Metal Ion Separation Mediated by Organized Molecular Assemblies

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METAL ION SEPARATION MEDIATED BY ORGANIZED MOLECULAR ASSEMBLIES

by

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Hong Cao
METAL ION SEPARATIONS MEDIATED BY ORGANIZED MOLECULAR ASSEMBLIES

Hong Cao, M.S.
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We have investigated the separation of Co(II), Ni(II), Cu(II) and Zn(II) by the ligands 8-hydroxyquinoline-5-sulfonic acid and 7-iodo-8-hydroxy-quinoline-5-sulfonic acid in the presence of the anionic surfactant sodium dodecyl sulfite (SDS) and the cationic surfactant octyltrimethylammonium bromide (OTAB). Baseline separations have been achieved by HPLC employing an octadecylsilanized silica (ODS) column. The underlying mechanisms of separation have been determined.

The significant new results of our studies are: (1) separation is not possible without the presence of surfactants and hence organized assemblies; (2) separations can be achieved under submicellar concentrations; (3) the separations are not driven by micelles; and (4) several competing equilibria must be taken into account to understand the mechanism of separation.
TABLE OF CONTENTS

ACKNOWLEDGMENTS .................................................. ii
LIST OF TABLES ..................................................... vi
LIST OF FIGURES ................................................... vii

CHAPTER

I. INTRODUCTION .................................................. 1
II. EXPERIMENTAL ................................................ 8
   Chemicals ....................................................... 8
   HPLC Column Packing ....................................... 9
   Apparatus ..................................................... 10
      HPLC System ............................................... 10
      UV-visible Spectrophotometer ........................... 11
      pH Meter .................................................. 11
      Transmission Electron Microscope (TEM) ............ 11
      Fluorescence Microscope ............................... 12
   Preparation of Sample, Mobile Phase and Indicator .... 12
   Procedure .................................................... 14

III. RESULTS AND DISCUSSION .................................. 16
   Selection of Detection Wavelength ....................... 16
   Separation in the Absence of Surfactants ............ 20
## Table of Contents

### CHAPTER

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS Systems</td>
<td>23</td>
</tr>
<tr>
<td>The SDS System in the Absence of Ligand</td>
<td>23</td>
</tr>
<tr>
<td>SDS and HQS System</td>
<td>24</td>
</tr>
<tr>
<td>SDS and HIQS System</td>
<td>50</td>
</tr>
<tr>
<td>Conclusions</td>
<td>60</td>
</tr>
<tr>
<td>Separation Mediated by OTAB</td>
<td>62</td>
</tr>
<tr>
<td>The OTAB System in the Absence of Ligand</td>
<td>63</td>
</tr>
<tr>
<td>Dependence of Separation on pH</td>
<td>65</td>
</tr>
<tr>
<td>Ligand Dependence</td>
<td>67</td>
</tr>
<tr>
<td>OTAB Dependence</td>
<td>69</td>
</tr>
<tr>
<td>The Stability of OTAB Adsorption</td>
<td>73</td>
</tr>
<tr>
<td>The Effect of Anion on OTAB Mediated Separation</td>
<td>73</td>
</tr>
<tr>
<td>Mechanism of OTAB Mediated Separation</td>
<td>75</td>
</tr>
<tr>
<td>Conclusions</td>
<td>84</td>
</tr>
<tr>
<td>Imaging Experiments</td>
<td>86</td>
</tr>
<tr>
<td>Transmission Electron Microscope</td>
<td>86</td>
</tr>
<tr>
<td>Fluorescence Microscopy Studies</td>
<td>94</td>
</tr>
</tbody>
</table>

### IV. CONCLUSIONS AND SUMMARY

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conclusions</td>
<td>101</td>
</tr>
<tr>
<td>Summary</td>
<td>104</td>
</tr>
</tbody>
</table>
Table of Contents-Continued

CHAPTER

V. FUTURE DIRECTIONS ................................................. 108

ABBREVIATIONS AND SYMBOLS ..................................... 109

APPENDICES

A. The Species Fraction of HQS, HIQS and Metal Ligand Complexes ........................................ 110

B. The Derivation of Equation 5 ................................. 124

C. The Derivation of Equation 20 ............................... 128

D. D' vs. [HL']/[H+] in OTAB + HQS System .............. 134

REFERENCES ............................................................. 139

BIBLIOGRAPHY ............................................................ 142
LIST OT TABLES

1. Stability Constants for Metal Ligand and PAR Complexes ................................. 20

2. Comparison of Machersy-Nagel Nucleosil® and Waters Spherisorb® ODS .................. 33

3. Equilibrium Constants for SDS + HQS System from Regression Analysis of Equation 5 .......... 42

4. Equilibrium Constants for SDS + HQS System from Regression Analysis of Equations 5, 8 and 9 .... 43

5. Equilibrium Constants for SDS + HQS System from Regression Analysis of Equations 10, 11 and 12 .... 45

6. Equilibrium Constants for SDS + HIQS System from Regression Analysis of Equations 5 and 9 ........ 55

7. Equilibrium Constants for SDS + HIQS System from Regression Analysis of Equations 9 and 13 ........ 56

8. Equilibrium Constants for SDS + HIQS System from Regression Analysis of Equations 12 and 14 ........ 59

9. Average Equilibrium Constant $K_2$ for OTAB + HQS System ........................................ 80
LIST OF FIGURES

1. The Structure of PAR ............................................. 16
2. UV-visible Spectra of PAR, HQS and HIQS .............. 18
3. Absorbance Spectra for PAR-Metal Complexes ........... 18
4. The UV-visible Spectra of Metal HQS Complexes ...... 19
5. The UV-visible Spectra of Metal HIQS Complexes ... 19
6. The Structures of 8-Hydroxyquinoline-5-sulfonic acid (HQS, $H_2L$) ........................................... 21
7. Chromatogram of Ni(II), Co(II) and Zn(II) in the Absence of Surfactants ......................... 22
8. The Structure of Sodium Dodecyl Sulfate (SDS) .... 23
9. Separation of Ni (II), Co (II) and Zn (II) with SDS and no Ligand ............................. 25
10. Separation of Cu(II), Ni(II), Co(II) and Zn(II) Chromatogram ........................................... 26
11. Separation of Ni(II), Co(II) and Zn(II) as a Function of pH ............................................. 28
12. Dependences of Capacity Factors of Ni(II), Co(II) and Zn(II) on Mobile Phase pH .............. 29
13. Separation of Ni(II), Co(II) and Zn(II) at Different HQS Concentrations ....................... 30
14. Dependences of Capacity Factor on HQS Concentration ............................................. 31
15. SDS Dependences ............................................... 34
List of Figures-Continued

16. Separation Chromatograms of Ni(II), Co(II) and Zn(II) .......................... 36

17. Influence of the Nature of Anion on SDS Mediated Separation ................. 37

18. Proposed Mechanism for SDS Mediated Metal Ion Separation on ODS Stationary Phase ....... 39

19. 1/D vs. [HL−]/[H+] for Ni(II) in SDS + HQS ........... 47

20. 1/D vs. [HL−]/[H+] for Co(II) in SDS + HQS ........... 48

21. 1/D vs. [HL−]/[H+] for Zn(II) in SDS + HQS ........... 49

22. The Acid Dissociation Equilibria of 8-Hydroxy-7-iodoquinoline-5-sulfonic acid .......... 50

23. Separation of Ni(II), Co(II) and Zn(II) with SDS + HQS System at Different pH .......... 51

24. Plot of D vs. pH for the Separation of Co(II) and Zn(II) with SDS and HQS .......... 52

25. Separations of Ni(II), Co(II) and Zn(II) with HQS + SDS at Different HQS Concentration .... 53

26. D vs. Concentration of Ligand HQS for the Separation of Co(II) and Zn(II) with SDS + HQS ........................ 53

27. 1/D vs. [HL−]/[H+] for Co(II) in SDS + HQS ........... 57

28. 1/D vs. [HL−]/[H+] for Zn(II) in SDS + HQS ........... 58

29. The Structure of Octyltrimethylammonium Bromide (OTAB) ......................... 62

30. The Chromatogram of Ni(II) and Zn(II) in OTAB in the Absence of HQS .............. 64
List of Figures-Continued

31. The Chromatograms of Zn(II) and Ni(II) at Different pH Values with the OTAB and HQS System .................... 66

32. Dependence of Capacity Factor D of Ni(II) and Zn(II) on Mobile Phase pH in the OTAB + HQS System ....................... 67

33. The Chromatograms of Zn(II) and Ni(II) Separation at Different HQS Concentrations with the OTAB and HQS System .................. 68

34. D vs. Concentrations of Ligand for the Separation of Ni(II) and Zn(II) OTAB+HQS .................. 69

35. The Chromatograms of Zn(II) and Ni(II) Separation with and without OTAB .................. 71

36. Dependence of D on the Concentration of OTAB .................. 72

37. The Chromatograms of Zn(II) and Ni(II) Separation in the Absence of OTAB .................. 74

38. Separations in the Presence of Different Sodium Salts .................. 75

39. Proposed Stepwise Mechanism for OTAB Metal Ion Separation on ODS Stationary Phase .................. 76

40. Proposed Mechanism for OTAB Metal Ion Separation on ODS Stationary Phase .................. 77

41. D’ VS. [HL⁻]/[H⁺] for 1 × 10⁻⁴ M OTAB .................. 81

42. D’ vs. [HL⁻]²/[H⁺]² for 1 × 10⁻⁴ M OTAB .................. 82

43. The TEM Image of Untreated ODS .................. 87

44. The TEM Image of ODS Equilibrated with SDS .................. 88
List of Figures-Continued

45. The TEM Image of ODS Equilibrated with SDS and HQS ......................... 89
46. The TEM Image of ODS Equilibrated with SDS, HQS and Zn(II) ...................... 90
47. The TEM Image of ODS Equilibrated with OTAB ................ 91
48. The TEM Image of ODS Equilibrated with OTAB and HQS ......................... 92
49. The TEM Image of ODS Equilibrated with OTAB, HQS and Zn(II) .................... 93
50. The Fluorescence Microscopy Image of ODS Equilibrated with SDS + HQS .......... 96
51. The Fluorescence Microscopy Image of ODS Equilibrated with SDS + HQS + Zn(II) 97
52. The Fluorescence Microscopy Image of ODS Equilibrated with OTAB and HQS .......... 98
53. The Fluorescence Microscope Image of ODS Equilibrated with OTAB, HQS and Zn(II) 99
54. The Fluorescence Microscopy Image of ODS Treated with OTAB, HQS and Cu(II) .... 100
55. The Fraction of Ligand Species of HQS vs. pH ..... 117
56. The Fraction of Co(II) Species vs. pH for Complexation with HQS .................. 118
57. The Fraction of Ni(II) Species vs. pH for the Complexation with HQS ............... 119
58. The Fraction of Zn(II) Species vs. pH for the Complexation with HQS ............... 120
59. The Fraction of Species of HIQS vs. pH .............. 121
List of Figures-Continued

60. The Fraction of Co(II) Species vs. pH for
the Complexation with HIQS ............................... 122

61. The Fraction of Zn(II) Species vs. pH for
the Complexation with HIQS ............................... 123

62. D’ vs. [HL\textsuperscript{-}]/[H\textsuperscript{+}] in 2 \times 10^{-4} M
OTAB + HQS System ......................................... 135

63. D’ vs. [HL\textsuperscript{-}]/[H\textsuperscript{+}] in 3 \times 10^{-4} M
OTAB + HQS System ......................................... 136

64. D’ vs. [HL\textsuperscript{-}]/[H\textsuperscript{+}] in 4 \times 10^{-4} M
OTAB + HQS System ......................................... 137

65. D’ vs. [HL\textsuperscript{-}]/[H\textsuperscript{+}] in 8 \times 10^{-4} M
OTAB + HQS System ......................................... 138

66. D’ vs. [HL\textsuperscript{-}]/[H\textsuperscript{+}] in 1.2 \times 10^{-3} M
OTAB + HQS System ......................................... 138
CHAPTER I

INTRODUCTION

Transition metal ion separation has been of particular interest due to its potential application in environmental remediation and restoration, the recovery of valuable metals from their natural sources, and spent ores and catalysts. Transition metal ion separation is difficult and complicated, especially for various cations having the identical charge and similar hydration energies. The complexity of matrices that metal ions are encountered in such as in brines, pickles, and sludges, and other factors such as high temperature, acidity, and the presence of target metal ions in low concentrations often in the presence of high concentrations of other metal ions demand metal ion selectivities [1].

