic strength, temperature, and pH, as well as undergoing deformation at high flow rates.[15] The net effect is the occurrence of column voids and/or resin degradation. Pellicular ion exchange materials do not shrink or swell, but possess much lower capacities than ordinary resins. It can thus be seen that a rigid support for chromatographic packings incorporating a selective functionality is desirable.

Bonded phases, developed to overcome bleeding of the organic phase in liquid-liquid chromatography, are currently playing an important role in modern chromatography. The organic phase is chemically bonded to silica through siloxane linkages and is thus quite stable. A general review dealing with the preparation and utility of bonded phases has been written by Cox.[23] Although the greatest

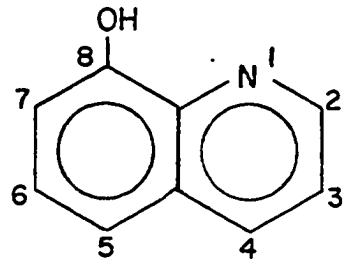
application of bonded phases has been directed toward organic separations, some work has been done with metal ions. The concept of a bonded phase containing a selective chelating group for the metal ions of interest appears to possess several advantages over other chromatographic types of separation methods and therefore is the subject of this study.

Approach Taken

The desired characteristics for an immobilized chelating group are: long-term stability, pH dependence of chelation, and suitable reactivity toward the immobilization reaction. The first criterion needs little further discussion, except to point out that some, otherwise excellent, chelating agents, such as dithizone and diethyldithiocarbamate, are disqualified by this requirement. The need for pH dependence of chelate formation arises from the desire for a simple method of elution. In this respect, it should be noted that silica begins to undergo significant dissolution at pH values greater than 8; consequently, an acidic region of pH dependence is required. However, the mobile phase must not be too acidic if corrosive attack on the metallic components of the chromatographic apparatus is to be avoided. The third criterion arises from the fact that it is impossible, in most cases, to directly bond the chelating agent to silica because of the high reactivity of the chloro- and alkoxysilanes used for the preparation of bonded phases. This necessitates a series of reactions which must proceed cleanly and in high yield for the production of a useful chromatographic

material. Immobilization by means of diazotization of an aromatic amine followed by coupling of the chelating group is a relatively common approach to meeting this requirement and the one chosen for this study.

With the above criteria in mind, the literature was thoroughly searched and 8-hydroxyquinoline (I) was selected for the present investigation. This compound has long been recognized as a nearly universal precipitant for metal ions[5] and thus is likely to have a broad range of applicability.



(I)

The pH dependence of chelate formation for this ligand is nearly ideal, with pK_a values of 5.0 and 9.65 for the aromatic nitrogen and hydroxyl groups respectively.[24] The phenolic hydroxyl group is strongly electron-releasing, making 8-hydroxyquinoline quite reactive toward diazo-coupling at the 5 position. While these highly desirable characteristics have been recognized by previous workers, they have not been fully exploited.

Vernon and Eccles[25] have reviewed the literature on resins containing 8-hydroxyquinoline and have reported that these resins

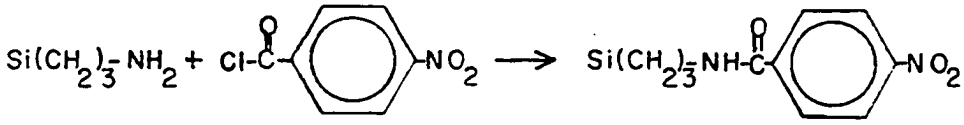
have some utility, but also that their characteristics varied greatly depending upon the method of preparation. Some of the limitations discussed included low capacity, slow equilibration, and high degrees of swelling. An indication of the potential of chemically bonded 8-hydroxyquinoline was given by Slovak, et al.,[26] who diazo-coupled 8-hydroxyquinoline to a glycomethacrylate polymer. They found that mixtures of zinc(II) and cadmium(II), as well as those of manganese(II), cobalt(II), iron(III), and copper(II) could be separated by elution with 0.2 M to 4 M HCl. Adequate stability up to 2 M acid concentrations was reported. The polymeric support, however, was not suitable for high pressure operation and consequently necessitated a slow flow rate of 0.3 ml/min. Freiser and Jezorek[17] have diazo-coupled 8-hydroxyquinoline to silica, using the procedure of Hill.[27] This material was shown to be capable of separating mixtures of zinc(II), manganese(II), cadmium(II), lead(II), cobalt(II), and nickel(II) using mild elution conditions and no auxiliary complexing agents. The capacity and efficiency of their packing were rather low, being about 50 microequivalents per gram and 0.2 to 0.5 mm per plate respectively.

