Determination of chlorine by fluorescence quenching

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DETERMINATION OF CHLORINE BY FLUORESCENCE QUENCHING

by

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Bachelor of Science
Peking University, Beijing, P. R. China
1987

A thesis submitted in partial fulfillment
of the requirements for the

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ABSTRACT

Determination of Chlorine by Fluorescence Quenching

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Chlorine is used extensively in the chemical industry and for disinfection of drinking water. Many methods have been reported for the determination of chlorine species. Fluorescence quenching to determine chlorine is a promising method because of its high sensitivity and selectivity. This study focuses on development of a sulfonamide-based collection system for low levels of chlorine in air. Quantitation of chlorine is based on the quenching of the fluorescence signal observed when chlorine reacts with the fluorescent sulfonamide compound. Both a direct fluorimetric and a fluorescence-HPLC method have been established. Linear relationships are found between the fluorescence intensity or HPLC peak area and the amount of chlorine. Detection limits are in the ppb range or better. These methods have been applied to water samples. Experiments have also shown that sulfonamide-treated dihydroxypropylated silica can effectively collect gas-phase chlorine.
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1. Chlorine and Its Effects on Health and Environment

Chlorine was first isolated in 1744 by a Swedish chemist, C. W. Scheele. It is a greenish yellow gas at normal temperature and pressure. Because of its high reactivity, chlorine is not found in elemental form in nature. Rather, it occurs as organochlorine compounds and chloride salts. Approximately two percent of the earth’s surface materials are chlorine, mostly in the form of sodium chloride in sea water and in natural deposits as carnallite ($\text{KMgCl}_3 \cdot 6\text{H}_2\text{O}$) and as sylvite ($\text{KCl}$). Active volcanoes emit some elemental gaseous chlorine (The Chlorine Institute, Inc., 1999), and aerosols mechanically generated from sea water are the major source of atmospheric chlorine (in the form of NaCl) worldwide (Cicerone, 1981).

Chlorine is used extensively in the chemical industry and for disinfection of drinking water. For example, chlorine can be used as a disinfectant and purifier in plastics and polymers, solvents, agrochemicals and pharmaceuticals, as well as an intermediate in the manufacturing of other substances. The chlorination of water supplies and polluted waters serves primarily to destroy or deactivate disease-producing microorganisms. Also because of the reactions of chlorine with ammonia, iron, manganese, sulfide, and some organic substances, overall water quality can be improved (American Public Health
Association et al., 1995). Although not a ubiquitous pollutant, elevated levels of chlorine can be found near industrial sources (e.g., chlor-alkali plants), where it can become a human health concern.

Chlorine gas is a respiratory irritant. The distinctive odor similar to household bleach is easily detectable at very low concentrations, e.g., 0.2-0.4 ppm — the “odor threshold.” Chlorine concentrations above 5 ppm are irritating to the nose, throat, and eyes. If a person is trapped for a long period in a high-chlorine-concentration atmosphere, loss of consciousness and possibly death can occur. Because chlorine is a respiratory irritant at higher concentrations, animals in the path of a significant amount of chlorine are affected the same way humans are. Plants in the path of a chlorine release may be damaged. Chlorine will bleach leaves. Pine trees and mature leaves of deciduous trees are most susceptible to damage. Leaves may turn brown and fall off, because chlorine stops the plant from producing chlorophyll. Healthy plants recover over time, although biomass yield and growth rate may be reduced (The Chlorine Institute, Inc., 1999).

Although associated with lower chlorine levels, chlorination of drinking water may also produce adverse effects. Taste and odor characteristics of phenols and other organic compounds present in a water supply may be intensified. Potentially carcinogenic chloroorganic compounds such as chloroform are formed. Combined chlorine formed on chlorination of ammonia- or amine-bearing waters adversely affects some aquatic life (American Public Health Association et al., 1995).

1.2 Atmospheric Chemistry of Inorganic Chlorine

The chloride (mostly as NaCl) in marine aerosols is volatized by HNO₃ and H₂SO₄ (Brimblecombe et al., 1988):

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\[
\text{NaCl} (\text{s}) + \text{HNO}_3 (\text{g}) \rightarrow \text{NaNO}_3 (\text{s}) + \text{HCl} (\text{g}) \quad (1)
\]
\[
2\text{NaCl} (\text{s}) + \text{H}_2\text{SO}_4 (\text{g}) \rightarrow \text{Na}_2\text{SO}_4 (\text{s}) + 2\text{HCl} (\text{g}) \quad (2)
\]

The Cl to Na ratio measured in collected aerosols has been observed to decrease in the presence of anthropogenic pollutants (Martens et al., 1973), qualitatively supporting this loss mechanism. However, ion balance data from the North Atlantic (Keene et al., 1990) and Arctic Oceans (Talbot et al., 1992) seem to suggest that the NO\text{}_3^\text{−} and SO\text{}_4^{2−} concentrations measured in marine aerosols cannot account for all the chlorine lost to volatilization. Before these measurement, some investigators had suggested the possibility of other chlorine species (i.e., active, defined as inorganic chlorine not in a −1 oxidation state) in the marine troposphere. Schroder et al (1974) and Finlayson-Pitts (1983) proposed an alternative mechanism for volatilization of chlorine based on the formation of nitrosylchloride by reaction with NO\text{}_2:

\[
2\text{NO}_2 (\text{g}) + \text{NaCl} (\text{s}) \rightarrow \text{NOCl} (\text{g}) + \text{NaNO}_3 (\text{s}) \quad (3)
\]

Later, Finlayson-Pitts et al (1989) reported observing laboratory reactions involving other volatile chlorine species:

\[
\text{ClNO}_3 (\text{g}) + \text{NaCl} (\text{s}) \rightarrow \text{Cl}_2 (\text{g}) + \text{NaNO}_3 (\text{s}) \quad (4)
\]
\[
\text{N}_2\text{O}_5 (\text{g}) + \text{NaCl} (\text{s}) \rightarrow \text{ClNO}_2 (\text{g}) + \text{NaNO}_3 (\text{s}) \quad (5)
\]
Recent studies show that there is an additional source of chlorine in the marine troposphere. Oum et al (1998) have indicated that molecular chlorine is generated from the photolysis of ozone in the presence of sea salt particles above their deliquescence point; this process may also occur in the ocean surface layer. This process involves oxidation initiated by OH in the aqueous phase:

\[
\begin{align*}
\text{O}_3 (\text{aq}) + \text{hv} + \text{H}_2\text{O} & \rightarrow \text{H}_2\text{O}_2 (\text{aq}) + \text{O}_2 \quad (\lambda = 254\text{nm}) \\
\text{H}_2\text{O}_2 (\text{aq}) + \text{hv} & \rightarrow 2\text{OH} (\text{aq}) \quad (\lambda = 254\text{nm}) \\
\text{OH} + \text{Cl}^- & \leftrightarrow \text{HOCl}^- \\
\text{HOCl}^- + \text{H}^+ & \leftrightarrow \text{Cl}^- + \text{H}_2\text{O} \\
\text{Cl}^- + \text{Cl}^- & \rightarrow \text{Cl}_2^- \\
\text{Cl}_2^- + \text{Cl}_2^- & \rightarrow \text{Cl}_2 + 2\text{Cl}^- \\
\text{Cl}_2 + \text{H}_2\text{O} & \leftrightarrow \text{HOCl} + \text{H}^- + \text{Cl}^- 
\end{align*}
\]

Experimental confirmation of the presence of tropospheric active chlorine has now been obtained. Molecular Cl\textsubscript{2} was identified and measured in a coast area at concentrations up to 150 ppt (Oum et al., 1998).

