Geochemical and environmental studies on chlorinated organic compounds based on chlorine stable isotope analysis

沼田，雅彦

(Degree)
博士（理学）

(Date of Degree)
2001-03-31

(Date of Publication)
2009-07-22

(Resource Type)
doctoral thesis

(Report Number)
甲2261

(URL)
https://hdl.handle.net/20.500.14094/D1002261

博士論文

GEOCHEMICAL AND ENVIRONMENTAL STUDIES ON CHLORINATED ORGANIC COMPOUNDS BASED ON CHLORINE STABLE ISOTOPE ANALYSIS

（塩素安定同位体分析による有機塩素化合物の地球化学的動態の解明）

平成13年1月

神戸大学大学院自然科学研究科
沼田 雅彦（Masahiko Numata）

Acknowledgements

I would like to thank my advisor Prof. Noboru Nakamura for his supervision and support. I also thank my colleagues in the cosmo-geochemistry laboratory for their help and encouragement. In particular, I acknowledge Dr. Katsuyuki Yamashita and Mr. Gabor Kondorosi for their helpful comments on the manuscript. Mr. Yusuke Hirota, Mr. Tadatsugu Murata, Ms. Miwa Yoshitake, Mr. Masanori Kawashima and Mr. Ken-ichiro Takahashi are thanked for their support to development of the chlorine isotopic analysis.

I thank late Dr. Mitsunobu Tatsumoto (U.S. Geological Survey) for kindly providing us the high purity graphite and also Prof. Toshitaka Gamo (Faculty of Science, Hokkaido University) for kindly providing us the Pacific Ocean seawater samples. Mr. Nobuyuki Kanazawa (ThermoQuest Co. Ltd.) is thanked for his advice on measurements using MAT262 mass spectrometers. Dr. Ying-Kai Xiao (Qinghai Institute of Salt Lakes, China) is appreciated for his advise on thermal ionization mass spectrometry of Cs_2Cl^+.

Support to treatments of chlorinated organic compounds and bacteria by Prof. Yutaka Terashima, Dr. Hiromoto Koshikawa and Dr. Masaharu Nagao (Graduate School of Engineering, Kyoto University) is highly acknowledged.

I thank Ms. Kumiko Yaguchi (Tama blanch Laboratory, The Tokyo Metropolitane Research Laboratory of Public Health), Prof. Masanori Fujita, Dr. Tae Ho Lee (Graduate School of Engineering, Osaka University) and Kennichi Matsuura (Dowa Mining Co., Ltd.) for kindly supply of tetrachloroethene degrading bacteria.

This research was partially supported by Grant-in-Aid (No. 11640489) from the Ministry of Education, Science and Culture of Japan.

Abstract

Determination of chlorine isotope ratios is a new tool for geochemical and environmental studies. An improved method for chlorine isotopic analysis using thermal ionization mass spectrometry (TIMS) of Cs_2Cl^+ has been investigated for precise measurement of $^{37}Cl/^{35}Cl$ isotopic ratios for small amount of samples.

Chlorine in inorganic samples was recovered as AgCl and converted to CsCl with metallic Mg and Cs^+-form ion exchange resin. The CsCl (containing 2 μg of Cl) was loaded onto a Ta filament together with graphite powder. The $^{37}Cl/^{35}Cl$ ratio was measured as Cs_2Cl^+ using Faraday cups in a static mode. Measurement for each sample took approximately 80 – 100 minutes, and the replicate analysis of sea water and laboratory standard (CsCl reagent) gave internal and external precision of 0.1-0.2 ‰ ($2\sigma_m$) and 0.1-0.2 ‰ ($1\sigma_{SD}$), respectively. This method has been tested on a variety of chemical reagents including metal chlorides and hydrochloric acid. It was found that the artificial chlorine compound show large variations of $^{37}Cl/^{35}Cl$ ratio (total ranges from -2.5 to +1.2 ‰ relative to standard mean ocean chloride). We suggest that the high sensitivity and precision technique established in this work would allow us to detect small isotopic variations of chlorine in small amounts of natural samples such as terrestrial materials (i.e. minerals, rocks, sediments, groundwater and various environmental materials), meteorites and other planetary materials.

Chlorinated aliphatic hydrocarbons (CAHs) are known as ubiquitous contaminants in aquifers and soil, but it is difficult to trace the source of pollution and identify their subsurface processes. The $\delta^{37}Cl$ values for 10 commercial CAHs provided by 6 suppliers are presented. The CAHs were treated with a sodium-biphenyl reagent. The liberated chloride ions were converted to CsCl and then their isotopic compositions were determined by TIMS. Replicate

analysis of the CAHs gave an internal precision of 0.1-0.4 ‰ ($2\sigma_m$) and external precision of 0.1-0.4 ‰ ($1\sigma_{SD}$). The CAHs showed larger variations in δ^{37}Cl values (−5.0 to +2.9 ‰ relative to standard mean ocean chloride) than inorganic compounds. As each compounds and each supplier (i.e. manufacturer) may have distinctive δ^{37}Cl value, Cl isotope data can be used to trace a specific source of pollutants in subsurface environment.

Trichloroethene (TCE) and tetrachloroethene (PCE) were extracted from aqueous solutions with toluene and their chlorine isotope compositions were analyzed. Because the differences in δ^{37}Cl values between the pure-phase and the extracted chloroethens were insignificant, the solvent extraction method may be applicable to analysis of actual contaminated water samples. Organic chlorine was recovered as inorganic chloride from unsaturated chlorinated aliphatic compounds (TCE and PCE) by $KMnO_4$ oxidation, but saturated compounds were resistant to $KMnO_4$ oxidation. Chlorine atom binding to an intramolecular site in an asymmetric molecule TCE was eliminated by anaerobic dechlorination, and recovered as chloride ion. The isotopic compositions of the recovered chlorine were then determined by TIMS. Such compound-specific and intramolecular site-specific isotopic analyses may give much information of past record of each contaminant compared with bulk isotope compositions.

Chlorine isotope fractionation during reductive dechlorination of TCE and PCE to *cis*-1,2-dichloroethene (cDCE) by anaerobic bacteria was investigated. Mathematical models for one-step reaction (TCE to cDCE) and two-step reaction (PCE to cDCE) were developed. The change of δ^{37}Cl values of organic-chlorine during TCE dechlorination to cDCE can be explained by regioselective elimination of chlorine accompanied with the Rayleigh fractionation. The fractionation factors (α) of the TCE dechlorination by 3 kinds of anaerobic cultures were approximately 0.994 to 0.995 at 30 ℃. The enrichment of ^{37}Cl in organic chlorine during PCE dechlorination to cDCE can be explained by random elimination of one chlorine atom in PCE molecule and followed regioselective elimination of one chlorine atom in TCE molecule. The fractionation factors for the first step of PCE dechlorination with 3 kinds of anaerobic cultures were estimated to be 0.987 to 0.991 at 30 ℃ using the model. Isotope fractionation during the first step would be the primary factor of the chlorine isotope fractionation during the PCE dechorination to cDCE. More precise analytical technique is necessary to estimate the fractionation factor of the second step of the reaction (TCE to cDCE). These models can be utilized to evaluate the fractionation factors of regioselective and multi-step reactions for which the simple Rayleigh model is not applied. Chlorine isotope fractionation factors during biotic or abiotic degradation of CAHs can be applied to investigate the subsurface reaction mechanisms and to assess the clean-up efficiency of the contaminated sites.

Finally, the application of TIMS for isotopic dilution mass spectrometry (IDMS) of chlorine and isotopic analysis of bromine was investigated. The CsBr and ^{37}Cl spiked CsCl gave intense and stable signals (Cs_2X^+, X: Cl or Br). The preliminary results suggest that the TIMS of cesium halide molecular ion may be applicable to determination of halogen abundance and bromine isotope ratio in geochemical and cosmochemical samples.

Table of Contents

5. Application of halogen isotopic analysis for cosmo-geochemistry

1. General introduction

As one of the representative volatile elements, chlorine participates in many geochemical processes (e.g. degassing process of juvenile planet, hydrothermal activity, chemical evolution of ocean). Chlorine is ubiquitous in the atmosphere, hydrosphere and lithosphere as major anion (Cl^-) and minor gaseous components (HCl, CH_3Cl) (Table 1-1, Fig.1-1). Cl is not only essential for life on the Earth, but also important for industrial and household use in the form of inorganic and organic compounds. As a consequence, many artificial Cl compounds are released to air, water and soil and cause serious environmental problems. Therefore, understanding the behavior of Cl is important in solving problems in the field of geochemistry and environmental chemistry.

Isotopes are ideal tracer of chemical species. On the other hand, isotope fractionation is identical for physical, chemical or biological reactions. For these reasons, isotopic analysis has been applied to investigate geochemical and biochemical cycle of many elements. After the discovery of two stable Cl isotopes (^{35}Cl and ^{37}Cl), several techniques for the determination of Cl isotope composition have been developed and applied for geochemical investigations. Variations in the isotopic compositions of chlorine are regarded as useful diagnostic tool for processes associated with important geochemical reactions in the atmosphere, surface fluids and the lithosphere (Hoefs, 1997).

The chlorine isotopes are fractionated mainly by phase change of Cl-bearing materials, (e. g. evaporites crystallized from brine: Vengosh et al., 1989; Eggenkamp et al., 1995; Liu et al., 1997; Lyons et al., 1999; Eastoe et al., 1999, reaction between hydrous silicate minerals and aqueous phase: Eastoe and Guilbert, 1992; Magenheim et al., 1995; Ransom et al., 1995, volatilization of HCl from acidic aerosols: Volpe and Spivack, 1994; Volpe et al., 1998; Lyons et al., 1999). Although the isotopic abundances are generally utilized as conservative tracers in hydrology (Phillips, 1995), not only mixing of waters from different sources (Kaufmann et al., 1993) but also difference of diffusion rate between $^{35}Cl^-$ and $^{37}Cl^-$ (Desaulniers et al., 1986; Eggenkamp et al., 1994), and ion filtration effect in clay layers (Phillips and Bentley, 1987) can cause variation in Cl isotopic ratio of groundwater. The studies of nonconservative behavior of Cl have potential for constraining the distribution and chemical evolution of volatile elements on the Earth (Magenheim et al., 1995; Eggenkamp and Koster von Groos, 1997; Markl et al., 1997; Pillippot et al., 1998).

Many organic Cl compounds (freon, trihalomethane, chlorinated solvents, chlorinated pesticides, PCBs, dioxins and so on) have polluted the atmosphere, surface water, aquifers, soils and ecosystems for many years. Isotope ratios in organic Cl compounds have been investigated to trace the chemicals produced by different production procedures in different manufacturers and/or different period and the sources of pollutants (Tanaka and Rye, 1991; Jarman et al., 1998; Beneteau et al., 1999; Reddy et al., 2000). Also the reaction pathways and mechanisms of organic Cl compounds in environment can be constrained by magnitude of isotopic fractionation (e.g. biodegradation: Bloom et al., 2000; Hunkeler et al., 1999; Sturchio et al., 1998; Sherwood Lollar et al., 1999; Heraty et al., 1999; evaporation: Slater et al., 1999; Huang et al., 1999; abiotic degradation: Dayan et al., 1999).

However, the studies of Cl isotopes were limited compared to other light elements by small range of Cl isotopic variations in natural materials and lack of analytical precision and sensitivity. Because there are no redox reactions of Cl in natural system, the variation of Cl isotopic ratios is less significant than other light elements. Several techniques, including electron impact ionization mass spectrometry (EIMS: Boyd et al., 1955; Hoering and Parker, 1961; Taylor and Grimsrud, 1969), negative thermal ionization mass spectrometry (N-TIMS: Shields et al., 1962; Vengosh et al., 1989) have been developed to determine the isotopic composition of Cl. However, memory effect and mass discrimination during the ionization process limits the analytical precision of the EIMS and the N-TIMS respectively.

Recently more sensitive and precise techniques of isotopic analysis has been developed, and research of Cl isotopes has been accelerated. The electron impact ionization mass spectrometry of CH_3Cl (Long et al., 1993; Musashi et al., 1998), known as the most precise method for Cl isotopic analysis were applied to many fields. On the other hand, the thermal ionization mass spectrometry (TIMS) of Cs_2Cl^+ (Xiao, 1992; Magenheim et al., 1994) is less precise to some extent but requires relatively small quantities of Cl compared to the mass spectrometry of CH_3Cl. Sensitivity of isotopic analysis is important for the studies of materials such as meteorites and environmental pollutants because their size and concentration of Cl are sometimes limited.

In the chapter 2, conditions of Cl isotopic analysis such as sample preparation, sample loading and mass spectrometric condition are described. Also, the results for the measurements of seawater and inorganic reagents are described. Isotopic composition of chloride in seawater is the standard of Cl isotopes. And a CsCl reagent was established as a laboratory reference standard.

In the chapter 3, a method of Cl isotopic analysis in organic compound were established and analytical results of several chlorinated aliphatic hydrocarbons were described.

The chlorinated aliphatic hydrocarbons (CAHs: e.g. trichloroethene, tetrachloroethene and 1,1,1-trichloroethane) are utilized to degrease semiconductors, machinery parts and clothes. They are released into groundwater systems and soils, and cause serious pollution in many countries (Kawasaki, 1985; Fisher et al., 1987). Although, some CAHs are suspected as mutagen or carcinogen, much cost and long operation period are necessary to identify the pollution source and to cleanup contaminated sites.

As Cl isotopes in the CAHs would be discriminated in the production processes, different materials and the manufacturing processes will be reflected to isotopic compositions. Conventional chemical analysis such as gas chromatography can give only information about the chemical species and their concentration. On the other hand, it will be possible to trace pollution sources by using isotope ratio in CAHs as fingerprints. In this study, highly sensitive technique of Cs_2Cl^+ thermal ionization mass spectrometry was applied for the purpose.

In the chapter 4, isotope fractionation effects by dechlorination of chlorinated ethenes were described.

In subsurface environments, phases of CAHs change by evaporation, absorption, solubilization and so on. Also, they are degraded by biotic or abiotic processes. As each process causes identical change of isotopic compositions in CAHs, it is possible to identify reaction pathways and mechanisms of subsurface processes by isotopic analysis. In this study, isotope fractionation of chlorinated ethenes during reductive dechlorination was examined, because the reaction plays important rolls in remediation or natural attenuation of contaminated soil and aquifers.

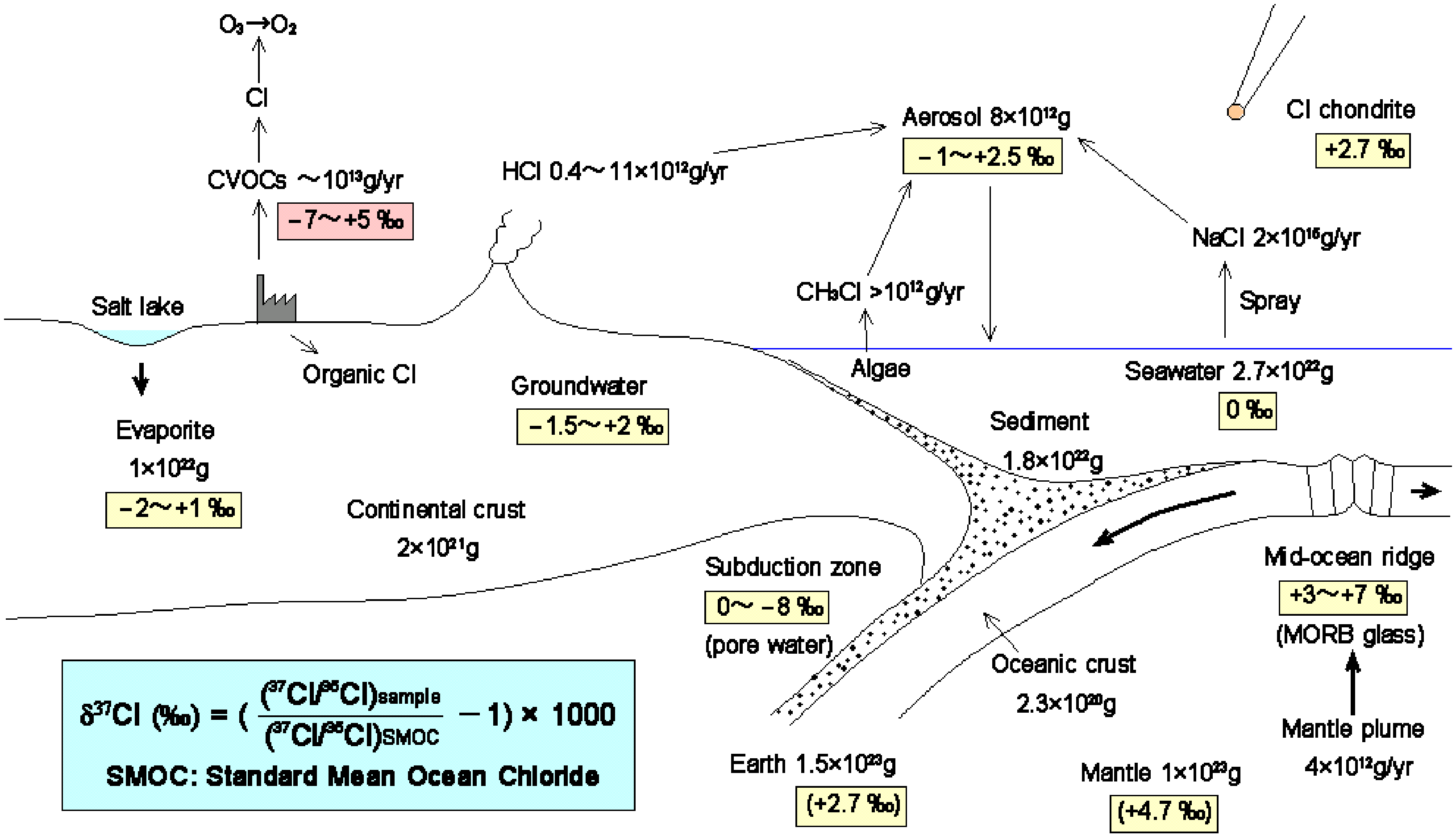
Finally, preliminary results of halogen analysis technique derived from Cs_2Cl^+ thermal ionization mass spectrometry are presented in the chapter 5.

Isotope dilution mass spectrometry for determination of Cl abundance and Br isotopic analysis by mass spectrometry of Cs_2Br^+ were investigated for possible use for research in the field of cosmochemistry.

Table 1-1. Concentrations and isotopic compositions of Chlorine in terrestrial and extraterrestrial systems

	Abundance (mg/kg)	δ^{37}Cl (‰)
Seawater	18000～20000	0.0
Crust	130（average）	-7.7～+7.5
Ignous Rocks	40～900	?
Sediments	―	-7.7～
Evaporites	～600000	-1.1～+1.2
Mantle	25?（average)	+4.7?（average）
(MORB Glass)	40～1000	+0.2～+7.5
Chondrites		
Carbonaceious	180～850	(CM:+3.4,CV:+4.0)
（C1)	580～850	+2.7
Ordinary	70～560	?
Achondrites	5～26	?
Moon (Basalt)	4～19	?
Martian Meteorites	14～1100	?
Martian 'Soil'	4000～8000	?

Data from Dreibus et al., 1977; Garrison et al., 2000; Magenheim et al., 1994; Ransom et al., 1995; Liu et al., 1997; etc.



$$\delta^{37}\mathrm{Cl}\ (‰) = \left(\frac{(^{37}\mathrm{Cl}/^{35}\mathrm{Cl})_{\mathrm{sample}}}{(^{37}\mathrm{Cl}/^{35}\mathrm{Cl})_{\mathrm{SMOC}}} - 1\right) \times 1000$$

SMOC: Standard Mean Ocean Chloride

Fig. 1-1. Distribution of Cl on the Earth Abundance: g, Flux: g/yr, δ^{37}Cl: ‰

Data from Schilling et al.(1978), Cicerone (1981), Hoef (1997), Volpe et al.(1998), Tanaka & Rye (1991) etc.

2. Measurements of chlorine isotopic ratios in inorganic compounds

Introduction

Variations in the isotopic compositions of chlorine ($^{37}Cl/^{35}Cl$) are useful diagnostic tool for processes associated with important geochemical reactions in the atmosphere, surface fluids and the lithosphere (Hoefs, 1997).

Several techniques, including electron impact ionization mass spectrometry (Boyd et al., 1955; Hoering and Parker, 1961; Taylor and Grimsrud, 1969; Kaufmann et al., 1984; Long et al., 1993; Musashi et al., 1998), negative thermal ionization mass spectrometry (Shields et al., 1962; Vengosh et al., 1989) and fast atom bombardment-mass spectrometry (Wastaway et al., 1998) have been developed to determine the isotopic composition of Cl. However, the study of Cl isotopes was limited by lack of precision and sensitivity. High sensitivity of isotopic analysis is important for samples like meteorites and environmental pollutants because their size and concentration of Cl are sometimes limited.

Thermal ionization mass spectroscopy (TIMS) of Cs_2Cl^+ has small mass dependent fractionation (Xiao and Zhang, 1992; Xiao et al., 1995), high sensitivity and low filament blank (Magenheim et al., 1994). Also, the analytical results of Cs_2Cl^+ mass spectrometry correlates closely with the results of electron impact mass spectrometry of CH_3Cl (Rosenbaum et al., 2000). It has been used successfully in the investigation of brines (Liu et al., 1997), aerosols (Magenheim et al., 1994; Volpe and Spivack, 1994; Volpe et al., 1998), rocks (Magenheim et al., 1994; Magenheim et al., 1995; Boudreau et al., 1997), fluid inclusion (Banks et al., 2000) and subduction-zone pore waters (Ransom et al., 1995) in magnetic scanning (peak jumping) mode.

In this work, a repeated analysis of ocean water and reagent standard have been carried out in different conditions in order to develop an improved method of the Cl isotopic analysis using TIMS. We have established a high sensitivity, precision method which enable us to analyze Cl isotopes with precision of ～0.1 ‰ for 2 μg-size Cl, demonstrated detailed methods and presented analytical results for ocean water and Cl-bearing commercial inorganic reagents.

Experimental Procedures

Presentation of isotopic data.

In this chapter, isotopic compositions are represented as isotopic ratios of ^{37}Cl to ^{35}Cl or in per mil (‰) deviation from those of the standard mean ocean chloride (SMOC: Long et al., 1993).

$$\delta^{37}Cl = \left[\left(\frac{R_{sample}}{R_{SMOC}}\right) - 1\right] \times 1000 \qquad R = \frac{^{37}\mathrm{Cl}}{^{35}\mathrm{Cl}} \tag{2-1}$$

where R_{SMOC} is the average of 25 ratios of the seawater chloride (Table 2-3).

Seawater Samples and Reagents

Seawater samples from the Pacific Ocean were taken during the KH-98-1 expedition (Fig. 2-1) by research vessel Hakuho-maru of the Ocean Research Institute (University of Tokyo). In addition, a coast water from Setouchi area of Japan (Setonaikai) was analyzed to find possible variation of Cl isotopic composition caused by human activities. The seawater samples were stored at -20 ℃ until use, and were filtered with a cellulose acetate membrane filter (pore size: 0.45 μm) before pretreatment for isotopic measurement.

Two high purity CsCl (99.999 %; Aldrich and 99.9 % Wako Chemical industries) and three commercial grade CsCl reagents (minimum assay 99 %;

Nacalai tesque, minimum assay 99 %; Wako Pure Chemical Industries: 1 g and 500 g bottles) were stored as 56.4 mM (2 g-Cl/L) solution. Water was purified using a Milli-RX12Plus and a Milli-Q SP systems (Millipore).

NaCl (99.99 %; Matsunaga Chemical), HCl (reagent grade hydrochloric acid, Wako Pure Chemical Industries; High-purity HCl gas, Sumitomo Seika Chemicals), KCl (minimum assay 99.5 %; Wako Pure Chemical Industries) were used for Cl isotopic measurements. Other chemicals were analytical or reagent grades.

Cs-form cation exchange resin was prepared from DOWEX 50Wx8 (200-400 mesh, Nakarai Chemicals). The resin in a polyethylene column was washed with 1.5 times resin volume of 3 M H_2SO_4 in order to remove possible contamination, and then washed with more than 10 times volume of water. Obtained H-form resin was mixed ca 0.5 M Cs_2CO_3 solution and converted into Cs-form resin. A quantity of the Cs_2CO_3 solution was used which supplied 1.2 times of ion exchange capacity of the resin. By this procedure, the amount of Cs-salt consumption was almost stoichiometric and much less than amount of neutral salt (e.g. $CsNO_3$) consumption for the in-column procedure (Xiao and Zhang, 1992), because counter anion of Cs^+ escaped as CO_2. Ba-form resin was prepared by washing the H-form resin with 10 times volume of 0.25 M $Ba(NO_3)_2$ solution in a polyethylene column. The Cs-form and Ba-form resins were washed in columns with at least 10 times volume of water before use.

H-form chelate resin was prepared from Diaion CR-11 (Na-form, 16-50 mesh; Supelco). The resin in a polyethylene column was washed with 1.5 times volume of 3 M H_2SO_4 in order to remove Na^+ and possible contamination, and then washed with more 10 times volume of water. Na-form chelate resin was used without any pretreatment.

Metal ribbons were obtained from Nilaco (Ta: 99.99 %, thickness: 0.025

mm, width: 0.75 or 1.5 mm) and Finnigan MAT (Ta, width: 0.75 mm or 1 mm; W, width: 0.75 mm; Re, width: 0.75 mm). The ribbons were washed with water in an ultrasonic oscillation bath (10 min) before use.

A graphite powder was supplied from late Professor Mitsunobu Tatsumoto (United States Geological Survey: USGS). Another high purity graphite powder (99.999 %) was obtained from Nilaco Co. Ltd.

Ion Chromatography

Analysis of chloride ion and other anions was performed using Hitachi ion chromatograph L-7000 (non-suppressor type). A 20 μl volume of the sample was injected into the mobile phase. Separations were performed with a Hitachi 2740 guard column and a Hitachi 2740 analytical column with a mixed eluent of 2.3 mM phthalic acid, and 2.5 mM 2-amino-2-hydroxymethyl-1,3-propanediol. A Shodex IC YK-421 guard column and a Shodex IC YK-421 analytical column with a mixed eluent of 1.0 mM 2,6-pyridinedicarboxylic acid, 5.0 mM L-tartaric acid, and 24 mM boric acid were used for analysis of alkali metal, alkali earth and ammonium ions.

Preparation of CsCl

Chloride ion in aqueous solution (seawater and dissolved inorganic reagents) was isolated as AgCl and converted into CsCl by the method of Hoering and Parker (1961) and Magenheim et al. (1994) with some modifications (Fig. 2-2).

Sample solutions containing 50 μg of Cl^- were placed into polypropylene centrifuge tubes. Fifty microliters of 1 M HNO_3 and 100 μL of 30 mM $AgNO_3$ solution were added to each tube, and then kept for 2 hours at room temperature in the dark. The solution was centrifuged at 12,000 g for 5 min, and the supernatant was discarded. Precipitation of AgCl was washed twice with 500 μL of 0.01 M HNO_3 and once with 50 μL of water by centrifugation, and then 50 μL of 1 M NH_3 was added to dissolve the AgCl. In order to remove Ag ion, ca. 1 mg of Mg powder was added to the solution. After one-hour reaction, the solution was centrifuged and the supernatant was recovered. The precipitate was washed twice with 50 μL of water. All supernatant fractions were combined and evaporated to dryness on a hot plate (120 ℃). The residue (NH_4Cl + $MgCl_2$) was dissolved in 50 μL of water and loaded onto a column (borosilicate glass, inner diameter: 2 mm) containing 0.1 mL of Cs-form cation exchange resin. The column was washed with 170 μL of water. The concentration of Cl^- in the eluent was measured by ion chromatography, and the eluent was evaporated to dryness on hot plate. The amount of Cl^- was calculated from the Cl^- concentration and volume of the eluent, and then water was added to the solid residue (CsCl) to form a 2 g-Cl/L solution.

Seawater samples were also treated by a different procedure as follows. Seawater (ca. 25 μL, containing 0.5 mg of Cl) was passed through Ba-form cation exchange resin (0.2 mL in a borosilicate glass column, inner diameter: 3mm), and Cs-form cation exchange resin (0.1 mL in a borosilicate glass column: inner diameter 2mm). The eluent (ca. 250 μL) was recovered, and concentrations of Cs^+ and Cl^- were measured by ion chromatography.

Mass Spectrometry

As preliminary study of isotopic ratio measurements, CsCl ionization efficiency and stability depending on loading or filament conditions were investigated using a JEOL JMS-05RB mass spectrometer (30 cm radius, 90^o geometry). The instrument has a Faraday cup detectors and a secondary electron multiplier.

The isotopic measurements of reagents and samples were performed using a Finnigan MAT Model 262 thermal ionization mass spectrometer (Fig. 2-3). This instrument has an equivalent radius of 64 cm and a 90° geometry. The instrument has five Faraday cup detectors, coupled to $10^{11}\ \Omega$ resistors, a secondary electron multiplier, and a retarding potential quadrupole system. Flat undegassed Ta ribbons (purity: 99.95 %; 0.025 x 1.5mm, Nilaco) were used for routine single filament mode measurements (Table 2-1).

The mass spectrometric procedure for routine Cl isotope measurements was the modified from Xiao and Zhang (1992), Magenheim et al. (1994), and Xiao et al. (1995). Two micrograms of chlorine as CsCl aqueous solution (56.4 mM, 1.0 μL) and graphite powder as a slurry in 80 % ethanol were mixed on a polytetrafluoroethylene sheet. The mixture was loaded on the center of the filament and then dried with a filament current of 1.5 A. The filaments were loaded into the ion source of the mass spectrometer and the analysis was started when the pressure in the ion source reached less than 10^{-5} Pa. The filament current was automatically increased to 1.2 A in 10min, and then increased manually to detect and focus the $Cs_2{}^{35}Cl^+$ ion beam. The intensity of $Cs_2{}^{35}Cl^+$ ion current was adjusted to (2 – 3) x 10^{-13} A by the filament current, which was typically between 1.4 - 1.5 A. In order to detect the target ions ($Cs_2{}^{35}Cl^+$: m/z = 301 and $Cs_2{}^{37}Cl^+$: m/z = 303), the accelerating voltage was set to 8 kV.

Data were acquired in a static (multi-Faraday cup) mode for less than 100 min. Typically 400 to 500 ratios were collected in blocks from 20 to 25.

Results and Discussion

Effects of graphite on CsCl ionization

A high m/z molecular ion Cs_2Cl^+, is suitable for isotope measurements because of its small mass discrimination during the thermal ionization process. The ratio of Cs^+ to Cs_2Cl^+ would be good indicator of the analytical conditions (Xiao and Zhang, 1992; Xiao et al., 2000; Banks et al., 2000; Rosenbaum et al, 2000).

Relationship of filament current and intensities of Cs_2Cl^+ and Cs^+ is shown in Fig.2-4. For this investigation, the JEOL mass spectrometer and Ta filaments (width: 0.75mm; Nilaco) were used. Besides, signal intensity of Cs_2Cl^+ reached plateau at more than 0.9 A of the filament current, Cs^+ intensity increased along with the filament current. As CsCl vaporized as dimeric molecule Cs_2Cl_2 at high temperature, the observed phenomena could be explained by dissociation of the molecule. At optimal filament current, Cs^+ / Cs_2Cl^+ ratio was smaller than 1 (approximately 0.3 at the lowest, during the early stage of ionization). As graphite is a reducing agent in high temperature, it has been used to generate ions at relatively low oxidation level (e.g. Nd^+ instead of NdO^+; Noble et al., 1989) for TIMS. Molecular ion emission from graphite loading filament at low temperature has been applied for isotope measurements of non-metallic elements (Xiao et al., 1988; Xiao and Zhang, 1992; Xiao et al., 1993). In the case of Cs_2Cl^+ mass spectrometry, graphite will not be a reducing agent because of low ionization temperature (<400℃; Xiao et al., 2000), and oxidation level of Cs_2Cl^+ is same to Cs^+. More studies are necessary to understand the mechanism of the molecular ion generation from graphite.

The graphite supplied from the USGS was mainly used for analyses in this chapter. This coarse graphite powder gave high ion current, but the mixture of

CsCl and graphite was fragile and easily dropped from the filaments. In order to prevent this, the graphite powder was crushed with an alumina mortar. High purity graphite (99.999 %, Nilaco) was finer, and did not have such a problem. Although obtained isotopic ratios were approximately 0.6 ‰ lower compared with result using graphite supplied from the USGS, relative $\delta^{37}Cl$ value of CsCl reagent (Nacalai tesque) did not change (Fig.2-5). Crushed carbon felt (Nihon Carbon) also gave Cs_2Cl^+ ion current comparable to that of graphite powder. The CsCl reagent was loaded on a graphite sheet (thickness: 0.15 mm, width: 1 mm; Nilaco) directly, the signal attenuated rapidly due to rapid evaporation of CsCl. Differences of isotopic ratios, ion current intensities, ratios of Cs_2Cl^+ to Cs^+ depending on graphite obtained from different sources may be caused by their crystal microstructure (distortion of crystal lattice), particle size and/or purity (Xiao et al., 2000).

The shape of CsCl and graphite on the filaments affected the intensity and duration of ion current (Fig. 2-4). Mixture of graphite slurry and CsCl solution was loaded on the center of the filament, whole surface of filament or V-shape concave (center of filament was bent to form V-shape concave with side 0.5 mm long). The highest and most durable ion current was obtained when sample and graphite was spotted on the center of the filament (width: 1.5 mm) about 1mm across in diameter.

Relationship between the amount of graphite and isotopic analysis results was also investigated. Without graphite Cs_2Cl^+ was not detected, and when the amount of graphite was ca.50 μg (with 2 μg of Cl: CsCl obtained from seawater chloride), Cs_2Cl^+ signal lasted long time (60 mV of $Cs_2{}^{35}Cl^+$ signal lasted for more than 120 min) and $^{37}Cl/^{35}Cl$ was relatively low (0.319681 ± 24). On the other hand, smaller amount of graphite (ca.15 μg) gave shorter signal duration (60 mV of $Cs_2{}^{35}Cl^+$ signal lasted for about 60 min) and higher isotope ratio (0.320075 ±

25). Larger amount of graphite (ca.200 μg) also gave shorter signal duration (30 mV of $Cs_2{}^{35}Cl^+$ signal lasted for about 50 min) and higher isotope ratio (0.319909 ± 53). As isotopic analysis results were sensitive to amount of graphite, amount of graphite should be reproducible for precise measurements of Cl isotope. For routine measurements, graphite slurry was taken into a teflon capillary mounted on the tip of a microsyringe, and the capillary (and syringe) was stood upright. By gentle strikes, graphite powder formed compact precipitation, and the height of precipitation was checked using a scale to confirm reproducibility of amount of graphite for each sample loading operations.

Xiao et al. (1992) loaded CsCl solution after loading the graphite on the filaments, while the procedure of Magenheim et al. (1994) was opposite. In the former case, graphite repels aqueous solution. In the latter case, ethanol easily spread on the filaments. In order to load the samples and graphite as small and homogeneous spot on the Ta filament, CsCl solution and graphite slurry were mixed before loading.

Effects of filament on CsCl ionization

Influence of materials and dimension of the filament was investigated using the JEOL mass spectrometer. The order of signal (Cs_2Cl^+) intensity and duration was as follows:

Ta(width:1.5 mm; Nilaco)≧Ta(width: 1.0 mm; Finnigan MAT) ＞Ta(width: 0.75 mm; Finnigan MAT, straight ribbon)＞Re(width: 0.75mm; Finnigan MAT)≧Ta(width:0.75 mm; Finnigan MAT, rolled ribbon)＞Ta(width: 1.5 mm; Nilaco)＞W(width: 0.75 mm; Finnigan MAT).

Cs_2Cl^+ was detected by all cases, but the cause of the filament effect is not clear. Because of difference of ion optics between the JEOL mass spectrometer and the Finnigan MAT mass spectrometer, the difference of signal intensities depending on filament width was not significant for isotopic analysis by using the Finnigan MAT mass spectrometer. For routine isotopic ratio measurements, Ta filaments (width: 1.5 mm, Nilaco) were used, because filament current control was easier.

