# University of Alberta

Graphitic Carbon Phases for Chelation Chromatography and Electrochemically Modulated Preconcentration

by

Wei Sun

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

Master of Science

Department of Chemistry


Spring 2012
Edmonton, Alberta

This thesis is dedicated to my parents, Jianyun Sun and Xiangzhen Li, and to my fiancée Yiman Wu. I am forever grateful for their guidance, help, inspiration, and encouragement.

**ABSTRACT**

The first part of this thesis explores the synthetic conversion of carbon clad zirconia particles into a chelation stationary phase. Oxine (8-hydroxyquinoline) groups were attached onto the graphitic carbon surface by *in situ* generated diazonium ions. X-ray photoelectron spectroscopy, Fourier transform infrared spectroscopy and scanning electron microscopy were utilized to characterize the functionalized particles, and optimum synthesis conditions were determined. The second part of this thesis applies the modified carbon clad zirconia particles to ion chromatographic separations. The effects of various factors such as eluent concentration, pH and temperature were studied. The last part of the work introduces electrochemically modulated metal preconcentration. After grafting oxine groups onto the surface of glassy carbon, the binding of calcium can be controlled by applying and electrical potential to the particle bed. Compared to the traditional preconcentration column, this technique can save analysis time and simplify the equipment.

**Acknowledgement**

I would first like to thank my supervisor, Dr. Charles Lucy, for his kind guidance, encouragement and inspiration over the past three years. His dedication made my graduate studies the most unforgettable experience in my life. I also want to thank the whole Lucy group for their help and advice. Especially, Amy, Stu, Ting, Chen, Donna, Nicole and Farooq, whose friendship and support have contributed to the most enjoyable educational experience in my life.

I would also like to thank Dionex for their funding and support during my research. The contributions of Glass Shop and Machine Shop in Department of Chemistry deserve a “thank you” as well.

I also thank my English teacher Patrece. She taught me a lot when I first came in Canada. A special “thank you” goes to Wenmin, Yidan, Liang Shi and Sheldon for their friendship and support.

Finally, I am forever grateful for my parents and my fiancée. Their support is my motivation.

**Table of Contents**

**List of Figures**

**List of Tables**

**List of Symbols**

A Multipath band broadening

B Longitudinal diffusion

$C_M$ The mass transfer for the mobile phase

$C_S$ Mass transfer related to the stationary phase

$d_c$ Diameter of the column

$d_f$ The average film thickness of the liquid stationary phase

$D_M$ Diffusion coefficient

$d_p$ Diameter of the particles

H Plate height

L Total column length

k' Retention factor

$K_c$ Distribution coefficient

N Plate number

Q Ion-exchange capacity of the column

$R_s$ Resolution

$t_R$ Retention time

$u$ Linear velocity

$w_b$ Peak width at baseline

$w_h$ Peak width at half height

$\beta$ Phase volume ratio

$\sigma$ Standard deviation or quarter peak width at baseline

$\lambda$ Packing factor

$\psi$ Obstruction factor

# CHAPTER ONE: Introduction

## 1.1 Motivation and Thesis Overview

High Performance Liquid Chromatography (HPLC) is a chromatographic technique that separates the components of a mixture. It is widely used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of a mixture [1]. Among the various types of HPLC, the analysis of ions in aqueous solution is one of the most common analyses performed. In Ion Chromatography (IC) the sample (e.g., drinking water, medicines) is transported by a flowing mobile phase into a particulate or monolith column. After being separated, the individual analytes are monitored using an on-line detector. The column is the heart of an HPLC. Typically, particulate columns are packed 3-5 μm silica or 5-10 μm polymer particles. However, both silica and polymer have drawbacks which limit their application in HPLC.

First of all, silica-based particles can only sustain pH 2-8 [2]. This narrow working pH prevents silica from being used for strongly acidified samples and/or using aggressive pH eluent. Polymers are an alternative which circumvents the pH stability issue associated with silica. However, columns packed with polymeric particles show lower separation efficiencies for small molecules compared to their silica counterparts [3]. Also, polymer particles provide poor mechanical strength which limits their application to lower efficiency separations using larger particles [2]. Consequently, exploration of new powerful stationary phases is necessary.

Carbon clad zirconia is a good candidate to overcome these problems. This new reverse-phase support is made from chemical vapor deposition of hydrocarbons onto porous microparticulate zirconia ($ZrO_2$). This type of carbon-overlaid zirconia particle is stable under extremes of pH (0-12) and temperature (80 ℃). Besides, the zirconia core provides the particles more mechanical and chemical stability than polymers [4].

This thesis focuses on covalent modification the 3 μm reverse phase carbon clad zirconia particles into a high performance chelation ion chromatography (HPCIC) phase. This chapter introduces basic concepts, ion chromatography (IC), and recent progress in the field. Chapter Two focuses on the synthesis of the modified particles. Various factors (time, temperature, and amount of chemicals) are optimized. Chapter Three studies the separation efficiency, and capacity of the particles. Factors such as temperature and pH effects are shown. Chapter Four investigates the electrochemical switching of retention on the modified graphitized carbon-based particles. Chapter Five summarizes the thesis and discusses possible future studies.

## 1.2 Introduction to Chromatography and Ion Chromatography

In modern science, chromatography is a laboratory technique which can separate the various components of a mixture both qualitatively and quantitatively. In liquid chromatography (LC), the analytes are carried by a liquid mobile phase (eluent) and pass through a column contains a solid stationary phase. Separation happens when each of the sample components interacts to a different extent with

the stationary phase. In 1950s, van Deemter and coworkers examined the mechanism of chromatography, and successfully developed a theory to explain the band broadening phenomenon [7]. They related the column efficiency to the linear mobile phase velocity, longitudinal diffusion, eddy diffusion, and mass transfer kinetics of the analyte between the mobile and stationary phases. From their proposed equations, they predicted smaller particles would achieve higher efficiency separations. However, the small particles cause higher back pressure due to the force needed to flush liquid through such tightly packed small particles. To achieve higher pressure, Horvath and coworkers designed a couple of new pumping systems in the 1960s [8]. Since then, "High Performance Liquid Chromatography" (HPLC) has become a widely used analytical instrument all around the world.

In 1975, Hamish Small and coworkers invented ion chromatography (IC) [9]. One ion exchange column was used to separate inorganic ions and a second column was used to suppress the background electrolyte in the eluent. This suppression allowed the ion exchange columns to be directly coupled with a conductivity detector cell and achieved continuous effluent monitoring. Currently, IC refers to the determination of trace ions on low capacity high efficiency columns possessing fixed ion-exchange sites [10]. These columns are commonly combined with suppressed conductivity detection to yield parts-per-billion detection of the common anions ($F^-$, $Cl^-$, $NO_2^-$, $Br^-$, $NO_3^-$, $HPO_4^{2-}$, and $SO_4^{2-}$), common cations ($Li^+$, $Na^+$, $NH_4^+$, $K^+$, $Mg^{2+}$, and $Ca^{2+}$), carboxylic acids and small amines.

IC retains ions and ionisable compounds through an ion-exchange mechanism. There are mainly three types of mechanisms. For anion-exchange, the stationary phase contains positively-charged sites such as quaternary amines. For cation-exchange, the surface of the stationary phase is negatively charged by functional groups such as sulfonate or carboxylate. The third type of IC, and that used in this thesis, is chelation exchange. In this technique the stationary phase carries a chelating group. This group can complex specific metal ions in the presence of other background metal ions. Unlike cation exchange columns, chelation can be used for samples possessing concentrated matrices.

### 1.2.1 Chromatographic terms

A "chromatogram" is the detector signal plotted versus the time since injection. Figure 1.1 shows a simple chromatogram.

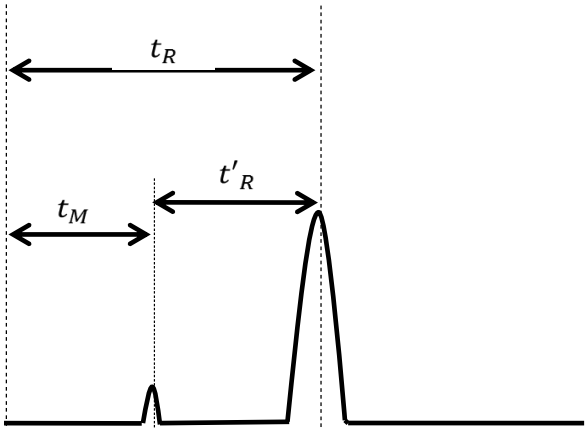


**Figure 1.1** A simple chromatogram

Compounds can be separated in chromatography only if they spend different amounts of time on the stationary phase. The time that a non-retained

molecule requires to pass through the column is called the dead time ($t_M$). The time that a retained compound requires to pass through the column is called the retention time ($t_R$). This retention time can be separated into two parts. One is the time required to go through the interstitial space in the column, which equals the dead time. The other is the time the molecule spends interacting with the stationary phase, which is known as the adjusted retention time ($t'_R$). Alternately the retention time can be described by:

$$t_R = t_M + K_c t_R \tag{1-1}$$

where $K_c$ is the distribution coefficient.

The distribution constant $K_c$ is defined as the equilibrium concentration of an analyte A in the stationary phase divided by equilibrium concentration in the mobile phase:

$$K_c = \frac{[A]_S}{[A]_M} \tag{1-2}$$

The distribution constant $K_c$ can be related to the retention factor (or capacity factor) $k'$ by accounting for the phase volume ratio, β.

$$K_c = \beta k' \tag{1-3}$$

In equation 1-3, the phase ratio is:

$$\beta = \frac{V_M}{V_S} \tag{1-4}$$

where $V_M$ and $V_S$ are the volumes of the mobile and stationary phases within the column. Finally:

$$k' = \frac{(\text{moles of A})_S}{(\text{moles of A})_M} \quad 1\text{-}5$$

By combining equations 1-2, 1-3, 1-4 and 1-5, we arrive at the relationship:

$$k' = \frac{t'_R}{t_M} \quad 1\text{-}6$$

The retention factor describes the elution of a molecule along the column, but it tells nothing about how sharp is the resultant peak. In chromatography, the term of plate number (N) is used to evaluate the performance (sharpness) of a separation.

$$N = \left(\frac{t_R}{\sigma}\right)^2 \quad 1\text{-}7$$

where $t_R$ is the total retention time as described above, and $\sigma$ is the standard deviation of the peak. Based on the assumption of a Gaussian peak, the standard deviation can be estimated as one quarter of the peak-width-at-baseline ($w_B$). Substituting the baseline width into equation 1-7, yields:

$$N = 16\left(\frac{t_R}{w_b}\right)^2 \quad 1\text{-}8$$

Equation 1-8 gives an algebraic method to calculate the plate number, and then quantitatively measure the quality of a given column.

The plate number depends on the length of the column, so it is difficult to compare the efficiency of different columns using the term N alone. In these circumstances, we use another parameter H, the plate height.

$$H = \frac{L}{N} \qquad 1\text{-}9$$

where L is the total column length. H has the unit of length. The smaller the H, the better the column efficiency. Ideal values of H are 2-3 times the diameter of the packing material.

Another measure of chromatographic performance is the term $R_s$, the resolution, which examines how well two peaks are separated with each other.

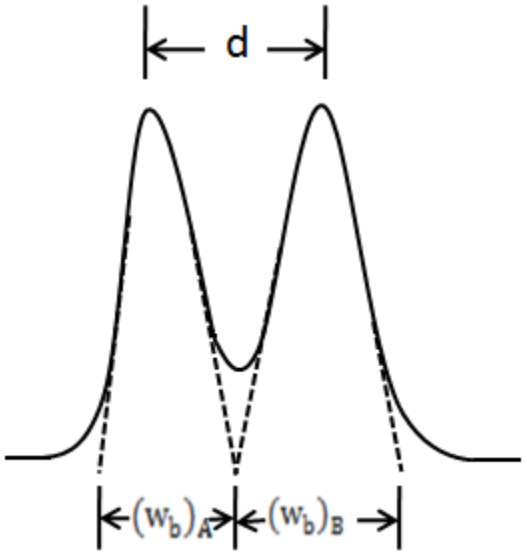


**Figure 1.2** The definition of resolution. Reproduced with permission of Wiley-Interscience. This figure illustrates a resolution of 1.0.

$R_s$ is defined as,

$$R_s = \frac{2d}{(w_b)_B + (w_b)_A} \quad 1\text{-}10$$

where d is the distance between the peak maxima and $w_b$ is the width of each peak at the baseline, as shown in Figure 1.2. The larger the resolution, the better the separation. A resolution of greater than 1.5 is referred to as "baseline separation".

Peak broadening in chromatography is most commonly described by the model published by van Deemter and co-workers in 1956 [7]. They examined parameters that influence the band broadening in packed GC columns, and pointed out three major effects: eddy diffusion (A term); longitudinal diffusion (B term); and the resistance to mass transfer between the mobile and stationary phases (C term).

$$H = A + \frac{B}{u} + Cu \quad 1\text{-}11$$

where u is the linear velocity of mobile phase, determined by equation 1-12.

$$u = \frac{L}{t_M} \quad 1\text{-}12$$

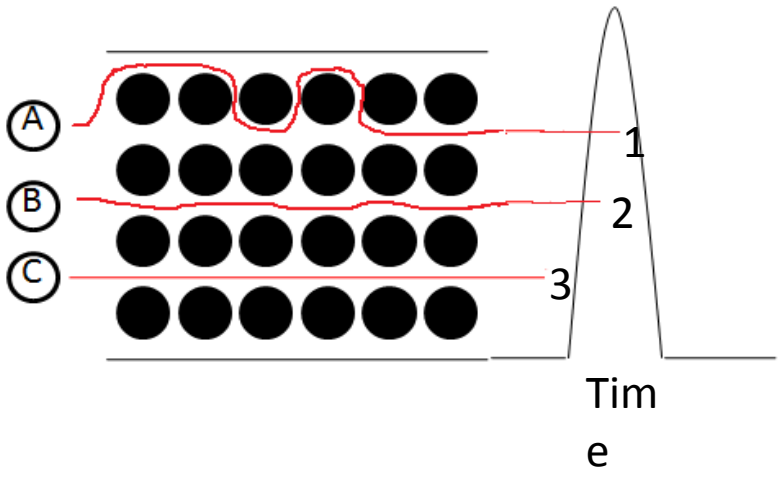


**Figure 1.3** Illustration of eddy diffusion or multipath effect. Reproduced with permission of Wiley-Interscience.

The A term is also called multipath term. As shown in Figure 1.3, three molecules start at the same initial position, they could follow different pathways through the packed column. Since the velocity of these three analytes are the same (flow rate is a constant), they will spend different amounts of time to reach the column end. Analyte C takes the most direct path, so it appears in the front part of the peak. In contrast, analyte A takes the most complex path through the column, and so takes longer to pass through the column and shows up at the end of the peak. Thus, eddy diffusion results in the band broadening in chromatographic system. The A term is independent upon the flow rate. It is mainly determined by the particle size ($d_p$) and the quality of the packing ($\lambda$):

$$A = 2\lambda d_p \qquad 1\text{-}13$$

Based on equation 1-13, smaller particle and tight packing will minimize the A term. In practice, smaller particle will increase the back pressure of the HPLC

system, so we should balance the eddy diffusion band broadening and pressure increase. Also smaller particles are more difficult to pack. This will discussed further in Chapter Three.

Longitudinal diffusion is the B term in the van Deemter equation. The B term refers to the molecular diffusion of the analyte sample in the mobile phase only and results in the time-dependent band broadening.

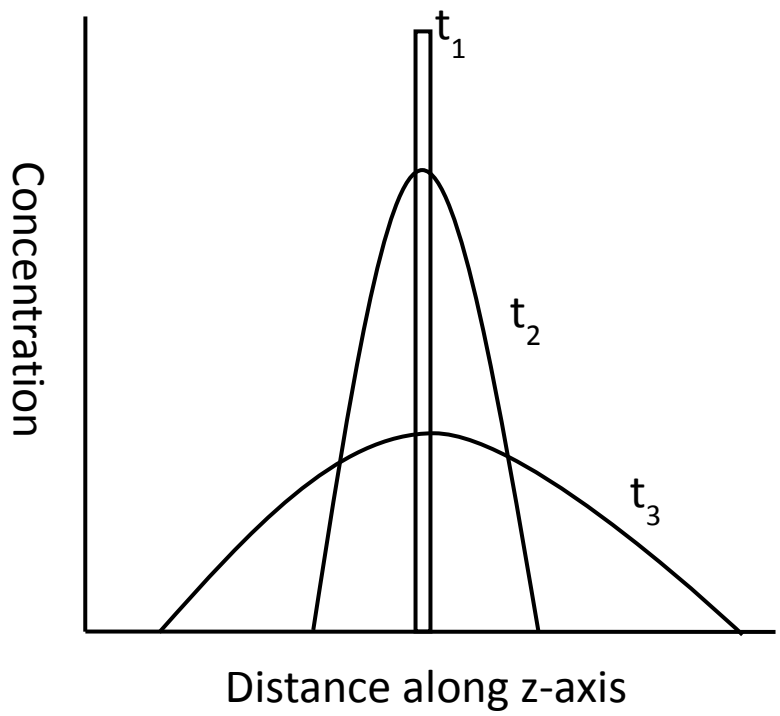


**Figure 1.4** Zone widening due to longitudinal diffusion. Three times are shown: $t_3 > t_2 > t_1$. Reproduced with permission of Wiley-Interscience.

Molecules will diffuse from a region of high concentration to that of a lower concentration. Thus, as shown in Figure 1.4, diffusion will cause the band

to widen the longer they are allowed to do so. The equation governing longitudinal diffusion is:

$$B=2\psi D_M \qquad 1\text{-}14$$

where $D_M$ is the diffusion coefficient for the solute in the mobile phase and $\psi$ is the obstruction factor which is related to the nature of the packed bed.

In the van Deemter equation (equation 1-11), the B term is divided by the linear velocity, such that a large flow rate or linear velocity minimizes the longitudinal diffusion. The higher the linear velocity, the less time the analyte spends in the column. Therefore, the sample molecule has less time to diffuse. HPLC increased the flow rate or linear velocity, such that the B term is not an important contributor to the overall band broadening. However, with the introduction of UHPLC, the contributions of other sources of band broadening have been decreased, so that the B term is once again a significant contributor to band broadening.