1.3 Current Determination Methods

Several methods have been reported for the determination of chlorine species; e.g., titrimetry, spectrophotometry, conductometry, and amperometry (American Public
Health Association et al., 1995; Dailey, 1984). The most widely accepted methods are based on the use of N, N-diethyl-p-phenylenediamine (DPD) (Jain et al., 1993; Bender, 1978). Among other spectrophotometric techniques are included measurement of light absorption by the chlorine in the air stream, measurement of a color change in a solution containing an appropriate reagent which reacts with chlorine, and detection of a color change in a continuous paper tape impregnated with a reagent. Conductometric methods make use of the measurable change in cell conductivity as a result of the presence of chlorine. Conductivity devices may use either liquid-filled cells or solid state sensors. The amperometric cell, as applied to chlorine gas detection, functions as an electrical current generator: the magnitude of the current (output) is controlled solely by the instantaneous quantity of chlorine to which it is exposed (Dailey, 1984). However, these methods are not simple, and their sensitivities are not adequate for atmospheric studies.

Cassinelli (1991) has reported a method based on collection of Cl₂ on a silver membrane, followed by release of the chlorine as chloride ion by reaction with Na₂S₂O₃ solution and analysis of the chloride by ion chromatography. The ion chromatographic method is subject to interference from any chloride (or hydrochloric acid) already present in the sample; the reagents used should also be free from chloride impurities (Keene et al., 1993).

A chemiluminescence method based on the reaction of ClO⁻ with H₂O₂ in alkaline solution has been incorporated into a continuous monitor for gas-phase chlorine (Takenaka et al., 1992), but limits of detection were only in the ppm range.

Jain et al (1993) have described an HPLC method involving precolumn derivatization to 4-bromoacetanilide. A mixed potassium bromide – acetanilide reagent
was used as a trapping agent for chlorine in air, and for its derivatization. This method is relatively sensitive and selective, but has not become widely used.

1.4 Fluorescence Quenching Method — Sulfonamides as Chlorine Collectors

1.4.1 Fluorescence Quenching

Recently, much attention has been paid to the application of fluorescence-based analysis in many branches of the chemical and biological sciences. Fluorescence quenching is the process in which the fluorescence intensity or quenching yield of luminescent species is decreased or even eliminated, by interaction with other chemical species. The principal advantages of this technique are high sensitivity, which allows the measurement of low analyte concentration, and selectivity, which is, in part, due to the two characteristic wavelengths (excitation and emission) of each fluorescent species.

Many chemical species can be determined by fluorescence quenching methods: e.g., ozone ($O_3$), chlorine dioxide ($ClO_2$), sulphur dioxide ($SO_2$), nitrogen dioxide ($NO_2$), and halothane etc. (Gong et al., 1994; Watanabe et al., 1992; Wolfbeis et al., 1988; Furuki et al., 1989; Wolfbeis et al., 1982).

Fluorescence quenching to determine active chlorine is a promising method. Potential fluorescent compounds have been studied, including polycyclic aromatic hydrocarbons (PAH). N-(6-methoxyquinolyl)-acetoethyl ester (MQAE), 2,3,7-trihydroxy-9-dibromohydroxyphenylfluorone (DBH-PF), Rhodamine B, and tetraphenylporphine (TTP) (Momin et al., 1992; Kar et al., 1995; Gong et al., 1993; Niessner et al., 1989; Beswich et al., 1987). Among these studies, the reported lowest detection limits were 0.10 µg/25ml for aqueous chlorine and 6 µg/m$^3$ for gaseous chlorine.
1.4.2 Chemistry of Sulfonamides

The homogeneous liquid phase reaction of sulfonamide compounds (and their N-alkyl-substituted derivatives) with active chlorine has been well established (Emerson, 1988). For example, the reaction of N-methyl-p-toluenesulfonamide with hypochlorous acid proceeds as follows:

\[
\text{H}_3\text{C}\text{C}_6\text{H}_4\text{SO}_2\text{N}^+\text{CH}_3 - \text{HOCl} \rightarrow \text{H}_3\text{C}\text{C}_6\text{H}_4\text{SO}_2\text{N}^+\text{CH}_3 + \text{H}_2\text{O} \quad (14)
\]

The reaction is thermodynamically favorable, and the chlorinated sulfonamide product retains oxidizing and biocidal properties similar to chloramines. When used as an oxidizer, the chlorinated sulfonamide regenerates the original sulfonamide and a chloride ion:

\[
\text{H}_3\text{C}\text{C}_6\text{H}_4\text{SO}_2\text{N}^+\text{CH}_3 - 2e^- + \text{H}^+ \rightarrow \text{H}_3\text{C}\text{C}_6\text{H}_4\text{SO}_2\text{N}^+\text{CH}_3^- + \text{Cl}^- \quad (15)
\]

The utility of such a reaction in solution is fairly limited, but incorporating the chemistry onto a surface or polymeric medium creates new possibilities. Emerson et al (1978 and 1982) immobilized a sulfonamide group on a polystyrene resin, creating a versatile class of materials that can strip active chlorine from aqueous solution (Emerson, 1988), can supply active chlorine upon demand (e.g., for biocidal water treatment; Emerson, 1990), and can be used to conduct chemical reactions (Emerson, 1991; Emerson 1993). These materials (called Haloscrubs) are also effective at removing active
bromine from water (Emerson, 1988) and, once depleted, can be regenerated by reducing agents such as HSO$_3^-$ and N$_2$H$_4$ (Emerson, 1990). The reaction with active chlorine or bromine is highly selective; aside from a reaction with HNO$_2$ to form a nitroso compound, few interferences are known (Emerson, 1990). The Haloscrubs are very stable and can be stored "on the shelf" without special precautions.

1.4.3 Purpose of Research

This research focuses on development of a sulfonamide-based collection system for low levels of active chlorine in ambient air. Quantitation of chlorine is based on the quenching of the fluorescence signal observed when chlorine reacts with the fluorescent sulfonamide compound. The analytical procedure was first studied in aqueous solution using a direct fluorimetric method. Since there are fluorescent interferences (e.g., humic and fulvic acids) in water samples, an HPLC method capable of separating fluorescence interferences was also investigated. To transfer the aqueous chemistry to gas-phase collection and analysis, two major areas of research were pursued: (1) selection of a suitable substrate material to be coated with sulfonamide compounds, and (2) selection and investigation of sampling conditions that would allow quantitative collection of chlorine in a laboratory environment.
CHAPTER 2

EXPERIMENTAL METHODS

2.1 Reagents

The compound N-(4-Butanoic acid) dansyl sulfonamide (BADS) (Figure 2.1) was synthesized in Dr. David W. Emerson’s laboratory. Stock solutions were prepared by dissolving appropriate amounts of BADS in 5.5% NaHCO₃ solution, and storing in a refrigerator, preferably in a brown glass-stoppered bottle.

\[
\text{SO}_2\text{N(CH}_3\text{)}_3\text{C}_2\text{H}_4\text{COOH}\text{.}
\]

Figure 2.1 Structural Formula of BADS

Chlorine standard solutions were prepared by the dilution of commercial bleach (5.25% available NaClO) and standardized via a standard titration method (American Public Health Association et al., 1995; Appendix 1). The chlorine standard gas was
commercially prepared by dilution of Cl₂ in nitrogen (10.2 ppm v/v; Scott Specialty Gases). Nitrogen gas was high purity grade.