Pretreatment procedures for filament cleanup were different by report on Cl isotopic analysis (degassed: Magenheim et al., 1994; Xiao et al., 2000; Banks et al., 2000, undegassed: Xiao and Zhang, 1992). In this study, the Ta filaments (width: 1.5 mm, Nilaco) were washed with water or 0.1 M HNO_3 in an ultrasonic oscillation bath (10 min) or degassed for 30 min at 5.0 A in vacuum. Compared with untreated filament, filaments washed with HNO_3 gave lower intensity of the signal. As the sample solution and graphite slurry easily spread on the degassed filaments, reproducible loading was difficult. In addition, the Cs_2Cl^+ signal was sometimes unstable using the degassed filaments. Thus filaments washed with water were used for routine isotope measurements.

Effects of other conditions on CsCl ionization

The relationship between amount of loaded Cl (CsCl obtained from seawater chloride) and measured isotopic ratio was shown in Fig.2-6. Isotope ratios of increased along with amount of loaded Cl. When the amount of Cl was 2 μg, Cs_2Cl^+ signal lasted long time (60 mV of $Cs_2{}^{35}Cl^+$ signal lasted for more than 120 min). But smaller amount of Cl (1 μg) gave lower but stable signal (35 mV of $Cs_2{}^{35}Cl^+$ signal lasted for more than 120 min). Larger amount of Cl (5 and 10 μg) gave shorter signal duration (40-50 mV of $Cs_2{}^{35}Cl^+$ signal lasted for less than 60 min). Rosenbaum et al. (2000) reported linear relationship between observed isotopic ratios and amount of loaded Cl in the range of 3 to 200 μg of Cl. It was suggested that Cs_2Cl^+ mass spectrometry could not be used as absolute measurement of Cl isotope ratio and amount of Cl should be reproducible for precise measurements of Cl isotope. From linear regression of the results in this study (Fig. 2-6), 0.2 μg (10 %) error of Cl amount might accompany with 0.04 ‰ of Cl isotope ratio error.

It was reported that optimum Cs_2Cl^+ beam was obtained for sample with a pH in the range 2-5 (Xiao and Zhang, 1992). On the other hand, it is possible that $H^{35}Cl$ preferential evaporation from acidic solution of Cl^- causes Cl isotope fractionation during sample loading and ionization (Volpe and spivack, 1994; Magenheim et al.,1994; Banks et al., 2000). In order to check the effect of acid in the sample solution on ionization of CsCl, non-volatile acids (H_3BO_3, H_3PO_4, H_2SO_4) were added to the sample solution. The Cs_2Cl^+ signal was sometimes enhanced by addition of 0.01-0.1 nmol of acids, but the effect was not reproducible. Thus, any non-volatile acid was not added to sample solution for routine isotope measurements.

For all reported mass spectrometry of CsCl, only single filament was used. In order to improve intensity of Cs_2Cl^+, isotopic measurements in double filament mode were examined (Table 2-2). Configurations of the double-filament measurements were as follows (EVA: evaporation filament – IONI: ionization filament; CsCl: Nacalai 56.4 nmol, graphite: Nilaco 50 μg):

1. CsCl+graphite (EVA) – CsCl+graphite (IONI)
2. CsCl+graphite (EVA) – CsCl (IONI)
3. CsCl+graphite (EVA) – none (IONI)
4. CsCl (EVA) – graphite+0.1 nmol of H_3BO_3 (IONI)

Only part of loaded CsCl is ionized as Cs_2Cl^+ during isotopic measurement of Cl by TIMS. It is possible that dissociation of Cs_2Cl^+ is suppressed under high partial pressure of CsCl vapor or Cs^+ on graphite surface. It was expected that partial pressure of CsCl vapor would be kept by the experimental setup 1. In addition, duplicate sample loading will level off ionization from uneven condition and reduce the deviation of analytical results. In the experiment 2, effects of Cs^+ supply from the ionization filament on Cs_2Cl^+ yield was also tested. Although relatively high and durable ion current was observed in the experiments 1 and 2 compared with the single filament ionization, repeated measurements will be necessary to assess the external error of this method. In the case of the experiment 3, the mixture of CsCl and graphite on the evaporation filament was heated by radiation from ionization filament, but Cs_2Cl^+ was not detected with indirect heating. When both filaments were heated, result same to result of single filament was obtained at relative low filament current. In order to remove possible non-volatile impurities that inhibit ionization of CsCl, sites of evaporation and ionization were separated in experiment 4. The Cs_2Cl^+ signal attenuated quickly after the intensity reached 10^{-13} A. As CsCl and graphite did not exist on the filaments after heating, amount of CsCl and H_3BO_3 (as binder of graphite) has to be larger to obtain stable signal.

Optimum conditions of Cl isotope measurement judged from above experiments were provided in Table 2-1. Typical time course of signal ($Cs_2{}^{35}Cl^+$) intensity and mass discrimination during isotopic analysis were shown in Fig. 2-7. Because mass discrimination sometimes progressed at early stage and rate stage of data acquisition, such data blocks were omitted for evaluation of data.

Procedure of CsCl preparation from chloride ion

For accurate and reproducible measurements of Cl isotopic ratios, the recovery yield for conversion process from Cl^- sample to CsCl should be high enough. Some impurities that suppress generation of Cs_2Cl^+ should be removed from samples (Magenheim et al., 1994).

Reported procedures of CsCl preparation were based on cation exchange techniques (Xiao and Zhang, 1992; Magenheim et al., 1994; Xiao et al., 2000;

Rosenbaum et al., 2000). In seawater, evaporites, aerosol and condensates obtained from pyrohydrolysis of silicate rocks, Cl^- is a major anion and concentrations of organic compounds are low. The reported procedures are not tedious but could be applicable for such simple composition samples. On the other hand, AgCl precipitation would be suitable to recover and concentrate Cl^- from samples having more complex contents.

In order to establish procedures of CsCl preparation from recovered AgCl, several procedures were examined. The reactions of conversion procedures were as follows:

1. $$2AgCl + M^0 \underset{\overset{\uparrow}{(H_2SO_4)}}{\rightarrow} M^{2+} + 2Cl^- (+ SO_4^{2-}) \underset{\overset{\uparrow}{\text{Ba-resin}}}{\rightarrow} Ba^{2+} + 2Cl^- \underset{\overset{\uparrow}{\text{Cs-resin}}}{\rightarrow} 2Cs^+ + 2Cl^-$$

   M: Zn or Mg, resin: DOWEX 50Wx8

2. $$2AgCl + 4NH_3 \rightarrow 2[Ag(NH_3)_2]^+ + 2Cl^- \underset{\overset{\uparrow}{M^0}}{\rightarrow} [M(NH_3)_n]^{2+} + 2Cl^-$$

   $$\overset{\Delta}{\rightarrow} M^{2+} + 2Cl^- \underset{\overset{\uparrow}{\text{Cs-resin}}}{\rightarrow} 2Cs^+ + 2Cl^-$$

   M: Zn or Mg, Δ: Hot plate (120 ℃)

3. $$2AgCl + 4NH_3 \rightarrow 2[Ag(NH_3)_2]^+ + 2Cl^- \underset{\overset{\uparrow}{\text{Na-form CR-11}}}{\rightarrow} 2Na^+ + 2Cl^- + 4NH_4^+ + 4OH^-$$

   $$\overset{\Delta}{\rightarrow} 2Na^+ + 2Cl^- \underset{\overset{\uparrow}{\text{Cs-resin}}}{\rightarrow} 2Cs^+ + 2Cl^-$$

   CR-11: chelate resin, Δ: Hot plate (120 ℃)

4. $$2AgCl + 4NH_3 \rightarrow 2[Ag(NH_3)_2]^+ + 2Cl^- \underset{\overset{\uparrow}{\text{H-form CR-11}}}{\rightarrow} 2H^+ + 2Cl^- \underset{\overset{\uparrow}{\text{Cs-resin}}}{\rightarrow} 2Cs^+ + 2Cl^-$$

5. $$2AgCl + 4NH_3 + Cs_2CO_3 \rightarrow 2[Ag(NH_3)_2]^+ + 2Cl^- + Cs_2CO_3 \overset{\Delta}{\rightarrow}$$

   $$2AgCl + Cs_2CO_3 \overset{\Delta}{\rightarrow} 2Ag^0 + CO_2 + 1/2O_2 + 2CsCl$$

   AgCl dissolved in NH_3 was loaded with Cs_2CO_3

   Δ: The mixture of AgCl and Cs_2CO_3 was heated on the filament

Except for the CsCl obtained by the method 2 using metallic Mg and the method 3, Cs_2Cl^+ signal intensities were lower than 10^{-13} A. There was no significant difference in isotopic ratios measured by the method 2 (using Mg) and 3 (0.31974: n=3, 0.31982: n=3; respectively). Because the batch treatment of $[Ag(NH_3)_2]^+$ with the chelate resin CR-11 was tedious, the method 2 using metallic Mg was adopted for routine isotopic analysis. Almost stoichiometric CsCl solutions (Cs^+:Cl^- ≒ 1:1, only 0.2 % of NH_4^+ and 0.5 % of Mg^{2+} in molar fraction of cations were detected) were obtained and the average of recovery yields of Cl^- as CsCl, was approximately 90 %.

Treatment with Ba-form resin of seawater sample gave higher signal intensities compared with treatment by $AgNO_3$ and Mg, also the former one gave insignificantly higher Cl isotope ratios (Table 2-3, Fig. 2-8). However, systematic shift of the Cl isotope ratios depending on signal intensities (Fig. 2-9) or the shift caused by recovery process was not observed in the analytical results of the Nacalai CsCl (Table 2-6).

Established CsCl preparation procedures for routine Cl isotope measurements are shown in Fig. 2-2.

Seawater

The results of Cl isotopic measurements of seawater (8°39.5’N, 153°56.6’E) chloride are shown in Table 2-3 and Fig. 2-8. The results of runs 1 to 11 were obtained for chloride recovered by treatment with Ba and Cs-form ion exchange resin, and the results of runs 12 to 25 were obtained for those recovered as AgCl. The mean $^{37}Cl/^{35}Cl$ ratios obtained by latter method appear to be lower, but the difference was not significant. The recovery yield of Cl as CsCl ranged from 79 to 94 % (average: 88 %) by the AgCl-precipitation procedure.

The ion current intensities for runs 1 to 11 were higher (4 - 7 x 10^{-13} A) than values of runs 12 to 25 (2 - 4 x 10^{-13} A). Although the internal errors ($2\sigma_m$: standard error of the mean of individual ratios in each run) of runs 12 to 25 were larger than errors of runs 1 to 11 by difference of ion current intensity, there was no difference in external reproducibility ($1\sigma_{SD}$: standard deviation of the mean of individual runs) between both pretreatment procedures. The average value (0.319794: run 1 - 25) was used as standard (standard mean ocean chloride: SMOC, δ^{37}Cl = 0 ‰; Long et al., 1993) in the following results.

Nine seawater samples from different locations were repeatedly analyzed (Table 2-4). Those samples were treated with Ba-form resin and Cs-form resin before isotopic measurement. The results revealed the isotopic composition of Cl in seawater is homogeneous as reported previously (Hoering and Parker, 1961; Kaufman et al., 1984). Hydrothermal activity was found near one of sampling point in the Pacific Ocean (18°12.9'N, 144°42.2'E; depth 992 m: minimum salinity layer; depth 3235 m: center of plume from hydrothermal vent; depth 3725 m: just above seafloor). Because the samples were not directly collected from hydrothermal vent, hydrothermal fluid would be highly diluted with seawater. Even if isotopic composition of chloride in hydrothermal fluid was different from seawater, the influence on Cl isotopic composition in the samples might be negligible. A sample from the In-land Sea (Setonaikai) must be affected by industrial and municipal discharge, but no difference of δ^{37}Cl was observed.

CsCl reagents

Because NIST SRM 975 (NaCl) is not available now, there is no international standard for Cl stable isotopic composition. Although Cl in seawater is isotopically homogeneous with variations less than the analytical precision, it is possible that the analytical methods could be improved to detect these variations (Xiao et al., 1995). In the case of mass spectrometry of CH_3Cl, reference CH_3Cl gas is introduced into the spectrometer, and ion beams are measured alternately in the sample and the reference for precise relative measurement (Long et al., 1993; Musashi et al., 1998). In order to compare precision and accuracy between laboratories and to check day-to-day reproducibility in a laboratory by thermal ionization mass spectrometry of Cs_2Cl^+, CsCl reagent would be suitable as a laboratory reference standard.

In order to establish a laboratory standard, 5 lots of commercial CsCl reagents were analyzed (Table 2-5, 2-6). We noted that mass spectrometric runs of a commercial grade reagent (Nacalai tesque) were most successful in obtaining more stable isotopic ratios over an extended time period (Fig. 2-8), while ion currents during a high purity CsCl (Aldrich, 99.999 %) and reagent grade CsCl (Wako, 1 g bottle) runs were occasionally unstable and hence some data which are not given in Table 2-4 were of poor quality. Also attenuation of Cs_2Cl^+ signal from other 2 reagents was too rapid to measure isotopic ratio. Therefore, we tentatively concluded that the Nacalai reagent was most safely employed as laboratory standard.

Xiao et al. have reported the pH of loading solution affect on efficiency and stability of ionization (Xiao and Zhang, 1992) and the pH of CsCl reagents may be different due to excess alkalinity remaining in reagents (Xiao, personal communication). The pH values of CsCl reagent solutions (56.4 mM) obtained by measurements with a pH electrode were 5.67 (Nacalai, reagent grade), 5.98 (Aldrich, 99.999%), 6.08 (Wako, reagent grade, 1 g bottle), 6.00 (Wako, high purity, 25 g bottle) and 6.33 (Wako, reagent grade, 500 g bottle). The differences were not so significant, but the order was roughly consistent with the order of the ion current stability.

Inorganic compounds

Isotopic composition of Cl recovered as AgCl from a CsCl reagent was measured to check isotopic fractionation by the procedure (Table 2-6). A significant difference was not observed between the composition obtained by this treatment and that of directly loaded CsCl reagent. The recovery yield of Cl ranged from 89 to 96 %.

Four kinds of inorganic chlorine compounds were used for Cl isotopic measurement. Chloride ion (50 μg) in each compound was recovered as AgCl and converted to CsCl (recovery yield: 88 to 96 %, average: 93 %). The range of δ^{37}Cl in the inorganic compounds, including CsCl, was -2.5 to +0.9 ‰ (Table 2-5, 2-6, 2-7). Difference in raw materials, processing of salts and production of Cl_2 and HCl gas would cause differences of δ^{37}Cl in inorganic compounds.

Main resources of Cl are seawater, brine and evaporites. Chlorine isotopic composition in seawater is homogeneous (δ^{37}Cl: 0 ‰), and the variations in brine and halite is small (in most of the case δ^{37}Cl: -1 to +1 ‰, Eggenkamp et al., 1995; Liu et al., 1997, Eastoe et al., 1999). Difference in raw materials would not be enough to explain the variation.

Salts, including NaCl and KCl, were purified by crystallization for industrial use (Grayson, 1985). The isotopic fractionation factor of chloride between NaCl crystal and its saturated solution is 1.0003, and δ^{37}Cl of NaCl crystals are expected to decrease from +0.3 to -0.6 ‰ during precipitation from seawater (Eggenkamp et al., 1995). Potassium chloride is obtained from evaporites (carnallite, $KCl\cdot MgCl_2\cdot 6H_2O$; sylvine, KCl) or bittern (residual brine after precipitation of NaCl) by fractional crystallization. Since the potassium salts crystallized at a later stage during the evaporation of brine, δ^{37}Cl of KCl would be more negative than δ^{37}Cl of NaCl. A small isotope fractionation factor of Cl between KCl crystal and its saturated solution (α = 0.9999, Eggenkamp et al.,

1995) would give KCl crystal depleted in ^{37}Cl at an early stage of the fractional crystallization. The repeated recrystallization for refinement could cause low δ^{37}Cl of KCl such as we can see it in case of this study. Other physical (e.g. flotation) and chemical (e.g. removal of divalent cations with chelate resin) processes for refinement would not affect the isotopic composition of the salts.

The most important intermediate of inorganic and organic chlorine compounds, Cl_2 gas is mainly produced by electrolysis of NaCl in aqueous solution (Grayson, 1985). After the tragic Minamata disease, Hg cells for the electrolysis were changed to ion exchange membrane cells, and more than 80 % of Cl_2 gas is produced using the membrane cells in Japan. Electrophoretic mobility in solution (Madorsky and Straus, 1947), and oxidation rate on anode are different between $^{35}Cl^-$ and $^{37}Cl^-$ in the electrolysis process. Johnston and Huchinson (1942) reported ^{35}Cl preferential Cl_2 gas generation and relatively large fractionation factor ($\alpha = 1.006$) for electrolysis of NaCl. Although the influence of cell type and electrode material on the fractionation factor has not been reported, electrolysis of NaCl can be the important step that causes the isotopic variation in manufacturing process of chlorine compounds.

Another important intermediate of chlorine compounds, HCl is mainly produced as by-product of organic chlorine compounds, and less than 1/3 is produced by the reaction of inorganic compounds (Cl_2 and H_2; NaCl and H_2SO_4, Grayson, 1985). In latter case, small isotopic fractionation in the step of manufacturing would result from high Cl-yields of these reactions.

Isotopic fractionation mechanisms during Cl_2 and HCl manufacturing processes were summarized in Fig. 2-10.

Most of cesium chloride is produced from pollucite ($CsAlSi_2O_6$) by digestion with acid (HCl) or decomposition with base ($CaCO_3$ + $CaCl_2$ or Na_2CO_3 + NaCl), and refined by precipitation as a complex double salt (Grayson, 1985). The δ^{37}Cl in three different CsCl regents ranged from -2.5 to +0.1 ‰ (Table 2-5, 2-6), but the information is not enough to qualify the procedure that causes such variations.

Comparison of mass spectrometric methods

Negative thermal ionization mass spectrometry (N-TIMS) of Cl^- from AgCl was developed by Shields et al. (1962). Vengosh et al. (1989) reported a simple procedure of N-TIMS using directly loaded geochemical samples. However, these techniques based on N-TIMS of Cl^- have high discrimination factor and large filament blank (Magenheim et al., 1994). Their high sensitivity is suitable for Cl qualification by isotope dilution mass spectrometry (Heumann et al., 1987; Shinonaga et al., 1994a,b), but they are not precise enough (uncertainty: 2 ‰) for isotopic analysis of most geochemical samples. This study showed that TIMS of Cs_2Cl^+ was sensitive and precise enough for some purpose.

The ion current intensity obtained in this study ($2 - 3 \times 10^{-13}$ A, 2 μg-Cl loaded) was much lower than the intensity reported by Xiao et al. (1995) (7×10^{-12} A, 10 μg-Cl loaded), but higher than the value reported by Magenheim et al. (1994) (4×10^{-14} A, 2 μg-Cl loaded). The Cl isotopic ratio of seawater measured in this study was 0.319794 ± 0.000051 (average of run 1 – 25, $\pm 1\sigma_{SD}$). This value is significantly higher than the one obtained by Xiao et al. (1995) (0.318988 ± 0.000049: $1\sigma_{SD}$), but similar to the ones reported by Magenheim et al. (1994) (0.31944 ± 0.00009 or 0.31970 ± 0.00008: $1\sigma_{SD}$). As described previously, many factors affect the ion current intensity, the reason of the differences of ion current and isotopic ratio is not clear. According to recent reports (Rosenbaum et al., 2000; Xiao et al., 2000), quantities of loaded samples and crystal microstructures of graphite would be most important factors on Cs_2Cl^+ signal intensity and Cl isotopic ratios. The modifications of loading condition and use of multi-Faraday cup mode employed in this work yielded precision (0.16 ‰) better than or comparable to the level of previously reported (0.15 ‰ using 10 μg of Cl: Xiao et al., 1995; 0.25 ‰ using 2 μg of Cl: Magenheim et al., 1994) using smaller size of samples (2 μg of Cl).

Although the precision of the measurements (0.12 to 0.29 ‰: external reproducibility, $1\sigma_{SD}$) was slightly lower compared to values obtained by electron impact ionization mass spectrometry of CH_3Cl (0.09 ‰: Long et al., 1993; 0.1 ‰: Musashi et al., 1998), purification process with a gas chromatograph is not necessary for the procedure described in this chapter. Also, the mass spectrometry of CsCl requires smaller amounts of sample as chloride ion (2 μg) for each measurement, and it is possible that the sample quantity for the measurements could be reduced further.

Conclusions

Thermal ionization mass spectrometry of Cs_2Cl^+ was applied for chlorine isotopic measurements of inorganic chlorine compounds. Representative results in this study are shown in Fig. 2-11. The $\delta^{37}Cl$ range of inorganic samples (-2.5 to +0.9 ‰) was similar to the range of that of most Cl in surface and subsurface water.

The precision (external reproducibility) of the analytical method (0.1 to 0.3 ‰: $1\sigma_{SD}$) was not better than the precision of the mass spectrometry of CH_3Cl, but was precise enough to distinguish Cl isotopic composition of chlorine compounds. In order to reduce the error of the method, improvement of sample preparation and loading procedure are necessary because many factors affect CsCl ionization process. Since it was possible to reveal Cl isotopic fractionation in most of the reagents and perhaps natural materials using small amounts of samples and without expensive equipment for pretreatment, this analytical procedure could be applied to solve geochemical, cosmochemical and environmental issues.

No variations in isotopic composition of seawater sample and commercial grade CsCl reagent from the corresponding average values were observed during the period of this study (more than 7months). Thus, CsCl reagent can be used as laboratory reference standard.

Table 2-1. Conditions of mass spectrometry

Filament	undegassed Ta
Sample Loading	
Amount of Chloride Ion	2 μg
pH	5 - 6
Cs^+ : Cl^- (molar ratio)	1 : 1
Drying (filament current)	1.5 A, 0.5 min
Filament current	
Preheat	0 → 1.2A（0.12A/min）
Measurement	1.42 - 1.48A
Ion current ($Cs_2{}^{35}Cl^+$)	2 - 5 × 10^{-13}A

Table 2-2. Results of double-filament methods

Loading		Filament current		Signal ($Cs_2{}^{35}Cl^+$)		^{37}Cl / ^{35}Cl
Evaporation	Ionization	Evaporation	Ionization	Intensity	Duration	
Double filament						
CsCl + G	CsCl + G	1.312A	1.312A	〜100mV	>1hr	0.3187 ± 2
CsCl	CsCl + G	1.335A	1.335A	40〜50mV	>1hr	0.3188 ± 5
CsCl + G	—	1.365A	1.470A	30〜35mV	<2hr	0.3187 ± 7
CsCl	H_3BO_3 + G	1.410A	1.366A	Max. 10mV	〜5min	—
Single filament						
CsCl + G		1.38〜1.45A	—	30〜40mV	>2hr	0.3188 ± 8*

*: mean of 10 measurements ± external error ($1\sigma_{SD}$), G: graphite

Table 2-3. Measured Cl isotopic compositions of the seawater sample (8°39.5'N, 153°56.6'E, Depth: 0m)

Run	Ion exchnage[a] $^{37}Cl/^{35}Cl$[c]	Run	Recovered as AgCl[b] $^{37}Cl/^{35}Cl$[c]
1	0.319852 ± 0.000018	12	0.319838 ± 0.000102
2	0.319806 ± 0.000031	13	0.319756 ± 0.000080
3	0.319885 ± 0.000027	14	0.319744 ± 0.000123
4	0.319760 ± 0.000033	15	0.319799 ± 0.000053
5	0.319821 ± 0.000021	16	0.319764 ± 0.000050
6	0.319734 ± 0.000015	17	0.319729 ± 0.000103
7	0.319849 ± 0.000027	18	0.319807 ± 0.000064
8	0.319797 ± 0.000022	19	0.319823 ± 0.000041
9	0.319856 ± 0.000026	20	0.319784 ± 0.000068
10	0.319811 ± 0.000041	21	0.319712 ± 0.000075
11	0.319857 ± 0.000029	22	0.319707 ± 0.000067
—	—	23	0.319811 ± 0.000050
—	—	24	0.319725 ± 0.000102
—	—	25	0.319834 ± 0.000070
average	0.319821 ± 0.000045[d]	average	0.319774 ± 0.000046[d]

[a] Seawater was treated with Ba-resin and Cs-resin

[b] Seawater was treated with $AgNO_3$, Mg and Cs-resin

[c] Error for the individual runs is $2\sigma_m$ (σ_m: standard error)

[d] Error for the average value is $1\sigma_{SD}$ (σ_{SD}: standard deviation)

Table 2-4. Measured Cl isotopic compositions of seawater samples

Location	Depth (m)	Salinity (‰)	Temp (℃)	Sampling date	^{37}Cl / ^{35}Cl *
Pacific Ocean					
8^{o}39.5'N, 153^{o}56.6'E	0	34.873	28.0	03/04/1998	0.319820 ± 0.000037 (n=7)
12^{o}06.9'N, 138^{o}52.3'E	0	34.387	26.4	03/08/1998	0.319894 ± 0.000171 (n=5)
12^{o}58.4'N, 143^{o}43.7'E	0	34.397	–	03/10/1998	0.319847 ± 0.000041 (n=3)
18^{o}12.9'N, 144^{o}42.0'E	0	34.843	27.0	03/11/1998	0.319829 ± 0.000079 (n=4)
18^{o}12.7'N, 144^{o}42.2'E	992	34.470	4.1	03/11/1998	0.319870 ± 0.000087 (n=4)
18^{o}12.7'N, 144^{o}42.2'E	3235	34.667	1.7	03/11/1998	0.319883 ± 0.000091 (n=5)
18^{o}12.7'N, 144^{o}42.2'E	3725	34.669	1.7	03/11/1998	0.319856 ± 0.000079 (n=6)
26^{o}30.2'N, 142^{o}39.4'E	0	34.947	22.9	03/13/1998	0.319853 ± 0.000117 (n=4)
Setonaikai					
34^{o}38.3'N, 135^{o}07.0'E	0	31.6	–	02/08/1998	0.319849 ± 0.000093 (n=4)

* Error: $2\sigma_m$ (σ_m: external standard error)

Period of the analysis: June 1998 - July 1998

Table 2-5. Measured δ^{37}Cl values of the CsCl reagents

Run	Aldrich (99.999%)	Wako (>99%)
	δ^{37}Cl (‰)	δ^{37}Cl (‰)
1	-0.74 ± 0.22	0.36 ± 0.15
2	-0.68 ± 0.41	0.15 ± 0.12
3	-0.81 ± 0.14	0.00 ± 0.14
4	-1.00 ± 0.15	-0.06 ± 0.15
5	-0.80 ± 0.22	
6	-0.66 ± 0.14	
7	-0.61 ± 0.43	
8	-0.96 ± 0.17	
9	-0.96 ± 0.23	
average	-0.80 ± 0.14 ($1\sigma_{SD}$)	0.11 ± 0.19 ($1\sigma_{SD}$)

Error for the individual runs is $2\sigma_m$ (σ_m: standard error)

CsCl was directly loaded on the filaments with graphite

Table 2-6.
Measured Cl isotopic compositions of the CsCl reagent
(Nacalai tesque, >99%)

Run	$^{37}\mathrm{Cl}/^{35}\mathrm{Cl}$	$\delta^{37}\mathrm{Cl}$ (‰)
Directly loaded[a]		
1	0.318913 ± 0.000039	-2.76 ± 0.12
2	0.318948 ± 0.000049	-2.65 ± 0.15
3	0.318981 ± 0.000071	-2.54 ± 0.22
4	0.319020 ± 0.000057	-2.42 ± 0.18
5	0.319015 ± 0.000044	-2.44 ± 0.14
6	0.319007 ± 0.000040	-2.46 ± 0.13
7	0.318955 ± 0.000043	-2.62 ± 0.13
8	0.319047 ± 0.000033	-2.33 ± 0.10
9	0.319096 ± 0.000061	-2.18 ± 0.19
10	0.318862 ± 0.000054	-2.91 ± 0.17
11	0.318977 ± 0.000060	-2.55 ± 0.19
12	0.319067 ± 0.000057	-2.27 ± 0.18
13	0.319007 ± 0.000075	-2.46 ± 0.24
14	0.319072 ± 0.000086	-2.26 ± 0.27
15	0.318973 ± 0.000090	-2.57 ± 0.28
16	0.318943 ± 0.000048	-2.66 ± 0.15
17	0.319005 ± 0.000044	-2.47 ± 0.14
18	0.318902 ± 0.000053	-2.79 ± 0.17
19	0.319012 ± 0.000090	-2.45 ± 0.28
20	0.319065 ± 0.000070	-2.28 ± 0.22
average	0.318993 ± 0.000061 ($1\sigma_{SD}$)	-2.49 ± 0.21 ($1\sigma_{SD}$)
Recovered Cl[b]		
1	0.318896 ± 0.000040	-2.81 ± 0.12
2	0.319001 ± 0.000041	-2.48 ± 0.13
3	0.319071 ± 0.000055	-2.26 ± 0.17
4	0.318851 ± 0.000046	-2.95 ± 0.14
5	0.319030 ± 0.000047	-2.39 ± 0.15
average	0.318970 ± 0.000093 ($1\sigma_{SD}$)	-2.58 ± 0.29 ($1\sigma_{SD}$)

Error for the individual runs is $2\sigma_m$ (σ_m: standard error)

[a] CsCl reagent was directly loaded on the filaments

[b] Cl^- was recovered from CsCl as AgCl,and converted to CsCl

Table 2-7. Measured δ^{37}Cl values of the inorganic compounds

Run	HCl (gas)	HCl	NaCl	KCl
	δ^{37}Cl (‰)	δ^{37}Cl (‰)	δ^{37}Cl (‰)	δ^{37}Cl (‰)
1	0.88 ± 0.14	-0.26 ± 0.33	-0.95 ± 0.34	-2.30 ± 0.31
2	1.03 ± 0.28	-0.24 ± 0.32	-1.04 ± 0.28	-2.26 ± 0.32
3	0.85 ± 0.16	-0.22 ± 0.25	-0.96 ± 0.47	-2.16 ± 0.37
4		0.05 ± 0.18	-0.70 ± 0.31	
average	0.92 ± 0.10	-0.17 ± 0.15	-0.91 ± 0.15	-2.24 ± 0.07
	($1\sigma_{SD}$)	($1\sigma_{SD}$)	($1\sigma_{SD}$)	($1\sigma_{SD}$)

Error for the individual runs is $2\sigma_m$ (σ_m: standard error)

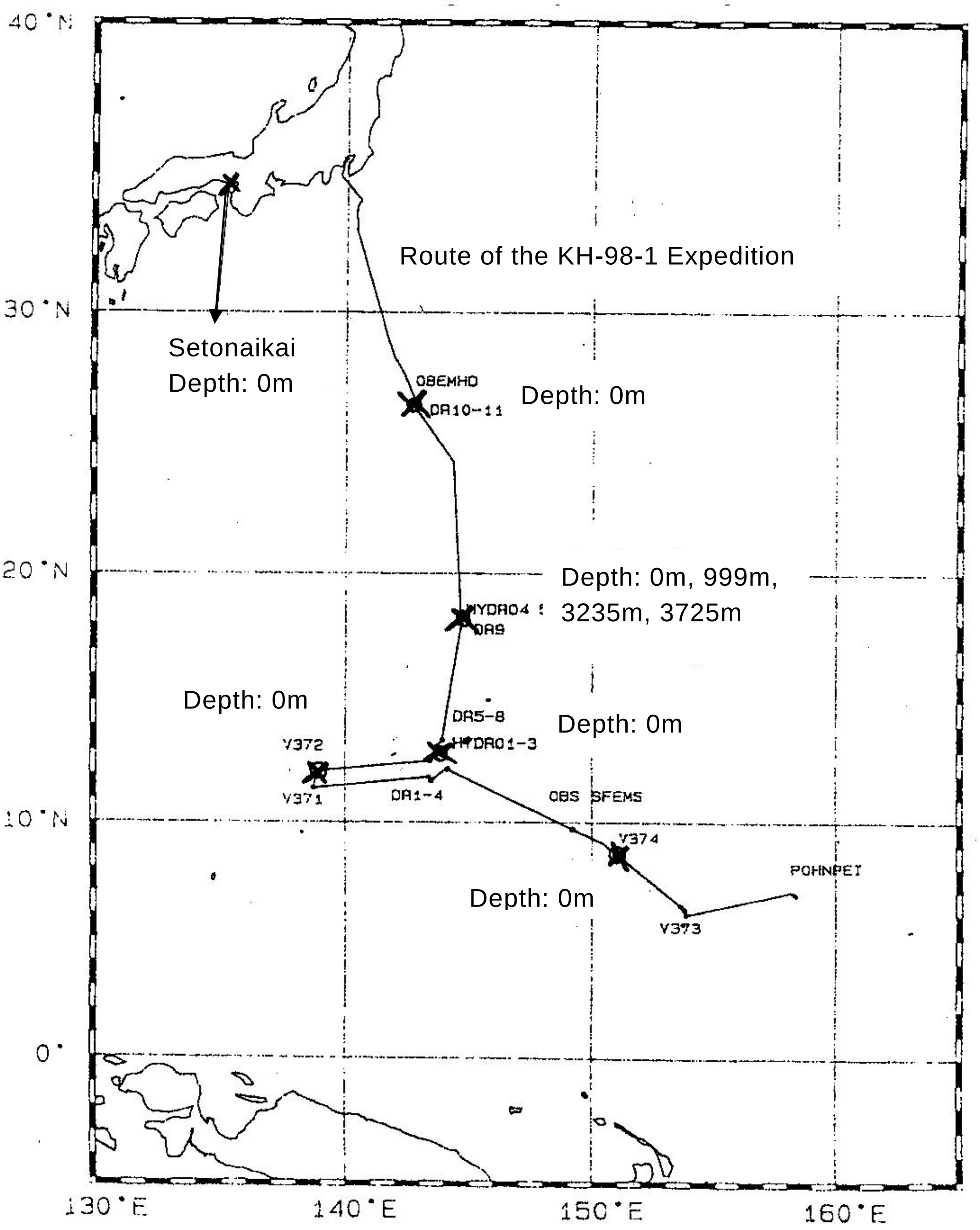


Fig. 2-1. Location map of the seawater sampling points.

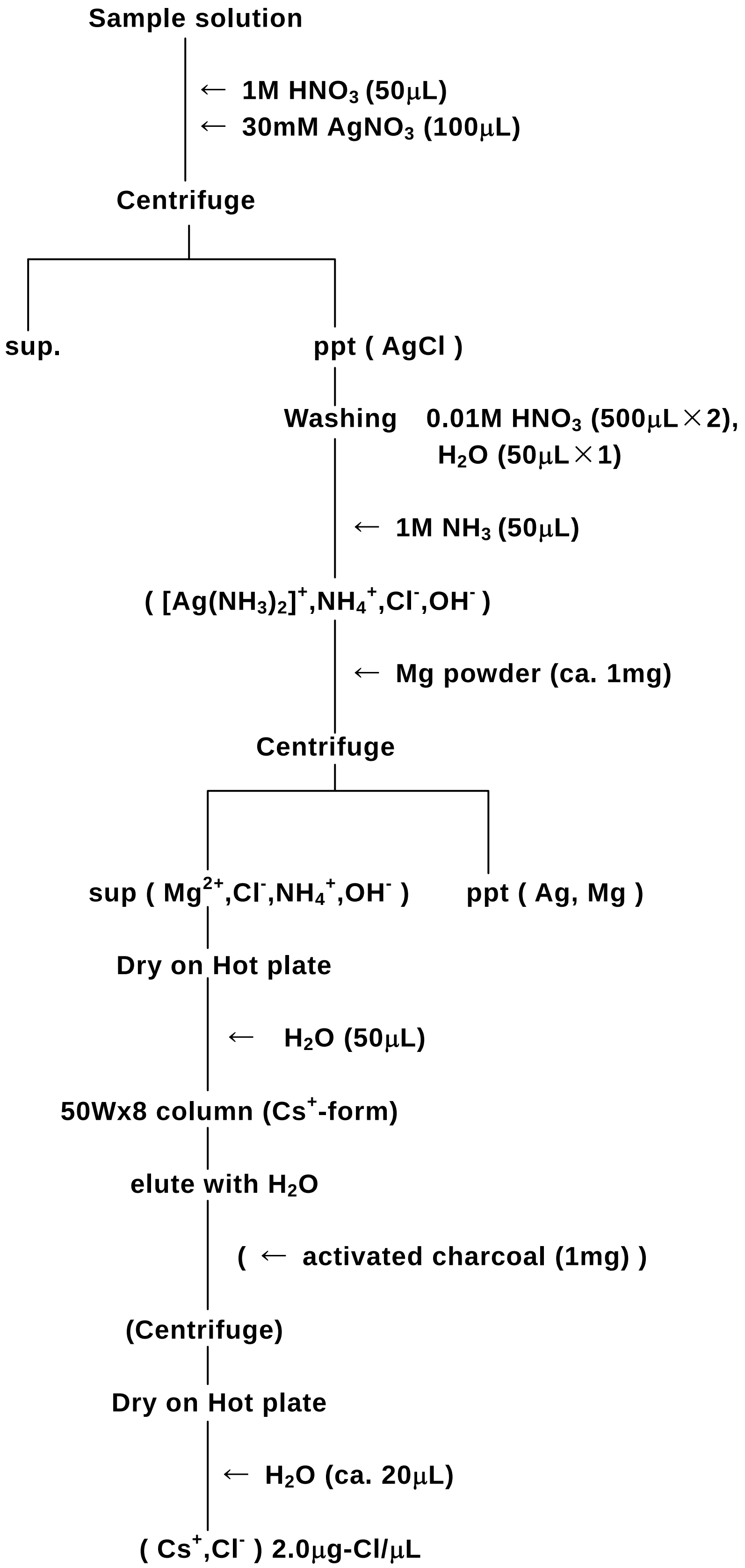


Fig. 2-2. Procedures of CsCl preparation from seawater, inorganic reagents and chloride ion recovered from organic compounds.

