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Aluminum Speciation Using Fluorescence Quenching

Aluminum Speciation Using Fluorescence Quenching

by

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A Thesis

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Abstract

A noninvasive method using fluorescence quenching (FQ) to determine the conditional stability constants (logK′) for aluminum with naturally occurring organic ligands has been developed. The method utilizes the Stern-Volmer equation to interpret data from ligand fluorescence suppression by aluminum. The total ligand concentration can also be determined using the measured stability constant and the Ryan-Weber equation. The method has been validated with the model ligand salicylic acid; logK′ was found to be 3.5 ± 0.01 vs. 4.0 from the literature. The method was applied to the reference ligand Armadale fulvic acid and chemically realistic values were obtained. In addition, the expected trend of increasing stability constant with decreasing pH was observed. The method was further validated by determining the stability constant for Armadale fulvic acid using an independent technique, PCV colourimetry; the results agreed very well logK′ = 4.7 vs. 4.65 for FQ analysis. Application of the method to whole filtered beaver pond water showed an increasing trend in the stability constant as the dissolved organic carbon (DOC) decreased. The Log of the stability constants were 3.15 ± 0.03, 3.26 ± 0.03, and 3.63 ± 0.02 for DOC concentrations 23, 14, and 10 ppm respectively. The method was also applied to size fractionated waters form lake Skjervatjern in Norway and the expected trend of increasing stability constant with increasing molecular weight was observed.

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1. INTRODUCTION

1.1 Motivation

Aluminum is ubiquitous in the environment; the crust of the earth is composed primarily of aluminosilicates and aluminum is mobilized from the rocks by weathering. Typical natural aqueous aluminum concentrations range from 0 to 400 ug/L (Kramer et al. 1981). Aluminum was once thought to be innocuous but recent evidence has revealed it as a potential environmental concern.

Increased weathering rates due to environmental acidification has increased concentrations of aqueous aluminum in some areas (Campbell 1992). This increased aluminum has been implicated in fish kills (Campbell 1992). In addition, high levels of aluminum can retard the growth of plant roots (Haug 1984). Several human illnesses have been attributed to aluminum; these include, dementia, and bone diseases (Nieboer et al. 1993). In addition, several other disorders may be caused by aluminum ie, alzheimer's disease (Nieboer et al. 1993).

Aqueous aluminum does not only come from weathering, there is also some anthropogenic input of aluminum. Aluminum can enter the environment as industrial particles or from the burning of fossil fuels (Lantzy et al. fide Schlesinger 1991). In addition, aluminum is used in drinking water treatment plants as a flocculant to remove colour from the water.

In aluminum toxicity studies it has been found that aluminum speciation is important to its toxicological effects. For example, the complexation of aluminum by organic ligands can reduce its toxic effects towards fish (Kramer 1993). It is necessary to understand aluminum speciation, especially with regard to naturally occurring organic ligands, in order to better understand and predict its potential toxicity. To determine aluminum speciation, its stability constant(s) with the ligand or ligands in question must be determined.

1.2 Objectives

The need for stability constants for aluminum with natural waters has been established. The aim of this research was to develop a dependable and convenient method to determine the stability constant of aluminum with organic ligands in natural waters. The method chosen was fluorescence quenching.

The objectives of this work were as follows:

(1) To develop a methodology using fluorescence quenching to determine the stability constant for aluminum with fluorescing organic ligands.

(2) To test the fluorescence quenching methodology with an independent methodology; the other method was pyrocatechol violet (PCV) colourimetry.

(3) To test the fluorescence quenching methodology with a model ligand; the ligand was salicylic acid.

(4) To test the fluorescence quenching methodology with a reference sample; the reference used was Armadale Fulvic Acid.

(5) To apply the fluorescence quenching methodology to a variety of natural samples.

1.3 Definition of Stability Constants

For a reaction of the form:

$$aA + bB \rightleftharpoons cC + dD \tag{1}$$

The stability constant K is defined as:

$$K = \frac{(C)^c(D)^d}{(A)^a(B)^b} \tag{2}$$

where the round brackets signify activities. For low ionic strength the activities are about the same as the concentrations; a correction can be made using the Debye-Huckel expression if necessary. In all the equation derivations and application within this work concentrations are used in lieu of activities.

A conditional stability constant (K′) is the stability constant for a given set of conditions of ionic strength, pH, solute and compounds present. Ringbom (1969) has extensively developed the theory of conditional stability constants. Conditional stability constants were determined in this research.

1.3.1 Calculation of Conditional Metal-Ligand Stability Constants

There are many ways to calculate conditional stability constants for natural waters, (Ruzic 1982, Neubecker et al. 1983, Fitch et al. 1984 and Sterrit et al. 1984). In general these methods involve manipulation of titration data where the ligand has been titrated with some metal and a response indicative of free or bound metal has been monitored. The result is a variety of graphical approaches to determine the stability constant and/or the total ligand concentration. These traditional methods have had to be partially abandoned in the present research because the fluorescence signal is an indication of free ligand not free metal. These approaches are still useful though because the PCV methodology can be thought of as measuring free metal. In fact equations derived by Ruzic (1982) were used to interpret PCV results within this report (see section 3.2).

The inorganic aqueous chemistry of aluminum has been extensively studied. There is, however, a disagreement in the hydrolysis of aluminum. In general, aluminum chemistry is often confused by slow kinetics and irreversibility; therefore, equilibration time is often arbitrarily chosen and the resultant stability constants are operationally defined. An excellent review of the chemistry, both organic and inorganic is given by Sposito (1989).

1.4 Aqueous Chemistry of Aluminum

1.4.1 Aluminum Hydrolysis

The hydrolysis of aluminum is reviewed by Pulfer and Kramer (1983). Aluminum hydrolysis is not completely understood but the major mononuclear species are likely Al^{+3}, $Al(OH)^{+2}$, and $Al(OH)_4^-$ (Pulfer and Kramer 1983). The free ion (hexahydrate) predominates at low pH and the tetrahydroxide ion will predominate at high pH. In addition polynuclear species also form such as $Al_{13}O_4(OH)_{24}^{+7}$; the formation of these is quite slow. the polynuclear structures are intermediate between the solution species and the solid species; ie, Keggin type structures. Experimental determination of equilibrium stability constants for aluminum hydroxide species, particularity polymeric forms, has been difficult due to slow kinetics. The formation of mononuclear aluminum species depends only on pH whereas the formation of polymeric species depend on pH and aluminum concentration.

The first hydrolysis of aluminum, the formation of $Al(OH)^{+2}$ occurs according to the reaction:

$$Al^{3+} + H_2O \rightleftharpoons Al(OH)^{2+} + H^{+}$$

The accepted value for the stability constant for the first hydrolysis of aluminum is $\log K \approx 5.0$ (Pulfer and Kramer 1983). Thus at a pH of 5.0 the concentration of free aluminum ion is equal to the concentration of $Al(OH)^{2+}$, ignoring all other species.

Taking into account the available data a useful predominance diagram can be developed for the aluminum hydroxide species mentioned here (Kramer 1993).

Polymeric forms of aluminum in solution can be viewed as initial forms of insoluble aluminum. There are many crystalline forms of aluminum including hydroxides, hydroxyoxides and oxides. If we assume that the most important form is gibbsite, $Al(OH)_3$, we can calculate the solubility of aluminum at a given pH, and using stability constant data can determine the predominate species for a given pH. The result of this, neglecting polynuclear species, see Figure 2, is the standard diagram showing the amphoteric nature of aluminum.

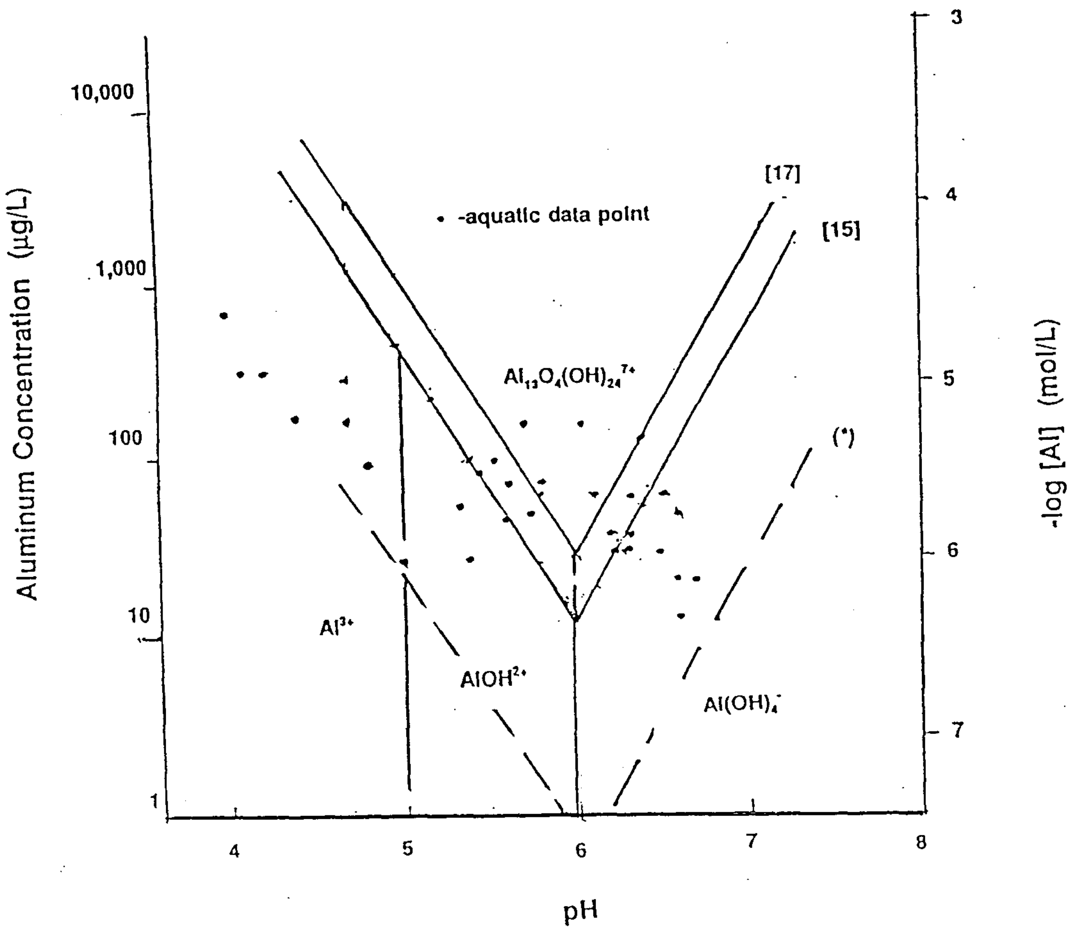

Figure 1: Predominance Diagram for Aluminum Hydrolysis at ionic strength I = 0.001 M. Including two estimates for the stability constant for one Polynuclear Species. (Kramer 1993).

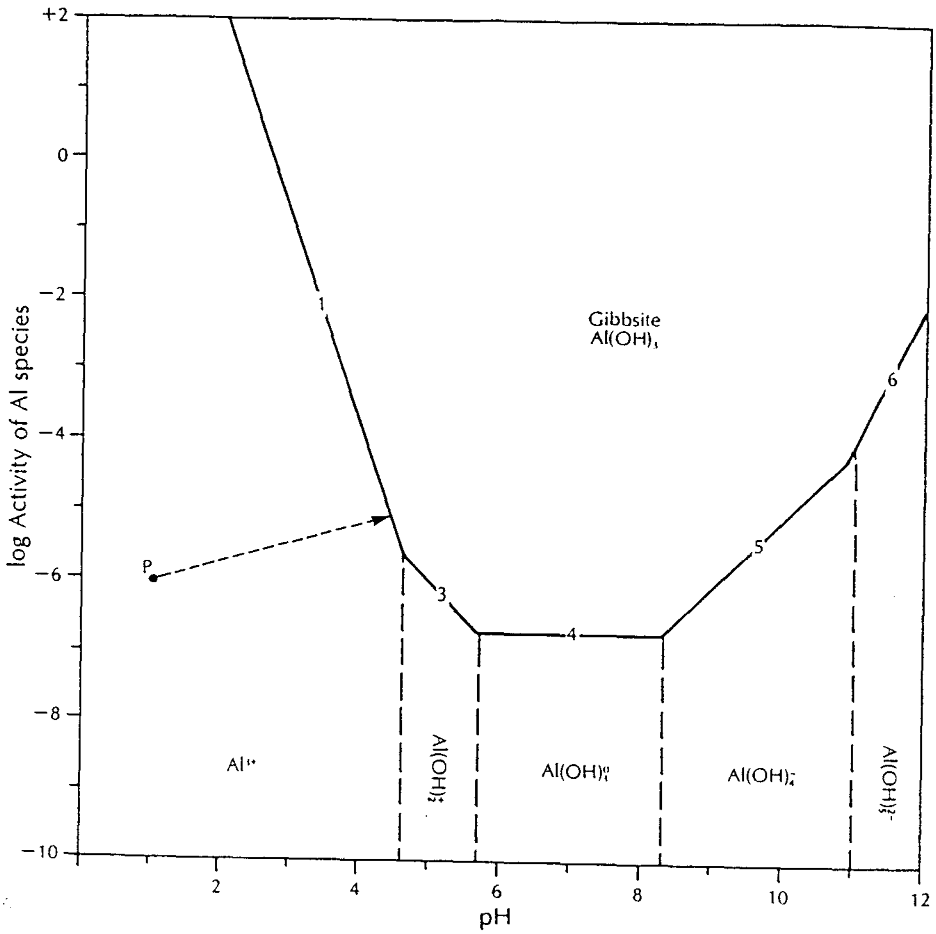

Figure 2: Solubility of Gibbsite, Showing the Amphoteric nature of Aluminum. Enviroments where Gibbsite is soluble have been subdivided to identify the dominant species depending only on pH. (Faure 1991)

1.4.2 Inorganic Ligands

Aluminum can form complexes with inorganic ligands other then hydroxide. Aluminum forms strong fluoro, and phosphato complexes, and weaker carbanato, silicato and sulfato complexes (Kramer 1993). Stability constants for fluoride complexes have been measured using a fluoride selective electrode (Baumann 1964) and for sulphate complexes using calorimetry (Izatt et al. 1969); these constants are summarized by Kramer et al (1981). In addition, the stability constants for iron and corresponding aluminum complexes are related according to the following equation (Kramer et al. 1981):

$$Log K_{Al} = 0.828 / log K_{Fe} - 0.325 \qquad (4)$$

1.4.3 Organic Ligands

Aluminum forms strong complexes with organic ligands, especially with carboxylic groups as in salicylic acid or phthalate. It also binds strongly to phenolic groups as in catechol. Aluminum can for chelate type complexes with ligands such as EDTA. Kramer et al. (1981) give a review of stability constants for aluminum with a variety of organic ligands.

In natural waters aluminum is complexed by organic ligands; these include simple ligands such as salicylate and more complex ligands such as humic and fulvic acid. Kribek et al suggested that high molecular weight organic carbon, ie, humic acid, is the dominant ligand for aluminum (fide Kramer et al. 1981). This is because of the large number of carboxylic groups in higher weight organic carbon. Stability constants for aluminum with ligands in natural waters are summarized in section 1.6.2.

1.5 Experimental Methods to Determine Aluminum Speciation

There are numerous operational methods to define aluminum speciation within a natural water sample. Within each method are defined fractions of the total aluminum; ie, mononuclear (monomeric) aluminum, polynuclear (polymeric) aluminum and organic aluminum. The methods are in general variations of different colourimetric techniques with or without an ion exchange column. Some of the colourimetric reagents that have been used are ferron, PCV and oxine. Sposito (1989) gives a good review of these different speciation methods; in addition an excellent classification of fractionation methods is presented by Clarke et al. (1992).

The ferron method to determine Al speciation has been used by Seip et al. (1984) and Driscoll (1980). It involves colorimetric determination of Al by ferron (8-hydroxy-7-iodoquinoline-5-sulfonic acid). To remove interferences by iron the first step is reduction of iron to Fe^{2+} by $NH_2OH \bullet HCl$ and complexation of this divalent cation by orthophenanthroline. Total Al is determined by acidification (pH 1.5) of the sample followed by addition of acetate buffer (pH 5) and ferron. This method also defines monomeric Al as the immediately reacting Al in an unacidified sample; the polymeric forms would take longer to react.

The PCV method is similar to the ferron method and in a comparison by Seip et al. (1983) was found to be the superior of the two methods. The PCV method has been automated (Rogeberg et al. 1985 and Royset 1986) and used to determine stability constants for model ligands (Kramer et al. 1994). Again orthophenanthroline is used to remove iron interferences and the pH is buffered; in this case hexamine (hexamethylene tetraamine) buffer (pH 6.0) is used. The main difference between different researchers has been in reaction time, ranging from 4 to 30 min. Total Al is defined by the colourimetric response of an acidified sample, monomeric Al is quickly measured in an unacidified sample. Organic monomeric aluminum is defined (Rogeberg et al. 1985 and McAvoy et al. 1992) using an ion-exchange column. Cation-exchange resins have been used alone (Pott et al. 1985) to determine bound and free Al in natural waters.

Polymeric vs. monomeric aluminum has been defined using timed reactions of 8-Hydroxyquinoline (McAvoy et al 1992 and Clarke et al. 1992). The quickly reacting fraction is monomeric whereas the polymeric forms react slower. Monomeric Al as determined from both the oxine method and the PCV method agrees very well (McAvoy 1992).