The C term in the van Deemter equation governs the resistance to mass transfer of the solute into and out of the stationary phase only. The mass transfer in the mobile phase was not significant initially since the equation was derived for GC. But when the van Deemter equation was applied to HPLC or open tubular capillary GC columns, it became necessary to also consider the C term for the mass transfer resistance in the mobile phase. Therefore, now the C term usually has two parts: $C_S$ which is the mass transfer related to the stationary phase; and

$C_M$ which depicts the mass transfer of the mobile phase only for those open tubular columns.

$$C_s = \frac{8}{\pi^2}\frac{k}{(1+k)^2}\frac{{d_f}^2}{D_s} \quad 1\text{-}15$$

$$C_M = \frac{(1+6k+11k^2)d_c^2}{96(1+k)^2 D_M} \quad 1\text{-}16$$

where $d_f$ is the average film thickness of the liquid stationary phase, $D_s$ is the diffusion coefficient of the solute in the stationary phase, $d_c$ is the diameter of the column, and $D_M$ is the diffusion coefficient in the mobile phase. From equation 1-15 and 1-16, thinner liquid stationary film and larger diffusion coefficient will minimize the band broadening coming from the C terms. In the case of open tubular columns, small column diameter relieves the effect of the C term since the mass transfer distance is smaller. In other words, minimizing the C term is equal to speeding up the mass transfer rate of the analyte into and out of both the mobile and the stationary phase.

From equation 1-11, the B term will dominate when the linear velocity is low, while the C term will dominate when the linear velocity is high. A typical van Deemter curve is shown in Figure 1.5.

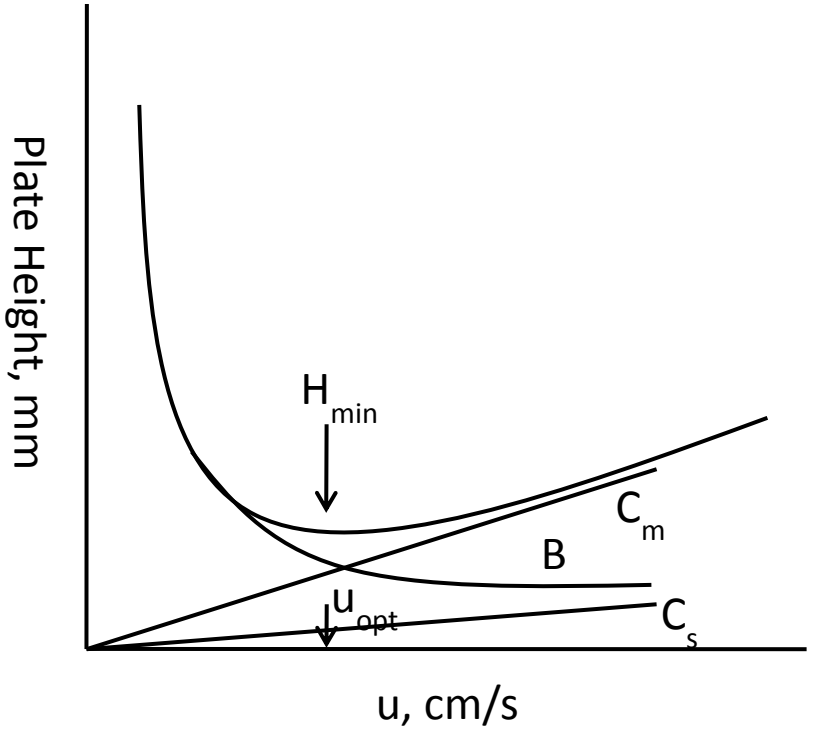


**Figure 1.5** Typical van Deemter plot. Reproduced with permission from Wiley-Interscience.

Figure 1.5 shows us how the B and C term change with the linear velocity individually. As we can see in the van Deemter plot, there is an optimum point in the graph. This optimum velocity is the linear velocity which provides the lowest H and thus the highest efficiency possible. Chromatographers usually consider the effect of velocity on both the efficiency and separation time, and so often will sacrifice efficiency to achieve fast separation.

### 1.2.2 Retention Theory of Cation Exchange IC

In the simplest case in cation-exchange IC [12], the eluent contains a single type of competing cation, such as hydronium ($H^+$) ions or

ethylenediammonium ions. The ion-exchange equilibrium for binding of an analyte cation $M^{x+}$ to the stationary phase in equilibrium with an eluent containing the competing cation $E^{y+}$ is given by:

$$yM_m^{x+} + xE_s^{y+} \rightleftharpoons yM_s^{x+} + xE_m^{y+} \qquad 1\text{-}17$$

where the subscripts m and s refer to the mobile and stationary phases; x and y stand for the charge of the individual ions, respectively. Figure 1.6 illustrates this ion-exchange equilibrium.

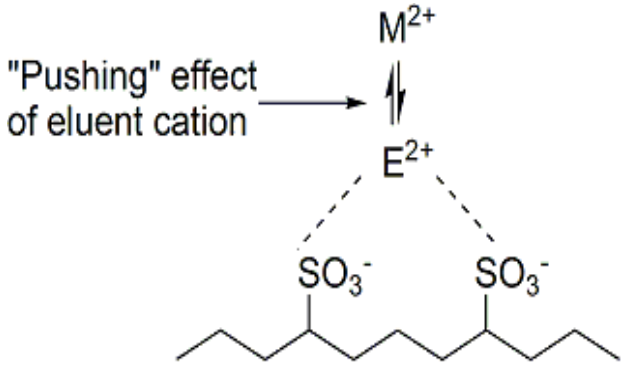


**Figure 1.6** Schematic representation of the elution of a solute $M^{2+}$ from a sulfonic acid cation exchanger. $E^{2+}$ stands for a single competing cation.

It can be seen that the competing eluent cation exerts a "pushing" effect on the solute cation (This is in contrast to the "pulling" effect that a complexing eluent has on the analyte). If activity coefficients are assumed to be unity, the equilibrium constant $K_{M,E}$ can be written:

$$K_{M,E} = \frac{\left[M_s^{x+}\right]^y \left[E_m^{y+}\right]^x}{\left[M_m^{x+}\right]^y \left[E_s^{y+}\right]^x} \qquad 1\text{-}18$$

The distribution coefficient for solute M is designated as $K_c$ and is given by:

$$K_c = \frac{[M_s^{x+}]}{[M_m^{x+}]} \quad 1\text{-}19$$

The distribution coefficient is related to the retention factor ( $k'_M$ ) for the solute $M^{x+}$ by:

$$k'_M = K_C \frac{w}{V_m} \quad 1\text{-}20$$

where w is the weight of the stationary phase and $V_m$ is the volume of the mobile phase.

From equation 1-19 and 1-20 we obtain:

$$\frac{[M_s^{x+}]}{M_m^{x+}} = k'_M \frac{V_m}{w} \quad 1\text{-}21$$

Substituting this into equation 1-18 gives:

$$K_{M,E} = (k'_M \frac{V_m}{w})^y \left(\frac{[E_m^{y+}]}{[E_s^{y+}]}\right)^x \quad 1\text{-}22$$

If we assume that the eluent ion, $E^{y+}$, occupies y ion-exchange sites on the stationary phase, then the ion-exchange capacity of the column, Q, is given by:

$$[E_s^{y+}] = \frac{Q}{y} \quad 1\text{-}23$$

Equation 1-22 now becomes:

$$K_{M,E} = \left(k'_M \frac{V_m}{w}\right)^y \left(\frac{Q}{y}\right)^{-x} [E_m^{y+}]^x \quad 1\text{-}24$$

which can be arranged to give:

$$k'_M = \frac{w}{V_m}\left(K_{M,E}\right)^{\frac{1}{y}}\left(\frac{Q}{y}\right)^{\frac{x}{y}}\left[E_m^{y+}\right]^{-\frac{x}{y}} \quad 1\text{-}25$$

Taking the logarithm of equation 1-25, yields:

$$\log k'_M = \frac{1}{y}\log K_{M,E} + \frac{x}{y}\log\frac{Q}{y} + \log\frac{w}{V_m} - \frac{x}{y}\log\left[E_m^{y+}\right] \quad 1\text{-}26$$

Equation 1-26 is fundamentally important to ion chromatography. A few of the conclusions that can be drawn from this equation are:

(1) The retention factor for $M^{x+}$ is determined by the equilibrium constant $K_{M,E}$, the ion-exchange capacity of the column Q, the ratio of stationary phase to mobile phases ($w/V_m$), and the concentration of competing cations in the eluent, $[E_m^{y+}]$.

(2) Increases in $K_{M,E}$, Q or $w/V_m$ lead to increased retention factors, while increasing $[E_m^{y+}]$ leads to decreased retention factors.

(3) Increased eluent charge results in decreased retention, while increased solute charge results in increased retention.

(4) A plot of $\log k'_M$ versus $\log[E_m^{y+}]$ will be a straight line of slope equal to –x/y.

### 1.3 Chelation Ion Chromatography and Recent Progress

Since Small and co-workers invented IC in 1975, the determination of trace anion has been revolutionized [9]. IC has also been widely used to the analyze trace metals due to its easy-automation and cheapness, especially compared to the spectrometric techniques [10].

In cation-exchange methods, sometimes weak complexing reagents are added into the eluent to optimize the speed and selectivity of the separation, as shown in Figure 1.7.

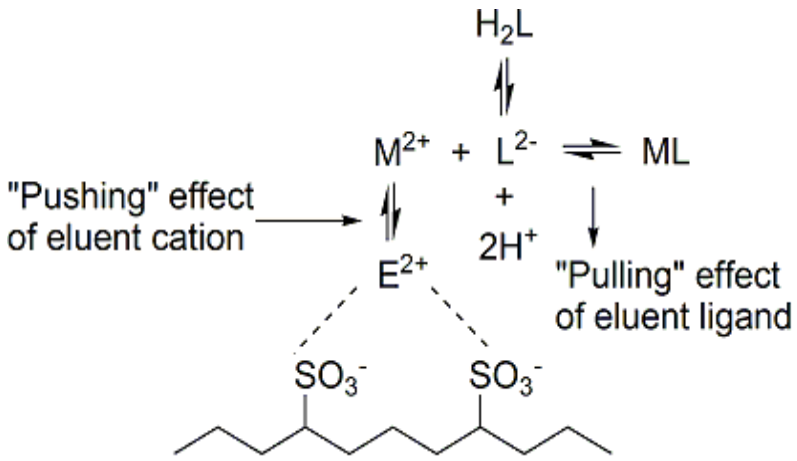


**Figure 1.7** Schematic representation of the elution of a solute $M^{2+}$ from a sulfonic acid cation exchanger. $E^{2+}$ and $L^{2-}$ stand for a single competing cation and weak chelation ligand, respectively.

However, traditional cation exchange fails in some applications of trace metal analysis, such as in the presence of large amount of other metals [11]. Chelation ion exchange has been explored as a solution to this problem. Chelating functional groups, such as iminodiacetate and oxine, can bind specific metals in the presence of complex matrices. For example, by utilizing a chelation ion exchange column, one can determine the amount of transition metal in sea-water where sodium is in high abundance [11]. Chelation ion exchange method was usually been considered a preconcentration technique due to its limited efficiency

(N) [15]. However since late 1980s, people have started to realize the potential power of this technique and tried to develop new high performance chelation ion chromatography (HPCIC) phases.

The basic idea is to covalently bond various chelation functional groups onto the surface of different substrates, such as polymer, control pore glass, or silica gel. By synthesizing these new types of materials people can obtain unique selectivity and avoid matrix effects in metal separation. Toei and co-workers attached iminodiacetic acid onto the surface of a hydrophilic polymer, and utilized this new chelation column to determine magnesium and calcium in sea-water [16]. Jonas and co-workers immobilized 8-hydroxyquinoline on a gel type poly(styrene-divinylbenzene) copolymer matrix, and achieved separation of zinc, copper, nickel, and cobalt in 30 minutes [17].

More recently, Haddad and co-workers combined a chelation and a cation ion-exchange column together to quantitatively determine aluminum in paper mill process water [18]. Brett Paull and co-workers dynamically coated a porous graphitic carbon column with *o*-cresolphthalein complexone and analyzed calcium and magnesium in sea-water by spectrophotometry without any addition of post column reagent [19]. Pavel Nesterenko and co-workers functionalized a silica column with iminodiacetate groups and determined transition metals ($Mn^{2+}$, $Fe^{2+}$, $Zn^{2+}$, $Cu^{2+}$, $Pb^{2+}$, $Fe^{3+}$) in fuel ethanol in 8 minutes [20].

**1.4 Instrumentation**

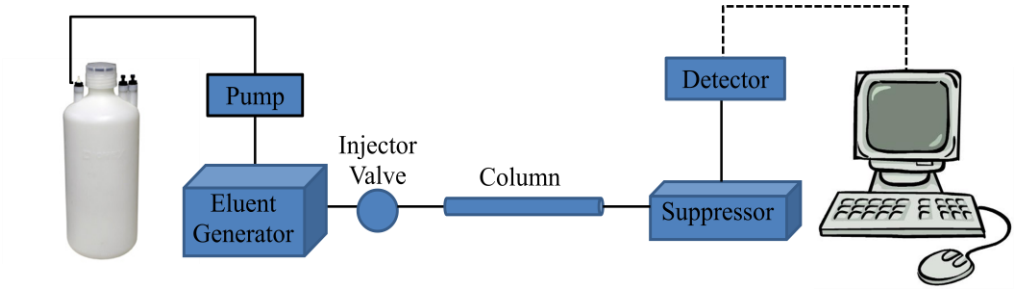


**Figure 1.8** Schematic diagram of a typical IC system

A traditional HPLC system includes a high-pressure eluent pump, injection valve, separation column, detector, and computer for data collection and analysis. Connecting tubing and fittings (solid lines) as well as network cables (dotted lines) are necessary components to link the individual parts together. As shown in Figure 1.8, instrumentation for ion chromatography is similar to a HPLC system, but has some special requirements. For example, the entire flow-path of IC must be metal-free, since the extreme pH eluents usually used in IC will result in severe metal contamination if stainless steel is used. Instead, IC systems are constructed of polyether ether ketone (PEEK), a chemically inert polymer material which can sustain high pressure and extremely alkaline conditions. In IC, eluents are usually generated on-line through electrodialysis using an eluent generator [21]. However, eluent generation is not used in this thesis and so will not be discussed further. Conductivity is the most popular detection mode in IC system because most ions do not absorb in the UV or fluoresce. Pumps for IC are usually isocratic, with a flow range between 0.1-1.5 ml/min. The typical injection volume is 20 μL, and conventional column dimension is 4.6 mm I.D. ×50, 100, or 250 mm length. Recently, Dionex released a capillary IC system (ICS-5000),

whose small dimensions improve the pressure capabilities of the eluent generator and enhance the suppression capacity. One can easily deduce the future of IC system will be in further shrinking the size of instrument, tubing and column.

### 1.4.1 Detection

In IC, conductivity detection is the most commonly used detection method, because all ionic species are electrically conductive. The conductivity detector cell contains two electrodes [19]. An electrical potential is applied between the electrodes. The ions in the eluent passing through the cell will hence respond to the potential and generate a corresponding current. The detector monitors this current. The current depends on the charge, concentration and ionic conductance of the individual ionic species. However, the eluent itself is highly conductive in IC, since it usually contains sodium hydroxide or sodium carbonate. Therefore, the background conductivity is always a big concern in IC. If the background is high, the signal-to-noise ratio will decrease, and hence the limit of detection will worsen. To solve this problem, an eluent suppressor is added to IC systems between the column and detector. The function of suppressors is discussed in Section 1.4.2.

Sometimes, UV absorbance detection is used in IC. However it can only detect UV-absorbing anions such as $IO_3^-$, $NO_2^-$, $NO_3^-$, $Br^-$, $I^-$ and $SCN^-$. The limited number of UV-absorbing anions limits its application. Post column derivatization detection is another type of detection which is mostly used in combination with a UV-Vis absorbance detector. Effluent from the column is

mixed with a colorimetric reagent such as 4-(2-Pyridylazo)-resorcinol (PAR) by means of a T-piece. The colorimetric reagent is delivered by a pump or a pressure applied to the reagent bottle. If longer reaction times are needed, a knitted PTFE capillary can serve as the flow-through reactor. PAR is the most widely used reagents for post-column detection of transition metals, and lanthanides [22]. In this thesis, since the metal ions do not have UV absorbance, most of the separations are achieved by post column derivatization detection with PAR.

### 1.4.2 Suppression

Suppression is a post column technique that eliminates the high background conductivity signal arising from the eluent. It converts hydroxide, carbonate or bicarbonate groups which are abundant in the eluent into their corresponding non-conductive form, e.g., $H_2O$ or $CO_2$. By suppressing the conductivity of the eluent ions, the limit of detection can be improved from parts-per-million (μg/mL) to pars-per-billion (ng/mL). Moreover, in the case of anion separation, suppression also increases the conductivity of analyte samples. It converts the counter-cation into $H^+$ (e.g, $Na^+Cl^-$ is converted into $H^+Cl^-$), which is more conductive than all the other cations, the signal of analyte is hence improved.

However, suppression has a few drawbacks. First, every suppressor has a finite capacity which limits the eluent concentration that can be used. Second, suppressors add additional flow volume, which increases the extra column band broadening of the system. Finally, some suppressors are based on a membrane

based structure, which limits the range of pressure and flow rate that can be applied onto the system.

There are two kinds of commercial suppressors: one is the packed-bed suppressor, and the others are membrane-based suppressors. A packed-bed suppressor is a short column which is a packed with ion-exchange resin. It requires frequently off-line regeneration and has relatively larger dead volumes. Membrane-based suppressor has a small dead volume, and can be regenerated continuously. Membrane-based suppressor has two types of design: one is chemical suppression, the other is electrolytic suppression. Currently, electrolytic suppression is the most widely used type of suppressor. The suppression technique is not applied in this work, so the mechanism of suppressor will not be discussed in detail.

**1.5 Eluent**

Eluent ions are necessary for the ion-exchange process, and their nature and concentration will affect the retention of the analyte ions. For anion separations, the most common eluents are hydroxide ($OH^-$), bicarbonate ($HCO_3^-$) and carbonate ($CO_3^{2-}$). These eluents are easily converted into non-conductive species ($H_2O$ and $CO_2$), and hence are compatible with the suppression technique. For cation separations, hydrogen cation ($H^+$) is the most widely used eluent ion. Researchers usually adjust the retention time of analyte by varying the pH. Sometimes, to optimize the performance, the addition of complexing agent such as salicylic acid or ethylenediaminetetraacetic acid (EDTA) is necessary. Haddad

and Foley have examined the effect of complexing agents and explained the mechanism in cation-exchange chromatography [14]. Their discussion has been illustrated in Figure 1.7.

## 1.6 Stationary Phase

The stationary phase is the heart of a chromatographic system, since that is the part where the actual separation of analytes takes place. The stationary phase can be made of polymer, silica, carbon, zirconia, or titania. Columns could also be classified based on their structure, e.g., monolithic or particulate columns [23]. This thesis is only concerned with particulate columns.