Traditional methods of metal ion separation employ ion-exchange or chelation with suitable monodentate and polydentate complexing agents (ligands) and macrocyclic systems. Separation by chelation and ion-exchange follow two main approaches, namely solid-liquid and liquid-
liquid systems. A combination of ion-exchange and chelation is also adopted such as in ion-exchange columns with complexing eluents to accelerate the elution of metal ions and/or to modify the selectivity [1-6]. Ion-exchange method is limited because of its poor selectivities. Although chelation provides higher selectivities than does ion-exchange, solid-liquid techniques are limited in capacity and liquid-liquid techniques are not environmentally friendly. A major limitation is that in both approaches ion-exchange and chelating sites are present in random macroenvironments. Higher selectivities than the existing approaches and environmentally friendly methods are critically needed for metal ion separations from complex matrices.

Surfactant-based separation processes could have less environmental impact, require less energy and be more economical than traditional separation methods [7]. In a surfactant solution, when the surfactant reaches a certain concentration, surfactant molecules would self-assemble to form a micelle. This concentration is called critical micelle concentration (CMC). The solution containing micelles is capable of dissolving compounds that are intrinsically insoluble or sparingly soluble in aque-
ous or nonaqueous media (normal and reverse micelles respectively) [8]. Due to the so-called micellar solubilization, surfactants were introduced into separation techniques. Micellar liquid chromatography (MLC) is the type of chromatography that uses surfactants in aqueous solutions at a concentration well above their CMC, so most of the surfactants are present as micelles. MLC uses no or low concentrations of organic solvents. It is one of the environmentally friendly methods for separation technology [9].

Surfactant mediated separations were initially developed for the separation of organic compounds. The application of MLC to metal ion separation is not common in comparison with its application to organic analytes. Ligand-modified micellar-enhanced ultrafiltration had shown that it could provide selectivity in the removal of cations from water [7].

The central hypotheses of this research are that: (1) Chelating ligands in organized nano- and micro-environments such as those in micelles, vesicles and dendrimers can provide high metal ion selectivities; (2) even higher selectivities could be achieved if such ligands are placed in predetermined locations in these
environments by chemical derivatization such as in chelating micelles, vesicles and dendrimers. The first hypothesis was investigated in the studies described in this thesis.

The selectivities could be achieved through interplay of factors such as the number of coordination sites of the metal ion, steric constraints, the stability constants of the metal complexes and kinetics of complex formation and dissociation. Successful demonstration of this hypothesis would provide high selectivities in metal ion separation in an environmentally friendly manner.

Surfactant based separations of metal ions have been mainly carried out in the presence of an anionic surfactant such as sodium dodecyl sulfate (SDS) at or above its CMC [10-14]. Separations under submicellar concentrations of anionic and cationic surfactants have rarely been investigated. Very little work has been performed with cationic surfactants such as octyltrimethylammonium bromide (OTAB) or with vesicles formed from a mixture of anionic and cationic surfactants. Although the separation by MLC has often been modelled as ion-exchange, mechanisms of surfactant based separations have not been fully elucidated [15-17]. It has been discussed whether or not
micellar mobile phase enhances the separation selectivity [18]. These studies have involved the use of polycarboxylic acid as ligand in the presence of sodium dodecyl sulfate (SDS) at or above its CMC in the mobile phase and an octadecylsilanized silica (ODS) as the stationary phase in HPLC [11,13]. A simple ion-exchange model was employed to rationalize the experimental results [10]. This model did not distinguish between mobile phase and stationary phase equilibria and did not rationalize the observed dependences of capacity factor, separation efficiencies and selectivities on pH, ligand concentration and ionic strength.

The objectives of this research are:

1. Understand the role of the surfactant assemblies in the separation. Surfactants at submicellar and micellar concentrations would be examined;

2. Employ HPLC with ODS as the stationary phase to discern the role of surfactant assemblies in metal ion separation;

3. Investigate the influence of the nature of ligands on metal ion separation;

4. Determine the mechanism of separation of the metal ions in the presence of surfactant assemblies by
varying ligand and surfactant concentration and pH of the solution;

5. Use the transition metal ion family as the model system for the fundamental understanding of metal ion separation mediated by organized molecular assemblies.

The metal ions studied were Co(II), Ni(II), Cu(II), and Zn(II). The surfactants examined were an anionic surfactant, SDS, and a cationic surfactant octyltrimethylammonium bromide (OTAB). The 8-hydroxyquinoline and its derivatives are well studied extractants for metal ions and are useful ligand systems for our research objectives. 8-hydroxyquinoline-5-sulfonic acid hydrate (HQS) and 8-hydroxy-7-iodoquinoline-5-sulfonic acid (HIQS) were used as ligands in these studies. The metal ion detection was achieved by post column complexation with 4-(2-pyridylazo) resorcinol (PAR), a metallochromic indicator; which avoided the interference from excess free ligand and buffer, and provided very high sensitivity in the visible region [19]. This sensitivity was essential to achieve the objectives of this study. In addition, PAR being a strong metal chelating reagent, it
could complex all the metal ions in the mobile phase and provide quantitative detection [20-22].

The understanding of metal ion separation mediated by organized molecular assemblies has both a fundamental and practical significance. Such an understanding is important towards employing them for environmentally friendly remediation of various metal contamination and wastes. Our long range goals are to employ molecular assemblies where chelating ligands are present in predetermined locations through appropriate derivatization of such assemblies. Such molecular assemblies that are of interest to us are chelating micelle, vesicle and dendrimers [23].
CHAPTER II

EXPERIMENTAL

Chemicals

Sodium Hydroxide (Food grade, 98.1%) and Formic Acid (88%, ACS grade) were obtained from Mallinckrodt. Sodium Nitrate (ACS grade) was provided by EM Science. SDS (99%), OTAB (98%), HQS (98%), HIQS (99%), Zinc(II) Nitrate Hexahydrate (98%), Copper(II) Nitrate Hemipentahydrate (98%), Cobalt(II) Nitrate Hexahydrate (98%), Nickle(II) Nitrate Hexahydrate, PAR (98%), Sodium Perchlorate (99%) and Chloroacetic Acid (99+%%) were research grade from Sigma-Aldrich Chemical Company. SDS, HQS and HIQS were recrystallized from water and methanol once. The other chemicals were used as received. MilliQ water was employed in HPLC studies. This was filtered through 0.2 µm nylon membrane and degassed with Helium prior to HPLC experiments.
HPLC Column Packing

Three 125 mm x 4.6 mm I.D. octadecyl-bonded silica (ODS, end capped with chlorotrimethyl silane to minimize residual silanols) reversed-phase columns were used in this research. The retention time of sodium chromate detected at 375 nm is considered as dead time because it is not retained on the column. For the SDS column 1, the packing material was Waters Spherisorb® 3-µm ODS; the dead volume was 1.18 mL. For the SDS column 2, the packing material was Macherey-Nagel Nucleosil® 3-µm ODS; the dead volume was 1.20 mL. For the OTAB column, the packing material was the same as SDS column 1; the dead volume was 1.07 mL. The difference in column dead volumes is because of different stationary phases, tiny difference in column length and column tightness (it is hard to pack the columns at exact the same tightness). Dedicated stationary phase were employed for each type of surfactant to avoid mixed micelle and vesicle formation. These columns were slurry packed in the laboratory at 42 MPa using methanol followed by water-methanol (50:50, v/v) and finally only water. Perkin-Elmer quaternary series 4 LC pump (Norwalk, CT, USA) was employed for column packing.
In this thesis, most of the experiments were performed on Waters Spherisorb® 3-µm ODS column except for experiments mentioned where Macherey-Nagel Nucleosil® 3-µm ODS column was employed.

Apparatus

HPLC System

The High Performance Liquid chromatograph (HPLC) consisted of a Perkin-Elmer quaternary series 200 LC pump (Norwalk, CT, USA), a Rheodyne (Cotati, CA, USA) Model 7725 injection valve equipped with a 20-µL sample loop and a Perkin-Elmer Series 200 UV/VIS detector. A short guard column packed with 3 µm ODS was placed between the pump and the injection valve. The mobile phase flow rate was 1.0 mL/min. The experiments were carried out at laboratory room temperature. Turbochrom Workstation 6.1.2 installed in a computer was used to perform instrument method control, data acquisition, and analysis. The computer is connected with HPLC pump and detector by Perkin-Elmer Model NELSON 600 Series Link. Post-column reaction detection method was used in this research. Altex Model
110A pump was used for delivering post-column derivative (PAR). The flow rate of PAR was 0.5 mL/min.

**UV-visible Spectrophotometer**

UV-vis spectra were recorded with a HP 8453 UV-visible Spectrophotometer (Agilent Technologies). Data collection and analysis were performed by Agilent UV-visible ChemStation Software.

**pH Meter**

Measurements of pH were performed by using an ORION® ROSS combination glass electrode and an ACCUMET® Basic Model AB15 (Fisher Scientific) pH meter. The pH meter was calibrated with standard buffer solutions (pH 2.0, pH 4.0 and pH 7.0) each day before measurements. Standard buffer solutions were supplied by Fisher Scientific.

**Transmission Electron Microscope (TEM)**

A transmission electron microscope was used to observe ODS surface. The TEM imaging experiments were performed by Western Michigan University imaging center with
JEOL 1230 TEM with a Gatan Digital Imaging System. This instrument has special lenses for high resolution-high contrast imaging of silica samples.

**Fluorescence Microscope**

A fluorescence microscope was used to study ODS surface adsorption behavior. The fluorescence microscope imaging experiments were performed by Western Michigan University imaging center with Nikon widefield fluorescence microscope with digital video camera. The digital camera has high resolution and low light (sub-visual) capability. These devices are coupled to a Datastor Pentium III processor through a Mutech Imaging board. Digital acquisition and processing is done utilizing Metamorph software from Universal Imaging Corporation.

**Preparation of Sample, Mobile Phases and Indicator**

Before running a separation experiment, all the de-ionized water and mobile phases were filtered by using Millipore filter kit with 0.2 μm nylon membranes.

The stock solutions of the test metal cations (0.1 M) were prepared from the corresponding nitrates with pH
3.5 deionized water. The pH 3.5 deionized water was adjusted by 0.1 M HCl. Before the measurement they were diluted to $1 \times 10^{-4} - 5 \times 10^{-3}$ M with pH 3.5 deionized water. The metal ion solutions were standardized by ICP/MS.

The mobile phases for HQS system were prepared with formic acid-NaOH buffer. The mobile phases for HIQS system were prepared with chloroacetic acid-NaOH buffer [24]. A 0.1 M stock solution of NaOH was standardized periodically with 0.1 M potassium hydrogen phthalate. The total ionic strength of a buffer was brought to 0.1 M by sodium nitrate except in the experiments of the effect of anion where sodium perchlorate was used.

The mobile phases consisted of surfactant (SDS or OTAB), ligand (HQS or HIQS) and buffer. The mobile phases were deaerated by vacuum degassing prior to HPLC runs and helium degassing during runs.

The metallochromic indicator PAR was prepared at $1 \times 10^{-4}$ M and kept at constant pH 4.0 with formic acid-NaOH buffer at a total ionic strength of 0.1 M adjusted with NaNO$_3$. 
Procedure

HPLC experiments were performed by first equilibrating the ODS stationary phase with the mobile phase for 2 to 8 hours. A data collection method was constructed with the Turbochrom software. The absorbance data were recorded once every two seconds. The metal ion mixture consisting of these metal ions, \([\text{Co(II)}] = 1 \times 10^{-4} \text{ M},\) \([\text{Ni(II)}] = 5 \times 10^{-4} \text{ M},\) and \([\text{Zn(II)}] = 5 \times 10^{-3} \text{ M}\) at pH 3.5, was employed for the SDS studies. A limited number of experiments were also conducted with a mixture of four metal ions which was prepared by introducing \(1 \times 10^{-3} \text{ M Cu(II)}\) to the three metal ion mixture. The metal ion mixture for the OTAB studies consisted of \([\text{Ni(II)}] = 5 \times 10^{-4} \text{ M}\) and \([\text{Zn(II)}] = 5 \times 10^{-3} \text{ M}\) at pH 3.5. A 20 µL sample of metal ion mixture was injected manually. The column was periodically cleaned with citric acid (pH = 3.5) to remove trace levels of metal ions left on the column. Column performance was monitored continuously through stability of the back pressure (typically around 15 MPa to 17 MPa), reproducibility of retention times and peak widths of the metal ion chromatograms. Post column deri-
vatization often gave rise to periodic noise in the chromatograms due to the post column pump. Effort was made to minimize this noise but it could not be completely avoided in every experiment.

When experimental conditions were changed for the SDS system such as in pH and ligand dependency studies, the column was equilibrated with the new mobile phase for an hour for the SDS studies. The equilibration times for OTAB were however much longer requiring 4 to 5 hours to attain complete equilibrium as indicated by the reproducibility of distribution ratios.

The chromatographic separations were performed a minimum of 2 to 4 times at each condition and the distribution ratios reported are an average of these runs. The reproducibility of the distribution constants was better than 5% as indicated by the calculated distribution ratios and visual inspection of the superimposed chromatographic runs.
CHAPTER III

RESULTS AND DISCUSSION

Selection of Detection Wavelength

Figure 1. The Structure of PAR [19].

