

Poster Presentation

Development of a highly sensitive and selective microplate chemiluminescence enzyme immunoassay for the determination of free thyroxine in human serum

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Thyroxine (3,5,3',5'-tetraiodothyronine, T4) is an important hormone secreted by the thyroid gland. More than 99.9% of T4 in blood is bound to carrier proteins, specially to thyroxine-binding globulin (TBG), and the free fraction of T4 (free T4, FT4) just accounts for $\sim 0.03\%$ ^[1]. But the FT4 represents the biologically active form of this compound. It is believed that FT4 could cross cell membranes and interact with receptors^[2], showing metabolically active and clinically relevant effect. Therefore, measurement of FT4 is of value in assessing thyroid function.

A microplate chemiluminescence enzyme immunoassay (CLEIA) with high sensitivity, selectivity and reproducibility was developed for the determination of FT4 in human serum. A competitive assay has been utilized with horseradish peroxidase (HRP) labeled thyroxine analog in the chemiluminescence (CL) detection. The CL signal produced by the emission of photons from luminol was directly proportional to the amount of analyte. The linear range was 0.45-7.5 ng dL⁻¹ and the detection limit was 0.09 ng dL⁻¹. Experimental conditions, such as temperature, pH, incubation time, titration level and other relevant variables upon the CL signal have been examined and optimized. A coefficient of variance of less than 16% was obtained for intra- and inter-assay precision. The present method has been successfully applied to the analysis of FT4 in human serum. The positive and negative coincidence ratios are satisfactory. Good correlations were obtained between the results by the proposed method and radioimmunoassay (RIA)^[3], as well as a Bayer ACS-180SE detection system.

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**Determination of phthalates in fruit jellies by dispersive solid-phase
extraction coupled with high performance liquid
chromatography-mass spectrometry**

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Phthalates (PAEs) are widely used as additives in the manufacture of plastics to increase the flexibility. PAEs are not chemically bound to plastics and can therefore be easily released into the environment. Consequently, these compounds are ubiquitous in environmental matrices such as water and soil. PAEs present in packaging materials may also be released into foods and beverages. Several animal studies have suggested that certain phthalates and their metabolites are potentially toxic to human health due to their endocrine-disruptive effects; therefore, presently PAEs have become a major public health concern. Fruit jellies are also packaged in plastic and during jelly production, contact between the food and plastic material may occur at various stages; therefore, fruit jellies may also be contaminated by phthalates. As fruit jellies are very popular in children, it's essential to develop phthalates analysis method in fruit jellies, and this is what the paper concerned.

In this study, a simple, rapid and sensitive method for the determination of 5 kinds of phthalates including dimethyl phthalate (DMP), diethyl phthalate (DEP), dipropyl phthalate (DPP), benzyl butyl phthalate (BBP) and dicyclohexyl phthalate (DcHP) in fruit jellies by liquid chromatography coupled with mass spectrometry has been developed. Samples were pretreated by a dispersive solid-phase extraction method, termed QuEChERS. The standard calibration curves were linear for all the analytes over the concentration range 10-250 ng ml⁻¹, and the correlation coefficients ranged from 0.9976 to 0.9991. The limits of detection and limits of quantification were in the ranges of 0.09-3.68 ng ml⁻¹ and 0.28-11.25 ng ml⁻¹, respectively. The accuracy of this method was evaluated by measuring the recovery from spiked samples. The recoveries of all five phthalates from samples spiked at three different concentrations (0.01 mg kg⁻¹, 0.1 mg kg⁻¹, and 1 mg kg⁻¹), were in the ranges of 103%-107%, 95%-102%, and 102%-107%, respectively. The relative standard deviation values (RSDs) for the samples spiked at 0.01mg kg⁻¹, 0.1 mg kg⁻¹ and 1.0 mg kg⁻¹ ranged from 2.0%-7.6%, 0.3%-2.2%, and 0.5%-1.2%, respectively. The method has been used for the analysis of real samples and the results are well.

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Yuwei Ma graduated from Dept. of Chemistry, Beijing Normal University, Beijing, China in 2007. In september of the same year, she was matriculated in Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China. Now she is in the second year of the postgraduate course. She majors in Environmental Analytical Chemistry and Prof. Jin-Ming Lin is her supervisor. In the past one year, she has worked at the determination of phthalates and estrogens in the environment.

Sensitive Protein Detection Based Magnetic Tunnel Junction Sensors

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Magnetoresistive sensors are robust, highly sensitive, have a high potential for integration, and can be fabricated in cost-effective silicon processes. In the last few years, biochips based on magnetoresistive sensors have been used for biomolecular recognition assays. In these assays biological probes are immobilized on the chip surface and complementary target biomolecules can be detected using a magnetic label. The detection of magnetic fringing fields of the nanoparticle labels is accomplished by a magnetoresistive sensor. The use of superparamagnetic particles as labels is considered a solution to the background-signal problems suffered by optical labeling methods employed in vitro disease marker identification. Because of its sensitivity, its reliability and its easiness of fabrication, GMR and spin valve sensors are the most commonly used sensor. In order to increase the sensitivity of this technique, an emerging interest in using magnetic tunnel junctions (MTJ) as magnetoresistive sensors is arising due to its higher magnetoresistance and its potential increase in sensitivity.