### 1.6.1 Polymer Based Stationary Phases

Polymeric particles are the first choice of material in IC because of its pH stability. However fully porous polymer particles suffer from poor mass transfer, making agglomerated particles the most popular type of polymer particle in IC. An agglomerated particle consists of a non-porous inner core and a thin outer layer of small particulate stationary phases. The thin layer of small (~0.1 μm diameter) latex particles are attached onto the surface of inner polymer bead by electrostatic and van-der-Waals interactions. The polymer core is usually made of sulfonated polystyrene or divinyl-benzene, while the latex particles are commonly polyvinylbenzyl chloride or polymethacrylate with quaternary amine functional groups. This charged monolayer of latex particles determines the selectivity and capacity of the stationary phase. The Dionex Ion Pac CS10 cation exchange column is based on this structure. More recent polymeric IC phases chemically

graft the ion exchange functionality onto the surface of the core particles. The grafted layer of functional groups is 1-5 nm thin. The Dionex Ion Pac AS14A and Ion Pac CS12A are good examples of this type of stationary phase.

### 1.6.2 Silica-based stationary phases for IC

Silica is the first choice for reversed or normal phase HPLC due to its excellent efficiency relative to its polymeric counterparts. Silica also shows good mechanical strength that will not shrink or swell in the presence of organic solvents [2]. The surface of silica can also be covalently modified using chlorosilanes [22]. This property provides silica with the potential to be tailored for a variety of applications. A wide array of silica particles are now commercially available. However, silica also has inevitable problems. The main drawback of silica particles is their pH stability. The bonded phase on silica hydrolyzes at pH lower than 2. Further, silica dissolves in eluents whose pH are higher than 8, such are commonly used in IC. This defect extremely limits the application of silica column in IC. Consequently, the development of new stationary phase materials which have both strong mechanical strength and wide range of pH stability remains a hotspot of IC research.

### 1.6.3 Graphitized carbon-based stationary phase and its application in IC

Graphitized carbon is an emerging stationary phase in IC. There are a few types of carbon particles commercially available such as carbon-clad zirconia and hypercarb. Carbon-clad zirconia was developed by Pete Carr and co-workers for the use of reverse phase HPLC [4]. Carbon-clad zirconia particle is made by

chemical vapour deposition of hydrocarbons on porous zirconia ($ZrO_2$) microparticles. In detail, toluene vapour first passed through the porous $ZrO_2$ particles in a tube furnace. A condition of high temperature (~700$^oC$) and low pressure (5 to 10 Torr) was then applied in the furnace. A uniform carbonaceous coating will form on the surface of particles with >97% coverage. Because of its graphitic carbon surface, this carbon-overlaid zirconia support is stable between pH 0-14. Given the strong zirconia core, it also has enough mechanical strength for high efficiency (high pressure) separations. Hypercarb (manufactured by Thermo) is another porous graphitic carbon-based stationary phase for HPLC. The surface of hypercarb is composed of flat sheets of hexagonally arranged carbon atoms. Previous study in our group has shown poor stability of hypercarb, so my research focussed on carbon-clad zirconia [27]. Similar to silica, the graphitized carbon surface could be easily grafted with various kinds of functional groups by electrochemical modification. Therefore, carbon-based stationary phases are a promising alternative to the traditional polymer and silica stationary phases in IC.

The Lucy group has pioneered paths to convert reverse phase carbon-clad zirconia into ion exchangers. In 2007, they created anion exchange phases from carbon-clad zirconia by coated it with a surfactant [26]. Three micron graphitic carbon and carbon clad zirconia particles were equilibrated with three different cationic surfactants such as didodecyldimethylammonium bromide (DDAB); cetyltrimethylammonium bromide (CTAB); and cetylpyridinium chloride (CPC). The surfactants adsorbed onto the carbon surface via hydrophobic interaction. The performance of each surfactant was examined using carbonate/bicarbonate eluents

with suppressed conductivity detection. Efficiencies as high as $5.0 \times 10^4$ plates/m were achieved. The surfactant coating was stable for more than $1.7 \times 10^3$ column volumes.

In 2009, Chambers et al. covalently modified carbon-clad zirconia particles via *in situ* diazonium generation and thermally grafted the tertiary amine N,N-dimethyl-*p*-phenylenediamine functionality onto the carbon surface [27]. A mixture of seven inorganic anions was separated in 12 minutes with efficiencies of 21,000 plates/m. The modified carbon phase showed stable retention over 33,000 column volumes when subjected to eluents ranging from pH 2-12. This material also exhibited comparable capacity to the commercial columns which had similar dimensions.

In 2011, Chambers et al. created agglomerated phases for IC [28]. Diazonium, chlorosulfonic acid, and nitric acid modification were explored as means to introduce a negative charge. The synthesized particles were packed into a 35 mm× 4 mm I.D. analytical column, and then coated with cationic AS12A latex particles. The new agglomerated particles separated common inorganic anions with efficiencies greater than 41,000 plates/m. Wahab et al. further optimized the diazonium method to modify 3 μm porous carbon clad zirconia particles [29]. He introduced benzene sulfonate functionalities on the carbon surface via diazonium chemistry and then coated with 70 nm triethylamine latex. An efficiency of 51,000 plates/m and RSD of 2% has been achieved via this method.

In summary, given the advantages of mechanical strength and pH stability, carbon clad zirconia is a good alternative to silica and polymer stationary phase in IC. Considering the conductivity of graphitized carbon, this new stationary phase also potentially opens the door to a new technique: electrically switched ion exchange. Creation of such a phase is the main objective of this thesis.

## 1.7 Summary

This thesis explores methodology to convert graphitized carbon particles into electrically switched ion exchangers. In Chapter Two, oxine groups are grafted onto the surface of 3 μm carbon clad zirconia particles. Various conditions are compared to obtain grafting. Chapter Three discusses the chromatographic performance of this new material. Capacity, reproducibility and efficiency are characterized. Chapter Four mainly explores the electrochemical functionality of the modified carbon particles, and discusses the possibility of using the modified particles into the electrochemically modulated preconcentration column. Chapter Five summarizes the thesis and briefly discusses potential future work.

## 1.8 References

[1] L. S. Ettre, *LC GC North America* **2003**, *21*, 458-467.

[2] U. D. Neue, *HPLC Columns: Theory, Technology, and Practice*, Wiley-VCH, Inc., New York, **1997**, p.

[3] S. Pelletier and C. A. Lucy, *Journal of Chromatography A* **2006**, *1125*, 189-194.

[4] T. P. Weber, P. T. Jackson and P. W. Carr, *Analytical Chemistry* **1995**, *67*, 3042-3050.

[5] L. S. Ettre, *Evolution of Liquid Chromatography: A Historical Overview in HPLC Advances and Perspectives*, Academic Press, New York, **1980**, p.

[6] A. J. P. Martin and R. L. M. Synge, *Biochemical Journal* **1941**, *35*, 1358-1368.

[7] J. J. Vandeemter, F. J. Zuiderweg and A. Klinkenberg, *Chemical Engineering Science* **1956**, *5*, 271-289.

[8] C. G. Horvath, B. A. Preiss and S. R. Lipsky, *Analytical Chemistry* **1967**, *39*, 1422-1428.

[9] H. Small, T. S. Stevens and W. C. Bauman, *Analytical Chemistry* **1975**, *47*, 1801-1809.

[10] C. A. Lucy, Journal of Chromatography A, **1996**, *739*, 3-13

[11] J. W. Mc Laren, A. P. Mykytiuk, S. N. Willie, and S. S. Berman, *Analytical Chemistry* **1985**, *57*, 2907-2911

[12] C. A. Lucy and P. Hatsis in *Ion Chromatography*, *Vol. 69A* (Ed. E. Heftmann), Elsevier, Amsterdam, **2004**, pp. 171-211.

[13] J. C. Giddings, *Analytical Chemistry* **1967**, *39*, 1027.

[14] P. R. Haddad and R. C. Foley, *Journal of Chromatography* **1990**, *500*, 301-312.

[15] P. Jones and P. N. Nesterenko, *Journal of Chromatography A* **1997**, *789*, 413-435.

[16] J. Toei and Z. Fresenius, *Analytical Chemistry* **1988**, *331*, 735-739.

[17] P. M. M. Jonas, D. J. Eve and J. R. Parrish, *Talanta* **1989**, *36*, 1021-1026.

[18] J. Tria, P. R. Haddad and P. N. Nesterenko, *Journal of Separation Science* **2008**, *31*, 2231-2238.

[19] B. Paull, M. Macka and P. R. Haddad, *Journal of Chromatography A* **1997**, *789*, 329-337.

[20] D. C. Jailson, K. T. Kubota, P. N. Nesterenko, G. W. Dicinoski, P. R. Haddad, *Analytical Methods* **2010**, 2, 1565-1570.

[21] Y. Liu, K. Srinivasan, C. Pohl and N. Avdalovic, *Journal of Biochemical and Biophysical Methods* **2004**, *60*, 205-232.

[22] W. W. Buchberger, *Journal of Chromatography A* **2000**, *884*, 3-22.

[23] F. C. Leinweber, U. Tallarek, *Journal of Chromatography A* **2003**, *1006*, 207-228.

[24] P. R. Haddad and P. T. Jackson, *Ion Chromatography: Principles and Applications*, Elsevior, New York, **1990**, p.

[25] J. M. Miller, *Chromatography Concepts and Contrasts*, John Wiley & Sons, Inc., Hoboken, New Jersey, **2005**, p. 82.

[26] S. D. Chambers and C. A. Lucy, *Journal of Chromatography A* **2007**, *1176*, 178-184.

[27] S. D. Chambers, M. T. McDermott and C. A. Lucy, Analyst **2009**, *134*, 2273-2280

[28] S. D. Chambers, C. A. Pohl and C. A. Lucy, *Journal of Chromatography A* **2011**, *1218*, 263-269.

[29] M. F. Wahab, C. A. Pohl, and C. A. Lucy, *Analyst*, **2011**, *136*, 3113-3120.

# CHAPTER TWO: Modification of Carbon Clad Zirconia Particles

## 2.1 Introduction

As discussed in the previous chapter, silica and polymeric stationary phases are the predominant media in the world of liquid chromatography. Silica can achieve higher efficiency for small molecules than polymers. In contrast, polymers can sustain a much wider pH range than silica. In IC separations, highly acidic and alkaline eluents are commonly used, so the poor pH stability limits the application of silica particles. Graphitic carbon can provide comparable efficiency with silica, and withstand pH 0-14 [1]. Therefore, significant research interests have focused on carbon as a chromatographic media [2-4].

Graphitic carbon's $sp^2$ hybridization provides excellent pH stability [5]. However, this hybridization also makes the graphitic carbon surface difficult to modify. Several modification approaches have been studied by different research groups. The oxidation of carbon surface is one of the most widely used methods. Rasheed and co-workers studied the yield and functionalization of carbon nanofibers after treatment with $HNO_3$, $KMnO_4$, $RuO_4$, and a mixture of concentrated $H_2SO_4/HNO_3$ [6]. Evans and co-workers utilized a radiofrequency plasma to introduce oxygen-containing functionality onto the surface of pyrolytic graphite [7]. Nystrom investigated electrochemical oxidation of graphitic electrode surface [8]. The synthetic routes above are all successful, but all introduce a mixture of functional groups (hydroxyl, carboxylic, ketonic groups) onto the carbon surface. For chromatographic applications, more uniform functionality is desirable. Thus, we have to explore alternative approaches.

By coating the graphitic carbon surface with cationic surfactants, Chambers et al. converted the reversed phase media into an ion exchanger [9]. The surfactant tail adsorbed onto the surface of the graphitic carbon through hydrophobic interactions. This method is cost-effective, and offers relative uniform functionality on the carbon surface. However, the coating is semi-permanent, and is gradually lost from the surface, resulting in decreasing retention [10].

Pinson and co-workers covalently grafted functionalized aryl radicals onto a carbon electrode by electrochemically reducing diazonium salts [11]. The diazonium salt accepts one electron from the conducting graphitic carbon, resulting in a covalent bond between the aryl group and the carbon surface. By choosing different functional groups in the para-position of the diazonium salt, various functionalities can be introduced onto the carbon surface. This method opens the door for the covalent modification of graphitic carbon for separation purposes.

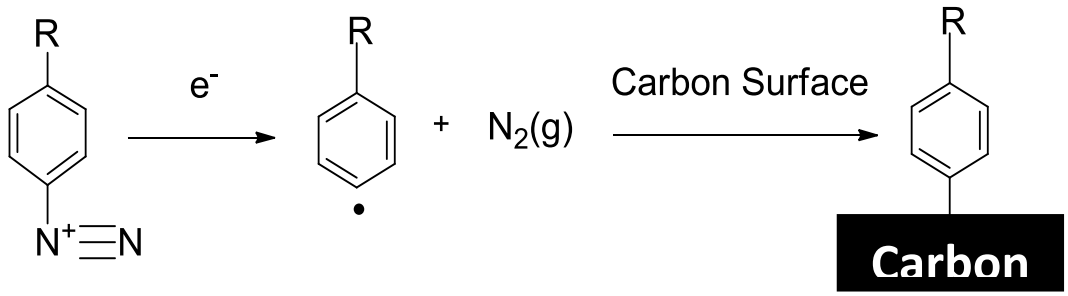


**Figure 2.1** General scheme for electrochemical reduction of diazonium salts onto carbon surfaces

In 2001, Porter and co-workers reported the successful on-column modification of carbon particles using electrochemical reduction of diazonium salts [12]. They introduced nitrobenzyl and hexylbenzyl group onto the surface of glassy carbon and porous graphitic carbon. The modified materials exhibit altered retention selectivity relative to pure carbon. This new material provided more stable retention than surfactant coated columns, and sustained 30 hours of eluent flush at pH from 1.5 to 11. However, the drawback of this method is that very few diazonium salts are commercially available, so they have to be synthetically prepared in very high purity. Any impurity in the product will yield a mixture of redox contaminants which would interfere with the final reaction. This defect severely limits the application of this methodology.

To broaden the application of electrochemical reduction of diazonium salts, B́langer and co-workers explored *in situ* generation of diazonium cations to modify carbon surfaces [11]. Under strongly acidic conditions, a primary arylamine reacts with sodium nitrite to form diazonium cations. These cations subsequently undergo electrochemical reduction, resulting in their attachment onto the graphitic carbon surface [14]. This synthetic route eliminates the necessity of custom synthesis of pure diazonium salts, but still requires expensive instrumentation to apply an electrical potential to the particles [12].

In 1998, Cabot Corporation patented a simpler way to modify carbon surfaces [15]. Diazonium ions are thermally reactive. At temperatures $> 5^{o}C$ diazonium compounds spontaneously bond to electron-rich moieties [16]. Cabot Corporation utilized this thermal property and *in situ* generation of diazonium

salts to modify various types of carbon. A few research groups have noticed the importance of this new synthetic approach and applied it in functionalization of carbon nanotubes [17] and electrodes [18]. However, very few researchers realized the chromatographic application potential of this technique. Previous work from our group used this approach to convert graphitic carbon media into anion exchangers [16].

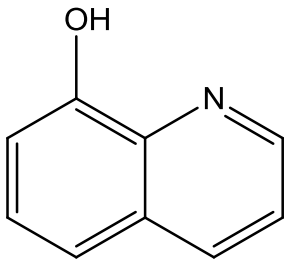


**Figure 2.2** The structure of 8-hydroxyquinoline

This chapter extends that previous work to create High Performance Chelation Ion Chromatography (HPCIC) media. By *in situ* generation and thermal deposition of diazonium salts, oxine (8-hydroxyquinoline) groups have been grafted onto the graphitic carbon surface. Various modification conditions were investigated and optimized to obtain optimum surface reaction.

## 2.2 Experimental

### 2.2.1 Reagents and Solution Preparation

Methanol, isopropanol (Fisher Scientific, Ottawa, ON) were of HPLC grade. Tetrahydrofuran (HPLC grade, EMD Chemicals, Gibbstown, NJ, USA), anhydrous ethanol (Fisher), hexane (98.5+%, Sigma-Aldrich), 30% hydrogen

peroxide (Fisher), concentrated hydrochloric acid (Fisher), and concentrated sulphuric acid (Fisher) were used as received. All reagent and eluent solutions were prepared using 18 MΩ nanopure water (Nanopure water system, Barnstead, Chicago, IL) and filtered through 0.22 μm nylon membrane filters (Millipore, Bedford, MA) prior to use. Three micron porous carbon clad zirconia (30 $m^2$/g, 300 Å, Zirchrom Separations. Inc., Anoka, MN), 5-amino-8-hydroxyquinoline dihydrochloride (95%, Sigma-Aldrich), sodium nitrite (99.9+%, Sigma-Aldrich), and acetone (HPLC grade, EMD) were used as received. All inorganic chemicals were reagent grade or better.

### 2.2.2 Reaction Vessel Cleaning

A new clean glass vial with a new screw cap top was used for every reaction. Glass vials (28×57 mm, Fisher) were cleaned with piranha (1 fold 30% $H_2O_2$, 3 fold sulfuric acid), rinsed with an abundance of filtered nanopure water, dried in a moisture free oven (>140$^o$C), and allowed to cool to room temperature before use as reaction vessels. The synthesis of 2 g carbon clad zirconia involved 200 ml round-bottle flasks, water condenser, and separatory funnel. They were all cleaned with copious amounts of water, ethanol and methanol, dried in a moisture free oven, and allowed to cool to the room temperature before use.

### 2.2.3 Diazonium Modification of Carbon-clad Zirconia

Carbon-clad zirconia was modified using *in situ* generated diazonium ions and thermally attached onto the particle surface. The synthetic route is shown in Figure 2.3. Optimization studies were performed using small scale (0.10 g)

batches of 3 µm porous carbon clad zirconia particles, as follows. The 1-5 mmol of 5-amino-8-hydroxyquinoline was transferred into a clean vial. The solid was dissolved into 8 ml 36% ethanol solution and sonicated for 10 minutes. The vial was placed in an oil bath and allowed thermally equilibrates. 0.1 g of 3 µm porous carbon-clad zirconia particles were introduced into the solution along with a magnetic stir bar. 1.27 mol/l $NaNO_2$ solution was added dropwise

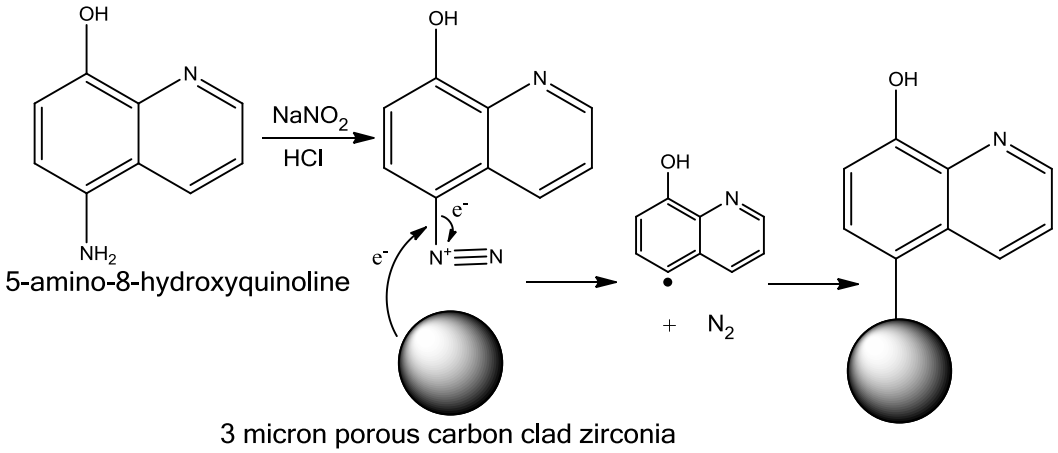


**Figure 2.3** Reaction scheme of carbon clad zirconia modification

into the vial, with the resultant release of $N_2$ bubbles. The vial was capped, and the mixture was allowed to react for 15 minutes to two hours. The reaction was stopped by placing the vial into an ice-bath. The particles were collected on a 0.22 µm nylon membrane filter, and washed with water, ethanol, tetrahydrofuran, acetone, methanol, and water/methanol blends until the filtrate becomes colorless. During this wash step, the membrane filter was changed if it became stained. The clean particles were suspended in 10 ml methanol and sonicate another 10 minutes. This mixture was rotovapped at 35 $^{o}$C to remove any remaining methanol. Modified sample powder was analyzed by AXIS ULTRA X-ray

photoelectron spectroscopy (XPS, Kratos Analytical, Wharfside, Manchester, UK) in the Alberta Centre for Surface Engineering and Science. The %N for each sample was measured at three different locations within the modified CCZ powders. The results are reported are the mean value of the triplicates. FTIR spectroscopy (Nicolet Magna 750, Analytical and Instrumentation Lab, Department of Chemistry) were also performed to determine sample composition. In detail, one mg of CCZ powder was mixed with 100 mg of dried KBr powder, and then this mixture was pressed in a die to yield a transparent pellet. The pellet was then held in the instrument for IR examination. Finally, Scanning Electron Microscope (Zeiss EVO MA 15, SEM Lab, Department of Earth & Atmospheric Sciences) were performed to determine particle structure.