All reagents were analytical grade or better. All reagent solutions were prepared using purified deionized water (18 MΩ-cm; Barnstead Thermolyne Corporation, Dubuque, IA).

Silica gel was obtained from SKC (Fullerton, CA). Solid phase extraction cartridges of dihydroxypropylated silica (20H) and strong anion exchanger (SAE) were obtained from Analytichem International. Solid phase extraction cartridges of C₁₈ were obtained from ANSYS Diagnostics, Inc.

Pumps used for sampling were a battery-powered universal flow sample pump (Model 224-PCXR7, SKC Inc.) and a vacuum pressure pump (Model 400-1901, Barnant Company).

2.2 Apparatus

2.2.1 Direct Fluorescence Method

For routine quantitation, the fluorimeter used was a Perkin-Elmer Model MPF-4 Fluorescence Spectrophotometer equipped with a 150 W Xenon discharge lamp. All experiments were conducted using 1 x 1 cm rectangular quartz cells. Excitation and emission slit-widths were fixed at 4.0 nm. The excitation wavelength was 332 nm; emission wavelength was 560 nm.

2.2.2 HPLC Method

High performance liquid chromatography (HPLC) was performed with a system consisting of a B-100-S pump (Eldex Laboratories, Inc.), a 20 μL loop injector, a 250
mm x 4.6 mm Macrosphere GPC 60Å 7μ column (Alltech Associates, Inc.), a fluorescence detector (Gilson, Model 121, excitation wavelength: 310-410 nm, emission wavelength: 480-520nm), and an IBM 286 computer with “Peak Simple” software (SRI). Peak area was used for quantitation. The mobile phase was 0.02 M NaH₂PO₄ (titrated to pH ~ 7 with 10 M NaOH) -10% CH₃OH at a flow rate of 0.5 ml/min.

Proton nuclear magnetic resonance (¹H NMR) spectra were obtained at 25 °C in CDCl₃, D₂O and DMSO using a BRUKER 400 MHz NMR.

2.3 Procedures

2.3.1 Aqueous BADS as Collectors

Chlorine in aqueous solution — Different volumes of 9.50 µg/ml chlorine standard or a known volume (1000- 4000 µl) of water samples were transferred to a series of 10 ml volumetric flasks, each containing 5.00 ml of 4.92 x 10⁻⁶ M BADS and buffer solution (0.2 M KH₂PO₄-0. 2M K₂HPO₄), and diluted to volume with pure water, making 2.46 x 10⁻⁶ M BADS solution in 0.1M buffer. Fluorescence intensity was measured, and a suitable volume (20 µl) was injected onto the HPLC column.

To make calibration curve of 2.46 x 10⁻⁷ M BADS solution, different volumes of chlorine standard solution were transferred to a series of 10 ml volumetric flasks, each containing 0.50 ml of 4.92 x 10⁻⁶ M BADS, 0.5 ml of 2 M KH₂PO₄ and 0.5 ml 2 M K₂HPO₄ solution, and diluted to volume with pure water.

Cl₂ in gaseous phase — As shown in Figure 2.2a, the sampling system was assembled and inspected to ensure that there were no leaks. Diluted Cl₂ standard gas (10.2 ppm Cl₂ in N₂) was bubbled into a sampling tube containing sulfonamide and
Figure 2.2 Sampling System for Gaseous Cl₂

(a) Aqueous BADS as Collectors
(b) Coated BADS as Collectors
buffer solution (as noted above). At the end of the sampling procedure, the solution was transferred to a 10 ml volumetric flask, and diluted to mark with pure water. By choosing different collection times at a fixed collection flow rate, an absorption series was obtained.

The amount of chlorine collected was calculated by the following formulas:

\[ C_M = \frac{M_w \cdot P \cdot C_V}{R \cdot T} \]  
(16)

\[ M = F \cdot t \cdot C_M \cdot r \cdot 10^{-3} \]  
(17)

Where:

- \( C_M \) = Mass concentration of standard Cl₂ gas (\( \mu \text{g/l} \))
- \( M_w \) = Molar mass of chlorine (70.9 g/mol)
- \( P \) = Pressure of atmosphere (atm)
- \( C_V \) = Volume concentration of standard Cl₂ gas (\( \mu \text{l/l} \))
- \( R \) = Gas law constant (0.0821 atm·l/K·mol)
- \( T \) = Temperature of atmosphere (K)
- \( M \) = Amount of chlorine collected (\( \mu \text{g} \))
- \( F \) = Collection flow rate (ml/min)
- \( t \) = Collection time (min)
- \( r \) = Dilution ratio

### 2.3.2 Coated BADS as Collectors

To coat the dihydroxypropylated silica columns with BADS, ~ 0.0860 – 0.0870 g of dihydroxypropylated silica was transferred to a 3.5 cm x 7.0 cm glass tube where one
end was plugged with glass wool. A 100 µl aliquot of $7.38 \times 10^{-5}$ M BADS solution was carefully added to the column, and the coating BADS solution was drained by gravity through the substrate material. The column was placed in a vacuum desiccator and the water was removed via vacuum, then was stored in a desiccator in a light free environment.

The sampling system was assembled and inspected to ensure that there were no leaks. The column was connected to the sampling train (Figure 2.2b). Different volumes of diluted Cl₂ standard gas and a known volume of air sample were drawn through coated columns (as noted above) using a vacuum pressure pump (Barnant Company), while the flow rate was controlled with a mass flow meter and a mass flow controller. At the end of the sampling procedure, the column was removed immediately from the sampling system and sealed with Parafilm. Sample analysis was usually conducted immediately after sampling.

To prepare samples for analysis, the sample column was rinsed by passing 3 ml of eluant (0.1 M KH₂PO₄ - 0.1 M K₂HPO₄) through the column into a test tube, while a Nalgene hand-operated vacuum pump was used to control the flow rate of the eluant (Figure 2.3). To obtain the corrected aqueous sample concentration, a measured blank value (obtained by passing eluant through uncoated dihydroxypropylated silica column) was subtracted from all samples. The solution in test tube was mixed and analyzed by the direct fluorimetric method.
Figure 2.3 Sample Preparation
CHAPTER 3

RESULTS AND DISCUSSION

3.1 Fluorimetric Method

3.1.1 Fluorescence Spectra

The excitation spectra of $2.46 \times 10^{-6}$ M BADS solution and chlorinated BADS were obtained by monitoring the fluorescence intensity at 530 nm while scanning the excitation wavelength from 250 to 500 nm. Emission spectra, on the other hand, were collected by using a fixed excitation wavelength of 325 nm and recording the emission intensity as a function of wavelength from 420 to 780 nm. As shown in Figure 3.1, the excitation and emission spectra of the BADS and chlorinated BADS systems are similar. The peak excitation and emission wavelengths are 332 nm and 560 nm, respectively. The fluorescence intensity of BADS decreases when chlorine is added.