The highly sensitive MTJ sensors which are an active electromagnetic chip with integrated CMOS and magnetic device have been studied for highly sensitive immunosensing over a wide range of target concentrations. The presence or absence of a single superparamagnetic bead was successfully detected by direct measurement of fringe fields of beads using MTJ sensors. The real-time detection was also successfully confirmed by the direct measurement of magnetic fields generated from the superparamagnetic beads passing an active sensing area of a MTJ sensors integrated with a microfluidic channel. Furthermore, we proposed the optimization of the magnetoimmuno assay for the highly sensitive and specific detection of carcinoembryonic antigen (CEA), a diagnostic marker for tumour. Finally, a dose-response curve was established that shows that our MTJ platform can specifically detect CEA down to 10 pg/mL. The MTJ sensors can be mass-produced at low cost and can detect superparamagnetic beads bound to the surface through specific antibody-antigen interactions, making it a potential platform for new simplified and rapid point-of-care diagnostic tests.

**Determination of Inorganic Anions in Ethyl Acetate
by In-line Hollow Fiber Membrane Extraction with Ion Chromatography**

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Ion chromatography is a common method to analyze inorganic anions. In the recent years, with the rapid advances in ion chromatography, it is no longer a difficult problem to determine inorganic anions even in some water-miscible solvents such as methanol, iso-propanol, acetone and N-methylpyrrolidone. However, it is still a conundrum to analyze inorganic anions in organic solvents which are slightly soluble in water.

In this work, a novel hollow fiber membrane extractor was set up to extract inorganic anions from ethyl acetate using deionized water. Inorganic anions in slightly soluble organic solvents can be determined by the in-line hollow fiber membrane extractor coupled with ion chromatography at first time. The analysis of inorganic anions in ethyl acetate consisted of three steps: (1) using the in-line hollow fiber membrane extractor to extract anions with deionized water from ethyl acetate meanwhile eliminate the matrix; (2) concentrating the target analytes using a concentration column; (3) eluting out the concentrated anions for analysis by ion chromatography.

Different aspects of the extraction procedure such as magnetic stirring speed, extraction flow rate and extraction time were optimized to achieve high extraction efficiency and good separation results. Satisfactory linear range (0.01-5 mg/L), limits of detection (0.001-0.005 mg/L) and good repeatability (0.35-7.63 %) were obtained. The procedure was applied to analyze inorganic anions in two commercial ethyl acetate samples.

Estimation of Emission and Analysis of PCDD/DFs, PCBs and Emerging POPs from Waste Incineration Facilities.

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Summary

In Korea, the levels of PCDD/DFs from the exhaust gas produced from residential, industrial waste incineration facilities are strictly controlled. However, the control for the Persistent Organic Pollutants other than PCDD/DFs, such as PCBs, PBDEs, and PCNs do not exist. Furthermore, the conditions regard to PCBs, PBDEs and PCN exhaustion and their influence to the environment and human health are not commonly discussed. In this study, the analytical methods for the Persistent Organic Pollutants released from the waste incineration facilities were discussed. Using the analytical methods suggested, the PCDD/DFs, PCBs, PBDEs and PCNs were analyzed from 30 selected incineration facilities. The size of incineration was determined based on the capacity of waste incinerated in an hour. According to the result, the TEQ-concentration of PCDD/DFs was 0.33ng/m³ for large facility, 1.12 ng/m³ for medium facility, and 1.54 ng.m³ for the small facility. The result also showed that the incineration facilities with stricter emission controls have lower emission concentrations. The TEQ-concentration of DL-PCBs had the similar pattern to PCDD/DFs. The TEQ-concentration of DL-PCBs was 0.06 ng.m³ for the small facility, 0.17 ng.m³ for the medium facility, and the 15.21 ng/m³ for the small facility. In case of PCBs, large incineration facilities produced lower PCB concentration.

Key word: PCDDs/DFs, DL-PCBs, POPs, PBDEs, PCNs, Incineration facilities, waste combustion, emission

1. Introduction

Dioxins and dioxin like compounds that are unintentionally produced during the incineration process are toxic to human health. Such chemicals are toxic, persistent, capable of long-range transfer and bio-accumulative. The common examples of these chemicals are pesticides, dioxins and Brominated Flame Retardants. POPs analyzed in this study, such as PCDD/DFs and DL-PCBs are carcinogenic and known to cause problems in metabolism, reproductive systems, endocrine systems and the genetic mutation. Internationally, the counter plans for the elimination of POPs are being requested by UNEP and other organizations. The sources of these POPs are usually industries and the waste incineration facilities, where the massive amounts of chemical solvents are used. Especially the waste incineration facilities are known to be the important source of PCDD/DFs, PCBs, PCNs and PBDEs. In this study, PCDDs, PCDFs, DL-PCBs and other new POPs, such as PBDEs and PCNs were monitored, in order to estimate the amount of emission, and to evaluate the overall danger of POPs. Furthermore, the result of this study will provide the nation wide data for the estimation of POPs emissions from the waste incineration facilities.