From an optical detection standpoint, PAR (pK$_1$ = 3.1, pK$_2$ = 5.6, pK$_3$ = 11.9) benefits from the capability for chelating a number of different transition and heavy metal cations with high molar absorptivities (the stability constants are listed in Table 1 [22]). Figure 2 shows the spectra of PAR, HQS and HIQS. Figure 3 displays the spectra of metal-PAR complexes formed from $5 \times 10^{-5}$ M each of metal ions and PAR where the spectra were recorded using $5 \times 10^{-5}$ M PAR as the reference. This was done to match the condition of HPLC detection where the baseline is adjusted to zero with PAR reagent mixed with
the mobile phase prior to the injection of the metal ion mixture.

Although the metal chelates of HQS and HIQS have strong absorbance by themselves at around 380 nm as shown in Figures 4 and 5, it is hard to avoid the interference from excess free ligand and buffer at this short wavelength. Post column complexation with PAR was chosen to detect the metal ions to obtain the total concentration of the metal ion as a function of time as it eluted from the column (the chromatogram). The concentration and flow rate of PAR ensured that PAR was always in excess (depending on the point on the chromatogram) over the concentration of the metal ion and the ligand employed for separation.

In order to prevent high background levels associated with absorption of the uncomplexed PAR ligand, the metal ions were detected at 540 nm, which is close to the absorbance maxima of the metal-PAR complexes.
Figure 2. UV-visible Spectra of PAR, HQS and HIQS. 

\[ [\text{PAR}] = 1 \times 10^{-4} \text{ M, pH} = 4.0; \quad [\text{HQS}] = 2 \times 10^{-4} \text{ M, pH} = 4.0; \quad \text{and} \quad [\text{HIQS}] = 2 \times 10^{-4} \text{ M, pH} = 3.0. \]

Figure 3. Absorbance Spectra for PAR-Metal Complexes. The concentration of each metal ions was $5 \times 10^{-5}$ M, pH 4.0.
Figure 4. The UV-visible Spectra of Metal HQS Complexes. The concentration of each metal ions was $1 \times 10^{-4}$ M, pH 4.0.

Figure 5. The UV-visible Spectra of Metal HIQS Complexes. The concentration of each metal ions was $1 \times 10^{-4}$ M, pH 3.0.
Table 1

Stability Constants for Metal Ligand and PAR Complexes [22,25].

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Log of</th>
<th>Cu(II)</th>
<th>Ni(II)</th>
<th>Co(II)</th>
<th>Zn(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta_1$</td>
<td>11.92</td>
<td>9.02</td>
<td>8.11</td>
<td>7.54</td>
</tr>
<tr>
<td>HQS</td>
<td>$\beta_2$</td>
<td>9.95</td>
<td>7.75</td>
<td>6.95</td>
<td>6.78</td>
</tr>
<tr>
<td></td>
<td>$\beta$</td>
<td>21.87</td>
<td>16.77</td>
<td>15.06</td>
<td>14.32</td>
</tr>
<tr>
<td></td>
<td>$\beta_1$</td>
<td>8.33</td>
<td>8.2</td>
<td>7.3</td>
<td>7.1</td>
</tr>
<tr>
<td>HQS</td>
<td>$\beta_2$</td>
<td>8.25</td>
<td>7.0</td>
<td>6.3</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>$\beta$</td>
<td>16.58</td>
<td>15.2</td>
<td>13.6</td>
<td>13.2</td>
</tr>
<tr>
<td></td>
<td>$\beta_1$</td>
<td>14.8</td>
<td>13.2</td>
<td>10.0</td>
<td>10.5</td>
</tr>
<tr>
<td>PAR</td>
<td>$\beta_2$</td>
<td>9.1</td>
<td>12.8</td>
<td>7.1</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>$\beta$</td>
<td>23.9</td>
<td>26.0</td>
<td>17.1</td>
<td>17.1</td>
</tr>
</tbody>
</table>

*$\beta_1$ and $\beta_2$ are the stepwise stability constants and $\beta$ is the overall stability constant ($\beta = \beta_1 \times \beta_2$).

Separation in the Absence of Surfactant

The ligand HQS was used as a chelating agent in these experiments. Metal ions and HQS could form 1:1 and 1:2 metal:ligand complexes. Their stability constants are listed in Table 1. As may be seen the stability constants
differ by seven orders of magnitude for HQS and three orders of magnitude for HIQS, which should facilitate metal ion separation.

Figure 6. The Structures of 8-Hydroxyquinoline-5-sulfonic acid (HQS, H$_2$L) [22].

The HPLC separation without any surfactants in the mobile phase and with only HQS ligand and buffer in the mobile phase was first examined. For this purpose, mobile phases, containing only $2 \times 10^{-3}$ M HQS, were prepared in formic acid-NaOH buffer. In the pH range 3.5 to 4.1, which was employed for metal ion separations with surfactants and ligand, it was found that when the metal ions were individually injected, Cu(II) was retained on the column indefinitely and Ni(II), Co(II) and Zn(II) were eluted close to the dead volume of the column. The retention times of Zn(II) and Co(II) were the same and the retention time of Ni(II) was a little bit longer than Zn(II) and Co(II). When the mixture of Ni(II), Co(II) and
Zn(II) was injected in this pH range, no separation was observed. Figure 7 is the chromatogram of Ni(II), Co(II) and Zn(II) at pH 3.8 without any surfactant added.

![Chromatogram of Ni(II), Co(II) and Zn(II)](image)

**Figure 7.** Chromatogram of Ni(II), Co(II) and Zn(II) in the Absence of Surfactants. Machersy-Nagel Nucleosil® 3 µm ODS. Mobile phase: 2 × 10⁻³ M HQS in pH 3.8.

When the pH was varied, the variations on the retention times were negligibly small. At pH = 4.1, the peak shapes for Co(II) and Zn(II) became broad and unsymmetrical. As a result, further variations of pH were not carried out.
Based on the retention behavior, we could conclude that in this pH range with an ODS column, no separation could be achieved in the absence of surfactants.

**SDS Systems**

![Figure 8. The Structure of Sodium Dodecyl Sulfate (SDS).](image)

Figure 8 is the structure of SDS, which is an anionic surfactant. Its critical micelle concentration is $1.4 \times 10^{-3}$ M at an ionic strength of 0.1 [26]. SDS was studied from submicellar concentration $5 \times 10^{-5}$ M to well above its CMC. Three systems were examined: The SDS system in the absence of ligand, SDS with HQS ligand and SDS with HIQS ligand.

**The SDS System in the Absence of Ligand**

We have established that no separation can be achieved in the absence of surfactants. We also examined the separation of metal ions with SDS but in the absence of ligands. This experiment was performed employing a mo-
bile phase containing $2 \times 10^{-3}$ M SDS at pH = 3.8 obtained with formic acid - NaOH buffer. When the mixture of Ni(II), Co(II) and Zn(II) was injected, as shown in Figure 9, a broad composite peak of all metal ions was obtained after 100 minutes. This clearly indicated that separation was not possible in the absence of ligands. The long retention time could be attributed to adsorption of the metal ions on the SDS monolayer through the electrostatic forces.

**SDS and HQS System**

Cu(II), Ni(II), Co(II) and Zn(II) Separation.

The separation of four transition metal ions was examined. Mobile phases containing $2 \times 10^{-3}$ M each of SDS and HQS in the pH range 3.4 to 4.3 were employed. The elution order was: Cu(II) < Ni(II) < Co(II) < Zn(II), which is the reverse order of the stability constants (Table 1). At low pH, the retention times of Co(II) and Zn(II) were too long, and the peak shape of Zn(II) was very broad and unsymmetrical. At high pH, although the retention times for the four metal ions were reasonable, Cu(II) and Ni(II) had very short retention time so that
they could not be completely separated. Based on these behaviors, a gradient method was applied to get the separation of four metal ions as shown in Figure 10. A very nice baseline separation of Cu(II), Ni(II), Co(II) and Zn(II) was achieved in less than 25 minutes.

Figure 9. Separation of Ni (II), Co (II) and Zn (II) with SDS and no Ligand. Column: Machersy-Nagel Nucleosil® 3 µm ODS. Mobile phase: 2 x 10^{-3} M SDS at pH 3.8.
Figure 10. Separation of Cu(II), Ni(II), Co(II) and Zn(II) Chromatogram. Gradient method: 0-2 min, $2 \times 10^{-3}$ M SDS + $2 \times 10^{-3}$ M HQS in pH 3.4; 2-25 min, $2 \times 10^{-3}$ M SDS + $2 \times 10^{-3}$ M HQS in pH 4.0. 1: Cu(II); 2: Ni(II); 3: Co(II); 4: Zn(II).
Dependence of Separation on pH.

Figure 11 shows that the retention of analytes was significantly influenced by the pH of the mobile phase. The concentrations of SDS and HQS were kept constant at $2 \times 10^{-3}$ M. The pH was varied in the range 3.7 - 4.1. Since in this pH range, Cu(II) and Ni(II) could not be baseline separated, only the separations of Ni(II), Co(II) and Zn(II) were studied.

Figure 11 shows that the retention time decreases with increasing pH. The capacity factor $D$ was used to express retention behavior. The capacity factor could be calculated from retention time as indicated in equation 1 [27]:

$$D = \frac{t_R - t_0}{t_0}$$

In equation 1, $t_R$ = the analyte retention time. $t_0$ = dead time, which is the retention time for an unretained analyte. In this research, the retention time of Na$_2$CrO$_4$ was used as dead time.

The dependence of $D$ on pH is displayed in Figure 12.
Figure 11. Separation of Ni(II), Co(II) and Zn(II) as a Function of pH. Mobile phase: $2 \times 10^{-3}$ M SDS + $2 \times 10^{-3}$ M HQS. (1): pH 3.8; (2): pH 3.9; (3): pH 4.1.
Figure 12. Dependences of Capacity Factors of Ni(II), Co(II) and Zn(II) on Mobile Phase pH.

Dependence of Separation on the Concentration of HQS

Dependences of the retention of analytes on the concentration of the ligand HQS were also examined. The SDS concentration was kept constant at $2 \times 10^{-3}$ M, HQS concentration was varied from $1 \times 10^{-3} - 3 \times 10^{-3}$ M. In order to get reasonable capacity factor $D$ of Ni(II) ($D > 1$), the pH was kept constant at 3.7. Figure 13 shows the chromatograms of ligand dependence.
Experimental values of the capacity factor $D$ versus ligand HQS concentration are shown in Figure 14. $D$ decreases with increasing HQS concentration.

Figure 13. Separation of Ni(II), Co(II) and Zn(II) at Different HQS Concentrations. pH = 3.7, [SDS] = $2 \times 10^{-3}$ M. A: [HQS] = $1 \times 10^{-3}$ M; B: [HQS] = $2 \times 10^{-3}$ M; C: [HQS] = $3 \times 10^{-3}$ M.
The influence of the SDS concentration in the mobile phase on the separation was also examined. In this study, the concentration of HQS was kept constant at $2 \times 10^{-3}$ M and at pH 3.8 (considering of the selectivities and the retention times). SDS concentration was varied from $5 \times 10^{-5}$ to 0.01 M. When there is no SDS in the mobile phase, three metal ions could not be separated (Figure 7). The dependence of D on the concentration of SDS is shown in Figure 15. At very low SDS concentration, the capacity
factors of metal ions increased with increasing SDS concentration until SDS concentration reached $5 \times 10^{-4}$ M. Thereafter, the capacity factors of metal ions were constant, i.e., independent of SDS concentration. This behavior may be rationalized by the formation of a SDS monolayer on the surface of ODS based on the work of Wirth [28-30]. The hydrophobic portion of a SDS molecule in the mobile phase adsorbs on the ODS surface. When the SDS concentration reached $5 \times 10^{-4}$ M, the ODS surface was saturated by the adsorbed SDS molecules. We could conclude that at this SDS concentration, a monolayer of the surfactant is formed. Once the monolayer is formed, ODS surface could not adsorb any more SDS molecules, and the separation becomes independent of SDS concentration even up to 0.01 M SDS. This suggested that the separation of metal ion was driven by the SDS monolayer on the ODS surface and not the micelle in the mobile phase.

The concentration of the metal ion Ni(II), Co(II) and Zn(II) on the SDS monolayer can be determined from the D values and the concentration of metal ions injected. The values are: Ni(II) = $2.42 \times 10^{-11}$ moles/m$^2$; Co(II) = $6.93 \times 10^{-12}$ moles/m$^2$; Zn(II) = $3.52 \times 10^{-10}$
moles/m². The concentration of SDS on the surface of ODS according to Wirth when the monolayer is formed is $3 \times 10^{-6}$ moles/m². This indicates that the concentration of metal ions on the SDS monolayer is well below the concentration of needed to saturate the monolayer.