3.1.2 pH Effects

Experiments have shown that fluorescence intensity of BADS is pH-dependent. Fluorescence disappears in strong acid solution (pH < 2.5), while high fluorescence intensity is observed in neutral and basic solution. In addition, chlorine fails to react with BADS at strong basic solution (pH > 12.9) (Fang et al., 1999). Therefore, 0.1 M KH$_2$PO$_4$ – 0.1 M K$_2$HPO$_4$ buffer solution (pH ≈ 7) was chosen.
Figure 3.1 Excitation and Emission Spectra of Sulfonamide Blank (1,1') and Sulfonamide - Chlorine (2,2')
3.1.3 Calibration Curves

Chlorine in aqueous solution — Different concentrations (2.46 x 10^-6 M and 2.46 x 10^-7 M) of BADS solutions were used to react with chlorine in aqueous solution. Calibration curves of fluorescence intensity (arbitrary units) against amount of chlorine (μg) are shown in Figure 3.2.

Although different concentrations of BADS resulted in different calibration curves, good linear relations between fluorescence intensity and amount of chlorine were found in the two calibration curves: the correlation coefficients (R^2) were 0.9923 and 0.9978, respectively. The linear range was ~ 0 – 9.1 μg/10ml with 2.46 x 10^-6 M BADS.

Chlorine in gaseous phase — N₂ was used to dilute Cl₂ standard gas (10.2 ppm in N₂) as shown in Figure 2.2. Different collection flow rates, e.g., 100, 200, 300, and 400 ml/min. were investigated.

Experimental results (Table 3.1. Figure 3.3 and Figure 3.4) showed that at different collection flow rates, calibration curves were all found to be linear over the range 0 – 9.0 μg/10ml with 6.14 x 10^-6 M BADS. If the X-axis is collection time (min), the calibration curves show different slopes. For absolute value of slope, S₁₀₀ mlimin ≡ ½ S₂₀₀ mlimin < S₃₀₀ mlimin < S₄₀₀ mlimin.

Table 3.1 Relation between Fluorescence Intensity and Chlorine Amount

<table>
<thead>
<tr>
<th>F=100ml/min</th>
<th>F=200ml/min</th>
<th>F=300ml/min</th>
<th>F=400ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl₂ (ug)</td>
<td>Emission (mV)</td>
<td>Cl₂ (ug)</td>
<td>Emission (mV)</td>
</tr>
<tr>
<td>0</td>
<td>20.0</td>
<td>0</td>
<td>20.0</td>
</tr>
<tr>
<td>1.39</td>
<td>18.0</td>
<td>1.17</td>
<td>18.3</td>
</tr>
<tr>
<td>2.44</td>
<td>16.4</td>
<td>2.23</td>
<td>16.4</td>
</tr>
<tr>
<td>3.71</td>
<td>13.1</td>
<td>3.95</td>
<td>11.0</td>
</tr>
<tr>
<td>5.80</td>
<td>7.5</td>
<td>5.75</td>
<td>5.0</td>
</tr>
<tr>
<td>8.04</td>
<td>2.9</td>
<td>7.97</td>
<td>1.0</td>
</tr>
</tbody>
</table>

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Figure 3.2 Calibration Curves for Aqueous Chlorine
Figure 3.3 Fluorescence Intensity vs. Chlorine Amount under Different Flow Rates
Fluorescence Intensity vs. Collection Time

Figure 3.4 Fluorescence Intensity vs. Collection Time under Different Flow Rates
3.1.4 Comparison of aqueous and gaseous calibration curves

As shown in Table 3.2, the two slopes and two intercepts were similar, indicating that BADS reacts with chlorine in both aqueous and gaseous phases in the same manner.

Table 3.2 Comparison of Aqueous and Gaseous Calibration Curves by Fluorimetric Method

<table>
<thead>
<tr>
<th>Cl₂ Standard State</th>
<th>Linear Equation</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>Y = -2.27X + 20.8</td>
<td>0.9923</td>
</tr>
<tr>
<td>Gaseous</td>
<td>Y = -2.31X + 21.0</td>
<td>0.9859</td>
</tr>
</tbody>
</table>

*a Conc. of sulfonamide: 2.46E-6M
Y: fluorescence intensity
X: amount of chlorine (μg)

3.2 HPLC Method

3.2.1 Reason for Developing HPLC Method

It is known that fluorescent substances (mainly humic and fulvic acids) are an important component of surface water (Allard et al., 1991; Suffet et al., 1989). They are present in most natural waters at concentrations of 0.1 – 200 mg/l dissolved organic carbon (DOC) (Kinniburgh et al., 1996). Our experiments have shown that these substances interfere with the chlorine measurement in water samples when using the direct fluorimetric method (see below). In order to separate these fluorescent interferences from BADS, an HPLC method was used.

3.2.2 Optimization of HPLC Conditions

Literature values for the molecular weight of humic substances range from a few hundred to a few hundred thousand daltons. The macromolecular nature of these...
substances makes them suitable candidates for analysis by size exclusion chromatography (SEC) (Miles et al., 1983; Allard et al., 1991). In this research, SEC was applied to the separation of humic substances from BADS. In addition, reversed phase chromatography, the most widely used chromatographic mode (Weston et al., 1997; Meyer, 1998), was also studied (Table 3.3).

A 0.02 M NaH₂PO₄ aqueous solution (titrated to pH~7 with 10M NaOH) was first used as mobile phase. The retention time of BADS was about 21 minutes at the flow rate of 0.65 ml/min: a small and broad peak was observed (Figure 3.5). To enhance sensitivity and reduce retention time, different chemicals were added to the buffer solution, e.g., surfactant sodium dodecyl sulfate (SDS), organic solvent tertiary-butyl alcohol (TBA), and methanol. Results (Table 3.3) indicate that SDS did not improve the peak shape and column efficiency because its T and HETP value all increased. There was an interaction of TBA with the chlorine in samples because of no peak area change after chlorination. Only methanol reduced retention time, improved peak shape and column efficiency, and had a less effect on chlorination reaction (Appendix 2). When placing two columns with different pore sizes (60 Å and 100 Å) in series, it was found that the second column (100 Å) did not improve resolution because both T and HETP value became bigger again compared with the results of single column (60 Å). And the major drawback was the increased analysis times.