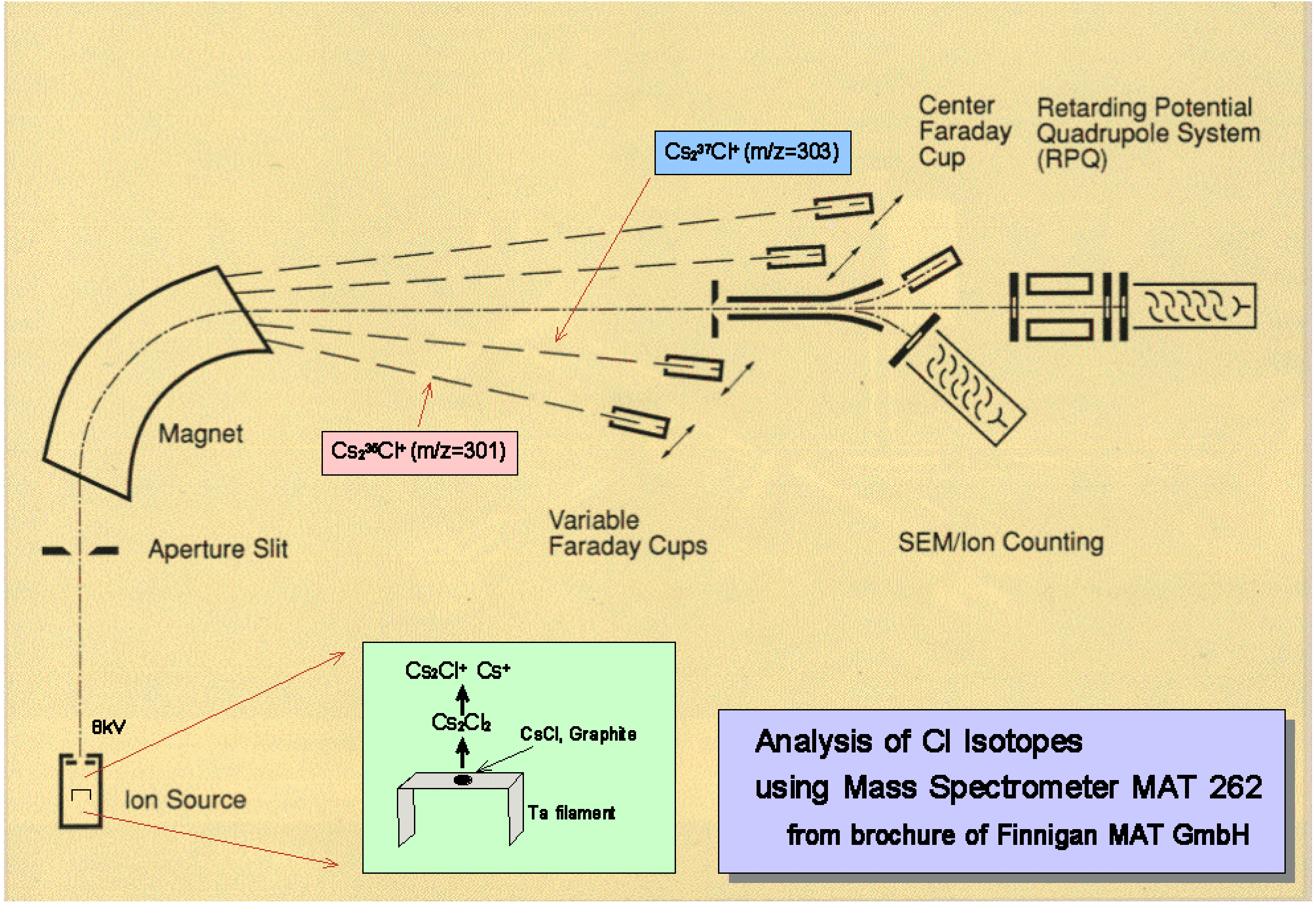


Fig. 2-3. Configuration of TIMS for Cl isotope measurements

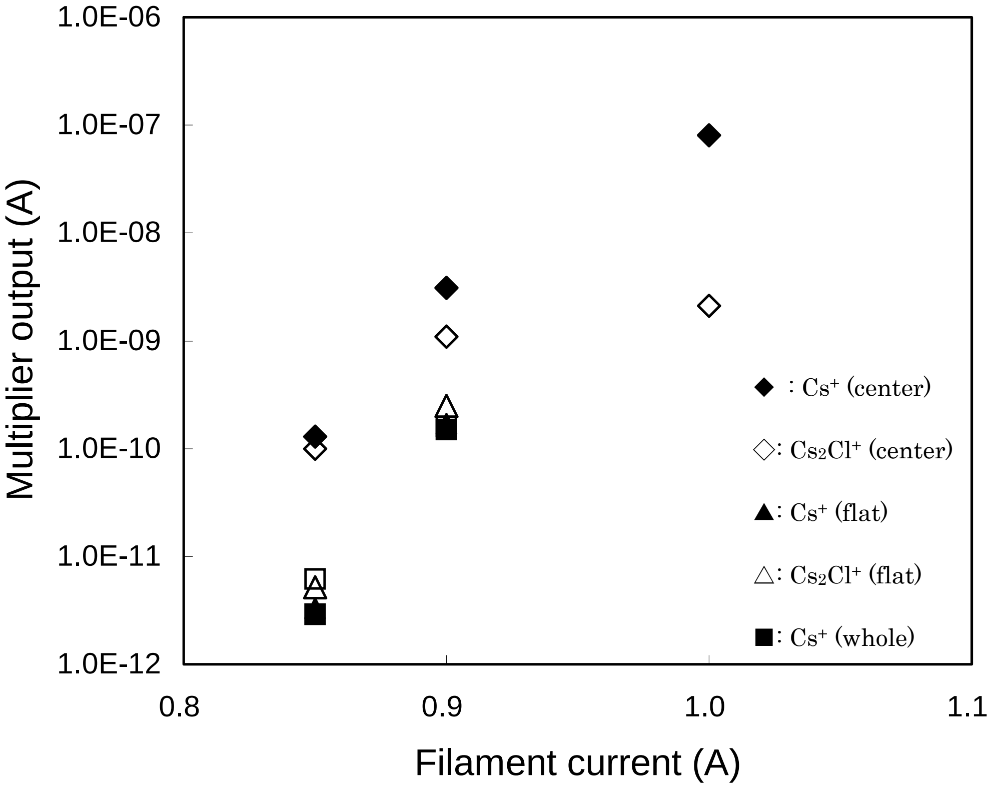


Fig. 2-4. The change of ion current intensities with filament current. A reagent grade CsCl (2 μg-Cl) was loaded on Ta filament (width 0.75mm; Nilaco) with graphite powder. Center, flat, whole: CsCl and graphite were loaded on center of the filament in ca. 1 mm, 2 mm, 7 mm of length respectively.

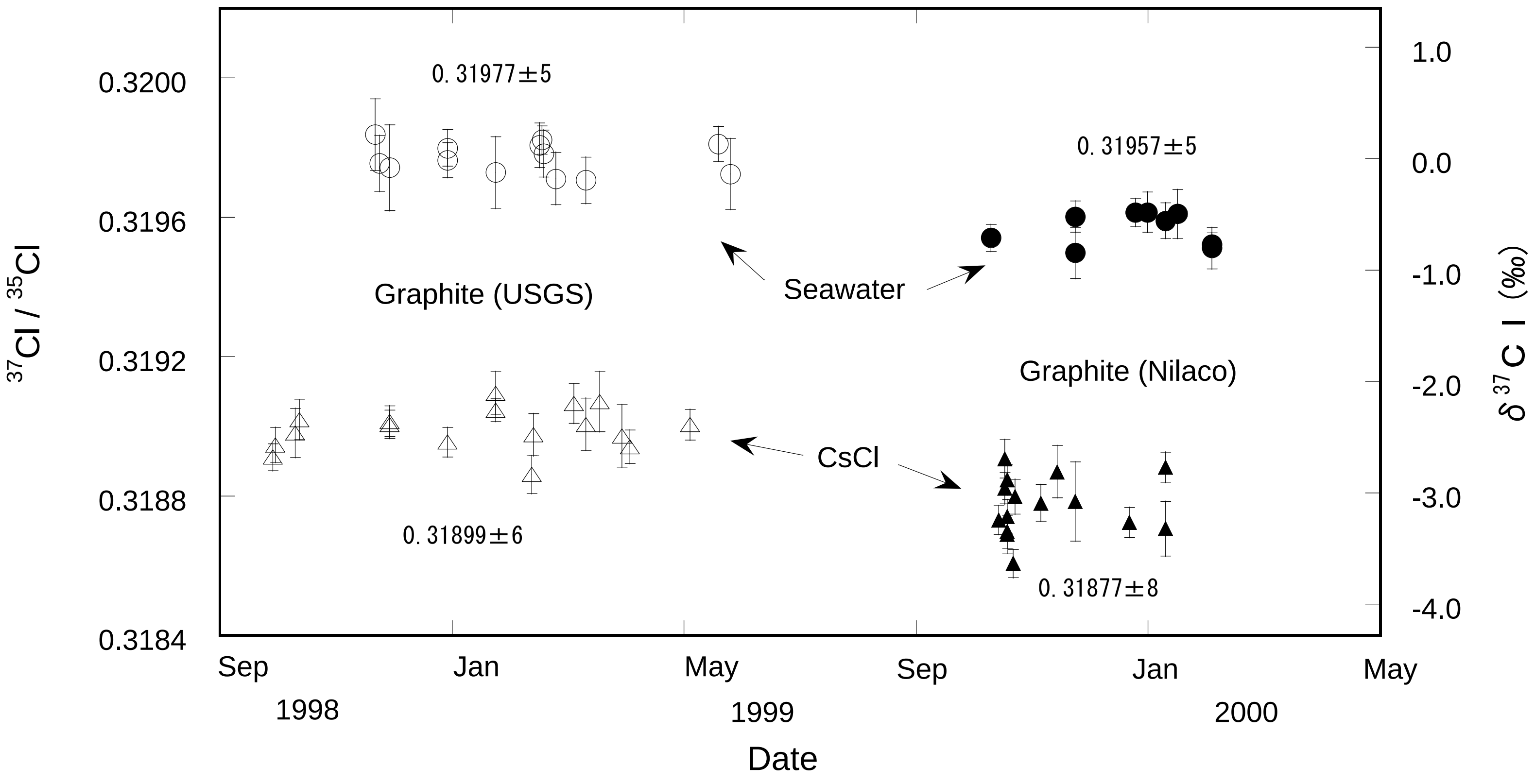


Fig. 2-5. Variation of ^{37}Cl / ^{35}Cl ratios of seawater chloride and Nacalai CsCl reagent with different kinds of graphite load.

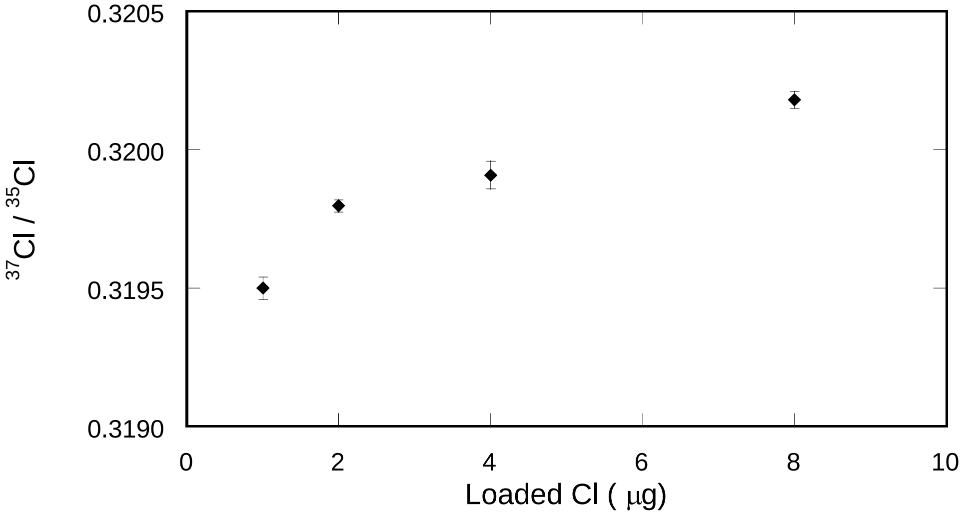


Fig. 2-6. Change of Cl isotopic ratio data with amount of loaded Cl. The sample was CsCl converted from seawater chloride (8°39.5'N, 153°56.6'E).

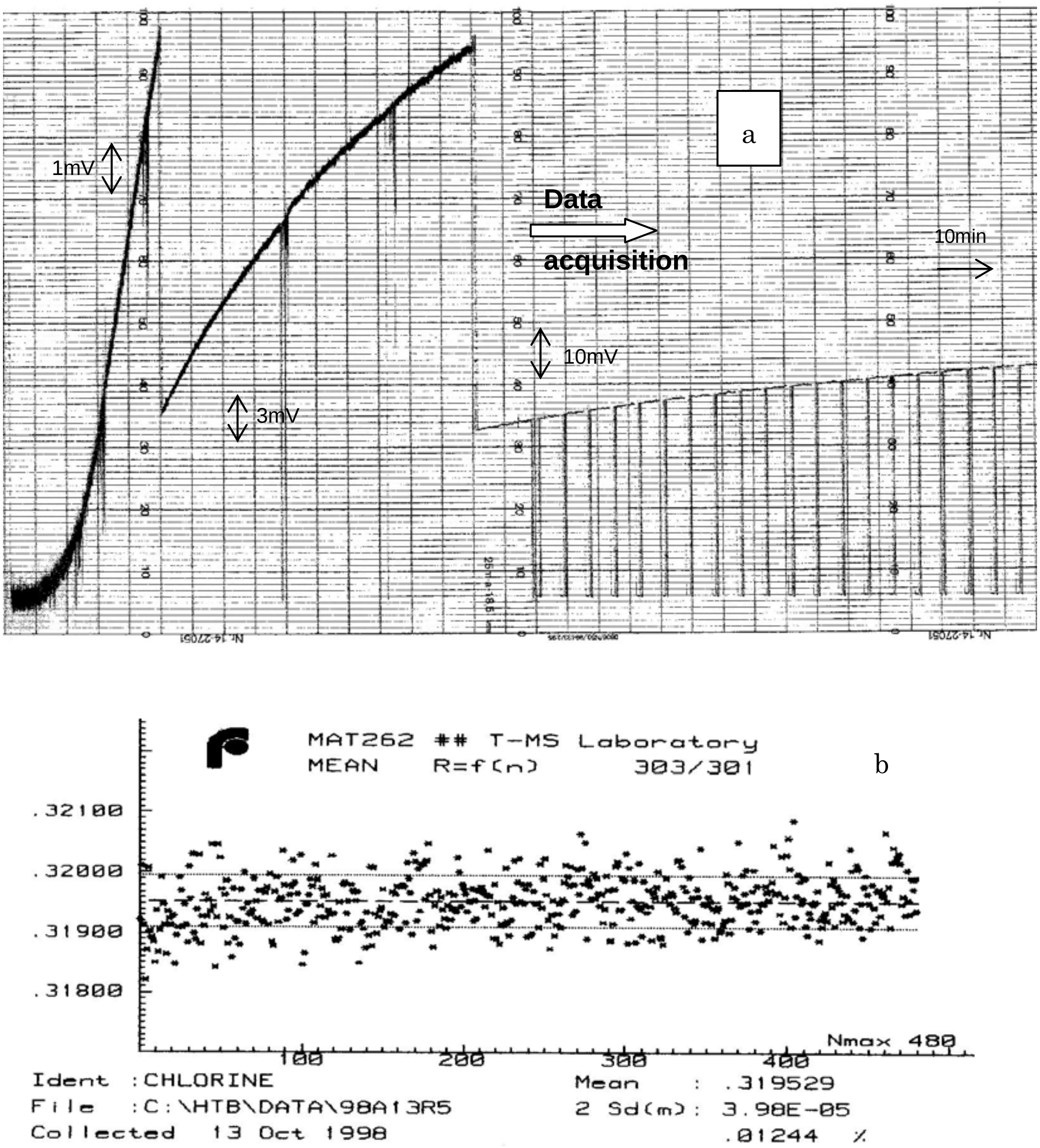


Fig. 2-7. Typical time course of Cs_2Cl^+ ion current (a) and mass discrimination (b) during TIMS analysis.

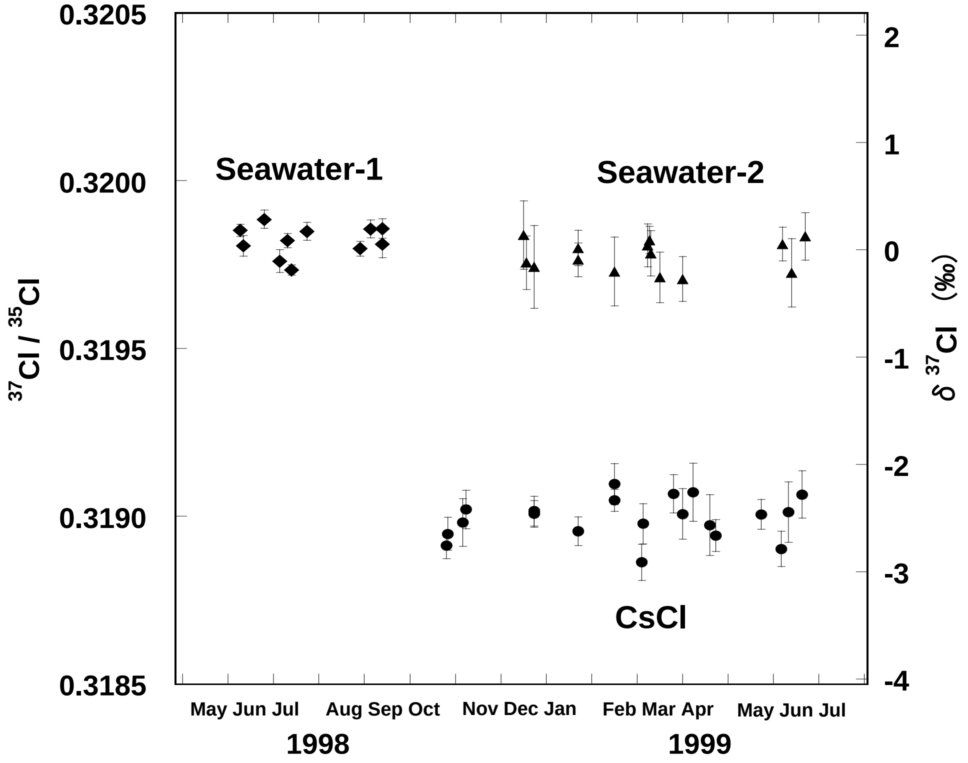


Fig. 2-8. Chlorine isotopic compositions of seawater and a CsCl reagent for the period between June 1998 and July 1999. The seawater was taken from the Pacific Ocean (8°39.5'N, 153°56.6'E) in March 1998 (Seawater-1: The seawater was treated with Ba-form resin and Cs-form resin; Seawater-2: The seawater was treated with $AgNO_3$, Mg, and Cs-form resin). The commercial grade (purity: >99%) CsCl reagent was obtained from Nacalai tesque, Kyoto. Error bars represent $2\sigma_m$ (σ_m: internal standard error).

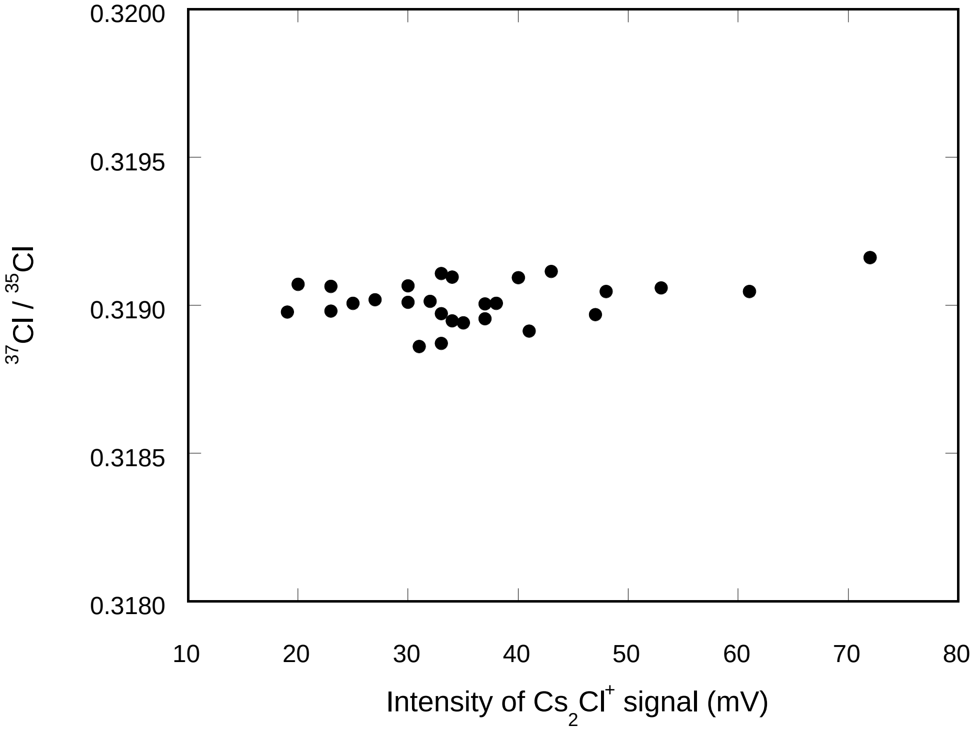


Fig. 2-9. Relationship between Cl isotopic compositions of the Nacalai CsCl and signal intensities of Cs_2Cl^+.

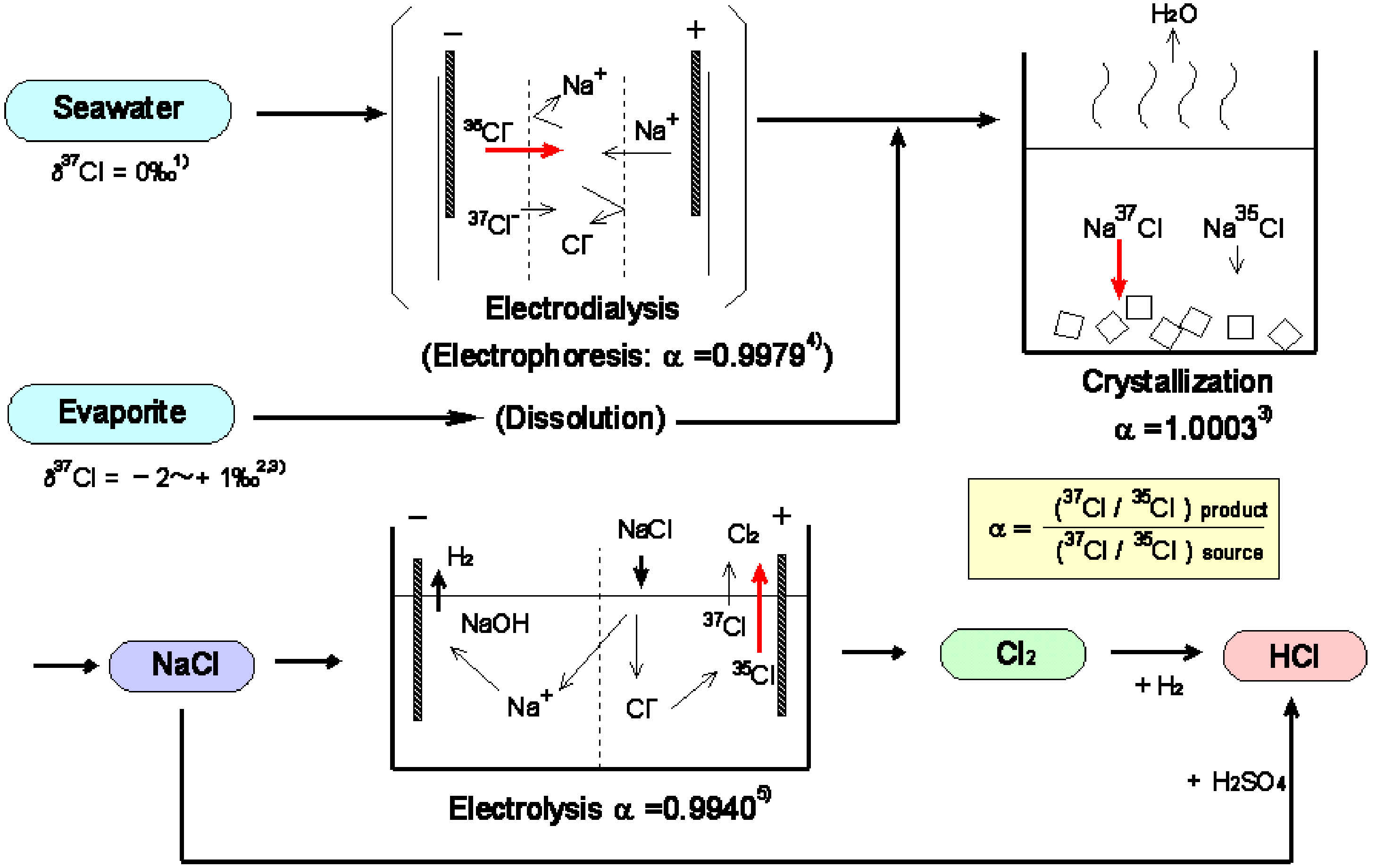


Fig. 2-10. Cl isotope fractionation during manufacturing processes of Cl_2 and HCl from Encyclopedia of Chemical Technology (John Weiley & Sons) etc., 1) Kaufman et al.(1984), 2) Liu et al.(1997), 3) Eggenkamp et al.(1995), 4) Madorsky & Straus (1947), 5) Herrick & Hutchison (1942)

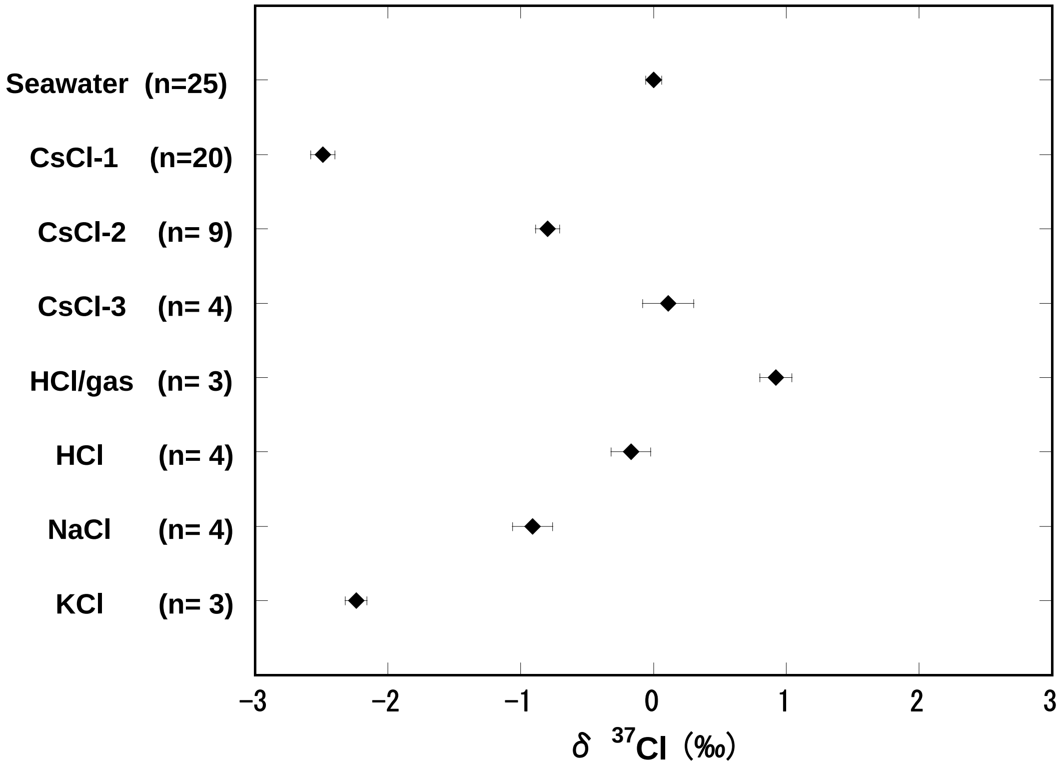


Fig. 2-11. Results of chlorine isotopic measurements of seawater and inorganic reagents. CsCl reagents were purchased from three companies (Nacalai tesque, Aldrich, Wako Pure Chemicals). Error bars are $2\sigma_m$ (σ_m: external standard error).

3. Measurements of chlorine isotopic ratio in chlorinated organic compounds

Introduction

Soil and groundwater contaminations with chlorinated aliphatic hydrocarbons (CAHs) are found in many industrial and residential areas (Kawasaki, 1985; Fisher et al., 1987). Although some of CAHs are suspected to be carcinogens and mutagens, it is difficult to identify the sources of subsurface pollutions and to evaluate effectiveness of clean-up technologies because of limited monitoring points and frequency, and complex behavior of CAHs in subsurface environments (Fig. 3-1).

Isotopic ratios of elements such as carbon, nitrogen, sulfur, boron, selenium, lead are useful indicators for tracing the sources of pollutants and their reactions in the field (Conrad et al., 1997; Aggarwal et al., 1997; Jarman et al., 1998; Yoshida and Toyoda, 2000; Nakai et al., 1991; Komor, 1997; Johnson et al., 2000; Strurges and Barrie, 1989). Ratios of two stable Cl isotopes (^{37}Cl / ^{35}Cl) can be used for similar purposes.

The range of Cl isotopic compositions in natural systems is smaller compared to that of other light elements (Hoef, 1997). The isotopic fractionation occurs in groundwater by the difference of diffusion rate between ^{35}Cl and ^{37}Cl (Desauliniers et al., 1986), ion filtration process (Phillips and Bentley, 1987), and mixing of different source of Cl (Kaufmann et al., 1993). The Cl isotopic composition of ground water typically range from –2 to +2‰ relative to standard mean ocean chloride (SMOC). In contrast, Tanaka and Rye (1991) reported a larger Cl isotopic variations in CAHs, and suggested that it may be used to quantify the flux of stratospheric chlorine derived from man-made chlorinated compounds apart from natural sources (e.g. Gschwend et al., 1985; Gribble, 1994; Hoekstra et al., 1998; Jordan et al., 2000; Robert et al., 2000; Yokouchi et al., 2000; Keppler et al., 2000). A similar study was also undertaken by van Warmerdam et al. (1995) who measured the Cl and C isotopic ratios in CAHs to distinguish the groundwater contaminants from different manufacturers and to trace them.

Electron impact mass spectrometry of CH_3Cl (Taylor and Grimsrud, 1969; Kaufmann et al., 1984; Long et al., 1993; Musashi et al., 1998) is known as the most precise method of Cl isotopic analysis, and has been used for investigation of Cl isotopes in CAHs (Tanaka and Rye, 1991; van Warmerdam et al., 1995; Holt et al., 1997; and Jendrzejewski et al., 1997; Beneteau et al., 1999) and PCBs (Jarman et al., 1998; Reddy et al., 2000). However, the technique of CH_3Cl preparation from chloride requires relatively large quantities of chlorine.

Thermal ionization mass spectroscopy of Cs_2Cl^+ has small mass dependent fractionation (Xiao and Zhang, 1992; Xiao et al., 1995), low filament blank, and high sensitivity (Magenheim et al., 1994; Rosenbaum et al., 2000). In this study, chlorine isotopic ratios in CAHs were determined using a high resolution mass spectrometer.

Experimental Procedures

Presentation of isotopic data.

The isotopic compositions of chlorine are represented in per mil (‰) deviation from those of the standard mean ocean chloride (SMOC: Long et al., 1993), and a reagent grade CsCl from Nacalai tesque was utilized as a routine laboratory standard of Cl isotopic composition during the course of this study (Numata et al., in press; see Chapter 2).

$$\delta^{37}Cl = \left[\left(\frac{R_{sample}}{R_{SMOC}}\right) - 1\right] \times 1000 \qquad R = \frac{^{37}\mathrm{Cl}}{^{35}\mathrm{Cl}} \tag{3-1}$$

One or two standards were typically loaded into the mass spectrometer together with the samples, and δ values of samples were calculated from the following equation.

$$\delta^{37}Cl \; = \left[1 - \frac{R_{sample}(1 + 10^{-3}\delta_{CsCl})}{\overline{R}_{CsCl}}\right] \times 1000 \tag{3-2}$$

where δ_{CsCl}: -2.49 (per mil deviation of isotopic ratios between the CsCl reagent and seawater, which was calculated from more than 25 isotopic measurements; Chapter 2), $\overline{R}$: average of standard CsCl isotopic ratios in the period of sample analysis.

Samples and Reagents.

The water used for this work, was purified using a Milli-RX12Plus and a Milli-Q SP system (Millipore). A commercial grade CsCl (minimum assay 99%; Nacalai tesque) were stored as 56.4 mM (2.0 g-Cl/L) solution and was used for laboratory reference standard (Numata et al., in press). An activated charcoal (GX-60: GL Sciences) was washed with water before use in order to remove chloride ion.

Dichloromethane (DCM: minimum assay 99 %; Wako, 99 %; Nacalai, pesticide analysis grade; Nacalai, pesticide analysis grade; Wako), trichloromethane (CF: assay 99 %; Nacalai), tetrachloromethane (CT: minimum assay 95%; Nacalai, assay 99 %; Nacalai, minimum assay 96 %; Nacalai), 1,2-dichloroethane (DCA: assay 99 %; Nacalai), 1,1,1-trichloroethane (MC: assay 97 %; Wako, assay 95 %; Nacalai), 1,1-dichloroethene (1,1-DCE: assay 99 %;GL), *cis*-1,2-dichloroethene (cDCE: assay 97 %; GL), *trans*-1,2-dichloroethene (tDCE: assay 99 %; GL), trichloroethene (TCE: minimum assay 99.5 %; Wako, minimum assay 99.5 %; Wako, assay 99.5 %; Nacalai), and tetrachloroethene (PCE: minimum assay 99 %; Wako, assay 98%; Nacalai, minimum assay 98 %, Nacalai, assay 99.7 %; Merck) were used for Cl isotopic measurements (where GL: GL Sciences; Merck: E. Merck ; Nacalai: Nacalai tesque; Wako: Wako Pure chemical Industries). Other chemicals were analytical or reagent grades.

Preparation of Cl^- fromCAHs.

The organic chlorine atoms were liberated as chloride ion with the sodium biphenyl reagent following the method of Liggett (1954) with some modifications (Numata et al., in press; Fig. 3-2).

Place 2.0 ml of organic solvents (toluene, n-decane or di-isopropyl ether) and 1.2 to 1.6 mg of chlorinated organic compounds containing 1.0 mg of Cl in test tubes with screw stoppers. Gas phase was replaced with nitrogen, and 0.2 ml of sodium biphenyl reagent (1 M solution in dimethoxyethane, Dojindo Laboratory) was added. After 30 min, 0.5 mL of water was added and shaken to extract chloride ion and to destroy the excess reagent. The lower aqueous layer was separated and the chloride ion was extracted twice with 1.0 mL of 1 M HNO_3 and 0.5 mL of water respectively. The extracted fractions were combined and diluted to 10 mL with water.