Techniques other than colourimetric have been used to determine Al speciation. Fluoride selective electrodes have been used (McAvoy et al. 1992), ^{27}Al NMR has been used to find Al speciation with hydroxy carboxylic acids (Thomas et al. 1993). In addition a suite of papers by Öhman et al (1982) have used potentiometric data to determine aluminum speciation with a variety of organic ligands.

1.6 Al-Stability Constant Determination

1.6.1 Methods to Determine Constants

Conditional stability constants for aluminum with naturally occurring organic ligands have been measured in many different ways. These methods include spectroscopic, potentiometric and column methods. The basic assumption, except where noted, is that the complex formed has one to one stoichiometry.

A classic paper by Schnitzer and Hansen (1970) determines the stability constant for a variety of metals, including aluminum with Armadale Fulvic Acid. They used Job's method of continuous variation and an ion-exchange equilibrium method. The experiments were performed at low pH (3.0) to avoid aluminum hydroxide precipitation. The dissolved organic carbon concentration was around 1000 mg/L which is realistic to what may be expected in soils (Schnitzer and Hansen 1970).

A cation exchange column was also used by Pott et al. (1985) to investigate the binding of aluminum with dissolved organic carbon (DOC) in the pH range from 3 to 5. They also investigated the stability constant for Aldrich humic acid in this pH range using the same method.

Potentiometric methods were utilized by Tipping et al. (1988) to investigate the stability constant for aluminum with aquatic humic substances. They modelled the data based on a aluminum competition with calcium and protons for two carboxylic

Table I. **Summary of LogK′ from the Literature.**

Method	Sample	pH	I	ppm DOC	logK′	Ref.
FQ	CLLE	4.0	0.1	100	4.21	BS
"	"	4.5	"	26	4.8	SS
"	"	"	"	100	4.6	BS
"	"	"	"	132	5.1	SS
"	"	"	"	150	4.8	SS
"	"	5-8	"	100	~ 4.7	BS
CV	ARFA	3	"	~ 1000	3.7	SH
"	"	2.35	0.15	"	5.3	"
"	"	"	0.0	"	2.9	"
IE	"	3	0.1	"	3.7	"
"	AIRH	3-5	0.0001	2	~ 6.8	PT
PT	HUMS	3-6	0.001-0.1	10-100	3.4-3.8	TH
"	WBGW	2.9-4.3	0.1	-	1. 4.4 2. 4.2	LO
"	ABGW	3.0-4.2	"	-	1. 4.1 2. 4.7	"

Method:

CV = continuous variation
FQ = fluorescence quenching
IE = ion equilibration column
PT = potentiometric titration

Sample:

CLLE = chestnut leaf litter extract
ARFA = Armadale fulvic acid
AIRH = Aldrich Humic Acid
HUMS = Humic Substance
WBGW = winter bog water
ABGW = autumn bog water

References

BS = Blaser and Sposito 1987
SS = Shotyk and Sposito 1990
SH = Schnitzer and Hansen 1970
PT = Pott et al. 1985
TH = Tipping and Hurely 1988
LO = Lövgren and Öhman 1987

type sites and two weakly-acidic groups. They performed their experiments as an acid base titration in the presence of aluminum over the pH range 3 to 6. Another model for aluminum binding to humic materials has been put forward by Taugbøl et al. (1994) where aluminum binds in a one to two ratio with the ligand (AlL_2) and competes with hydrogen ion.

Fluorescence quenching has been applied by to determine stability constants for aqueous chestnut leaf litter extract with aluminum by Shotyk and Sposito (1988). They interpreted their data using the Ryan-Weber equation.

1.6.2 Summary of Literature Values

Comparison of stability constants determined by different methods is difficult because the constant is operationally and conditionally defined. Table I summarizes the experimental results in determining logK′ for a variety of natural ligands.

1.7 Metal Humic Binding Using Florescence Quenching

1.7.1 Fluorescence

The concept of fluorescence is discussed fully by Guilbault (1990). Stokes fluorescence occurs when a molecule adsorbs a photon of a given wavelength, called the excitation wavelength and emits a photon of longer wavelength, called the emission wavelength. The concept can be understood from Figure 3. Where a molecule in a singlet ground state (S_o) is promoted to some excited singlet (S_2) and, after nonradiative transitions through closely spaced energy levels, arrives at the first

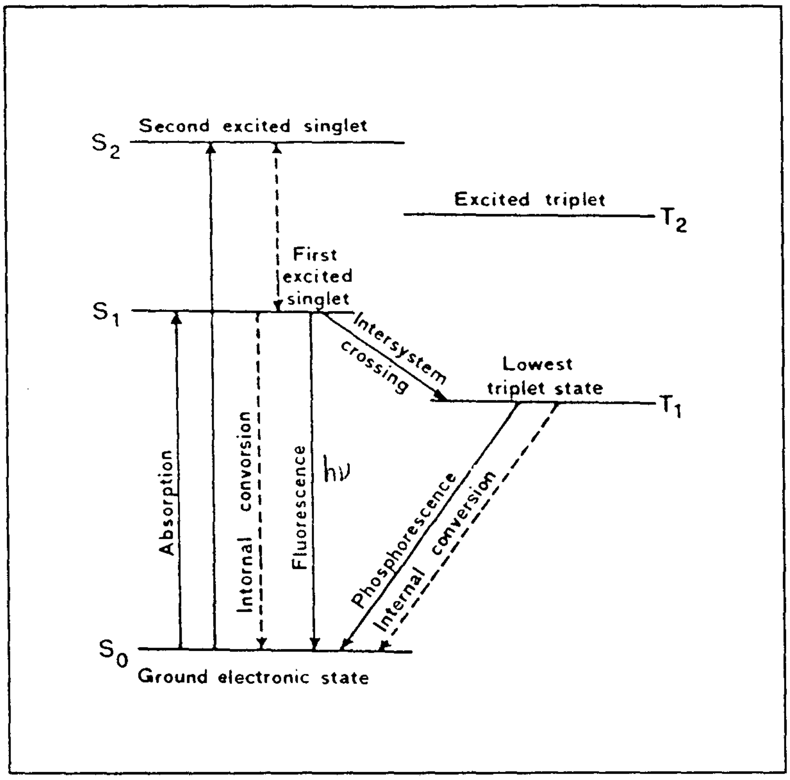

Figure 3: **Schematic representing molecular interactions with light. (Guilbault 1990)**

excited singlet (S_1) where a photon (hv) is emitted in transition back to the ground state. The emitted photon can in fact be the same wavelength as the incident photon in resonance fluorescence and of longer wavelength in Anti-Stokes fluorescence. Phosphorescence occurs when the excited singlet undergoes internal conversion (forbidden transition) to a triplet state (T_1) were the photon is then emitted as the molecule decays to the ground state; this is effectively time-delayed fluorescence because the internal conversion takes more time ie, minutes vs microseconds for fluorescence.

Most molecules (X) do not fluoresce because nonradiative relaxation from the excited state (X^{*}) occurs at a rate exceeding radiative relaxation, 10^{-15}s vs 10_{-5}s for fluorescence. The adsorption of a photon occurs:

$$X + h\nu \rightarrow X^{*} \tag{5}$$

and the energy is dissipated as heat through intermolecular collisions; including collisions with solvent molecules:

$$X^{*} \rightarrow X + heat \tag{6}$$

Alternatively when fluorescence occurs:

$$X^{*} \rightarrow X + h\nu' \tag{7}$$

some new photon with energy $h\nu'$ is emitted. Fluorescence phenomena only occurs in very special molecular structures that slow the rate of nonradiative relaxation. The structures that are most suited towards florescence are aromatic rings, especially when fused; in addition, highly conjugated double bonds and carbonyl compounds may fluoresce.

There are fluorophores in naturally occurring waters. These are fluorescing molecules and fluorescent groups within macromolecules. For example the simple ligand salicylic acid shows strong fluorescence, and groups within fulvic and humic acid show florescence. Humic and fulvic acid are operationally defined extractions of organic macromolecules from water or soil; humic acid is base but not acid soluble whereas fulvic acid is soluble in both acid and base. Humic acids are higher molecular weight. These organic acids contain phenolic (-OH) and carboxylic groups (-COOH). The fluorescence of humic acid can be understood by examining a possible structure proposed by Christman et al. (1989) for a hypothetical fresh water humic acid as shown in Figure 4.

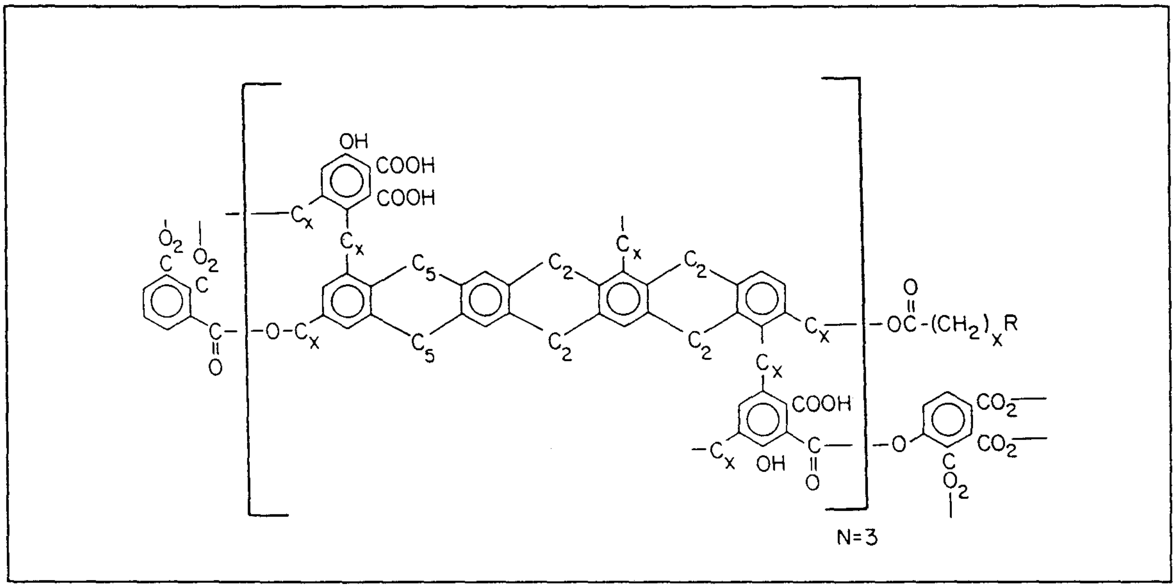

Figure 4: Model structure of humic acid demonstrating the presence of potentially fluorescing groups. (Christman et al. 1989)

The concept of fluorescence spectroscopy with regards to fulvic acid is reviewed comprehensibly by Senesi (1990).

1.7.2 Fluorescence Quenching

If fluorescence does occur it can be suppressed by many different mechanisms. Fluorescence decreases with increasing temperature because the number of intermolecular collisions increases and thus the nonradiative transitions increase. Also, if phosphorescence occurs it effectively quenches the fluorescence signal. In addition, the presence of quenching species can also reduce fluorescence. Quenching species include paramagnetic (unpaired electrons) metals, high atomic weight metals, some organic species and oxygen. Diamagnetic (paired electrons) metals do not quench fluorescence well unless they are heavy ions such as Pb^{2+}. There are three mechanisms whereby a quencher suppresses fluorescence; these are static, dynamic and apparent quenching. Apparent quenching is when the species added or a complex formed adsorbs at the excitation or emission wavelengths making it appear that fluorescence is suppressed; this is called the inner filter effect. Dynamic quenching is the result of a collision between the quencher and the excited fluorophore causing nonradiative relaxation. Static quenching is a result of a ground state association between the quencher and the fluorophore. Figure 5 illustrates the different potential quenching mechanisms.

In the case of aluminum the hydrated trivalent cation is diamagnetic and light (26.98154 g/mol vs 207.2 g/mol for lead) and therefore would not quench fluorescence well. Fluorescence can be suppressed by $Al(OH)_x$ compounds though because the aluminum oxygen bond causes paramagnetism. This can be demonstrated using a

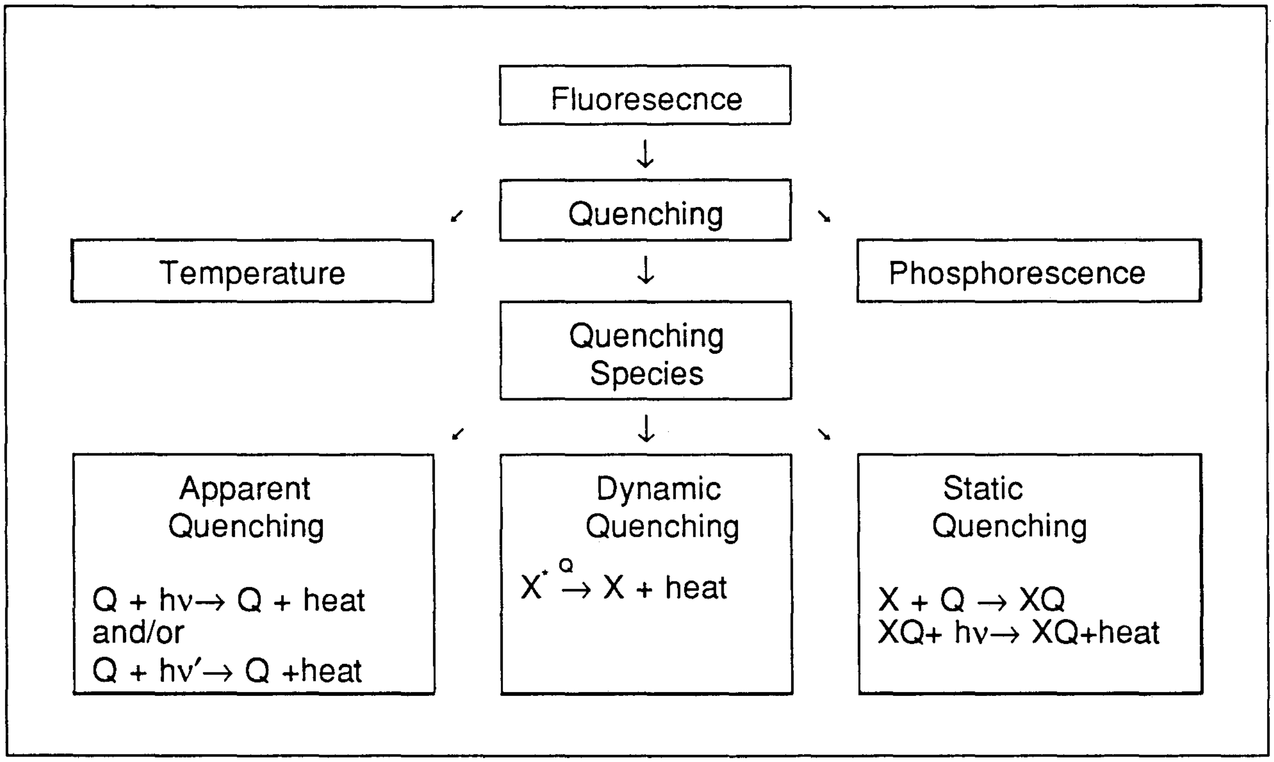

Figure 5. Schematic showing different quenching mechanisms.

simplified molecular orbital approach in which a minimal (valence) basis set is used in a linear combination of atomic orbitals. This is shown schematically in Figure 6 where the 3s and 3p orbitals on aluminum combine with the 2s and 2p orbitals on oxygen; there are 3 valence electrons from aluminum and six from oxygen to total eight electrons.

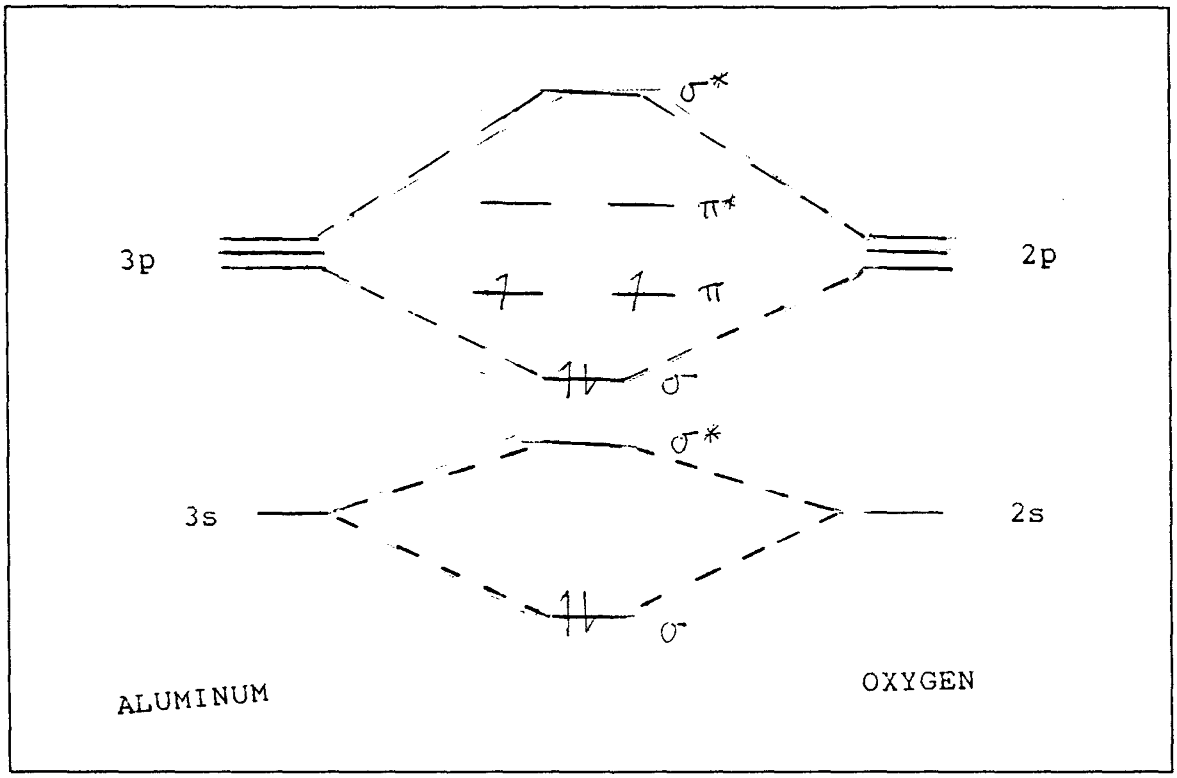

Figure 6: Simplified Molecular Orbital Approach to the Alumium Oxygen bond. Notice that the bond Al is paramagnetic.