The synthesis of packing particles was performed in 2 g batches of 3 μm porous carbon clad zirconia. The synthetic routes were shown in Figure 2.3.

### 2.2.4 Packing

Polyether etherketone (PEEK) columns (35 × 4 mm I.D., Dionex, Sunnyvale, CA) were packed at constant pressure using a Haskel air-driven liquid pump with a lower pressure air modification (DSF-122-87153, Haskel, Burbank, CA). The Haskel pump and all packing tubing and junctions were washed with 400 ml isopropanol. Two grams of modified carbon-clad zirconia were slurried in 50 ml 90/10 (v/v) isopropanol/hexane solvent, and sonicated for 30 minutes. The particles were then transferred in a 50 ml stainless steel solvent reservoir (Lab Alliance, State College, PA, USA), and packed in the downward direction using

isopropanol as the driving solvent. The pressure was increased from 0 kPa (0 PSI) to $4.1\times10^4$ kPa (6000 PSI) within 2 minutes, and then kept at $4.1\times10^4$ kPa for two hours. During the packing, 250 ml isopropanol were used. The column were then fitted with end fittings (0.5 μm polymer frits, Dionex, Sunnyvale, CA) quickly and flushed with copious amounts of water. The column were then capped and left overnight at $30^oC$ to allow packing bed expansion before use.

## 2.3 Results and Discussion

### 2.3.1 Optimization of reaction conditions

As mentioned in Section 2.1, previous work from our group proved that carbon-clad zirconia particles could be modified by *in situ* generation and thermal grafting of diazonium ions [19], as shown in Figure 2.4.

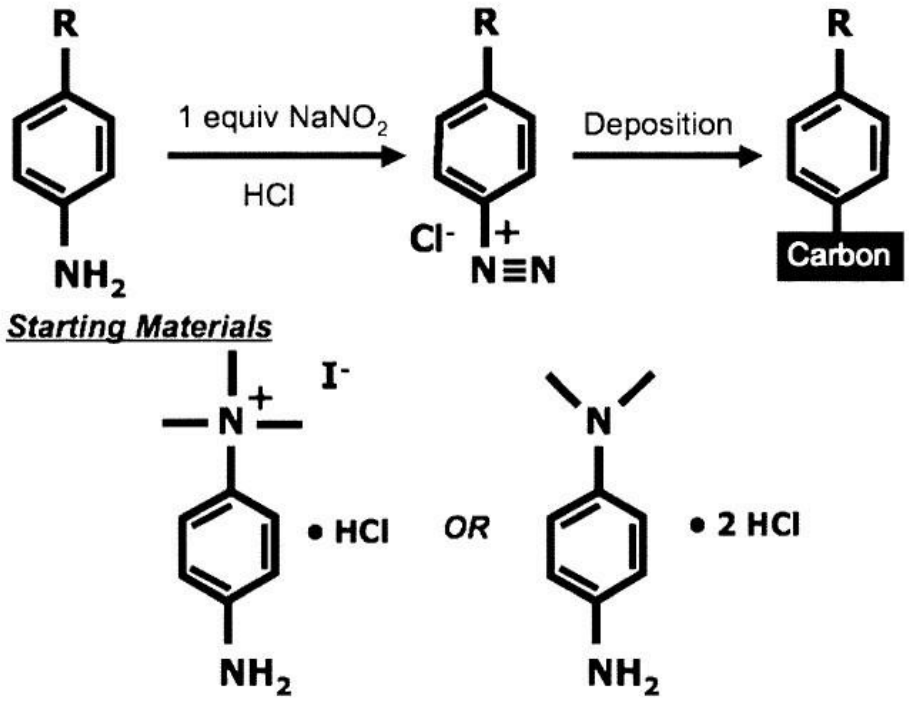


**Figure 2.4** Reaction scheme of diazonium modification of carbon surface in previous work done by our group.

To obtain optimized chelation functionality on the same material, the effect of reaction time, temperature, and amount of 5-amino-8-quinolinol are the three factors investigated. To determine how these factors influence modification, and which one is most important, a three factors, three levels orthogonal experiment table was established, as shown in Table 2.1.

**Table 2.1** Three factors three levels orthogonal experiment table

| Factors / Expt No. | A (Time/mins) | B (Temperature/℃) | C (Amount of quinoline) | Results |
|---|---|---|---|---|
| | 1 | 2 | 3 | Atomic conc% of N |
| **0** | 0 | 0 | 0 | 0.00 |
| **1** | 1（15） | 1 (room temperature) | 1 (1mmol) | 2.53 |
| **2** | 1 | 2 (60) | 2 (2.5mmol) | 4.62 |
| **3** | 1 | 3 (80) | 3 (5mmol) | 11.84 |
| **4** | 2 (60) | 1 | 2 | 5.27 |
| **5** | 2 | 2 | 3 | 10.04 |
| **6** | 2 | 3 | 1 | 8.96 |
| **7** | 3 (240) | 1 | 3 | 7.44 |
| **8** | 3 | 2 | 1 | 7.95 |
| **9** | 3 | 3 | 2 | 13.41 |

In these experiments, oxine functional groups were grafted onto the graphitic carbon surface under various conditions. XPS was utilized to determine the amount of nitrogen on the surface of the 3 μm carbon-clad zirconia particles. The blank sample (Expt. 0, Table 2.1) contained no detectable surface nitrogen. The atomic concentration of nitrogen was thus used to monitor the effectiveness of the modification. From Table 2.1, it can be seen that the oxine groups were successfully grafted onto the surface of graphitic carbon via *in situ* generation and thermal attachment of diazonium ions under all conditions studied. Under the most favoured conditions, considerable amounts of nitrogen ($>10\%$) were attached.

**Table 2.2** Analysis of results of orthogonal experiment

(Values were calculated based upon the %N of modified sample)

| | | | | | |
|---|---|---|---|---|---|
| **Average of 15 mins** | 6.33 | **Average of room temperature** | 5.08 | **Average of 1 mmol** | 6.48 |
| **Average of 60 mins** | 8.09 | **Average of 60 ℃** | 7.54 | **Average of 2.5 mmol** | 7.77 |
| **Average of 240 mins** | 9.60 | **Average of 80 ℃** | 11.40 | **Average of 5 mmol** | 9.77 |
| **Standard deviation of time** | 1.64 | **Standard deviation of Temperature** | 3.19 | **Standard deviation of amount** | 1.66 |
| **%RSD of time** | 0.20 | **%RSD of T** | 0.40 | **%RSD of amount** | 0.21 |

Table 2.2 is a deeper statistical analysis of the synthetic yield. Temperature shows the largest standard deviation which means increasing the reaction temperature causes the biggest increase in yield. The standard deviation of time and the amount of quinoline are lower than that for temperature. This means increasing time or amount cannot enhance synthetic yield to the same extent as temperature. Therefore, to maximize the yield, experiments should be conducted at the highest temperature possible.

The experiments above only compare the effects of the three factors. However, we also want to figure out how each factor individually affects the yield. Figure 2.5 shows the effect of the amount of quinoline on the modification. The atomic concentration of nitrogen increased considerably when the amount of quinoline was increased from 0 to 1 mmol. Once the amount of quinoline reaches

5 mmol, the nitrogen does not increase significantly. Based on Figure 2.5, 3 mmol of quinoline is optimal for modification.

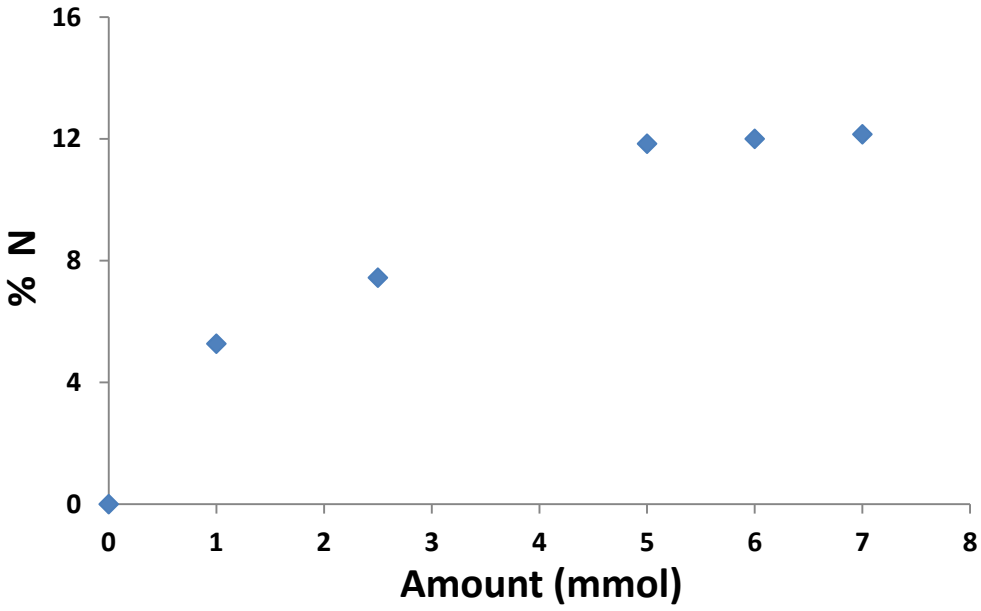


**Figure 2.5** Effect of 5-amino-8-quinolinol amount on the modification of 3 μm carbon-clad zirconia particles. Conditions: certain amount of 5-amino-8-quinolinol in 33% water/EtOH; 0.100 g $ZrO_2$-C 3 μm; reaction temperature: 80 $^oC$, 1 equiv. $NaNO_2$; 2 equiv. HCl; reaction time: 15 min.

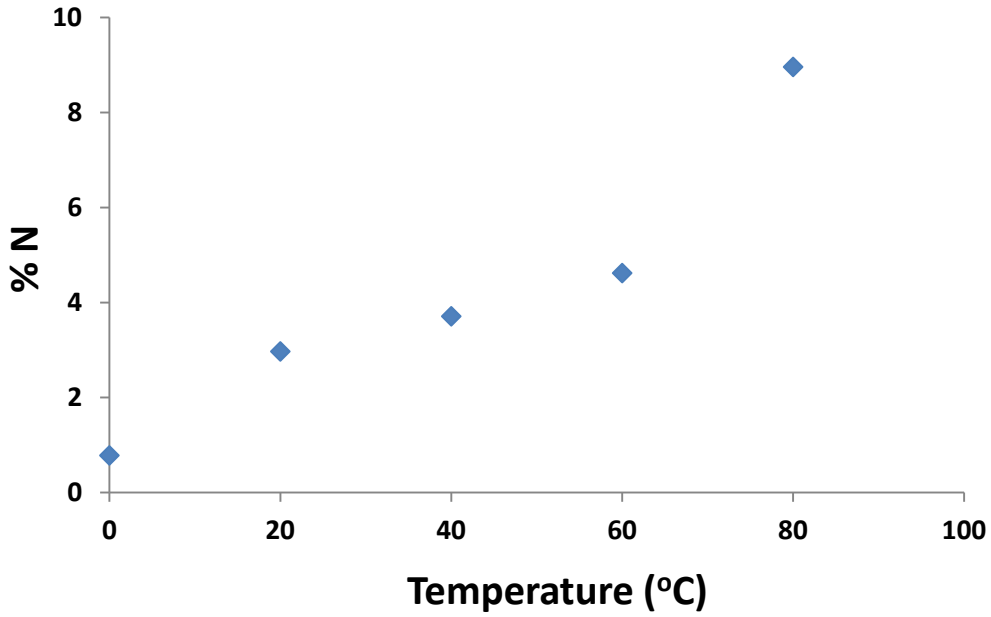


**Figure 2.6** Effect of temperature on the modification of 3 µm carbon-clad zirconia particles. Conditions: 2.5 mmol of 5-amino-8-quinolinol in 33% water/EtOH; 0.100 g $ZrO_2$-C 3 µm; 1 equiv. $NaNO_2$; 2 equiv. HCl; reaction time: 15 min.

Figure 2.6 shows the effect of temperature on the modification. The yield increases with increasing temperature. The atomic concentration jumps from 5% to 9% when the temperature increases from 60 to 80°C. The boiling point of the water and ethanol mixture is 80°C, which prohibits further temperature increases. Based on Figure 2.6, 80°C is favorable to maximize the yield.

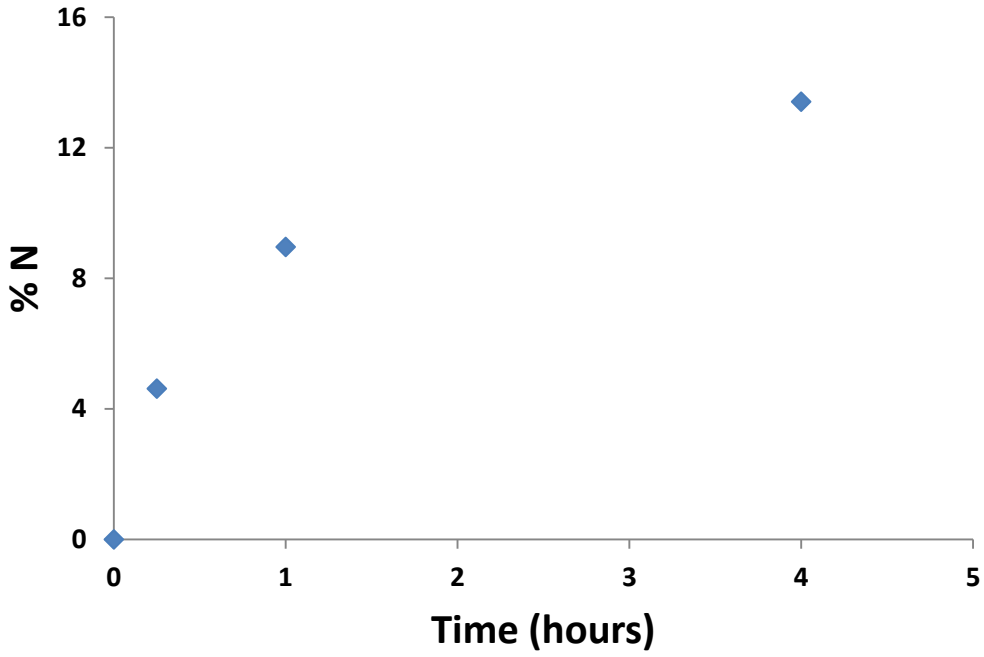


**Figure 2.7** Effect of time on modification of 3 μm carbon-clad zirconia particles. Conditions: reaction temperature: 80 $^{o}$C; 2.5 mmol of 5-amino-8-quinolinol in 33% water/EtOH; 0.100 g $ZrO_2$-C 3 μm; 1 equiv. $NaNO_2$; 2 equiv. HCl.

Figure 2.7 shows the effect of reaction time on the modification. Fifteen minutes provided ~ 4% nitrogen on the surface, and one hour of reaction doubles this yield. Increasing the reaction time further to 4 hours only provided ~ 12% nitrogen. It means the effect of time becomes weaker as the time increases.

In conclusion, by doing this series of experiments, the effects of each factor time, amount, and temperature on the synthetic yield were individually determined. Compared to the surface coverage (~2.5%) of previous works done in our group, the yield has been significantly improved [19]. There are two possible reasons for this improvement. Firstly, the double ring structure of oxine would

stabilize the diazonium radical relative to their single ring counterparts. Thus kinetically the radicals have longer time to react with the graphitic carbon surface. Secondly, as shown in Figure 2.6, the higher reaction temperature used here (80$^{o}$C compared to 60$^{o}$C) would increase the yield.

However, the XPS data only shows the atomic concentration of nitrogen on the carbon surface. To determine whether the grafted nitrogen contains the desired oxine chelation group, we investigated the surface using IR spectroscopy.

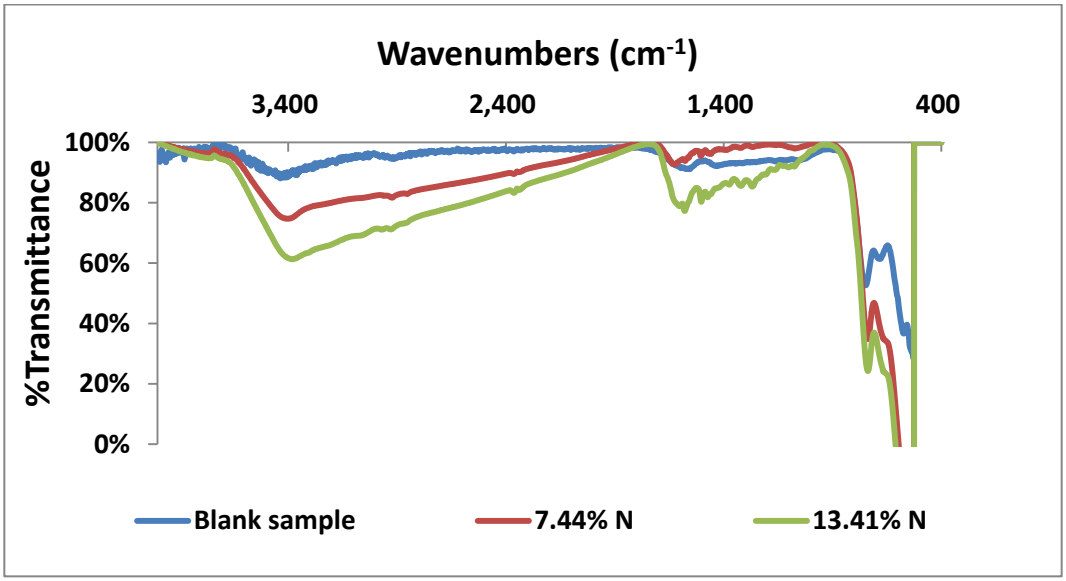


**Figure 2.8** IR spectra of modified 3 µm carbon-clad zirconia particles.

Figure 2.8 shows IR spectra of functionalized 3 µm carbon-clad zirconia particles. The increasing absorption at 3400cm$^{-1}$ represents the hydroxyl (OH) group. The modified sample also shows strong absorption at 1635 cm$^{-1}$ corresponding to a carbon nitrogen ring stretch. The presence of hydroxyl group and nitrogen on the graphitic carbon surface is consistent with the presence of oxine functionality on the carbon surface, suggesting that our experiments

successfully converted reverse phase carbon clad zirconia into a chelation ion exchanger.