CAHs (DCM, CT, TCE and PCE) were also treated with $KMnO_4$ and the Fenton reagent (H_2O_2 + Fe^{2+}) (Imagawa, 1990) in order to compare the recovery yield of Cl and isotopic ratio with the result of sodium biphenyl method. Place 100 mL of 0.01 M $KMnO_4$ and 15 mg of CAHs in a serum bottle, and the bottles were sealed with polytetrafluoroethene lined rubber septa and aluminum cups. After the reaction (30 ℃, 12 hr), 0.38 g of Na_2SO_3 was added and the bottles were shaken to reduce the excess permanganate ion. For the experiments of Fenton reaction, 45 mL of aqueous solution containing 50 mg of $FeSO_4 \cdot 7H_2O$, 25 mg of H_2SO_4 and 15 mg of CAHs were placed in a serum bottle. The bottles were then sealed with polytetrafluoroethene lined rubber septa and aluminum cups, and the solution was injected with 5 mL of 20% H_2O_2. After reacting for 12 hours at 30 ℃, 1.5 mL of 1 M Na_2SO_3 solution was added and the bottle was shaken to reduce the excess H_2O_2.

Preparation of CsCl from Cl^-.

Chloride ion in aqueous solution was isolated as AgCl and was converted into CsCl using the modified method of Hoering and Parker (1961) and Magenheim et al. (1994) (Numata et al., in press; see Chapter 2). The degradation products of CHAs containing 50 μg of Cl^- were placed in a polypropylene centrifuge tubes and water was added to produce 1.0 mL of solution. Fifty microliter of 1 M HNO_3 and 100 μL of 30 mM $AgNO_3$ solution were then added to each tube. After waiting for ~1 hour at room temperature, the solutions were centrifuged at 12,000 g for 5 min, and the supernatants were discarded. The AgCl precipitate was washed twice with 500 μL of 0.01 M HNO_3 and once with 50 μL of water by centrifugation, and 50 μL of 1 M NH_3 was subsequently added to dissolve the AgCl. Approximately 1 mg of Mg powder was then added to the solution. After one-hour of reaction, the solution was centrifuged and the supernatant was recovered. The precipitate was washed twice with 50 μL of water. All supernatant fractions were combined and evaporated to dryness on a hot plate. The residue (NH_4Cl + $MgCl_2$) was dissolved in 50 μL of water and was loaded onto a column that contained 0.1 mL of Cs-form cation exchange resin (DOWEX 50Wx8, 200-400mesh; Nakarai Chemicals). The column was washed with 150 μL of water. Activated charcoal suspension (20 μL, ca.1mg activated charcoal included) was added to the eluent in order to remove possible residual organic compounds. After 30 min, the solution was centrifuged (at 12,000 *g*, 5 min). The precipitate was washed with 50 μl of water, and was centrifuged again. The concentration of Cl^- in the combined supernatant was measured by ion chromatography. The eluent was evaporated to dryness on a hot plate. Amount of Cl^- was calculated from Cl^- concentration and weight of the eluent, and then water was added to the solid residue (CsCl) to form 2 g-Cl/L of solution.

Chloride ion concentrations were measured using a Hitachi ion chromatograph L-7000 with a Hitachi 2740 analytical column and a mixed eluent (2.3mM phthalic acid and 2.5mM 2-amino-2-hydroxymethyl-1,3-propanediol) at 1 mL min^{-1}.

CAHs recovery from aqueous solution

To establish an extraction method of Cl in chlorinated solvent dissolved in water, reagent grade TCE (Wako) and PCE (Wako) were recovered from aqueous solution by solvent extraction and air purge. The extraction experiments were performed in 120-mL serum bottles. Place 100 mL of water and 1.5 mg of TCE or PCE were placed in serum bottles, and then the bottles were sealed with polytetrafluoroethene lined rubber septa and aluminum cups. After 24 hours, 3.0 mL of toluene was added through the septum in order to extract TCE. The bottles were then shaken vigorously to extract TCE from water and headspace. After transferring the toluene layer containing chloroethenes to another test tube, 2.0 mL of toluene was added to the bottle and extraction of chloroethenes was repeated. TCE extracted in toluene was dried with 1.0 g of anhydrous Na_2SO_4 and then treated with the sodium biphenyl reagent by the above method. Because remaining water consumed the sodium-biphenyl reagent at the degradation of TCE, 400 μL of the sodium-biphenyl reagent was added instead of 200 μL.

The TCE solution was also purged with air flow (30 mL / min, 1 hr) and the effluent was passed through in PbO_2+H_2SO_4 layer in a commercial gas detector tube (Gastec No.132M: for TCE detection). Released HCl gas was bubbled through 10 ml of water in test tube.

Regioselective Cl elimination from TCE

TCE (Wako) was degraded by three kinds of culture of anaerobic bacteria (Table 4-1) to eliminate Cl_c in the TCE molecule (Fig. 3-5). From regressions of Cl isotopic ratio trajectories with fraction of TCE, Cl isotopic ratio in Cl_c and average of ratio in Cl_a and Cl_b were calculated (Fig. 4-7).

The Cl_b atom in the TCE molecule was liberated as chloride ion by β-elimination (dehydrochlorination) with KOH. The reaction was performed in 68-mL serum bottles with 1.0 mL of 5 M KOH. The solution was purged with N_2 gas for 5 min to remove oxygen in the media and headspace gas. The bottles were then sealed with polytetrafluoroethylene lined rubber septa and aluminum cups. The solution was injected with 10 mg of the TCE, and then heated in an autoclave for 8 hors at 121 ℃. Progress of the reaction was monitored by headspace analysis using GC-FID after cooling. The headspace was purged with N_2 gas for 5 min to remove oxygen and dichloroethyne, a reaction product, because dichloroethyne is unstable in oxic atmosphere. The solution was neutralized with 1 M HNO_3 and the eliminated Cl^- was recovered as AgCl.

Mass Spectrometry

Isotopic determinations were performed using a Finnigan MAT Model 262 thermal ionization mass spectrometer. The mass spectrometric procedure (see Chapter 2) was modified from Xiao and Zhang (1992), Magenheim et al. (1994) and Xiao et al. (1995). Two micrograms of chlorine as CsCl aqueous solution (56.4 mM, 1.0 μL) and ca. 70 μg of graphite powder (purity: 99.999 %; Nilaco) as a slurry in 80 % ethanol were mixed on a polytetrafluoroethylene sheet. The mixed slurry was loaded onto the center of a Ta filament (purity: 99.95 %; 0.025 × 1.5 mm, Nilaco) and was dried with a filament current of 1.5 A. The filaments were placed into the ion source of the mass spectrometer and the analysis began when the pressure in the ion source was better than 10^{-5} Pa. The filament current was first increased to 1.2 A in 10 min, and then increased manually to detect the $Cs_2{}^{35}Cl^+$ ion beam. The intensity of $Cs_2{}^{35}Cl^+$ ion current was adjusted to $(2 - 3) \times 10^{-13}$ A by adjusting the filament current, which was typically between 1.35 - 1.43 A. Because mass range of the target ions was high ($Cs_2{}^{35}Cl^+$: m/z=301 and $Cs_2{}^{35}Cl^+$: m/z=303), the accelerating voltage was set to 8 kV.

Results and Discussion

Effects of degradation procedures on Cl recovery yield

In general, CAHs containing hydrogen atom(s) and/or double bond(s) degraded easily by abiotic or biotic oxidation. On the other hand, reaction rate of reductive dechlorination is higher according with number of Cl atoms in molecules (Vogel et al., 1987). In order to avoid mass discrimination during sample pretreatment, Cl recovery yields should be as high as possible. The representative CAHs (DCM, CT, TCE, PCE) were degraded by various methods, and the yields were compared (Table 3-1). The yields of Cl by dechlorination with the sodium-biphenyl reagent were the highest among tested reactions, but CT was resistant to $KMnO_4$ and the Fenton reagent. The Cl yield from DCM was low in the case of $KMnO_4$ oxidation.

Cl isotopic ratios in Cl^- recovered from TCE (Wako) and PCE (Wako) were measured (Table 3-2, 3-3). Although $KMnO_4$ oxidation was a relatively slow reaction (Fig. 3-3), the recovery yield from TCE was high for both sodium-biphenyl and $KMnO_4$ treatments. The $KMnO_4$ oxidation gave slightly lower $\delta^{37}Cl$ values compared with sodium-biphenyl treatment. However, because the difference in $\delta^{37}Cl$ values between the two treatments was insignificant, it would be proper to use both degradation procedures for Cl recovery from CAHs. On the other hand, the Fenton reaction gave low Cl yield and higher isotopic ratio. When this reaction was performed, the pressure of the reaction vessels increased. Part of TCE may have escaped from the reaction vessels as the pressure of the vessel increased with oxygen generated from H_2O_2 and relatively heavier TCE isotopomers may have degraded. The results of PCE degradation experiments were similar to the results of TCE.

As any technique of compounds specific Cl isotopic measurements has not been reported, it will be difficult to interpret Cl isotopic data of samples containing complex mixture of organic chlorine compounds. Although the

GC/C/MS is a powerful tool to trace natural or artificial organic compounds by compound specific $\delta^{13}C$ or $\delta^{15}N$ fingerprints (e.g. Hunkeler et al., 1999; Hunkeler and Aravena, 2000a; Bloom et al., 2000), such on-line isotope ratio mass spectrometry for Cl has never been developed. Separation (e.g. preparative chromatography) or compound (group) specific degradation (e.g. $KMnO_4$ oxidation) techniques might be used for the purpose.

Effect of solvents on Cl recovery from CAHs

Dehalogenation of chlorinated organic compounds with sodium-biphenyl reagent has been performed in benzene (Pecherer et al., 1950), toluene (Liggett, 1954) or diethyl ether (Venkateswarlu, 1982). Effects of solvents (n-decane, toluene, di-isopropyl ether) on Cl recovery yields from CAHs were examined to obtain high Cl recovery yield (Table 3-4). The yield was the highest in the case of toluene extraction. The Cl recovery rates from CT and DCM were lower using di-isopropyl ether. Thus, toluene was used for routine analysis of CAHs.

Comparison of Cl isotopic ratios in CAHs

The sodium biphenyl reagent, known as a reactive reductant, is used for complete extraction and qualification of organic chlorine in fuels and industrial waste. Although yield of reductive dechlorination from ca. 1 mg of chlorinated organic solvents was high (96 to 99 %, average, 98 %), yield of Cl recovery as CsCl (78 to 97 %, average: 85 %) was lower than the yield of CuO-oxidation methods used for CH_3Cl preparation (Holt et al., 1997; Jendrzejewski et al., 1997). Since isotopic fractionation during the CsCl preparation procedure from Cl^- is negligible (Table 2-6), low recovery yield at this step would not be a problem during the analysis. Chloride ion was not detectable by ion chromatography during blank test of the pretreatment procedure. Possible contamination in the CsCl preparation step was less than 0.05 % of Cl^- from organic compounds.

Ten different kind of chlorinated organic solvents (chlorinated methanes, ethanes and ethenes; 20 lots) obtained from several agents of chemical products were used for isotopic measurements, and the results are shown in Table 3-5, 3-6. The variation of isotopic compositions (-5.0 to +2.9‰) is consistent with reported values, e.g. -6.8 to +2.6 (Tanaka and Rye, 1991), -2.9 to +4.1(van Warmerdam et al., 1995), -2.9 to +2.1 (Holt et al., 1997), +1.0 to +3.9 (Jendrzejewski et al., 1997). The chlorinated organic solvents are produced adding of Cl_2 or HCl to carbon from CH_4, CH_3OH, C_2H_4, C_2H_2, C_3H_8 (Grayson, 1985). Difference in raw materials, processing of salts and production of Cl_2 and HCl gas would cause differences of $\delta^{37}Cl$ in organic compounds (Fig. 2-10).

In addition to the difference in raw materials and intermediates, the isotope effects on C-Cl covalent bond formation or Cl elimination from carbon atoms are important. Chlorine kinetic isotope effects (k_{35} / k_{37}) on nucleophilic substitution by S_N1 and S_N2 reaction mechanisms showed that ^{37}Cl is bound more firmly to the carbon atom than ^{35}Cl (Barthlomew et al., 1954; Turnquist et al., 1973; Wastaway et al., 1998). Therefore the isotopic fractionation during the synthesis of chlorinated compounds would have a cumulative effect on isotopic variations of Cl_2 and HCl. Radical reaction, electrophilic addition, β-elimination, and other reactions of Cl_2 or HCl are utilized to manufacture the chlorinated compounds ranging in temperature from 20 to 650 ℃ (Fig 3-4). The high $\delta^{37}Cl$ value of 1,1-dichloroethene can be explained by faster elimination of $H^{35}Cl$ from 1,1,2-trichloroethane at relatively low reaction temperature (< 100℃). However, only speculations on the fractionation mechanisms are possible, because the manufacturers did not open the information about actual manufacturing procedures of each reagent. More information on the manufacturing processes of the chlorinated organic compounds is necessary to qualify fractionation effects.

CAHs recovery from aqueous solution

In order to determine Cl isotopic ratio of actual groundwater samples, low concentration CAHs need to be extracted from aqueous solution and the Cl in CAHs has to be converted to CsCl. The purge-and-trap (Struchio et al., 1998; Beneteau et al.,1999; Heraty et al., 1999) and solid-phase microextraction (SPME: Hunkeler and Aravena, 2000a) techniques have been applied successfully for isotopic analysis of CAHs in aqueous solution. In this study, solvent extraction and air purging methods were examined.

Difference between Cl isotopic ratios in TCE pure phase and TCE recovered by toluene extraction were insignificant (Table 3-7). Also TCE (Wako) and PCE (Wako) were extracted from a medium for bacteria culture (MMY medium for anaerobic bacteria; see Chapter 4). The δ^{37}Cl value in recovered TCE and PCE were -1.66±0.22 ‰ (n=3, error: $1\sigma_{SD}$) and -2.46±0.08 ‰ (n=4, error: $1\sigma_{SD}$) respectively. The differences between Cl isotopic ratios in pure phase and extracted TCE and PCE were insignificant.

Instead of aqueous solution of CAHs, 1 M NaCl solution was extracted with toluene and the recovered toluene layer was treated with the sodium-biphenyl reagent. The reaction product was extracted with water and HNO_3 and then Cl^- concentration was measured by ion chromatography to determine the procedural blank. Because the Cl blank was less than 0.1 μg, inorganic chloride in sample solution will have negligible effect on the measurement of Cl isotopic ratios in CAHs of more than 10 microgram.

Detection tubes are known as simple tools for determination of gaseous pollutants in work-environments and soil gas in contaminated site. Chlorinated ethenes can also be detected with a kind of detection tube. In the detection tubes, chlorinated ethenes are oxidized PbO_2 / H_2SO_4 mixture, and the HCl produces change of the color of pH indicator layer. In order to recover HCl from TCE vapor for isotopic analysis, the air was bubbled in the TCE aqueous solution and then passed through the PbO_2 / H_2SO_4 layer and water. However, the recovery yield was low and Cl isotopic ratio in recovered HCl was higher than the value in TCE pure phase. As low efficiency of TCE oxidation in PbO_2 / H_2SO_4 layer might give lighter HCl, low recovery on the step of trapping in water could be the cause of the results.

Intramolecular site specific Cl isotopic ratio in TCE

Intramolecular distributions of isotopes (i.e. compositions of isotopomers) constrains geochemical cycles of substances more strictly compared with bulk isotopic composition (Yoshida and Toyoda, 2000). Because the CAHs consist of combinations of C, H, Cl isotopes, they are actually complex mixture of isotopomers. Even if the existence of ^{13}C and ^{2}H are neglected to simplify the discussion, there are theoretically 8 TCE isotopomers (Fig. 3-5). Perhaps it is not practical to determine the abundances of isotopomers and to interpret the data. TCE isotopomers are categorized into 4 isobaric molecules (e.g. $C_2H^{35}Cl_3$, $C_2H^{35}Cl_2{}^{37}Cl$, $C_2H^{35}Cl^{37}Cl_2$, $C_2H^{37}Cl_3$) and the compositions may be determined by gas-phase mass spectrometry. However, it would be difficult to withdraw information about past records of the molecules from the compositions. On the other hand, synthesis or degradation processes would reflect to intramolecurar site specific isotope distribution, because reactivity of the atoms on each sites are not equivalent in asymmetric molecules.

TCE is the only asymmetric molecule among chlorinated ethenes containing plural Cl atoms. This means each Cl atoms are distinguishable by their intramolecular sites. Several anaerobic bacteria cultures (e.g. Yaguchi et al., 1994; Lee et al., 1997; Numata et al.,1999a,b) mediate reductive dechlorination (hydrodechlorination) of TCE to cDCE. In this case, only Cl_c is eliminated by the reaction (Fig. 3-6). The difference between the initial and final isotopic composition of the organic Cl (TCE+cDCE), would indicate difference of isotopic compositions of Cl_c and Cl_a+Cl_b. The compositions were calculated by three regression curves (Fig. 4-7).

In order to determine $\delta^{37}Cl$ value in Cl_b (and Cl_a), TCE was treated with KOH (Otto, 1942). The dehydrochlorination product will be dichloroethyne and Cl^- (Cl_b), if the reaction mechanism is anti-elimination of H and Cl atoms in the molecule. Although 47 % of TCE was lost from the reference reaction vessel and 9.1 % of TCE remained in the reaction vessel, 31.8 % of Cl was recovered from TCE as Cl^-. This means that the labile dichloroethyne was degraded and the recovered Cl^- included not only Cl_a but also Cl_b and Cl_c. Assuming that the $\delta^{37}Cl$ value (+2.6±0.3 ‰, n=9, error: 95 % confidential limit) of recovered Cl^- is the isotopic composition of Cl_a, the $\delta^{37}Cl$ value of Cl_b may be 2.0 ‰. The provisional results in intramolecular Cl isotope distribution are shown in Fig. 3-6. However other site-specific and high-yield elimination procedure should be applied to obtain accurate isotopic ratio in Cl_a and Cl_b.

Comparison of mass spectrometric methods

Impurities or degradation products of some chlorinated organic solvent inhibited the ionization of CsCl without activated charcoal treatment. Also, small amount of impurity affected ionization of CsCl regent even after activated charcoal treatment. This effect may be more serious for organic compounds than inorganic compounds (external reproducibility: inorganic Cl, 0.1 to 0.2 ‰; organic Cl, 0.1 to 0.4 ‰; see Chapter 2). Although the precision of the measurements (0.12 to 0.43 ‰: external reproducibility, $1\sigma_{SD}$) was slightly worse compared to values obtained by electron impact ionization mass spectrometry of CH_3Cl (0.07 ‰: Holt et al., 1997; 0.04 to 0.32 ‰: Jendrzejewski et al., 1997), vacuum lines and furnaces are not necessary for the sodium biphenyl degradation procedure. Also, the mass spectrometry of CsCl requires smaller amounts of sample as chloride ion, and it is possible that the amount of organic chlorine sample for the measurements could be reduced further.

Conclusions

Thermal ionization mass spectrometry of Cs_2Cl^+ was applied for chlorine isotopic measurements of organic chlorine compounds. The results in this study are shown in Fig. 3-7, 3-8. The range of $\delta^{37}Cl$ in inorganic samples (-2.5 to +0.9 ‰, Chapter 2) was similar to the range of most Cl in surface and subsurface water. On the other hand, the range of $\delta^{37}Cl$ in organic compounds (-5.0 to +2.9 ‰) was almost twice as large.

The external reproducibility of the analytical method (0.1 to 0.4 ‰: $1\sigma_{SD}$) was slightly worse than the precision of the CH_3Cl mass spectrometry, but it enough to distinguish Cl isotopic composition of organic chlorine compounds. Since it was possible to reveal Cl isotopic fractionation in most of the reagents and natural materials using small amounts of samples for pretreatment without vacuum line, this analytical procedure could be applied to solve environmental issues.

CAHs in aqueous solution were recovered by solvent extraction and the mass discrimination during pretreatment processes was negligible. Also compound specific and intramolecular site specific Cl isotopic ratio measurements were investigated and preliminary results were obtained. These techniques have potential to clarify subsurface behavior of CAHs.

Table 3-1. Effects of degradation procedures on Cl recovery yields

	DCM	CT	TCE	PCE
Fenton				
TCE→Cl^-	77%	0.2%	80%	82%
Cl^-→CsCl	100%	—	98%	98%
Total	77%	—	79%	80%
$KMnO_4$				
TCE→Cl^-	0%	0%	96%	78%
Cl^-→CsCl	—	—	91%	90%
Total	—	—	87%	70%
Na-biphenyl				
TCE→Cl^-	96%	98%	99%	97%
Cl^-→CsCl	88%	88%	87%	86%
Total	84%	86%	86%	83%

Table 3-2. Comparison of TCE degradation procedures

Degradation	Na-Biphenyl	$KMnO_4$	Fenton
Recovery yield			
TCE→Cl^-	97 - 99%	94 - 98%	80%
Cl^-→CsCl	82 - 92%	89 - 93%	98%
Total	80 - 91%	84 - 91%	79%
δ^{37}Cl (‰)[1)]	-1.72 ± 0.10 (n=5)	-2.03 ± 0.17 (n=3)	-0.90 ± 0.30 (n=2)

1) Error : 1σ (external reproducibility, σ: standard deviation)

Table 3-3. Comparison of PCE degradation procedures

Degradation	Na-Biphenyl	$KMnO_4$	Fenton
Recovery yield			
PCE→Cl^-	98%	96%	82%
Cl^-→CsCl	82 - 98%	88 - 95%	98%
Total	82 - 96%	83 - 92%	80%
δ^{37}Cl (‰)[1]	-1.97 ± 0.36 (n=5)	-1.63 ± 0.38 (n=3)	-1.1 ± 0.7 (n=2)

1) Error：1σ (external reproducibility, σ: standard deviation)

Table 3-4. Effect of solvents on Cl recovert yields of CAHs dechlorination with Na-biphenyl reagent

Solvents	DCM	CT	TCE	PCE
toluene	96%	98%	99%	97%
n-decane	82%	96%	98%	98%
DIPE	88%	89%	97%	99%

DIPE: di-isopropyl ether

Table 3-5. Chlorine recovery yields and δ^{37}Cl of chlorinated methans and ethanes

CAHs (Abbreviation)	Company	Recovery of Cl (%)		δ^{37}Cl (‰)	δ^{37}Cl (‰)	δ^{37}Cl (‰)	δ^{37}Cl (‰)	Mean δ^{37}Cl (‰)	σ_{SD} ($n \geqq 3$)
		Dechlorination	Total (as CsCl)						
CH_2Cl_2 (DCM)	Nacalai	98	84-90	+0.49 ± 0.14				+0.5	—
	Nacalai	97	75-92	+0.64 ± 0.26				+0.6	—
	Wako	95	80-88	-0.26 ± 0.17				-0.3	—
$CHCl_3$ (CF)	Nacalai	98	80-93	-2.83 ± 0.25	-2.92 ± 0.18	-2.92 ± 0.39		-2.9	0.05
$CHCl_4$ (CT)	Nacalai	103	87-97	-2.39 ± 0.27				-2.4	—
	Nacalai	98	91	-4.33 ± 0.12				-4.3	—
	Nacalai	99	83-95	-0.23 ± 0.16	-0.20 ± 0.19	-0.79 ± 0.35	-0.11 ± 0.20	-3.3	0.31
CH_2Cl-CH_2Cl (DCA)	Nacalai	99	89-96	-1.11 ± 0.26				-1.1	—
CCl_3-CH_3 (MC)	Nacalai	99	89	-2.83 ± 0.25	-3.08 ± 0.23			-3.0	—
	Wako	96	73-91	-4.89 ± 0.33	-4.95 ± 0.25	-5.20 ± 0.30		-5.0	0.16

Internal error of δ^{37}Cl: $2\sigma_m$ (σ_m: standard error)

Table 3-6. Chlorine recovery yields and δ^{37}Cl of chlorinated ethens

CAHs	Company	Recovery of Cl (%)		δ^{37}Cl	δ^{37}Cl	δ^{37}Cl	δ^{37}Cl	δ^{37}Cl	Mean δ^{37}C	σ_{SD}
(Abbreviation)		Dechlorination	Total (as CsCl	(‰)	(‰)	(‰)	(‰)	(‰)	(‰)	(n≧3)
CCl_2=CH_2 (1,1-DCE)	GL	98	84-86	+2.93 ± 0.15	+3.20 ± 0.29	+2.41 ± 0.25			+2.9	0.40
CHCl=CHCl (cDCE)	GL	100	84	+0.08 ± 0.20					+0.1	—
CHCl=CHCl (tDCE)	GL	93	86	-0.14 ± 0.23					-0.1	—
CCl_2=CHCl (TCE)	Nacalai	97	81-87	-0.36 ± 0.21	-0.54 ± 0.27				-0.5	—
	Wako	97-99	85-91	-1.76 ± 0.16	-1.80 ± 0.18	-1.55 ± 0.23	-1.72 ± 0.23	-1.79 ± 0.27	-1.7	0.10
	Wako	98	86	+0.05 ± 0.21					+0.1	—
CCl_2=CCl_2 (PCE)	Nacalai	98	75	-1.33 ± 0.20					-1.3	—
	Nacalai	98	80-87	-2.08 ± 0.18					-2.1	—
	Wako	97	82-96	-1.74 ± 0.17	-1.50 ± 0.19	-1.96 ± 0.30	-2.35 ± 0.27	-2.29 ± 0.27	-2.0	0.36
	Merck	99	85	-0.67 ± 0.30	-1.04 ± 0.26				-0.9	—

Internal error of δ^{37}Cl: $2\sigma_m$ (σ_m: standard error)

Table 3-7. Comparison of TCE recovery procedures

	TCE 1.5mg	TCE extracted from aqueous solution (15mg/L)	
Extraction	―	with Toluene	Purge with air
Degradation	Na-Biphenyl	Na-Biphenyl	PbO_2/H_2SO_4
Recovery yield			
Extraction	―	88～95%1)	98%2)
TCE→Cl^-	97～99%	(98%)	33%
Cl^-→CsCl	82～92%	89～93%	92%
Total	80～91%	80～83%	30%
δ^{37}Cl (‰)3)	-1.79 ± 0.12 (n=3)	-1.84 ± 0.30 (n=3)	-0.26 ± 0.19 (n=3)

1) Assuming recovery rate of TCE dechlorination is 98%
2) Calculated from residual TCE concentration after purging
3) Error：1σ (external reproducibility, σ: standard deviation)

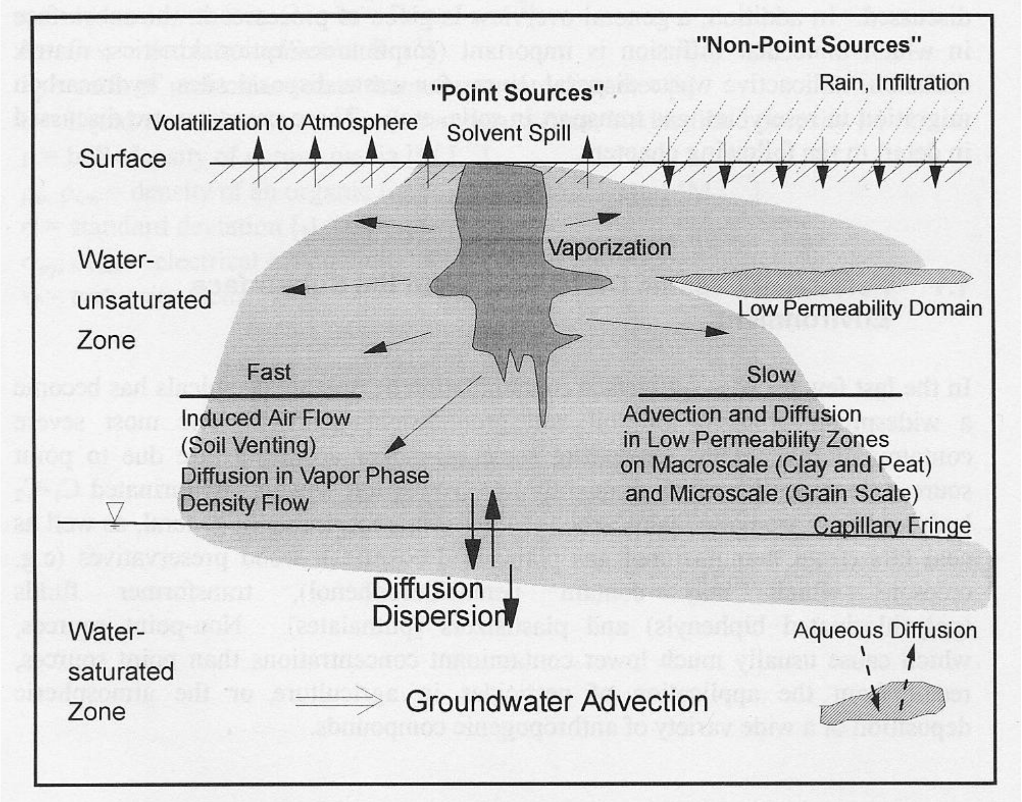


Fig. 3-1. Processes of CAHs in the subsurface environment. *Source:* From *Diffusion in Natural Porous Media,* Peter Grathwohl, Kluwer Academic Publishers, Boston

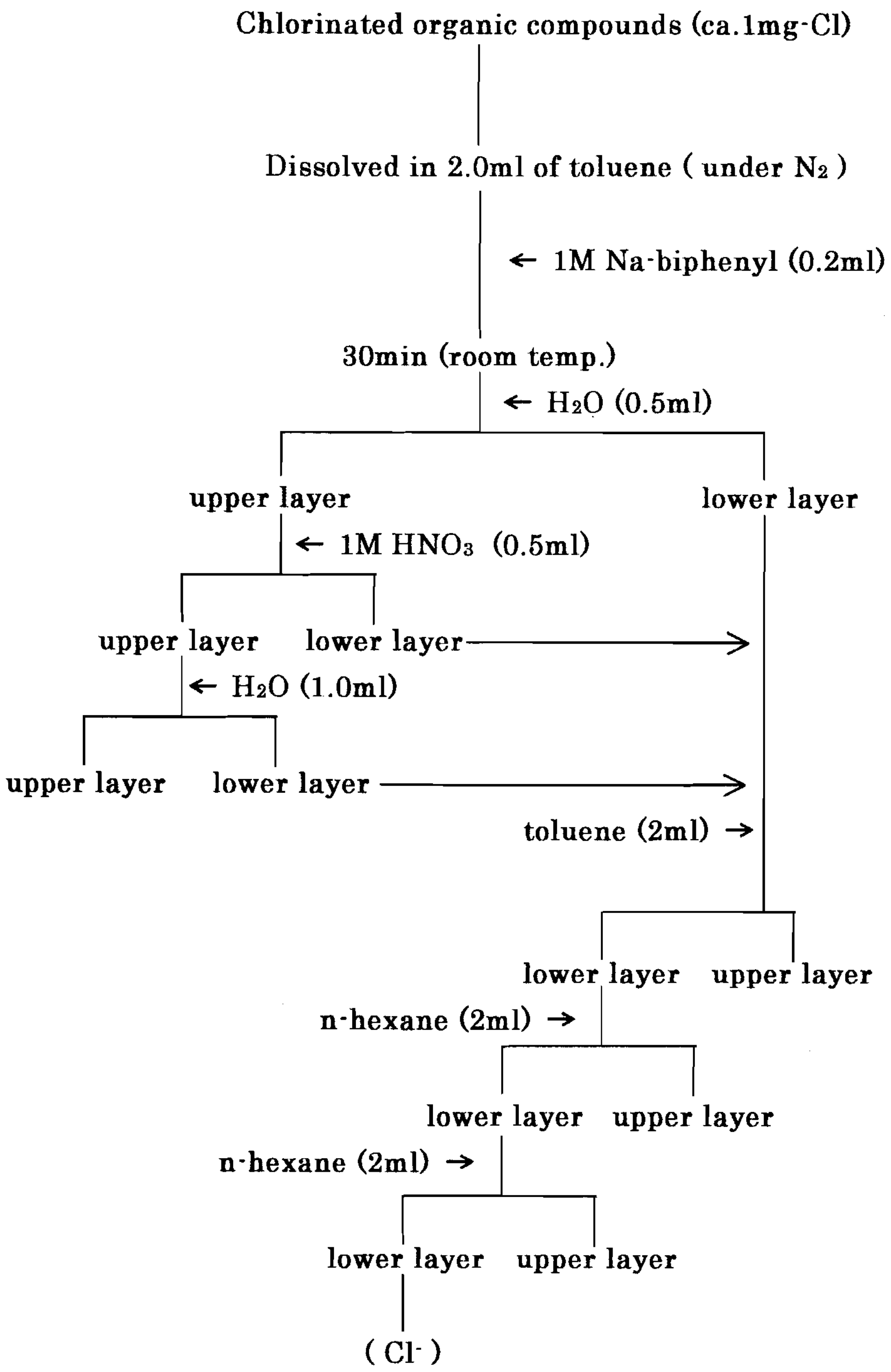


Fig. 3-2. Procedures of reductive dechlorination of chlorination organic compounds with sodium-biphenyl reagent.

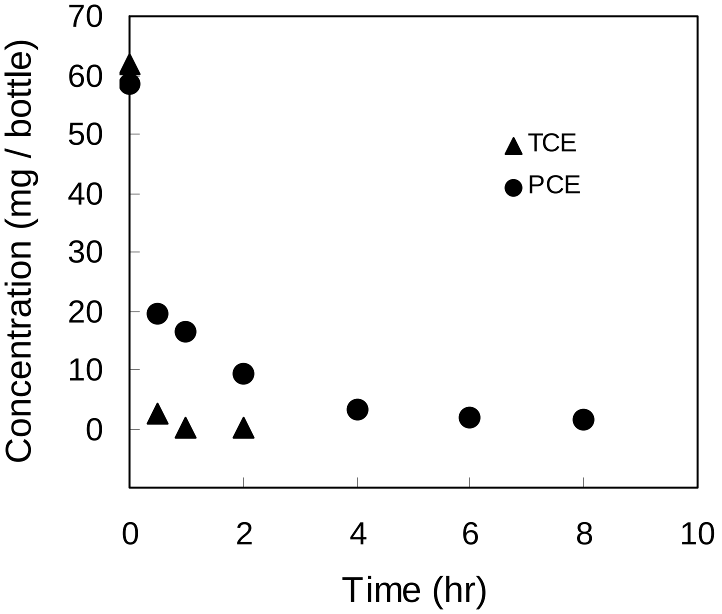


Figure 3-3. Concentrations of chloroethenes (PCE, TCE) in sample bottles over time during the course of $KMnO_4$-oxidation experiments.