2. Materials and Methods

2.1 Sample grouping

Each sample was collected based on air pollution process control method. More than 3m³ of samples were collected for more than 4 hours. The collected samples were categorized based on the amount of waste incinerated in an hour. The large waste incineration treated 3ton/hr, medium incineration treated 0.25 to 3 ton/hr, and the small incinerator treated 0.05 to 0.25 ton/hr. The incineration facilities handle industrial waste, directed waste and medical waste. However, the majority of wastes treated are the industrial waste. The large incineration facilities are equipped with exhausted gas absorption systems and the filtration systems, while the small incineration facilities are furnished with cyclone dust collection system.

2.2 Sample pretreatment

Among the collected exhausted gas sample, the solid samples on the absorbent, such as XAD were extracted by Soxhlet extraction using 300ml of Toluene for 16 hours. The extractions of liquid phased samples were processed by liquid-liquid extraction for twice, using 100ml of Toluene. The solvents of the extracted samples were transferred to n-hexane for the clean up. For the analysis of DL-PCBs, PCNs and PBDEs, multilayer silica gel column (multi silica-gel, 70-230 mesh, neutral, Merck) was used during the clean up process. For the analysis of PCDD/DFs, both multilayer silica gel column and the alumina column (activity I, 70-230 mesh, Merck) were used. The instrumental analyses of the samples were completed by High Resolution Gas Chromatography and Mass Spectrometer (HRGC/HRMS; Agilent GC/ JEOL-800D).

3. Result and Discussion

The recovery rate obtained from the PCDD/DFs, Co-PCBs, PBDEs and PCNs analytical methods in this study was over 85% for all the compounds. The total average dioxin concentration in large incineration facility was 14.6 ng/m³, 44.3 ng/m³ in medium, and 107.2ng/m³ in small incineration facilities. The large incineration facilities that have strict control processes produced low concentration of dioxins, while the smaller facilities produced higher concentrations of dioxins. The average concentration of total PCBs in large, medium and small facilities were 191.3 ng/m³, 135.3 ng/m³ and 211.7 ng/m³, respectively.

According to the result, the size of the incineration facility did not cause great impact on the concentration of total PCB produced. However, since the large incineration facilities produce more emission, and have longer operation hours compare to the small and medium sized incineration facilities, the large incineration facilities can make high contribution to the total nation wide POPs emissions.

Furthermore, in all the facilities, PBDEs and PCNs were also monitored from the emission. From the result, the need for the process control on Co-PCBs and other dioxin like compound productions were observed. It could be also concluded that the control of POPs and emerging POPs sources, and the plans for the POPs eliminations are strongly required in waste incineration facilities.

Application of Polydimethylsiloxane Mini-Disk Extraction to the Analysis of Floral Aroma by Gas Chromatography and Mass Spectrometry

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This paper reports application of a novel sampling method called headspace polydimethyl-siloxane (PDMS) mini-disk extraction (HS-PDE) combined with GC/MS for the analysis of floral aroma from *Prunus mume*, *Lantana camara*, and *Albizia julibrissin*. The technique uses a prototype PDMS mini-disk (diameter: 8 mm, thickness: 0.157 mm, weight: 9.4 mg) as a sorption device and very small volume (0.03 mL) of acetonitrile as an organic solvent for extraction. The relative extraction efficiencies of proposed method have been compared with other sampling techniques based on headspace solid phase micro-extraction (HS-SPME) and mulberry paper bag micro-solid phase extraction (MPB- μ -SPE). This new HS-PDE method is very simple to use, inexpensive, rapid, requires small sample amounts and solvent consumption.

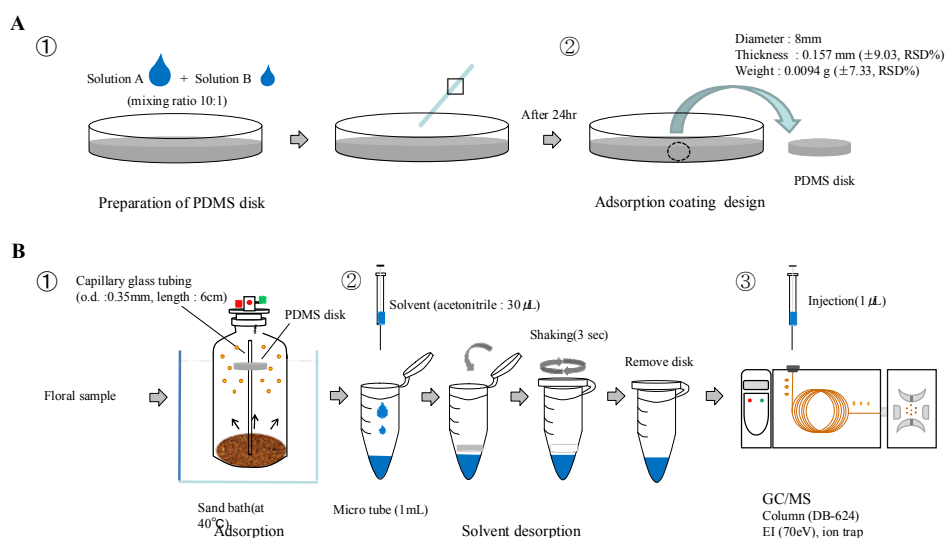


Fig. Schematic illustration showing how to mold a PDMS disk and headspace PDMS disk extraction. A, molding method of PDMS disk. B, Procedure of HS-PDE and GC-MS.