Two different sources of ODS, Macherey-Nagel Nucleosil® ODS and Waters Spherisorb® ODS, were used in SDS dependence study. The characteristics of the ODS are listed in Table 2. From Figure 15, we notice that in the SDS concentration range $1 \times 10^{-3} \text{ M} - 2 \times 10^{-3} \text{ M}$, when different sources of ODS were used, the capacity factors were not significantly different under identical experimental conditions.

Table 2

Comparison of Machersy-Nagel Nucleosil® and Waters Spherisorb® ODS [31]

<table>
<thead>
<tr>
<th>ODS</th>
<th>Particle Size</th>
<th>Pore Size</th>
<th>Surface Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spherisorb®</td>
<td>3 µm</td>
<td>80 Å</td>
<td>220 m²/g</td>
</tr>
<tr>
<td>Nucleosil®</td>
<td>3 µm</td>
<td>100 Å</td>
<td>350 m²/g</td>
</tr>
</tbody>
</table>

33
Figure 15. SDS Dependences. Column 125 x 4.6 mm, Mobile phase SDS + 2 x 10^{-3} M HQS, pH 3.8. (A): Macherey-Nagel Nucleosil® 3 μm ODS. (B): Waters Spherisorb® 3 μm ODS.
The Stability of SDS Monolayer.

The Stability of SDS monolayer was also examined. After SDS column 2 had been working with SDS mobile phase for about 3 weeks, we may expect a stable monolayer to be present on the ODS surface. The column was washed with water for about 2 hours then equilibrated with a mobile phase in the absence of SDS, which contained only HQS and buffer. A very nice separation of Ni(II), Co(II) and Zn(II) was achieved, as shown in Figure 16. Comparison with Figure 7 clearly indicates the role of the adsorbed monolayer of SDS in mediating the separation of the metal ions. This indicates that the SDS adsorption is very strong, and once a SDS monolayer is formed, it is very stable. This behavior also suggests that the separation of metal ions is driven by the SDS monolayer, and the micelle does not play a significant role.

The retention times of metal ions in Figure 16 are less than the retention times of same analytes under the condition where the SDS concentration greater than $5 \times 10^{-4}$ M is employed in mobile phase, indicating that some of the adsorbed SDS is removed from the stationary phase during equilibration with the mobile phase without SDS.
Therefore, SDS should be present in the mobile phases to keep monolayer stable and it should be present at a concentration level of $5 \times 10^{-4}$ M.

![Figure 16. Separation Chromatograms of Ni(II), Co(II) and Zn(II). Machersy-Nagel Nucleosil® 3 µm ODS, equilibrated with SDS. Mobile phase $2 \times 10^{-3}$ M HQS, pH 3.8.](image)

**HQS and pH Dependences at Other SDS Concentrations.**

Dependences of retention upon pH and ligand were examined at SDS concentrations of $4 \times 10^{-4}$ M, $1 \times 10^{-3}$ M and $2 \times 10^{-3}$ M with SDS column 2. Their retention behaviors are the same as already discussed: when the SDS concentration and ligand HQS concentration are constant, capac-
ity factors $D$ decrease with increasing $pH$; when the SDS concentration and $pH$ are constant, capacity factors $D$ decrease with increasing ligand concentration.

The Effect of Anion on SDS Mediated Separation

To keep the ionic strength of mobile phase at 0.1, NaClO$_4$ or NaNO$_3$ could be used to adjust the ionic strength. Figure 17 (a) and 17 (b) are the chromatograms with NaClO$_4$ with NaNO$_3$, respectively. The NaNO$_3$ provided better selectivity in the separation even though separation was still possible with NaClO$_4$.

![Chromatograms](image)

**Figure 17. Influence of the Nature of Anion on SDS Mediated Separation. Column: Machersy-Nagel Nucleosil® 3 µm ODS. Mobile phase $1 \times 10^{-4} M$ SDS + $2 \times 10^{-3} M$ HQS, pH 4.2.**
Proposed Mechanisms for SDS Mediated Separation

In order to understand the separation mechanism, first we need to understand the various species that exist in the mobile phase. Based on the acid dissociation constants and stability constants for metal ligand complexes, we could determine the various ligand and metal species at different pH values and ligand concentrations. The derivation of the equations is provided in Appendix A [32]. The plots of fraction of ligand species versus pH and of the fractions of metal-ligand complex species versus pH are also included in the Appendix A. From these plots, we found that in the working pH range pH 3.4 to pH 4.2, HQS mainly exists as HL\(^-\) and neutral H\(_2\)L, and the metal ions mainly exist as free M\(^{2+}\) and the 1:1 and 1:2 metal:ligand complexes ML, ML\(_2\)^{2-} respectively. Considering that a correlation exists between the species present and the retention behavior of the metal ions, the following three equilibria are proposed to exist between the mobile phase and the stationary phase containing an adsorbed monolayer of SDS:
Figure 18. Proposed Mechanism for SDS Mediated Metal Ion Separation on ODS Stationary Phase.
It may be seen from equation 3 and 4 that when the pH of the mobile phase is increased, resulting in a decrease in the concentration of $H^+$, the equilibrium would shift to the left, and the capacity factor $D$ would decrease; when the total concentration of ligand HQS is increased, resulting in an increase in the concentration of $HL^-$, the equilibrium would shift to the left, and the capacity factor $D$ would decrease. These mechanisms could explain the dependence of the retention behaviors of Co(II), Ni(II) and Zn(II) on pH and ligand concentration.

Based on the definition for the capacity factor $D$ and the proposed three equilibria, the following equation 5 could be derived (the derivation is shown in Appendix B):

$$\frac{1}{D} = \frac{1}{K_0} + \frac{1}{K_1} \times \frac{[HL^-]}{[H^+]} + \frac{1}{K_2} \times \frac{[HL^-]^2}{[H^+]^2}$$

(5)

Where $K_0$, $K_1$ and $K_2$ are equilibrium constants defined in equation 2-4. $[HL^-]$ and $[H^+]$ are the concentrations of $HL^-$ and proton in the mobile phase. According to the definition of pH:

$$[H^+] = 10^{-pH}$$

(6)

$[HL^-]$ could be calculated from equation 7:
\[ [HL^-] = \alpha_{HL^-} \times [H_2L]_t \]  

(7)

Where \( \alpha_{HL^-} \) is the fraction of \( HL^- \), which depends on pH, \( pK_a \) values of \( H_2L \) (Figure 6), and total ligand concentration \([H_2L]_t\). Multiple regression analysis was performed with \( 1/D \) as the dependant variable, the terms \([HL^-]/[H^+]\) and \([HL^-]^2/[H^+]^2\) as the independent variables. The constants \( K_0, K_1 \) and \( K_2 \) could be calculated from this regression analysis. The constants could be used to calculate \( 1/D \) and determine the fit between calculated and experimental \( 1/D \) values.

In order to get accurate values of the various equilibrium constants, as many data points as possible must be used in the regression. Multiple regression analysis was performed with all the experimental data for SDS concentration \( 1 \times 10^{-3} \) M and \( 2 \times 10^{-3} \) M. Table 3 lists mean equilibrium constants from regression analysis based on equation 5 and all the experimental data. From Table 3, it was found that the \( K_0 \) of \( Ni(II) \) and the \( K_2 \) of \( Zn(II) \) were not reasonable because their standard deviations were greater than the mean values. This indicates that the equilibria in equations 2 and 4 are not significant for \( Ni(II) \) and \( Zn(II) \) respectively.
Table 3

Equilibrium Constants for SDS + HQS System from Regression Analysis of Equation 5.

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>Ni(II)</th>
<th>Co(II)</th>
<th>Zn(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(K_0)</td>
<td>34(±38)</td>
<td>76(±25)</td>
<td>77(±16)</td>
</tr>
<tr>
<td>(K_1)</td>
<td>30(±15)</td>
<td>136(±18)</td>
<td>211(±27)</td>
</tr>
<tr>
<td>(K_2)</td>
<td>60(±6)</td>
<td>(2.5(±0.3) \times 10^3)</td>
<td>(4.8(±5.4) \times 10^4)</td>
</tr>
</tbody>
</table>

This may be rationalized from Figure 57 and 58 which indicate the fractions of Ni(II) and Zn(II) species as a function of pH. These indicate that free Ni(II) and ZnL\(_2\)\(^{2-}\) are minor species in the pH range of the experiments and as a result the equilibria involving them are insignificant. Based on these facts, the capacity factor \(D\) of Ni(II) should follow equation 8 and the capacity factor \(D\) of Zn(II) should follow equation 9:

\[
\frac{1}{D} = \frac{1}{K_1} \times \frac{[HL^-]}{[H^+]} + \frac{1}{K_2} \times \frac{[HL^-]^2}{[H^+]^2}
\]  

(8)
\[
\frac{1}{D} = \frac{1}{K_0} + \frac{1}{K_1} \frac{[HL^-]}{[H^+]} \tag{9}
\]

Regression analysis for the experimental data of Ni(II) was performed with 1/D as the dependant variable, the terms \([HL^-]/[H^+]\) and \([HL^-]^2/[H^+]^2\) as the independent variables and the constant forced to zero; regression analysis for the experimental data of Zn(II) was performed with 1/D as the dependant variable, the term \([HL^-]/[H^+]\) as the independent variable. Table 4 lists the average equilibrium constants from the new regression analysis for all the experimental data based on equation 5, 8 and 9 respectively.

**Table 4**

Equilibrium Constants for SDS + HQS System from Regression Analysis of Equation 5, 8 and 9.

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>Ni(II)</th>
<th>Co(II)</th>
<th>Zn(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equation</td>
<td>8</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>(K_0)</td>
<td>/</td>
<td>76(±25)</td>
<td>92(±12)</td>
</tr>
<tr>
<td>(K_1)</td>
<td>21(±2)</td>
<td>136(±18)</td>
<td>190(±5)</td>
</tr>
<tr>
<td>(K_2)</td>
<td>66(±4)</td>
<td>2.5(±0.3) x10^3</td>
<td>/</td>
</tr>
</tbody>
</table>
The constants in Table 4 were used in equations 5, 8 and 9 respectively to calculate 1/D and determine the fit between calculated and experimental 1/D values. Multiple regression analysis were also performed with pH and ligand dependence results at SDS concentrations of $1 \times 10^{-3}$ M and $2 \times 10^{-3}$ M separately.

Figures 19, 20 and 21 show that experimental data 1/D fit very well with calculated 1/D based on equilibrium constants from different regression analyses. These figures indicate that the proposed equilibria could explain well the retention behavior in the SDS system.

Equation 5, 8 and 9 could also be written in the form of equation 10, 11 and 12 respectively:

\[
\frac{[H^+]^2}{[HL^-]^2} \times \frac{1}{D} = \frac{1}{K_0} \times \frac{[H^+]^2}{[HL^-]^2} + \frac{1}{K_1} \times \frac{[H^+]}{[HL^-]} + \frac{1}{K_2} \quad (10)
\]

\[
\frac{[H^+]^2}{[HL^-]^2} \times \frac{1}{D} = \frac{1}{K_1} \times \frac{[H^+]}{[HL^-]} + \frac{1}{K_2} \quad (11)
\]

\[
\frac{[H^+]^2}{[HL^-]^2} \times \frac{1}{D} = \frac{1}{K_0} \times \frac{[H^+]^2}{[HL^-]^2} + \frac{1}{K_1} \times \frac{[H^+]}{[HL^-]} \quad (12)
\]
Based on the experimental capacity factor D, multiple regression analysis was performed with \( \frac{[H^+]^2}{[HL^-]^2} \times \frac{1}{D} \) as the dependant variable, the terms \( \frac{[H^+]^2}{[HL^-]^2} \) and / or \( \frac{[H^+]}{[HL^-]} \) as the independent variables. The constants \( K_0, K_1 \) and \( K_2 \) could be calculated from this regression analysis also as shown in Table 5.

Table 5

Equilibrium Constants for SDS + HQS System from Regression Analysis of Equation 10,11 and 12.

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>Ni(II)</th>
<th>Co(II)</th>
<th>Zn(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equation</td>
<td>11</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>( K_0 )</td>
<td>/</td>
<td>76(±14)</td>
<td>74(±5)</td>
</tr>
<tr>
<td>( K_1 )</td>
<td>18(±1)</td>
<td>138(±24)</td>
<td>214(±14)</td>
</tr>
<tr>
<td>( K_2 )</td>
<td>77(±8)</td>
<td>2.5(±0.8) \times 10^3</td>
<td>/</td>
</tr>
</tbody>
</table>

From Table 4 and 5, we find that the average equilibrium constants are similar from different regression analyses. We also find that the \( K_0 \) values are about the same for Co(II) and Zn(II) but the \( K_1 \) and \( K_2 \) values in-
crease from Ni(II) to Zn(II) in the reverse order of their stability constants (Table 1). The similarity in $K_0$ values for all transition metal ions is not surprising as this corresponds to an ion-exchange equilibrium constant that is determined by electrostatic attraction between $RSO_4^-$ and $M^{2+}$. Equations 3 and 4 indicate that $K_1$ and $K_2$ will increase as the stabilities of the complexes ML and $ML_2^{2-}$ decrease. Complexes with small stability constants will facilitate equilibria in equations 3 and 4 than those with large stability constants resulting in larger $K_1$ and $K_2$ values for the former than for the latter.