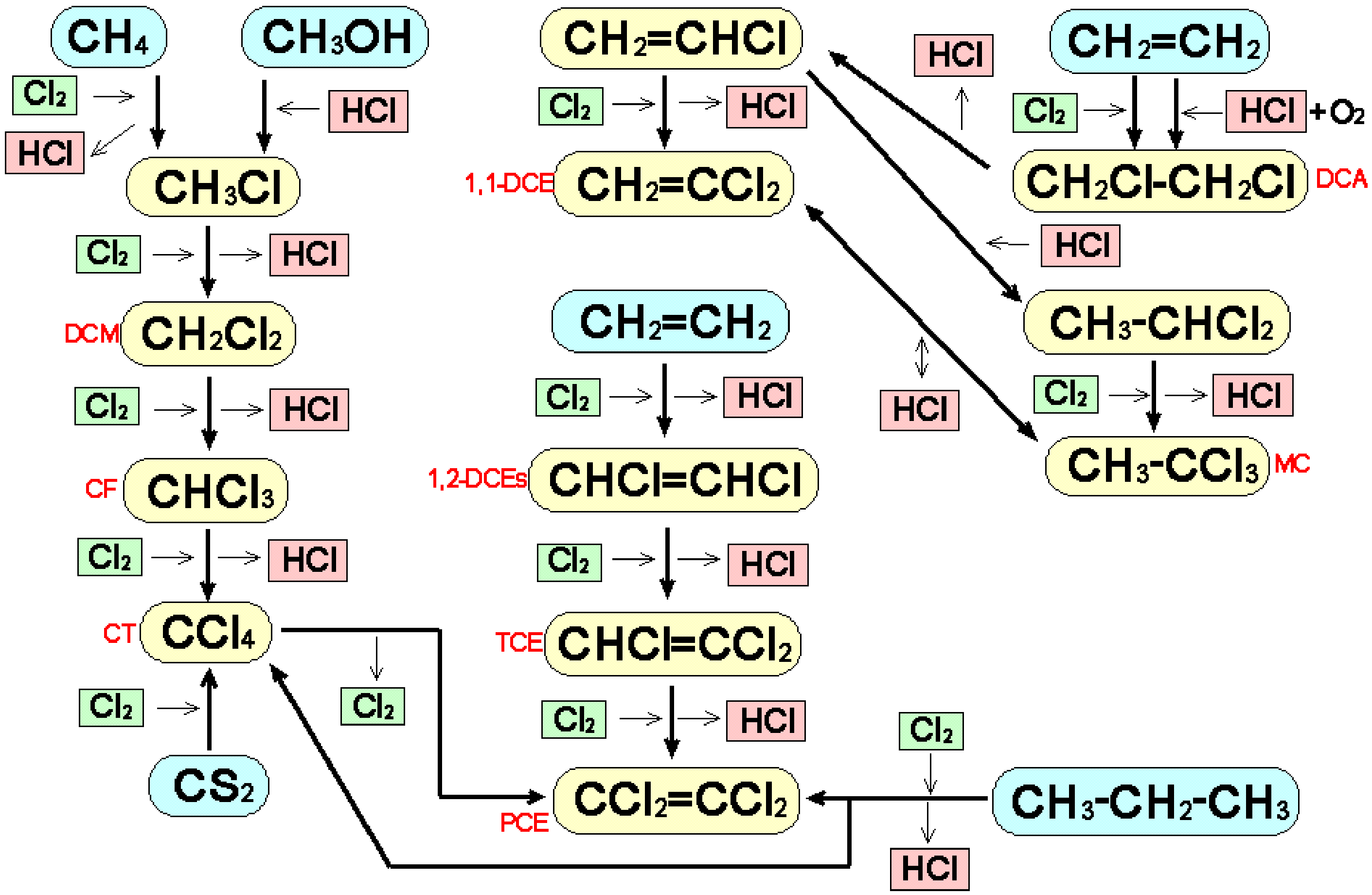


Fig. 3-4. Manufacturing processes of CAHs

from Encyclopedia of Chemical Technology (John Weiley & Sons) etc.

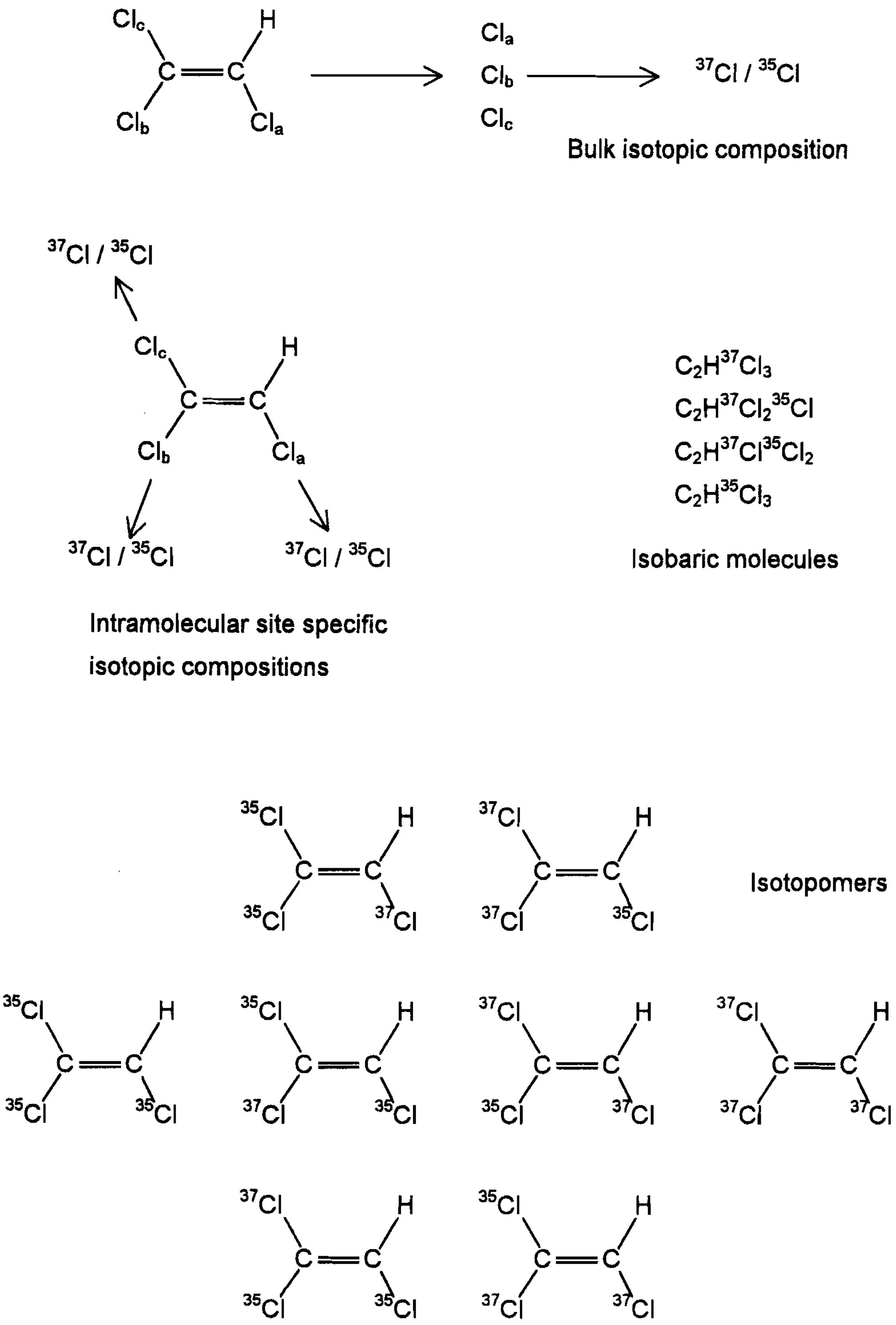
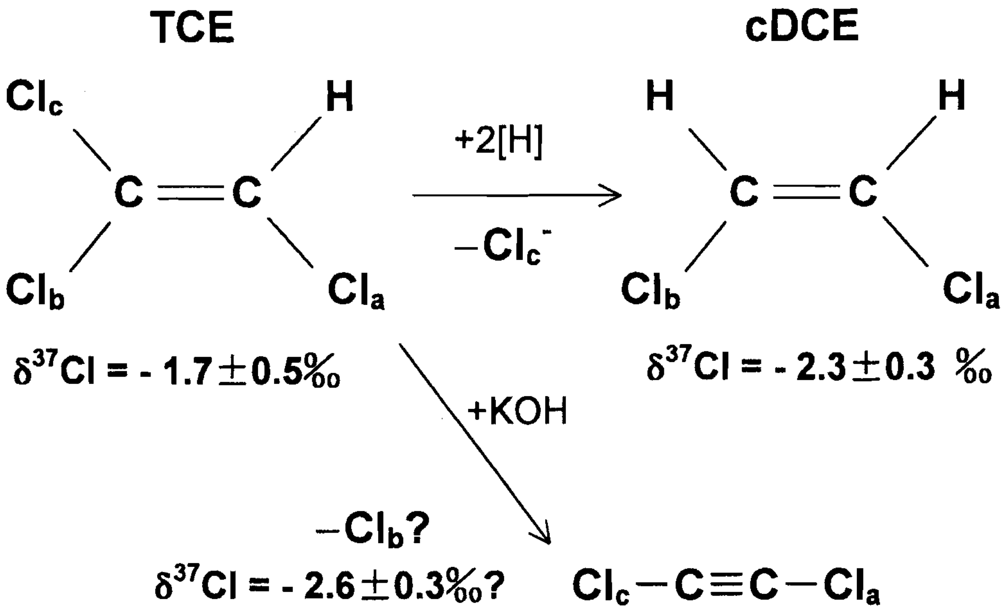


Fig. 3-5. Concepts of Cl isotope distribution in TCE molecule



$\delta^{37}Cl_a$ = - 2.0‰?

$\delta^{37}Cl_b$ = - 2.6‰?

$\delta^{37}Cl_c$ = - 0.3‰

Fig. 3-6. Intramolecular distribution of Cl isotopes within the TCE molecule (Wako, reagent grade).

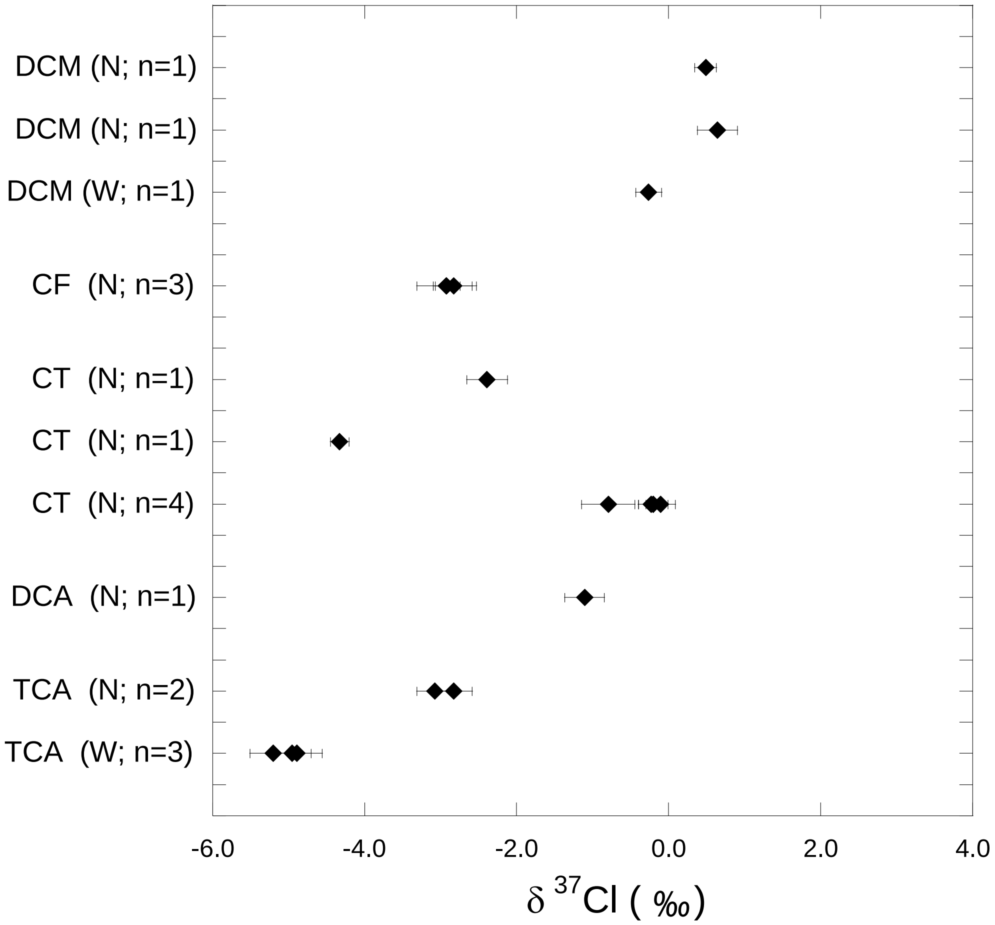


Fig. 3-7. Results of chlorine isotopic measurements of chlorinated methanes and ethanes. Error bars are $2\sigma_m$ (σ_m: external standard error). N: Nacalai tesque, W: Wako Pure Chemical Industries.

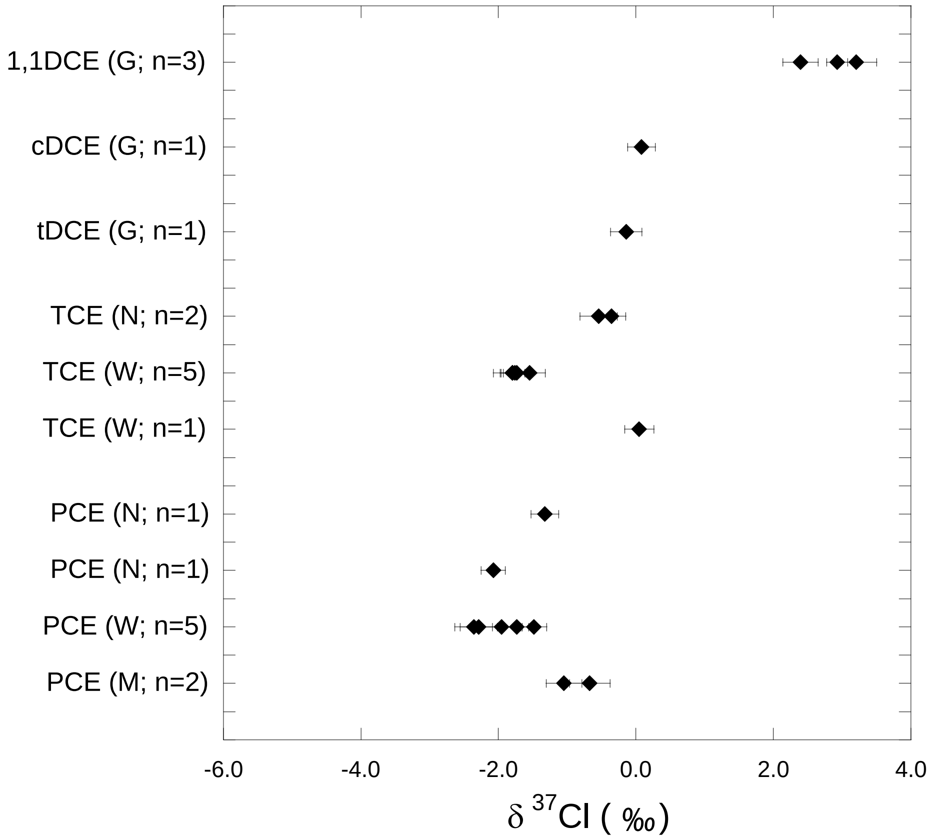


Fig. 3-8. Results of chlorine isotopic measurements of chlorinated ethenes. Error bars are 2σm (σm: external standard error). G: GL Sciences, N: Nacalai tesque, W: Wako Pure Chemical Industries, M: Merck.

4. Isotope fractionation during reductive dechlorination of chlorinated ethenes

Introduction

Chlorinated aliphatic hydrocarbons (CAHs) have been widely utilized as solvent and degreasing agents for many years. Once they enter subsurface environment, they migrate as soil gas and aqueous solution in aquifer with supplying from the dense non-aqueous phase liquids (DNAPL, free product, pure phase) and absorbed phase on soil particles. Coexistence of such mobile and less mobile phases of CAHs causes wide and durable pollution of surface soils, unsaturated zones and aquifers (Fig. 3-1). Although, CAHs are degraded by abiotic or biotic reactions (e.g. oxidation, hydrolysis, dehydrohalogenation, substitution Vogel et al., 1987; Fetzner, 1998), the reaction rates are usually low in natural systems. Because number of sampling points and frequency of monitoring are limited, investigation of the pollution source and behavior of CAHs in subsurface is difficult.

Isotope analysis is valuable tool to investigate not only to investigate the inorganic pollutants (e.g. Nakai et al., 1991; Sturges and Barrie, 1989; Johnson et al., 2000) but also organic ones. Carbon isotope analysis of hydrocarbons has been successfully used for such purposes (e.g. Conrad et al., 1997). Significant progress in this field was made by development of gas chromatograph /combustion/isotope ratio mass spectrometry (GC/C/MS), rapid, sensitive and compound-specific technique (e.g. Dempster et al., 1997). As isotope analysis also has potential for research of organic chlorine compounds, several analytical techniques for isotopes in CAHs have been developed Tanaka and Rye, 1991; van Warmerdam et al., 1995; Holt et al., 1997; Jendzejewski et al., 1997; Slater et al., 1999; Hunkeler and Aravera, 2000a). The quantitative information about isotope fractionation would be necessary to (1) judge feasibility of using isotopes to trace the sources of pollutant, (2) evaluate effectiveness of in-situ cleanup techniques and (3) investigate mechanisms of subsurface processes.

Tanaka and Rye (1991) reported relatively large Cl isotope variations in CAHs, these authors suggested Cl isotope can be used to quantify the flux of stratospheric chlorine derived from man-made chlorinated compounds. In addition, isotopic compositions of chlorinated solvents (Beneteau et al., 1999) and PCBs (Jarman et al., 1998; Reddy et al., 2000) were measured to distinguish contaminants from different manufacturers and to trace them in the environment. Although, isotopes are regarded as idealistic tracer of elements or compounds, sometimes isotopes of Cl^- are not regarded as conservative tracer in aquatic system because they would be discriminated during migration by interaction with solid phases (Desaulniers et al., 1986; Pillips and Bentley, 1987). In order to use unique isotopic compositions of pollutants to fingerprint their source, magnitude of isotopic fractionation during migration should be known quantitatively.

In-situ cleanup techniques (e.g. bioremediation, natural attenuation, permeable reactive barrier) would be cost effective and suitable for contaminated plume widely spread (Fig. 4-1). However, practical uses of these techniques are limited by difficulty of proving their effectiveness, because the contribution of both biotic or abiotic degradation from contribution of other physical processes (e.g. dilution, migration) by chemical analysis of samples obtained from limited number of boring cores or monitoring wells. Carbon isotope analysis has successfully been utilized to investigate contribution of biotic degradation of hydrocarbons to inorganic carbon in contaminated soil (e.g. Suchomel et al., 1990; Conrad et al., 1997). Compared with petroleum hydrocarbons, relatively few measurements of isotope fractionation during reactions of CAHs have been made. Because trajectories of C and Cl isotope composition of residual dichloromethane are different in the case of evaporation and aerobic biodegradation (Heraty et al., 1999; Huang et al., 1999), it was suggested that isotopic change could be used to distinguish contribution of biodegradation from evaporation in bioremediation processes. In order to interpret the actual isotopic changes in CAHs contaminated aquifer (Sturchio et al., 1998; Hunkeler et al., 1999), more information on isotope fractionation is necessary (e.g. anaerobic degradation: Hunkeler et al., 1999; Bloom et al., 2000; Sherwood Lollar et al., 1999, aerobic biodegradation: Heraty et al., 1999; Hunkeler and Aravera, 2000b, abiotic degradation: Dayan et al., 1999, evaporation: Slater et al., 1999; Huang et al., 1999, absorption, solubilization).

In general, it is difficult to investigate pathway and mechanism of subsurface processes because soil is complex chemical and biological system. Changes in isotope ratios have been successfully exploited to investigate metabolism processes of compounds containing C (Sugimoto and Wada, 1993), N (Mariotti et al., 1988), O (Aggarwal et al., 1997) and S (Kemp and Thode, 1968). Studies of isotopes in CAHs would be a useful tool to investigate mechanisms of reactions mediated by complex consortium or non-culturable microorganisms.

Chlorinated ethenes are representative CAHs. In paticuller, trichloroethene (TCE) and tetrachloroethene (PCE) are among the most serious groundwater contaminants (Kawasaki, 1985; Fisher et al., 1987). Reductive dechlorination (hydrogenolysis) of chlorinated ethenes mediated by abiotic (Liggett, 1954; Gantzer and Wackett, 1991) and biotic (Holliger et al., 1993; Maymó-Gatell et al., 1997) systems plays important roles for wastewater treatment (de Bruin et al., 1992; Numata et al., 1999a,b), remediation and natural attenuation of contaminated aquifers and soils (Holliger, 1995; Bloom et al., 2000; Gillham and O'Hannesin, 1994) (Fig. 4-2).

In this study, the degree of Cl isotope fractionation during biotic dechlorination of TCE or PCE to *cis*-1,2-dichloroethene (cDCE) has been investigated in laboratory experiments. Mathematical models for the change of isotopic ratios were developed and we attempted to determine isotope fractionation factors for each dechlorination step. And also isotope effect in abiotic dechlorination of PCE mediated with a transition-metal coenzyme cyanocobalamin (vitamin B_{12}) was examined as a model of electron transport enzymes.

Experimental Procedures

Presentation of isotopic data

The isotopic compositions of chlorine are represented in per mil (‰) deviation from those of the standard mean ocean chloride (SMOC: Long et al., 1993), and a reagent grade CsCl from Nacalai tesque was utilized as a routine laboratory standard of Cl isotopic composition during the course of this study (Numata et al., in press; see Chapter 2).

$$\delta^{37}Cl = \left[\left(\frac{R_{sample}}{R_{SMOC}}\right) - 1\right] \times 1000 \qquad R = \frac{^{37}\mathrm{Cl}}{^{35}\mathrm{Cl}} \tag{3-1}$$

One or two standards were typically loaded into the mass spectrometer together with the samples, and δ values of samples were calculated from the following equation.

$$\delta^{37}Cl = \left[1 - \frac{R_{sample}(1 + 10^{-3}\delta_{CsCl})}{\overline{R}_{CsCl}}\right] \times 1000 \tag{3-2}$$

where δ_{CsCl}: -2.49 (per mil deviation of isotopic ratios between the CsCl reagent and seawater, which was calculated from more than 25 isotopic measurements; Chapter 2), $\overline{R}$: average of standard CsCl isotopic ratios in the period of sample analysis.

Microorganisms

The strain T (a sulfate-reducing bacterium belongs a novel genus, isolated from a drainage of a laundry, (Yaguchi et al., 1991; Yaguchi et al., 1994), consortium N (an enrichment culture N-YE obtained from TCE contaminated soil, Numata et al., 1999a) and consortium F (an enrichment culture obtained from PCE contaminated soil, Lee et al., 1997) were used for biological dechlorination of PCE and TCE. The cultures dechlorinated PCE and TCE to *cis*-1,2-dichloroethene (cDCE).

The cultures were sustained in 120-mL serum bottles with 50 mL of liquid media containing 10 mg/L of PCE under N_2 atmosphere. The MMY medium (K_2HPO_4 7 g, KH_2PO_4 2 g, $MgSO_4 \cdot 7H_2O$ 0.1 g, $(NH_4)_2SO_4$ 1 g, Yeast Extract 2 g, trisodium citrate 0.5 g in distilled water 1 L, pH 7.2; Yaguchi et al., 1991) was used for cultivation of consortium F. For cultivation of strain T, 0.3 g/L of L-cysteine hydrochloride was added to the medium. For cultivation of consortium N, trisodium citrate was omitted from the medium.

Samples and reagents

Water used for this work was purified using a Milli-RX12Plus and a Milli-Q SP systems (Millipore). A commercial grade CsCl (minimum assay 99 %; Nacalai tesque) was used for laboratory reference standard (Numata et al., in press; see Chapter 2). Activated charcoal (GX-60: GL Sciences) was washed with water before use in order to remove chloride ion.

TCE (minimum assay 99.5 %; Wako Pure Chemical Industries) and PCE (minimum assay 99 %; Wako Pure Chemical Industries) were used for dechlorination experiments. The $\delta^{37}Cl$ values of the TCE and PCE were -1.72± 0.10 ‰ and −1.97±0.36 ‰ (n=5, error: $1\sigma_{SD}$) respectively (see Chapter 2). Other chemicals were analytical or reagent grades.

Chemical analysis

Concentrations of chlorinated ethenes in liquid media were determined by headspace gas analysis using a Shimadzu GC-9A gas chromatograph equipped with a Shimadzu CBP1 Hi-Cap column (0.2 mm x 25 m, film thickness 0.25 μm) and a flame-ionization detector. The 50 μL of headspace gas was sampled with a gastight syringe through rubber septum and was injected into the GC-FID system and analyzed in isothermal mode (column temperature: 40 °C). As internal standard, 50 μmol of toluene dissolved in methanol was added to each reaction vessels. Molar fractions of chlorinated ethenes were the mole amount of each compound normalized with initial PCE or TCE mole amount. As ethene, ethane and methane were not separated with the column, the corresponding chromatogram peaks were simply regarded as ethene (ETE). Also separation of ethene and vinyl chloride (VC) was incomplete and quantification of VC might not be so accurate.

Chloride concentrations were measured using a Hitachi ion chromatograph L-7000 with Hitachi 2740 analytical column.

Dechlorination of chlorinated ethenes with microoraganisms

The biotic dechlorination experiments were performed in 68-mL serum bottles with 25 mL of liquid media for consortium F and N. Because strain T could not degrade high concentration of PCE, the experiments for strain T were performed in 120-mL serum bottles with 50 mL of liquid media. Several bottles with the media (23.8 mL for consortium F or N, 47.5 mL for strain T) were sterilized with autoclave (121 ℃, 15 min). After cooling, the media were purged with N_2 gas for 5 min to remove oxygen in media and headspace gas, and 1.2 mL (consortium F or N) or 2.5 mL (strain T) of subcultures were inoculated in the bottles. N_2 purge was continued for 5min to remove chlorinated ethenes dissolved in subculture, and then the bottles were sealed with polytetrafluoroethylene lined rubber septa and aluminum cups. The cultures were injected with 10 μL of PCE or TCE stock solution (in methanol) containing 10 μmol of PCE or TCE, and then incubated at 30 ℃. Progress of the reaction was monitored by headspace analysis by GC-FID. Toluene (3 mL) was added through the septa in order to stop the reaction. The bottle was then shaken vigorously to extract chlorinated ethenes from medium and headspace. More than 7 bottles were prepared for a series of experiment, and one of which was sacrificed for extraction at each reaction stage. After moving the toluene layer containing chlorinated ethenes to another test tube, 2mL of toluene was added to the bottle and chlorinated ethenes were extracted repeatedly. The procedures were shown in Fig. 4-3.

Anaerobic chloroethene dechlorination mediated with cyanocobalamin

The procedure was modified method of Gantzer and Wackett (1991). The chemical dechlorination experiments were performed in 68-mL serum bottles with 25 mL of Tris-HCl buffer (1 M, pH8.2) containing 0.75 μmol of cyanocobalamin and 0.75 mmol of trisodium citrate. After purging the bottle with N_2 gas to remove oxygen in media and headspace gas, the bottles were sealed with polytetrafluoroethene lined rubber septa and aluminum cups. The solution were injected 10 μL of PCE stock solution (in methanol) containing 10 μmol of PCE, and then incubated at 30 ℃. Partition of PCE between the solution and the headspace gas equilibrated for at least 1 hr, and the reactions were started by injecting 470 μL of $TiCl_3$ solution containing ca.0.75 mmol of Ti(III) (20 % $TiCl_3$ in 1 M HCl, Wako Pure Chemical Industries) into the bottles. Progress of the reaction was monitored by headspace analysis by GC-FID, and toluene was added through the septum in order to extract chlorinated ethenes same as biotic reaction.

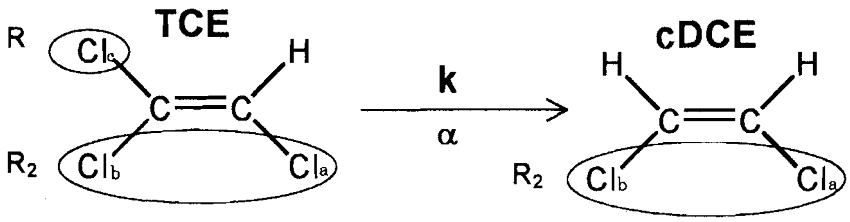
Isotopic analysis of chloroethenes

Chloroethenes extracted in toluene was dried with 1 g of anhydrous Na_2SO_4. They were treated with the sodium biphenyl reagent by the method of (Liggett, 1954) with some modifications described in Chapter 3.

Chloride ion in aqueous solution was isolated as AgCl and was converted into CsCl by a method modified from (Hoering and Parker, 1961) and (Magenheim et al., 1994) (see Chapter 2, 3).

Isotopic determinations were performed using a Finnigan MAT 262 thermal ionization mass spectrometer (see Chapter 2, 3).

Isotopic fractionation model for TCE declorination



The biotic reductive dechlorination of TCE is a regioselective reaction. Only the Cl_c is substituted with a hydrogen atom, and cDCE is produced. Thus, isotopic composition of the Cl_a and the Cl_b would be kept constant during dechlorination. On the other hand, isotopic composition of the Cl_c will change by mass discrimination. The isotope ratio in organic-chlorine (TCE + cDCE) is represented as a binary mixing relationship between R_2 (isotope ratio in Cl_a + Cl_b) and R (isotope ratio in Cl_c):

$$R_t = \frac{N_c R(1+R_2) + (1-N_c)R_2(1+R)}{N_c(1+R_2) + (1-N_c)(1+R)} \qquad (4\text{-}3)$$

R_t : Cl isotopic ratio of organic-Cl (TCE+cDCE), N_c: molar fraction of Cl_c

When the change of isotope ratio is small enough, it means that ratio of $(1+R_2)$ and $(1+R)$ approach unity. Thus eq 4-3 reduced to

$$R_t \cong N_c R + (1-N_c)R_{ab} \qquad (4\text{-}4)$$

Using molar fraction of TCE remaining (f_T), N_c becomes

$$N_c = \frac{f_T}{f_T + 2} \qquad (4\text{-}5)$$

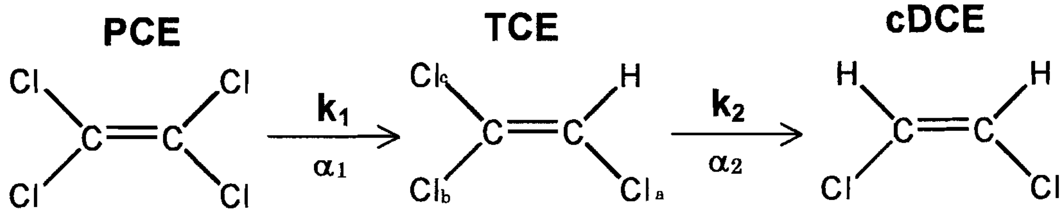
If the isotopic fractionation of Cl_c by dechlorination follows the Rayleigh equation,

$$R = R_1 f_T^{(\alpha-1)} \qquad (4\text{-}6)$$

where R_1 is the initial isotope ratio of Cl_c and α is the fractionation factor. Substituting eq 4-5 and eq 4-6 into eq 4-4 gives,

$$R_t = \frac{R_1 f_T^{\alpha} + 2R_2}{f_T + 2} \qquad (4\text{-}7)$$

Isotopic fractionation model for PCE declorination



Dechlorination of PCE to cDCE will be regarded as a sequential first-order reaction (Ninomiya and Sakai, 1993). Theoretically, time course of chloroethenes concentrations change on time represented as follows (f_P, f_T, f_D: fraction of PCE, TCE, cDCE):

$$f_P = e^{-k_1 t} \tag{4-8}$$

$$f_T = \frac{k_1}{k_1 - k_2}\left(e^{-k_2 t} - e^{-k_1 t}\right) \tag{4-9}$$

$$f_D = 1 - f_P - f_T \tag{4-10}$$

During the first step of the PCE dechlorination to TCE, one-fourth of Cl atom may be eliminated randomly. If the second dechlorination step does not occur and isotope fractionation follows the Rayleigh equation, isotope ratio change of Cl in PCE and TCE will be represented as follows:

$$R_{PTD}(f_P) = R_0\{(3 + f_P)/4\}^{(\alpha_1 - 1)} \tag{4-11}$$

where α_1 is isotope fractionation factor for the first dechlorination step.

During the second dechlorination step, Cl_c in TCE molecule would be eliminated and Cl_a and Cl_b would remain in cDCE molecule. Namely, total isotope composition of Cl_a + Cl_b in TCE molecule; and Cl in PCE and cDCE molecules will change as eq 4-11. Meanwhile, isotopes of Cl_c in TCE might be fractionated more. The fractionation model of Cl_c was calculated as follows.

The material balance of ^{37}Cl in the system is derived as eq 4-12 (left hand side is quantity of ^{37}Cl in Cl_c at time=t, right hand side is distribution of ^{37}Cl at time=$t+\Delta t$).

$$f_T X_{37} = (f_T + \Delta f_i - \Delta f_e)(X_{37} + \Delta X_{37}) - \Delta f_i X_{37i} + \Delta f_e X_{37e} \tag{4-12}$$

where f refers to the fraction of Cl; X_{37} is the molar fraction of ^{37}Cl [^{37}Cl / ($^{35}Cl+^{37}Cl$)]; subscript letters T, e and i represent TCE (Cl_c), efflux and influx respectively.

Since the PCE dechlorination is a sequential first-order reaction, the amount of influx to TCE in Δt would be equal to degradation of PCE in Δt. Also, the ratio of the first reaction rate and second reaction rate ($\Delta f_e / \Delta f_i$) would be $k_2 f_T / k_1 f_P$. If new parameters $F\,(=1-f_P)$ and $b\,(=k_2/k_1)$ are introduced, the Δf_i and Δf_e would be represented as follows:

$$\Delta f_i = \Delta F \quad \Delta f_e = \frac{b f_T}{1-F}\Delta F \tag{4-13}$$

The molar fraction of ^{37}Cl can be written as follows using isotopic ratio R_c and isotopic fractionation factor of the second reaction α_2.

$$X_{37} = \frac{R_c}{1+R_c} \qquad X_{37_e} = \frac{\alpha_2 R_c}{1+\alpha_2 R_c} \tag{4-14}$$

Substituting eqs 4-13 and 4-14 into eq 4-12 gives

$$f_T \cdot \frac{R_c}{1+R_c} = (f_T + \Delta F - \frac{b f_T}{1-F}\Delta F)(\frac{R_c}{1+R_c} + \Delta \frac{R_c}{1+R_c})$$

$$- \Delta F \cdot X_{37i} + \frac{b f_T}{1-F}\Delta F \cdot \frac{\alpha_2 R_c}{1+\alpha_2 R_c} \tag{4-15}$$

If Δt is an infinitely short time,

$$\Delta F \to dF,\ \Delta(R_c/(1+R_c)) \to d(R_c/(1+R_c)),\ \Delta F \cdot \Delta(R_c/(1+R_c)) \to 0$$

Thus,

$$\left(X_{37i} - \frac{1-F-b f_T}{1-F} \cdot \frac{R_c}{1+R_c} - \frac{b f_T}{1-F} \cdot \frac{\alpha_2 R_c}{1+\alpha_2 R_c} \right) \cdot df = \frac{f_T}{(1+R_c)^2} \cdot dR_c \tag{4-16}$$

From eq 4-8, eq 4-9 and eq 4-11, f_T and X_{37i} are represented as function of F.

$$f_T = \frac{f_P^b - f_P}{1-b} = \frac{(1-F)^b - (1-F)}{1-b} \tag{4-17}$$

$$X_{37i} = \frac{R_{PTD}}{1+R_{PTD}} = \frac{R_0 \{(4-F)/4\}^{(\alpha_1 - 1)}}{1+R_0 \{(4-F)/4\}^{(\alpha_1 - 1)}} \tag{4-18}$$

Eq 4-16 becomes a differential equation by substitution with eq 4-17 and eq 4-18:

$$\frac{dR_c}{dF} = \left(\frac{R_0 \{(4-F)/4\}^{(\alpha_1 - 1)}}{1 + R_0 \{(4-F)/4\}^{(\alpha_1 - 1)}} - \frac{1 - b(1-F)^{(b-1)}}{1-b} \cdot \frac{R_c}{1+R_c} + \frac{1-(1-F)^{(b-1)}}{1-b} \cdot \frac{\alpha_2 R_c}{1 + \alpha_2 R_c} \right)$$

$$\left(\frac{1-b}{(1-F)^b - (1-F)} \right) (1+R_c)^2 \qquad (4\text{-}19)$$

The equation was solved numerically using a commercially available software MATLAB (Math Works) with the initial condition $R_c(0.001)=R_0$, because denominator of the right hand side becomes 0 when F equals to 0.