In addition, NaH₂PO₄ - CH₃CN gradient elution was performed with reversed phase chromatography, the problem was still the reaction of mobile phase with the chlorine in samples (Table 3.3 and Appendix 2).
Table 3.3 Comparison of Different HPLC Conditions

| Type                        | Col. No. | Mobile Phase       | \( t_r \) (min) | \( T^a \) | HETP\(^b\) | Peak Area Change (%)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Size-exclusion</strong></td>
<td>1(^d)</td>
<td>0.02M NaH(_2)PO(_4)</td>
<td>21</td>
<td>2.6</td>
<td>0.40</td>
<td>–</td>
</tr>
<tr>
<td>(Macrosphere GPC)</td>
<td>1</td>
<td>0.02M NaH(_2)PO(_4)-0.1g/L SDS(^e)</td>
<td>17</td>
<td>2.8</td>
<td>0.68</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.02M NaH(_2)PO(_4)-10%TBA(^f)</td>
<td>7</td>
<td>1.0</td>
<td>0.04</td>
<td>~100</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.02M NaH(_2)PO(_4)-10%CH(_3)OH</td>
<td>10</td>
<td>1.0</td>
<td>0.06</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>2(^g)</td>
<td>0.05M NaH(_2)PO(_4)-10%CH(_3)OH</td>
<td>27</td>
<td>1.8</td>
<td>0.20</td>
<td>79</td>
</tr>
<tr>
<td><strong>Reversed-phase</strong></td>
<td>1</td>
<td>NaH(_2)PO(_4)-CH(_3)CN (gradient elution)</td>
<td>11.17</td>
<td>1.0</td>
<td>–</td>
<td>~100</td>
</tr>
</tbody>
</table>

\(^a\) The peak asymmetry, \( T \), is determined at 10% of the total peak height, \( h \):

\[
T = \frac{b_{0.1}}{a_{0.1}}
\]

where \( a_{0.1} \) is the distance between the peak front and the peak maximum, measured at 0.1\( h \), and \( b_{0.1} \) is the distance between the peak maximum and the peak end, measured at 0.1\( h \) (Meyer, 1998).

\(^b\) Height equivalent to a theoretical plate (HETP):

\[
H = \frac{\sigma^2}{L}
\]

where \( \sigma \) is approximately the peak width at 50% of the peak height, and \( L \) is the column length (Miller, 1988).

\(^c\) Percent of original signal after chlorination.

\(^d\) 60 Å column.

\(^e\) Sodium Dodecyl Sulfate.

\(^f\) Tertiary-butyl alcohol.

\(^g\) 60 Å and 100 Å columns.
Figure 3.5 Chromatogram of BADS using 0.02 M NaH$_2$PO$_4$ as Mobile Phase
For all of the cases examined, the best conditions were found to be a 250 mm x 4.6 mm Macrosphere GPC 60Å column with 7µ packing, using a mobile phase of 0.02 M NaH₂PO₄- 10% CH₃OH at a flow rate of 0.5 ml/min. A chromatogram of BADS under the optimum conditions is shown in Figure 3.6. The retention time of BADS is about 10 minutes, and the peak shape is symmetrical.

Figure 3.6 Chromatogram of BADS under Optimum Conditions
3.2.3 Calibration Curve

Calibration curve for aqueous chlorine by HPLC was obtained according to the procedures mentioned in Chapter 2. As shown in Figure 3.7, the X-axis is the amount of chlorine (µg), and Y-axis is peak area. The linear range is from 0 – 7.6 µg/10ml with 2.46 x 10^-6 M BADS. The correlation coefficient (R^2) is 0.9962.

![Figure 3.7 Calibration Curve for Aqueous Chlorine by HPLC](image)

3.2.4 Reproducibility of HPLC Method

Table 3.4 and Table 3.5 show the results of reproducibility experiments for the HPLC method. The relative standard deviations of the peak area and the retention time for BADS on same day (i.e., short-term reproducibility) were 2 %, and 3 %, respectively; the results from different days (i.e., long-term reproducibility) were 12 %, and 6 %, respectively.
Table 3.4 Short-term Reproducibility of HPLC on 5/15/2000

<table>
<thead>
<tr>
<th>Item</th>
<th>Inj. 1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>6th</th>
<th>Avg. ± Std.</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Area</td>
<td>479.2</td>
<td>482.2</td>
<td>491.2</td>
<td>491.1</td>
<td>493.3</td>
<td>505.9</td>
<td>490.5±9.4</td>
<td>1.9</td>
</tr>
<tr>
<td>Retention</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(min)</td>
<td>10.26</td>
<td>10.10</td>
<td>10.92</td>
<td>10.30</td>
<td>10.73</td>
<td>10.31</td>
<td>10.44±0.32</td>
<td>3.1</td>
</tr>
</tbody>
</table>

*a* Average.  
*b* Standard deviation.  
*c* Relative standard deviation.

Table 3.5 Long-term Reproducibility of HPLC on Different Days

<table>
<thead>
<tr>
<th>Item</th>
<th>11-May</th>
<th>15-May</th>
<th>15-May*</th>
<th>16-May</th>
<th>17-May</th>
<th>Avg. ± Std.</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Area</td>
<td>410</td>
<td>490</td>
<td>452</td>
<td>543</td>
<td>533</td>
<td>486±56</td>
<td>12</td>
</tr>
<tr>
<td>Retention</td>
<td>9.28</td>
<td>10.44</td>
<td>9.57</td>
<td>10.54</td>
<td>10.71</td>
<td>10.11±0.64</td>
<td>6.3</td>
</tr>
</tbody>
</table>

*a* Second time to start HPLC with new-treated eluant on that day.  
*b* Average.  
*c* Standard deviation.  
*d* Relative standard deviation.

3.2.5 Selection of Internal Standard

Although reproducibility results shown in Table 3.4 and Table 3.5 allow accurate determinations in the 0 – 1 ppm range, it was desired to improve reproducibility and to lower the detection limit. Instrumental variability was observed during a sequence of analysis. Therefore, the use of the internal standard method was considered. The requirements for an internal standard in this reaction system are that it must be pure, have similar chromatographic properties to BADS, and must not react with chlorine or affect the fluorescence of BADS. Seven available compounds were investigated for their potential as internal standards (Table 3.6, Figure A-1 to Figure A-7). Experiments
indicated that none of compounds tested was found to be suitable as an internal standard, because some compounds reacted with chlorine; some compounds were not pure enough; and some compounds even did not dissolve in buffer solution. Therefore, finding a suitable internal standard compound is a goal for future work.

3.2.6 Detection Limit

The detection limit is defined as the concentration of analyte that produces a signal equal to three times the standard deviation of the background noise (Willard et al., 1988). In this study, blank BADS solution was injected repeatedly, and the standard deviation of the background noise was calculated (Table 3.7). The detection limit was determined as 0.052 ppm by converting the peak area value (3 x Std.) to ppm according to the calibration curve (Figure 3.7). This limit could be lowered with better sample reproducibility.