Development of New Ultra Violet Photo-Reactor and Its Application to the Determination of Total Nitrogen and Total Phosphorus

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Summary

A new ultra violet photo-reactor was developed to enhance the simplicity, rapidity, safety and low-cost as a pre-treatment device for the oxidative decomposition method, and was applied to the determination of total nitrogen and total phosphorus in river water and waste water samples coupled with a simplified measurement method. The analytical results for practical samples obtained by the proposed reactor showed a good correlation with those obtained by JIS method.

Key word: Ultra violet photo-reactor, low pressure mercury lamp, total nitrogen and total phosphorus determination, simplified measurement method

1 Introduction

Kyoritsu Chemical-check Lab., Corp. developed a simplified measurement kits of Pack Test[®], which can be used to check the water quality. Pack Test[®] has been used widely in the practical analysis, because it can be handled easily and quickly, moreover, it is well known to be cost-effective. However, very little pre-treatment device for a simplified measurement method is available such as distillation, separation, concentration, decomposition and so on. Therefore, it is desired to develop a new type of pre-treatment device coupled with simplified measurement method. In this work, an ultra violet photo-reactor was developed for the oxidative decomposition of organic compounds instead of an autoclave and heating devices, and it was applied to the determination of total nitrogen and total phosphorus in the practical water samples by using Pack Test[®] after the decomposition.

2 Experimental

The ultra violet photo-reactor consisted of a low pressure mercury lamp (14 mm ϕ , 13.4 cm length, 4W), two pieces of PTFE tubing (2 mm i.d., 3 mm o.d., 105 cm length) and two sample injection ports. Two pieces of reaction tubing were wound directly around the mercury lamp. Potassium peroxodisulfate was used as a decomposition reagent. TiO₂ was added the sample solution as a photo-catalyst. Two kinds of Pack Test[®] were used to detect for nitrate and phosphate, which were based on the Zn reduction - diazotization/coupling and molybdenum blue spectrophotometric methods. The sample solution of 2 mL was mixed with decomposition reagent and TiO₂ solution before irradiation, and then the mixed solution of 1.5 mL was injected into the reaction tubing by a disposable syringe. The decomposition reaction occurred in the PTFE tubing during irradiation of ultra violet for 20 min. The resulting nitrate and phosphate in the treated sample solutions were analyzed.

3 Result and Discussion

Generally, a quartz coil has been used for the ultra violet irradiation, however, it was reported PTFE tubing could be also used as a reaction coil in the photo chemical reaction. Therefore, PTFE tubing was selected in this work, because of being easy to handle and safely. It was found that TiO₂ could be effective to work as a photo catalyst in this photo chemical reaction using the mercury lamp (254 nm) as well as black light (365 nm). Various practical samples of river and waste water were analyzed by both proposed photo-reactor / Pack Test[®] method and autoclave / spectrophotometric method (JIS method). There were good correlations between both methods with correlation coefficient of 0.980 (n=70) for total phosphorus

Determination of Total Arsenic in Environmental Samples with Decomposition by UV-Irradiation using Hydride Generation Atomic Absorption Spectrometry

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Summary

It is well known that the sensitivity of arsenic measurement by hydride generation atomic absorption spectrometry (HG-AAS) depends on its chemical form. The measurement of the concentration of total arsenic often results in considerable errors in a sample containing various arsenic compounds. Therefore, the arsenic compounds need to be reduced to a homogeneous form. The optimum conditions for ultraviolet irradiation as a pretreatment have been investigated in this study. The main aspects examined were reagent concentrations and time of exposure to ultraviolet radiation. The sample was mixed with $K_2S_2O_8$ and NaOH, and ultraviolet light was irradiated using a UV reactor (Model WA-UVR, KYORITSU CHEMICAL-CHECK Lab., Corp.). This pretreatment method was applied to the analysis of total arsenic in a standard reference material (The Japan Society for Analytical Chemistry, JSAC0302). Analytical results were in good agreement with the certified values. The recovery of each of the five arsenic compounds tested (methylarsonic acid, dimethylarsinic acid, trimethylarsine oxide, arsenobetaine and arsenocholine) was about 100%.

Key word:

Arsenic (As), UV irradiation, Hydride generation, Atomic absorption

1 Introduction

The sensitivity of arsenic measurement by hydride generation atomic absorption spectrometry (HG-AAS) depends on its chemical form. Thus the arsenic compounds present need to be reduced to a homogeneous form using a microwave digestion system or similar procedure prior to measurement. However, the procedure for microwave digestion is difficult and time consuming. We tried to use an ultraviolet (UV) irradiation method for the decomposition of arsenic compounds. The merits of this pretreatment were its ease of use and time-saving potential. We applied this procedure to the determination of arsenic in environmental samples.

2 Materials and Methods

Five arsenic compounds (methylarsonic acid, dimethylarsinic acid, trimethylarsine oxide, arsenobetaine and arsenocholine) were investigated. Sample solutions containing $K_2S_2O_8$ and NaOH as oxidation reagents were UV irradiated for a few minutes using a UV reactor (Model WA-UVR, KYORITSU CHEMICAL-CHECK Lab., Corp.). After UV irradiation, the concentration of total arsenic was measured in the sample solutions by hydride generation atomic absorption spectrometer.