### 2.3.2 SEM Characterization

In Section 2.3.1 the synthetic conditions were optimized to maximize the nitrogen content on the carbon clad zirconia particles. However numerous attempts to pack the 13.4% N modified particles into a 35×4 mm I.D. PEEK column failed. The pressure during the packing process was extremely difficult to control, with the high pressure (>6500 psi) actually bursting the column jacket. Therefore, we have to go back and redesign our synthetic conditions.

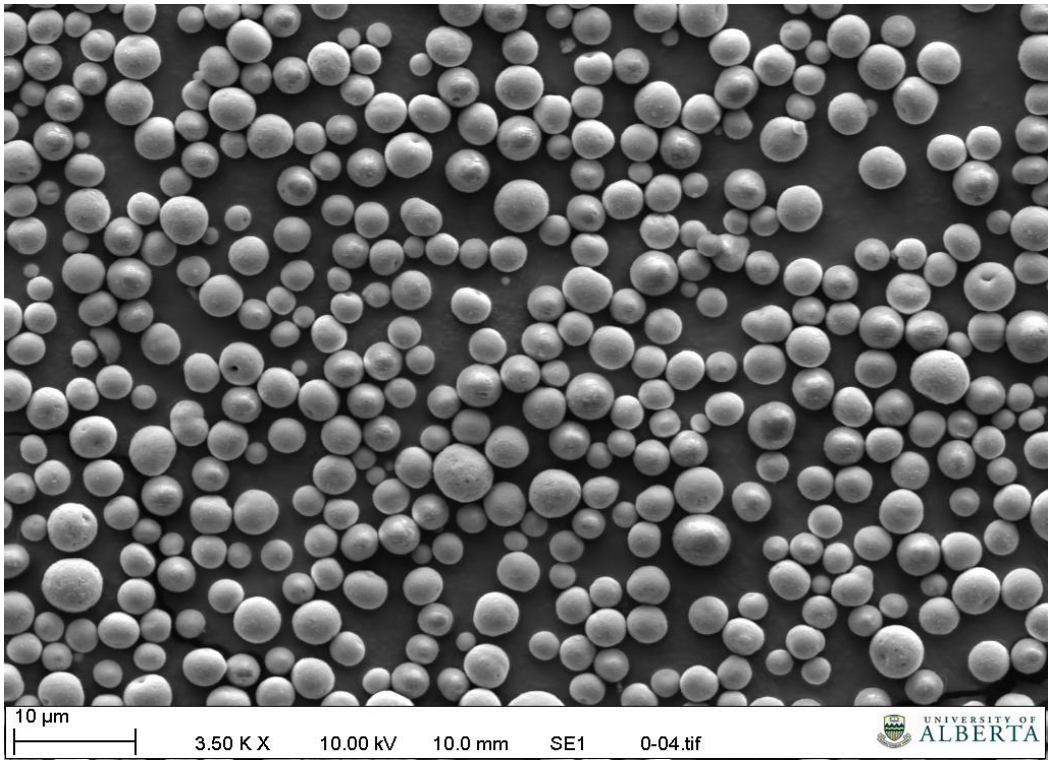


**Figure 2.9** Scanning electron microscope of blank carbon clad zirconia particles

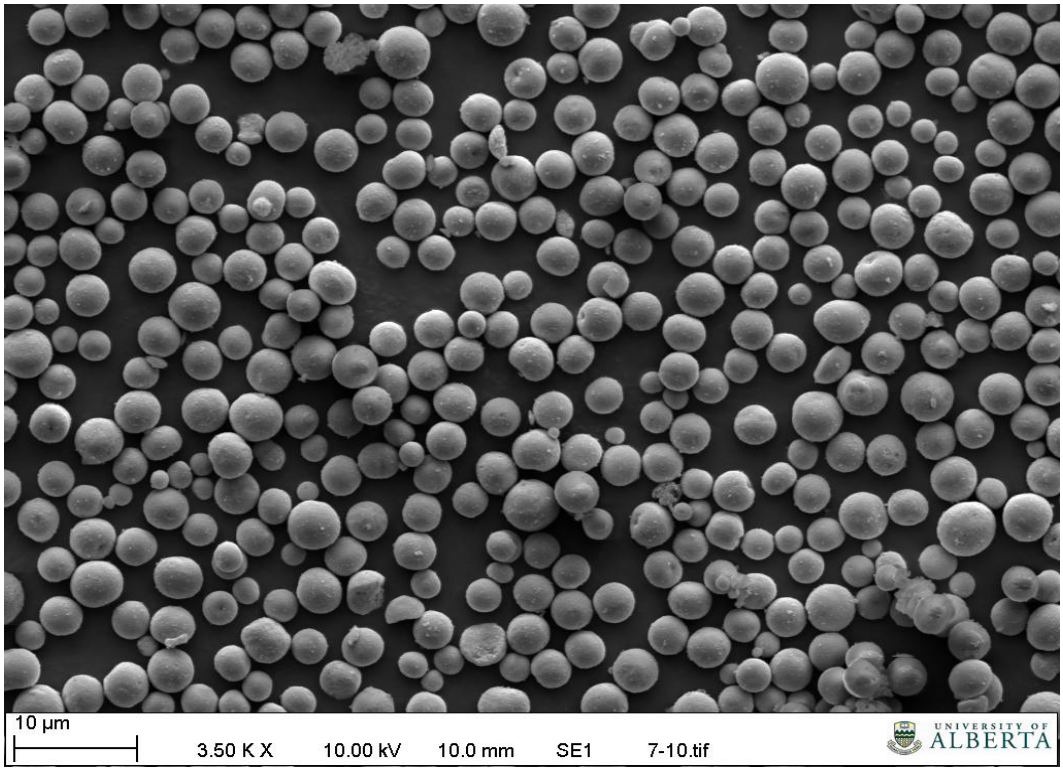


**Figure 2.10** Scanning electron microscope of modified carbon clad zirconia particles with 7.44% atomic concentration of nitrogen

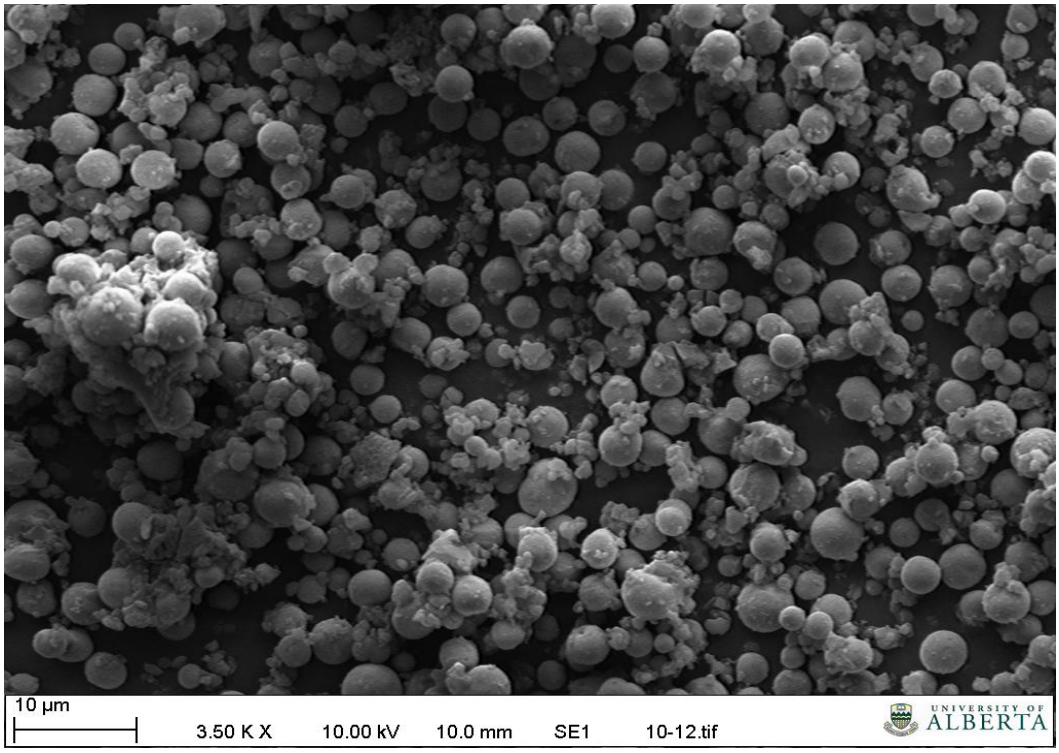


**Figure 2.11** Scanning electron microscope of modified carbon clad zirconia particles with 13.41% atomic concentration of nitrogen

Figure 2.9 to 2.11 compare scanning electron micrographs (SEM) of particles with different extents of modification. Figure 2.9 shows that unmodified particles vary considerably in size and are not uniformly spherical. Most important herein, the surfaces are smooth, and no debris or agglomeration is evident. Figure 2.11 showing a sample of carbon clad zirconia with 13.41% nitrogen provides a sharp contrast. Most of the particles are now irregular in shape. They are agglomerated together and there is substantial debris evident. It is believed that the small debris was responsible for plugging the column during packing, resulting in the high pressure which burst the column jacket. Finally, Figure 2.10 points out the appropriate condition we should use. The modified

particles in Figure 2.10 have a high surface coverage of nitrogen (7.44%), but the particles remain similar in shapes to the original particles. The spherical shape will provide sufficient efficiency in the future separation. Therefore, the moderate modification conditions (80 $^{o}$C, 15 minutes, with 2.5 mmol of 5-amino-8-hydroxyquinoline dihydrochloride) that yield 7.44% N will be used in the future synthesis.

## 2.4 Conclusions

This chapter discussed the covalent modification of 3 μm porous carbon-clad zirconia particles into chelation ion-exchange phases utilizing *in situ* generation and thermal deposition of diazonium ions. The effects of time, amount, and temperature are investigated and compared. Temperature is the most important factor that contributes to the final yield. The optimum conditions are 80$^{o}$C, 15 minutes, with 2.5 mmol of 5-amino-8-hydroxyquinoline dihydrochloride. By applying this optimum condition, we can obtain a high surface coverage of nitrogen element (5.31%-7.44%) without distorting the particle size and shape. Too high nitrogen concentration on the surface results in substantial debris and gluing the particles together, so that excessive back pressure observed in packed columns. Consequently, too much amount of 5-amino-8-quinolinol or too long reaction time is not recommended.

The next step of this project will be testing the performance of this new chelation ion-exchanger. Chapter Three focuses on capacity measurement and cation separation.

## 2.5 References

[1] T. P. Weber, P. T. Jackson and P. W. Carr, *Analytical Chemistry* **1995**, *67*, 3042-3050.

[2] M. Pabst, J. S. Bondili, J. Stadlmann, L. Mach and F. Altmann, *Analytical Chemistry* **2007**, *79*, 5051-5057;

[3] N. Helali and L. Monser, *Journal of Separation Science* **2008**, *31*, 276-282;

[4] L. M. Ponton and M. D. Porter, *Analytical Chemistry* **2004**, *76*, 5823-5828.

[5] C. A. Coulson and M. D. Poole, *Carbon* **1964**, *2*, 275-279.

[6] A. Rasheed, J. Y. Howe, M. D. Dadmun and P. F. Britt, *Carbon* **2007**, *45*, 1072-1080.

[7] J. F. Evans and T. Kuwana, *Analytical Chemistry* **1977**, *49*, 1632-1635.

[8] W. A. Nystrom, *Journal of the Electrochemical Society* **1969**, *116*, 17-&.

[9] S. D. Chambers and C. A. Lucy, *Journal of Chromatography A* **2007**, *1176*, 178-184.

[10] K. M. Glenn and C. A. Lucy, *Analyst* **2008**, *133*, 1581-1586.

[11] M. Delamar, R. Hitmi, J. Pinson and J. M. Saveant, *Journal of the American Chemical Society* **1992**, *114*, 5883-5884.

[12] J. A. Harnisch, D. B. Gazda, J. W. Anderegg and M. D. Porter, *Analytical Chemistry* **2001**, *73*, 3954-3959.

[13] S. Baranton and D. Belanger, *Journal of Physical Chemistry B* **2005**, *109*, 24401-24410.

[14] M. F. W. Dunker, E. B. Starkey and G. L. Jenkins, *Journal of the American Chemical Society* **1936**, *58*, 2308-2309.

[15] J. Belmont, R. Amici and C. Galloway, *Reaction of carbon black with diazonium salts, resultant carbon black products and their uses*, Carbot Corporation (Boston, MA), **1998**.

[16] D. F. Detar and S. V. Sagmanli, *Journal of the American Chemical Society* **1950**, *72*, 965-969.

[17] M. S. Strano, C. A. Dyke, M. L. Usrey, P. W. Barone, M. J. Allen, H. W. Shan, C. Kittrell, R. H. Hauge, J. M. Tour and R. E. Smalley, *Science* **2003**, *301*, 1519-1522.

[18] D. Shewchuk, M. McDermott, *Langmuir* **2009**, 8, 4556-4563.

[19] S. D. Chambers, M. T. McDermott and C. A. Lucy, *Analyst* **2009**, *134*, 2273-2280.

# CHAPTER THREE: Capacity Measurement and Column Separation

## 3.1 Introduction

Chapter Two introduced the methodology that converts reverse phase carbon-clad zirconia into a chelation ion exchanger. Various conditions have been optimized. In this chapter I will focus on the application of this new functionalized stationary phase.

8-Hydroxyquinoline (oxine) is a well known ligand. Over 60 metal ions form complexes with oxine with aqueous phase formation constants ranging from $10^4$ ($Ba^{2+}$) to $10^{38}$ ($Fe^{3+}$) [1]. Moreover, oxine has been immobilized on different substrates such as polymers [2-5], silica gel [6-12], and controlled pore glass [9-12]. These functionalized materials have been utilized to remove trace concentrations of heavy metal ions from electrolyte solutions [13], to preconcentrate trace metal ions prior to their quantitative determination [5, 7, 9], and for batch and chromatographic separations [2, 4, 12]. In 1988, Cantwell and co-workers covalently grafted 8-hydroxyquinoline onto controlled pore glass (CPG), and used this material to determine the concentration of free $Ca^{2+}$ in aqueous solution [14]. But CPG-oxine has low metal-complexing capacities and narrow pH range due to the hydrolysis of silanol groups [8, 15]. Moreover, the ionization of silanol groups on the glass surface introduces a negative charge, so that the CPG-oxine possesses some cation-exchange property in addition to its complexing character [14, 16]. To solve these problems, oxine has been bound

onto styrene/divinylbenzene copolymers [17]. But such polymer phases suffer from low mechanical strength and low efficiency [2, 4].

Carbon-clad zirconia is an ideal alternative to the substrates above. Its zirconia core provides high mechanical strength, and its graphitic carbon surface sustain pH from 0-14 [18, 19]. Our lab has demonstrated the use of surfactant coatings [20], covalent modification by diazonium chemistry [21], and latex coating by electrostatic interaction [19] to convert graphitic carbon-based stationary phase into an anion exchanger. However, to the best of our knowledge, no one has functionalized graphitic carbon-based particles to create a chelation ion exchanger.

In this chapter, the complexing capacity of modified carbon-clad zirconia synthesized in Chapter Two is measured by an established literature method based on $Cu^{2+}$ uptake [16]. Chromatographic separations are performed to determine a mixture of $Na^{+}$, $Ca^{2+}$, and $Mg^{2+}$. Finally, the effects of ionic strength, temperature and pH on retention and chromatographic efficiency are studied.

## 3.2 Experimental

### 3.2.1 Apparatus

Measurements of pH were made with glass and calomel electrodes using a Corning model 445 pH meter (Fisher Scientific Co.).

Copper concentrations were measured at 224.7 nm and 213.6 nm using an Optima 2100DV inductively coupled plasma optical emission spectrophotometer

(Perkin-Elmer). A calibration curve was prepared from electrolytic copper (Anachemia) for each measurement. The concentration of the copper (II) standard solution buffered by 0.2 M sodium acetate at pH=5 ranged from 0.1 to 100 ppm, and the correlation coefficient of the calibration curve was $\geqslant$ 0.9999.

The modified particles (as described in Sec. 2.2.3) were packed in our lab into a 35$\times$4 mm I.D. PEEK column jacket (Dionex, Sunnyvale, CA) at constant pressure using a Haskel air-driven liquid pump with lower pressure air modification (DSF-122-87153, Haskel, Burbank, CA). The Haskel pump and all packing tubings and junctions were washed with 400 ml isopropanol. Two grams of modified carbon-clad zirconia were slurried in 50 ml 90/10 (v/v) isopropanol/ hexane solvent, and sonicated for 30 minutes. The particles were then transferred into a 50 ml stainless steel solvent reservoir (Lab Alliance, State College, PA, USA) and packed in the downward direction using isopropanol as the driving solvent. The pressure was increased from 0 kPa (0 PSI) to $4.1\times10^4$ kPa (6000 PSI) within 2 minutes, and then kept at $4.1\times10^4$ kPa for two hours. During the packing, 250 ml isopropanol were used in total. The column were then quickly fitted with end fittings (0.5 μm polymer frits, Dionex) quickly and flushed with copious amounts of water. Finally, the column was capped and left overnight at 30 $^{o}$C to allow packing bed expansion before use.

Separations were performed using a Model 709 dual-piston pump (Metrohm, Herisau, Switzerland) , a 6-port Cheminert CCP0140 injection valve with a 20 μl loop (Valco Instruments, Houston, TX), a post-column reaction

system includes a PC10 pneumatic controller (Dionex), a zero dead volume poly (tetrafluoroethylene) (PTFE) tee followed by a 375 µl knitted reaction coil (Dionex, P/N 043700) and a Lambda-Max Model 481UV detector set at 510 nm (Waters, Milford, MA). The post column reaction system was operating at 30 psi for delivery of 4-(2-pyridylazo) resorcinol (PAR) post-column reagent. Data was collected at 30 Hz using a Metrohm 762 data acquisition system with IC Net 2.1 software.