In the present investigation it was felt that diazo-coupling of 8-hydroxyquinoline to silica was the most promising method for obtaining a high performance chromatographic column material capable of separating the metal ions of interest from interferants. The reaction scheme employed is outlined in Figure 1. Several improvements on the previously reported methods of preparation seemed to be appropriate. For example, it was felt that the capacity of the

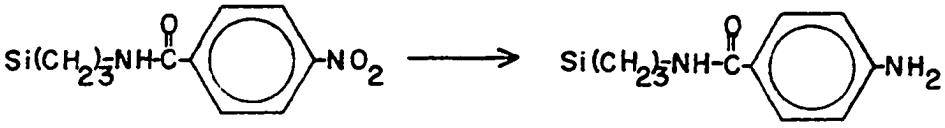
Figure 1

Reaction Sequence for the Preparation of
8-Hydroxyquinoline-Bonded Silica

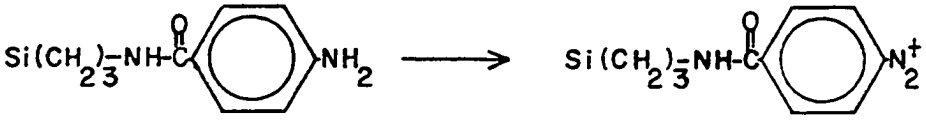
Benzoylation



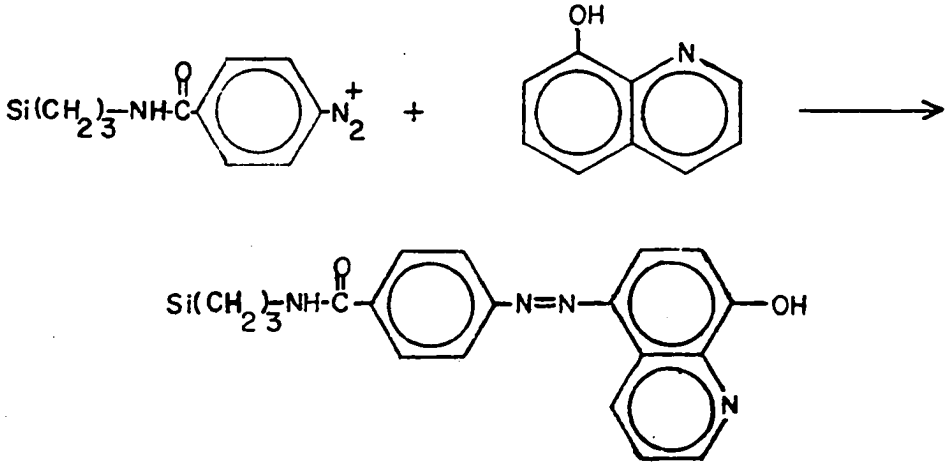
Reduction



Diazotization



Coupling



column could be significantly increased through optimization of the reactions leading to the immobilization of 8-hydroxyquinoline. Consequently, model compounds were prepared utilizing the reactions to permit evaluation of procedures in terms of the yield of desired product and possible complications. One such complication, noted by Freiser and Jezorek,[17] is the formation of elemental sulfur when using dithionite in the reduction step. Preliminary experiments were conducted for the purpose of selecting a reducing agent effective at lower temperatures to minimize possible hydrolysis of the amide linkage. In addition, the optimum conditions for diazo-coupling 8-hydroxyquinoline had not, as far as can be ascertained from the literature, been employed. The rate of the coupling reaction, and thus the yield and purity of product, is highly dependent upon the pH and the temperature.[28]

A second approach taken toward the optimization of column performance was from a chromatographic viewpoint. It is well-known that column efficiency increases with decreasing particle size of the packing and that spherical particles having a narrow distribution of sizes contribute significantly to column bed uniformity.[15] For these reasons a five micron spherical packing material having a narrow distribution of sizes was selected. Through implementation of the above approaches it was hoped that the full potential for metal ion separations with 8-hydroxyquinoline-bonded silica might be evaluated.

Chapter II

EXPERIMENTAL

Reagents

Inorganic chemicals were of analytical reagent grade (J.T. Baker Co.). The organic solvents used were ACS reagent grade (Mallinckrodt) with the exceptions of ethyl acetate and 95% ethanol, which were of the type commonly encountered in the chemical laboratory. Organic reagents were used as received unless otherwise noted.

Preliminary Experiments

A solution of Ti(III) was prepared by allowing a 0.02 M solution of $Ti(SO_4)_2$ to stand in contact with zinc metal. The excess zinc metal was removed by the addition of sulfuric acid immediately prior to use. Fifty-milligram portions of 4-nitrobenzamide and 2-nitroaniline (both obtained from Eastman Organic Chemicals) were placed in separate test tubes, 20 ml of Ti(III) solution added to each, and the tubes stoppered. The tubes were then shaken intermittently and allowed to stand overnight at room temperature. The purple color of the Ti(III) ion was observed to fade slowly and was discharged overnight in the case of 4-nitrobenzamide, whereas the 2-nitroaniline solution did not undergo any change in color. The Ti(III) reduction reactions were thus inefficient at room temperature. Siggia[29] has found that temperatures of 70 to 100 °C are

often necessary for the quantitative reduction of aromatic nitro compounds to the corresponding amine with Ti(III).

A 1.0-g portion of 4-nitrobenzamide was mixed with 75 ml of a 0.5 M solution of $SnCl_2$ which was also 2 M in HCl in a 100-ml round bottom flask. The mixture was heated at 50 °C with stirring for two hours. After cooling, 5 ml of concentrated HCl was added and the solution was extracted with 100 ml of ethyl acetate. The reaction mixture was then made strongly basic with 50% NaOH and re-extracted with a fresh 100-ml portion of ethyl acetate. The evaporation of this organic layer gave 0.34 g of tan crystals having a melting point of 179 °C, which corresponded to a 41% yield of impure 4-aminobenzamide (literature[30] melting point 183 °C). Apparently, higher temperatures are necessary in order for stannous ion to be an efficient reducing agent.