3 Result and Discussion

Organic arsenic compounds were able to be transformed into inorganic arsenic by UV irradiation for a few minutes. In this study, we decided that the optimal concentration of $K_2S_2O_8$ was 2% and that of NaOH was 1%. The total concentration of arsenic in a standard reference material (The Japan Society for Analytical Chemistry, JSAC0302) and JSAC0302, to which a known concentration of each arsenic compound was added, was measured using this method. Analytical results were in good agreement with the certified values. The recovery of each of the five arsenic compounds was about 100%.

Source apportionment of hazardous elements of SPM aerosol in Yokohama, Japan

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Summary

An extensive chemical analysis of Suspended particulate matter (SPM) mass was conducted to measure metals (Mg, Al, Ca, V, Cr, Mn, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Ag, Cd, Cs, Ba, Pb and Bi), water soluble ions (Na⁺, NH₄⁺, K⁺, Ca²⁺, Cl⁻, NO₃⁻ and SO₄²⁻) and carbonaceous mass (EC and OC). PCA-APCS technique was employed to apportion the sources. Automobile was a significant source at urban site and rapidly evolving industries in association with secondary aerosol was predominant at sub-urban site. Moreover, many hazardous elements existed into the sources may bolster the exposure of human health in current locations.

Key words: SPM, aerosol, source apportionment, anthropogenic

1 Introduction

Suspended particulate matter (SPM) is increasingly received attention and broadly accepted as a potent air pollutant in urban locations and released from wide ranges of sources. SPM triggers respiratory diseases [1, 2]. In addition, SPM causes visibility reduction [3] in urban atmosphere. Thus, it was considered as a most significant pollutant to be routinely monitored in ambient air. However, inadequate studies have been investigated in apportionment of SPM in Japan [4, 5]. Therefore, particular emphasis was given to be conducting comprehensive source analysis of hazardous elements in SPM contemplating substantial number of compositions and several locations in Yokohama, Japan.

2 Materials and Methods

SPM aerosol samples were collected at an urban (Hiranuma) and a sub-urban (Nagahama) site of Yokohama, Japan from April 1999 to May 2005. The SPM samples were subjected to analysis for metals, water soluble ions and carbonaceous mass using ICP-MS, ion chromatography and CHN analyzer, respectively. Principle Component Analysis and Absolute Principle Component Score (PCA-APCS) along with Multiple Linear Regression (MLR) were employed to resolve the contributing sources.

3 Result and Discussion

SPM concentrations were measured as 34.2 $\mu\text{g}/\text{m}^3$ and 22.9 $\mu\text{g}/\text{m}^3$ at urban and sub-urban sites, respectively. PCA-APCS were appropriately applied and identified four distinct sources as shown in Figure 1. Soil dust accounted for 3.6 and 4.6 $\mu\text{g}/\text{m}^3$ of SPM mass at urban and sub-urban sites. Road traffic contributed significantly larger at urban (7.2 $\mu\text{g}/\text{m}^3$) than at sub-urban (5.7 $\mu\text{g}/\text{m}^3$). Industrial contribution rendered 7.4 and 1.4 $\mu\text{g}/\text{m}^3$ at urban and sub-urban sites. Moreover, mineral resuspension (6.5 $\mu\text{g}/\text{m}^3$) source was predicted at urban and secondarily formed SPM combining with refuse burning

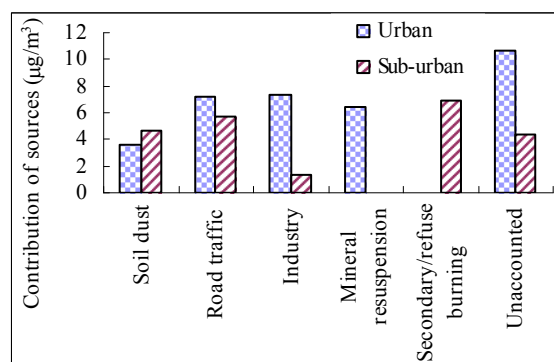


Fig. 1. Contribution of sources in SPM

(6.9 $\mu\text{g}/\text{m}^3$) was identified at sub-urban site. Unlike sub-urban site, tracers of traffic were profoundly interfering into SPM pollution at urban site. In contrast, industrial combustion in association with secondarily formed aerosol mass was greatly contributed at sub-urban site. Major tracer of mineral resuspension being widely used into cement and other construction materials was found in significant quantities at urban site.

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Examination of VOC Analysis in soil by Direct Head Space-GC/MS

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Summary

The prompt method for analyzed VOC in soil was required. We developed the Direct Head Space-GC/MS method that is very easy and quickly. Initially, soil sample was put into the vial. Next, cap was tightened and internal standard was added to the sample using micro-syringe. Lastly, the vial with sample set to the auto-sampler for GC/MS. GC/MS system carried out agitation with heating, injection sample gas from head space of vial and SIM and SCAN measurement. Linearity from 10ng to 500ng was recognized for 23 VOCs containing benzene. Relative standard division for each compound was less than 10%. These results were similar to containing test, and reflected to soil pollution.