1.7.3 Applications of Fluorescence Quenching

Fluorescence quenching is a very useful analytical tool for the investigation of the interaction of potential pollutants with fluoresceing materials, e.g. humic substances. Two classes of anthropogenic materials have been investigated via fluorescence quenching; polycyclic aromatic hydrocarbons and aqueous metals. In the case of polycyclic aromatic hydrocarbons the fluorophore is the hydrocarbon and its fluorescence is quenched by humic materials. In the case of metals the fluorophore is the humic material and the quencher is the metal cation.

Quenching data have been interpreted by the Stern-Volmer approach (Stern and Volmer 1919) and by the nonlinear parameter fitting model of Ryan and Weber (1982). There have been varying degrees of success with both methods. Polycyclic aromatic hydrocarbon fluorescence quenching experiments have been interpreted more often via the Stern-Volmer equation and metal experiments have been interpreted more often by the Ryan-Weber equation. In addition, biological macromolecule fluorescence quenching has been interpreted via the Stern-Volmer equation (Carraway et. al 1991 and Eftink et al. 1976).

The Stern-Volmer Equation is:

$$\frac{F_o}{F} = 1 + K[M] \tag{8}$$

Where F_o is the fluorescence level of the system before quencher [M] is added. F is the fluorescence intensity and K is the stability constant for a one to one association of quencher and the fluorophore.

The Ryan-Weber equation is:

$$F = \left(\frac{(F_{ML}{}^{o} - 100)}{(2KL_T)}\right)\left[(KL_T + KM_T + 1) - \sqrt{(KL_T + KM_T + 1)^2 - 4K^2L_TM_T}\right] + 100 \tag{9}$$

Where F is the fluorescence, $F_{ML}{}^{\circ}$ is the fluorescence of the bound fluorophore when [ML] is equal to the total ligand concentration, L_T. The stability constant is again K and the total metal concentration is M_T.

1.7.4 Derivation of the Stern-Volmer and Ryan-Weber Equations

The two equations are both based upon developing relationships among ligands, metals and fluorescence signals. The derivations of each equation are presented below along with a comparison of the two methods.

Static quenching is caused by a ground state association (bond) between the fluorophore and the quencher. This is obviously the most useful quenching mechanism in the determination of stability constants. Neglecting the inner filter effect, quenching will, in general, be caused by a combination of static and dynamic mechanisms. In the derivation of both equations, quenching is assumed to be purely static and the inner filter effect is assumed to be negligible.

The derivation of the Stern-Volmer equation is as follows:

Assumption 1: Assume a 1:1 ground state association of the fluorophore (L) and the quencher (M).

$$M + L + \rightleftharpoons ML \tag{10}$$

The conditional stability constant K′ is defined as:

$$K' = \frac{[ML]}{[M][L]} \tag{11}$$

and a mass balance for the fluorophore species can be written:

$$L_T = [L] + [ML] \tag{12}$$

Combine (11) and (12) and rearrange:

$$\frac{L_T}{[L]} = 1 + K'[M] \tag{13}$$

Fluorescence intensity is proportional to concentration, and for nonconcentrated solutions the relationship is linear. **Assumption 2:** The fluorophore concentration is within the linear range of fluorescence intensity vs. concentration. This is a valid assumption for dilute solutions.

Thus the original fluorescence (F_o) is related to the original fluorophore concentration (L_T) by the proportionality constant k.

$$F_o = kL_T \tag{14}$$

Assumption 3: The fluorescence at any time during the titration is proportional only to the unbound fluorophore; the bound fluorophore must not affect the fluorescence.

$$F = k[L] \tag{15}$$

Equation (13) becomes the Stern-Volmer equation on substituting equations (14) and (15):

$$\frac{F_o}{F} = 1 + K'[M] \tag{16}$$

A Stern-Volmer plot is a plot of F_o/F vs [M]; if the Stern-Volmer plot is linear the quenching is either purely static or purely dynamic. For purely static quenching the slope is the conditional stability constant (K′).

In practice if no independant method exists to determine the concentration of the free quenching species then an addtitional assumption may have to be used: **Assumption 4:** The concentration of the free quencher is approximately equal to the concentration of the added quencher. The amount of bound quencher is negligible. This assumption is valid for small values of K′.

In dynamic quenching the stability constant K is replaced by a product of the bimolecular quenching rate constant (κ_q) and the fluorescence lifetime (τ_q) of the fluorophore in the absence of quencher. The Stern Volmer equation becomes:

$$\frac{F_o}{F} = 1 + \kappa_q \tau_q [M] \tag{17}$$

The derivation of this is shown in Parker (1986).

The equation derived by Ryan and Weber (1982) has been used many times in interpretting fluorescence quenching data (see section 1.7.7). The derivation of this equation is presented below:

Assumption 1: The ligand and the metal form a simple 1 to 1 complex according to equation (10) and thus the stability constant is defined as in equation (11) except the free ligand concentration ([L]) is expressed as L_T - [ML].

$$K = \frac{[ML]}{[M][L_T - [ML]} \qquad (18)$$

and mass balance for the metal

$$[M] = M_T - [ML] \qquad (19)$$

Combination of equations (18) and (19) yields a quadratic equation in [ML].

$$[ML] = \frac{K(M_T + L_T) + 1}{2K} \pm \frac{\sqrt{K^2(L_T^2 + 2L_TM_T + L_T^2) + 2K(M_T + L_T) + 1 - 4K^2M_TL_T}}{2K} \qquad (20)$$

In equation (20) the second term is chosen to be negative because if it were positive the value for [ML] would exceed M_T and be physically meaningless. Thus the equation can be rewritten as:

$$[ML] = \frac{K(M_T + L_T) + 1}{2K} - \frac{\sqrt{K^2(L_T^2 + 2L_TM_T + L_T^2) + 2K(M_T + L_T) + 1 - 4K^2M_TL_T}}{2K}$$

(21)

Ryan and Weber (1982) observed that fluorescence is not necessarily quenched completely on metal binding to the fluorophore. In fact, there is a maximum amount of quenching beyond which fluorescence will not decrease; Ryan and Weber define this as $F_{ML}°$.

Assumption 2: The overall fluorescence intensity is given by the sum of the fluorescence from the free and the bound ligand, F_L and F_{ML} respectively.

$$F = F_L + F_{ML} \tag{22}$$

Fluorescence is related to concentration (c) by the following equation:

$$F = \phi I_o(1 - e^{\varepsilon bc}) \tag{23}$$

Where Φ = the quantum efficiency of the fluorophore, I_o is the incident power, b is the path length and ε is the molar absorbtivity. This equation becomes linear for dilute solutions, $\varepsilon bc < 0.5$. This can be shown by using a polynomial expansion of the exponential term:

$$F = \Phi\ I_o \left[1 - \left(1 - (-\varepsilon bc) + \frac{(-\varepsilon bc)^2}{2!} + \frac{(-\varepsilon bc)^3}{3!} + \ldots\right)\right] \tag{24}$$

and equation (24) reduces to

$$F \sim \Phi I_o \varepsilon bc \tag{25}$$

for $\varepsilon bc \leq 0.5$.

Assumption 3: That the concentration for L and ML is within the linear fluorescence range. If this is true than the following is true:

$$\begin{aligned} &F = k_1[L] + k_2[ML] \\ &\text{where } k_1 = \Phi_L I_o \varepsilon_L b \\ &\text{and } \quad k_2 = \Phi_{ML} I_o \varepsilon_{ML} b \end{aligned} \tag{26}$$

The fluorescence of the end members can now be defined. The fluorescence of the ligand alone is:

$$\begin{aligned} &F_L{}^o = \Phi_L I_o \varepsilon_L L_T \\ &\text{so } \frac{F_L{}^o}{L_T} = \Phi_L I_o \varepsilon_L b = k_1 \end{aligned} \tag{27}$$

The fluorescence of the bound ligand alone; when $L_T = [ML]$, is given by:

Now that k_1 and k_2 are known equation (26) can be rewritten:

$$F_{ML}{}^{o} = \Phi_{L} I_{o} \epsilon_{L} b L_{T}$$

$$\text{so } \frac{F_{ML}{}^{o}}{L_{T}} = \Phi_{L} I_{o} \epsilon_{L} b = k_{2} \tag{28}$$

$$F = \frac{[L]}{L_{T}} F_{L}{}^{o} + \frac{[ML]}{L_{T}} F_{ML}{}^{o} \tag{29}$$

This equation can then be rearranged to yield:

$$\frac{[ML]}{L_{T}} = \frac{F_{L}{}^{o} - F}{F_{L}{}^{o} - F_{ML}{}^{o}} \tag{30}$$

Finally equation (21) is substituted into equation (29), (30) to yield the Ryan-Weber equation when $F_L{}^{\circ}$ is set to 100%:

$$F = \left(\frac{(F_{ML}{}^{\circ} - 100)}{(2KL_{T})}\right)\left[(KL_{T} + KM_{T} + 1) - \sqrt{(KL_{T} + KM_{T} + 1)^{2} - 4K^{2}L_{T}M_{T}}\right] + 100 \tag{31}$$

1.7.5 Comparison of the Stern-Volmer and Ryan-Weber Equations

If the fluorescence of the bound ligand is negligible then equation (29), (30) of the Ryan-Weber derivation becomes:

$$F = \frac{[L]}{L_T} F_L{}^{o} \tag{32}$$

Upon substitution of the mass balance equation (12) for the ligand and the stability constant as defined in equation (11) this becomes the Stern-Volmer equation.

The main difference between the two is that Stern-Volmer equation ignores the potential fluorescence of the bound ligand. In addition, the Stern-Volmer equation is a function of free metal (assumed approximately equal to total added metal) whereas the Ryan-Weber equation is a function of metal added; this brings in the quadratic term.

The two equations have several assumptions in common when they are applied to complicated organic ligands such as fulvic acid. The assumptions for application of either equation to naturally occurring organic ligands are (after Ryan and Weber 1982):

1. The material in the sample that fluoresces is representative of the sample.
2. The bulk of the material behaves as one ligand with average properties represented by K and L_T.
3. One to one complex stoichiometry.
4. The fluorescence is a linear function of fluorophore concentration.

1.7.6 Fluorescence Quenching of Polycyclic Aromatic Hydrocarbons

Fluorescence quenching of polycyclic aromatic hydrocarbons by humic material has been interpreted with varying degrees of success by the Stern-Volmer equation (Gauthier et al. (1986), and Puchalski et al. (1992)). The quenching of anthracene, phenanthrene and pyrene with humic acid resulted in linear Stern-Volmer plots (Gauthier et al. (1986)) but experiments on difenzoquat and 1-naphthol by Puchalski et al. (1992) resulted in nonlinear Stern-Volmer plots. The stability constant of complex formation can be determined with greater confidence from linear Stern-Volmer plots than if the plot is nonlinear. Special Techniques can be invoked to interpret nonlinear Stern-Volmer plots (Eftink et al. (1976) but the results can still be ambiguous (Puchalski (1992)).

1.7.7 Fluorescence Quenching of Humic Materials with Metals

The Ryan and Weber equation is generally used to investigate metal - humic interactions (Ryan and Weber 1982, 1983); the metals analyzed have been Cu, Mn, Co and Pb. The equation has also been used to investigate aluminum by Sposito et al. (1987, 1988, 1990). The Ryan and Weber equation has been criticized by Cabaniss and Shuman (1988) as being invalid and not in fact predicting a stability constant but just finding parameters to fit fluorescence data. They also believe that the three parameters are not independent. Ryan (1990) defended the use of the equation.

Fluorescence has also been used to investigate electrostatic properties of humic substances (Green et al. 1992) and the kinetics of aluminum-fulvic acid complexation (Plankey et al. 1987). Fluorescence has been used to investigate alumium interactions with organic matter. Cabaniss (1992) utilizes synchronous scan fluorescence to investigate metal (including Al) fulvic interactions. Tam and Sposito (1993) investigate the effects of humification and aluminum complexation on aqueous pine litter extracts.

1.8 PCV Method

Pyrocatechol violet (PCV), 3,3′,4-trihydroxyfuchsone-2″-sulfonic acid, is a colourimetric reagent for aluminum. PCV is a tetraprotic acid; the structure and of the PCV molecule and its ionization from H_4L to H_3L^- is illustrated in Figure 6. According to the CRC Handbook of Organic Analytical Reagents (1982) the coloured complex with aluminum is limited to a narrow pH range about 6.0 and an adsorbance maxima occurs at 580 nm. PCV is a strong acid with respect to its first ionization; the IUPAC chemical data series No. 22 (1979) gives its pK_a values at 25°C with respect to further ionization as follows: $H_3L^- = 7.8$, $H_2L^{2-} = 9.8$ and $HL^{3-} = 11.7$. In addition the stability constants for stepwise coordination of Al(III) to L^{4-} are given as $\log K_1 = 25.1$, $\log K_2 = 22.3$ and $\log K_3 = 20.7$, for ionic strength 0.2 (KCl) and room temperature. The CRC handbook of Organic Analytical Reagents (1982) gives $\log K_1 = 19.3$ and $\log K_2 = 4.95$.

The basis for the determination of the stability constant using PCV is a ligand competition reaction between the PCV and the aqueous ligands in the sample for the

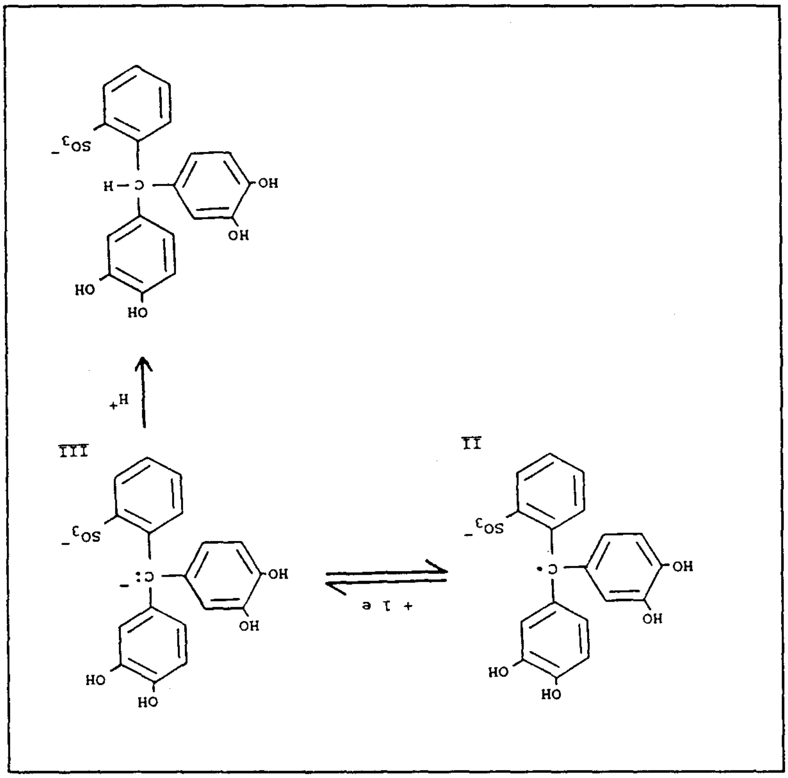

Figure 7: Structure and ionization of Pyrocatechol Violet (PCV) (Vukomanovic et al. 1991).

aluminum (Kramer 1994). The form of the resultant equation, referred to as the JRK equation, is identical to that of Ruzic (Ruzic 1982); a comparison is presented below. Experimentally a solution of PCV with the sample of interest is titrated with aluminum. For a review of PCV usage in aluminum speciation refer to chapter 1.5.

1.8.1 Derivation of JRK Equation

Assumption 1: The aluminum forms complexes with one to one stoichiometry. In a natural water sample there may be more than one ligand so the reaction is defined as:

$$Al + L' \rightleftharpoons AlL' \tag{33}$$

for the total ligands L′ and complexes AlL′. The conditional stability constant (K′) is defined as:

$$K' = \frac{[AlL']}{[Al'][L']} \tag{34}$$

where [L′] is the total ligand concentration minus [AlL′] which is the sum of the aluminum complexed ligand species. [Al′] is the concentration of free reactive Al.