Several 1.0-g portions of 4-nitrobenzamide were reacted, at room temperature, with a 250% excess of Cr(II) solutions of varying concentration and acidity. These solutions were prepared by treatment of aqueous $CrCl_3$ solutions with excess zinc metal. The solutions were then stored under hydrogen and filtered just prior to use. The color of the chromous ion solution was observed to change rapidly from blue to green upon the addition of the solution to the nitro compound. The acid-insoluble reactant was completely dissolved over a period of several hours. Attempts to extract the product by various means were not fruitful.

Chromous ion was selected for further study on the basis of the rapid color change and the dissolution of the nitro compound. Also, Siggia[29] has descibed the determination of aromatic nitro compounds utilizing chromous ion as a quantitative reducing agent at room temperature in many cases.

Synthesis of Model Compound

Chloroform was freed of ethanol by extraction with sulfuric acid, followed by washing with water and distillation from $CaCl_2$. A 50-g portion (0.27 mole) of 4-nitrobenzoyl chloride (Eastman Organic Chemicals) was dissolved in 500 ml of ethanol-free chloroform containing 10 ml of triethylamine (Aldrich Chemical Co.). The resulting solution was added to 25 ml (0.15 mole) of n-octylamine (97%, Aldrich Chemical Co.) in a one-liter round bottom flask and an additional 40 ml of triethylamine was added. The reaction was allowed to proceed overnight with stirring at a temperature of 40 °C. The product, 4-nitro-N-(octyl)-benzamide, was recovered by evaporation of the chloroform, followed by treatment with aqueous silver nitrate. The solid was extracted by refluxing with diethyl ether and subsequently filtered. The filtrate was sucessively extracted with 5% NaOH, water, 1% HCl, and water. The ether layer, after evaporation, gave 36.7 g (a 90% yield) of crude product. The product was recrystallized from dilute ethanol and found to melt at 86.5 °C. The infrared spectrum (KBr) of the purified product exhibited the following bands: 3312 cm^{-1} (amide N-h), 1641 cm^{-1} (C=O), and 1345 cm^{-1} (NO_2). The proton NMR spectrum of a

$CDCl_3$ solution of the product did not give detailed structural information, but the intensity ratios observed were consistent with the expected structure. The major fragments of the 70 eV mass spectrum, m/e (relative intensity), were 278 (5), 277 (21), 221 (19), 207 (12), 193 (21), 180 (48), 179 (26), 167 (17), 150 (100), 120 (22), and 104 (34). The spectrum was obtained using a probe temperature of 100 °C. Elemental analysis (performed by Midwest Microlabs) gave 64.72% C, 8.29% H, and 10.03% N. The calculated values for $C_{15}H_{22}N_2O_3$ are 64.72% C, 7.97% H, and 10.07% N.

A 13.1-g portion of the product of the benzoylation reaction was placed in a 500-ml filtering flask. The flask was flushed with CO_2 for ten minutes. The CO_2 pressure was then used to force about 500 ml of 1.6 M Cr(II) solution (about 1 M in HCl) from a reservoir containing zinc metal, through a sintered glass crucible, into the flask. About 15 ml of concentrated HCl was added and the reaction mixture was stirred for 40 hours at room temperature in the CO_2 atmosphere. The reaction mixture was then extracted with 500 ml of diethyl ether. This resulted in the formation of a white solid at the interface of the aqueous and organic layers. The ether layer was withdrawn and the aqueous layer, containing the solid, filtered. An infrared spectrum (KBr) of the solid gave the following bands: 3360 cm^{-1} (amide N-H), 1920 cm^{-1} (amine hydrochloride N-H), and 1645 cm^{-1} (C=O). The solid was dissolved in 95% ethanol and reprecipitated by the addition of dilute NaOH. This gave 9.7 g of product, which corresponded to an 83% yield. A portion of this material was recrystallized from benzene in a glove box containing

a nitrogen atmosphere. The melting point of the purified product, 4-amino-N-(octyl)-benzamide, was 120 °C. The infrared spectrum (Fluorolube mull) of the recrystallized material exhibited the following bands: 3420 cm^{-1} (amine N-H), 3340 cm^{-1} (amide N-H), 3225 cm^{-1} (amine N-h), and 1650 cm^{-1} (C=O). The proton NMR spectrum of a hexadeutero-dimethyl sulfoxide solution of the product gave intensity ratios consistent with the expected structure. The major fragments of the 70 eV mass spectrum, obtained using a probe temperature of 200 °C; m/e (relative intensity), were: 248 (43), 150 (31), 136 (48), 121 (17), 120 (100), and 96 (26). Elemental analysis (performed by Midwest Microlabs) gave 70.95% C, 9.53% H, and 10.71% N. The calculated values for $C_{15}H_{24}N_2O$ are 72.54% C, 9.74% H, and 11.28% N.