The change of isotope ratio in total organic-Cl was then calculated as binary mixing equation. If the reaction started from one mole of PCE, the sum of Cl atomic abundance in the molecule PCE and cDCE; Cl_a and Cl_b in TCE is represented as follows:

$$n_{PTD} = 4f_P + 2f_T + 2f_D \qquad (4\text{-}20)$$

Substituting eq 4-5 into eq 4-20 gives

$$n_{PTD}(f_P) = 4f_P + 2f_T + 2(1 - f_P - f_T) = 2f_P + 2 \qquad (4\text{-}21)$$

The atomic abundance of Cl_c is,

$$n_c(f_P) = f_T = \frac{f_P^b - f_P}{1-b} \qquad (4\text{-}22)$$

The binary mixing equation with Cl_c and other Cl isotopic composition is similar to eq 4-3.

$$R_{total}(f_P) = \frac{n_{PTD}(f_P) \cdot R_{PTD}(f_P) \cdot (1 + R_c(f_P)) + n_c(f_P) \cdot R_c(f_P) \cdot (1 + R_{PTD}(f_P))}{n_{PTD}(f_P) \cdot (1 + R_c(f_P)) + n_c(f_P) \cdot (1 + R_{PTD}(f_P))} \qquad (4\text{-}23)$$

Change of organic Cl isotopic ratio (R_{total}) was calculated by substituting eq 4-11, eq 4-21, eq 4-22 and numerical solution of eq 4-19 into eq 4-23.

Results

Biotic reductive dechlorination of TCE and PCE

Fig.4-4 shows time courses of reductive TCE dechlorination with 3 kinds of the anaerobes. Although, these results may be the first-order reactions on the concentrations of TCE, lag phase of bacteria growth gave sigmoid curves of chlorinated ethenes concentration instead of logarithmic curves. In the case of PCE dechlorination, features of the curves correspond to the theoretical sequential first-order reaction (eqs 4-3,4-4,4-5) except existence of lag phase (Fig.4-5). The peaks of 1,1-DCE were also detected on the chromatograms, but its amounts were negligible against cDCE (strain T, 0.1 – 0.2 %; consortium N and consortium F, 0.1 % or less). Therefore, the simultaneous reaction of TCE dechlorination will not affect the discussions below. Rate constants of dechlorination calculated from regressions of late reaction stage are shown in Table 4-1.

Isotope fractionation associated with TCE dechlorination

The covalent bonds formed by the lighter isotopes are usually weaker than bonds formed by heavier isotopes. Due to preferential elimination of ^{35}Cl from C-Cl bonds, the residual Cl in precursors becomes enriched in ^{37}Cl. However in the case of TCE dechlorination to cDCE, only part of Cl isotope in the molecule is fractionated. Consequently, change of bulk isotopic ratio will be relatively small.

The mathematical model of Cl isotopic shift (eq 4-7) plotted against fraction of TCE remaining is shown in Fig. 4-6. The initial isotopic composition is given by weighted mean of isotopic compositions of Cl_c and Cl_a + Cl_b, and final isotopic composition is equal to composition of Cl_a + Cl_b. The smaller isotope fractionation factor (i.e. the larger isotope effect) gives the larger curvature of trajectories.

The Rayleigh equation (eq 4-6) is an approximation when the abundance of minor isotope is much smaller than unity. The equation must be modified as eq 4-24 for cases where the concentration of minor isotope is substantial such as ^{37}Cl (Criss, 1999).

$$R(1+R)^{(\alpha-1)} = R_1\{(1+R_1)f_T\}^{(\alpha-1)} \qquad (4\text{-}24)$$

As differences between the calculated values of R_t calculated from eq 4-3 and eq 4-24 and the values calculated from eq 4-7 were less than 0.01 ‰, it will be unnecessary to use eq 4-24 for interpretation of the data.

The results of isotopic measurements during biotic TCE dechlorination by 3 kinds of anaerobic bacteria culture are shown in Fig. 4-7. The changes of isotopic composition during biotic dechlorination were small but the trend of depletion in ^{37}Cl at the later stage of dechlorination was observed in all cases. The difference of the isotopic ratio trajectories was not significant because of relatively large error on isotopic analysis. The regressions through the data shown in Fig. 4-7 yield best fit isotope fractionation factors (α) of 0.9943 to 0.9945 for 3 bacterial cultures utilizing the eq 4-7.

Isotope fractionation associated with PCE dechlorination

The mathematical model for Cl isotopic change (eq 4-23) plotted against the fraction of PCE remaining is shown in Fig. 4-8 and Fig. 4-9. The isotopic shift is controlled by fractionation factors α_1 and α_2 (Fig. 4-8); and ratio of rate constant of the reactions, k_2/k_1 (Fig. 4-9). Same α_1 values give same isotopic ratio at the end of the reaction. The effect of the α_2 value on the trajectories is small, but it shifts the curves upward as the α_2 value decreases. Clearly shift of trajectories from simple Rayleigh fractionation curve (eq 4-11) depends on fraction of the TCE (i.e. k_2/k_1 ratio).

From the numerical solution of eq 4-19, the isotopic ratio of Cl_c increases as the F value increases, and then reach to plateau. The higher k_2/k_1 ratio gives the wider interval of plateau and the smaller α_2 gave higher Cl isotopic ratio at the end. Also, higher k_2/k_1 ratio gives higher TCE fraction. The shifts of the theoretical curves from simple Rayleigh model curves ($\alpha_2=1$) can be explained by these effects (Fig. 4-10).

Compared with TCE dechlorination, changes in Cl isotopic compositions were larger. The general trends toward enriched $\delta^{37}Cl$ values with degradation of PCE were consistent with the theoretical models (dotted curves in Fig. 4-10), and shifts from the simple Rayleigh model were slightly larger when the k_2/k_1 ratio was smaller. The isotope fractionation factors for PCE dechlorination to TCE were 0.991 (strain T), 0.990 (consortium N) and 0.987 (consortium F) at a rough estimate from $\delta^{37}Cl$ values at initial and final reaction stage. The order of isotope effects coincided with order of rate constants for dechlorination, but the causality is unclear. The precision of Cl isotopic measurements was not high enough to determine values of α_2.

Abiotic reductive dechlorination of PCE mediated with cyanocobalamin

In this work, the reaction ingredient was slightly different from conditions of Gantzer and Wackett (1991). We used the mixture of titanium(III) chloride and sodium citrate and N_2 gas instead of titanium(III) citrate and Ar gas used in the original report. Fig.4-11 shows time course of PCE dechlorination mediated with cyanocobalamin. Features of the reaction were different from biotic ones in some respects. One of important diffecences was fast ethene generation. The molar fraction of ethene was represented in Fig. 4-11 as 'ETE' but it is possible that the quantities of 'ETE' include ethane and C_1-compounds. The ethene accumulated faster than isomers of dichloroethenes (DCEs) and VC. The other difference was the lower regiospecificity during the TCE dechlorination to DCEs. Two isomers of dichloroethenes, *trans*-1,2-dichloroethene (tDCE) and 1,1-dichloroethene (1,1-DCE) accounted for 10 % and 7 % respectively of DCEs. These rates were higher some extent but consistent with the results of previous paper (Gantzer and Wackett, 1991). Despite of the complex change in composition of chlorinated ethenes, the trend of isotope composition in organic-Cl during dechlorination was similar to the change during biotic reactions (Fig 4-12).

Discussion

Highly chlorinated organic compounds are usually nonbiodegradable by aerobes. And supply of electron acceptors, especially molecular oxygen is limiting factor of in-situ biodegradation of organic pollutants in subsurface environments. However, microbial communities and isolates that can reductively dechlorinate high concentration of chlorinated organic compounds under anaerobic conditions (Fetzner, 1998), have potential for bioaugmentation and natural attenuation. PCE and TCE are the most ubiquitous contaminants in aquifers and soils. Numbers of enriched culture of anaerobic bacteria that can dechlorinate PCE and TCE have been reported, and there are on going research on isolated microorganism (e.g. Yaguchi et al., 1994; Maymó-Gatell et al., 1997) and molecular biological studies of dechlorination enzyme system (Neumann et al, 1996; Schumacher et al, 1997; Miller et al., 1998; Magnuson et al., 2000).

Isotopic data in chlorinated organic compounds can be used to constrain degradation mechanisms in subsurface environments. It might be possible to evaluate contribution of biotic or abiotic degradation to attenuation of pollutants, and to identify microorganisms (or metabolic pathway) mediating degradation of pollutants by using quantitative information on isotope effects in various subsurface processes.

The Rayleigh model (Criss, 1999) is usually used to quantify fractionation factor. However it is difficult to apply such model to the data obtained from multi-step reactions. Although compound specific $\delta^{13}C$ data are available, a series of microcosm experiments starting with the each intermediate product in every step is necessary to determine the enrichment factor of ^{13}C for each step of PCE dechlorination to ethene (Hunkeler et al., 1999; Bloom et al., 2000). Fractionation during sequential reactions: bacterial reduction of selenate [Se(VI)] to elemental selenium [Se(0)] via selenite [Se(IV)] have been investigated using a numerical model (Herbel et al., 2000), but each Se species contains only one Se atom. As compound specific techniques has never been developed for Cl isotopic analysis, and elimination of Cl atom(s) from organic compounds is sometimes regiospecific reaction, interpretation of Cl isotope data is more difficult. Therefore, relatively simple one-step process (TCE to cDCE) and two-step process (PCE to cDCE) were investigated in this study. Isotopic fractionation factors calculated for PCE dechlorination to TCE were ca. 0.987 to ca. 0.991 and for TCE to cDCE were ca. 0.9943 to ca. 0.9945 using the mathematical models.

A few studies have been published on Cl isotope fractionation during biotic and abiotic reactions of chlorinated organic compounds. The isotope fractionation factor for 2-chloro-1-methyl-propane solvolysis (S_N1 reaction) and for 1-chlorobutane substitution (S_N2 reaction) were reported (α=0.991 and 0.996 respectively, at room temperature) (Bartholomew et al., 1954; Turquist et al., 1973). Isotope fractionation factor for the abiotic reductive dehalogenation of benzyl chloride with sodium borohydride (S_N2 reaction) derived from measurement using FABS-mass spectrometry was 0.992 (Wastaway et al., 1998). Heraty et al. (1999) reported isotopic fractionation factor for aerobic biodegradation of dichloromethane (α=0.9962). The fractionation factors for PCE and TCE dechlorination calculated from the isotope data using the mathematical models developed in this study were consistent with those reported values.

Although two Cl atoms existed in dichloromethane molecule, the Cl isotopic fractionation during aerobic biodegradation can be explained by a simple Rayleigh model (Heraty et al., 1999). The rate-determining step of the dichloromethane by methylotrophs is a glutathione-dependent nucleophilic substitution on one of Cl atoms, and then another Cl atom eliminates spontaneously. Because only one of Cl atoms participates in isotopic fractionation, the change in isotopic composition follows the Rayleigh equation. In contrast, Cl isotopic fractionations during dechlorination of PCE and TCE are more complicated. In this study, mathematical models to determine isotopic fractionation factors using non-compound specific Cl isotope data were developed. Although, the actual changes in isotope compositions were roughly consistent with the models, more precise and/or compounds specific isotopic measurement technique should be developed to interpret the small isotopic change like case of TCE dechlorination and to verify suitability of the model. Also isotopic change in the eliminated Cl may be more sensitive against progress of degradation. However the isotopic analysis of Cl^- in reaction medium will be difficult because high concentration of Cl^- and organic compounds exist in the medium.

Carbon isotope fractionation during biotic and abiotic dechlorination of chlorinated ethenes has been investigated (Sherwood Lollar et al., 1999; Hunkeler et al., 1999; Bloom, et al., 2000; Dayan et al., 1999). The reported ^{13}C enrichment factors for each step of the dechlorination decreased monotonically as a function of number of Cl atoms in precursor molecules in the case of dechlorination by anaerobic microorganisms (Hunkeler et al., 1999; Bloom et al., 2000). In contrast, such correlation was not found in reductive dechlorination of PCE, TCE and cDCE by metallic iron (Dayan et al., 1999). The authors explained the relatively small isotope effect on TCE dechlorination by metallic iron by simultaneous dechlorination pathways starting from asymmetric TCE molecule. Apart from this case, the reason for the dependence of isotope change on degree of chlorination has not been explained. The calculated Cl isotopic enrichment factors for PCE dechlorination were larger than the factors for TCE dechlorination. The reason for the opposite trend of enrichment factors depending upon degree of chlorination between ^{13}C and ^{37}Cl is unclear.

However, further studies on factors that may possibly affect isotopic fractionation during dechlorination of CAHs will be necessary to make the models more precise and to apply the model for actual contaminated sites. In this study, the possibilities of isotope exchange between chlorinated ethenes and Cl^- in the media (Bantysh et al., 1962; Harper et al., 2000), rearrangement of Cl atoms in the molecules, and isotope effects on neighboring Cl atoms in the molecules were neglected during the development of the mathematical models. Furthermore, bacteria population (Flynn et al., 2000) change in consortiums and rate constant and isotope fractionation factor change depending on concentration of CAHs or growth stage of the bacteria would affect trajectories of isotopic composition. During PCE dechlorination, actual trajectories of TCE fraction along with PCE fraction change were slightly shifted from theoretical curves (Fig. 4-10) and also the rate constants for TCE dechlorination to cDCE were different in the cases of PCE dechlorination and TCE dechlorination (Table 4-1). This means that dechlorination was inhibited by high concentration of TCE or the bacteria population has changed depending upon growth stage or chlorinated ethenes concentration. Such factors may disturb simple assumptions for development of the models.

The Cl isotopic composition of Cl_c in a TCE reagent (Wako) was significantly different from the composition of Cl_a + Cl_b. The reactivity of the atoms on each sites are not equivalent in asymmetric molecules like TCE. Such intramolecular distributions of isotopes (i.e. compositions of isotopomers) constrains geochemical cycles of substances more strictly compared with bulk isotopic composition (Yoshida and Toyoda, 2000), because synthesis or degradation processes would reflect to intramolecurar site specific isotope ratios. (see Chapter 3).

The transition-metal coenzyme cyanocobalamin mediates dechlorination of chlorinated organic compounds (Gantzer and Wakett, 1991) and it works as active center of dehalogenase in anaerobic bacteria (Schumacher et al., 1997; Holliger et al., 1999). Therefore the cyanocobalamin mediating dechlorination are regarded as simple model to investigate the enzymatic systems participating in biotic dechlorination.

In the dechlorination of chlorinated ethenes mediated by several transition-metal coenzymes, the displacement of each Cl atom decreased the rate constant for the removal of subsequent Cl atom by approximately 1 order of magnitude (Gantzer and Wakett, 1991). However, in this study, stoichiometric quantities of TCE or DCEs did not accumulate but another product (possibly ethene) was formed (Fig. 4-11). The relatively higher rate constants for dechlorination of DCEs and VC or existence of bypass pathway to ethene may be the possible cause of the results. Although, the sum of molar fractions of chlorinated ethenes and 'ETE' were almost unity during the reaction, it is possible that the quantities of 'ETE' include ethane and C_1-compounds. Therefore, identification of the compounds having same retention time to ethane will be needed to examine the inconsistency of this and previous works.

The interpretation of isotopic data for dechlorination of PCE to ethane is difficult, because it is a multi-step reaction and the regiospecificity of the TCE dechlorination is lower than biotic reaction. It was expected that the isotope fractionation factors for each dechlorination step may be calculated if the rate constants for the each step decrease by large magnitude along with decrease in number of Cl atoms as reported by Gantzer and Wackett (1991). Despite of the complex change in composition of chlorinated ethenes, the trend of isotope composition in organic-Cl during dechlorination was similar to the biotic reaction (Fig 4-12). The larger isotopic change at final stage of the reaction would be due to progress of dechlorination of DCEs. Further experiments will need to explain the trajectory of isotopic compositions.

Conclusions

Biotic and abiotic reductive dechlorination of TCE and PCE caused significant isotope fractionation of organic-form Cl. The mathematical models derived from the Rayleigh equation were developed to estimate the fractionation factors of Cl isotope for regiospecific reaction (TCE to cDCE) and two-step reaction (PCE to cDCE). The measured change in Cl isotope roughly followed the calculated trajectories. The values of the kinetic fractionation factor (α) for the TCE dechlornation, estimated from series of experiments using 3 kinds of anaerobe culture conducted at 30 ℃, were 0.9943 to 0.9945. The isotope fractionation in the first step of PCE dechlorination was larger than fractionation in TCE dechlorination, the values of the kinetic fractionation factor (α_1), estimated from experiments conducted at 30 ℃, were 0.987 to 0.991.

However, more precise and/or compounds specific isotopic measurement technique should be developed to interpret small isotopic change (e.g. TCE dechlorination) and multi-step reactions (e.g. PCE dechlorination by cyanocobalamin).

Heterogeneous intramolecular distribution of isotopes (i.e. compositions of isotopomers) was found in a TCE reagent. It would constrain geochemical cycles of substances more strictly compared with bulk isotopic composition.

The analytical techniques and the mathematical models developed in this study will be a preliminary step to apply isotopic data to evaluation of possible disturbance on tracing pollution sources by their isotopic fingerprint, quantification of in-situ cleanup effectiveness, and investigation of mechanisms in subsurface processes.

Table 4-1. Properties of anaerobic bacteria used for the biotic dechlorination experiments.

Anaerobic Bacteria	Strain T	Consortium N	Consortium F
nitrate reduction	n.d.	n.d.	+
sufate respiration	+	—	n.d.
methanogenesis	—	—	—
PCE→cDCE			
k_1 (PCE→TCE)	0.061 hr^{-1}	0.11 hr^{-1}	0.12 hr^{-1}
k_2 (TCE→cDCE)	0.53 hr^{-1}	1.7 hr^{-1}	3.4 hr^{-1}
α_1 (PCE→TCE)	0.991	0.990	0.987
TCE→cDCE			
k (TCE→cDCE)	0.16 hr^{-1}	0.14 hr^{-1}	0.25 hr^{-1}
α (TCE→cDCE)	0.9945±9	0.9944±7	0.9943±10

n.d.: not determined

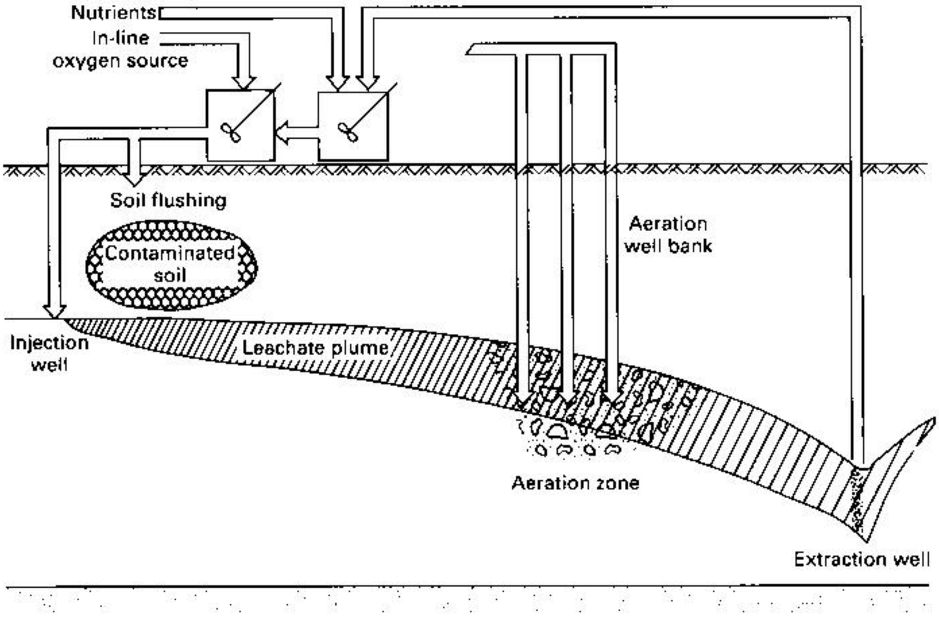


Fig. 4-1. The concept of in-situ bioremediation. (*Source:* From *Hazardous Waste Management,* C. A. Wenz, McGraw-Hill, New York)

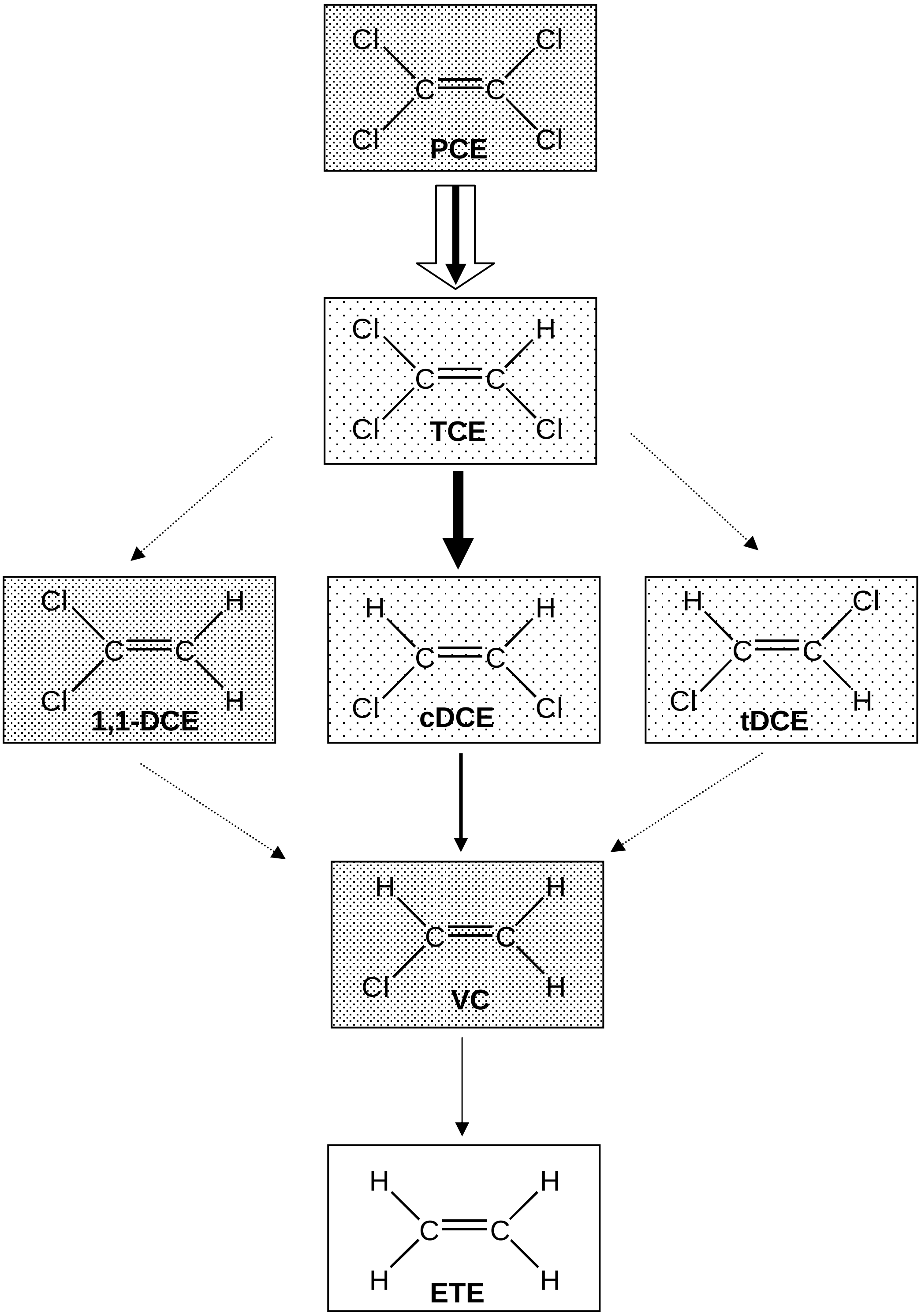


Fig. 4-2. Biotic and abiotic reductive dechlorination pathway of chlorinated ethenes

▓ > ░ > □ : toxicity

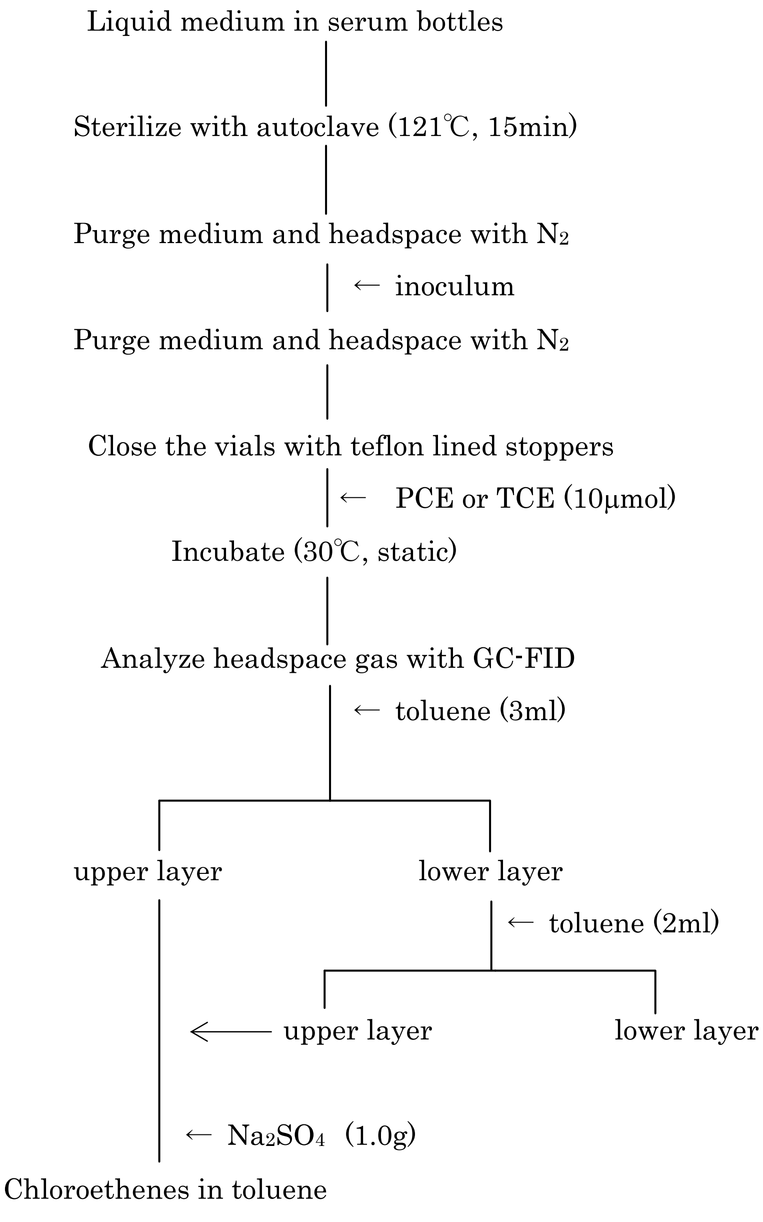


Fig. 4-3. Procedures of PCE and TCE dechlorination by anaerobic bacterial cultures.

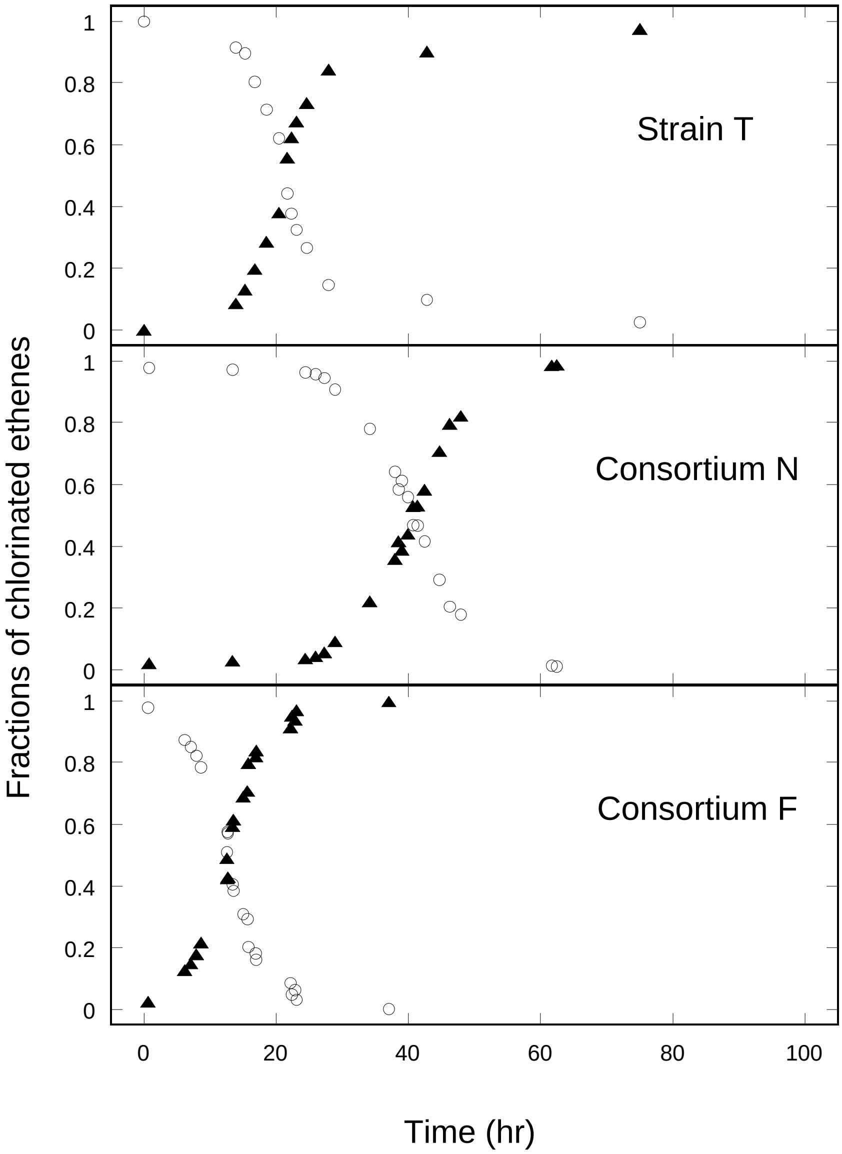


Fig. 4-4. Molar fractions of chloroethenes (TCE, cDCE) in sample bottles over time during the course of biological TCE dechlorination experiments. ○: TCE, ▲: cDCE

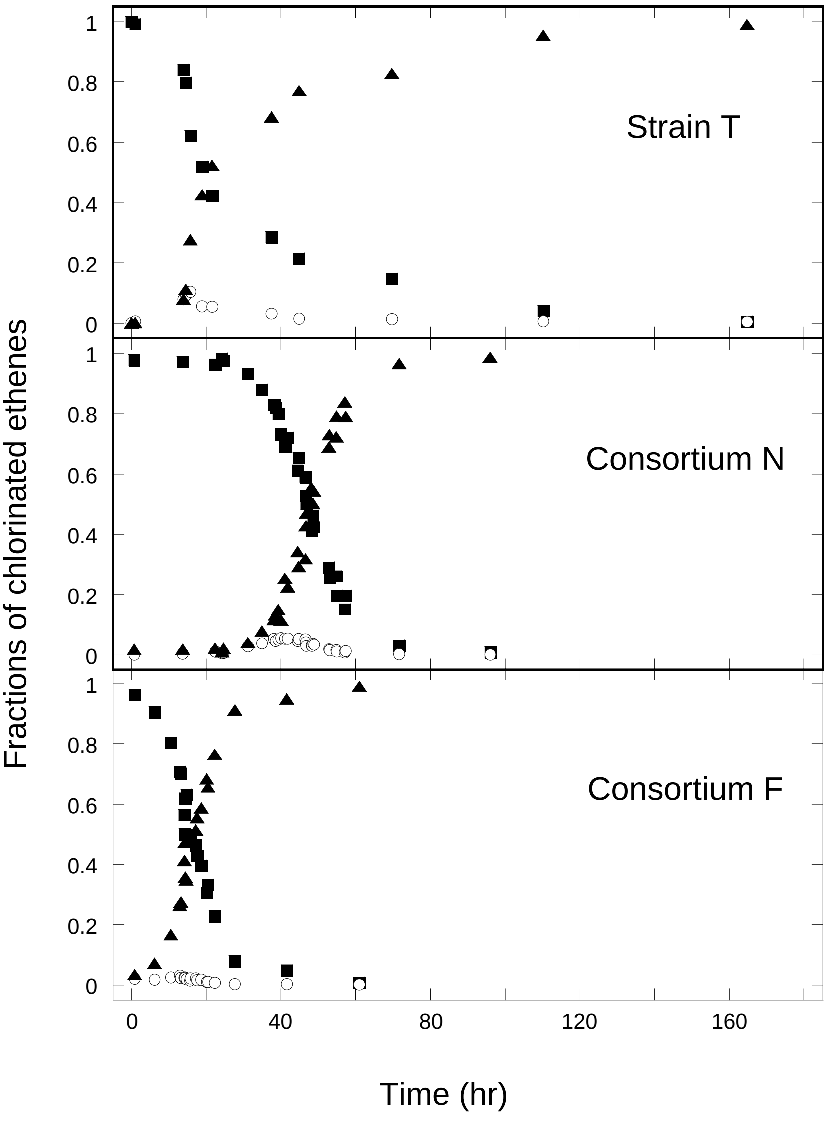


Fig. 4-5. Molar fractions of chloroethenes (PCE, TCE, cDCE) in sample bottles over time during the course of biological PCE dechlorination experiments. ■: PCE, ○: TCE, ▲: cDCE

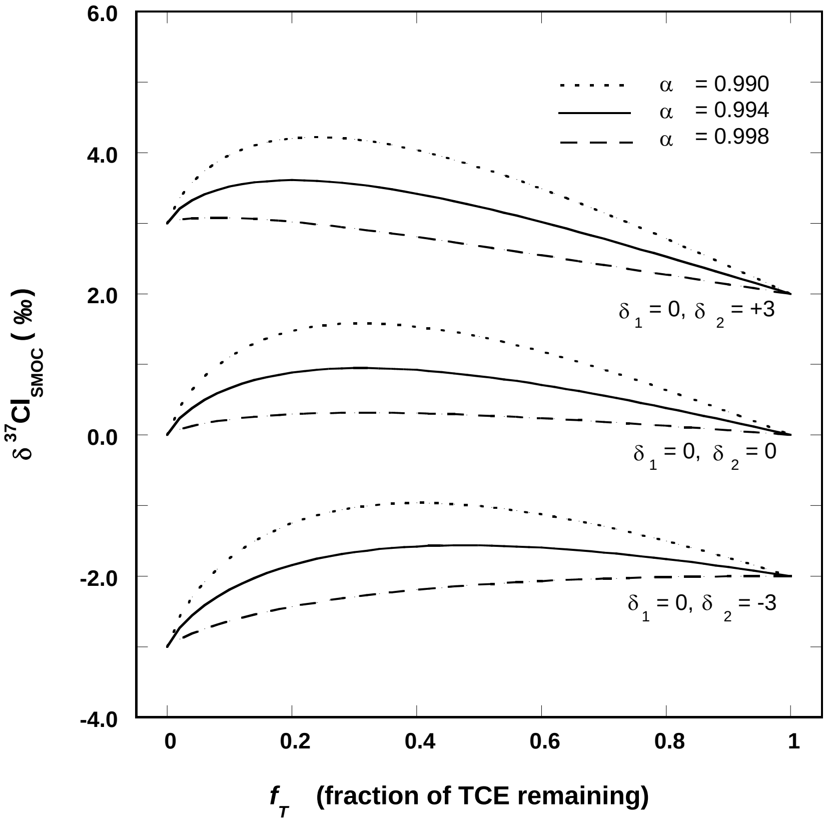


Fig. 4-6. Calculated trajectories of δ^{37}Cl value of organic-chlorine (TCE + cDCE) versus fraction of TCE remaining (eq 4-7; see text). δ_1 and δ_2 represent initial δ^{37}Cl value of Cl_c and $Cl_{a,b}$ respectively. α represents isotope fractionation factor.