The difference in the $K_1$ and $K_2$ values between the metal ions is of the same order of magnitude as the differences in their stability constants $\beta_1$ for ML and $\beta$ for $ML_2^{2-}$. It may also be seen that larger $\beta_1$ and $\beta$ values, the smaller are the $K_1$ and $K_2$ values.
Figure 19. $1/D$ vs. $[HL^-]/[H^+]$ for Ni(II) in SDS + HQS.
Figure 20. 1/D vs. [HL\textsuperscript{-}]/[H\textsuperscript{+}] for Co(II) in SDS + HQS.
Figure 21. $1/D$ vs. $[\text{HL}^-] / [\text{H}^+]$ for Zn(II) in SDS + HQS.
SDS and HIQS System

To better understand the separation mechanism mediated by SDS, the separation of Ni(II), Co(II) and Zn(II) was investigated with another ligand 8-hydroxy-7-iodoquinoline-5-sulfonic acid (HIQS). Figure 22 shows its structure.

![Figure 22. The Acid Dissociation Equilibria of 8-Hydroxy-7-iodoquinoline-5-sulfonic acid (HIQS) [17].](image)

The structures of HQS and HIQS are similar, but the pK$_a$ values are very different. It might be expected that the mechanism of SDS mediated separation with this ligand should be similar to HQS. pH dependence and ligand dependence were studied for HIQS. Since reasonable capacity factor D for Ni(II) could be only obtained at pH less than 2.5, which is detrimental to the ODS stationary phase [20], only the separations of Co(II) and Zn(II) were examined in the pH range 2.7 - 3.1. The ODS column
degraded with use in this pH range and had to be discarded.

**pH Dependence**

Figure 23 shows changes in the retention with pH of the mobile phase. The concentrations of SDS and HIQS were kept at $2 \times 10^{-3}$ M. The pH was varied in the range 2.7-3.1. Experimental data are presented in Figure 24. The retention times decreased with increasing pH.

![Graph showing changes in retention time with pH](image)

**Figure 23.** Separation of Ni(II), Co(II) and Zn(II) with SDS + HIQS System at different pH. Mobile phase $2 \times 10^{-3}$ M SDS + $2 \times 10^{-3}$ M HIQS.
Figure 24. Plot of D vs. pH for the Separation of Co(II) and Zn(II) with SDS and HIQS.

HIQS Dependences

Figure 25 shows the influence of ligand HIQS concentration on the separation of Ni(II), Co(II) and Zn(II). The SDS concentration was kept constant at $2 \times 10^{-3}$ M and pH was kept constant at 2.9. Experimentally determined variation of capacity factor with the concentration of HIQS is shown in Figure 26. Capacity factors decrease with increasing concentration of HIQS.
Figure 25. Separations of Ni(II), Co(II) and Zn(II) with HIQS + SDS at Different HIQS Concentration. Mobile phase $2 \times 10^{-3}$ M SDS + $1 \times 10^{-3} - 3 \times 10^{-3}$ M HIQS, pH 2.9.

Figure 26. D vs. Concentration of Ligand HIQS for the Separation of Co(II) and Zn(II) with SDS + HIQS.
Proposed Mechanism

Based on the HIQS pK$_a$ values and chelate stability constants for metal HIQS complexes, we can generate the fraction of species vs. pH plots. The HIQS and metal HIQS complex fraction diagrams are listed in Appendix A. From the fraction diagram, we find that in the working pH range pH 2.7 to pH 3.1, HIQS mainly exists as HL$^-$ and neutral H$_2$L; Co(II) mainly exists as M$^{2+}$, ML and ML$_2^{2-}$; Zn(II) mainly exists as M$^{2+}$ and ML. We could suggest that the separation mechanism in SDS with HIQS system follows the same equilibria as those in SDS with HQS.

The same data treatment could be performed for the SDS + HIQS system. We supposed the capacity factor D of Co(II) still followed equation 5 and the capacity factor D of Zn(II) followed equation 9. We could get the equilibrium constants from regression analysis. Table 5 shows the mean equilibrium constants obtained based on equations 5 and 9 respectively. It may be seen from this table that the equilibrium constants K$_0$ and K$_2$ for Co(II) are not significant, suggesting that equilibria in equations 2 and 4 are not important.
Table 6

Equilibrium Constants for SDS + HIQS System from Regression Analysis of Equations 5 and 9

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>Co(II)</th>
<th>Zn(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equation</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>$K_0$</td>
<td>$89(\pm82)$</td>
<td>$74(\pm8)$</td>
</tr>
<tr>
<td>$K_1$</td>
<td>$25(\pm11)$</td>
<td>$49(\pm3)$</td>
</tr>
<tr>
<td>$K_2$</td>
<td>$181(\pm225)$</td>
<td>/</td>
</tr>
</tbody>
</table>

If only equation 3 was a significant equilibrium for Co(II) in SDS with HIQS system, the capacity factor $D$ of Co(II) should follow equation 13:

$$\frac{1}{D} = \frac{1}{K_1} \times \frac{[HL^-]}{[H^+]}$$  \hspace{1cm} (13)

Regression analysis for the experimental data of Co(II) was performed with $1/D$ as the dependent variable and $[HL^-]/[H^+]$ as the independent variable, the constant was forced as zero. The equilibrium constants are listed in Table 7.
Table 7

Equilibrium Constants for SDS + HIQS System from Regression Analysis of Equations 9 and 13

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>Co(II)</th>
<th>Zn(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equation</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>$K_0$</td>
<td>/</td>
<td>74(±8)</td>
</tr>
<tr>
<td>$K_1$</td>
<td>18(±1)</td>
<td>49(±3)</td>
</tr>
</tbody>
</table>

We could then compare the calculated $1/D$ from equations 9 and 13 with the experimental $1/D$. Figures 27 and 28 show that experimental data fit very well with the calculated results. We could conclude that the proposed mechanism explains the retention behavior in SDS with HIQS system as well.

Equation 13 could also be written as equation 14:

$$\frac{[H^+]^2}{[HL^-]^2} \times \frac{1}{D} = \frac{1}{K_1} \times \frac{[H^+]}{[HL^-]} \quad (14)$$
Figure 27. $1/D$ vs. $[\text{HL}^-]/[\text{H}^+]$ for Co(II) in SDS + HIQS.
Figure 28. $1/D$ vs. $[\text{HL}^-]/[\text{H}^+]$ for Zn(II) in SDS + HIQS.
The experimental data could also be treated in another way based on equations 12 and 14 respectively. Based on the experimental capacity factor $D$ of Zn(II), regression analysis was performed with $\frac{[H^+]^2}{[HL^-]^2} \times \frac{1}{D}$ as the dependant variable, the terms $\frac{[H^+]^2}{[HL^-]^2}$ and $\frac{[H^+]}{[HL^-]}$ as the independent variables and the constant was forced as zero. For Co(II), regression analysis was also performed with $\frac{[H^+]^2}{[HL^-]^2} \times \frac{1}{D}$ as the dependent variable, $\frac{[H^+]}{[HL^-]}$ as the independent variable and the constant was forced as zero. The constants $K_0$ and $K_1$ calculated from this regression analysis are shown in Table 8.

Table 8

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>Co(II)</th>
<th>Zn(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equation</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>$K_0$</td>
<td>/</td>
<td>77(±7)</td>
</tr>
<tr>
<td>$K_1$</td>
<td>17(±1)</td>
<td>48(±3)</td>
</tr>
</tbody>
</table>
The $K$ values in Table 7 and Table 8 are similar. This suggested that the proposed mechanism for SDS mediated separation applies to both ligands.

The $K_0$ values for Co(II) and Zn(II) determined with HQS and HIQS as might be expected are similar (Table 4 and 7). However, the $K_1$ values for Co(II) and Zn(II) are one order of magnitude smaller for HIQS than for HQS. It may be seen from Table 1 that the stepwise formation constants $\beta_1$ for Co(II) and Zn(II) with HIQS are smaller by about one order of magnitude than the formation constants with HQS. These differences with stability constants are reflected with separation equilibrium $K$ values.

Conclusions

1. Separation cannot be achieved in the absence of SDS. Mixture of metal ions injected into a mobile phase containing HQS equilibrated with ODS do not separate.

2. SDS in the mobile phase could form an adsorbed monolayer on the ODS by the adsorption of the hydrocarbon portion of the SDS on the C$_{18}$ chains of ODS. The minimum concentration of SDS required to form
the monolayer is $5 \times 10^{-4}$ M. It is well below the CMC of SDS (the CMC of SDS = $1.4 \times 10^{-3}$ M).

3. The adsorption of SDS on the ODS surface is very strong.

4. The influence of SDS concentration on HPLC separations of metal ions indicates that the critical concentration for reproducible separations is the concentration at which a monolayer of the SDS is formed and not the critical micelle concentration as reported in the literature. Separations are driven by the monolayer of SDS adsorbed on ODS. The micelle itself does not play a role in the separation mechanism of SDS system.

5. The order of elution of the metal ions in the SDS separations is Cu(II), Ni(II), Co(II) and Zn(II) which is exactly in the reverse order of their stability constants with HQS.

6. The figures of the fraction of metal species versus pH indicate that in the working pH range that metal:ligand complexes and metal ions coexist in the separation system.
7. The proposed mechanism of separation with SDS is based on the competition for the metal ions by the sulfate groups of SDS on the stationary phase surface and the ligand in the mobile phase. This can result in the various metal complexes observed. This also helps to rationalize the need for only sub-micellar concentrations of surfactant to achieve separations.

8. The order of equilibrium constants for the distribution of metal ions from the mobile phase into the adsorbed monolayer of SDS is the opposite of the order of the stability constants for the 1:1 and 1:2 metal:ligand complexes.

Separations Mediated by OTAB

Figure 29. The Structure of Octyltrimethylammonium Bromide (OTAB)
Figure 29 is the structure of OTAB, which is a cationic surfactant. Its critical micelle concentration is 0.269 M at an ionic strength 0.1 [18]. Separation with OTAB and HQS were studied at far below CMC from $5 \times 10^{-5}$ M to $1.2 \times 10^{-3}$ M. Cu(II) was indefinitely retained on the column at all concentrations of OTAB most likely due to the large formation constant with HQS. Co(II) could be detected only at low pH (about pH 3). When pH was higher than 3.3, Co(II) could not be detected by post column reaction derivatization with PAR. The behavior of Co(II) is not clear. The retention behaviors of Cu(II) and Co(II) were not examined further. The separation of Ni(II) and Zn(II) was examined along the lines of separations with SDS systems by varying pH, ligand concentration, OTAB concentration and the nature of the anion in the sodium salt used for adjusting ionic strength.

The OTAB System in the Absence of Ligand

We have established that no separation can be achieved in the absence of surfactants. We also examined the separation of metal ions with OTAB in the absence of ligands. The experiment was performed employing a mobile
phase containing $8 \times 10^{-4}$ M OTAB at pH 3.8 obtained with formic acid-NaOH buffer. When the mixture of Ni(II) and Zn(II) was injected, as shown in Figure 30, a sharp composite peak of the metal ions was obtained at a retention time corresponding to the column dead time. This clearly indicated that separation was also not possible in the absence of ligands with the OTAB surfactant.

![Chromatogram]

Figure 30. The Chromatogram of Ni(II) and Zn(II) in OTAB in the Absence of HQS. Mobile phase: $8 \times 10^{-4}$ M OTAB at pH 3.8.
Dependence of Separation on pH

The effect of the pH of the mobile phase on the retention behavior was also examined in OTAB system. The concentration of HQS was kept constant at $2 \times 10^{-3}$ M and OTAB was kept at $5 \times 10^{-5}$ M. The pH was varied in the range 4.0 - 4.5. Three chromatograms, which are representative of the separations obtained at different pH, are shown in Figure 31. It shows that the order of retention was always Zn(II) < Ni(II). The retention times increased with increasing pH.

The experimentally determined capacity factors versus pH for Zn(II) and Ni(II) separation are shown in Figure 32.

The dependence of D on pH was also examined at other OTAB concentrations. At each OTAB concentration, the capacity factors increased with increasing pH.
Figure 31. The Chromatograms of Zn(II) and Ni(II) at Different pH Values with the OTAB and HQS System. Mobile phase: $5 \times 10^{-5}$ M OTAB + $2 \times 10^{-3}$ M HQS.
Figure 32. Dependence of Capacity Factor D of Ni(II) and Zn(II) on Mobile Phase pH in the OTAB + HQS System.