A 3.9-g portion of the product from the reduction reaction was dissolved in a solution consisting of 20 ml of concentrated HCl, 75 ml of distilled water, and 200 ml of methanol. This solution and another containing 2.07 g of $NaNO_2$ in 20 ml of distilled water were chilled to 5°C in an ice bath. The two solutions were mixed and allowed to stand in the ice bath for an additional 20 minutes with intermittent shaking. During this time, a third solution containing 4.385 g of 8-hydroxyquinoline (Baker analytical reagent), 5.5 g of NaOH, and 20 g of $Na_3PO_4 \cdot 12\ H_2O$ in 500 ml of distilled water was also chilled. The 8-hydroxyquinoline solution, initially at a pH of 13.5, was placed in a one-liter beaker in an ice bath. The diazotized amine solution was poured slowly into the 8-hydroxyquinoline solution whereupon large amounts of a deep red solid

were formed. The temperature rose from 5 °C to 10 °C and it was necessary to add 2 M NaOH dropwise near the end of the addition in order to maintain a pH of 10. The reaction mixture was stirred for 30 minutes at 10 °C and then allowed to warm to 30°C over a one hour period. The mixture was acidified to a pH of 1 with HCl and the product was recovered by filtration. The crude product was purified by successive washings with water, 5% NaOH, water, 10% HCl, water, and diethyl ether. The compound was observed to dissolve partially upon treatment with ether and, for this reason, no determination of yield was attempted. The infrared spectrum (KBr) exhibited the following bands: 3300 cm^{-1} (O-H) 1630 cm^{-1} (C=O), and 1395 cm^{-1} (N=N). The major fragments of the 70 eV mass spectrum, m/e (relative intensity), were: 404 (7), 403 (22), 248 (23), 160 (26), 150 (16), 144 (27), 136 (25), 120 (100), and 44 (73). This spectrum was obtained from the solid product using a probe temperature of 350 °C. Attempts to obtain a satisfactory proton NMR spectrum were not successful, as a consequence of the limited solubility of the product, 4-(5'-azo-8'-hydroxyquinolinyl)-N-(octyl)-benzamide.

Preparation of 8-Hydroxyquinoline-Bonded Silica

The 4-nitrobenzoyl chloride was recrystallized from petroleum ether (bp 60-110 °C) and found to melt in the range 72 to 74 °C (literature[30] melting point 75 °C). A 2.5-g (1.5 mmole) portion of aminopropyl silica (Spherisorb S5-NH_2, Phase Separations Ltd.) was suspended in 25 ml of ethanol-free chloroform and transferred to a

3-necked 100-ml round bottom flask fitted with a mechanical stirrer, a condenser, and a thermometer. A solution containing 0.77 g (4.1 mmole) of 4-nitrobenzoyl chloride in 25 ml of ethanol-free chloroform was added. One milliliter of triethylamine was added and the mixture was stirred and heated at 45 °C for three days. During this time three 0.1-g portions of 4-nitrobenzoyl chloride and 5-drop portions of triethylamine were added. The silica was recovered by centrifugation and washed with five 50-ml portions of ethanol-free chloroform, three 50-ml portions of 95% ethanol, and one 50-ml portion of water.

A Jones reductor (2 cm x 27 cm) was prepared by allowing 40-mesh zinc metal to stand in a saturated $HgCl_2$ solution. A 3-necked 100-ml round bottom flask was equipped with mechanical stirring and an inlet for CO_2. The benzoylated silica was transferred to the flask with a minimum amount of 1 M HCl. The flask was flushed with CO_2 for ten minutes prior to the addition of 70 ml of 0.45 M $CrCl_3$ (1 M in HCl) into the reductor and then directly into the flask. The blue color of the Cr(II) solution was observed to change immediately to green as it entered the stirred reaction mixture until about 30 ml had been added. The reaction was allowed to continue for three hours, after which the silica was recovered by centrifugation and washed with two 50-ml portions each of 1 M and 0.1 M HCl.

A saturated solution of 8-hydroxyquinoline in a buffer consisting of 167 ml of 1 M $KHCO_3$ and 80 ml of 1 M KOH was prepared.

About 100 mg of aminopropyl silica starting material was added and the solution was allowed to equilibrate for one hour before filtration. The filtrate was cooled to 3 °C in a 3-necked 250-ml round bottom flask fitted with mechanical stirring, thermometer, and a pH electrode. The arylamino silica from the reduction step was then mixed with 15 ml of 1 M HCl and chilled in an ice bath. After cooling to 3 °C, 0.32 g of KNO_2 was added and the mixture was shaken and held in the ice bath for 15 minutes. At this point a solution consisting of 0.23 g of urea in 5 ml of distilled water was added. The resulting mixture was shaken and held in the ice bath until gas evolution had subsided. The diazotized silica was added to the buffered 8-hydroxyquinoline solution that was initially at a pH of 10.4 and a temperature of 4.0 °C. During the addition the pH decreased to 10.0 and the temperature increased to 6.1 °C. The color of the reaction mixture changed from yellow to red immediately upon the addition of the diazotized silica. The reaction was allowed to proceed, with vigorous stirring, at 6 °C for 30 minutes. The mixture was then allowed to warm to room temperature over a period of one hour. The mixture was neutralized to a pH of 6.8 with concentrated HCl and the product silica recovered by centrifugation. The 8-hydroxyquinoline-bonded silica was then washed with two 50-ml portions of distilled water and three 50-ml portions of methanol. Dry samples of the product silica were obtained by evaporation of the methanol, while that used for the packing of the column was stored under the methanol.