### 3.2.2 Reagents and solution preparation

The cupric sulphate pentahydrate ( ＞ 98.0%, Anachemia), 4-(2-pyridylazo)-resorcinol monosodium salt mono hydrate (PAR, Janssen Chimica), and zinc chloride (98.5%, Fisher) were used as received. All reagent and eluent solutions were prepared using 18 MΩ water (Nanopure water system, Barnstead, Chicago, IL) and filtered through 0.22 µm nylon membrane filters (Millipore, Bedford, MA) prior to use. Isopropanol (Fisher Scientific, Ottawa, ON), hexane (98.5%, Sigma-Aldrich), concentrated hydrochloric acid (Fisher), concentrated nitric acid (Fisher) and glacial acetic acid (99.7%, Caledon) were used as received. The ethylenediaminetetraacetic acid disodium salt (EDTA, >99.0%, BDH) and sodium acetate anhydrous (99.0%, Caledon) were used as received. Unless otherwise noted, all chemicals were analytical reagent grade quality.

PAR solutions were prepared by adding 30 ml $1\times10^{-3}$ M ZnEDTA reagent to the mixture solution of $3\times10^{-5}$ M PAR and 0.05 M $CH_3COONH_4$, as well as sufficient concentrated $NH_3$ to adjust the pH to 11.

ZnEDTA were prepared by weighing out $10^{-4}$ mol of zinc chloride and EDTA and adding them into 100 ml 18 MΩ distilled deionized water.

The $CuSO_4$ solution in the capacity measurement was prepared by adding a certain amount of $CuSO_4 \cdot 5H_2O$ into 0.2 M acetate buffer (pH=5) to obtain 150 ppm standard solution.

All solution and water containers were soaked overnight in 1:1 $H_2O$-$HNO_3$, rinsed, and soaked overnight again in a 0.1 mM EDTA wash solution at about pH 8-9. These were periodically rinsed again with the $HNO_3$ wash solution before being finally rinsed with 18 MΩ water.

### 3.2.3 Capacity determinations

The complexing capacity of modified carbon-clad zirconia is measured by the established literature method based on $Cu^{2+}$ uptake [16].

0.1 g of modified 3 μm porous carbon clad zirconia particles were equilibrated with 10.00 ml 150 ppm copper (II) buffer for 30 minutes. After centrifuging the mixture at 4500 rpm for 10 min, the remaining copper (II) solution was removed. The particles were filtered, and washed with 1 l of distilled deionized water. The particles were then transferred into a clean vial, and rinsed again by 10 ml 1 M HCl and 0.1 M $HNO_3$. After centrifugation at 4500 rpm for 10 min, an aliquot of supernatant was taken. The Cu (II) concentration was measured by ICP-OES, and this concentration is Cu (II) elution.

## 3.3 Results and Discussion

Silica and polymer are the most commonly stationary phases used in chromatography nowadays, but both have their drawbacks. Bonded phase silica can only sustain pH with the range 2-8, and polymer suffers from low mechanical strength. The objective of this study is to explore an alternative stationary phase which is strong and stable enough for preconcentration or IC separations.

The mechanical stability of carbon clad zirconia is well documented in the literature [18]. In my work, the column bed was stable even when working at pressures as high as 6500 PSI. Likewise, my modified carbon clad zirconia showed excellent pH stability, ranging from 1-11.

A key parameter of a preconcentrator is the capacity, so we measured the capacity of my modified particles (as described in Sec. 2.2.3) using established literature methods [16]. Next, chromatographic performance of chelation phase was assessed by using separations of alkali and alkaline earth metals with both conductivity detection and post-column derivatization detection. The effects of ion strength, temperature and pH were studied. Based on these results, conclusions regarding the effectiveness of oxine on carbon clad zirconia as a preconcentrator and high performance IC media will be made.

### 3.3.1 Complexing capacity

There are three reasons to choose copper as the capacity marker in this experiment. First, copper binds with oxine to form a stable complex. Second, copper is less abundant than magnesium and calcium in the environment, and so there is less chance of contamination affecting the background. Third, copper

method was well documented by literatures [9], so our use of copper will make it more convenient to compare our results with literature.

Copper may bind either non-specifically to the carbon clad zirconia matrix or specifically through chelation by oxine. Thus during the capacity measurement experiments, it is important to wash the phase to remove any loosely adsorbed copper before measuring the concentration. Table 3.1 compares the copper capacity of oxine modified carbon clad zirconia with the other oxine bonded substrates.

**Table 3.1** The results of complexing capacity measurement

| Sample Name | Cu(II) elution μmol/g |
|---|---|
| 3 μm Porous Carbon-Clad Zirconia | |
| Blank Sample | 7 |
| Modified Sample | 57 |
| Literature Results [9] | |
| CPG-ox | 46 |
| Porasil-ox | 89 |
| silica gel-ox | 188 |

The bare CCZ particles had measureable uptake of Cu (II). Previous work from our group also observed the same effect [21]. The reason is that the porous surface of CCZ loosely adsorbs some copper solution. These interactions are not chelation and can be easily washed off, so the copper elution of bare particle is

only 7 μmol/ g. In contrast, modified particles can bind much more copper. After washing by the same amount of water, there were still 57 μmol/ g copper bound to the carbon surface. This means the interaction between copper and modified particles are much more stable than the bare particles. Therefore, the bonded oxine groups on the carbon surface are still functional and able to chelate metal ions. This is further confirmed by the studies of the effect of ionic strength (Sec 3.3.3) and pH (Sec. 3.3.4) discussed below.

Table 3.1 also shows the capacity of 8-hydroxyquinoline phases based on control pore glass (CPG), porasil, and silica gel, the most commonly used substrates in chromatography. These functionalized materials have been used in metal ions measurement [16], preconcentration [5, 7, 9], and extraction [15]. CCZ-ox shows comparable complexing capacity with the other oxine bonded substrates. It has more capacity by mass than CPG-ox, but less than that of Porasil-ox or silica gel-ox. Due to the low surface area of CPG material, CPG-ox suffers from low exchange capacity [15]. Silica gel has larger surface area, so the capacity is higher than CCZ-ox [18].

### 3.3.2 Isocratic separation of alkali and alkaline earth metal

Bare carbon clad zirconia retains metals because there is some zirconia exposed on the particle surface, and the zirconia itself is an ion exchanger. Figure 3.1 shows that by grafting oxine groups on the graphitic carbon surface, the efficiency improved and the selectivity changed. However, like CPG-oxine, the separation is still mix-mode (cation/chelation exchange). To obtain pure chelation

separation, in Sec. 3.3.3, we suppress the cation exchange mechanism by increasing the ionic strength of the eluent.

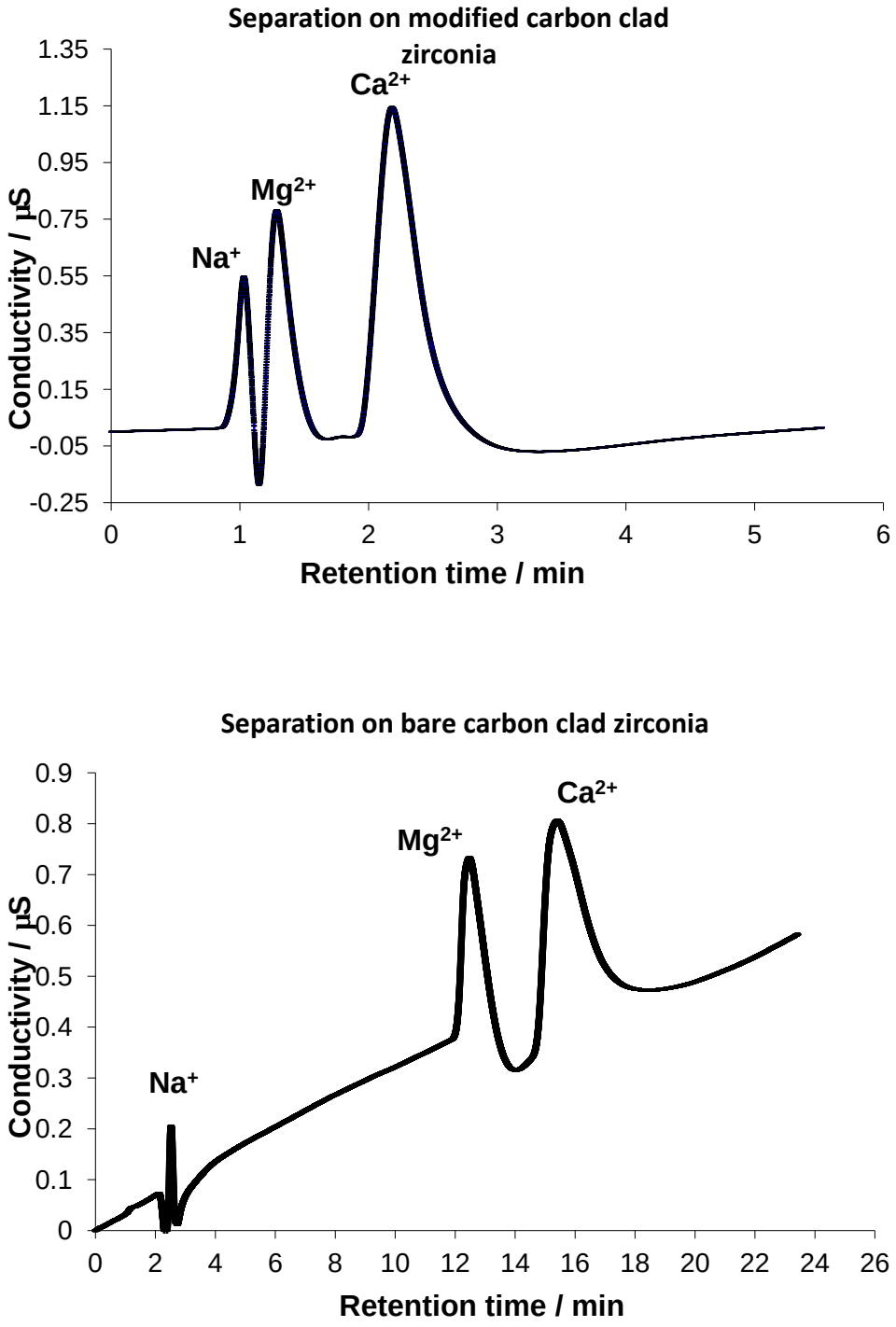


**Figure 3.1** Isocratic separation of alkaline and alkaline earth metals on modified 3 µm porous carbon-clad zirconia particles. Conditions: Flow: 0.4 ml/min; Eluent:

50 μM sulfosalicylic acid; pH=5.64; Analytes: $Na^+$ 0.07 mM, $Mg^{2+}$ 0.06 mM, $Ca^{2+}$ 0.2 mM; Injection volume: 20 μl; Conductivity detection.

The separation of alkaline earth metals has been conducted. The elution order is in agreement with literature binding constants [27]. The efficiency for magnesium is 1.4 $\times 10^3$ plates/m which is comparable to that in the literature [1, 2, 4], but still lower than the cation exchange resins (~$10^4$ plates/m). For one thing, the mixing chamber in the post-column reaction detection increases the extra-column band broadening. For another, chelation exchange is kinetically slower than cation exchange, and causes lower efficiency. The retention time RSD is < 2% over 2.0 $\times 10^3$ column volumes using sodium acetate buffer in pH 5.5. Moreover, the modified particles were stable for half year under chromatographic separations. One advantage of this material is the pH stability (demonstrated in Sec. 3.3.1). We can condition this column in high pH to optimize the separation. Moreover, due to the binding specificity of oxine ligand, in Sec 3.3.3 alkaline earth metals have been separated in the high background of non-retained cations such as sodium. In contrast, normal cation exchange columns overload in this situation [26]. Most of commercial chelation columns are using ＞10 μm divinylbenzene substrates and iminodiacetate ligands. Three micron oxine bonded carbon clad zirconia provides different selectivity and better mechanical strength. Therefore it is an ideal alternative to the current preconcentrators such as Dionex MetPac CC-1. Furthermore, to better understand the functionality of the CCZ-oxine, we did a series of experiments to investigate the effect of ionic strength, pH, and temperature.

### 3.3.3 The effects of ionic strength on alkaline earth metals

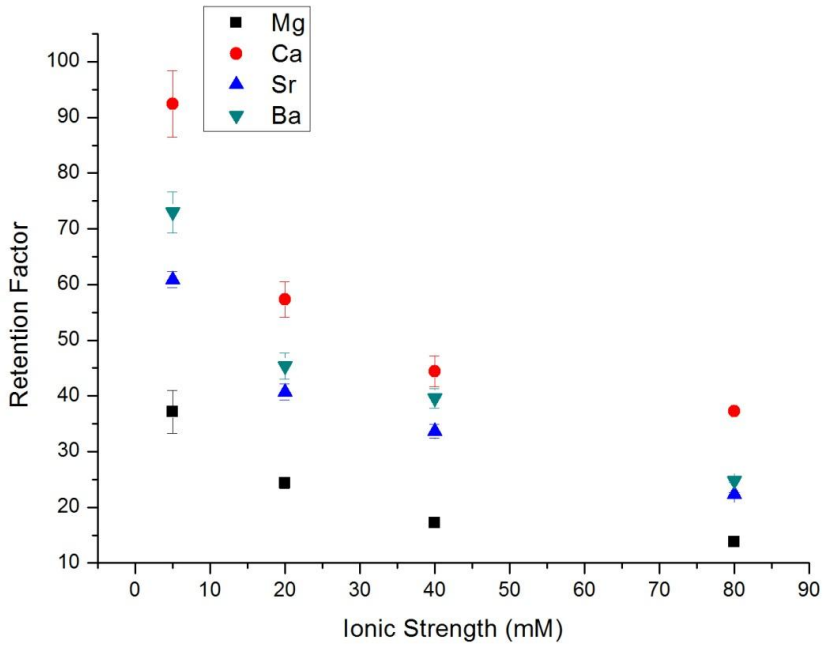


**Figure 3.2** The effects of ionic strength on alkaline earth metals. Conditions: Flow rate: 0.4 ml/min; Eluent: various concentration of sodium acetate buffer at pH=5.7; Analytes: 30 µM $Mg^{2+}$, 30 µM $Sr^{2+}$, 60 µM $Ca^{2+}$, 60 µM $Ba^{2+}$; Injection volume: 20 µl. Post-column derivatization UV-Vis detection, wavelength=510 nm; Error for each reading is the standard deviation of the triplicate data.

Figure 3.2 shows that the retention factors of alkaline earth metals decrease with increasing ionic strength. The column can still separate metals without overloading when the concentration of sodium acetate is 80 mM which is at least one thousand times higher than the analytes. In contrast, bare carbon clad zirconia loses retention in this case. The retention factor of magnesium is ~0 when using 80 mM sodium acetate buffer in the same pH. For cation exchange column,

high background cations will either overload the column or co-elute all the analytes. The tolerance of high ionic strength proves that CCZ-oxine is dominated chelation exchange mechanism. This study demonstrates its potential to be used as a preconcentrator.

### 3.3.4 pH effects on alkaline earth metals

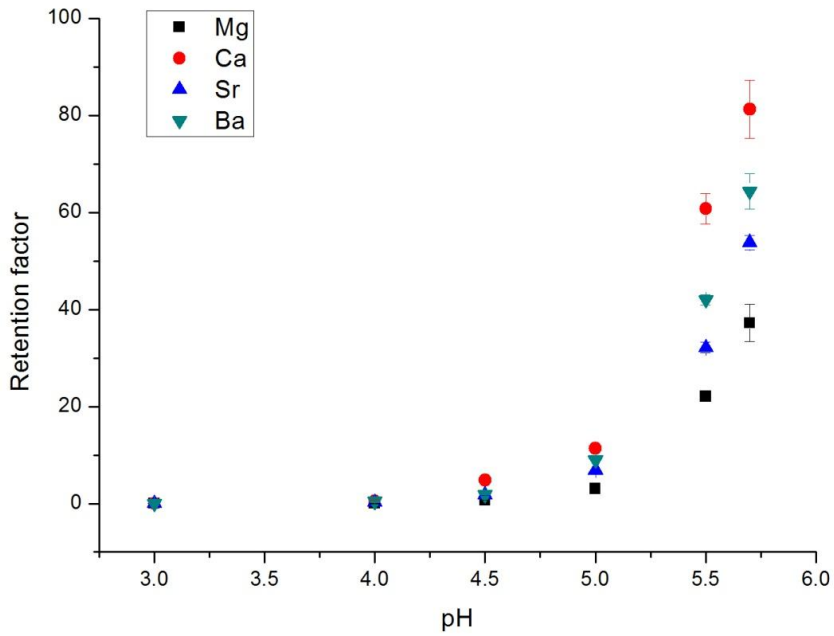


**Figure 3.3** The effect of pH on alkaline earth metal retention on CCZ-ox; Conditions: as in Figure 3.2; Error for each reading is the standard deviation of the triplicate data.

The $pKa_1$ of oxine in solution is 4.97 [14]. However, binding a ligand to a surface alters the pKa [16]. Therefore the effect of pH on retention of alkaline earth metals is studied. In Figure 3.3, in low pH (<4), the oxine groups do not bind metals, because all the nitrogen atoms on the quinoline ring are protonated.

Retention starts becoming significant at pH=5.0, and increases drastically up to pH=5.7. Figure 3.3 demonstrates the trend of each metal is the same, so higher pH is only studied on magnesium because the retention time of the other metals are too long.

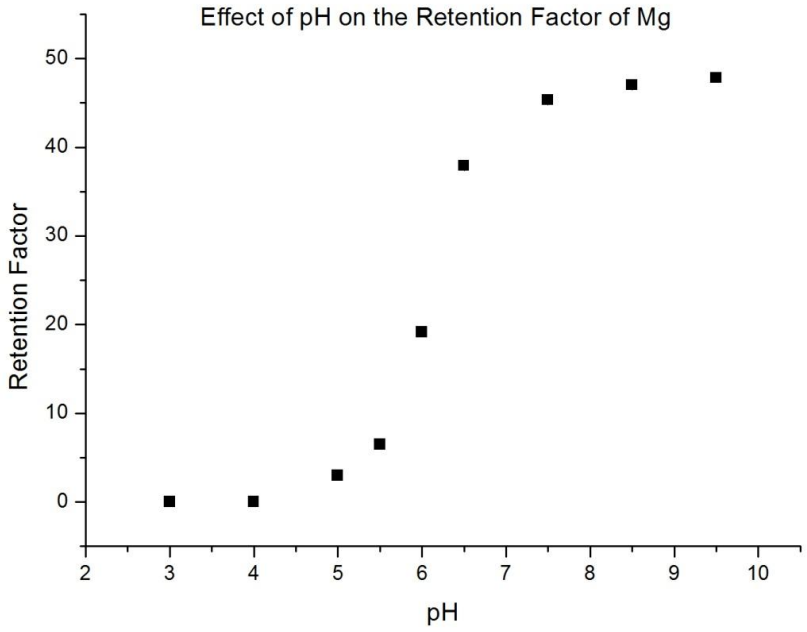


**Figure 3.4** The effect of pH on the retention factor of magnesium; Conditions: as in Figure 3.2; Error for each reading is equal to or smaller than the size of each point.