<table>
<thead>
<tr>
<th>Item</th>
<th>Inj. 1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>6th</th>
<th>Avg.</th>
<th>Std.</th>
<th>3 x Std.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Area</td>
<td>591.9</td>
<td>592.7</td>
<td>585.4</td>
<td>611.7</td>
<td>596.9</td>
<td>608.7</td>
<td>597.9</td>
<td>10.3</td>
<td>30.9</td>
</tr>
<tr>
<td>Name</td>
<td>Structural Formula</td>
<td>F.W.</td>
<td>HPLC Results</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>-------------------</td>
<td>-------</td>
<td>------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium salt of 2-naphthalenesulfonic acid</td>
<td><img src="image" alt="Structural Formula" /></td>
<td>230.22</td>
<td>Reacts with chlorine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naphthalenesulfonic acid sodium salt</td>
<td><img src="image" alt="Structural Formula" /></td>
<td>230.22</td>
<td>Several peaks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromotropic acid disodium salt dihydrate</td>
<td><img src="image" alt="Structural Formula" /></td>
<td>400.29</td>
<td>Reacts with chlorine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,6-Naphthalene-disulfonic acid, disodium</td>
<td><img src="image" alt="Structural Formula" /></td>
<td>332.26</td>
<td>Two peaks, non-reproducible peak ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-Quinolinol</td>
<td><img src="image" alt="Structural Formula" /></td>
<td>145.15</td>
<td>Hard to dissolve in buffer, poor peak shape</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinine sulfate merck</td>
<td>$(\text{C}<em>{26}\text{H}</em>{24}\text{N}<em>{2}\text{O}</em>{2})_2 \cdot \text{H}_2\text{SO}_4 \cdot 2\text{H}_2\text{O}$</td>
<td>782.92</td>
<td>Hard to dissolve in buffer, several peaks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinoline (synthetic)</td>
<td><img src="image" alt="Structural Formula" /></td>
<td>129.17</td>
<td>Hard to dissolve in buffer, several peaks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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3.3 Reaction of Sulfonamide with Chlorine

As introduced in Chapter 1, it has been known that sulfonamide compounds (and their N-alkyl-substituted derivatives) react with active chlorine (e.g., hypochlorites, hypochlorous acid, chloramine, dichloramine, and trichloramine, etc.) (Emerson, 1988). NMR studies of the reaction product of free (i.e., nonimmobilized) N-propylmethanesulfonamide with excess chlorine (aqueous) showed no detectable chlorination of the alkyl side groups, suggesting that this pathway is not responsible for loss of the chlorine (Johnson et al., 1999).

By analogy to these studies, the reaction of N-(4-Butanoic acid) dansyl sulfonamide sodium salt with active chlorine was predicted to be:

\[
\begin{align*}
\text{N(CH}_3\text{)}_2 & \quad \text{NlCH}_3 \quad \text{Cu(OCl)}_2 \\
\text{SO}_2\text{N(CH}_3\text{)}_2\text{COO}^+\text{Na}^- & \quad \text{HCl(H}_2\text{O)} \\
\end{align*}
\]

3.3.1 NMR Spectra

To investigate the predicted chlorine addition product, NMR spectra were taken of the original sulfonamide and the chlorinated sulfonamide product.

The spectrum of sulfonamide in CDCl3 is shown in Figure 3.8. It is easy to find the coincidence of sulfonamide structure with its NMR spectrum. There are three peaks from right to left at \( \delta 2.50 \), \( \delta 2.85 \) and \( \delta 3.15 \); the "integration steps" are in the
Figure 3.8 $^1$H NMR Spectrum of Sulfonamide

Figure 3.9 $^1$H NMR Spectrum of Chlorinated Sulfonamide
ratios 2:6:2, corresponding to CH₂, N(CH₃)₂, and CH₂. The small and broad peak at ~ δ 5.95 is the proton on the sulfonamide. Four groups of peaks are also found at ~ δ 7.15, ~ δ 7.50, ~ δ 8.25 and ~ δ 8.50; the relative integrations are 1:2:2:1, corresponding to the six aromatic protons.

When acquiring the NMR of chlorinated sulfonamide product, various factors were investigated, e.g., the effects of different solvents (such as D₂O, CDCl₃, DMSO), sulfonamide concentration, chlorine concentration and its states (aqueous and gaseous phases), pH and matrixes, etc. However, compared with the spectrum of original sulfonamide, the spectra of chlorinated products are difficult to explain on the basis of Equation 18. Not only does the proton on sulfonamide disappear, but also the NMR signals of the aromatic protons are profoundly changed; the N(CH₃)₂ group is being chlorinated. The exact position of chlorine after the chlorination reaction is still unknown. One of the spectra is shown in Figure 3.9 (sample preparation method is shown in Appendix 4).

3.3.2 Reversibility of Chlorination Reaction

Previous studies have indicated that chlorinated BADS can be regenerated by adding excess reducing agents such as hydrazine or sodium sulfite to the chlorination reaction system, the chlorinated BADS regenerates the original BADS and creates a chloride ion (Fang et al., 1999). This is proved by results shown in Table 3.8.
Table 3.8 Reversibility of Chlorination Reaction

<table>
<thead>
<tr>
<th>Method</th>
<th>Chlorination$^a$</th>
<th>Reduction$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorimeter</td>
<td>84%</td>
<td>99%</td>
</tr>
<tr>
<td>HPLC</td>
<td>79%</td>
<td>96%</td>
</tr>
</tbody>
</table>

$^a$ Percent of original signal after chlorination.

Place 5.00 ml of $4.92 \times 10^{-6}$ M BADS and buffer solution in a 10 ml volumetric flask, add 4.00 ml of tap water, then dilute to volume with pure water.

$^b$ Percent of original signal after chlorination and reduction with excess sulfite.

After 4.00 ml of tap water, add 100 μl of 0.1 M Na$_2$SO$_3$ solution, then dilute to volume with pure water.

3.4 Applications

3.4.1 Determination of Chlorine in Water

Tap water and swimming pool water samples were analyzed by fluorimetric and HPLC methods. A standard chlorine titration method (American Public Health Association et al., 1995) was also conducted. The results obtained are given in Table 3.9.

Table 3.9 Determination of Chlorine in Water Samples and Comparison of Results by Different Methods

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fluorimeter</th>
<th>HPLC</th>
<th>Titration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap Water (ppm)</td>
<td>$0.38 \pm 0.02^a$</td>
<td>$0.71 \pm 0.01^b$</td>
<td>$0.65 \pm 0.02^c$</td>
</tr>
<tr>
<td>Swimming Pool Water (ppm)</td>
<td>$1.69 \pm 0.08^a$</td>
<td>$2.35 \pm 0.05^b$</td>
<td>$2.70 \pm 0.02^c$</td>
</tr>
</tbody>
</table>

$^a$ The uncertainty is about ± 5%.

$^b$ The uncertainty is about ± 2%.

$^c$ The uncertainty is estimated considering ± 0.02 ml of the uncertainty of titration.

The HPLC results agree more closely to those of the titration method than the fluorimetric method, because there are fluorescent interferences, such as humic acids, in
water samples (as noted earlier); and the HPLC method can effectively separate these interferences from BADS (Figure 3.10).

During water disinfection, chlorine can combine with aquatic humic substances to form chlorinated organic compounds, such as chloroform, other low-molecular-weight disinfection byproducts (e.g., haloacetonitriles, haloacids, haloaldehydes, haloketones, chlorophenols, chloropicrin, and cyanogen chloride) and complex high-molecular-weight chlorinated compounds (Allard et al., 1991; Suffet et al., 1989). Also because of the presence of ammonia in water (Lawrance, 1996; EPA, 1984; U.S. Department of Health & Human Services, 1990), chlorine can react with it to form the chloramines: monochloramine, dichloramine, and nitrogen trichloride (American Public Health Association, 1995). In this study, to investigate the effect of ammonia on the fluorescence quenching reaction, diluted ammonia (0.0015 M) was added to the reaction system. The preliminary results are shown in Figure 3.11. It can be seen that the magnitude of slope of the curve with ammonia is smaller than the one without ammonia, i.e., the fluorescence intensity with ammonia is higher. The result indicates that at high concentration, ammonia competes with BADS for reacting with chlorine. The concentration of ammonia used here is almost 100 times higher than is encountered in tap water, however, so it should not be a significant interference.
Figure 3.10 Chromatogram of a Tap Water Sample by HPLC

1. Humic Acids
2. BADS
3.4.2 Determination of Chlorine in Gaseous Phase

Selection of Suitable Substrate Materials

As noted earlier, gaseous chlorine uptake was successfully demonstrated by using aqueous BADS solution in an impinger. This result suggests that it would be possible to use BADS-treated solid substrates to absorb chlorine from gaseous phase.