Capacity Measurement

The metal ion capacity of the 8-hydroxyquinoline-bonded silica was determined by the cupric ion uptake method described by Freiser and Jezorek,[17] with several modifications. Centrifugation, rather than decantation and filtration, was the primary means of phase separation. This was followed by filtration through a 0.45 micron Type HA membrane (Millipore Corporation). The equilibration was performed in a 0.1 M acetate buffer, at a pH of 4.05, for one hour. The result of this measurement of capacity, 0.25 meq/g, was slightly higher than that obtained using unbuffered solutions because a significant amount of hydronium ion is released by the metal ion chelation reaction.

Column Packing

About 1.5 g of 8-hydroxyquinoline-bonded silica was slurried with about 150 ml of methanol in a Model 705 (Micrometrics Instrument Corporation) stirred-slurry column packer. The solvent inlet of the packer was connected to the pump of a Model 5020 (Varian/ Instrument Division) liquid chromatograph and the packer was purged of air. A 3 mm x 30 cm stainless steel column (Glenco Scientific) was connected to the outlet of the packer. The initial flow rate of methanol was 10 ml/min. The flow rate was decreased as the maximum pressure limit (350 atm) of the chromatograph was exceeded. The pressure was maintained between 300 and 350 atm throughout the packing procedure.

Chromatography

A Model 5020 (Varian/Instrument Division) liquid chromatograph was used with the column and appropriate 0.010 in i.d. stainless steel tubing and connectors. The water used for chromatography was house distilled water that was deionized by passage through two mixed-bed ion exchange columns and redistilled; this is hereafter referred to as ddd H_2O. Solvents were prepared from analytical reagent grade nitric acid and sodium nitrate using the ddd H_2O. All solvents were repeatedly passed through a Type HA 0.45 micron membrane until no further discoloration of the membrane was observed, followed by vacuum degassing for about 5 minutes.

Metal ion solutions for chromatography were prepared by dissolution of the analytical reagent grade nitrate salts in ddd H_2O, followed by acidification to a pH of 1.0 with concentrated nitric acid. The $Ca(NO_3)_2$ solution was prepared by dissolution of calcium carbonate in dilute nitric acid. The $Mn(NO_3)_2$ solution was prepared by dilution of a 51.6% solution (Baker, analytical reagent). Each injected metal ion solution was 0.01 M in each metal ion. A 10-microliter injection loop was used for most experiments, with the exception of some involving cadmium and zinc, which were done using a somewhat larger loop to enhance detectability.

The column was cleaned with about 50 ml of pH 1.0 HNO_3 and then equilibrated with about 50 ml of pH 2.3 HNO_3 daily prior to metal ion experiments. Care was also taken to flush the injection loop thoroughly between injections. A 10-microliter sample of 0.01 M

$Pb(NO_3)_2$ was chromatographed isocratically at a pH of 2.1 as a routine check on column performance.

Detection of eluted metal ions was accomplished by collecting small fractions, usually 0.4 ml, manually in test tubes to which three drops of a 3×10^{-4} M solution of 4-(2'-pyridylazo)-resorcinol (PAR), obtained from the G. Frederick Smith Chemical Co., buffered at a pH of 10 with ammonia, had been added. PAR has been reported to be a nearly universal metal ion indicator.[17] Other indicators were tried, including Eriochrome Black T and a Mg-EDTA-Calmagite solution, but they were found to be generally inferior to PAR. The minimum detectable amount of metal ion by visual means was found to be about 10 nmole. The retention volumes and apparent bandwidths were estimated visually. Most chromatography was conducted at a nominal flow rate of 2.0 ml/min and a column temperature of 30 °C.

The volume of the system tubing was determined by the injection of 10 microliters of 0.01 M $Cd(NO_3)_2$ solution with the column removed from the system. Fractions of 0.05 ml were collected into test tubes containing 3 drops of PAR indicator. The flow rate was 0.1 ml/min and the pH of the eluent was 2.0. This resulted in a value of 0.1 ml. The retention volume of Mg^{2+} was measured in a similar fashion, using a 0.05% Calmagite solution in place of the PAR solution, with the column in place. The difference between this volume, 1.15 ml, and the volume of the system tubing was taken to be the column void volume.

Chapter III

RESULTS AND DISCUSSION

Synthetic Procedures

The data gathered from the model compound synthesis indicate that the reaction sequence is indeed an effective method for the incorporation of 8-hydroxyquinoline functionality. The benzoylation step was shown to proceed cleanly and in high yield using essentially the procedure of Hill.[27] A major outcome of the preliminary experiments and subsequent reduction reactions was the effective use of chromous ion as the reducing agent at room temperature. Reduction at ambient temperatures is advantageous because hydrolysis of the amide linkage is minimized. Chromous ion cannot be recommended for homogeneous synthetic work because of the inherent difficulty in recovering the product amine. It was fortunate that the amine hydrochloride was precipitated in the model compound experiment. This allowed verification of structure and estimation of the yield. The amino compound appeared to be susceptible to decomposition to a brownish substance when exposed to air, which may partially account for the discrepancy in elemental analysis. The product of the diazotization and coupling reactions was suspected to be impure on the basis of liquid chromatographic data. The presence of a 7'-azo- isomer is of particular concern because of the mixed retention mechanism effect that it could have on metal ion chromatography. Attempts to determine this isomerism by proton

nuclear magnetic resonance spectroscopy were not successful, as a result of the limited solubility of the compound in the common solvents. The presence of the 8-hydroxyquinoline functionality was confirmed by infrared spectroscopy. Furthermore, a potentiometric titration in 60% aqueous dioxane media indicated two acidic protons.