**Ligand Dependence**

The effect of the concentration of HQS on the capacity factors was also examined. In this study, the concentration of OTAB was kept at $1 \times 10^{-4}$ M and ligand HQS concentration was varied from $1 \times 10^{-3} - 5 \times 10^{-3}$ M at a constant pH = 3.9. Figure 33 shows Zn(II) and Ni(II) chromatograms at different HQS concentrations.
Figure 33. The Chromatograms of Zn(II) and Ni(II) Separation at Different HQS Concentrations with the OTAB and HQS System. Mobile phase: $1 \times 10^{-4}$ M OTAB + HQS, pH 3.9.

Experimental values of the capacity factor $D$ versus ligand HQS concentration in OTAB system are displayed in Figure 34. $D$ increased with increasing ligand concentration.

The dependence of $D$ on ligand concentration was also examined at other OTAB concentrations. At each OTAB con-
centration, capacity factor $D$ increased with increasing ligand concentration.

![Graph showing $D$ vs. Concentrations of Ligand for the Separation of Ni(II) and Zn(II) OTAB + HQS.](image)

**Figure 34. D vs. Concentrations of Ligand for the Separation of Ni(II) and Zn(II) OTAB + HQS.**

**OTAB Dependence**

The effect of OTAB concentration on the capacity factors was also examined. Figure 35 shows the chromatograms of Zn(II) and Ni(II) separation with and without OTAB in the mobile phase. When there was no surfactant in the mobile phase, Zn(II) and Ni(II) could not be separated. It is obvious that the addition of OTAB to the mo-
bile phase could increase the separation selectivity. Further studies were performed to elucidate the effect of OTAB concentration on the retention of Ni(II) and Zn(II).

In these experiments, the concentration of HQS was kept constant at $2 \times 10^{-3}$ M and the Ni(II) and Zn(II) were individually examined. In order to get longer retention for Zn(II) and shorter retention for Ni(II), the pH of the mobile phase was kept constant at 4.2 for Zn(II) retention studies and 3.6 for Ni(II) retention studies. The OTAB concentration was varied in the range $5 \times 10^{-5}$ M - $1.2 \times 10^{-3}$ M. Figure 36 shows the experimental data of $D$ versus the concentration of OTAB. At very low OTAB concentrations, capacity factors $D$ of Ni(II) and Zn(II) increased linearly with the concentration of OTAB. When the concentration of OTAB was higher than $5 \times 10^{-4}$ M, the capacity factors of Zn(II) and Ni(II) increased much more slowly compared to the change below this concentration. A clear plateau in the $D$ values above the OTAB concentration of $5 \times 10^{-4}$ M as in the case of SDS was not observed. While the formation of OTAB monolayer at the OTAB concentration of $5 \times 10^{-4}$ M may be indicated by the retention behavior of Zn(II), Ni(II) data is unclear and does not
support the monolayer formation. This may be due to the adsorption of OTAB on ODS is very much weaker than that of SDS leading to the observed dependencies of D values of Zn(II) and Ni(II) on the concentration of OTAB. The weak adsorption was also borne out by experiments on the stability of the OTAB monolayer.

Figure 35. The Chromatograms of Zn(II) and Ni(II) Separation with and without OTAB. Mobile phase: $2 \times 10^{-3}$ M HQS with or without OTAB. pH 4.1.
A: [OTAB] = $5 \times 10^{-5}$ M; B: [OTAB] = 0
Figure 36. Dependence of D on the Concentration of OTAB. A: Zn(II) at pH = 4.2; B: Ni(II) at pH = 3.6. Mobile phase: OTAB + $2 \times 10^{-3}$ M HQS.
The Stability of OTAB Adsorption

The stability of the OTAB monolayer on the stationary phase was also examined. A stable monolayer might have been expected on the ODS surface after the column had been equilibrated with the mobile phase for about 2 months. This column was washed with water for about 2 hours and then equilibrated with a mobile phase, which contained $2 \times 10^{-3}$ M HQS at pH 3.8 and no OTAB. Injection of a Ni(II) and Zn(II) resulted in no separation as shown in Figure 37. This indicates that the OTAB adsorption is much weaker than that of SDS. This may be attributable to the shorter alkyl chain ($C_8$) and bulkier head group (quaternary ammonium) in OTAB compared to SDS. This behavior suggests that the separation of metal ions could be only achieved if the surfactant and the ligand are both present.

The Effect of Anion on OTAB Mediated Separation

$NaClO_4$ or $NaNO_3$ could be used to adjust the ionic strength in OTAB separations. Figure 38 (a) and (b) are the chromatograms with $NaNO_3$ and $NaClO_4$ respectively. The $NaNO_3$ provided much better selectivity in the separation.
The influence of anion can be understood based on the mechanism proposed for OTAB mediated separation discussed below.

Figure 37. The Chromatograms of Zn(II) and Ni(II) Separation in the Absence of OTAB. Mobile phase: $2 \times 10^{-3}$ M HQS, pH 3.8.
Mechanisms of OTAB Mediated Separation

The retention behavior of metal ions in OTAB system is the opposite of that in SDS system. The retention times increase with decreasing the concentration of $\text{H}^+$ or increasing the concentration of ligand. The following equilibria are proposed to rationalize the observed behavior.
Figure 39. Proposed Stepwise Mechanism for OTAB Metal Ion Separation on ODS Stationary Phase.
The overall equilibria involving the formation of 1:1 and 1:2 metal:ligand complexes on the adsorbed OTAB layer on ODS are given in equations 18 and 19 respectively in Figure 40.

![Chemical structures and equations](image)

Figure 40. Proposed Overall Mechanism for OTAB Metal Ion Separation on ODS Stationary Phase.
When the concentration of ligand is increased, the equilibria would shift to the right (D increases). When the concentration of proton is increased (pH decreases), the equilibrium would shift to the left (D decreases).

Based on these two equilibria and the definition of the capacity factor, the equation 20 could be derived (the derivation is shown in Appendix C):

\[
D' = K_1 \times \frac{[HL^-]}{[H^+]} + K_2 \times \frac{[HL^-]^2}{[H^+]^2}
\]  

(20)

Where \( D' = D \times (1 + \beta_1 \times [L^{2-}] + \beta_2 \times [L^{2-}]^2) \), \( \beta_1 \) and \( \beta_2 \) are stepwise stability constants of metal:ligand 1:1 and metal:ligand 1:2 complexes respectively. Multiple regression was performed with \( D' \) as the dependent variable, the terms \( [HL^-]/[H^+] \) and \( [HL^-]^2/[H^+]^2 \) as the independent variables and the constant was forced as zero. The equilibrium constants could be determined from the regression analysis.

Multiple regression analysis were performed with pH and ligand dependence results at OTAB concentration of 1 \( \times 10^{-4} \) M, 2 \( \times 10^{-4} \) M, 3 \( \times 10^{-4} \) M, 4 \( \times 10^{-4} \) M, 8 \( \times 10^{-4} \) M and
$1.2 \times 10^{-3}$ M. While a monolayer maybe considered to be formed at a concentration of OTAB of $5 \times 10^{-4}$ M, it is not conclusive as in the case of SDS. As a result the dependence of D values on pH and ligand concentration at the various OTAB concentration had to be individually analyzed using equation 20. The D values above OTAB concentration $5 \times 10^{-4}$ M could not be combined as in the case of SDS. It was also found from the regression that the equilibrium involving the formation of 1:1 metal:ligand complex on the OTAB layer, equation 18 is not a significant pathway. This was indicated by poor regression fits of experimental data and negative $K_1$ values. This equilibrium was neglected and only the formation of 1:2 metal:ligand complex on the ODS surface was considered as the significant pathway leading to equation 21.

$$D' = K_2 \times \frac{[HL^-]^2}{[H^+]^2}$$

Regression was performed with $D'$ as the dependent variable, the term $[HL']^2/[H']^2$ as the independent variable and the constant was forced to zero. The equilibrium
constants $K_2$ at different OTAB concentrations are listed in Table 9.

Equation 21 was used to calculate $D'$ from the $K_2$ values and determine the agreement between calculated and experimental $D'$ values. Figures 41 and 42 for an OTAB concentration of $1 \times 10^{-4}$ M show that the experimental $D'$ data fit very well with calculated $D'$ based on the $K_2$ value in Table 9. Similar plots at different OTAB concentrations are included in the Appendix D. The $K_2$ values for each OTAB concentration are shown in Table 9.

Table 9

<table>
<thead>
<tr>
<th>OTAB Conc. M</th>
<th>Zn(II), $K_2$</th>
<th>Ni(II), $K_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1 \times 10^{-4}$</td>
<td>0.1007(±0.0024)</td>
<td>32.45(±0.67)</td>
</tr>
<tr>
<td>$2 \times 10^{-4}$</td>
<td>0.1757(±0.0049)</td>
<td>70.70(±2.07)</td>
</tr>
<tr>
<td>$3 \times 10^{-4}$</td>
<td>0.1934(±0.0095)</td>
<td>69.38(±5.23)</td>
</tr>
<tr>
<td>$4 \times 10^{-4}$</td>
<td>0.3154(±0.0114)</td>
<td>139.79(±5.29)</td>
</tr>
<tr>
<td>$8 \times 10^{-4}$</td>
<td>0.2187(±0.0102)</td>
<td>Not determined</td>
</tr>
<tr>
<td>$1.2 \times 10^{-3}$</td>
<td>0.2878(±0.0102)</td>
<td>Not determined</td>
</tr>
</tbody>
</table>
Figure 41. $D'$ VS. $[\text{HL}^-]/[\text{H}^+]$ for $1 \times 10^{-4}$ M OTAB. 
A: Zn(II); B: Ni(II)
Figure 42. D' vs. $[HL^-]^2/[H^+]^2$ for $1 \times 10^{-4}$ M OTAB. A: Zn(II), B: Ni(II)
The $K_2$ values for Ni(II) were not determined at OTAB concentration higher than $4 \times 10^{-4}$ M due to the very large D values.

It may be seen that the equilibrium constants for 1:2 metal:ligand complex formation on the OTAB monolayer is 2 orders of magnitude larger for Ni(II) than for Zn(II). The stability constants of Ni(II) and Zn(II) complexes also differ by about 2 orders of magnitude (Table 1).

The equilibrium constant $K_2$ for the formation of 1:2 metal:ligand complex increase with the concentration of OTAB on the surface of ODS clearly indicating that the formation of the complex on ODS is facilitated by the absorbed OTAB.

The effect of anion on the retention behavior can be rationalized based on the proposed mechanism. Since ClO$_4^-$ can be expected to form a much stronger ion pair with the adsorbed OTAB than NO$_3^-$, this anion will compete with the ligand and reduce the retention of metal ions which are returned as their complexes.

The retention order was always Zn(II) < Ni(II). This order could be explained by the complex formation on the
column surface. Ni(II) forms stronger complex with HQS than does Zn(II) and as a result Ni(II) is retained on the column more strongly than Zn(II). Since Cu(II) has the largest stability constant among those metal ions, it was retained indefinitely on the column as its complex.

Conclusions

1. Separation cannot be achieved in the absence of OTAB.
2. OTAB in the mobile phase would be adsorbed on the ODS surface by the adsorption of the hydrocarbon moiety of the OTAB on the C$_{18}$ chains of ODS. The minimum concentration of OTAB required to form the monolayer is not clear. The data for Zn(II) suggest that the monolayer is formed at an OTAB concentration of about $5 \times 10^{-4}$ M.
3. The adsorption of OTAB on the ODS surface is much weaker than that of SDS.
4. The separation of metal ions occurs well below the critical micelle concentration of OTAB (the CMC of OTAB is 0.269 M). This indicates that the presence
of micelle in the mobile phase is not critical for the separation of metal ions.

5. Separations are driven by the OTAB adsorbed on ODS. Micelle itself does not play a role in the separation mechanism.

6. The order of elution in the case of OTAB follows the order of the stability constants of the metal ions with HQS. The chromatographic behavior of Cu(II) and Co(II) is also complicated by their poor efficiencies resulting in very broad peaks which at pH values > 3.3 become undetectable.

7. The mechanism of separation with OTAB is based on the competition for the HQS ligand by the adsorbed cationic surfactant OTAB and the free metal ions in the mobile phase solution. The experimental data support the formation of 1:2 metal:ligand complex on the adsorbed OTAB layer. The mechanism may also be regarded as the distribution of the 1:2 metal:ligand complex between the mobile and stationary phases. This is in contrast to the distribution of the metal ion between the mobile and stationary phases in case of SDS.
Imaging Experiments

In order to better understand the proposed mechanism for SDS and OTAB mediated separations, imaging experiments were performed to characterize ODS surfaces, which were equilibrated with different surfactants and ligand solutions.

Transmission Electron Microscope (TEM)

Machersy-Nagel Nucleosil® 3 µm ODS was used for TEM experiments. Surface porosity is clearly evident as in Figure 43. TEM images of the silica equilibrated for 4 days with surfactant, surfactant + ligand and surfactant + ligand + metal ion were also recorded.