Key word: Direct Head Space GC/MS, benzene, VOC, soil,

1 Introduction

Recently, soil pollution by harmful compounds was increased in Japan. Ministry of Environmental said numbers of soil survey was increased to 1371 in 2007 from 64 in 1997. The cases of over criteria were 732 cases. These cases contained heavy metal pollutions, organic solvent contaminations, PCB and DIOXIN contaminations. Soil Contamination Countermeasures Act had been worked from 2003.

VOC compounds are typical contents of soil pollution. For example, the land around cleaning factory had been contaminated by cleaning agent containing trichloroethylene and tetrachloroethylene. The countermeasure for soil pollution is very expensive because the survey of land circumstances, analysis of harmful compound in soil and land clean-up. Therefore, simple and quick techniques for analysis are needed for cost down and short term of survey. In this study, we developed new VOC analysis technique in soil that called Direct Head Space GC/MS.

2 Materials and Methods

2-1: Preparation of soil contaminated by VOCs.

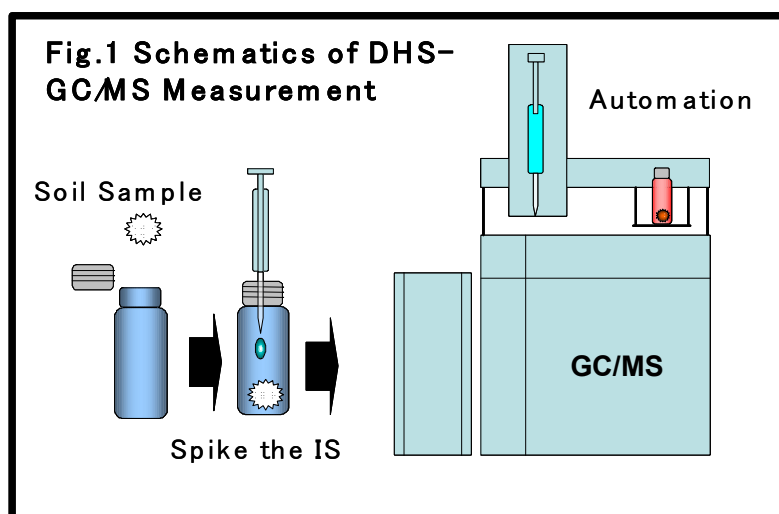
Black soil was filtrated by 5mm mesh sieve. VOC mixture was spiked to black soil and soil was mixed well. High and low contaminated soils were prepared.

2-2: Leaching test

Soil sample was put into the glass bottle. Mineral water was added into this bottle without space in the bottle. Sample in the bottle was stirred gently for four hours. Test elution sample was prepared after filtration that sample. VOC containing in this elution sample was analyzed by Head Space GC/MS system.

2-3: VOC concentration in soil

VOC was extracted from soil with methanol for 3 times. After



centrifuge, methanol was move to another bottle. Using this methanol sample, VOC was analyzed by Head Space GC/MS system.

2-4: Head Space GC/MS measurement

Liquid sample was put into the vial and added salts. After cap was tighten, added internal standard. This vial was hold at 60°C for 30 minutes. Then, gas from vial was injected to GC/MS.

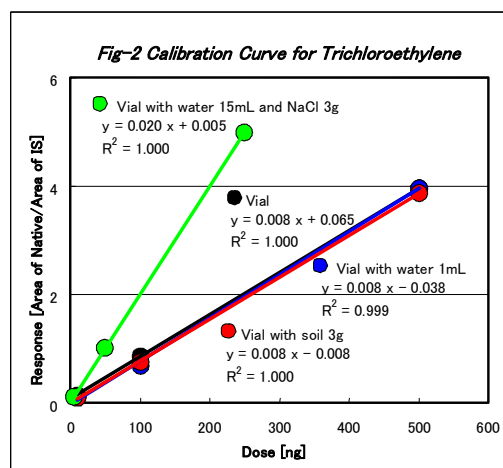
2-5: Direct Head Space GC/MS measurement

The soil sample is put into the vial. And internal standards were added in this vial. This vial was hold at 60°C for 30 minutes. Then, gas from vial was injected to GC/MS. Fig.-1 shows the head space GC/MS system.

3 Result and Discussion

3-1: Dose-Response relationship and accuracy

Initially, linearity of calibration curve and accuracy for this method were checked. Four cases of calibration curves were tried. In the case A, this is the normal method, only standard solution with IS was added after cap was tightened. In the case B, influence of water was considered in agitation. In the case of high volatile and high water solubility compounds that like methanol, it was expected that vaporization of those compounds were influence by water contents. Then, water (1mL) was added in vial initially, and cap was tightened, standard solution with IS was added in the vial at last. In the case C, it was similar condition of soil sample. Soil sample without VOCs was added in vials at first. Standard solution with IS was added in the vial after cap was tightened. In the case D, this is the same method for water sample. In the all cases and 23 VOCs, good linearity were recognized at ranged from 10ng to 500ng (Fig.-2).



The lowest standard solutions (10ng) were repeated for 5 times because of accuracy for DHS-GC/MS method was confirmed. Those results from 4 cases were compared with elution test and containing test. The standard deviations for these cases were less than 10%.