The reaction of PCV with aluminum must also be considered:

$$Al + PCV \rightleftharpoons AlPCV \tag{35}$$

The stability constant for (35) is:

$$K_{AlPCV} = \frac{[AlPCV]}{[Al'][PCV]} \tag{36}$$

and the value of K_{AlPCV} is known. Combination of the reverse of reaction (33) and the forward direction of reaction (35) yields the overall reaction:

$$PCV + AlL \rightleftharpoons AlPCV + L \tag{37}$$

with the overall stability constant:

$$K_{over} = \frac{1}{K'} K_{AlPCV} = \frac{[L][AlPCV]}{[AlL][PCV]} \qquad (38)$$

which contains the conditional constant for aluminum with the unknown ligand(s):

$$\frac{1}{K'} = \frac{[L'][AlPCV]}{[AlL'][PCV]K_{AlPCV}} \qquad (39)$$

To solve for K′ we need to solve for [L′] and [AlL′]; this is done through mass balance considerations. A mass balance for aluminum can be written:

$$[Al_o] + [Al_a] = [AlPCV] + [AlL'] + [Al'] \qquad (40)$$

where: Al_o = the original Al present Al_a = the aluminum added

Assumption 2: Assume that Al_o is negligible.

Assumption 3: Assume that the concentration of free aluminum is negligible compared to the other terms in the mass balance expression.

Using assumptions 2 and 3 equation (40) can be rewritten:

$$[AlL'] = [Al_a] - [AlPCV] \qquad (41)$$

A ligand mass balance can also be written:

$$L_T = [L'] + [AlL'] \tag{42}$$

Equations (41) and (42) can be combined and rearranged to give:

$$[L'] = L_T - [Al_a] + [AlPCV] \tag{43}$$

[AlL'] from (41) and [L'] from (43) can now be substituted into the definition of the stability constant (39) to yield:

$$\frac{1}{K'} = \frac{(L_T - [Al_a] + [AlPCV])[AlPCV]}{([Al_a] - [AlPCV])[PCV]K_{AlPCV}} \tag{44}$$

If we substitute (36), the definition for the stability constant K_{AlPCV}, then (44) can be rewritten:

$$\frac{1}{K'} = \frac{(L_T - [Al_a] + [AlPCV])[Al']}{([Al_a] - [AlPCV])} \tag{45}$$

If we define Y = [Al'] and X = $[Al_a]$ - [AlPCV] then (45) becomes a linear equation of the form:

$$Y = L_T\frac{Y}{X} - \frac{1}{K'} \tag{46}$$

and a plot of Y vs Y/X will yield a straight line with slope L_T and intercept -1/K′. In order to plot this equation we still need the value of [Al′], and can be calculated from the stability constant for AlPCV.

1.8.2 Comparison of JRK and Ruzic Equations

The equation derived by Ruzic (1982) has mathematically the same form as the JRK equation. The form of the equation is identical to (45) but Y is equal to the free aluminum as defined by [AlPCV] and X is equal to the bound aluminum as defined by the difference between the added and free aluminum. The equation is more often stated in the form:

$$\frac{[M_F]}{[M_B]} = \frac{[M_F]}{L_T} + \frac{1}{K'L_T} \tag{47}$$

A plot of free metal over bound metal concentration versus free metal concentration will result in a line with slope $1/L_T$ and intercept $1/(L_TK')$.

Free aluminum is defined as being proportional to the colourimetric signal for [AlPCV] because PCV can be used to operationally define monomeric, labile aluminum (section 1.5). The assumption will tend to overestimate the free aluminum because some organic forms of aluminum will be complexed by the pyrocatechol reagent.

If the stoichiometry of the complex is not one to one then for low metal concentrations the line will deviate from linearity. The equation can be extended to apply to 1:2 metal to ligand complex formation. The total ligand concentration of both ligands then is the reciprocal of the slope of the linear portion of the curve at high metal concentrations and the intercept is one over the product of the total ligand concentration and the overall conditional stability constant. In addition, the two stability constants and ligand concentrations can be found if they are sufficiently different (Ruzic 1982).

2. EXPERIMENTAL METHODS

2.1 Stock Solution Preparation

Solutions of Hexamine, and PCV had to be prepared monthly because they degrade slowly with age; PCV in particular is sensitive to light. They were prepared according to the method given by Røgeberg et al. (1985). PCV was made to be about 10 mM with MilliQ water and stored in polyethylene containers in a dark fridge. Hexamine was made to be 2M by dissolving the solid in MilliQ water and adding ammonium hydroxide to a concentration of 0.25 M; the ammonium ions are the source of the ionic strength, and conc HCl was added for final pH adjustment. Usually the Hexamine solution had to be filtered to remove particulate matter. Hexamine was used as a buffer in both the PCV and the fluorescence quenching methodology. It does not complex aluminum (Røyset 1986) and its pK_a is very near 6.0 so it is an excellent buffer. Its structure is shown in Figure 8.

Stock aluminum solution was prepared by dissolving 0.400 g of aluminum wire in 20 mL of conc HCl and dilution to 1000 mL. Dilutions of this solution (400 mg/L) were used in the titrations.

Armadale fulvic acid solution was obtained by dissolving the solid, stored in a desiccator, in MilliQ water. The usual stock concentration was 30 mg of fulvic acid in 1000 mL of water.

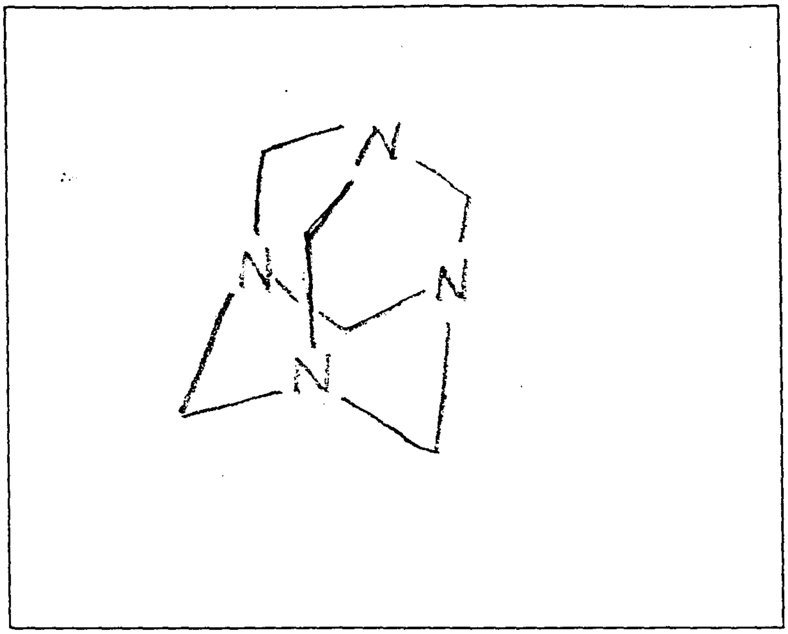

Figure 8: Structure of Hexamine (Hexamethylenetetraamine)

2.2 Description of Samples

2.2.1 Lake Skjervatjern Samples

Lake Skjervatjern is a small lake in Norway (see Figure 8); it is the site of the Humex project (Humex/Humor Newsletter 1993). The Humex project involves artificially dividing a lake with a physical boundary and then acidifying half the lake and its catchment.

Samples were obtained from both sides of the lake; the control and the acidified sides. Two large (about 20 L) samples were taken from the surface of both sides. These were filtered through Millipore Pellicon filtration apparatus on site, and the retentates of different sizes were retained. In addition raw sample was obtained and

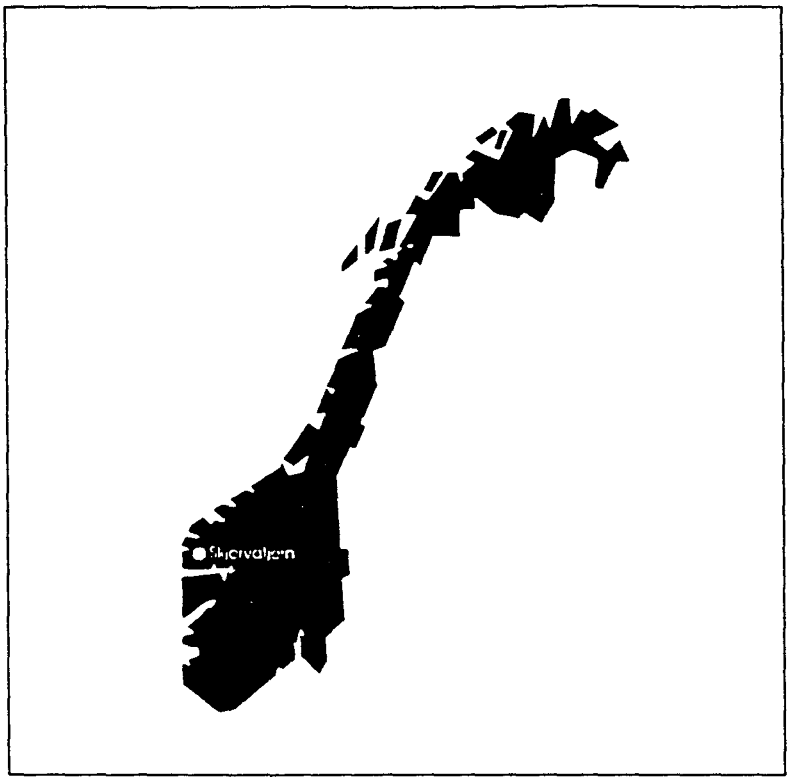

Figure 9: Location of Lake Skjervatjern in Norway.

not processed. The raw samples were filtered through either a 0.45 µm filter (control side) or a 100 000 MW (acidified side). The retentate was reduced by filtration to 0.5 L or less. The water that passed the filter was then processed through the next smaller pore size (sequence 0.45 µm, 100 000 MW, 10 000 MW). Finally a 1 L sample was taken from the water that passed the 10 000 MW membrane.

2.2.2 Manitoba Beaver Pond Samples

Three different beaver ponds near Thompson Manitoba were sampled. The sampling was performed by Dr. R.A. Bourbonniere from the Canada Centre for Inland Waters. They were named GBP-D, MBP-AD and CBP-C according to their source. The locations are as follows:

GBP-D	55°53′N 98°45′E
MBP-AD	50°51′N 98°01′E
CBP-C	55°53′N 98°30′E

Air photos were available for the MBP-AD and the GBP-D sites. The air photos are shown in Figure 9 and Figure 10 the sample locations are marked.

The pH's of the samples were quite high; they were 6.9, 8.0, 9.0 for GBP-D, MBP-AD and CBP-C respectively. Dissolved organic carbon (DOC) decreased from GBP-D to CBP-C; the values were 23, 14, 10 mg/L. The calcium concentration increased from GBP-D to CBP-C; the values were 21.3, 25.1, 27.1 mg/L which correspond to moderately hard waters and could explain the high pH.

2.3 PCV Methodology

Specific experiments are discussed in sections 3.1 and 3.2. Adsorption measurements were made using an autoanalyzer system with a flow-through colorimeter. The sample solutions were mixed with a reagent solution of PCV and hexamine buffer at pH 6.0; the solutions equilibrated in time delay coils for about 15

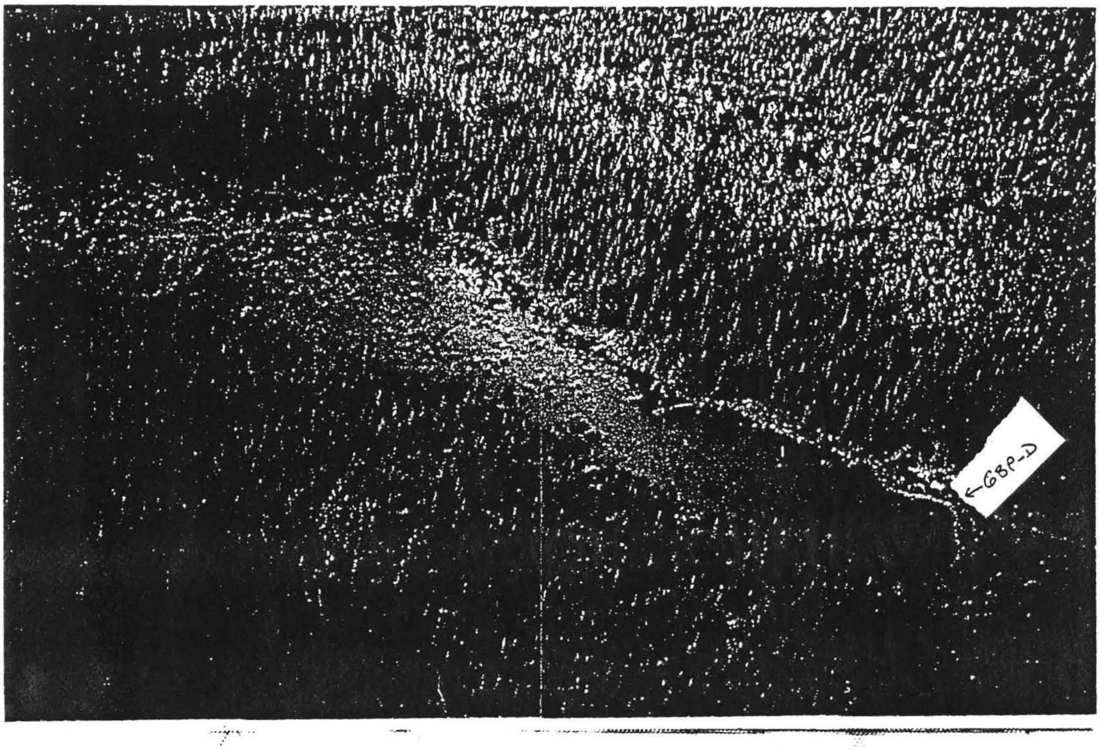

Figure 10: Air Photo of The GBP-D site. Notice the trees still standing at the west end; this is a relatively young beaver pond.

minutes and were then pumped to the detector and through to the waste. For solution preparation see section 2.2 for a more detailed discussion of the PCV method see section 3.2.

2.4 Fluorescence Quenching Methodology

A Turner 110 fluorometer was used equipped with a uv lamp. The excitation wavelength always used was 300 nm corresponding to the corning filter 7-54. The emission wavelengths used were 436nm (4-36 Corning filter) for salicylic acid, Armadale fulvic acid and for the Manitoba beaver pond samples. The Norway samples required a 460 nm filter which had a very narrow range. The wavelengths of the filters have been determined by Sill (1961).

The fluorometer was equipped with a flow through cell and the output was a strip chart recorder. Zero percent fluorescence was defined as the buffer solution at pH 6.0 alone and 100% florescence was defined as the sample alone. The scale was maximized on the strip chart by varying the aperture size and by the use of 1% and 10% neutral density filters. Once the scale was maximized and the 100% fluorescing ligand passing through the ligand solution was titrated with aluminum. Aluminum was delivered via a 10 or 20 µL pipette and pH was monitored using a glass electrode.

The Lake Skjervatjern fractions were diluted to their concentration in the unfractionated water and the stability constant determined. The other samples were titrated at approximately their natural concentration.

3. RESULTS

3.1 Results for Preliminary PCV experiments

The PCV experiments involved measuring the colourimetric response at 590 nm for increasing amounts of aluminum in both blank and fulvic acid solutions. The assumption being that the decreased response in the presence of fulvic acid would be proportional to the amount of aluminum complexed.

The wavelength chosen for absorbance measurement was 590 nm because the aluminum complex adsorbs more than the PCV alone at this wavelength. This was proven in an absorption spectra experiment. Spectra were obtained using a Spectronic 20 colourimeter; 100% transmission was determined with MilliQ water, and equilibration time was about 15 minutes. Spectra were obtained for the following solutions, all at pH 6.0: PCV alone, PCV with Al (1.5 x 10^{-6} mol/L), PCV and Al with Armadale Fulvic Acid (15 mg acid/L) and finally a solution of 15ppm fulvic acid alone. The resultant spectra are shown in Figure 11.

The spectra show that PCV alone has an adsorbance maxima at 445 nm and adsorbs very little at 590 nm but in the presence of aluminum the 445 nm peak decreases and a 590 nm peak appears. In addition, the spectra show that fulvic acid adsorbs very little at 590 nm and that the fulvic acid does not affect the position of the adsorbance maxima for Al-PCV.