The procedures developed in the synthesis of the model compound were readily adaptable to the preparation of 8-hydroxyquinoline-bonded silica. Care was taken to exclude oxygen from the arylamino silica to minimize the decomposition observed with the model compound. The only difficulty encountered in the preparation was that of obtaining an excess of 8-hydroxyquinoline in a reasonable volume of solution for coupling at the proper pH (9.6 to 10.6) and temperature (0 to 5 °C) because of its limited solubility. An experimental detail to be noted is the equilibration of the 8-hydroxyquinoline solution with some silica prior to the addition of the diazotized silica in order to minimize dissolution of the product silica at the high pH employed for coupling.

The metal ion capacity of the product silica is about five times greater than that reported by Freiser and Jezorek.[17] This is due primarily to the higher bonded phase coverage of the commercial aminopropyl silica starting material, although higher conversion to the 8-hydroxyquinoline functionality was achieved. The 42% conversion obtained in this study is to be compared with a figure of about 30%, which was estimated from a combination of Freiser and Jezorek's[17] and Hill's[27] data. The capacity value,

when combined with the manufacturer's value for the surface area of the starting material, implies an 8-hydroxyquinoline surface density of 68 groups per 10^4 square angstroms. This suggests that the capacity of the 8-hydroxyquinoline-bonded silica may be limited by steric factors.

Column Characteristics

A consequence of the higher capacity of the column packing material is that longer periods of time are required for equilibration following a change in eluent pH. Longer time periods are required at pH values nearer to the pK_a of 3.3. An equation has been derived for calculating the number of column void volumes of eluent necessary to equilibrate the column at the new pH following a stepwise change in the pH of the eluent by using a batch equilibration treatment. With the column initially at a given pH, a fraction, α, of the total amount of 8-hydroxyquinoline groups is present as unprotonated, i.e., electrostatically neutral, 8-hydroxyquinoline. Equation (1) may readily be derived from mass balance and equilibrium relationships;

$$\alpha_i = \frac{[HQ]}{C_Q} = \frac{K}{K + [H^+]} \qquad (1)$$

where $[HQ]$ represents the concentration of unprotonated 8-hydroxyquinoline, C_Q represents the total amount of 8-hydroxyquinoline in both protonated and unprotonated forms, and K is the acid dissociation constant of the protonated form. The second dissociation

step is ignored. When the solution in contact with the column is removed and a fresh portion of eluent that is at a lower pH is added, the total concentration of H^+ supplied by the eluent, C_{H^+}, is distributed as follows:

$$C_{H^+} = [H^+]_f + [H_2Q^+]_{formed} \qquad (2)$$

where $[H^+]_f$ is the final concentration of H^+ after equilibration and $[H_2Q^+]_{formed}$ is the amount of protonated 8-hydroxyquinoline that is formed as a result of the lower pH. By a mass balance relationship,

$$[H_2Q^+]_{formed} = [HQ]_i - [HQ]_f \qquad (3)$$

where i and f stand for initial and final, respectively. By combining Equations (1), (2), and (3) one obtains

$$C_{H^+} = [H^+]_f + \alpha_i C_Q - \frac{KC_Q}{K + [H^+]_f} \qquad (4)$$

Then, by rearrangement and quadratic solution, the final concentration of H^+ is given by Equation (5).

(5)

$$[H^+]_f = \frac{C_{H^+} - K - \alpha_i C_Q + \sqrt{(K + \alpha_i C_Q - C_{H^+})^2 - 4K(\alpha_i C_Q - C_Q - C_{H^+})}}{2}$$

Equation (5) may be used in a repetitive manner, substituting a new value of α_i calculated from the final concentration of H^+ each time, to calculate the number of column void volumes of eluent required to equilibrate the column at the pH of the eluent. The parameter C_Q was evaluated empirically by fitting experimental data involving a stepwise change in eluent pH and subsequent metal ion elution. A value of 0.15 gave good agreement with the experimental results. This value does not necessarily have any precise physical significance because of the manner in which it was evaluated and the nature of the heterogeneous system. Equation (5) was found to be useful for gradient construction and prediction of metal ion retention volumes when employing a gradient generated by a stepwise change in eluent pH. It is to be expected that if larger columns than that used in the present work were to be packed, the equilibration periods would be proportionally longer.

Although the column appeared to be well-packed, as evidenced by the low column void volume, the efficiencies observed were quite low by conventional high performance chromatography standards, being on the order of 150 plates per meter. The apparent low efficiencies are thought to arise from kinetic factors in the metal ion-ligand exchange reactions. This is substantiated by the differences in the apparent column efficiencies for zinc and lead. These metal ions exhibit very similar retention behavior under identical elution conditions, but the number of plates for lead is greater than that for zinc, i.e., the lead band is consistently less wide. It is unlikely that this phenomenon is an artifact of the visual detection,

since the lead reaction with PAR is the more sensitive of the two. The phenomenon may be caused by a faster rate of exchange for lead than for zinc, but the kinetics have not been established. This type of behavior might also occur if the column was significantly overloaded, since the amount of zinc injected was somewhat greater than the amount of lead. The inherent lack of sensitivity of visual detection prevented a systematic investigation into the degree of column loading.