Figure 3.4 shows retention factor plateaued at pH$\geqslant$8. It means all the nitrogen atoms on the oxine groups deprotonated at pH=8. From this experiment, CCZ-ox is still working at high pH, however, bonded phase silica will dissolve when pH>8. The pH stability makes CCZ-oxine an ideal alternative to the silica-based stationary phases. Our experiments stopped at pH 9.5, because the $pKa_2$ of

the oxine is 9.97 [14]. Further increasing the pH will cause the hydroxyl groups on oxine deprotonated and introduce cation exchange site on the surface.

### 3.3.5 van't Hoff curves and temperature effects

Previous studies have shown that temperature has a very different effect upon the retention of metal ions depending upon the exact mode of retention. Fortier and Frits investigated temperature effects on a strong cation-exchange resin and showed retention times for divalent metal ions decreased with increasing temperature [22]. Hatsis and Lucy observed the same trends using a carboxylate/phosphonate stationary phase with a methanesulphonic acid eluent [23]. However, the reverse trend has been observed in chelation-exchange by Brett Paull's group [24, 25], and they attributed this phenomenon to the separation mechanism. Therefore endothermic behaviour (increasing retention with increasing temperature) generally indicates chelation as the dominant retention mechanism.

The van't Hoff equation relates equilibrium constants such as the retention factor with the reciprocal of temperature.

$$\ln k' = -\frac{\Delta H^{\circ}}{RT} + \frac{\Delta S^{\circ}}{R} + \ln\varphi \qquad 3\text{-}1$$

In pure ion exchange interactions it is reasonable to assume $\Delta S$ to be a constant [23], and so the above equation can be simplified as follows:

$$\ln k' = -\frac{\Delta H^{\circ}}{RT} + \text{constant} \qquad 3\text{-}2$$

where k' is the retention factor, T is the column temperature, R is the gas constant, $\Delta H^{\circ}$ is the enthalpy of the sorption process, $\Delta S^{\circ}$ is the change in entropy and $\varphi$ is the phase ratio.

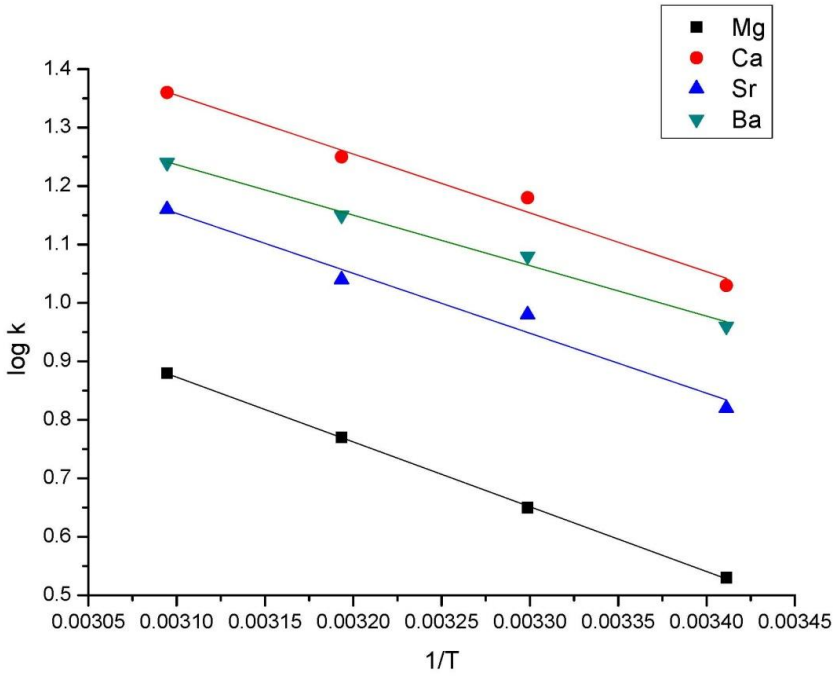


**Figure 3.5** The effect of temperature on alkaline earth metal retention on CCZ-ox (van't Hoff Curve); Conditions: as in Figure 3.3; Error for each reading is equal to or smaller than the size of each point.

Figure 3.5 shows the van't Hoff plot for the alkaline earth metals on CCZ-ox. Firstly, retention increases with increasing temperature, indicating that the alkaline earth metals are being retained via chelation. Second, the plots are linear (as shown in Table 3.2, $R^2$>0.96), as predicted by equation 3-2. Therefore our observations are corresponding with theory and the other literatures.

In Table 3.2, our calculated ΔH values are comparable with those reported by Neterenko et al. [28], Paull et al. [29], Barron et al. [30]. Fortier and Fritz found for pure ion exchange values of ΔH to be small, being less than 3 kJ • $mol^{-1}$ for divalent metal ions on a cation exchange resin [22]. In Table 3.2, all the observed ΔH values are at least two times larger than 3 kJ • $mol^{-1}$. Relatively high ΔH values confirmed chelation ion-exchange kinetics is dominant in the system [30].

**Table 3.2** Thermodynamic parameters from van't Hoff plots

| | Slope | ΔH/ kJ • $mol^{-1}$ | $R^2$ |
|---|---|---|---|
| Mg | $-1.108 \times 10^3$ | 9.212 | 0.9997 |
| Ca | $-1.006 \times 10^3$ | 8.364 | 0.9769 |
| Sr | $-1.025 \times 10^3$ | 8.522 | 0.9641 |
| Ba | $-0.863 \times 10^3$ | 7.175 | 0.9884 |

## 3.4 Conclusions

The modified carbon clad zirconia has been packed into a column and chromatographic separations have been run. Both conductivity detection and post-column derivatization detection were utilized to monitor the metal ions. Performance of bare carbon clad zirconia was investigated and compared with modified particles. CCZ-oxine showed improved peak shape and efficiency, and retention dominated by a chelation mechanism. The effects of ionic strength, pH,

and temperature have been studied. The work in this chapter further proved oxine groups have been grafted onto the carbon surface by diazonium chemistry and the modified particles are able to separate metal ions via chelation mechanism. The 3 μm CCZ-oxine also shows better pH stability than silica-based substrates, which means CCZ-oxine can be used for preconcentrating alkaline earth metals in the condition of extreme pH.

However, since the graphitic carbon is conductive, the new chelation functionalized material opens a door to the Electrochemically Modulated Ion Chromatography (EMIC). EMIC means we can control the retention time of analytes by varying the potential that applies on the stationary phase. This technique will allow users to retain and elute desired analytes by the same eluent, and initiate a novel type of preconcentrator. Therefore, Chapter Four will explore the electrochemical modification on oxine bonded graphitic carbon.

### 3.5 References

[1] J. R. Jezorek and H. Freiser, *Analytical Chemistry* **1979**, *51*, 366-373.

[2] F. Vernon and H. Eccles, *Analytica Chimica Acta* **1973**, *63*, 403-414.

[3] J. R. Parrish and Stevenso.R, *Analytica Chimica Acta* **1974**, *70*, 189-198.

[4] F. Vernon and K. M. Nyo, *Journal of Inorganic & Nuclear Chemistry* **1978**, *40*, 887-891.

[5] S. N. Willie, R. E. Sturgeon and S. S. Berman, *Analytica Chimica Acta* **1983**, *149*, 59-66.

[6] R. E. Sturgeon, S. S. Berman, S. N. Willie and J. A. H. Desaulniers, *Analytical Chemistry* **1981**, *53*, 2337-2340.

[7] D. K. Ryan and J. H. Weber, *Talanta* **1985**, *32*, 859-863.

[8] M. A. Marshall and H. A. Mottola, *Analytica Chimica Acta* **1984**, *158*, 369-373.

[9] M. A. Marshall and H. A. Mottola, *Analytical Chemistry* **1983**, *55*, 2089-2093.

[10] J. R. Jezorek, K. H. Faltynski, L. G. Blackburn, P. J. Henderson and H. D. Medina, *Talanta* **1985**, *32*, 763-770.

[11] C. Fulcher, M. A. Crowell, R. Bayliss, K. B. Holland and J. R. Jezorek, *Analytica Chimica Acta* **1981**, *129*, 29-47.

[12] J. M. Hill, *Journal of Chromatography* **1973**, *76*, 455-458.

[13] E. D. Moorhead and P. H. Davis, *Analytical Chemistry* **1974**, *46*, 1879-1880.

[14] P. Y. T. Chow and F. F. Cantwell, *Analytical Chemistry* **1988**, *60*, 1569-1573.

[15] K. F. Sugawara, H. H. Weetall and G. D. Schucker, *Analytical Chemistry* **1974**, *46*, 489-492.

[16] A. K. Kolstad, P. Y. T. Chow and F. F. Cantwell, *Analytical Chemistry* **1988**, *60*, 1565-1569.

[17] G. Persaud and F. F. Cantwell, *Analytical Chemistry* **1992**, *64*, 89-94.

[18] T. P. Weber, P. T. Jackson and P. W. Carr, *Analytical Chemistry* **1995**, *67*, 3042-3050.

[19] S. D. Chambers, C. A. Pohl and C. A. Lucy, *Journal of Chromatography A* **2011**, *1218*, 263-269.

[20] S. D. Chambers and C. A. Lucy, *Journal of Chromatography A* **2007**, *1176*, 178-184.

[21] S. D. Chambers, M. T. McDermott and C. A. Lucy, *Analyst* **2009**, *134*, 2273-2280.

[22] N. E. Fortier and J. S. Fritz, *Talanta* **1987**, *34*, 415-418.

[23] P. Hatsis and C. A. Lucy, *Analyst* **2001**, *126*, 2113-2118.

[24] W. Bashir and B. Paull, *Journal of Chromatography A* **2002**, *942*, 73-82.

[25] W. Bashir, E. Tyrrell, O. Feeney and B. Paull, *Journal of Chromatography A* **2002**, *964*, 113-122.

[26] P. Jones, P. Nesterenko, *Journal of Chromatography A*, **1997**, 789, 413–435.

[27] Stability Constants of Metal Ion Complexes Part A. Inorganic Ligands, Pergamon Press, Oxford, **1982**

[28] A. Elefterov, M. Kolpachnikova, P. Nesterenko, and O. Shpigun, *Journal of Chromatography A* **1997**, *769*, 179-188.

[29] B.Paull, W. Bashir, *Analyst* **2003**, *128*, 335-344.

[30] L. Barron, M. O'Toole, D. Diamond, P. Nesterenko, and B. Paull, *Journal of Chromatography A*, **2008**, 1213, 31–36

# CHAPTER FOUR: Electrochemically Modulated Preconcentrator

## 4.1 Introduction

Electrochemically modulated liquid chromatography (EMLC) is a technique that combines both electrochemistry and chromatography. Its mechanism is to alter the retention of analytes by changing the potential ($E_{app}$) applied to a conductive stationary phase such as porous graphitic carbon (PGC) [1]. In EMLC, the packed particles have two functions: to serve as stationary phase of a chromatographic system and to act as the working electrode of an electrochemical cell.

The history of EMLC can be traced back to the 1960s. In 1964, Blaedel and Strohl first introduced the concept of a union of liquid chromatography and electrochemistry for removal of the heavy metal ions from water [2]. However, this and subsequent studies suffered from poor chromatographic efficiency (10-200 plates/m) [3]. Among the obstacles, one of the most challenging points is to adjust the ratio of column dead volume to the surface area of the stationary phase. Chromatographically, this ratio must be small to obtain high capacity and high efficiency. Electrochemically, this ratio must be large to minimize complications due to high solution resistance.

In the 1990s, Marc Porter and co-workers redesigned the structure of the column and utilized PGC and glassy carbon (GC) as the stationary phase [4]. Since then, EMLC has been utilized for applications such as: separating aromatic sulfonates [4], corticosteroids [5], and enantiomers [6]; interfacing with mass

spectrometry to achieve complex sample determination under isocratic eluent conditions [5, 7]; and electro-synthesis of a bonded phase on the carbon support [8]. Most recently, researchers have begun to explore broader applications such as liquid-liquid extraction of ions [9], and separation of inorganic anions [10] and triazines [11]. However, to the best of our knowledge, no one has reported electrochemically modulated cation separations or preconcentration.

Traditional preconcentration columns possess chelating functional groups that bind specific metal ions in a complex matrix. To elute the retained ions, the column must be flushed with acidic solution to protonate the ligand. The need of this elution step slows the overall process and requires expensive instrumentation to perform gradient elution. In an electrochemically modulated preconcentrator (EMP) the capacity of the preconcentrator is dynamically controlled by adjusting the potential applied ($E_{app}$) to the PGC concentrator bed. It is envisaged that development of EMP will save the analysis time and simplify the equipment.

This chapter explores the materials that can be used for electrochemically modulated preconcentrator. Chelating glassy carbon (GC) particles were synthesized by *in-situ* generation and thermal deposition of diazonium ions, as was discussed in Chapter 2. Next, a specifically designed electrochemical cell was constructed to enable application of a potential to the packed bed of modified GC particles. The effects of various experimental conditions were studied, and the chelation capacity of the modified material was studied under applied voltage conditions.

## 4.2 Experimental

### 4.2.1 Reagents and chemicals

All reagent and eluent solutions were prepared using 18 MΩ nanopure water (Nanopure water system, Barnstead, Chicago, IL) and filtered through 0.22 μm nylon membrane filters (Millipore, Bedford, MA) prior to use. Tetrahydrofuran (HPLC grade, EMD Chemicals, Gibbstown, NJ, USA), anhydrous ethanol, methanol, (Fisher Scientific, Ottawa, ON), acetone (HPLC grade, EMD) were used as received. Calcium chloride dihydrate (99%, Sigma), sodium acetate anhydrous (99%, CALEDON, Georgetown, ON), 30% hydrogen peroxide, concentrated sulphuric acid (Fisher) and concentrated hydrochloric acid (≥37%, for trace analysis, Fluka Analytical, Oakville, ON) were used as received. The glassy carbon (99.95%, Sigma-Aldrich) is 2-12 μm spherical powder and is kept in a desiccator after received. Unless otherwise noted, all chemicals were analytical reagent grade quality.

Measurements of pH were made with glass and calomel electrodes using a Corning model 445 pH meter (Fisher Scientific Co.).

### 4.2.2 Oxine Modified Glassy Carbon Synthesis

A new clean glass vial with a new screw cap top was used for every reaction. Glass vials (28×57 mm, Fisher Scientific, Ottawa, ON) used to collect calcium solutions were cleaned with piranha (1 fold 30% $H_2O_2$, 3 folds sulfuric acid), rinsed with an abundance of filtered 18.0 MΩ nanopure water, dried in a

moisture free oven ($>140^{o}$C), and allowed to cool down to room temperature before use. The synthesis of 2 g glassy carbon particles involved 200 ml round-bottle flasks, water condenser, and separatory funnel. They are all cleaned by copious amounts of water, ethanol and methanol, dried in a moisture free oven, and allowed to cool down to the room temperature before use.

As discussed in Sec. 2.2.3, modified glassy carbon was synthesized under the optimized conditions using *in situ* generated diazonium ions and thermally grafted onto the particle surface. Briefly, 4.66 g of 5-amino-8-hydroxyquinoline was transferred into a clean round bottle. The solid was dissolved into 50 ml 36% ethanol solution and sonicated for 10 minutes. The round bottle was placed in an oil bath and allowed thermally equilibrates at 80 ℃. 2 g of glassy carbon particles were introduced into the solution along with a magnetic stir bar. 15.75 ml 1.27 mol/l $NaNO_2$ solution was added dropwise into the round bottle by a separatory funnel, with the resultant release of $N_2$ bubbles. The round bottle was connected to a water condenser, and the mixture was allowed to react for 15 minutes while stirring. The reaction was stopped by placing the round bottle into an ice-bath. The particles were collected on a 0.22 μm nylon membrane filter, and washed with water, ethanol, tetrahydrofuran, acetone, methanol, and water/methanol blends until the filtrate becomes colorless. During this wash step, the membrane filter was changed if it became stained. XPS results shows 7% nitrogen coverage on the surface.

### 4.2.3 Apparatus

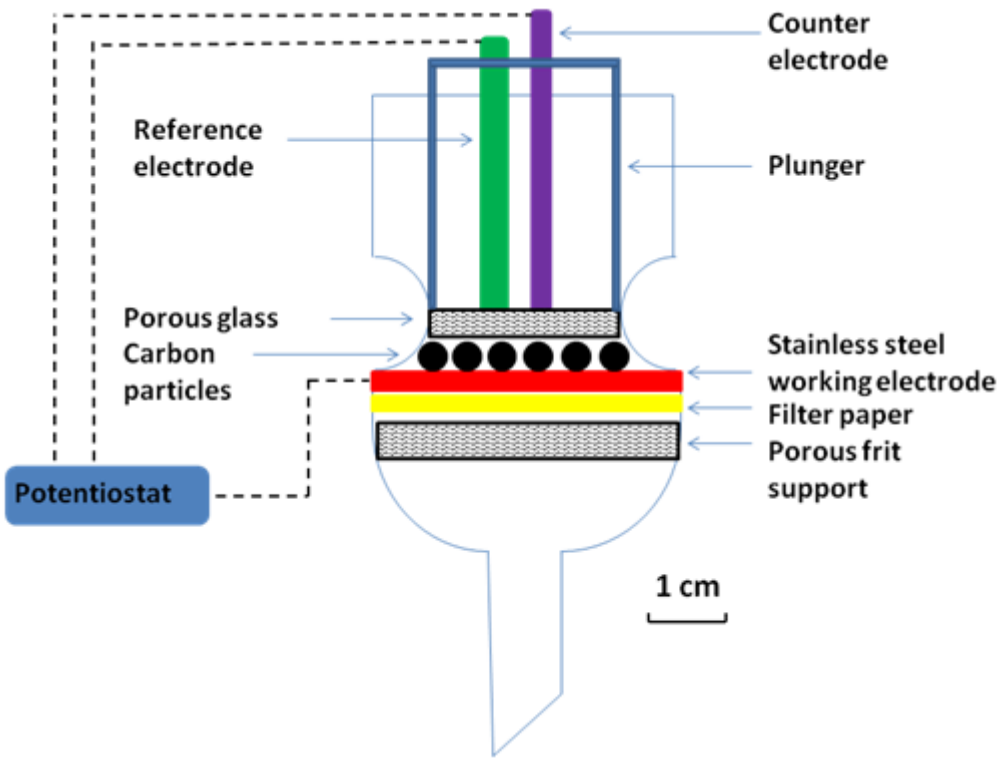


**Figure 4.1** Schematic illustration of the electrochemical cell designed in collaboration with Md. Farooq Wahab.

The electrochemical cell was constructed by the Department of Chemistry Glass Shop and was based upon the general design of a Millipore filtration device. The cell consisted of three glass components. The bottom of the cell was 5.5 cm diameter filtration outlet with a 4.0 cm porous glass support (type D porous glass, pore size: 10-20 μm). The upper component was a 4.0 cm I.D. cylinder with a flanged bottom. The upper and lower components of the cell are held together by a blue Millipore clamp. A 4.0 cm diameter cylinder with a porous glass frit (4.0 cm diameter, type D porous glass frit, pore size: 10-20 μm) could slide as a plunger within the top portion of the cell to compress the GC particles into a bed.