The colour formation in the PCV experiments was initially very rapid but its rate decreased slowly with time. In fact, to achieve a constant colourimetric signal the

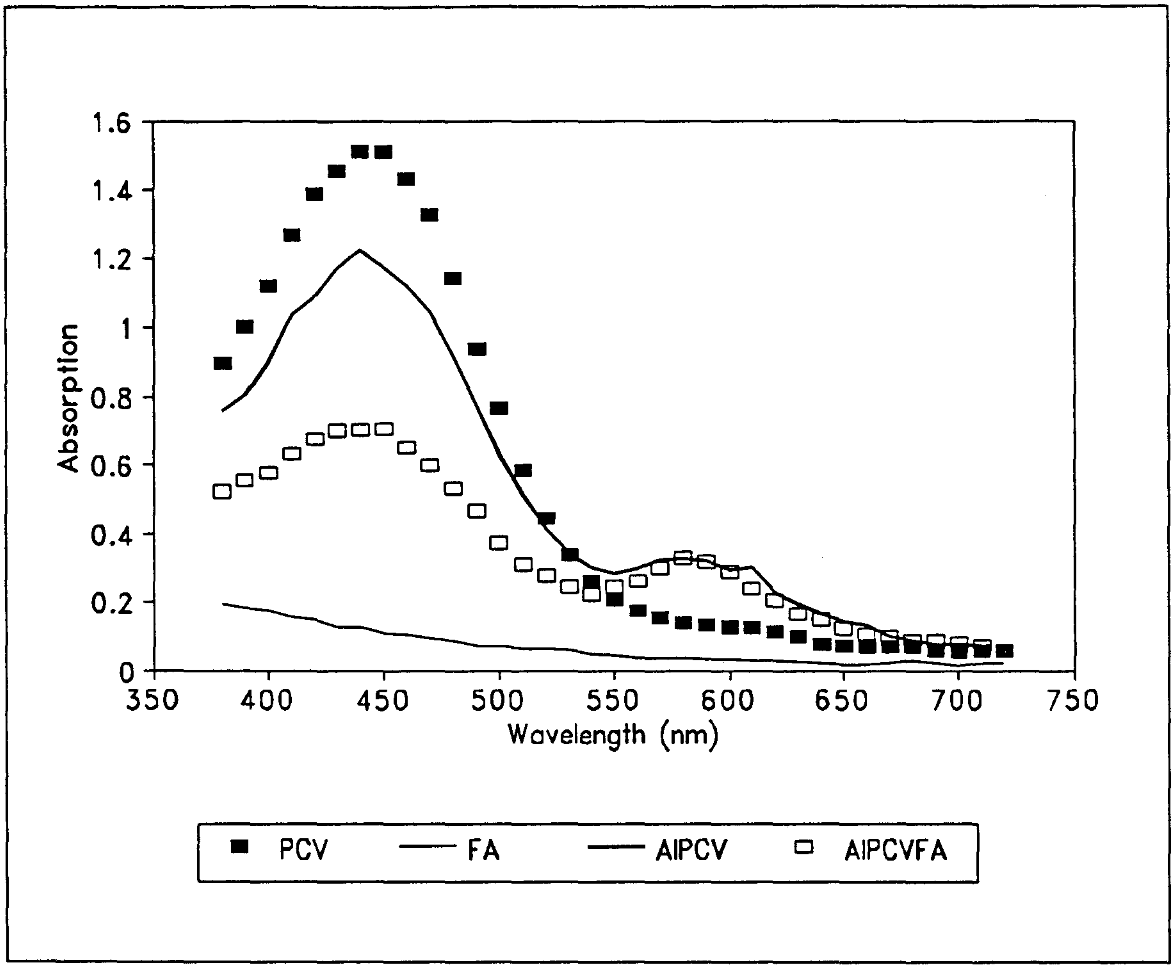

Figure 11: Adsorption Spectra Showing PCV-Al adsorbance at 590 nm. Including the following spectra: PCV alone; PCV and Al; PCV, Al and Fulvic Acid and Fulvic acid alone.

solution had to be equilibrated for 24 hours. When experiments were performed with 24 hr equilibration time it was found that solutions containing fulvic acid adsorbed more than blank solutions; this is opposite the expected trend. This result is possibly explained by PCV slowly binding, via Vanderwaals interactions, to the fulvic acid and the new complex adsorbing more at 590 nm. Alternatively aluminum present in the fulvic acid may have slowly been removed by the stronger PCV ligand. Whatever the explanation a more appropriate equilibration time had to be found.

The rate of colour formation with and without fulvic acid (FA) was measured; the results are shown in Figure 12.

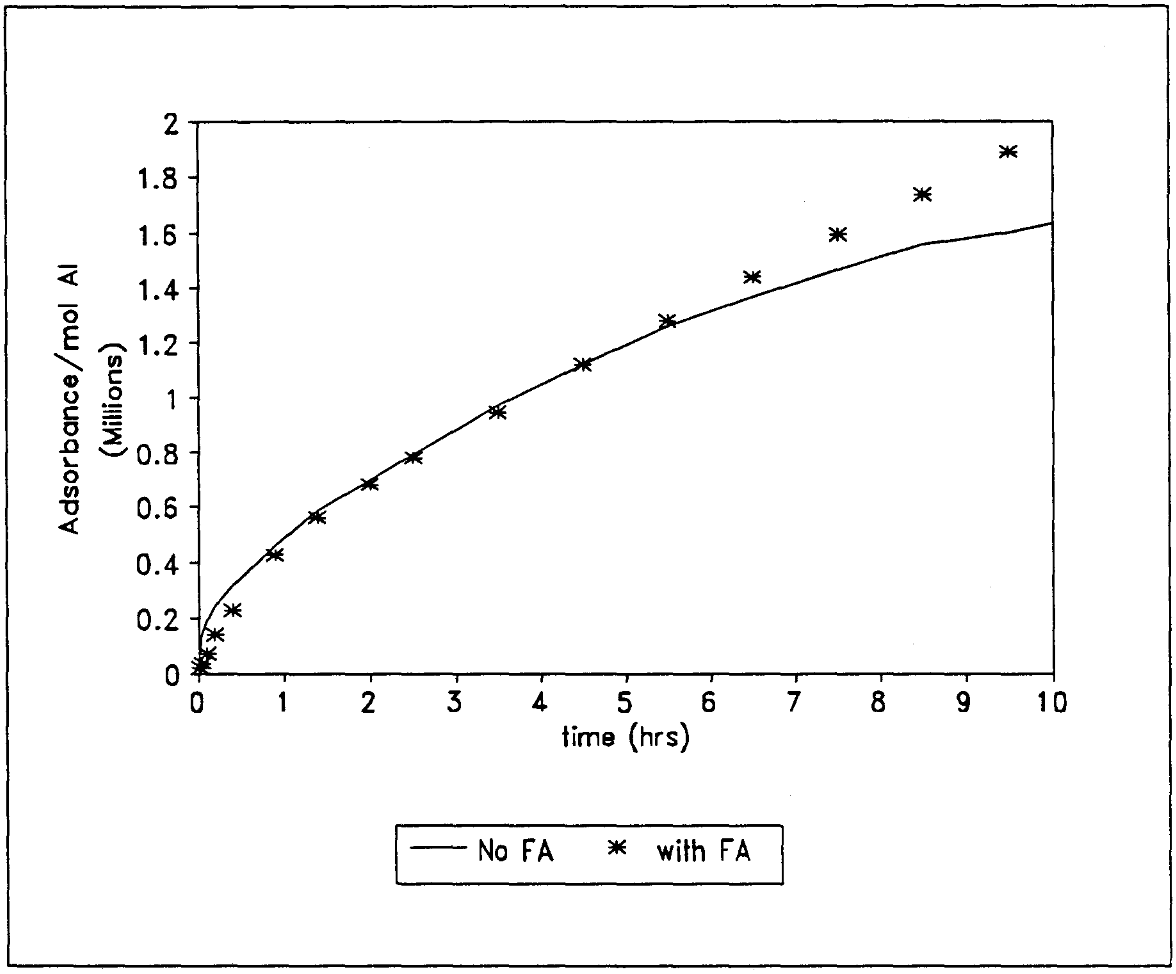

Figure 12: Adsorbance of PCV-Al solutions normalized to the amount of aluminum added, versus time (hrs) for solutions with and without Armadale Fulvic Acid.

Figure 12 shows the expected trend, ie, the fulvic acid solution adsorbing less than the blank solution, for the first hour only. After about one hour the solutions adsorb the same amount of light per mole of aluminum present. Finally after 6 hours the fulvic acid solution continues to have increased adsorption but the blank's adsorbance starts to level off; this is opposite the expected trend. The conclusion from this experiment was to perform all colour measurements after a set equilibration time that was less than an hour.

3.2 PCV method for Al-Fulvate Interactions

An autoanalyser (Gilson Sample Changer model TD1 5T/SC 5) was used in the PCV method to determine the conditional stability constant for aluminum and Armadale Fulvic Acid. The instrument set up is shown in Figure 13; it involves sampling of an aluminum solution, with or without fulvic acid and mixing that solution with buffered PCV reagent and, after a time delay (15 minutes), adsorbance measurement.

The experiment that was performed with fulvic acid involved aluminum concentrations in the range 0 to 3.75 μM in 25.00 mL test tubes. The sampling time was 5 minutes and the rinsing time, with dilute (5%) nitric acid, was 5 minutes. The reagent solution was a 70 (21) μM solution of PCV in a 0.71 (0.21) mol/L hexamine solution. The ionic strength was 0.08 (0.02) mol/L. The values in parenthesis represent the diluted values after mixing with the sample. The dilution was by 0.30 because the sample tube was 0.081″, the reagent tube was 0.045″ and the air tube was 0.030″. Since volume in a tube is proportional to the radius squared we can see that the reagent is diluted by a factor of 0.30 in mixing with the sample.

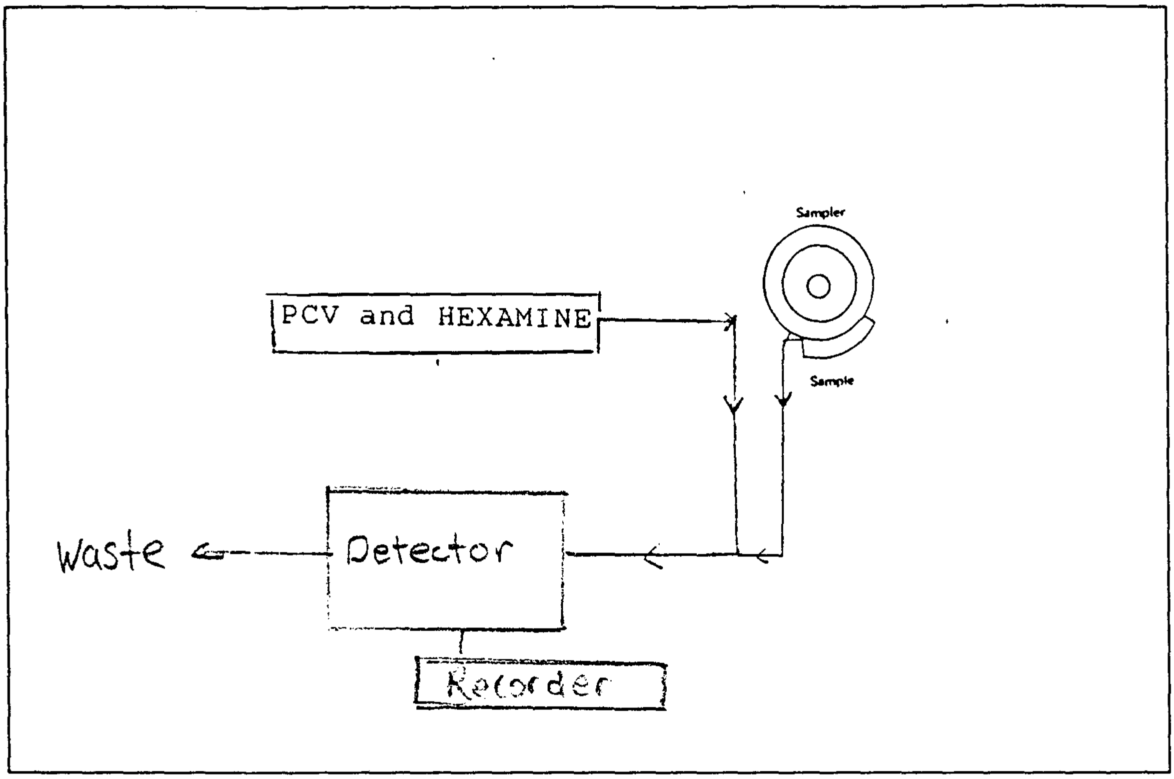

Figure 13: Automatic Method for Measuring [Al-PCV] using a flow-through colourimeter and an autoanalyser.

The colourimetric signal was recorded on a strip chart recorder moving at 10 cm/hr, 100% transmission was set by the PCV solution alone and 0% transmission was set by closing the shutter in the colourimeter. The result was a series of peaks corresponding to the different solutions in the autosampler; the peak height was taken as being proportional to the Al-PCV concentration.

The reproducibility of the apparatus was determined by loading the autoanalyser with aqueous aluminum solutions; the resultant calibration plot along with the 95% confidence interval about the line and the points (see Appendix for statistical formulas used) is shown in Figure 14.

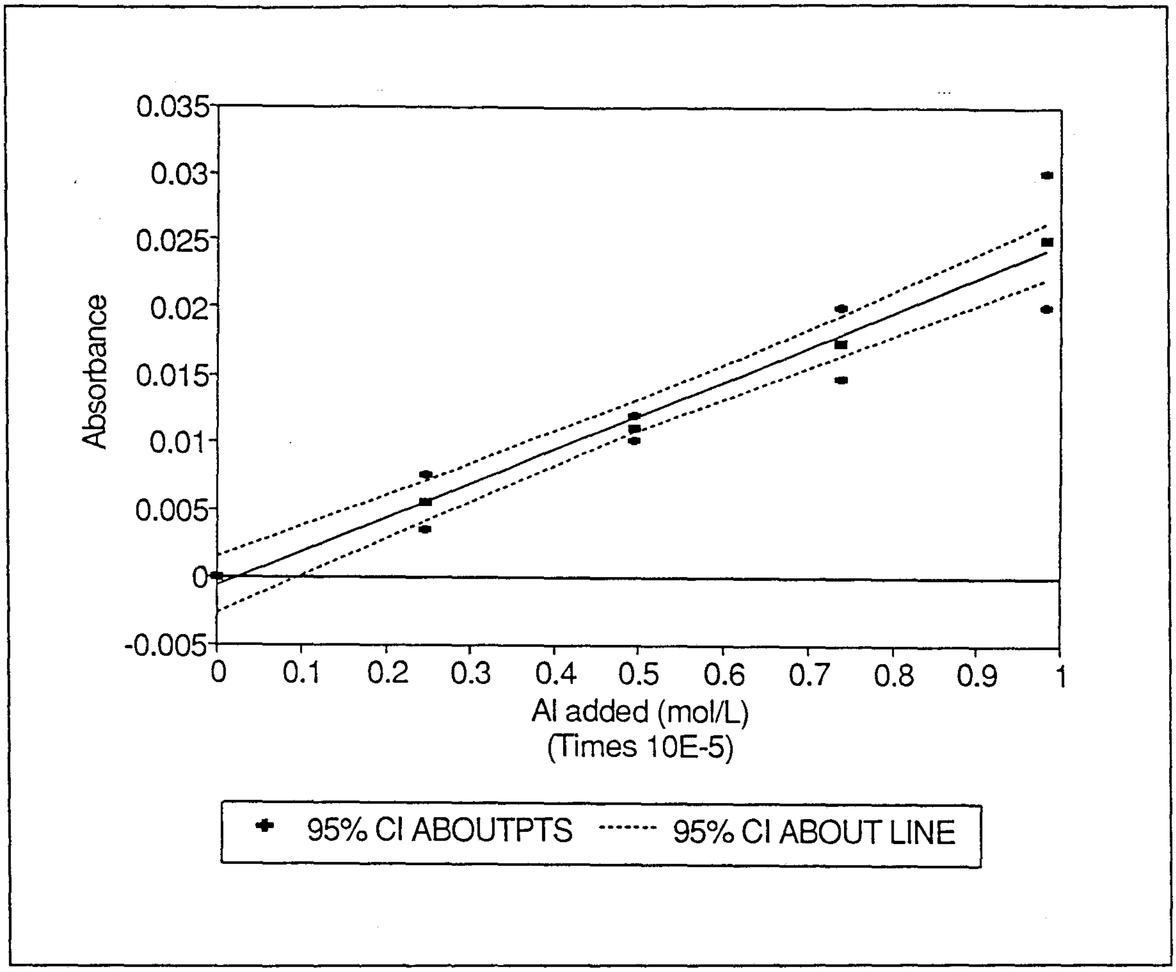

Figure 14: Calibration of the PCV method using the autoanalyser and showing reproducibity for solutions in MilliQ water.

Figure 14 demonstrates that the method is quit reproducible for low aluminum concentrations. The linear dynamic range (LDR) is at least 1 to 5 µM; the detection limit is 0.5 µM; defined as aluminum concentration for response of largest 95% CI in the LDR.

An experiment, with a new calibration plot, was performed with fulvic acid concentrations of 6 mg acid/L. Figure 15 shows the result of this experiment. The expected trend of lower adsorbance for the fulvic acid solution with the same aluminum concentration as the blank was observed. The conditional stability constant was calculated from this plot using both the JRK and Ruzic equations. The results are shown in the calculation section 3.2.1.