3-2: Comparison for test method

Soil sample contaminated with VOCs was measured by three Methods (Leaching test, solvent extraction and DHS-GC/MS) and results were compared. Table shows the results from three methods. The results from DHS-GC/MS were similar with solvent extraction method. As leaching test, because sample was dilution with water, results were ten times lower than other methods. Thus, DHS-GC/MS was very useful for VOC analysis in soil.

Comparison between three analytical methods for VOC in soil

	n		Bz	TCE	PCE
Leaching Test→HS-GC/MS	2	(mg/L)	0.49	0.54	0.81
Solvent Extraxtion→HD-GC/MS	5	(mg/kg)	5.1±0.23	5.8±0.28	9.7±0.95
DHS-GC/MS	5	(mg/kg)	5.6±0.70	6.6±0.88	8.2±1.9

Development of Continuous Monitoring System for Ammonium ion in Industrial Waste Brine Water by Using FIA Technique

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Summary

A Fully automated continuous monitoring system by using conventional flow injection spectrophotometry was developed, and applied to the determination of ammonium ion in industrial waste brine water. The system enables one to monitor highly concentrated ammonium ion without any manual dilution procedure. The chemistry is based on the reaction of ammonium ion with salicylate and hypochlorite to form a colored indophenol blue derivative. The monitoring of ammonium ion was continuously carried out for 14 days in the drains in iodine and natural gas manufacturing factories.

Key word: ammonium ion, brine water, continuous monitoring, flow injection spectrophotometry.

1 Introduction

The national effluent standards are uniformly applied in Japan, especially for ammonia, ammonium compounds, nitrate and nitrite compounds. When the provisional effluent standard is applied, the permissible limit is regulated to be 100 mg/L as nitrogen, which value means total of N-NH_4^+ multiplied by 0.4, N-NO_2^- and N-NO_3^- . Brine water flowing underground in Chiba area contains natural gas and iodine, which are separated from brine and made into a product. The waste brine water contains highly concentrated ammonium ion (approximately 200 mg/L N-NH_4^+). After separating gas and iodine, it is necessary to monitor the concentration of ammonium ion continuously in industrial waste brine water. In this work, a fully automated continuous monitoring system was developed by using FIA technique and applied to the determination of ammonium ion in the practical waste brine water.

2 Experimental

A peristaltic micro pump was used for propelling the reagent solutions instead of double-plunger pump used in conventional FIA. Six-way rotary valve and multi-positions valve were used for sample injection and sample changing. A detector was consisted of PTFE tubing (2 mm.i.d., 3 mm.o.d.) flow cell and LED of 660 nm. A newly designed sampling pot was used to transport a brine sample from a waste drain. A sample solution from the sampling pot is introduced into a sample loop on a six-way injection valve after passing through a suction filter (10 μm). The sampling and analytical conditions can be controlled by a microcomputer installed in a FIA system. The data processing was also carried out by the same microcomputer.

3 Result and Discussion

The chemistry was based on the reaction of ammonium ion with salicylate and hypochlorite to form a colored indophenol derivative. In this work, the FIA system and the composition of reagent solutions were changed to reduce the sensitivity to the concentration range of approximately 200 mg/L (N-NH_4^+) without any manual dilution procedure. A flow cell and all of flow lines were placed in a temperature - controlled air oven, because the temperature was changeable at sampling sites. The data obtained is printed out for each analysis, and at least 500 analyses can be stored as electric data. In addition, they can be sent to other computers by e-mail. The monitoring of ammonium ion was continuously carried out in the practical drains of iodine manufacturing factories.

Flow injection determination of hydrogen peroxide based on fluorescence quenching of chromotropic acid catalyzed with iron(II)

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Summary

Flow-injection analysis system (FIA system), which was based on Fe(II)-catalyzed oxidation of chromotropic acid with hydrogen peroxide, was developed for the determination of hydrogen peroxide. The chromotropic acid has a fluorescence measured at $\lambda_{em} = 440$ nm with $\lambda_{ex} = 235$ nm, and the fluorescence intensity at $\lambda_{em} = 440$ nm quietly decreased in the presence of Fe(II), which was caused by Fe(II)-catalyzed oxidation of chromotropic acid with hydrogen peroxide. By measuring the difference of its fluorescence intensity, hydrogen peroxide ($1.0 \times 10^{-8} - 1.0 \times 10^{-3}$ M) could be determined by the proposed FIA system, whose analytical throughput was 40 samples h^{-1} . The relative standard deviation (RSD) was 1.0% ($n = 10$) for 4.0×10^{-8} M hydrogen peroxide. The proposed FIA technique could be applied to the determination of hydrogen peroxide in rain water samples.

Key word: flow-injection analysis, H_2O_2 determination, Fe(II)-catalyzed oxidation, chromotropic acid

1 Introduction

In the wide field of biochemistry, clinical chemistry, food chemistry, and environmental chemistry, hydrogen peroxide must be simply and sensitively determined in the samples in order to analyze the behavior of hydrogen peroxide, such as oxidation processes and interaction mechanisms. Therefore, the development of the novel analytical method for hydrogen peroxide is one of the aims in the analytical science.

2 Materials and Methods

The novel FIA system was developed for the determination of hydrogen peroxide. The hydrogen peroxide was detected on the fluorescence quenching of chromotropic acid, which is based on the oxidation reaction of chromotropic acid with hydrogen peroxide using Fe(II) as a catalyst.