The chromatographic studies of metal ion retention produced the elution order: $Ca^{2+} < Mn^{2+} < Cd^{2+} < Pb^{2+} = Zn^{2+} < Co^{2+}$. This order is in accord with the order of homogeneous 1:1 chelate formation constants for 8-hydroxy-5-(phenylazo)-quinoline.[31] Zinc and lead could not be adequately resolved under any elution conditions tried. The isocratic retention volumes for these two metal ions were measured as a function of eluent pH. The retention volume, V_R, was converted to a distribution ratio, D, by the relationship

$$D = \frac{V_R - V_m}{V_s} \qquad (6)$$

where V_m is the volume of the mobile phase, or the column void volume, and V_s is the volume of the stationary phase. The V_R and log D data, as well as the observed bandwidths, are presented in Table 1.

Table 1

Chromatographic Data for Lead(II) and Zinc(II)

	Metal Ion					
	Pb			Zn		
pH	V_R(ml)	V_B(ml)	log D	V_R(ml)	V_B(ml)	log D
1.8	3.8	2.0	0.39	3.6	2.1	0.36
1.9	5.0	2.8	0.56	4.8	2.8	0.53
1.95	5.7	2.8	0.63	5.6	3.6	0.62
2.0	6.4	2.4	0.69	6.3	3.0	0.68
2.05	7.5	3.0	0.77	7.3	3.4	0.76
2.1	8.6	3.2	0.84	- -	- -	- -
2.15	- -	- -	- -	10.8	6.4	0.96
2.2	12.9	5.2	1.0	13.0	7.2	1.0

The data for the variation of log D with pH indicate a good correlation to straight lines and that the lines intersect at a pH of about 2.0. The difference in the slopes of the lines is not felt to be significant due to the potential for error in visual detection. The slopes of 1.6 and 1.7 for lead and zinc respectively, differ markedly from those reported by Freiser and Jezorek[17] for zinc(II), cadmium(II), and nickel(II). They stated that the slopes should be equal to one in accord with a single proton displacement, but they also noted that cobalt(II) exhibited an inexplicably high slope. The data from this present investigation suggests that, since the 8-hydroxyquinoline groups are protonated at the aromatic

nitrogen to a significant degree, the slope ought to reflect the fraction of 8-hydroxyquinoline groups that are not protonated and thus available for chelation. The slope would be predicted to change with pH in pH ranges near the pK_a. It should be noted that α is small in the pH range studied and that a small amount of curvature might well escape detection. It was not possible to obtain distribution data over a wide range of pH values with any single metal ion as a result of difficulties in detecting the diffuse bands encountered at high values of the distribution ratio and also in accurately measuring the sharp bands encountered at low values of the distribution ratio.

Separations

The range of distribution ratios is such that an isocratic separation of the metal ions of interest is impractical.[17] Therefore, a large number of gradients were tried to obtain a separation of Ca^{2+}, Mn^{2+}, Cd^{2+}, Pb^{2+}, and Co^{2+}. The retention volumes and bandwidths corresponding to one of the more successful gradients are given in Table 2. A reconstruction of the gradient employed is depicted in Figure 2. The separation of the metal ions, with the exception of lead and cadmium, is quite good. It is to be expected that the resolution will be improved at lower levels of column loading.