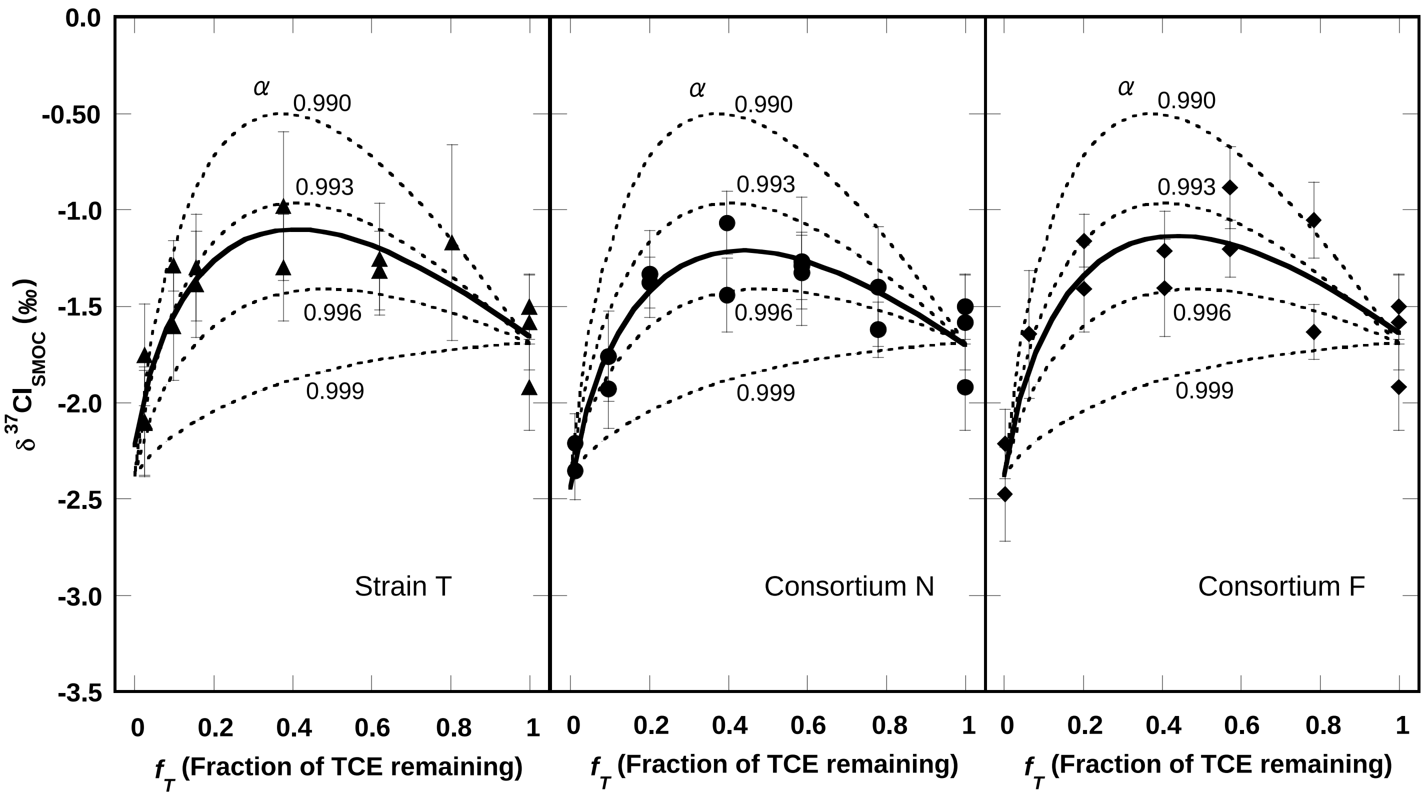


Fig. 4-7. δ^{37}Cl value of organic-chlorine (TCE + cDCE) versus fraction of TCE remaining from the biological TCE dechlorination experiments. Dotted lines are calculated trajectories of δ^{37}Cl value of organic-chlorine (eq 4-7; see text). Error bars represent the internal error of each run of Cl isotopic measurement ($2\sigma_m$). δ_1 and δ_2 for calculated trajectories are average of δ_1 and δ_2 in three regressions (δ_1 and δ_2 represent initial δ^{37}Cl value of Cl_c and $Cl_{a,b}$).

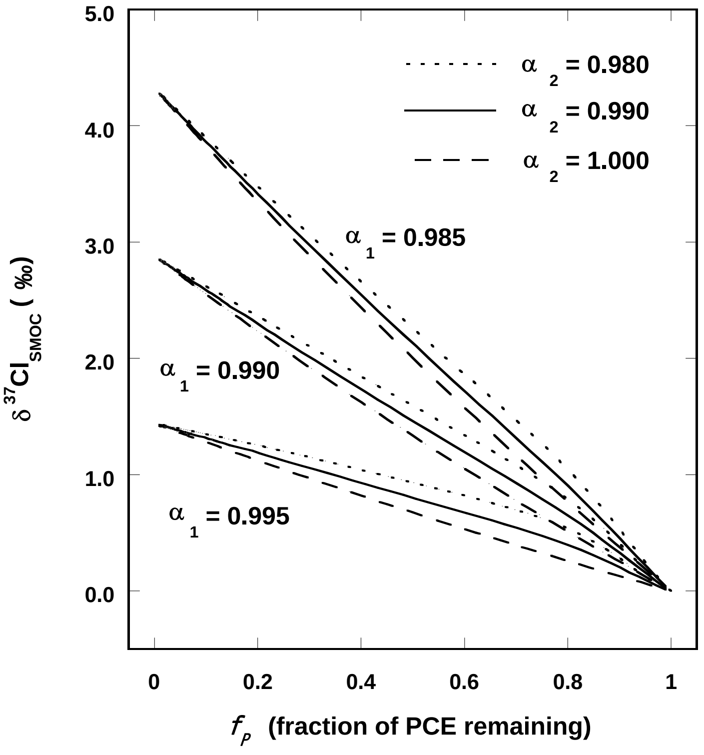


Fig. 4-8. Calculated trajectories δ^{37}Cl value of organic-chlorine (PCE + TCE + cDCE) versus fraction of PCE (eq 4-23; see text) for different values of isotope fractonation factor with constant k_2/k_1 value. δ_0 represents initial δ^{37}Cl value of PCE (0‰). α_1 and α_2 represents isotope fractionation factor for PCE and TCE dechlorination respectively. k_2/k_1 represents ratio of rate constants of TCE dechlorination and PCE dechlorination (k_2/k_1=15).

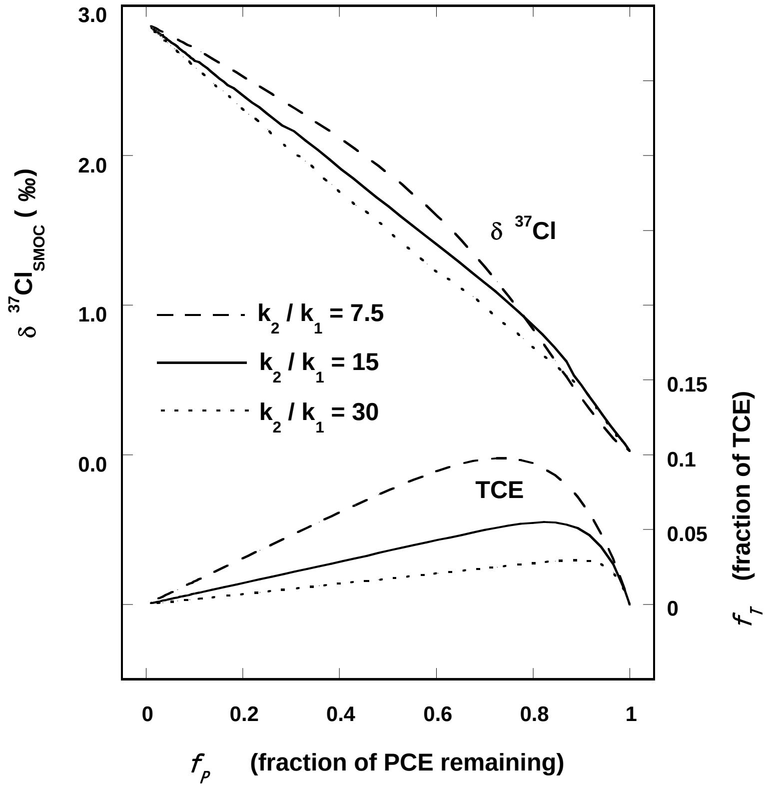


Fig. 4-9. Calculated trajectories δ^{37}Cl value of organic-chlorine (PCE + TCE + cDCE) and fraction of TCE versus fraction of PCE (eq 4-23 and eq 4-17; see text) for different k_2/k_1 values with constant values of isotope fractonation factors. δ_0 represents initial δ^{37}Cl value of PCE (0‰). α_1 and α_2 represents isotopic fractionation factor for PCE and TCE dechlorination respectively (α_1=0.990, α_2=0.980). k_2/k_1 represents ratio of rate constants of TCE and PCE dechlorination.

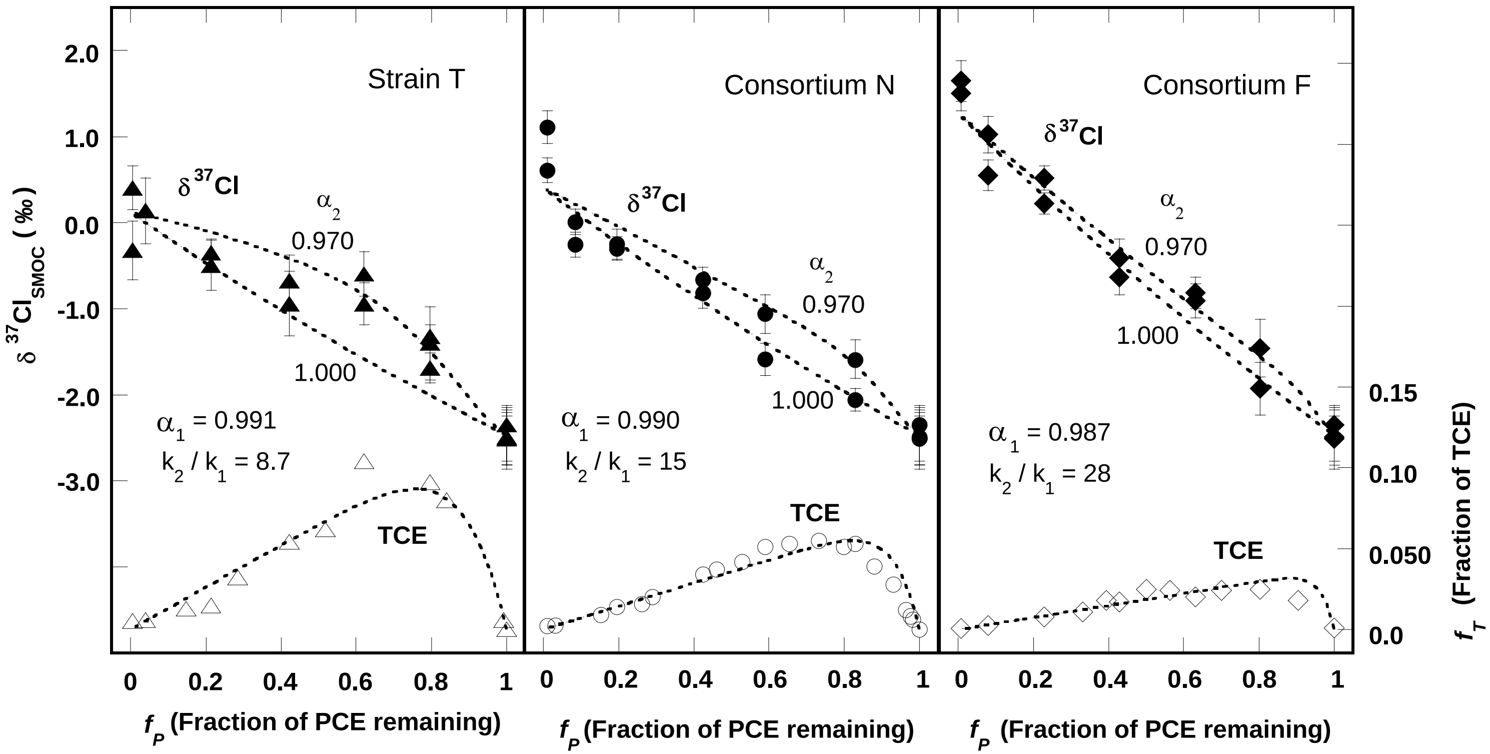


Fig. 4-10. δ^{37}Cl value of organic-chlorine (PCE + TCE + cDCE) and fraction of TCE versus fraction of PCE remaining from the biological PCE dechlorination experiments. Dotted lines are calculated trajectories of δ^{37}Cl value of organic-chlorine and fraction of TCE (eq 4-23 and eq 4-17; see text). Error bars represent the internal error of each run of Cl isotopic measurement ($2\sigma_m$). δ_0 for calculated trajectories is average of initial δ^{37}Cl value of PCE (-2.47‰). α_1 and α_2 represents isotopic fractionation factor for PCE and TCE dechlorination respectively. k_2/k_1 represents ratio of rate constants of TCE and PCE dechlorination.

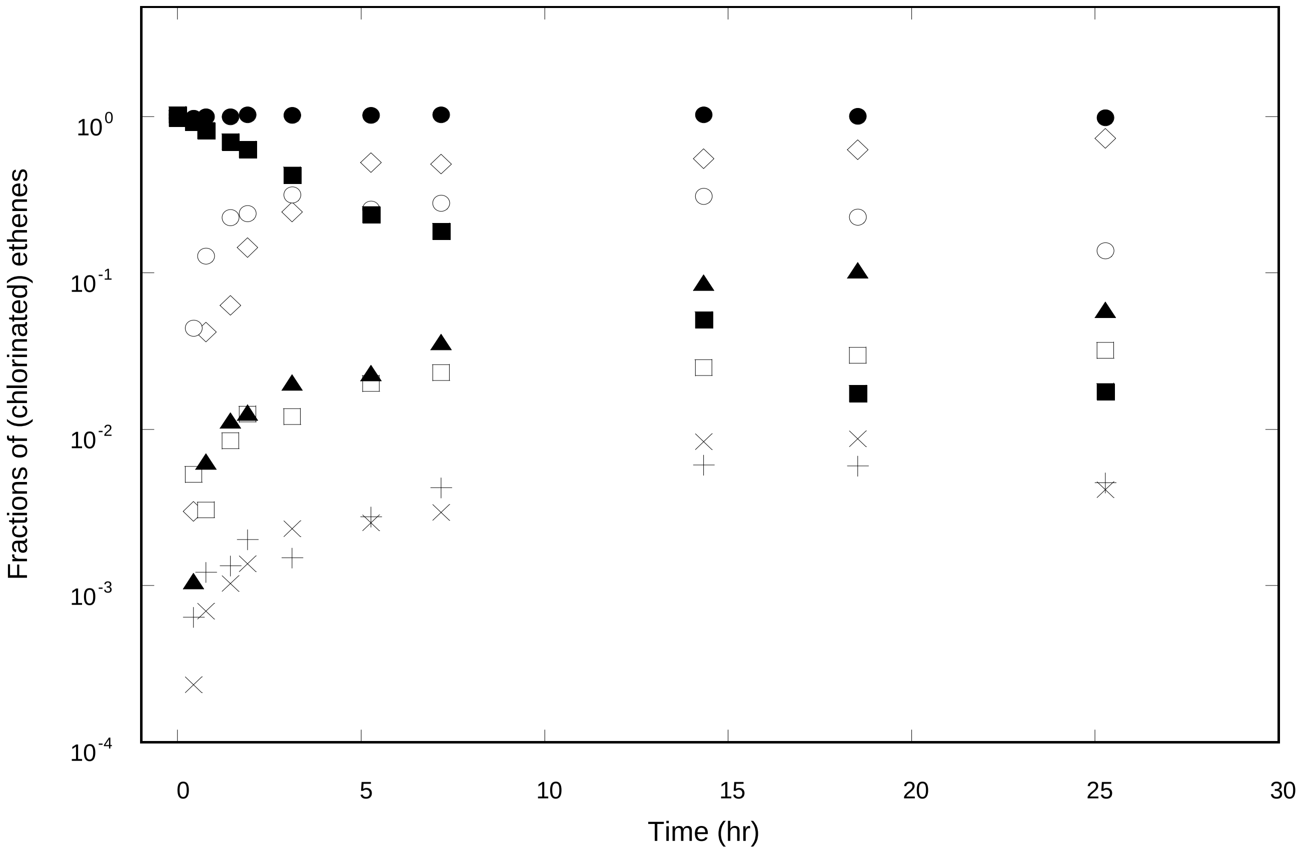


Fig. 4-11. Molar fractions of (chlorinated) ethenes in sample bottles over time during the course of an abiotic PCE dechlorination experiment. ■: PCE, ○: TCE, ▲: cDCE, ×: tDCE, +: 1,1-DCE, □: VC, ◇: (ETE), ●: sum of (chlorinated) ethenes.

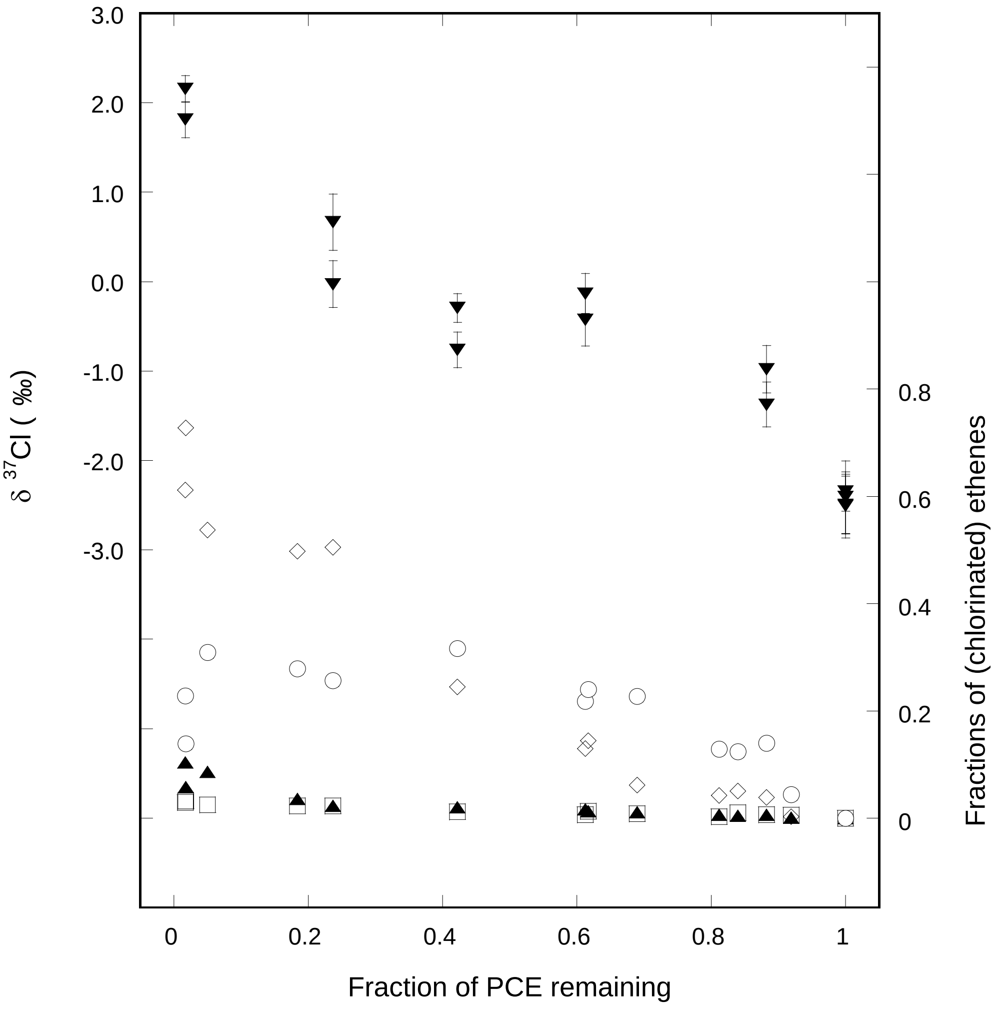


Fig. 4-12. δ^{37}Cl value of organic-chlorine (PCE + TCE + cDCE +VC) and fraction of TCE, cDCE, VC and ETE versus fraction of PCE remaining from the abiotic PCE dechlorination experiment. Error bars represent the internal error of each run of Cl isotopic measurement ($2\sigma_m$). ▼: δ^{37}Cl, ○: TCE, ▲: cDCE, □: VC, ◇: ETE

5. Application of chlorine isotopic analysis for cosmo-geochemistry

Introduction

Halogens participate in many geochemical processes on the Earth as volatile elements (e.g. hydrothermal activity, chemical evolution of ocean; Eastoe et al., 1989; Eastoe and Guilbert, 1992; Seyfield et al., 1986; Ransom et al., 1995; Ito et al., 1983; Philippot et al., 1998; Boudreu et al., 1997; Schilling et al., 1978; Campbell et al., 1988; Berndt and Seyfried; 1990). Halogens also exist in meteorites (Reed, 1971; Dreibus et al., 1977; Garrison et al., 2000) as components of silicates (e.g. sodalite, scapolite, glass; Bridges et al., 1997), chloroapatite (Bridges and Grady, 1999), lawrencite, and halite (Zolensky et al., 1999; Whitby et al., 2000; Bridges and Grady, 1999; Bridges and Grady, 2000; Sawyer et al., 2000).

Although isotopic analysis is a useful tool for the determination of geochemical and cosmochemical processes of many elements, a few investigations on Cl isotopic composition in meteorites have been reported. The small range of bulk δ^{37}Cl values (-0.7 to +0.7 ‰) in 4 ordinary chondrites was reported in Hoering and Parker (1961). The bulk δ^{37}Cl values in representative carbonaceous chondrites Orgueil (CI), Murchison (CM), Allende (CV) were also measured (+2.7 to +4.0 ‰; Magenheim et al., 1994; Magenheim et al., 1995). The distributions of Cl among mantle and an exogenic reservoir (atmosphere, ocean and crust) can be calculated from mass balance of Cl isotopes. By assuming CI chondrite (Orgueil) and MORB glass represents the bulk Earth and degassed mantle respectively, Magenheim et al. (1995) calculated that approximately 40 % of Cl resides in the exogenic reservoir. Although this result is rough estimation due to a small number of measurements of Cl isotopic composition in meteorite and uncertainty of Cl isotopic composition in MORB (i.e. mantle), it constrains evolution of ocean and atmosphere on the Earth. The climate of ancient Mars would be warm enough to sustain liquid water on the surface. The martian 'soil' containing much amount of sulfates and chloride ranges all over the surface of the mars. These salts in the martian soil (Clark and Van Hart, 1981; Banin et al., 1992; Rieder et al., 1997) and martian meteorites (Bridges and Grady, 1999; Bridges and Grady, 2000; Sawyer et al., 2000) may be residues of ancient seawater. If isotopic composition of Cl in the salts is homogeneous enough and martian meteorites retains volatile elements in the mantle component, distribution of Cl between martian mantle and exogenic reservoir may be calculated.

Evidences of aqueous alteration have been found in CI and CM chondrites, but their evolution processes have not been clarified (Tomeoka, 1990; Brandenburg 1996). The isotopic composition has never used to investigate chemical evolution of meteorites. Heavier halogen isotopes distribute to solid phase than fluid (Eastoe and Guilbert, 1992; Magenheim et al., 1995; Ransom et al., 1995; Philippot et al., 1998), aqueous solution than gas phase (Volpe and Spivack, 1994). Distribution of halogen and its isotopes in minerals may constrain evolution of meteorites and their parent bodies.

The radioactive and radiogenic isotopes of halogens (^{36}Cl → ^{36}Ar,^{36}S: $t_{1/2}=3.01\times10^{5}$ yr; ^{129}I→^{129}Xe: $t_{1/2}=1.57\times10^{7}$ yr, ^{81}Kr→^{81}Br: $t_{1/2}=2.29\times10^{5}$ yr) are utilized for not only dating of groundwater (e.g. Phillips, 1995; Lyons et al., 1998; Hendry et al., 2000; Collon et al., 2000) but also dating of meteorites. A cosmogenic isotope ^{36}Cl is used to determine terrestrial residence age (Nishizumi et al., 1989). An extinct isotope ^{129}I is utilized to determine relative age of meteorites (e.g. Ott, 2000). Chlorine bearing minerals are important as carriers of these isotopes (Bridges et al., 1997; Whitby et al., 2000). As the I-Xe age of halite in an ordinary chondrite is one of the oldest value in the solar system, trace of shorter life time isotope ^{36}Cl is searched in the halite (Ott, 2000). It was thought that the half-life of ^{81}Kr is not long enough to survive the time necessary for the bodies to cool to temperatures low enough to retain noble gases (Wyttenbach et al., 1965), decay of ^{81}Kr may cause δ^{81}Br anomaly.

In general, analysis of halogens in silicate rocks is more difficult than metal elements due to their volatility. In particular, this is more serious problem for limited sample size of meteorites.

Halogens are extracted from silicate rocks and meteorites for determination of their abundances and isotopic compositions by alkali fusion (Hoering and Parker, 1961; Wyttenbach et al., 1965; von Gunten et al., 1965; Maxwell, 1968; Jeffery, 1975; Wilson and Gent, 1983; Potts, 1987; Shinonaga et al., 1994a; Shinonaga et al., 1994b; Black, 1997), HF decomposition (Heumann et al., 1987; Magenheim et al., 1995; Musashi et al., 1998), HCl distillation (Geijer, 1960; Robinson, 1959), Cl_2 volatilization (Aruscavage and Campbell, 1983), combustion (Evans and Moore, 1980), pyrohydrolysis (Dreibus et al., 1977; Potts, 1987; Magenheim et al., 1994; Magenheim et al., 1995). Techniques of mineral specific extraction have been also reported. Cl in water soluble phases (e.g. halite, fluid inclusion) is extracted with boiling water (von Gunten, 1965; Eastoe and Maxwell, 1968; Jeffery, 1975; Guilbert, 1992; Bank, et al., 2000) and ‘acid soluble Cl’ is extracted from sodalite, chlorapatite and carbonates with dilute nitric acid (Maxwell, 1968; Eastoe and Guilbert, 1992; Eggenkamp and Koster van Groos, 1997).

The abundances of halogens are determined by INAA (Wyttenbach et al., 1965; von Gunten, 1965; Dreibus et al., 1977; Shinonaga et al., 1994a; Shinonaga et al., 1994b; Garrison et al., 2000), EPMA (Bridges et al, 1997), gravimetric methods (Jeffery, 1975; Maxwell, 1968), photometric methods (Jeffery, 1975; Blackwell, 1997), ion chromatography (Potts, 1987; Blackwell et al., 1997; Evans and Moore, 1980; Wilson and Gent, 1983), ion selective electrode (Aruscavage and Campbell, 1983; Jeffery, 1975; Potts, 1987), isotope dilution mass spectrometry (Heumann et al., 1987; Heumann, 1988; Shinonaga et al., 1994a; Shinonaga et al., 1994b).

Measurement of isotopic ratio is conducted by electron impact mass spectrometry of CH_3Cl (Markl et al., 1997; Eastoe and Guibert, 1992; Eastoe et al., 1989; Musashi et al., 1998) and thermal ionization mass spectrometry of Cs_2Cl^+ (Magenheim et al., 1994; Magenheim et al., 1995; Banks et al., 2000).

As described previously, it is important to develop techniques for determination of halogens abundances and isotopic compositions in meteorites for investigation of their history. In general, isotope dilution mass spectrometry (IDMS) gives accurate results for trace analysis of many elements. The IDMS is regarded as a definitive analytical method and utilized for dating of meteorites by isochron methods and determination of rare earth elements pattern in meteorites (Heumann, 1988; Potts, 1987). The IDMS by negative thermal ionization mass spectroscopy (N-TIMS) of halogens is highly sensitive and has been used successfully in the investigation of meteorites (Heumann et al., 1987; Shinonaga et al., 1994a; Shinonaga et al., 1994b). Although sensitivity of thermal ionization mass spectroscopy of Cs_2Cl^+ is lower than the N-TIMS method in some extent, the precision may be higher.

In this work, in order to develop an improved method of IDMS for determination of Cl and Br abundances, and the precise Br isotopic ratio using TIMS which is applicable to tiny planetary materials, ^{37}Cl spiked CsCl and CsBr prepared from a commercial KBr reagent were analyzed. Preliminary results of

IDMS for Cl abundance determination and isotopic analysis of Br were described in this chapter.

Experimental Procedures

Seawater Sample and Reagents

A seawater sample from the Pacific Ocean (8°39.5'N, 153°56.6'E) was used for isotopic dilution mass spectrometer (see Chapter 2).

A ^{37}Cl enriched spike (NaCl: ^{37}Cl 98.21 %; Oak Ridge National Laboratory) was stored as 6.85 and 1.37 μmol/g aqueous solution (24.7 mg of ^{37}Cl enriched NaCl was dissolved with water). A commercial grade CsCl reagent (minimum assay 99 %; Nacalai tesque) was stored as 56.4 mM (2 g-Cl/L) solution. Water was purified with a Milli-RX12Plus and a Milli-Q SP system (Millipore).

Cs-form and Ba-form cation exchange resin was prepared from DOWEX 50Wx8 (see Chapter 2).

Measurements of isotopic composition in the ^{37}Cl spike

The ^{37}Cl spike solution containing 3.2 μmol of Cl was evaporated to dryness on hot plate (120 ℃). The residue was dissolved in 50 μL of water and loaded on a column (borosilicate glass, inner diameter: 2 mm) that contained 0.1 mL of Cs-form cation exchange resin. The column was washed with 200 μL of water. The eluent was evaporated to dryness on a hot plate (120 ℃). The residue was dissolved in 60 μL of water, and 1.0 μL of the solution was loaded on a Ta filament with graphite. The isotopic ratios were measured by using a Finnigan MAT Model 262 thermal ionization mass spectrometer (see Chapter 2).

Isotope dilution mass spectrometry for determination of Cl concentration in seawater

The spike solution containing 0.3 or 0.6 μmol of ^{37}Cl was added to 50 μL of the dilute seawater sample containing 0 to 0.25 μmol of Cl. And then the mixed solutions were evaporated to dryness on a hot plate (120 ℃). The residue was dissolved in 50 μL of water and loaded on a column (borosilicate glass, inner diameter: 4 mm) that contained 0.2 mL of Ba-form cation exchange resin. The column was washed with 250 μL of water, and the eluent was loaded on a column (borosilicate glass, inner diameter: 2 mm) that contained 0.1 mL of Cs-form cation exchange resin. The column was washed with 200 μL of water. The eluent was evaporated to dryness on a hot plate (120 ℃). The residue was dissolved in 3.0 μL of water, and 1.0 μL of the solution was loaded on a Ta filament with graphite. The isotopic ratios were measured by using a JEOL JMS-05RB mass spectrometer (30 cm radius, 90° geometry) as described in Chapter 2.

Concentration of Cl^- in the seawater was also determined with a Hitachi ion chromatograph L-7000.

Isotope mass spectrometry of Br

Fifty microliters of KBr solution (56.4 mM) was loaded on a column (borosilicate glass, inner diameter: 2 mm) that contained 0.1 mL of Cs-form cation exchange resin. The column was washed with 200 μL of water. The eluent was evaporated to dryness on a hot plate (120 ℃). The residue (CsBr) was dissolved in 50 μL of water, and 1.0 μL of the solution containing 56.4 nmol of CsBr was loaded on a Ta filament with graphite. The ratios of $Cs_2{}^{79}Br^+$ (m/z=345) and $Cs_2{}^{81}Br^+$ (m/z=347) were measured by using a JEOL JMS-05RB mass spectrometer. Also mixture of CsCl solution (56.4 mM, 0.5 μL) and the CsBr solution (56.4 mM, 0.5 μL) were was analyzed by same procedure.

Results and Discussion

Isotope dilution mass spectrometry for determination of Cl concentration in seawater

Isotopic composition in the ^{37}Cl spike was determined using the Finnigan MAT 262 mass spectrometer. The calculated ^{37}Cl abundance was 98.1 % (^{37}Cl / ^{35}Cl = 51.53). The isotopic abundance of spike NaCl observed by us were in agreement within 0.2 % with the value given by Oak Ridge National Laboratory.

Results of Cl abundance in diluted seawater determined by isotope dilution mass spectrometry were shown in Fig. 5-1. The internal error ($2\sigma_m$) was ca. 0.6 % by measurement of 9 scans ca. 3×10^{-10} A of signals. The calculated Cl abundance in seawater was 528 mmol-Cl/kg. This value was slightly higher than the value determined by ion chromatography (488 mmol-Cl/kg). The spike solution should be calibrated with chloride standard solution which has natural isotopic composition.

Advantage of isotope dilution mass spectrometry is independence of accuracy on recovery yield of the target element after addition of spikes. Because quantitative recovery of Cl from silicate rocks is recovery, this is important. However, isotope dilution by Cs_2Cl^+ mass spectrometry requires relatively large samples (50 nmol-Cl). Although the sensitivity of by ion chromatography is much higher than this IDMS (detection limit of Cl^-: approximately 0.02 nmol), the accuracy depends on recovery yields. However less than 1 nmol of Br can be measured by negative ion mass spectrometry of Br^-(Shinonaga et al., 1994a). In order to apply isotope dilution mass spectrometry of Cs_2Cl^+ to actual meteorites samples, sample size should be reduced further.

Isotope mass spectrometry of Br

When 56 nmol of CsBr with ca.50 μg of graphite was loaded on the filament, Cs_2Br^+ signal lasted long time enough to measure isotopic ratio (3.5×10^{-10}A of $Cs_2{}^{79}Br^+$ signal lasted for more than 60 min). The obtained isotopic ration was 0.9745 ± 0.0037 (9 scans, error: $2\sigma_m$). When 28 nmol of CsCl and 28 nmol of CsBr with ca.50 μg of graphite were loaded on the filament, duration of Cs_2Cl^+ and Cs_2Br^+ signals became shorter (2×10^{-10}A of $Cs_2{}^{79}Br^+$ signal lasted for 60 min and 7×10^{-10}A of $Cs_2{}^{37}Cl^+$ signal lasted for 60 min).

In this study, Cl^- in aqueous solution is recovered as AgCl for Cl isotopic analysis. Due to difficulty of handling of trace amount of AgCl precipitaion and solubility of AgCl, the recovery yield of Cl from low concentration of solution will be reduced. As graphite powder adhered to the surface of filament with CsCl, graphite and CsCl will drop from the filament if amount of CsCl is too small. Mixture of CsCl and CsBr will be obtained by co-precipitation of trace Cl^- with AgBr. As mixture of CsCl and CsBr gave Cs_2Cl^+ and Cs_2Br^+ signals, the co-precipitation procedure may solve the problems.

The electron impact mass spectrometry of CH_3Br (Willey and Taylor, 1978) and the thermal ionization mass spectrometry of Cs_2Br^+ (Xiao et al., 1993) have been developed and applied to samples processed Br from reagents suppliers.

However, the isotope geochemistry of Br has not been exploited, due to expected analytical difficulties (e.g. rarity of Br in natural samples and separation from relatively large amount of Cl). There are a few reports in which attempts were made to measure natural Br isotope variations. Cameron and Lippert (1955) applied mass spectrometry of HBr for measurement of Br isotope composition in natural samples, but no significant variations were found. Wyttenbach et al. (1965) reported Br isotopic compositions in 5 meteorites, but the anomaly was not found because of low precision of isotopic analysis by the neutron activation. Eggenkamp and Coleman (2000) have developed the method for Br separation from Cl, conversion into CH_3Br and electron impact mass spectrometry of CH_3Br. They measured two NaBr reagents, seawater bromide as a standard (Standard Mean Ocean Bromide: SMOB), Br separated from 11 formation water samples.

It has been expected that the mechanisms of isotopic fractionation of Br in natural systems resemble the case of Cl isotopic fractionation (e. g. crystallization: Vengosh et al., 1989; Eggenkamp et al., 1995; Liu et al., 1997; Lyons et al., 1999; Eastoe et al., 1999, aqueous alteration of silicate minerals: Eastoe and Guilbert, 1992; Magenheim et al., 1995; Ransom et al., 1995, volatilization: Volpe and Spivack, 1994; Volpe et al., 1998; Lyons et al., 1999, diffusion: Desaulniers et al., 1986; Eggenkamp et al., 1994; Phillips and Bentley, 1987), because the chemistry of Br is very similar to Cl. However difference processes of isotopic fractionation between Cl^- and Br^- in the formation water samples was suggested due to coexistence of positive $\delta^{81}Br_{SMOB}$ values and negative $\delta^{37}Cl_{SMOC}$ values (Eggenkamp and Coleman, 2000). Different from $\delta^{37}Cl$, $\delta^{81}Br$ anomalies may be caused by oxidation of Br^- to Br_2 at high redox potential circumstances (e.g. evaporites in the Chilean Atacama desert; Ericksen, 1981), formation or decomposition of natural or artificial brominated organic compounds (Harper, 1985) or radioactive decay of ^{81}Kr (Wyttenbach et al.,1965; Collon et al., 2000).

Conclusions

The isotope dilution mass spectrometry (IDMS) of Cs_2Cl^+ for determination of Cl abundance was developed. The results of measurement of a seawater sample by IDMS were consistent with results of ion chromatography. The sensitivity of IDMS should be improved for practical use for meteorite samples.