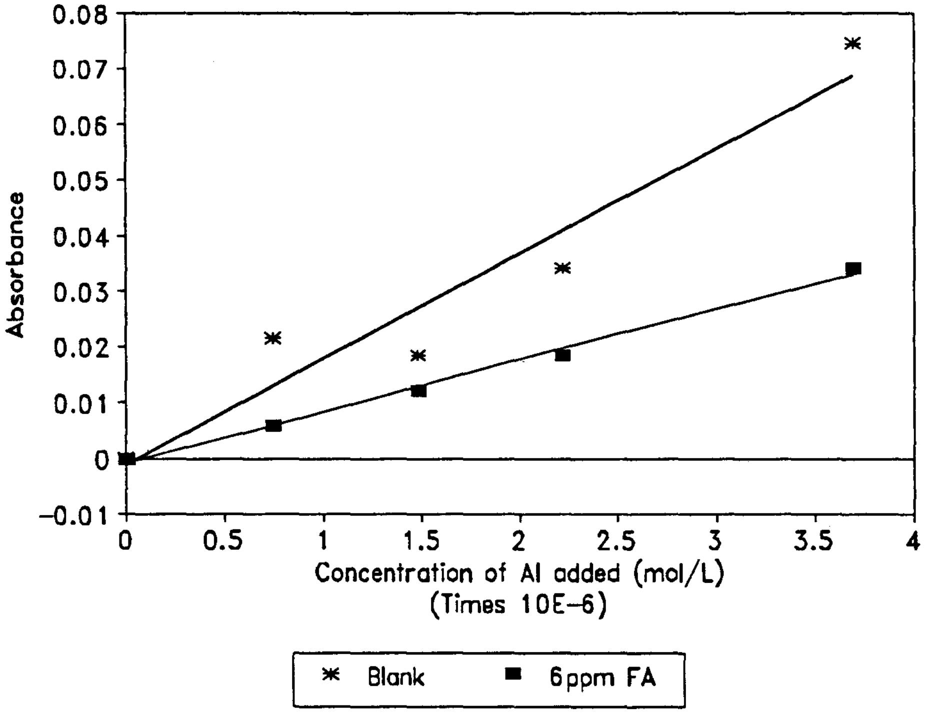

Figure 15: Results using PCV method and an autoanalyser showing decreased Al-PCV adsorbance in the presence of Armadale Fulvic Acid.

3.2.1 Calculations Using PCV Results

The JRK equation (see section 1.8.1) was used to determine the stability constant from the results in Figure 15. The resultant JRK plot is shown in Figure 16; the stability constant, $logK_{AlPCV}$, was taken as 19.3 from the CRC Handbook of Organic Analytical Reagents (1982).

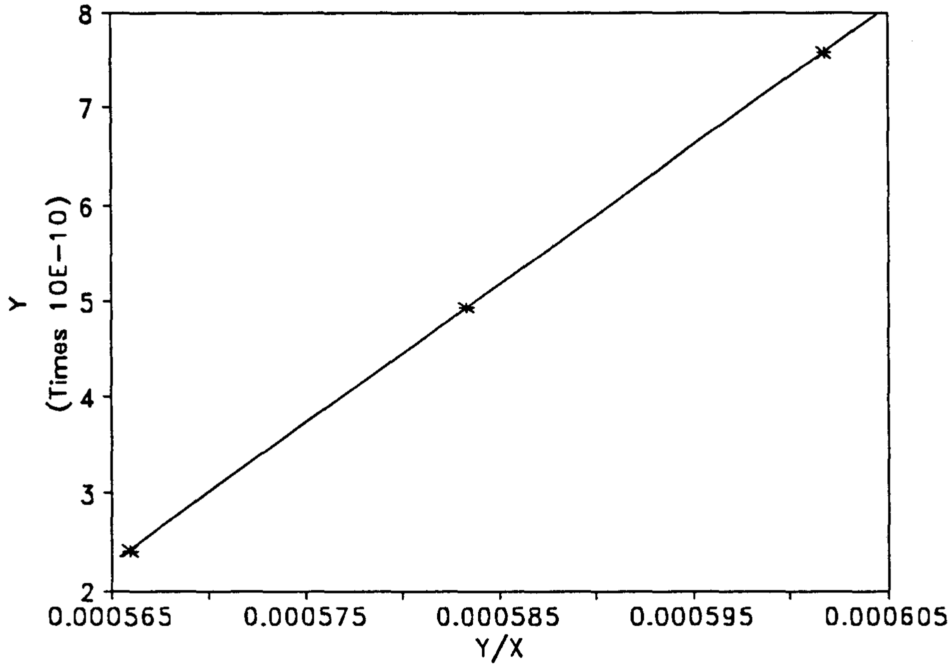

Figure 16: JRK plot to determine the stability constant of aluminum and armadale fulvic acid.

In Figure 16 Y = [Al'], the concentration of free aluminum and

X = [AlPCV] - [Ala], the AlPCV concentration from the analytical signal minus the

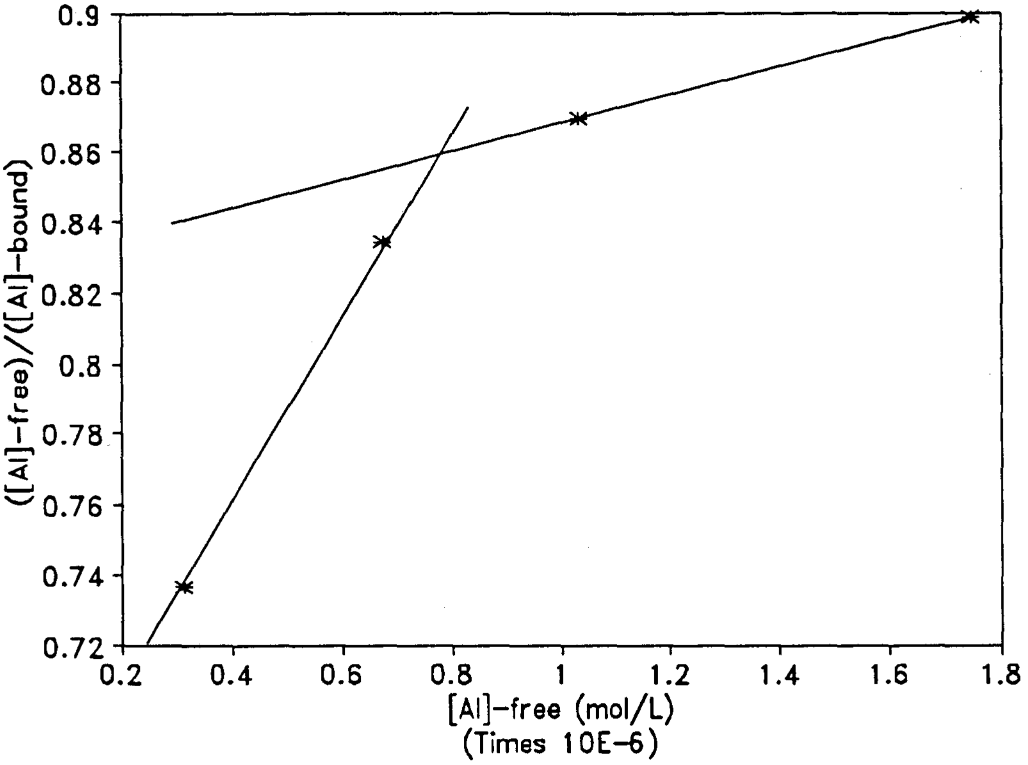

Figure 17: Ruzic plot for determination of Al stability constant with Fulvic acid. The overall stability constant is logK'. = 4.7 and total ligand concentration is 2.5 x 10^{-5} mol/L.

concentration of the aluminum added. The intercept of this graph is -1/K' and is equal to -7.9 x 10^{-9} which yields logK' = 8.1. Also the total ligand concentration L_T is the slope of the graph = 1.44 x 10^{-5} mol/L. Note a fourth point was omitted off this graph because it deviated from linearity; the justification in its removal was that it was at an aluminum concentration outside the linear dynamic range of the calibration; this point was included in the Ruzic plot though.

The Ruzic equation (1.8.2) was also used to analyze this same set of data. The resultant Ruzic plot is shown in Figure 17. In performing the data analysis a two ligand model had to be invoked because the plot was not a straight line. The overall stability constant was calculated via the method given in section 1.8.2 to be logK'. = 4.7 and the total ligand concentration was calculated to be 2.5 x 10^{-5} mol/L. The individual constants K_1 and K_2 could not be evaluated because one would have to be negative and the stability constant, by definition, must be positive.

3.3 Preliminary Fluorescence Quenching Experiments

The preliminary experiments in fluorescence quenching involved obtaining fluorescence emission spectra for the samples with and without aluminum at a pH of 6.0. The emission spectra for Armadale fulvic acid, raw water from the control side of lake Skjervatjern and CBC-C beaver pond were obtained (refer to section 2 for details on samples). The excitation wavelength was 300 nm in all cases and the slit width of excitation and emission was 5 nm in all cases except CBC-C had slit width of 3 nm excitation and emission.

The emission spectra for Armadale Fulvic acid shows a broad peak at 430 nm; this agrees well with the spectra determined by Lévesque (1972). Figure 18 shows suppression of fluorescence in the presence of 50 ppb Al. The suppression is to the left of 430 nm and is approximately a constant amount. For quenching experiments (see section 3.4) the emission filter was chosen as 436 nm based on this figure and

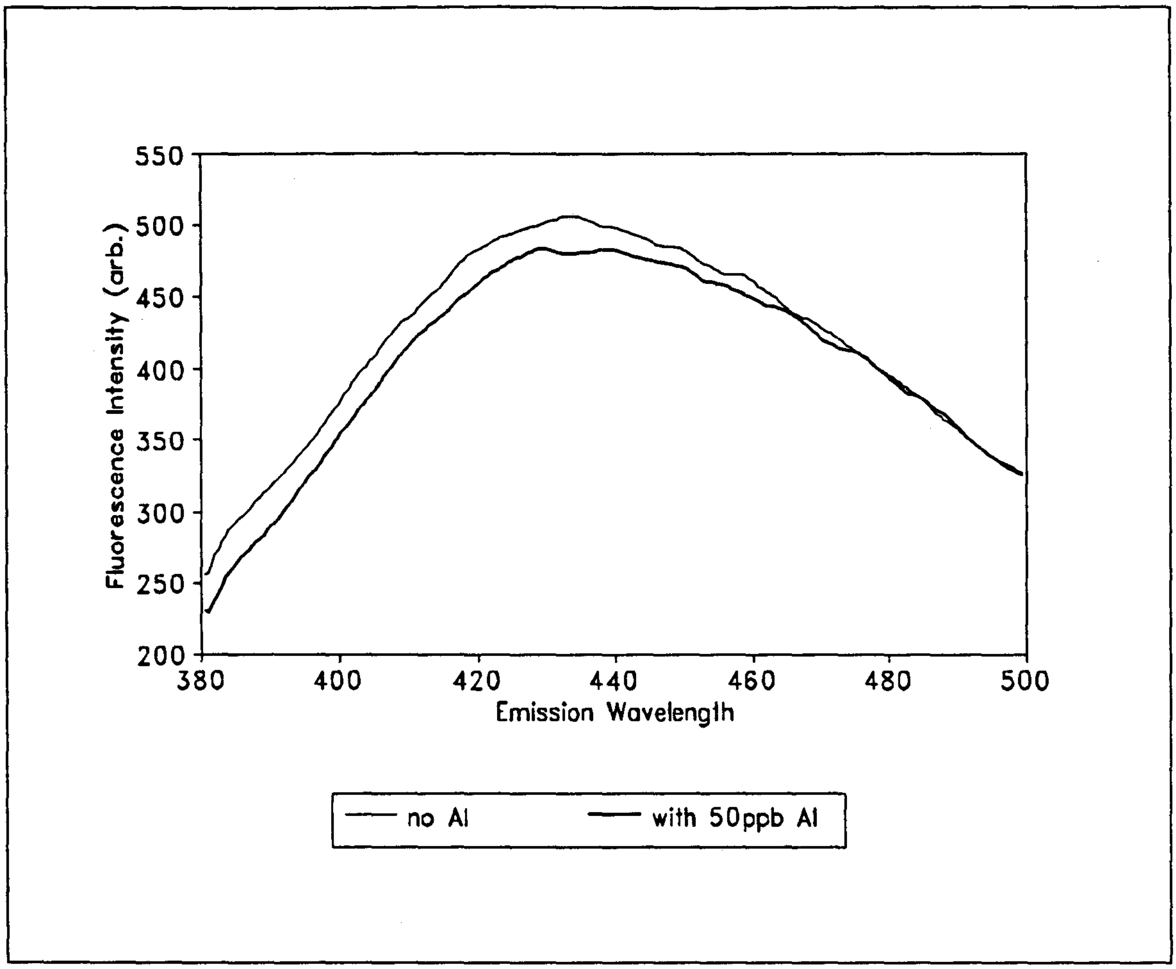

Figure 18: Emission spectra for Armadale fulvic acid with and without 50 ppb Al. Notice that fluorescence is suppressed an approximately constant amount right of 430 nm. Excitation was 300 nm and slit width was 3 nm, and pH was 6.0.

filter availability.

Figure 19 is the emission spectra for lake Skjervatjern waters at pH 6.0 with and without 50 ppb Al. The spectra is similar to that for Armadale fulvic acid; it is a broad band about 430 nm. It differs from the Armadale spectra in that fluorescence is suppressed to the right to 430 nm in this case. In the quenching experiments a 460 nm filter was chosen.

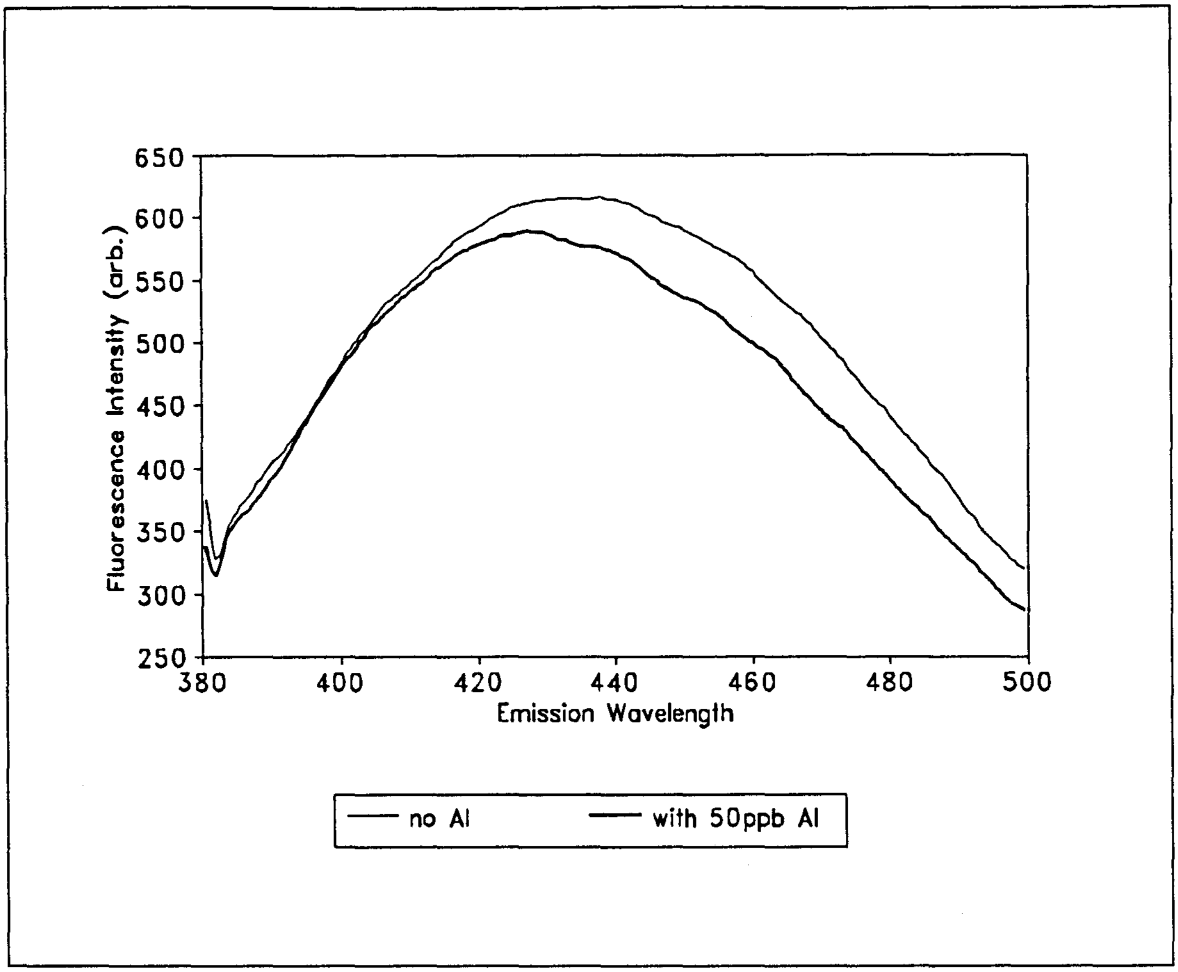

Figure 19: Emission spectra (excitation 300 nm) for Control Side Raw Water Sample from Lake Skjervatjern with and without 50 ppb Al. Notice that fluorescence suppression is approximately constant to the right of 430 nm.

The final emission spectra is shown in Figure 20; it is for CBC-C beaver pond in Manitoba. It is a much more complicated spectra than the other two but it still shows obvious suppression of fluorescence in the presence of 50 ppb Al. A 436 nm filter was chosen for use in quenching experiments based on this spectra and filter availability.