Table 2

Chromatographic Data for Metal Ion Separation

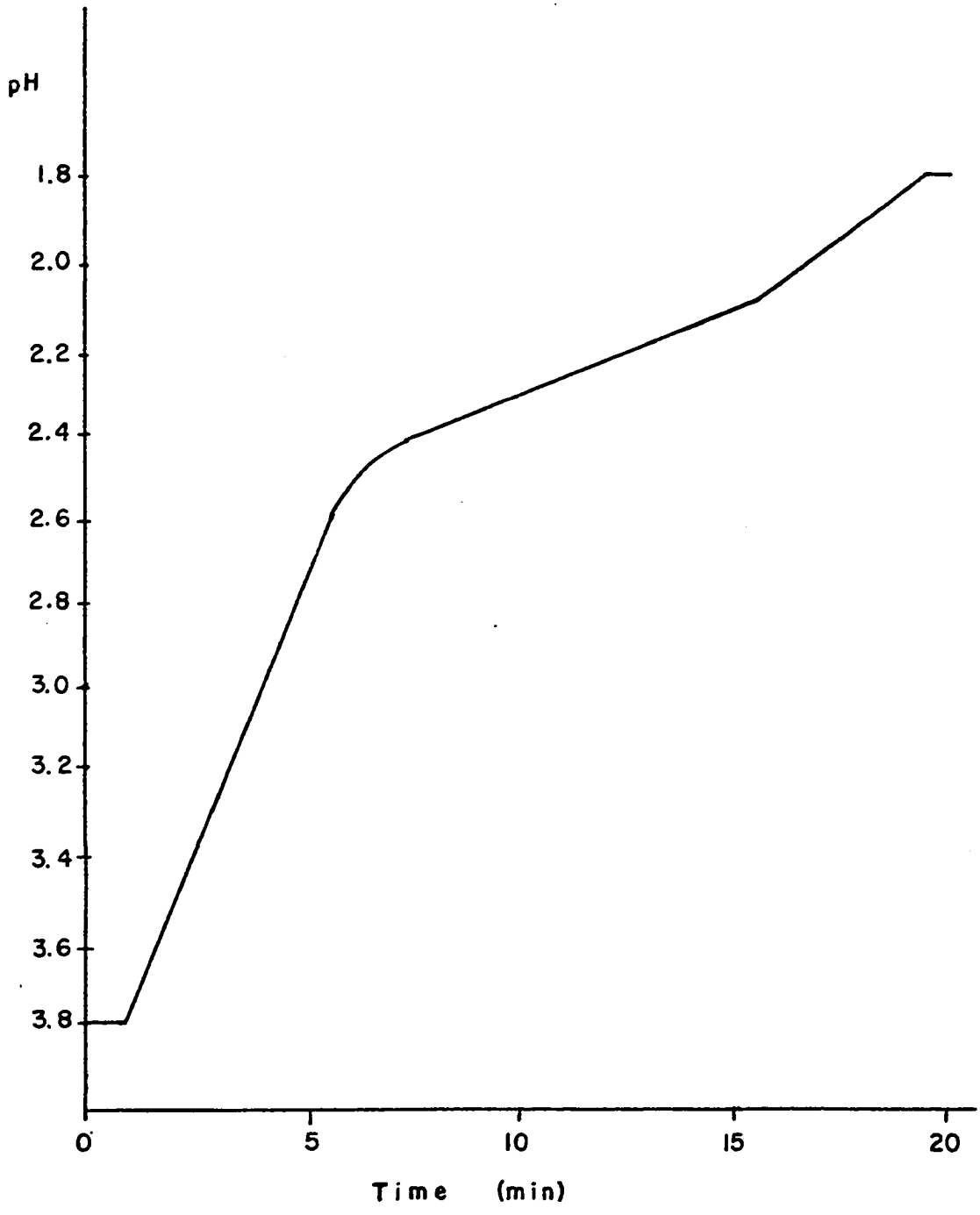
Metal Ion	V_R(ml)	W_B(ml)
Ca^{2+}	1.2[a]	0.8
Mn^{2+}	9.8	1.6
Cd^{2+}	29.5	3.0
Pb^{2+}	32.5	2.4
Co^{2+}	43.0	8.0

[a]This volume corresponds to the void volume of the column and system tubing.

The fact that calcium is unretained at an eluent pH of 3.8 suggests that gradient chromatography of the type shown in Figure 2 could find application as a simple and rapid means of separating various transition metal ions from calcium. Sample matrix effects could be greatly reduced by using the method simply as a clean-up procedure. Another promising application of the chromatographic method described is that of preconcentration of the metal ions of interest from very dilute solutions, followed by selective elution. The capacity of the column is the limiting factor in this case and requires that the sample be free of large amounts of metal ions more strongly retained than those of interest. The higher capacity obtained by the synthetic procedure developed in this investigation can be seen to be of great value for this application.

Figure 2

Gradient for Metal Ion Separation



The selectivity of 8-hydroxyquinoline-bonded silica does not appear to be great enough for the analytical separation of certain pairs of metal ions, e.g., lead and zinc, however, others can be adequately resolved. The recent development of a highly sensitive inductively coupled plasma emission detector for liquid chromatography[32] may enable direct quantitation of the chromatogram. In the event the detector is applied, even metal ions that are not completely resolved may be quantitated, since the detector is metal-specific. In any event, the collection of fractions, followed by analysis by the current best method for the particular metal should result in lower detection limits and better precision through the elimination of interferences.

An important limitation to the range of applicability of 8-hydroxyquinoline-bonded silica is to be noted. Metal ions such as Cu(II) and Fe(III) are not eluted from the column when using eluents of pH 1.8 or greater. Even when using nitric acid solutions of pH 1.0 for column cleaning, these metal ions are removed only gradually. This was manifested as a gradual increase in the effective column capacity (k') after cleaning procedures were implemented. This cleaning process also introduces contamination through corrosive attack on the components of the chromatograph, as was evidenced by the positive PAR tests obtained when using pH 1.0 nitric acid solutions. These effects masked any loss of bonded phase that may have occurred. This limitation might be overcome and corrosion of the chromatograph reduced by the use of a solution of a complexing

agent for the elution of strongly retained metal ions. This, however, could have a detrimental effect on the column performance if partitioning of the complexing agent were to occur. It is apparent, nevertheless, that a better cleaning procedure is needed.

In summary, we report a synthetic procedure which results in an 8-hydroxyquinoline-bonded silica chromatographic material of significantly higher capacity than those previously reported in the literature. The higher capacity allows method development to proceed more rapidly by enabling the injection of sufficient amounts of metal ion that visual detection may be employed. A column packed with the material has been demonstrated to be suitable for high pressure liquid chromatography and to be capable of a number of analytical separations of certain potentially toxic metal ions. It is hoped that some of the analytical applications suggested by this investigation will be taken up by other workers in trace metals analyses in the future.

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