Silica gel was first tested as a substrate material. Weighed amounts (2.8 g) of silica gel were transferred to three different sizes of glass tubes with one end plugged with glass wool. A 100 µl aliquot of $7.38 \times 10^{-5}$ M BADS solution was added to the column and dried. To recover the original BADS coated on silica gel, column rinse and extraction methods were conducted. The results are given in Table 3.10.
Table 3.10 Recovery of BADS Coated on Silica Gel

<table>
<thead>
<tr>
<th>Column Type</th>
<th>Column Rinse (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Extraction (%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.70 x 7 cm</td>
<td>14</td>
<td>64</td>
</tr>
<tr>
<td>0.35 x 7 cm</td>
<td>70</td>
<td>78</td>
</tr>
<tr>
<td>0.35 x 15 cm</td>
<td>30</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>a</sup> Add 3 ml of eluant (0.1M KH₂PO₄ - 0.1M K₂HPO₄) directly onto column packed with sulfonamide-treated silica gel

<sup>b</sup> Transfer sulfonamide-treated silica gel to a clean test tube. (1) add 1 ml of eluant, shake and centrifuge; (2) transfer the decant to another test tube; (3) repeat (1) and (2) twice; (4) combine all decant together, and mix it.

The highest recovery (70%, 78%) was obtained using 0.35 x 7 cm glass tubes. However, this recovery is marginal for air sampling. Because silica is amorphous with a heterogeneous surface and all functional groups (e.g., free silanols, geminal silanols, associated silanols, siloxanes, etc.) at the surface act as adsorptive centers, untreated materials have the potential to produce unwanted interactions with BADS and retain BADS. Therefore, silica gel pretreatment and chemically modified silicas were investigated (Table 3.11).

Silica gel was first washed with 0.1 M NaOH solution, then repacked in glass tubes. Its recovery was 76%, and there was no difference compared with untreated silica. Then non-polar C<sub>18</sub>, mid-polar dihydroxypropylated silica, and strong anion exchanger bonded silica were tested in original cartridges or in repacked glass tubes. These investigations demonstrated that the dihydroxypropylated silica showed an acceptable recovery (99.5%), and can be utilized as a suitable substrate material.
Table 3.11 Selection of Substrate Materials

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Treatment Method</th>
<th>Recovery(%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silica gel</td>
<td>Pretreated with 0.1 M NaOH&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>Repacked in glass tubes&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;18&lt;/sub&gt;</td>
<td>In original cartridges&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>Dihydroxypropylated silica</td>
<td>In original cartridges</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>In original cartridges</td>
<td>85&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Repacked in glass tubes</td>
<td>99.5</td>
</tr>
<tr>
<td>Strong anion exchanger bonded silica</td>
<td>In original cartridges</td>
<td>105</td>
</tr>
</tbody>
</table>

<sup>a</sup> Obtained by column rinse method with 3 ml of 0.1M KH<sub>2</sub>PO<sub>4</sub> - 0.1M KH<sub>2</sub>HPO<sub>4</sub>

<sup>b</sup> Add 6.4 ml of 0.1M NaOH to 3.2 g silica gel, stir and decant solution, wash silica three times with water, then filter and dry silica.

<sup>c</sup> Pack 0.28 g pretreated silica gel in a glass tube (0.35 x 7 cm), transfer 100 μl of 7.38 x 10<sup>-3</sup> M BADS solution into the column and dry it by passing through N<sub>2</sub>.

<sup>d</sup> Directly add BADS solution into cartridges, and dry the cartridges.

<sup>e</sup> Rinse with 3 ml of 0.1M KH<sub>2</sub>PO<sub>4</sub> - 0.1M KH<sub>2</sub>HPO<sub>4</sub> - 10% CH<sub>3</sub>OH

Calibration Curves by Using Coated Dihydroxypropylated Silica

Diluted Cl<sub>2</sub> standard gas was collected into sampling columns, each containing BADS–treated dihydroxypropylated silica. By choosing different collection periods (e.g., 5 min, 10 min, 15 min, 20 min, and 25 min) at a fixed collection flow rate, a calibration curve was obtained. There is a linear relationship between fluorescence intensity and amount of chlorine collected. Figure 3.12 shows the calibration curve at a collection flow rate of 100 ml/min.
Fluorescence Intensity vs. Chlorine Amount

\[ y = -2.13x + 19.3 \]
\[ R^2 = 0.9886 \]

Figure 3.12 Calibration Curve for Gaseous Chlorine by BADS-treated Dihydroxypropylated Silica

**Breakthrough Test**

To ensure that collection of the gaseous chlorine is concentrated on the first sampling column, indoor air was drawn through two packed columns connected in the sampling system. Analysis of the two columns showed that at 100 ml/min of collection flow rate, and sampling periods of 4.5 hours, there was no detectable breakthrough of chlorine (Table 3.12).

<table>
<thead>
<tr>
<th>Test Date</th>
<th>First Column Chlorine Amount (µg)</th>
<th>Second Column Chlorine Amount (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10/12/2000</td>
<td>3.84</td>
<td>&lt; D. L.</td>
</tr>
<tr>
<td>10/16/2000</td>
<td>5.86</td>
<td>&lt; D. L.</td>
</tr>
</tbody>
</table>
CHAPTER 4

CONCLUSIONS AND FUTURE WORK

4.1 Conclusions

The purpose of this research is to develop a BADS-based collection system for low levels of active chlorine in ambient air. A direct fluorimetric method and a pre-separation method utilizing HPLC have been developed which are based on the quenching of the fluorescence signal observed when chlorine reacts with the fluorescent BADS. Aqueous BADS reacts with both aqueous and gaseous active chlorine in a similar manner. A linear decrease in the fluorescence intensity or HPLC peak area and the amount of chlorine over a range from 0 – 9.1 µg/10ml or 0 – 7.6 µg/10ml were observed. Detection limit was found to be 52 ppb. The chlorination reaction retains its reversibility as excess reducing agents exist. When these methods are applied to water samples, HPLC results agree more closely to those of the standard titration method than the fluorimetric method. This is because of fluorescent interferences (e.g., humic acids) in water samples. The HPLC method can effectively separate these interferences from BADS. Laboratory experiments have also shown that BADS-treated dihydroxypropylated silica can effectively collect gas-phase chlorine. The recovery of BADS coated on the substrate material is 99.5 %: the calibration curve for gaseous chlorine is linear over the range from 0 – 7.31 µg, and the correlation coefficient ($R^2$) is 0.9886.
4.2 Future Work

Work will continue on the collection of atmospheric data, the effects of potential interferences (e.g., ammonia) on the fluorescence quenching reaction, and the choice of a suitable internal standard for the HPLC method. Also, the mechanism of fluorescence quenching will be further investigated by NMR, and this fluorescence quenching method will be extended to bromine determination.