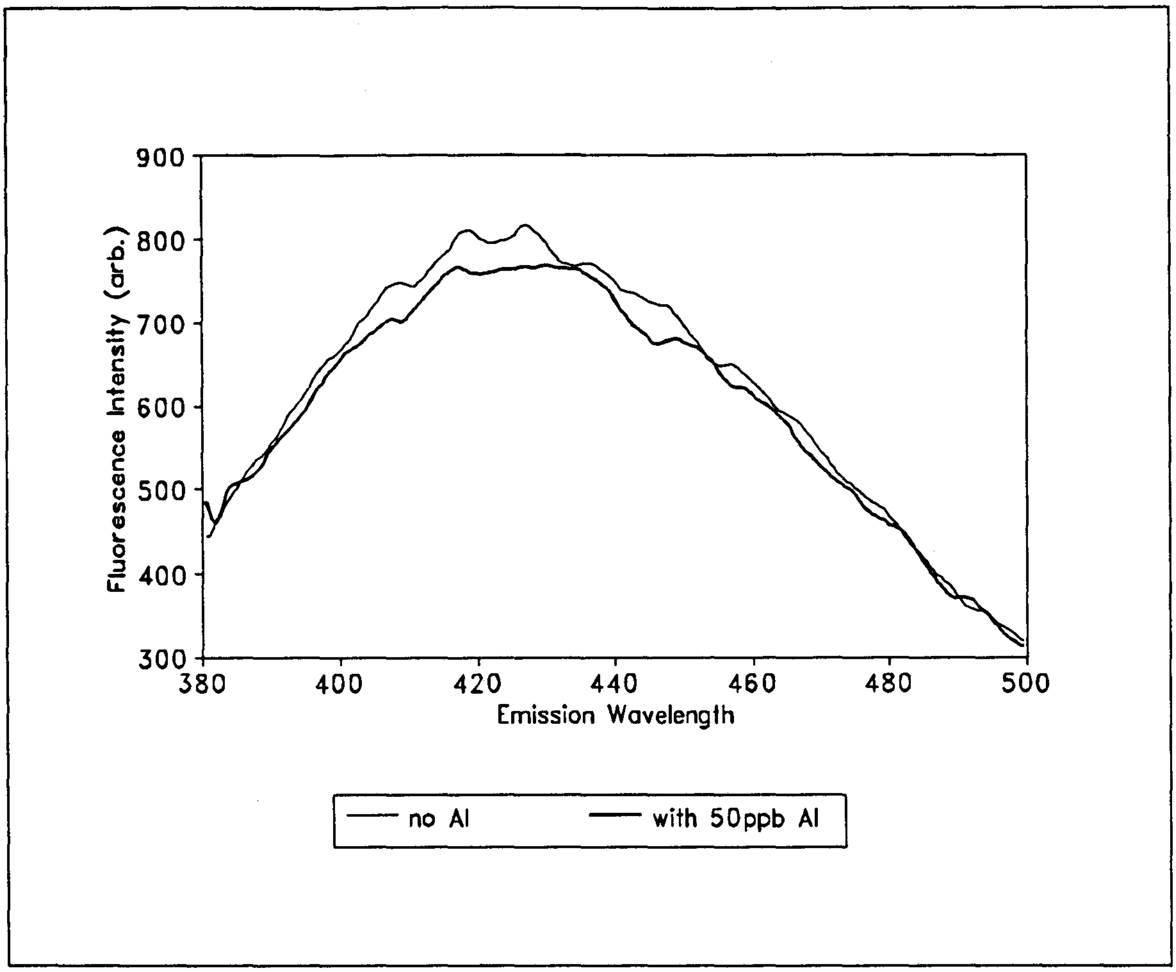

Figure 20: Emission spectra for beaver pond CBC-C. Excitation 300nm, slit widths 3nm. At pH 6.0 with and without 50ppb Al.

3.4 Fluorescence Quenching Results

The following sections present the results for Stern-Volmer analysis of salicylic acid, Armadale fulvic acid, sized fractionated samples from lake Skjervatjern and raw beaver pond waters from Manitoba. For details on these samples refer to section 2. All of the plots are not shown but the raw data is compiled in the Appendix.

3.4.1 Stern-Volmer Results

A full statistical analysis (for equations used refer to appendix) was performed on a raw sample from the acidified side of lake Skjervatjern. The results for a Stern-Volmer plot is a plot of original fluorescence vs fluorescence in the presence of aluminum vs added aluminum (mol/L); the slope is the stability constant, if we assume static quenching (see section 1.7.4. The method used was the same as is described in section 2.5 and the resultant Stern-Volmer plot is shown in Figure 21.

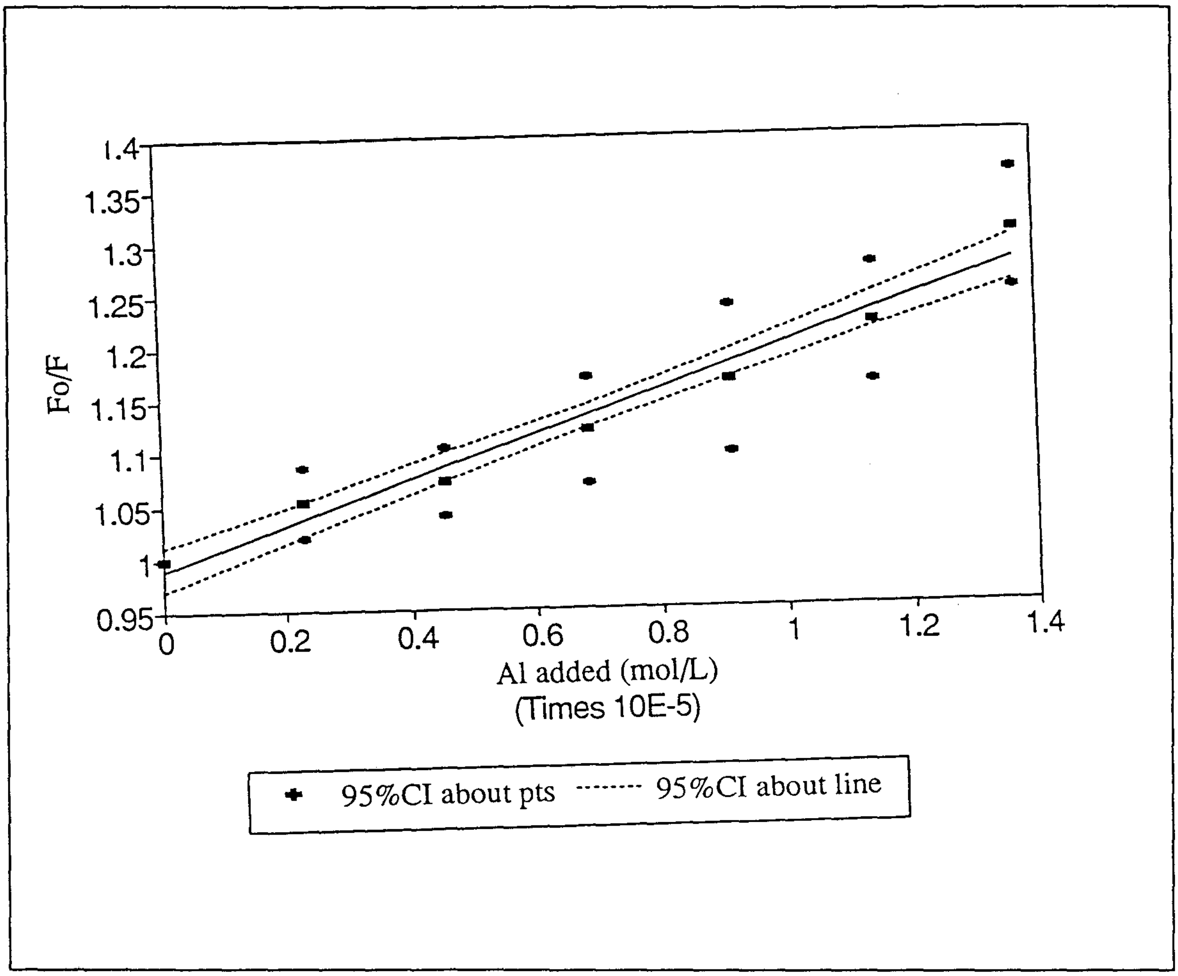

Figure 21: Stern-Volmer Plot for Acidified Side of Lake Skjervatjern (raw sample). The pH was 6.0, excitation wavelength was 300nm, emission wavelength was 460nm and resultant logK = 4.32.

The Stern-Volmer plot for lake Skjervatjern acidified water at pH 6.0 has a stability constant of logK′ = 4.32. The error from the regression estimate of the slope is ± 0.03. The linear dynamic range is from 0 to 0.8 x 10^{-5} mol/L aluminum. The detection limit is 1 μM, or 20 μg/L aluminum. This low detection limit is applicable to most natural and potable waters.

Stern-Volmer plots for the other samples could be made as well. The regression analysis has been done and the resultant stability constants are shown in section 3.5. The raw data is in the appendix.

3.4.2 Ryan-Weber Results

The Ryan-Weber equation is derived in section 1.7.4; a Ryan-Weber plot is a plot of fluorescence vs metal added where the free ligand is set to 100% fluorescence. A program was written in Matlab to perform the nonlinear regression and to plot the results; these programs are included in the appendix. Unfortunately the curve fitting resulted in physically meaningless answers for most of the samples. The usual difficulty was a negative fluorescence of the bound species parameter F°_{ML}. A Ryan-Weber Plot was possible for the salicylic acid experiment because the parameter L_T, the total ligand concentration was known; 1.4 x 10^{-4} mol/L was added to the reaction vessel. The resultant Ryan-Weber plot is shown Figure 22 where the parameters logK′ and F°_{ML} were determined as 4.0 and 30% respectively.

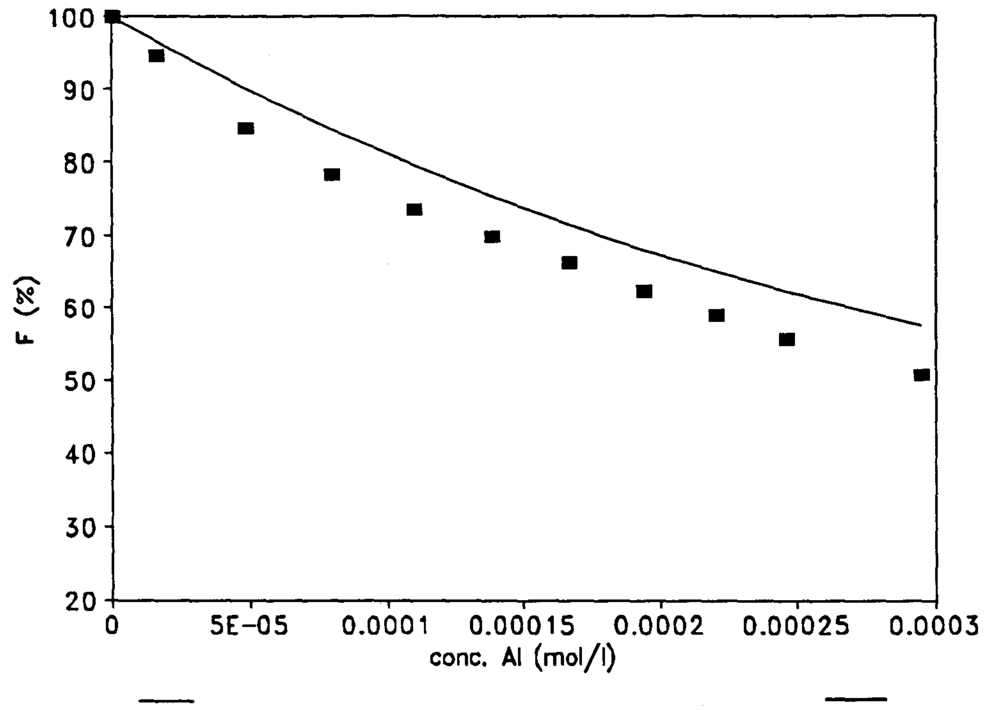

Figure 22: Ryan-Weber Plot for Salicylic Acid fitting only the two parameters logK′ = 4.0 and F°_{ML} = 30% and not L_T which was set at 1.4×10^{-4} mol/L.

3.4.3 Combined Ryan-Weber and Stern-Volmer Results

The Ryan-Weber equation is difficult to curve fit unless one or more parameters are independently fixed. This can be done by using the Stern-Volmer results in the Ryan-Weber equation. From the Stern-Volmer results the stability constant can be found under the assumption that F°_{ML} is equal to zero; the only parameter left to fit is the total ligand concentration. This has been done for the salicylic acid experiment except all three parameters were fixed if the Stern-Volmer results were assumed to be true. The result is shown in Figure 23.

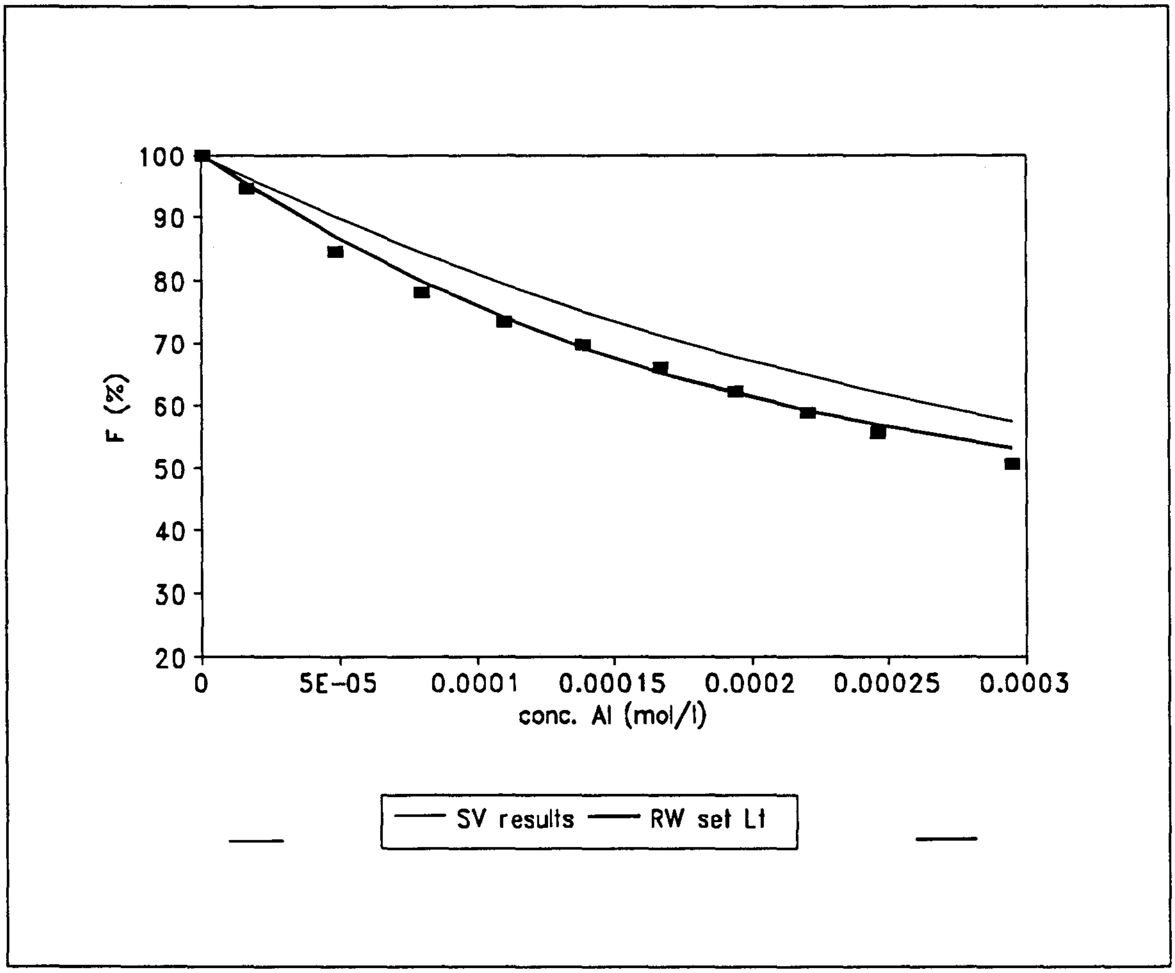

Figure 23: Stern-Volmer results as a Ryan-Weber plot for Salicylic Acid at pH 6.0.

The Stern-Volmer Results for Salicylic acid do not fit the Ryan and Weber plot. This is not due to some residual fluorescence F°_{ML} because to get a better fit the residual fluorescence must be negative.

A better result was obtained when performing a Ryan-Weber analysis using the results from the Stern-Volmer plot of the acidified lake Skjervatjern water (Figure 21). The result of this fit is good and is shown in Figure 24.