Also isotope ratio mass spectrometry of Br was examined. The stability and duration of Cs_2Br^+ signals were enough for isotopic analysis.

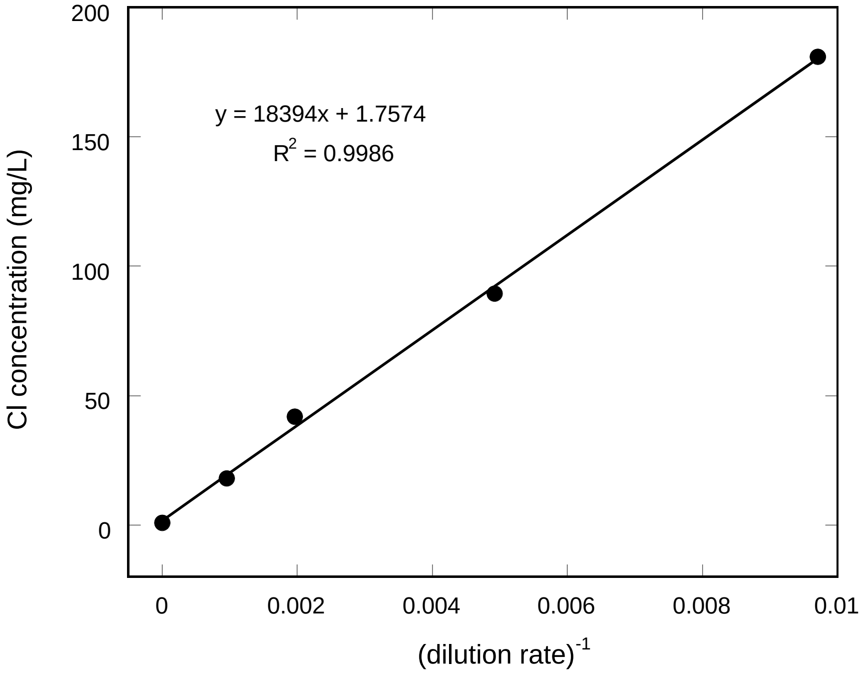


Fig. 5-1. Results of isotope dilution mass spectrometry for determination of Cl concentration in a seawater sample.

6. References

Aggarwal, P. K., Fuller, M. E., Gurgas, M. M., Manning, J. F. and Dillon, M. A. (1997) Use of oxygen and carbon isotope analyses for monitoring the pathways and rates of intrinsic and enhanced in situ biodegradation. *Environ. Sci. Technol.*, 31, 590-596.

Aruscavage, P. J. and Campbell, E. Y. (1983) An ion-selective electrode method for determination of chlorine in geological materials. *Talanta*, 30, 745-749.

Banin, A., Clark, B. C. and Wänke, H. (1992) Surface chemistry and mineralogy, *In* Kieffer, H. H., Jakosky, B. M., Snyder, C. W. and Matthews, M. S. (eds.) *Mars.* pp. 594-625. The University of Arizona Press. Tuscon.

Banks, D. A., Green, R., Cliff, R. A. and Yardley, B. W. D. (2000) Chlorine isotopes in fluid inclusions: Determination of the origins of salinity in magmatic fluids. *Geochim. Cosmochim. Acta*, 64, 1785-1789.

Bantysh, A. N., Zel'venskii, Y. D., and Shalygin, V. A. (1962) Isotopic exchange of the chloride ion with some organic chloro-compounds. *Rus. J. Phys. Chem.*, 36, 30-33.

Bartholomew, R. M., Brown, F., and Lounsbury, M. (1954) Chlorine isotope effect in reactions of tert-butyl chloride. *Can. J. Chem.*, 32, 979-983.

Beneteau,K.M., Aravena, R. and Frape, S.K. (1999) Isotopic characterization of chlorinated solvents - laboratory and field results. *Org. Geochem.*, 30, 739-753.

Berndt, M. E. and Seyfried Jr., W. E. (1990) Boron, bromine, and other trace elements as clues to the fate of chlorine in mid-ocean ridge vent fluids. *Geochim. Cosmochim. Acta*, 54, 2235-2245.

Blackwell, P. A., Cave. M. R., Davis, A. E. and Malik, S. A. (1997) Determination of chlorine and bromine in rocks by alkaline fusion with ion chromatography detection. *J. Chromatogr. A.*, 770, 93-98.

Bloom, Y., Aravena, R., Hunkeler, D., Edward, E. and Frape, S. K. (2000) Carbon isotope fractionation during microbial dechlorination of trichloroethene, *cis*-1,2-dichloroethene, and vinyl chloride: Implications for assessment of natural attenuation. *Environ. Sci. Technol.*, 34, 2768-2772.

Boudreau, A. E., Stewart, M. A. and Spivack, A. J. (1997) Stable Cl isotopes and origin of high-Cl magmas of the Stillwater Complex, Montana. *Geology*, 25, 791-794.

Boyd, A. W., Brown, F. and Lounsbury, M. (1955) Mass spectrometric study of natural and neutron-irradiated chlorine. *Canadian J. Phys.*, 33, 35-42.

Brandenburg, J. E. (1996) Mars as the parent body of the CI carbonaceous chondrites. *Geophys. Res. Lett.*, 23, 961-964.

Bridges, J. C., Alexander, C. M. O'D., Huchison, R., Franchi, I. A. and Pillinger, C. T. (1997) Sodium-, chlorine-rich mesostases in Chainpur (LL3) and Parnallee (LL3) chondrules. *Meteorit. Planet. Sci.*, 32, 555-565.

Bridges, J. C. and Grady, M. M. (1999) A halite-siderite-anhydrite-chlorapatite assemblage in Nakhla: Mineralogical evidence for evaporites on Mars. *Meteorit. Planet. Sci.*, 34, 407-415.

Bridges, J. C. and Grady, M. M. (2000) Evaporite mineral assemblages in the naklite (martian) meteorites. *Earth Planet. Sci. Lett.*, 176, 267-279.

Cameron, A. E. and Lippert, E .L. (1955) Isotope composition of bromine in nature. *Science*, 121, 136-137

Campbell, A. C., Palmer, M. R., Klinkhammer, G. P., Bowers, T. S., Edmond, J. M., Lawrence, J. R., Casey, J. F., Thompson, G., Humphris, S., Rona, P. and Karson, J. A. (1988) Chemistry of hot springs on the Mid-Atlantic Ridge. *Nature,* 335, 514-519.

Cicerone, R. J. (1981) Halogens in the atmosphere. *Rev. Geophys. Space Phys.*, 19, 123-139

Clark, B. C. and Van Hart, D. C. (1981) The salts of Mars. *ICARUS*, 45, 370-378.

Collon, P., Kutschera, W., Loosli, H. H., Lehmann, B. E., Purtschert, R., Love, A., Sampson, L., Anthony, D., Cole, D., Davids, B., Morrissey, D. J., Sherrill, B. M., Steiner, M., Pardo, R. C. and Paul, M. (2000) ^{81}Kr in the Great Artesian Basin, Australia: a new method for dating very old groundwater. *Earth Planet. Sci. Lett.*, 182, 103-113.

Conrad, M. E., Daley, P. F., Fisher, M. L., Buchanan, B. B., Leighton, T., and Kashgarian, M. (1997) Combined ^{14}C and δ^{13}C monitoring of in situ biodegradation of petroleum hydrocarbon. *Environ. Sci. Technol.*, 31, 1463-1469.

Criss, R. E. (1999) *Principles of stable isotope distribution*, Oxford University press, New York. pp.105-110.

Dayan, H., Abrajano, T., Sturchio, N. C. and Winsor, L. (1999) Carbon isotopic fractionation during reductive dehalogenation of chlorinated ethenes by metallic iron. *Org. Geochem.*, 30, 755-763.

de Bruin, W. P., Kotterman, M. J. J., Posthumus, M. A. Shraa, G. and Zehnder, A. J. B. (1992) Complete biological reductive transformation of tetrachloroethene to ethene. *Appl. Environ. Microbiol.*, 58, 1996-2000.

Dempster, H. S., Sherwood Lollar, B. and Feenstar, S. (1997) Tracing Organic Contaminants in groundwater: A new methodology using compound-specific isotopic analysis. *Environ. Sci. Technol.*, 31, 3193-3197.

Desaulniers, D. E., Kaufmann, R. S., Cherry, J. A., and Bentley, H. W. (1986) ^{37}Cl-^{35}Cl variations in a diffusion-controlled groundwater system. *Geochim. Cosmochim. Acta*, 50, 1757-1764.

Dreibus, G., Spettel, B. and Wänke, H. (1977) Halogens in meteorites and their primordial abundances. *In* Ahrens, L. H. (ed.) *Origin and distribution of the* *elements*, pp. 33-38, Pergamon Press, Oxford.

Eastoe, C. J., Guilbert, J. M. and Kaufmann, R. S. (1989) Preliminary evidence for fractionation of stable chlorine isotopes in ore-forming hydrothermal systems. *Geology*, 17, 285-288.

Eastoe, C. J. and Guilbert, J. M. (1992) Stable chlorine isotopes in hydrothermal processes. *Geochim. Cosmochim. Acta*, 56, 4247-4255.

Eastoe, C. J., Long, A. and Knauth, P. (1999) Stable chlorine isotope in the Palo Duro Basin, Texas: Evidence for preservation of Permian evaporite brines. *Geochim. Cosmochim. Acta*, 63, 1375-1392.

Eggenkamp, H. G. M., Middelburg, J. J., and Kreulen, R. (1994) Preferential diffusion of ^{35}Cl relative to ^{37}Cl in sediments of Kau Bay, Halmahera, Indonesia. *Chem. Geol (Isotope Geosci. Sect.)*, 116, 317-325.

Eggenkamp, H.G., Kreulen, R., and Koster van Groos, A. F. (1995) Chlorine stable isotope fractionation in evaporite. *Geochim. Cosmochim. Acta*, 59, 5169-5175.

Eggenkamp, H.G. and Koster van Groos, A. F. (1997) Chlorine stable isotopes in carbonatites: evidence for isotopic heterogeneity in the mantle. *Chem. Geol.*, 140, 137-143.

Eggenkamp, H. G. M., and Coleman, M. L. (2000) Rediscovery of classical methods and their application to the measurement of stable bromine isotopes in natural samples. *Chem. Geol.*, 167, 393-402.

Erickson, G. E. (1981) Geology and origin of the Chilean nitrate deposits. *USGS Prof. Paper*, 1188, pp. 37.

Evans, K. L. and Moore, C. B. (1980) Combustion-ion chromatographic determination of chlorine in silicate rocks. *Anal. Chem.*, 52, 1908-1912.

Fetzner, S. (1998) Bacterial dehalogenation. *Appl. Microbiol. Biotechnol.*, 50, 633-657.

Fisher, A. J., Rowan, E. A. and Spalding, R, F. (1987) VOCs in ground water influenced by large scale withdrawals. *Ground Water*. 25, 407-413

Flynn, S. J., Löffler, F. E. and Tiedie, J. M. (2000) Microbial community changes associated with a shift from reductive dechlorination of PCE to reductive dechlorination of *cis*-DCE and VC. *Environ. Sci. Technol.*, 34, 1056-1061.

Gantzer, C. J. and Wackett, L. P. (1991) Reductive dechlorination catalyzed by bacterial transition-metal coenzymes. *Environ. Sci. Technol.*, 25, 715-722.

Garrison, D., Hamlin, S. and Bogard, D. (2000) Chlorine abundances in meteorites. *Meteorit. Planet. Sci.*, 35, 419-429.

Geijer, P. (1960) Halogens in skarn amohiboles. *Arkiv. Mineral. Geol.*, 2, 482-484.

Gillham, R. W. and O'Hannesin, S. T. (1994) Enhanced degradation of chlorinated aliphatics by zero-valent iron. *Groundwater*, 32, 958-971.

Grayson, M. eds. (1985) *Kirk-Othmer Concise Encyclopedia of Chemical Technology*, John Wiley and Sons, New York

Gribble, G. W. (1994) The natural production of chlorinated compounds. *Environ. Sci. Technol.*, 28, 310A-319A.

Gschwend, P. M., MacFarlane, J. K., and Newman, K, A. (1985) Volatile halogenated organic compounds released to seawater from temperate marine macroalgae. *Science*, 227, 1033-1035.

Harper, D. B. (1985) Halomethane from halide ion – a highly efficient fungal conversion of environmental significance. *Nature*, 315, 55-57.

Harper, D. B., Kalin, R. M., Larkin, M. J., Hamilton, J. T. G. and Coulter, C. (2000) Microbial transhalogenation: A complicating factor in determination of atmospheric chloro- and bromomethane budgets. *Environ. Sci. Technol.*, 34, 2525-2527.

Hendry, M. J., Wassenaar, L. I. and Kotzer, T. (2000) Chlorine and chlorine isotopes (^{36}Cl and δ^{35}Cl) as tracers of solute migration in a thick, clay-rich aquitard system. *Water Resour. Res.*, 36, 285-296.

Heraty, L. J., Fuller, M. E., Huang, L., Abrajano Jr., T. and Sturchio, N. C. (1999) Isotopic fractionation of carbon and chlorine by microbial degradation of dichloromethane. *Org. Geochem.*, 30, 793-799.

Herbel, M. J., Johnson, T. M., Oremond, R. S. and Bullen, T. D. (2000) Fractionation of selenium isotopes during bacterial respiratory reduction of selenium oxyanions. *Geochim. Cosmochim. Acta*, 64, 3701-3709.

Heumann, K. G., Gall, M. and Weiss, H. (1987) Geochemical investigation to explain iodine-overabundances in Antarctic meteorite. *Geochim. Cosmochim. Acta*, 51, 2541-2547.

Heumann, K. G. (1988) Isotope dilution mass spectrometry. *In* Adams, F., Gijbels R. and van Grieken, R. (eds.) *Inorganic Mass Spectrometry*, pp. 301-368. John Wiley and Sons, New York.

Hoefs, J. (1997) *Stable Isotope Geochemistry.* Springer-Verlag, Berlin, pp. 62-63.

Hoekstra, E. J., de Leer, E. W. B. and Brinkman, U. A. T. (1998) Natural formation of chloroform and brominated trihalomethanes in soil. *Environ. Sci. Technol.*, 32, 3724-3729.

Hoering, T. C., and Parker, P. L. (1961) The geochemistry of the stable isotopes of chlorine. *Geochim. Cosmochim. Acta*, 23, 186-199.

Holliger, C., Schiraa, G., Stams, A. J. M. and Zehnder, A. J. B. (1993) A highly purified enrichment culture coupled the reductive dechlorination of tetrachloroethene to growth. *Appl. Environ. Microbiol.*, 59, 2991-2997.

Holliger, C. (1995) The anaerobic microbiology and biotreatment of chlorinated ethenes. *Curr. Opin. Biotechnol.*, 6, 347-351.

Holliger, C., Wohlfarth, G. and Diekert, G. (1999) Reductive dechlorination in the energy metabolism of anaerobic bacteria. *FEMS Microbiol. Rev.*, 22, 383-398.

Holt, B. D., Sturchio, N. C., Abrajano, T. A., and Heraty, L. J. (1997) Conversion of chlorinated volatile organic compounds to carbon dioxide and methyl chloride for isotopic analysis of carbon and chlorine. *Anal. Chem.*, 69, 2727-2733.

Huang, L., Sturchio, N. C., Abrajano Jr., T., Heraty, L.J. and Holt, B.D. (1999) Carbon and chlorine isotope fractionation of chlorinated aliphatic hydrocarbons by evaporation. *Org. Geochem.*, 30, 777-785.

Hunkeler, D., Aravena, R. and Butler, B. J. (1999) Monitoring microbial dechlorination of tetrachloroethene (PCE) in groundwater using compound-specific carbon isotope ratios: Microcosm and field studies. *Environ. Sci. Technol.*, 33, 2733-2738.

Hunkeler, D. and Aravena, R. (2000a) Determination of compound-specific carbon isotope ratios of chlorinated methanes, ethanes, and ethenes in aqueous samples. *Environ. Sci. Technol.*, 34, 2839-2844.

Hunkeler, D. and Aravena, R. (2000b) Evidence of substantial carbon isotope fractionation among substrate, inorganic carbon, and biomass during aerobic mineralization of 1,2-dichloroethane by *Xanthobacter autotrophicus*. *Appl. Environ. Microbiol.*, 66, 4870-4876.

Imagawa, T. (1990) 化学反応による有機塩素化合物の分解. Kagaku-Kougyou, (1), 24-27 (in Japanese).

Ito, E., Harris, D. M. and Anderson Jr., A. T. (1983) Alteration of oceanic crust and geologic cycling of chlorine and water. *Geochim. Cosmochim. Acta*, 47, 1613-1624.

Jarman, W. M., Hilkert, A., Bacon, C. E., Collister, J. W., Ballschmiter, K., and Risebrough, R. W. (1998) Compound-specific carbon isotopic analysis of Aroclors, Clophens, Kaneclors, and Phenoclors. *Environ. Sci. Technol.*, 32, 833-836.

Jeffery, P. G. (1975) *Chemical methods of rock analysis*, Pergamon Press, Oxford. pp. 190-197.

Jendrzejewski, N., Eggenkamp, H. G. M., and Coleman, M. L. (1997) Sequential determination of chlorine and carbon isotopic composition in single microliter samples of chlorinated solvent. *Anal. Chem.*, 69, 4259-4266.

Johnson, T. M., Bullen, T. D. and Zawislanski, P. T. (2000) Selenium stable isotope ratios as indicators of sources and cycling of selenium: Results from the Northern Reach of San Francisco Bay. *Environ. Sci. Technol.*, 34, 2075-2079.

Johnston, H. L., and Hutchison, D. A. (1942) Efficiency of the electrolytic separation of chlorine isotopes. *J. Chem. Phys.*, 10, 469-472.

Jordan, A., Harnisch, J., Borcher, R., Guern, F. L. and Shinohara, H. (2000) Volcanogenic hydrocarbons. *Environ. Sci. Technol.*, 34, 1122-1124.

Kaufmann, R. S., Long, A., Bentley, H., and Davis, S. (1984) Natural chlorine isotope variations. *Nature*, 309, 338-340.

Kaufmann, R. S., Frape, S. K., McNutt, R., and Eastoe, C. (1993) Chlorine stable isotope distribution of Michigan Basin formation waters. *Appl. Geochem.*, 8, 403-407.

Kawasaki, T. (1985) Present condition and countermeasure with groundwater pollution. *Jpn. J. Water Pollut. Res.*, 8, 265-268.

Kemp, A. L. W. and Thode, H. G. (1968). The mechanism of the bacterial reduction of sulfate and of sulfite from isotopic fractionation studies. *Geochim. Cosmochim. Acta*, 32, 71-91.

Keppler, F., Elden, R., Niedan, V., Pracht, J. and Schöler, H. F. (2000) Halocarbon produced by natural oxidation processes during degradation of organic matter. *Nature*, 403, 298-301.

Komor, S. C. (1997) Boron contents and isotopic compositions of hog manure, selected fertilizers, and water in Minnesota. *J. Environ. Qual.*, 26, 1212-1222.

Lee, T., Yoshimi, M., Ike, M., and Fujita, M. (1997) Characterization of an anaerobic soil enrichment capable of dechlorinating high concentrations of tetrachloroethylene . *Water Sci. Technol.*, 36, 117-124.

Liggett, L. M. (1954) Determination of organic halogen with sodium biphenyl reagent. *Anal. Chem.*, 26, 748-750.

Liu, W. G., Xiao, Y. K., Wang, Q. Z., Qi, H. P., Wang, Y. H., Zhou, Y. M., and Shirodkar, P. V. (1997) Chlorine isotopic geochemistry of salt lakes in the Qaidam Basin, China. *Chem. Geol. (Isotope Geosci. Sect.)*, 136, 271-279.

Long, A., Eastoe, C. J., Kaufmann, R. S., Martin, J. G., Wirt, L., and Finley, J. B. (1993) High-precision measurement of chlorine stable isotope ratios. *Geochim. Cosmochim. Acta*, 57, 2907-2912.

Lyons, W. B., Welch, K. A. and Sharma, P. (1998) Chlorine-36 in the waters of the McMurdo Dry Valley lakes, Southern Victoria Land, Antarctica: Revisited. *Geochim. Cosmochim. Acta*, 62, 185-191.

Lyons, W. B., Frape, S. K. and Welch, K. A. (1999) History of McMurdo Dry Valley lakes, Antarctica, from stable chlorine isotope data. *Geology*, 27, 527-530.

Madorsky, S. L., and Straus, S. (1947) Concentration of isotopes of chlorine by the counter-current electromigration method. *J. Res. Natl. Bur. Stand.*, 38, 185-189.

Magenheim, A. J., Spivack, A. J., Volpe, C., and Ransom, B. (1994) Precise determination of stable chlorine isotopic ratios in low-concentration natural samples. *Geochim. Cosmochim. Acta*, 58, 3117-3121.

Magenheim, A. J., Spivack, A. J., Michael, P. J., and Gieskes, J. M. (1995) Chlorine stable isotope composition of the oceanic crust: Implications for earth's distribution of chlorine. *Earth Planet. Sci. Lett.*, 131, 427-432.

Mangnuson, J. K., Romine, M. F., Burris, D. R. and Kingsley, M. T. (2000) Trichloroethene reductive dehalogenase from *Dehalococoides ethenogenes*: Sequence of *tceA* and substrate range characterization. *Appl. Environ. Microbiol.*, 66, 5141-5147.

Mariotti, A., Landreau, A., and Simon, B. (1988) ^{15}N isotopic biogeochemistry and natural denitrification process in groundwater: Application to the chalk aquifer of northern France. *Geochim. Cosmochim. Acta*, 52, 1869-1878.

Markl, G., Musashi, M., and Bucher, K. (1997) Chlorine stable isotope composition of granulites from Loften, Norway: Implications for the Cl isotopic composition and for the source of Cl enrichment in the lower crust. *Earth Planet. Sci. Lett.*, 150, 95-102.

Maxwell, J. A. (1968) *Rock and mineral analysis*, Intersience Publishers, New York.

Maymó-Gatell, X., Chien, Y. T., Gossett, J. M. and Zinder, S. H. (1997) Isolation of bacterium that reductively dechlorinates tetrachloroethene to ethene. *Science*, 276, 1568-1571.

Miller, E., Wohlfarth, G. and Diekert, G. (1998) Purification and chracterization of the tetrachloroethene reductive dehalogenase of strain PCE-S. *Arch. Microbiol.*, 169, 497-502.

Musashi, M., Marl, G., and Kreulen, R. (1998) Stable chlorine-isotope analysis of rock samples: New aspects of chlorine extraction. *Anal. Chim. Acta*, 362, 261-269.

Nakai, N., Tsuji, Y., and Takeuchi, U. (1991) Stable isotopes- Natural and anthropogenic sulfur in the environment. *In* Krouse, H. R. and Grinenko, V. G. (eds.) *SCOPE*, 43, pp. 344-361, John Wiley & Sons, New York.

Neumann, A., Wolfarth, G. and Diekert, G. (1996) Purification and characterization of tetrachloroethene reductive dehalogenase from

Dehalospirillum multivorans. J. Biol. Chem., 271, 16515-16519.

Ninomiya, K. and M. Sakai (1993) Estimation on behavior of chlorinated hydrocarbons in groundwater using mathematical model. *J. Jpn. Soc. Water Environ.*, 16, 742-746 (in Japanese, with English abstract).

Nishizumi, K., Elmore, D. and Kubik, P. W. (1989) Update on terrestrial ages of Antarctic meteorites. *Earth Planet. Sci. Lett.*, 93, 299-313.

Noble, S. R., Lightfoot, P. C. and Schärer, U. (1989) A new method for single-filament isotopic analysis of Nd using in situ reduction. *Chem. Geol. (Isotope Geosci. Sect.)*, 79, 15-19.

Numata, M., Matsuura, K. and Yuki, Y. (1999a) Continuous degradation of tetrachloroethene by a two-stage anaerobic/aerobic bioreactor system. *J. Jpn. Soc. Water Environ.*, 22, 485-490 (in Japanese with English abstract).

Numata, M., Matsuura, K. and Yuki, Y. (1999b) Degradation of tetrachloroethene and trichloroethene by immobilized anaerobic and aerobic microorganisms. *J. Jpn. Soc. Water Environ.*, 22, 479-484 (in Japanese with English abstract).

Numata, M., Nakamura, N. and Gamo, T. Precise measurement of chlorine stable isotopic ratios by thermal ionization mass spectrometry. *Geochem. J.*, (In Press).

Ott, E. (1942) *Ber.*, 75, 1517

Ott, U. (2000) Salty old rocks. *Science*, 288, 1761-1762.

Pecherer, B., Gambrill, C. M. and Wilcox, G. W. (1950) Determination of bromine and chlorine in gasoline. *Anal. Chem.*, 22, 311-315.

Philipott, P., Agrinier, P. and Scambelluri, M. (1998) Chlorine cycling during subduction of altered oceanic crust. *Earth Planet. Sci. Lett.*, 161, 33-44.

Phillips, F. M. and Bentley, H. W. (1987) Isotopic fractionation during ion filtration: I. Theory. *Geochim. Cosmochim. Acta*, 51, 683-695.

Phillips, F. M. (1995) The use of isotopes and environmental tracers in subsurface hydrology. *Rev. Geophys.,* (Supplement), 1029-1033.

Potts, P. J. (1987) *A handbook of silicate rock analysis,* Blackie Academic and Professional, London.

Ransom, B., Spivack, A. J., and Kastner, M. (1995) Stable Cl isotopes in subduction-zone pore waters: Implications for fluid-rock reactions and the cycling of chlorine. *Geology,* 23, 715-718.

Reddy, C. M., Heraty, L. J., Holt, B. D., Sturchio, N. C., Eglinton, T. I., Drenzek, N. J., Xu, L., Lake, J. L. and Maraya, K. A. (2000) Stable isotopic compositions of Aroclors and Arochlor-contaminated sediments. *Environ. Sci. Technol.,* 34, 2866-2870.

Reed Jr., G. W. (1971) Chlorine, In Muson, B. (ed.) *Handbook of elemental abundances in meteorites.* pp. 143-148. Gordon and Bereach Science Publishers, New York.

Rieder, R., Economou, T., Wanke, H., Turkevich, A., Crisp, J., Bruckner, J., Dreibus, G. and McSween Jr., H. Y. (1997) The chemical composition of martian soil and rocks returned by the mobile alpha proton X-ray spectrometer: Preliminary results from the X-ray mode. *Science,* 278, 1771-1774.

Robert, R. C., Miller, B. R. and Weiss, R. F. (2000) Natural methyl bromide and methyl chloride emissions from coastal salt marshes. *Nature,* 403, 292-295.

Robinson, J. W. (1959) Rapid determination of chloride in silica or other solids. *Anal. Chim. Acta,* 20, 2014-2026.

Rosenbaum, J. M., Cliff, R. A. and Coleman, M. L. (2000). Chlorine stable isotopes: A comparison of dual inlet and thermal ionization mass spectrometric measurements. *Anal. Chem.,* 72, 2261-2264.

Sawyer, D. J., McGehee, M. D., Canepa, J. and Moore, C. B. (2000) Water soluble ions in the Nakhla martian meteorite. *Meteorit. Planet. Sci.*, 35, 743-747.

Schilling, J. -G., Unni, C. K. and Bender, M. L. (1978) Origin of chlorine and bromine in the oceans. *Nature*, 273, 631-636.

Schumacher, W. and Holiger, C., Zehnder, A. J. B. and Hagen, W. R. (1997) Redox chemistry of cobalamin and iron-sulfur cofactor in the tetrachloroethene reductase of *Dehalobacter restricus*. *FEBS Lett.*, 409, 421-425.

Seyfried Jr., W. E., Berndt, M. E. and Janecky, D. R. (1986) Chloride depletions and enrichments in seafloor hydrothermal fluids: Constraints from experimental basalt alteration studies. *Geochim. Cosmochim. Acta*, 50, 469-475.

Sherwood Lollar, B., Slater, G.F., Ahad, J., Sleep, B., Spivack, J., Brennan,M. and MacKenzie, P. (1999) Contrasting carbon isotope fractionation during biodegradation of trichloroethylene and toluene: Implications for intrinsic bioremediation. *Org. Geochem.,* 30, 813-820.

Shields, W. R., Murphy, T. J., Garner, E. L., and Dibeler, V. H. (1962) Absolute isotopic abundance ratio and the atomic weight of chlorine. *J. Am. Chem. Soc.*, 84, 1519-1522.

Shinonaga, T., Ebihara, M., Nakahara, H., Tomura, K. and Heumann. K. G. (1994a) Cl, Br and I in igneous standard rocks. *Chem. Geol.*, 115, 213-225.

Shinonaga, T., Endo, K., Ebihara, M., Heumann, K. G. and Nakahara, H. (1994b) Weathering of Antarctic meteorites investigated from contents of Fe^{3+}, chlorine, and iodine. *Geochim. Cosmochim. Acta*; 58, 3735-3740.

Slater, G. F., Dempster, H. S., Lollar, B. S., and Ahad, J. (1999) Headspace analysis: A new application for isotopic characterization of dissolved organic contaminants. *Environ. Sci. Technol.*, 33, 190-194.

Sturchio, N. C., Clausen, J. L., Heraty, L. J., Huang, L., Holt, B. D., and

Abrajano, Jr., T. A. (1998) Chlorine isotope investigation of natural attenuation of trichloroethene in an aerobic aquifer. *Environ. Sci. Technol.*, 32, 3037-3042.

Strurges, W. T., and Barrie, L. A. (1989) The use of stable lead 206/207 isotope ratio and elemental composition to discriminate the origins of lead in aerosols at a rural site in eastern Canada. *Atmos. Environ.*, 23, 1645-1657.

Sturchio, N. C., Clausen, J. L., Heraty, L. J., Huang, L., Holt, B. D., and Abrajano, Jr., T. A. (1998) Chlorine isotope investigation of natural attenuation of trichloroethene in an aerobic aquifer. *Environ. Sci. Technol.*, 32, 3037-3042.

Suchomel, K. H., Kleamer, D. K. and Lomg, A. (1990) Production and transport of carbon dioxide in an contaminated vadose zone: A stable and radioactive carbon isotope study. *Environ. Sci. Technol.*, 24, 1824-1831.

Sugimoto, A. and Wada, E. (1993) Carbon isotopic composition of bacterial methane in a soil incubation experiment: Contribution of acetate and CO_2/H_2. *Geochim. Cosmochim. Acta*, 57, 4015-4027.

Tanaka, N., and Rye, D. M. (1991) Chlorine in the stratosphere. *Nature*, 353, 707.

Taylor, J. W., and Grimsrud, E. P. (1969) Chlorine isotopic ratios by negative ion mass spectrometry. *Anal. Chem.*, 41, 805-810.

Tomeoka, K. (1990) Phyllosilicate veins in a CI meteorite: evidence for aqueous alteration on the parent body. *Nature*, 345, 138-140.

Turnquist, C. R., Taylor, J. W., Grimsrud, E. P., and Williams, R. C. (1973) Temperature dependence of chlorine kinetic isotope effects for aliphatic chlorides. *J. Am. Chem. Soc.*, 95, 4133-4138.

van Warmerdam, E. M., Frape, S. K., Aravena, R. Drimmie, R. J., Flatt, H., and Cherry, J. A. (1995) Stable chlorine and carbon isotope measurements of selected chlorinated organic solvents. *Appl. Geochem.*, 10, 547-552.

Vengosh, A. Chivas, A. R., and McCulloch, M. T. (1989) Direct determination of boron and chlorine isotopic compositions in geological materials by negative thermal-ionization mass spectrometry. *Chem. Geol.*, 79, 333-343.

Venkateswarlu, P. (1982) Sodium biphenyl method for determination of covalently bound fluorine in organic compounds and biological materials. *Anal. Chem.*, 54, 1132-1137.

Vogel, T. M., Criddle, C. S. and McCarty, P. L. (1987) Transformation of halogenated aliphatic compounds. *Environ. Sci. Technol.*, 21, 722-736.

Volpe, C. and Spivack, A. J. (1994) Stable chlorine isotopic composition of marine aerosol particles in the western Atlantic Ocean. *Geophys. Res. Lett.*, 21, 1161-1164.

Volpe, C., Wahlen, M., Pszenny, A. A. P., and Spivack, A. J. (1998) Chlorine isotopic composition of marine aerosols: Implications for the release of reactive chlorine and HCl cycling rates. *Geophys. Res. Lett.*, 25, 3831-3834.

von Gunten, H. R., Wyttenbach, A. and Scherle, W. (1965) Determination of chlorine in stony meteorites by neutron activation. *Geochim. Cosmochim. Acta*, 29, 475-480.

Wastaway, K. C., Koemer, T., Fang, Y. R., Rudzinski, J., and Paneth, P. (1998) A new method of determining chlorine kinetic isotope effect. *Anal. Chem.*, 70, 3548-3552.

Whitby, J., Burgess, R., Turner, G., Glimour, J. and Bridges, J. (2000) Extinct ^{129}I in halite from a primitive meteorite: Evidence for evaporite formation in the early solar system. *Science*, 288, 1819-1821.

Willey, J. F. and Taylor, J. W. (1978) Capacitive integration to produce high precision isotopic measurement on methyl chloride and bromide. *Anal. Chem.*, 50, 1930-1933.

Wilson, S. A. and Gent, C. A. (1983) Determination of chlorine in geological samples by ion chromatography. *Anal. Chim. Acta*, 148, 299-303.

Wyttenbach, A., von Gunten, H. R. and Scherle, W. (1965) Determination of bromine content and isotopic composition of bromine in stony meteorites by neutron activation. *Geochim. Cosmochim. Acta*, 29, 467-474.

Xiao, Y. K., Beary, E. S. and Fassett, J. D. (1988) High precision isotopic measurement of boron by thermal ionization mass spectrometry of the Cs_2Cl^+ ion. *Int. J. Mass Spectrom. Ion Proc.*, 85, 203.

Xiao, Y. K., and Zhang, C. G. (1992) High precision isotopic measurement of chlorine by thermal ionization mass spectrometry of the Cs_2Cl^+ ion. *Int. J. Mass Spectrom. Ion Proc.*, 116, 183-192.

Xiao, Y. K., Liu, W. G., Qi, H. P. and Zhang, C. G. (1993) A new method for the high precision isotopic measurement of bromine by thermal ionization mass spectrometry. *Int. J. Mass Spectrom. Ion Proc.*, 123, 117-123.

Xiao, Y. K., Zhou, Y. M., and Liu, W. G. (1995) Precise measurement of chlorine isotopes based on Cs_2Cl^+ by thermal ionization mass spectrometry. *Anal. Lett.*, 28, 1295-1304.

Xiao, Y. K., Wei, H. Z., Zhou, Y. M., Wang, Y. H. and Liu, W. G. (2000) Investigation of characteristics of non-reductive thermal ion emission of various graphites in thermal ionization mass spectrometry. *Anal Chim Acta*, 420, 95-101.

Yaguchi, K., Watanabe, S., Hirata, I., Itoh, T. and Hamada, A. (1991) Degradation of tetrachloroethylene by an anaerobic bacterium from soil. *Suishituodakukenkyu*, 14, 479-486 (in Japanese with English abstract).

Yaguchi, K., Suzuki, T., Hirata, I., Itoh, T. and Hamada, A. (1994) Biological and chemical characterization of the tetrachloroethylene-degrading bacterium, strain T. *Jpn. J. Toxicol. Environ. Health*, 40, 178-1184 (in Japanese with English abstract).

Yokouchi, Y., Nojiri, Y., Barrie, L. A., Toom-Sauntry, D., Machida, T., Inuzuka, Y., Akimoto, H., Li, H. –J., Fujinuma, Y. and Aoki, S. (2000) A strong source of methyl chloride to the atmosphere from tropical coastal land. *Nature*, 403, 295-298.

Yoshida, N. and Toyoda, S. (2000) Constraining the atmospheric N_2O budget from intramolecular site preference in N_2O isotopomers. *Nature*, 405, 330-334.

Zolensky, M. E., Bodnar, R. J., Gibson Jr., E. K., Nyquist, L. E., Reese, Y, Shih, C-Y. and Wiesmann, H. (1999) Asteroidal water within fluid inclusion-bearing halite in an H5 chondrite, Monahans (1998). *Science*, 285, 1377-1379.