Another area of research is to pursue compounds other than BADS, which are easier to crystallize, have stronger fluorescence and higher purity.
APPENDIX 1

FAS Titration Method for Determination of Aqueous Chlorine

(Adapted from American Public Health Association et al., 1995)

Titration of Standard Chlorine Solution

A stock standard solution was prepared by mixing 1.0 ml of commercial bleach (5.25 % w/w NaClO) and 1.0 ml of glacial acetic acid in 500 ml of water to make a solution of nominally 100 ppm chlorine (as Cl₂) at about pH 7. Before titration, 2.00 ml of the stock solution was diluted to a total volume of 100 ml.

Place 5 ml of phosphate buffer reagent (24 g of Na₂HPO₄, 46 g of KH₂PO₄ and 0.8 g of Na₂EDTA in 1 l), 5 ml of EDTA solution (40 g of Na₂EDTA in 1 l), and 5 ml of N. N-diethyl-p-phenylenediamine (DPD) indicator solution (1.1 g of DPD sulfate, 2 ml of concentrated H₂SO₄ and 0.2 g of Na₂EDTA in 1 l) in a titration flask and mix. Add 100 ml diluted sample, and 1 g of KI crystals. Mix. Titrate rapidly with standard ferrous ammonium sulfate (FAS, 1.106 g of Fe(NH₄)₂(SO₄)₂·6H₂O and 1 ml 1+3 H₂SO₄ in 1 l) titrant until red color is discharged.

Titration of Water Samples

Place 5 ml of phosphate buffer reagent, 5 ml of EDTA solution, and 5 ml of DPD indicator solution in a titration flask and mix (see above). Add 100 ml tap water or
swimming pool water samples, and 1 g of KI crystals, mix. Titrate rapidly with standard FAS titrant until red color is discharged.
## APPENDIX 2

Table A-1 Effects of CH$_3$OH and CH$_3$CN on Fluorescence Intensity

<table>
<thead>
<tr>
<th>Sample Components</th>
<th>Fluorescence Intensity (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfonamide</td>
<td>20.0</td>
</tr>
<tr>
<td>Sulfonamide+CH$_3$-OH (10%)</td>
<td>20.1</td>
</tr>
<tr>
<td>Sulfonamide+CH$_3$-CN (10%)</td>
<td>20.6</td>
</tr>
<tr>
<td>Sulfonamide+Cl$_2$</td>
<td>9.4</td>
</tr>
<tr>
<td>Sulfonamide+Cl$_2$+CH$_3$-OH (10%) (pure)</td>
<td>9.9</td>
</tr>
<tr>
<td>Sulfonamide+Cl$_2$+CH$_3$-CN (10%) (pure)</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
<td>26.6</td>
</tr>
<tr>
<td></td>
<td>28.8</td>
</tr>
</tbody>
</table>
APPENDIX 3

Chromatograms of Potential Internal Standards

Figure A-1 – Figure A-7

Figure A-1 Chromatogram of Sodium Salt of 2-Naphthalenesulfonic Acid
Figure A-2 Chromatogram of Naphthalenesulfonic Acid
Figure A-3 Chromatogram of Chromotropic Acid Disodium Salt Dihydrate
Figure A-4 Chromatogram of 2,6-Naphthalenedisulfonic Acid, Disodium
Figure A-5 Chromatogram of 8-Quinolinol
Figure A-6 Chromatogram of Quinine Sulfate
Figure A-7 Chromatogram of Quinoline (Synthetic)
APPENDIX 4

Sample Preparation for NMR Spectra

**Original Sulfonamide**

The original sulfonamide solution was prepared by dissolving 0.3568 g of N-(3-propanoic acid) dansyl sulfonamide (M.W. 322) in 5 ml of CDCl₃. The concentration of sulfonamide was about 0.22 M. Then an appropriated volume of the solution was transferred into a NMR tube, and ¹H NMR spectrum was taken (Figure 3.8).

**Chlorinated Sulfonamide**

July 11, 2000. A 0.5 ml aliquot of 0.22 M sulfonamide solution (as noted above) was transferred into a NMR tube, and 3.15 ml of pure chlorine gas (10% excess) were slowly bubbled into the solution using a syringe with a long hypodermic needle. White fume formed and then turned to be a yellow-brown precipitate. After separating the liquid layer, deuterated DMSO was added to dissolve the precipitate. Then ¹H NMR was taken (Figure A-8).

July 12, 2000. To prevent precipitate, a 0.5 ml aliquot of 0.22 M sulfonamide solution (as noted above) was transferred into a small test tube, and 0.0205 g of titramethylammonium hydroxide pentahydrate (Me₄NOH • 5H₂O) was added to react with the sulfonamide. To absorb the water produced, molecular sieve (4A) was added,
followed by a few drops of CDCl$_3$. Pure chlorine gas (3.15 ml) was carefully bubbled into the solution (as noted above). A very small amount of orange-yellow precipitate formed when Cl$_2$ was added, and the solution turned to be milky. Then molecular sieve was added again to clear the solution. Finally, $^1$H NMR spectrum was taken of the clear solution (Figure 3.9).

Figure A-8 $^1$H NMR Spectrum of Chlorinated Sulfonamide in DMSO

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Table A-2 Concentration of Chlorine in Water Samples

<table>
<thead>
<tr>
<th>Date</th>
<th>Titration (ppm) TW&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Fluorimeter (ppm) TW&lt;sup&gt;a&lt;/sup&gt;</th>
<th>HPLC (ppm) TW&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tap water SPW&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Swimming pool water SPW&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>4/28/00</td>
<td>0.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/02/00</td>
<td>1.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/05/00</td>
<td>1.03</td>
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<td>5/08/00</td>
<td>0.79</td>
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<tr>
<td>5/10/00</td>
<td>0.73</td>
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<tr>
<td>5/11/00</td>
<td>0.90</td>
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<td></td>
</tr>
<tr>
<td>5/15/00</td>
<td>0.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/16/00</td>
<td>0.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/17/00</td>
<td>0.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/12/00</td>
<td>0.75</td>
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<td></td>
</tr>
<tr>
<td>6/14/00</td>
<td>0.65</td>
<td>2.70</td>
<td>0.38</td>
</tr>
<tr>
<td>6/29/00</td>
<td>1.01</td>
<td>3.40</td>
<td>0.70</td>
</tr>
<tr>
<td>7/14/00</td>
<td>1.13</td>
<td>3.95</td>
<td>0.62</td>
</tr>
<tr>
<td>7/18/00</td>
<td>1.16</td>
<td>2.94</td>
<td>0.54</td>
</tr>
</tbody>
</table>

<sup>a</sup> Tap water

<sup>b</sup> Swimming pool water
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Zhao, B.; Wei, Y. Development and Application of a Multifunctional Automatic Sampler for Cigarette Smoke. Indoor Environment (to be published, in English).


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Determination of Chlorine by Fluorescence Quenching

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