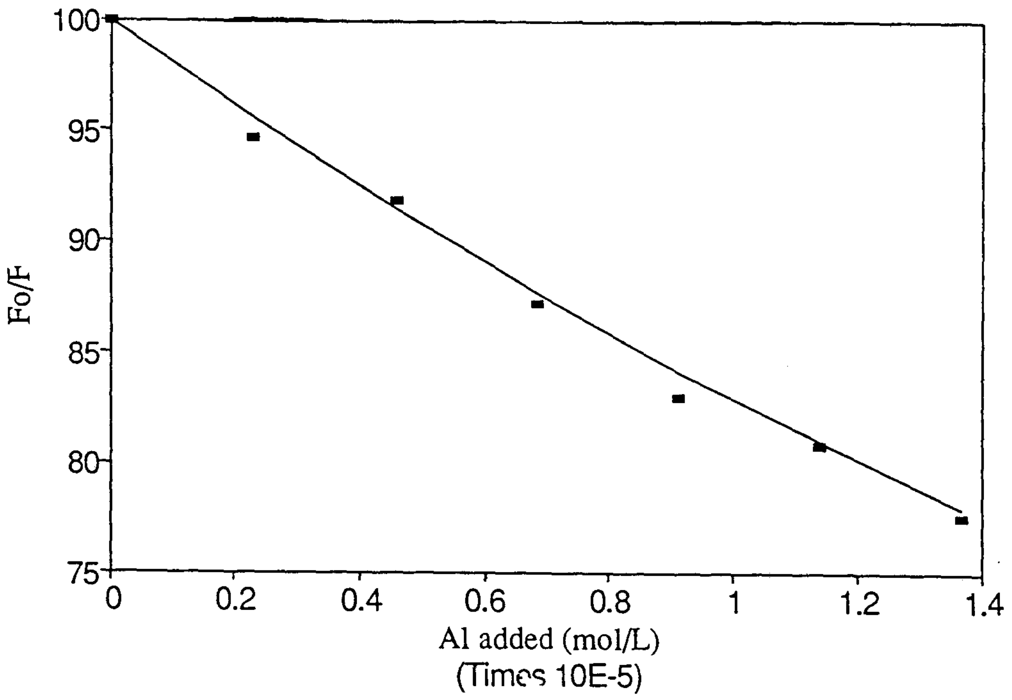

Figure 24. Ryan-Weber analysis for Lake Skjervatjern using Stern-Volmer results. L_t = 1 μM; at pH of 6.0

3.5 Summary of logK′ Values

The following tables summarize the conditional stability constants obtained in this series of experiments.

Table III: **Results for Reference Materials:** I = 0.04 for all analysis.

Sample	Method	pH	logK′	L_T (μmol/L)
Armadale fulvic acid	FQSV	6.0	4.65 ± 0.07	-
"	FQSV	7.0	3.85 ± 0.01	-
"	PCVR	6.0	4.7	25
"	PCVJ	"	8.1	14
Salicylic acid	FQSV	"	3.5 ± 0.01	140
	FQRW	"	4.0	"
	S&M	"	4.0 (I = 0.02)	"

FQSV refers to fluorescence quenching interpretted via the Stern-Volmer Equation.
FQRW refers to fluorescence quenching interpretted via the Ryan Weber equation.
PCVR refers to PCV data interpretted with the Ruzic equation.
PCVJ refers to data interpreted using the JRK equation.
S&M refers to literature value from Smith and Martell (1982).

From Table III it can be seen that the logK′ results from the JRK method differs greatly from the fluorescence quenching results at pH 6.0 (8.1 vs 4.7), but the Ruzic results do not differ substantially (4.7 vs 4.65). This leads to the conclusion that some of the assumptions in the JRK equation may not be valid. In addition, two methods arriving independently at the same result improves our confidence in each method. There is an increasing trend in logK′ with decreasing pH; this is the expected trend which also improves our confidence in the Stern-Volmer approach.

The stability constant for salicylic acid agrees very well with the literature in the Ryan-Weber analysis (both are 4.0) and the Stern-Volmer results are reasonable (3.5 vs 4.0). In general fluorescence quenching may underestimate the stability constant because aluminum is diamagnetic and diamagnetic materials do not quench fluorescence well (see section 1.7.2) whereas paramagnetic metals do quench fluorescence. Fluorescence is suppressed at pH 6.0 because $AlOH^+$ is paramagnetic, as determined from a simplified molecular orbital diagram and linear combination of atomic orbitals. At pH 6.0 there is still some Al^{3+} (about 10%) and thus all the aluminum is not quenching fluorescence and the stability constant could be underestimated. Another possible explaination in the case of salicylic acid is the formation of a two to one (ML_2) complex. At a pH of 6.0, according to the stability constants in Martell and Smith (19), $[ML_2] \approx [ML]$ and fluorescence quenching would only measure an average stability constant which would in fact be lower. The second explaination does not predict an underestimation of the fulvic acid and natural water stability constants if they are assummed to only have one to one stoichiometry.

The results for the Manitoba beaver ponds show increasing stability constant for decreasing dissolved organic carbon; a lower stability constant implies that less aluminum is bound and that the bond is weaker. Dissolved organic carbon tends to decrease in older beaver ponds (Bourbonniere 1989), because this decreased organic carbon becomes more refractory than in younger beaver ponds; thus, more humic material would predominate in old beaver ponds and more fulvic material in younger beaver ponds. The humic material would have a higher molecular weight and be

Table IV **Summary of Results for Manitoba Beaver Ponds from Stern-Volmer Analysis**: pH = 6.0, ionic strength = I = 0.04 and DOC = dissolved organic carbon

Sample	DOC (ppm)	LogK′
GBP-D	23	3.15 ± 0.03
MBP-AD	14	3.26 ± 0.03
CBP-C	10	3.63 ± 0.02

dominated by carboxylic and polycarboxylic groups that could potentially strongly bind aluminum. The observed trend than actually mirrors the trend of increasing humic material which aluminum has a stronger affinity for. Thus, the Stern-Volmer approach yields the expected result of increasing stability constant with increasing molecular weight.

The relatively small stability constants compared to that of armadale fulvic acid could be explained by the high levels of calcium (section 2.3.2) competing for the aluminum sites. Calcium is diamagnetic and would not suppress fluorescence.

Table V **Results for Size Fractionated Waters from Lake Skjervatjern (Humex Project).**

Fraction	% of wtr by volume	DOC* ppm	LogK′ CONTROL	LogK′ ACIDIFIED
RAW	100	8.7	4.18 ± 0.01	4.32 ± 0.03
< 10 000 MW	95.9	6.7	4.44 ± 0.02	4.42 ± 0.02
10 000 - 100 000 MW	2.5	0.9	4.91 ± 0.06	5.67 ± 0.02
>100 000 MW	1.6	1.1	4.9 ± 0.1	4.86 ± 0.01

* DOC values are approximate and were determined by assuming proportionallity between the average molecular weight for each fraction and its volume with the fraction's DOC concentration.

The results for the size fractionated waters from Lake Skjervarjern again show the expected trend of increasing stability constant with increasing molecular weight. The higher weight fractions complex more aluminum on both the acidified and control sides, although the middle molecular weights on the acidified side have a very large stability constant (5.67) vs the smaller constant (4.86) for the highest molecular weight fraction.

This could possibly be explained by the acidification causing, for some reason, an increase of carboxylic and polycarboxylic groups in the middle molecular weight fractions. The constants are otherwise very comparable for the two sides; actually within experimental error they are the same for the lowest and highest molecular weights. The raw sample has a slightly larger stability constant (4.32 vs 4.18) because of the middle molecular weight fraction's very large affinity for aluminum. Thus, although the middle molecular weights are only 2.5 % by volume of the sample they still have a significant impact on the overall stability constant.

In addition, the results also show the extreme sensitivity of the method; it works for even small amounts of dissolved organic carbon. In this case the DOC was as low as 0.9 mg/L and a fluorescence signal could still be detected and its suppression monitored.

4. Conclusions

A. PCV CONCLUSIONS

1) The PCV method is better interpretted using the Ruzic equation than the JRK equation. The Ruzic equation assumes that the colourimetric signal is proportional to the free monomeric aluminum in the definition of the stability constant K′ whereas the JRK equation is based on a ligand competition reaction between the ligand and PCV for the aluminum.

2) The PCV technique is an invasive technique and will potentially change the sample and thus is not a particularly good method for stability constant determination.

3) The Ruzic equation will overestimate the stability constant because it assumes taht the [AlPCV] os equal to the free aluminum. PCV has been used to define monomeric labile aluminum but that speciation fraction is operationally defined and not necessarily true.

B. Fluoresecence quenching and conditional stability constants for Al.

1) Fluorescence quenching is validated by the good agreement in the Stern-Volmer determined stability constant and the literature value for salicylic acid (3.5 vs 4.0).

2) Fluorescence quenching is validated by excellent agreement between the Stern-Volmer and the Ruzic values for the stability constant (4.65 vs 4.7)

3) Fluorescence quenching is limited to pH's where aluminum hydroxide species predominate because these are paramagnetic and can thus quench fluorescence, whereas Al^{3+} is diamagnetic and does not suppress fluorescence.

C Observations in applications of fluorescence quenching

1) When the Stern-Volmer equation is used a trend of increasing stability constant with decreasing pH was expected and observed for Armadale fulvic acid.

(2) Complexation of aluminum is greater by higher molecular weight fractions as exemplified by the Manitoba Beaver Ponds and size fractionated waters from Lake Skjervajern in Norway.

D. Advantages of fluorescence quenching

1) Fluorescence quenching is a good technique for the determination of conditional stablity constants for aluminum and fluorescing ligands:

- **a)** It is a non-invasive technique; it involves no prechemistry or treatment of the sample or steps that would alter the nature of the disssolved organic carbon.
- **b)** Sensitive; can work at low, environmenttaly realistic levels of ligand and aluminum. The aluminum detection limit is 20 µg/L and for the ligand the detection limit is 0.9 mg C/L.
- **c)** Wide range of applicability; because of sensitivity could work in concentrated or dilute solutions - from bog water to potable water.

d) Easy technique to perform and easy data manipulation under a wide range of applications.

e) Potential to determine total ligand concentration if the Ryan-Weber equation is used along with the Stern-Volmer equation.

f) Precise method; can determine logK′ to ± 0.0X where X is between 1 and 10.

The fluorescence quenching methodology, through utilization of the Stern-Volmer equation, is a powerful technique to be used in stability constant, and hence, speciation determination, for aluminum with fluorescing natural ligands. This project has only shown the potential of this method and further research would involve reworking the Ryan-Weber equation, applying the method to more known ligands and unknown samples - including potable waters.

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Appendix A Raw Fluorescence Quenching Data

RAW FLUORESCENCE DATA

all samples are at 0.04 mol/L ionic st
and pH of 6.0. The method is descri
in the experimental section.

SAMPLE	Al Conc added (mol/L)	Fluoresce Intensity (%)
Lake Skj...em acidified side >100 000 M	0	100
	2.28E-06	73.75
	4.56E-06	52.5
	6.84E-06	37.5
Lake Skj...em control side >100 000 M	0	100
	2.28E-06	71.42857
	4.56E-06	60.71429
	6.84E-06	64.28571
	9.12E-06	57.14286
Lake Skj...em control raw sample	0	100
	2.28E-06	97.18182
	4.56E-06	94.54545
	6.84E-06	91.09091
	9.12E-06	88.63636
	1.14E-05	85.63636
	1.37E-05	82.81818
Lake Skj...em control 10 000 MW-100 000 MW	0	100
	2.28E-06	87.90698
	4.56E-06	83.72093
	6.84E-06	65.11628
	9.12E-06	58.13953
	1.14E-05	41.86047
	1.37E-05	27.90698
	1.6E-05	20.93023
MBP-AD	0	100
	2.28E-06	99.52607
	4.56E-06	99.2891
	6.84E-06	99.14692
	1.25E-05	97.81991
	1.82E-05	96.77725
Lake Skj...em control side <10 000 MW	0	100
	2.28E-06	96.38298
	4.56E-06	91.91489
	6.84E-06	87.76596
	9.12E-06	82.97872
	1.14E-05	77.65957
	1.37E-05	74.68085
	1.6E-05	69.68085
Lake Skj...em acid side <10 000 MW	0	100
	2.28E-06	95.53073
	4.56E-06	91.06145
	6.84E-06	87.3743
	9.12E-06	81.89944
	1.14E-05	77.98883
	1.37E-05	73.18436
CBP-C	0	100
	2.28E-06	99.91489
	4.56E-06	99.53191
	6.84E-06	99.10638
	1.25E-05	98.34043
CBP-C	0	100
	2.28E-06	94.65
	4.56E-06	91.75
	6.84E-06	87.18
	9.12E-06	82.95
	1.14E-06	80.83
	1.37E-06	77.6

SAMPLE	Al Conc Added (mol/L)	Fluorescence intensity (%)
Salicylic	0	100
Acid	1.65E-05	95.45455
1.4x10e-4 mol/	4.88E-05	86.81818
	7.99E-05	81.81818
	0.00011	78.18182
	0.000139	75.45455
	0.000167	72.72727
	0.000194	69.54545
	0.000221	66.81818
	0.000246	64.09091
	0.000295	60.22727
	0.000341	56.59091
	0.000384	53.40909
	0.000425	51.13636
	0.000463	48.63636
	0.0005	46.59091
	0.000535	45.22727
	0.000568	43.72727
	0.000599	42.95455
	0.000629	40.90909
	0.000657	40.22727
CBP-C	0	100
	2.28E-06	96.6
	2.96E-06	93.91
	4.45E-06	91.09
	5.93E-06	87.59
	7.41E-06	84.32
	8.89E-06	77.57
Armadale	0	100
Fulvic	2.28E-06	92.85714
Acid	4.56E-06	88.92857
	6.84E-06	79.64286
	9.12E-06	71.78571
	1.14E-05	66.07143
	1.37E-05	35.71429

SAMPLE	Al conc Added (mol/L)	Fluorescence Intensity (%)
Lake Skj...ern	0	100
Acidified	2.25E-06	52.37571
side	4.49E-06	33.32667
10 000 MW	6.74E-06	23.80238
-100 000 MW	8.98E-06	19.04
	0	100
	2.25E-06	62.216
	4.49E-06	48.87911
	6.74E-06	42.20956
	8.98E-06	26.656
	0	100
	2.25E-06	52.62632
	4.49E-06	26.31053
	6.74E-06	26.3079
	8.98E-06	21.04421

SAMPLE	Al conc Added (mol/L)	Fluorescence intensity (%)
Armadale	0	100
Fulvic acid	1.85E-06	98.23789
at pH 7.0	3.7E-06	96.9163
	5.54E-06	96.03524
	7.38E-06	94.71366
	9.22E-06	93.8326
	1.11E-05	92.51101

Appendix B Matlab Programs

```
 function for fluorescence intensity I

unction I=inten(p)
=p(1); C=p(2); M=p(3);
=F(:,1); y=F(:,2);

 setting up the equation piece by piece to allow square matrices
 but only to have them exist for squaring, not multi or add!

 = (M-100)/(2*K*C);
 = (K*C+K*y+1); v = diag(V);
 = (4*K^2*C*y); z = diag(Z);

 calculating the intensity but not subtracting 100 because this is still
 a square matrix

 = U*(v-((v^2)-z)^0.5);

 make R the calculated intensity into a vector (r)
 and add 100 component wise

 = diag(R);
 = o+100;

 take into accout actual intensity data by subtraction
 this is the function to be minimized

 = r-t;
 = sum(I.^2);

 try to plot the function

lot (r,y,':',t,y,'o')
t=max(t)/2;
t=max(y)/2;
ext(xt,1.1*yt,['K= 'num2str(K) ' C= 'num2str(C) ' M= 'num2str(M)])
ext(xt,1.0*yt,['err sum of squares= 'num2str(I)])
```

```
% making plots to see good guesses for K, C, M

function R = piotr(K, C, M)
t=F(:,1); y=F(:,2);

% make y and t into square matrices

Y=diag(y);
T=diag(t);

% the function

U=(M-100)/(2*K*C); % U is a scaler constant

V=(K*C+K*y+1); v=diag(V); % V is a vector and v is a square matrix
Z=(4*K^2*C*y); z=diag(Z); % Z is a vector and z is a square matrix

R=(U)*(v-((v^2)-z)^0.5); % R is a square matrix with the answer on the diagonal

% finding the vector of R

o=diag(R); % now just have to add 100 to find the intensity
r=o+100;

% plotting the function

plot (y,r,':',y,t,'o')
```

Appendix C Statistics and Error Equations Used

The linear model used in curve fitting was:

$$\hat{R}_p = \beta_o + \beta_1 Q_p \tag{1}$$

where a linear regression analysis in Quattro-Pro calculated the best estimate of the parameters β_o and β_1 using the average response (R_p) and aluminum concentration (Q_p).

The 95% confidence interval about this line was defined as:

$$95\%\ C.I. = (t^{0.05}{}_{n-2}) \sqrt{\left[\frac{1}{n} + \frac{(Q_p - \bar{Q}_p)^2}{\sum_{p=1}^{n} (Q_p - \bar{Q}_p)^2}\right] \left[\frac{\sum_{p=1}^{n} (\bar{R}_p - \hat{R}_p)^2}{(n-2)}\right]} \tag{2}$$

where:

t^{05}_{n-2} is the "student t" value from "t" tables for n-2 degrees of freedom and 0.05 probability

n is the number of calibration solutions including the blank

p is an index running from 1 to n

$\bar{Q}_p$ is the average value of the concentration of the calibration solutions including the blank

$\bar{R}_p$ is the average peak height observed for the pth calibration solution

R_p, β_o, β_1 and Q_p are as defined above.

In addition, the 95% confidence interval about the data points was defined as twice the standard deviation of the response for a given aluminum concentration.

In error analysis the error of a logarithm was calculated as

$$\sigma(\log(x)) = \frac{0.43\alpha\sigma(x)}{x} \tag{3}$$

$$for\ y = \alpha\log(x)$$