To assemble the electrochemical cell, a 0.22 μm nylon filter (47 mm diameter, MAGNA, Middlesex, MA, USA) was placed onto a mesh support (type D porous glass frit, pore size: 10-20 μm) of the bottom glass component. A stainless steel mesh working electrode (grade 316 stainless steel, 5.5 cm diameter, 35.56 nm thick, 30.5 % open area, 43.18 nm square size) was on top of the nylon filter, and then the upper and lower components of the cell were clamped together. A slurry of 2 g of GC particles in 50 ml water was placed in the cell and vacuum was applied to suck down the solution. Next the plunger with the porous glass bottom was used to compress the GC particle bed so that the particles were in intimate contact with the stainless steel mesh electrode. The Pt rod counter electrode and a Ag/AgCl reference electrode (Accumet, Fisher Scientific, Ottawa, ON) were placed inside the plunger. Electrical connection was also made to the stainless steel mesh electrode. Potentials were applied to the packed GC bed using a Bipotentiostat (Model AFCBP1, Pine Instrument Company, Grove City, PA, US) with the software of AfterMath (Version 1.2.4532, Pine Research Instrumentation, Inc).

### 4.2.4 Electrochemically Modulated Preconcentration Procedure

2 g of glassy carbon particles were functionalized by oxine groups using the method described in Sec. 4.2.2. The particles (2 g) were loaded into the electrochemical cell illustrated in Figure 4.1, as described in Sec. 4.2.3. The particle bed and electrodes were washed by 10 ml 0.1 M HCl followed by 10 ml DD water to avoid any metal ion contamination.

The bed was equilibrated with analyte by rinsing the GC particle bed with 10 ml of 50 ppm $CaCl_2$ solution in 0.2 M sodium acetate (pH 5.5) over 5-30 minutes. A potential was applied ($E_{app}$, vs Ag/AgCl) during this loading procedure. The effluent was collected and analyzed by ICP-OES, as described below. 50 ppm minus the concentration we measured here is the total calcium uptake by the carbon surface including the non-specific interaction and chelation binding.

Next while maintaining the potential, the particle bed was rinsed and washed with 10 ml 0.2 M sodium acetate (pH=5.5) over 10 min. This wash solution was collected and analyzed by ICP-OES. This concentration shows how much non-specific interaction binding on the carbon surface.

Finally, the voltage was turned off, and column was eluted with 10 ml of 0.1 M HCl. The effluent was analyzed by ICP-OES. The concentration here shows the amount of calcium that strongly bound to the oxine groups on the carbon surface (i.e., chelated).

Calcium concentrations were measured at 317.9 nm and 315.8 nm using a Model Optima 2100DV inductively coupled plasma optical emission spectrophotometer (Perkin-Elmer). A calibration curve was prepared from electrolytic calcium for each measurement. Four standard concentrations were used: 0.1 ppm, 1 ppm, 10 ppm, 100 ppm.

### 4.3 Results and Discussion

As shown in Figure 3.3, the binding of alkaline earth ions is a function of the charge state of the oxine bound to the carbon surface. The hypothesis

underlying our electrochemically modulated preconcentrator is that the charge state of oxine can be altered by application of a voltage to the GC onto which the oxine is covalently bound. That is, the voltage should affect the induction into or electron withdrawal of the oxine groups, and hence controls the charge state of the nitrogen. Therefore, the $E_{app}$ acts as an adjustable Hammett parameter, as shown in Figure 4.2.

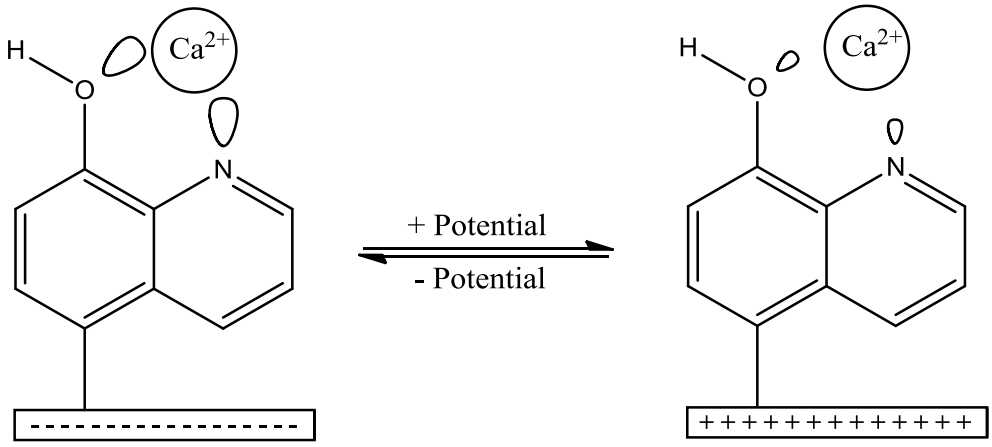


**Figure 4.2** Methodology of electrochemically modulated preconcentrator

### 4.3.1 Preliminary studies of the effects of potential, pH, and time

In Figure 3.4, magnesium retention of oxine modified carbon clad zirconia showed the drastic change at pH 5.5. Therefore initial studies of the effect of applied voltage on oxine modified GC was performed at this pH. Table 4.1 shows the binding behaviour of oxine functionalized glassy carbon for applied potentials of +1 V to -2.5 V vs. Ag/AgCl. The $E_{app}$ cannot go over ± 2.5 V due to electrolysis of water, and the voltage stability of the nylon membrane. The "total adsorption of calcium" in Table 4.1 is due to both non-specific (e.g., ion exchange)

and specific (chelation) binding. The total uptake increased significantly as applied potential was made more negative. With the potential continuing to be applied, the preconcentrator was rinsed with buffer to determine how much calcium was weakly bound, as 0.2 M $Na^+$ constitutes strong cation exchange elution conditions. Significant amounts of calcium were removed from the preconcentrator simply by rinsing with 0.2 M sodium acetate buffer: from 89% at +1.0 V down to 36 % at -2.5 V.

**Table 4.1** The effect of potential on the binding capacity of oxine modified glassy carbon. Conditions: 0.2 M sodium acetate (pH=5.5). Time: 5 min. The mean and standard deviation were obtained based on triplicate whole experiments.

| | Total Ca binding (μmoles Ca/ g of C) | Non specific adsorption (μmoles Ca/ g of C) | Chelation binding (μmoles Ca/ g of C) |
|---|---|---|---|
| 1V | 1.56 ±0.06 | 1.38 ±0.06 | 0.07 ±0.01 |
| 0V | 1.88 ±0.05 | 1.36 ±0.06 | 0.09 ±0.01 |
| -1V | 1.88 ±0.07 | 1.29 ±0.02 | 0.11 ±0.01 |
| -1.5V | 2.62 ±0. 09 | 1.21 ±0.05 | 0.21 ±0.01 |
| -2V | 2.38 ±0.04 | 1.07 ±0.09 | 0.29 ±0.01 |
| -2.5V | 2.51 ±0.02 | 0.91 ±0.02 | 0.37 ±0.01 |

Finally to determine how much calcium was strongly bound (i.e., chelated) to the modified oxine particles, the potential was turn off and the packed bed was eluting with 0.1 M HCl. From +1 to -1 V applied only small amounts of calcium are strongly bound by the oxine modified GC (Table 4.1). But when the applied voltage is greater than -1 V substantial increases in the bound calcium is observed. These results support our hypothesis that the voltage applied to the oxine modified GC can cause induction into or electron withdrawal from the oxine groups, and thereby controls the charge state of the nitrogen of oxine and consequently the conditional formation constant of metal complexes.

In Chapter Three (Figure 3.4), it was observed that pH altered magnesium retention on carbon clad zirconia possessing oxine sites. Table 4.2 shows the effect of pH on calcium binding on oxine modified GC under a constant applied potential of -1.0 V vs. Ag/AgCl. At pH 3.0 the total calcium binding and the chelation binding are low, relative to other pH behaviour. This is consistent with the effect of pH on the retention of magnesium on carbon clad zirconia (Figure 3.4). The amount of weak interaction, presumably due to ion exchange, remains essentially constant: independent of pH. In general, the effect of increasingly negative applied voltages is comparable to the effect of increasing the solution pH.

**Table 4.2** The effect of pH on the binding capacity of modified glassy carbon particles. Conditions: 0.2 M sodium acetate solution. Voltage: -1.0 V. Time: 10 min.

| | Total Ca binding (μmoles Ca/ g of C) | Non specific adsorption (μmoles Ca/ g of C) | Chelation binding (μmoles Ca/ g of C) |
|---|---|---|---|
| pH=3.0 | 1.38 ±0.07 | 1.11 ±0.03 | 0.07 ±0.01 |
| pH=3.5 | 1.52 ±0.05 | 1.11 ±0.07 | 0.09 ±0.02 |
| pH=4.0 | 1.65 ±0.05 | 1.17 ±0.07 | 0.10 ±0.01 |
| pH=4.5 | 1.55 ±0.07 | 1.23 ±0.01 | 0.13 ±0.01 |
| pH=5.0 | 1.87 ±0.04 | 1.12 ±0.03 | 0.13 ±0.02 |
| pH=5.5 | 1.95 ±0.07 | 1.20 ±0.01 | 0.17 ±0.02 |

Table 4.3 shows the effect of period over which 10 ml of 50 ppm $Ca^{2+}$ was passed through the GC bed. The loading time did not significantly affect the total $Ca^{2+}$ uptake, but the amount of non-specific interaction slowly decreases with time. After 10 minutes, strong binding becomes constant, which means chelation interactions achieve equilibrium. Compare to the effects of potential and pH, time is the weakest factor governing the binding capacity of oxine.

**Table 4.3** The effect of time on the binding capacity of modified glassy carbon particles. Conditions: 0.2M sodium acetate solution buffer in pH=5.5. Applied voltage= -1.0 V

| | Total Ca binding (μmoles Ca/ g of C) | Non specific adsorption (μmoles Ca/ g of C) | Chelation binding (μmoles Ca/ g of C) |
|---|---|---|---|
| 5 min | 1.88 ±0.06 | 1.29 ±0.02 | 0.11 ±0.01 |
| 10 min | 1.95 ±0.05 | 1.20 ±0.01 | 0.17 ±0.02 |
| 20 min | 1.99 ±0.01 | 1.23 ±0.02 | 0.16 ±0.01 |
| 30 min | 1.92 ±0.07 | 1.28 ±0.02 | 0.17 ±0.01 |

### 4.3.2 Electrical switched ion exchange

Using the optimum conditions determined by the preliminary studies in Sec 4.3.1, the strong binding of calcium by the oxine modified GC was compared to unmodified GC (Figure 4.3).

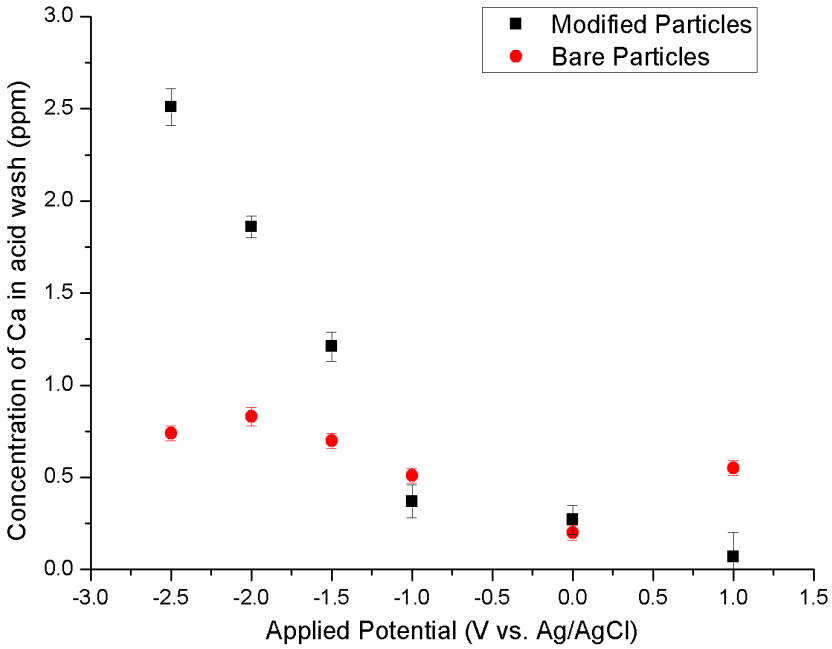


**Figure 4.3** Effect of potential on bare and modified glassy carbon particles (background deducted). Conditions: 50 ppm $Ca^{2+}$ buffered by 0.2 M $CH_3COONa$ solution in pH 5.5; time=10 min; 2 g of each carbon sample; Error is 1σ of repeating the whole experiment (n=3).

The amount of calcium strongly bound to the unmodified glassy carbon was low and essentially unaffected by the applied potential. In contrast, the oxine modified GC binds 3 times more $Ca^{2+}$ when the potential is -2.5 V. Moreover, when the $E_{app}$ is positive, the modified oxine actually binds less $Ca^{2+}$ than unmodified GC. Therefore, by applying proper voltage on the oxine functionalized graphitic carbon, we not only modulate the binding capacity of stationary phase, but turn it into an electrically modulated preconcentrator.

## 4.4 Conclusions

This chapter compares the effect of electric potential on the binding capability of bare and oxine modified glassy carbon. The effects of potential, time and pH on weak and strongly binding of calcium were observed and compared. We demonstrated that oxine functionalized glassy carbon can be used as an electrical modulated preconcentrator. This technique can be used for electrochemically modulated ion chromatography, 2-D LC, or electrically switched water softener.

## 4.5 References

[1] J. A. Harnisch and M. D. Porter, *Analyst* **2001**, *126*, 1841-1849.

[2] W. J. Blaedel and J. H. Strohl, *Analytical Chemistry* **1964**, *36*, 1245-&.

[3] H. L. Ge, P. R. Teasdale and G. G. Wallace, *Journal of Chromatography* **1991**, *544*, 305-316.

[4] E. Y. Ting and M. D. Porter, *Analytical Chemistry* **1998**, *70*, 94-99.

[5] E. Y. Ting and M. D. Porter, *Analytical Chemistry* **1997**, *69*, 675-678.

[6] M. K. Ho, S. J. Wang and M. D. Porter, *Analytical Chemistry* **1998**, *70*, 4314-4319.

[7] C. G. Enke, *Analytical Chemistry* **1997**, *69*, 4885-4893.

[8] J. A. Harnisch, D. B. Gazda, J. W. Anderegg and M. D. Porter, *Analytical Chemistry* **2001**, *73*, 3954-3959.

[9] a) A. Berduque, A. Sherburn, M. Ghita, R. A. W. Dryfe and D. W. M. Arrigan, *Analytical Chemistry* **2005**, *77*, 7310-7318; b) A. Berduque and D. W. M. Arrigan, *Analytical Chemistry* **2006**, *78*, 2717-2725.

[10] L. M. Ponton and M. D. Porter, *Journal of Chromatography A* **2004**, *1059*, 103-109.

[11] B. J. Yakes, D. W. Keller and M. D. Porter, *Journal of Chromatography A* **2010**, *1217*, 4395-4401.

# CHAPTER FIVE Summary

## 5.1 Conclusions

This thesis explored the methodology that converts graphitic carbon based stationary phases (glassy carbon and 3 μm porous carbon clad zirconia) into a chelation ion exchanger. 8-Hydroxyquinoline (oxine) had been covalently attached onto the carbon surface by *in situ* generation of diazonium ions at high temperature (80 ℃).

Chapter Two demonstrated that by applying optimum conditions (80 $^{o}$C, 15 minutes, with 2.5 mmol of 5-amino-8-hydroxyquinoline dihydrochloride), oxine groups can be covalently attached onto the carbon surface (% nitrogen is 7.44) without changing the particle shape. Importantly, it was illustrated that the reaction favoured relatively high temperature. Also, too long reaction time crushed the carbon phases while too much reactant (5-amino-8-hydroxyquinoline dihydrochloride) agglomerated the particles. Compared to the previous work from our group [1], my optimized conditions increased yield three fold. This improvement was attributed to the stability of oxine radicals, so that kinetically they had longer time to react with the carbon surface.

Chapter Three examined the chelation capacity and separation efficiency of the particles synthesized in Chapter Two. CCZ-oxine showed higher capacity than the control pore glass-oxine (CPG-oxine), but still lower than silica gel-oxine [2]. However, considering the wider pH range (1-12) and high mechanical strength of CCZ-oxine, it was a good alternative to traditional oxine based

chelators. CCZ-oxine also exhibited comparable separation efficiency ($1.4 \times 10^3$ plates/m for Mg) to the other oxine based stationary phases [3-5]. The retention time RSD is <2% over $2.0 \times 10^3$ column volumes using sodium acetate buffer in pH 5.5, which means the covalent modification is more stable than those with semi-permanent coatings such as surfactant coatings [6]. The effects of ionic strength, pH, and temperature on retention were studied. These results further demonstrated that retention on CCZ-oxine was dominated by chelation mechanism. Chapter Four utilized the synthetic routes of Chapter Two to introduce oxine onto glassy carbon (GC) particles. With a specially designed electrochemical cell, the effect of applied potential on oxine bonded GC was investigated. The effects of time, eluent pH were also studied. By applying proper conditions (0.2 M sodium acetate buffer in pH 5.5, 10 min), binding of calcium could be controlled through application of an applied potential. These results demonstrate the feasibility of Electrochemically Modulated Preconcentration.

**5.2 References**

[1] S. D. Chambers, M. T. McDermott and C. A. Lucy, *Analyst* **2009**, *134*, 2273-2280.

[2] M. A. Marshall and H. A. Mottola, *Analytical Chemistry* **1983**, *55*, 2089-2093.

[3] F. Vernon and K. M. Nyo, *Journal of Inorganic & Nuclear Chemistry* **1978**, *40*, 887-891.

[4] P. Y. T. Chow and F. F. Cantwell, *Analytical Chemistry* **1988**, *60*, 1569-1573.

[5] G. Persaud and F. F. Cantwell, *Analytical Chemistry* **1992**, *64*, 89-94.

[6] S. D. Chambers and C. A. Lucy, *Journal of Chromatography A* **2007**, *1176*, 178-184.