All the TEM imagings were taken under 300,000 magnifications. Figures 44 - 49 show the images of ODS surfaces for the various equilibrations. It was found that after the ODS was equilibrated with the various surfactant solutions, its surface became much more smooth than the untreated ODS due to the adsorption of the surfactant on the surface of ODS. It was difficult to distinguish between the adsorption of OTAB and OTAB + ligand as the
ligand adsorption does not significantly alter the surfactant monolayer.

Figure 43. The TEM Image of Untreated ODS.
Figure 44. The TEM Image of ODS Equilibrated with SDS. $[\text{SDS}] = 2 \times 10^{-3}$ M, pH 4.0.
Figure 45. The TEM Image of ODS Equilibrated with SDS and HQS. [SDS] = 2 \times 10^{-3} \text{ M, [HQS]} = 2 \times 10^{-3} \text{ M, pH 4.0.}
Figure 46. The TEM Image of ODS Equilibrated with SDS, HQS and Zn(II). [SDS] = $2 \times 10^{-3}$ M, [HQS] = $2 \times 10^{-3}$ M, [Zn(II)] = $1 \times 10^{-3}$ M, pH 4.0.
Figure 47. The TEM Image of ODS Equilibrated with OTAB. 
[OTAB] = 1.2 \times 10^{-3} \text{ M}, \text{ pH } 4.0.
Figure 48. The TEM Image of ODS Equilibrated with OTAB and HQS. \([\text{OTAB}] = 1.2 \times 10^{-3} \text{ M}, \]
\([\text{HQS}] = 2 \times 10^{-3} \text{ M}, \text{ pH 4.0}.\)
Figure 49. The TEM Image of ODS Equilibrated with OTAB, HQS and Zn(II). \([\text{OTAB}] = 1.2 \times 10^{-3} \text{ M},\]
\([\text{HQS}] = 2 \times 10^{-3} \text{ M, } [\text{Zn(II)}] = 1 \times 10^{-3} \text{ M,}\]
\(\text{pH 4.0.}\)
Fluorescence Microscopy Studies

Fluorescence Microscopy Studies were performed to obtain a direct evidence for the adsorption of HQS on the OTAB monolayer during the separation process. The ligand HQS has strong fluorescence emission with a maximum at 450 nm when excited in the 325 - 400 nm range. The HQS adsorbed on the monolayer of OTAB may be detectable by fluorescence microscopy. The equilibrated Machersy-Nagel Nucleosil® 3 µm ODS silica were also used for fluorescence microscopy experiments. The equilibrated ODS were excited in the 360 - 390 nm range with a mercury lamp.

Figures 50 and 51 show the fluorescence microscopy images for ODS equilibrated with SDS + HQS, and for ODS equilibrated with SDS + HQS + Zn(II) respectively. Both images did not show the fluorescence. This is in line with the proposed mechanism where HQS is not adsorbed on the SDS monolayer.

Figures 52 and 53 show the fluorescence microscopy images for ODS equilibrated with OTAB + HQS, and for ODS equilibrated with OTAB + HQS + Zn(II) respectively. Both images show the fluorescence from the adsorbed HQS and Zn-HQS complexes respectively. This again agrees with the
proposed mechanism that involves the adsorption of free ligand and its complexes on the OTAB monolayer (equations 15 and 17).

The adsorption of the ligand and its complexes on OTAB was further supported by fluorescence microscopy experiments with Cu(II)-HQS complex. Cu(II) has 9 electrons in its highest occupied d orbits. When Cu(II) complexes with HQS ligand, the unpaired d electron would couple with the π orbital of HQS quenching the fluorescence of HQS. It may be seen from Figure 54 that the fluorescence of HQS is quenched when Cu(II)-HQS complex is adsorbed on the monolayer of OTAB on ODS.
Figure 50: The Fluorescence Microscopy Image of ODS Equilibrated with SDS + HQS.

\[ [\text{SDS}] = 2 \times 10^{-3} \text{ M}, \quad [\text{HQS}] = 2 \times 10^{-3} \text{ M}, \quad \text{pH} \ 4.0 \]
Figure 51. The Fluorescence Microscopy Image of ODS Equilibrated with SDS + HQS + Zn(II).

\([\text{SDS}] = 2 \times 10^{-3}\) M, \([\text{HQS}] = 2 \times 10^{-3}\) M,
\([\text{Zn(II)}] = 1 \times 10^{-3}\) M, pH 4.0.
Figure 52. The Fluorescence Microscopy Image of ODS Equilibrated with OTAB and HQS. [OTAB] = 1.2 × 10^{-3} M, [HQS] = 2 × 10^{-3} M, pH 4.0.
Figure 53. The Fluorescence Microscope Image of ODS Equilibrated with OTAB, HQS and Zn(II). [OTAB] = 1.2 \times 10^{-3} \text{ M}, [HQS] = 2 \times 10^{-3} \text{ M}, [\text{Zn}^{2+}] = 1 \times 10^{-3} \text{ M}, \text{pH 4.0}.
Figure 54. The Fluorescence Microscopy Image of ODS Treated with OTAB, HQS and Cu(II).
[OTAB] = $1.2 \times 10^{-3}$ M, [HQS] = $2 \times 10^{-3}$ M, [Cu(II)] = $1 \times 10^{-3}$ M, pH 4.0.
CHAPTER IV

CONCLUSIONS AND SUMMARY

Conclusions

The major conclusions of the separations mediated by SDS and OTAB surfactant assemblies are:

1. Separation cannot be achieved in the absence of surfactants. Mixture of metal ions injected into a mobile phase containing HQS equilibrated with ODS do not separate.

2. A significant finding of the HPLC studies is that the cationic surfactant in the mobile phase adsorb on the ODS in an analogous manner to anionic surfactants.

3. A further significant finding is that the minimum concentration of SDS required to form the monolayer is $5 \times 10^{-4}$ M. At this concentration OTAB may also form monolayer. This minimum concentration is well below the critical micelle concentration of either surfactant.
4. Separations are driven by the monolayer of surfactant adsorbed on ODS. The micelle itself does not play a role in the separation mechanism.

5. Micellar concentrations may be required only in cases where the micelle is necessary to solubilize the ligand.

6. HPLC separations as a function of SDS and OTAB concentrations clearly indicate that the critical concentration for reproducible separations is the concentration at which a monolayer of the surfactant is formed and not the critical micelle concentration as reported in the literature. This clearly has great practical significance as metal ion separations can be achieved under submicellar concentrations for a variety of surfactants.

7. The order of elution of the metal ions in the SDS separations is Cu(II), Ni(II), Co(II) and Zn(II) which is exactly in the reverse order of their stability constants with HQS.

8. The mechanism of separation with SDS is based on the competition for the metal ion by the sulfate groups of SDS on the stationary phase surface and ligand in the mobile phase. This helps to rationalize the need...
for only submicellar concentrations of surfactant to achieve separations. The micelle itself does not play a role in the separation mechanism.

9. The order of elution in the case of OTAB follows the order of the stability constants of the metal ions with HQS.

10. The mechanism of separation with OTAB is based on the competition for the ligand by the adsorbed cationic surfactant and the free metal ions in the mobile phase. It may also be viewed as the distribution of the metal complex between the mobile and stationary phases.

11. This is the first demonstration of the presence of competing equilibria on the self-assembled monolayers on the stationary phase and the surfactant mobile phase that result in the separation of the metal ions.

12. The imaging experiments support the proposed mechanisms for both SDS and OTAB mediated separation.
Summary

The SDS mediated separations are driven by the monolayer of SDS on C18 stationary phase. The adsorbed monolayer of SDS thus resembles a polymeric cation exchange sulfonate resin. In summary, the significant difference between a monolayer of anionic surfactant and sulfonate resin are:

1. Anionic surfactant monolayer can be generated on a variety of reverse phase stationary phases when the alkyl chain length can be varied both on the stationary phase and the surfactant. This provides the possibility for separation with mixed anionic surfactant systems. Such stationary phase for separation of cations can be readily generated compared to sulfonate resins which must be synthesized.

2. The cation exchange sites on the adsorbed monolayer are in an extremely ordered microenvironment compared to the sulfonate groups of polymeric resins which are present in random macro environments dictated by the synthetic procedure used.
3. The characterization at the molecular level of metal ion separations employing separation, spectroscopic, and imaging techniques can be systematically performed more readily on anion surfactant monolayer stationary phases than on sulfonate resin stationary phase.

4. The surfactant monolayer as demonstrated in the studies can be reproducibly generated and are stable. In general, silica stationary phases are more robust and can be employed over extended periods of time compared to polymeric stationary phase which crush more readily and do not possess the mechanical strength of silica stationary phases.

5. Generally polymeric stationary phases can be employed over much wider pH range (1-14) compared to silica stationary phases (2.5-7.5).

6. Metal ion chelating ligands must be employed to obtain separation with anionic surfactant and sulfonate resin stationary phases. Sulfonate resins are polystyrene based which limits the ligands that can be employed with them to aliphatic systems, as aromatic ligands interact with the poly-
meric matrix primarily through π - π and Van der Waals forces, adversely affecting separation efficiency and selectivities.

This research has demonstrated that highly selective metal ion separations can be achieved by ordered surfactant layers on reverse phase silica. This substantiates the hypotheses of the research that high metal ion selectivities can be achieved by ligands in organized microenvironments. Surfactants adsorbed on surfaces such as reverse phase silica provide ordered microenvironments that are ideal for the investigation of the fundamental mechanisms of metal ion and molecular recognition mediated by such structures. They also provide great versatility and flexibility to optimize experimental conditions to achieve maximum selectivities. They are also excellent model systems to discern separation mechanisms at the molecular level by employing a variety of spectroscopic and imaging techniques. They also provide environmentally friendly (green) approaches to separations and environmental remediation. The current research has also provided several new directions for this research to further
substantiate the central hypotheses and these are discussed in the following section on future directions.
CHAPTER V

FUTURE DIRECTIONS

The future directions for this research subject are:
1. Mixing anionic surfactant with cationic surfactant to form vesicles, and then using vesicles in mobile phase, and comparing vesicles mediated separations with single surfactant mediated separations.
2. The separation of other metal ions such as lanthanides.
3. Separations with self-assembling ligand systems.
4. Other imaging studies such as AFM, STM and small angle X-ray scattering to characterize the surface adsorbates at the molecular level.
ABBREVIATIONS AND SYMBOLS

Abs.: absorbance
AFM: atomic force microscope
CMC: critical micelle concentration
D: capacity factor
HIQS: 8-hydroxy-7-iodo-5-quinoline-sulfonic acid (HIQS)
HPLC: high performance liquid chromatograph
HQS: 8-hydroxyquinoline-5-sulfonic acid
H₂L: ligand (HQS or HIQS)
MLC: micellar liquid chromatography
ODS: octadecysilanized silica
OTAB: octyltrimethylammonium bromide
PAR: 4-(2-pyridylazo) resorcinol
SDS: sodium dodecyl sulfate
STM: scanning tunneling microscope
TEM: transmission electron microscope
Appendix A

The Species Fraction of HQS, HIQS and Metal Ligand Complexes
For a $H_2L$ ligand, the following two acid dissociation equilibria are existed:

$$H_2L \rightleftharpoons K_{a1} HL^- + H^+ \quad (22)$$

$$HL^- \rightleftharpoons K_{a2} L^2^- + H^+ \quad (23)$$

Here $K_{a1}$ and $K_{a2}$ are the acid dissociation constants corresponding to equilibria 22 and 23, respectively. The $K_{a1}$ and $K_{a2}$ are given by

$$K_{a1} = \frac{[HL^-][H^+]}{[H_2L]} \quad (24)$$

$$K_{a2} = \frac{[L^2^-][H^+]}{[HL^-]} \quad (25)$$

Equation 24 and 25 could be rewritten as:

$$[HL^-] = \frac{K_{a1}[H_2L]}{[H^+]} \quad (26)$$
If a solution contained an acid, we know equation 28 should exist:

\[
[H_2L]_t = [H_2L] + [HL^-] + [L^{2-}]
\]  

Equation 28 applies under all the experimental conditions employed since the ligand is in large excess like metal ions in a dynamic separation experiment which has a fixed concentration of ligand in the mobile phase. Here \([H_2L]_t\) represents the total acid concentration. The fractions of \(H_2L\), \(HL^-\) and \(L^{2-}\) species are given by:

\[
\alpha_{H_2L} = \frac{[H_2L]}{[H_2L]_t}
\]  \hspace{1cm} (29)

\[
\alpha_{HL^-} = \frac{[HL^-]}{[H_2L]_t}
\]  \hspace{1cm} (30)

\[
\alpha_{L^{2-}} = \frac{[L^{2-}]}{[H_2L]_t}
\]  \hspace{1cm} (31)
Here $\alpha$ represents fraction. Substitution of equation 26, 27 and 28 into 29, 30 and 31 gives:

\[
\alpha_{[H_2L]} = \frac{1}{1 + \frac{K_{a1}}{[H^+]} + \frac{K_{a1} \times K_{a2}}{[H^+]^2}}
\]

(32)

\[
\alpha_{[HL^-]} = \frac{\frac{K_{a1}}{[H^+]}}{1 + \frac{K_{a1}}{[H^+]} + \frac{K_{a1} \times K_{a2}}{[H^+]^2}}
\]

(33)

\[
\alpha_{[L^2-]} = \frac{\frac{K_{a1} \times K_{a2}}{[H^+]^2}}{1 + \frac{K_{a1}}{[H^+]} + \frac{K_{a1} \times K_{a2}}{[H^+]^2}}
\]

(34)

Based on equation 32, 33 and 34, Figures 55 and 59 could be generated by using a spread sheet program.

The equilibria involved in metal chelate formation are:

\[
M^{2+} + L^{2-} \rightleftharpoons ML
\]

(35)

This yields

\[
[ML] = \beta_1 \times [M^{2+}] \times [L^{2-}]
\]

(36)
\[ ML + L^2- \xrightarrow{\beta_2} ML_2^{2-} \]  

(37)

The overall equilibrium is

\[ \beta \]

\[ M^{2+} + 2L^2- \xleftrightarrow{\beta} ML_2^{2-} \]  

(38)

This yields

\[ [ML_2^{2-}] = \beta \times [M^{2+}] \times [L^2^-]^2 \]  

(39)

Here \( \beta_1 \) and \( \beta_2 \) are the stepwise stability constants and \( \beta \) is the overall stability constant \( (\beta = \beta_1 \times \beta_2) \). \([M^{2+}]_t \) represents all the metal species

\[ [M^{2+}]_t = [M^{2+}] + [ML] + [ML_2^{2-}] \]  

(40)

The fractions of \( M^{2+} \), \( ML \) and \( ML_2^{2-} \) species are given by:

\[ \alpha_{M^{2+}} = \frac{[M^{2+}]}{[M^{2+}]_t} \]  

(41)

\[ \alpha_{ML} = \frac{[ML]}{[M^{2+}]_t} \]  

(42)
Substitution of equations 36, 39 and 40 into equations 41, 42 and 43 gives

\[ \alpha_{M^{2+}} = \frac{1}{1 + \beta \times [L^{2-}] + \beta \times [L^{2-}]^2} \]  

(44)

\[ \alpha_{ML} = \frac{\beta \times [L^{2-}]}{1 + \beta \times [L^{2-}] + \beta \times [L^{2-}]^2} \]  

(45)

\[ \alpha_{ML^{2-}} = \frac{\beta \times [L^{2-}]^2}{1 + \beta \times [L^{2-}] + \beta \times [L^{2-}]^2} \]  

(46)

Equation 31 could be rewritten as

\[ [L^{2-}] = \alpha_{L^{2-}} \times [H_2L]_i = \frac{K_{a1} \times K_{a2} \times [H^+]^2}{K_{a1} + K_{a1} \times K_{a2}} \times [H_2L]_i \]  

(47)

At constant ligand concentration, \([L^{2-}]\) is a function of pH. Based on equation 44, 45, 46 and 47, Figures
56-58, 60 and 61 could be generated by using a spreadsheet program.
Figure 55. The Fraction Of Ligand Species of HQS vs. pH.
Figure 56. The Fraction Of Co(II) Species vs. pH for Complexation with HQS. 
[HQS] = 2 \times 10^{-3} \text{ M.}
Figure 57. The Fraction of Ni(II) Species vs. pH for the Complexation with HQS. 
$[\text{HQS}] = 2 \times 10^{-3}$ M.
Figure 58. The Fraction of Zn(II) Species vs. pH for the Complexation with HQS. 
\([\text{HQS}] = 2 \times 10^{-3} \text{ M.}\)
Figure 59. The Fraction of Species of HIQS vs. pH
Figure 60. The Fraction Of Co(II) Species vs. pH for the Complexation with HIQS. [HIQS] = 2 × 10⁻³ M.
Figure 61. The Fraction Of Zn(II) Species vs. pH for the Complexation with HIQS. 
[HIQS] = 2 × 10^{-3} M.
Appendix B

The Derivation of Equation 5
The following three equilibria were proposed to describe the retention of metal ions in SDS with ligands (H₂L) system:

$$\text{CH}_3\text{Si--CH}_3\text{H}_3\text{C--SO}_4^- + \text{M}^{2+} \rightleftharpoons \text{K}_0$$

$$\text{CH}_3\text{Si--CH}_3\text{H}_3\text{C--SO}_4^- \rightleftharpoons \text{ML} + \text{H}^+ \rightleftharpoons \text{K}_1$$

$$\text{CH}_3\text{Si--CH}_3\text{H}_3\text{C--SO}_4^- + 2\text{H}^+ + \text{ML}_2 \rightleftharpoons \text{K}_2$$

$$\text{CH}_3\text{Si--CH}_3\text{H}_3\text{C--SO}_4^- + 2\text{H}^+ + 2\text{HL}^- \rightleftharpoons \text{K}_2$$
Where $K_0$, $K_1$ and $K_2$ are the equilibrium constants corresponding to equilibria 2, 3 and 4, respectively. The $K_0$, $K_1$ and $K_2$ are given by

$$K_0 = \frac{[M^{2+}]_s}{[M^{2+}]_m} \quad (48)$$

$$K_1 = \frac{[M^{2+}]_s \times [HL^-]}{[ML]_m \times [H^+]} \quad (49)$$

$$K_2 = \frac{[M^{2+}]_s \times [HL^-]^2}{[ML_2^{2-}]_m \times [H^+]^2} \quad (50)$$

Where subscripts $s$ and $m$ represent stationary and mobile phases and $ML$ and $ML_2^{2-}$ are metal:ligand 1:1 and 1:2 complexes respectively; $[H^+]$ is the concentration of protons in the mobile phase. ($[H^+] = 10^{-pH}$); $[HL^-]$ is the concentration of $HL^-$ species in the mobile phase. ($[HL^-] = \alpha_{HL^-} \times [H_2L]_{total}$; $\alpha_{HL^-}$ is the fraction of $HL^-\). Equations 49 and 50 could also be rewritten as:

$$\frac{[M^{2+}]_s}{[ML]_m} = K_1 \times \frac{[H^+]}{[HL^-]} \quad (51)$$

$$\frac{[M^{2+}]_s}{[ML_2^{2-}]_m} = K_2 \times \frac{[H^+]^2}{[HL^-]^2} \quad (52)$$
The capacity factor of a metal ion is given by

\[
D = \frac{[M^{2+}]_{s}}{[M^{2+}]_{m} + [ML]_{m} + [ML_{2}^{2-}]_{m}} \quad (53)
\]

Here the subscript total represents the total concentration of all the metal species. The capacity factor of an analyte should consider the phase ratio also. Since the phase ratio is a constant, it is incorporated in the equilibrium constants. Equation 53 can be rewritten as:

\[
\frac{1}{D} = \frac{[M^{2+}]_{m} + [ML]_{m} + [ML_{2}^{2-}]_{m}}{[M^{2+}]_{s} + [ML]_{s} + [ML_{2}^{2-}]_{s}} \quad (54)
\]

Combination of equations of 48, 51, 52 and 54, gives an equation for \(1/D\)

\[
\frac{1}{D} = \frac{1}{K_{0}} + \frac{1}{K_{1}} \times [H^{+}] + \frac{1}{K_{2}} \times [H^{+}]^{2} \quad (5)
\]

This expression was used in analysing the experimental data. The derivation of equations 8 and 9 is similar with the derivation of equation 5.
Appendix C

The Derivation Of Equation 20
The following two equilibria were proposed to describe the retention of metal ions in OTAB with ligand HQS system:

\[ \text{HQS} + M^{2+} + HL^- \rightleftharpoons \text{HQS} \cdot M + HL^- \]  
(18)

\[ \text{HQS} \cdot M + H^+ \rightleftharpoons \text{HQS} + LM + M^{2+} + 2HL^- \]  
(19)

Where $K_1$ and $K_2$ are the equilibrium constants corresponding to equilibria 18 and 19, respectively.
The $K_1$ and $K_2$ are given by

$$K_1 = \frac{[ML_s] \times [H^+]}{[M^{2+}]_m \times [HL^-]}$$  \hspace{1cm} (55)$$

and

$$K_2 = \frac{[ML_{2-}]_s \times [H^+]^2}{[M^{2+}]_m \times [HL^-]^2}$$  \hspace{1cm} (56)$$

Here subscript s and m represent stationary and mobile phases and ML and ML$_{2-}$ represent metal:ligand 1:1 and 1:2 complexes respectively. $[H^+]$ is the concentration of protons in the mobile phase ($[H^+] = 10^{-pH}$); $[HL^-]$ is the concentration of HL$^-$ species in the mobile phase ($[HL^-] = \alpha_{HL^-} \times [H_2L]_{total}$. $\alpha_{HL^-}$ is the fraction of HL$^-$ present).

Equation 55 and 56 could be rewritten as:

$$\frac{[ML_s]}{[M^{2+}]_m} = K_1 \times \frac{[HL^-]}{[H^+]}$$ \hspace{1cm} (57)$$

$$\frac{[ML_{2-}]_s}{[M^{2+}]_m} = K_2 \times \frac{[HL^-]^2}{[H^+]^2}$$ \hspace{1cm} (58)$$

The capacity factor of a metal ion is given by equation

130
\[
D = \frac{[M^{2+}_{\text{total}}]_s}{[M^{2+}_{\text{total}}]_m} = \frac{[ML]_s + [ML_2^{2-}]_s}{[M^{2+}]_m + [ML]_m + [ML_2^{2-}]_m} \quad (59)
\]

Here subscript total means the total concentration of all the metal species in stationary phase or mobile phase. As before since the phase volume ratio is constant, it is incorporated into the equilibrium constants.

In the mobile phase, the equilibria for the metal chelate formation are the same as we mentioned in Appendix A:

\[
\begin{align*}
M^{2+} + L^2- & \rightleftharpoons ML \quad (35) \\
\text{This yields} & \quad [ML] = \beta_1 \times [M^{2+}] \times [L^2-] \quad (36) \\
ML + L^2- & \rightleftharpoons ML_2^{2-} \quad (37) \\
\text{The overall equilibrium is} & \quad M^{2+} + 2L^2- \rightleftharpoons ML_2^{2-} \quad (38)
\end{align*}
\]

This yields \([ML_2^{2-}] = \beta \times [M^{2+}] \times [L^2-]^2 \quad (39)\)
Here $\beta_1$ and $\beta_2$ are the stepwise stability constants and $\beta$ is the overall stability constant ($\beta = \beta_1 \times \beta_2$).

Combination of equations 59, 36 and 39 gives an equation for $D$

$$D = \frac{[ML]_s + [ML_2^{2-}]_s}{[M^{2+}]_m} \times \left( \frac{1}{1 + \beta_1 \times [L^{2-}] + \beta \times [L^{2-}]^2} \right) \quad (60)$$

We define $D'$ as

$$D' = D \times (1 + \beta_1 \times [L^{2-}] + \beta \times [L^{2-}]^2) \quad (61)$$

Substitution of equations 57, 58 and 61 into equation 60 gives

$$D' = K_1 \times \frac{[HL^-]}{[H^+]} + K_2 \times \frac{[HL^-]^2}{[H^+]^2} \quad (20)$$

When only equilibrium in equation 19 is significant, the capacity factor is given by:
\[ D = \frac{\left[ M^{2+}_{\text{total}} \right]_s}{\left[ M^{2+}_{\text{total}} \right]_m} = \frac{[M_{2}^{2-}]}{[M^{2+}]_m + [ML]_m + [ML_{2}^{2-}]_m} \quad (62) \]

Substitution of equation 36 and 39 into equation 62 gives an equation for \( D \)

\[ D = \frac{[ML_{2}^{2-}]}{[M^{2+}]_m} \times \frac{1}{1 + \beta \times [L^{2-}] + \beta \times [L^{2-}]^2} \quad (63) \]

Combination of equation 58, 61 and 63 gives

\[ D' = K_2 \times \frac{[HL^{-}]^2}{[H^{+}]^2} \quad (21) \]
Appendix D

D' vs. $[HL^-]/[H^+]$ In OTAB+ HQS System
Figure 62. \( D' \) vs. \([\text{HL}^-]/[\text{H}^+]\) in 2 \(\times\) 10\(^{-4}\) M OTAB+ HQS System.
Figure 63. $D'$ vs. $[\text{HL}^-]/[\text{H}^+]$ In $3 \times 10^{-4}$ M OTAB+ HQS System.
Figure 64. $D'$ vs. $[\text{HL}^-]/[\text{H}^+]$ in $4 \times 10^{-4}$ M OTAB+ HQS System.
Figure 65. D' vs. [HL⁻]/[H⁺] In 8 x 10⁻⁴ M OTAB+ HQS System.

Figure 66. D' vs. [HL⁻]/[H⁺] In 1.2 x 10⁻³ M OTAB+HQ System.
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