3 Result and Discussion

The chromotropic acid has a fluorescence, which can be measured at $\lambda_{em} = 440$ nm with $\lambda_{ex} = 235$ nm, whereas the fluorescence intensity of chromotropic acid at $\lambda_{em} = 440$ nm is quenched by its oxidation with hydrogen peroxide. The difference of the fluorescence intensity between non-oxidized chromotropic acid (base line) and oxidized chromotropic acid (signals) provided the negative peaks, which was used as a peak height for the detection of hydrogen peroxide by the proposed FIA system. It was caused by the oxidation of chromotropic acid by the hydrogen peroxide. The relative standard deviation (RSD) of ten measurements of 4.0×10^{-8} M hydrogen peroxide was 1.0%, and the limit of detection (LOD) was 5.0×10^{-9} M. The analytical throughput was 40 samples h^{-1} .

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Using a Capillary GC Backflush Device for Rapid Analysis

-Analysis of Acrylonitrile in ABS Resin -

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Summary

This study reports the results of analyzing acrylonitrile rapidly by backflushing such solvent and high-boiling-point contaminants. Shimadzu GC-2010-backflushing system equipped with FID-2010 and FTD-2010 was used as gas chromatograph. Backflushing after eluting acrylonitrile reduced the analysis time significantly; including the time for elimination unwanted substances, and analysis time was saved from about 1 hour for normal analysis using GC/FID to 12.4 minutes. For GC/FTD, backflushing N,N-dimethylformamide allowed rapid, high-sensitivity analysis of acrylonitrile, while simultaneously preventing deterioration of detector stability. Since using the backflush GC system no need to flush out contaminants for long time at high temperatures, it can also help to extend the life of columns.

Key word:

Gaschromatography, GC, Backflush, Acrylonitrile, FTD,

Introduction

Testing of materials, such as containers, packaging, cooking implements, or other such items, which has become an increasing concern in recent years, is analyzed substances with relatively low boiling points. However, extracts from the plastic materials contain large amounts of substances with high boiling points. One example is the analysis of acrylonitrile, which has attracted attention as a carcinogen and is regulated by the U.S. Food and Drug Administration (FDA) and European Community (EC). It is measured using a flame ionization detector (FID) or flame thermionic detector (FTD), but these analysis require a long time to flush out the high-boiling-point substances that come from solvents or plastics. Furthermore, Some solvents have possibility of causing reduced stability or deterioration of FTD detectors. This study reports the results of analyzing acrylonitrile rapidly by backflushing such solvent and high-boiling-point contaminants.

Materials and Methods

As gaschromatograph, Shimadzu GC-2010-backflushing system equipped with FID-2010 and FTD-2010 was used. As Data processing and control of backflushing GC system, Shimadzu GC work station GC-solution was used.

Result and Discussion

Backflushing after eluting acrylonitrile reduced the analysis time significantly; including the time for elimination unwanted substances, and analysis time was saved from about 1 hour for normal analysis using GC/FID to 12.4 minutes.

For GC/FTD, backflushing N,N-dimethylformamide allowed rapid, high-sensitivity analysis of acrylonitrile, while simultaneously preventing deterioration of detector stability.

Since using the backflush GC system no need to flush out contaminants for long time at high temperatures, it can also help extend the life of columns.

RAPID CHARACTERISATION AND INVESTIGATION OF OIL SHALES BY THERMAL DESORPTION-PYROLYSIS-GC/MS USING MULTI-FUNCTIONAL PYROLYZER

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Industry burn oil shale directly as a low-grade fuel for power generation and heating purposes and can use it as a raw material in chemical and construction-materials processing. Oil shale has been gained attention as a one of the future energy resources as the price of conventional sources of petroleum has risen and as a way for some areas to secure independence from external suppliers of energy. However, oil-shale mining and processing involve a number of environmental issues, such as land use, waste disposal, water use, waste-water management, especially greenhouse-gas emissions and air pollution. The conventional analytical methods of oil shales has been used with Fischer Assay and thermogravimetric analysis but these are not enough for the fully evaluation of complex composition of oil-shales. In order to minimize these issues, rapid, reliable and precise analytical methods have been requested for long time.

The analytical system using a vertical type of micro-furnace pyrolyzer combined with capillary column GC/MS has been demonstrated itself as one of powerful tool for rapid characterization of various artificial and natural polymers. [1, 2]. In this work, the multi-functional pyrolyzer (Frontier Laboratories; Japan) was introduced with oil shale sample for the first time to estimate its potential in investigation of the oil shale organic matter (kerogen) generally known as a natural complex biogeopolymer and ancient precursor of natural petroleum in Earth's crust. The analytical system consists of pyrolyzer with GC/MS used for testing of the two representative Estonian Kukersite and Dictyonema oil shales is described as follows.

The system enables both flash pyrolysis and stepwise programmed thermal desorption/pyrolysis using deactivated stainless steel capillary column (Ultra ALLOY column; Frontier Laboratories Ltd. UA1-30M-1.0F), and evolved gas analysis of the oil shales in widely varied temperature region as from 100 to 800 °C using simultaneous detection mode of GC/MS and pulse flame photometric detectors (PFPD). As a specific feature for the Kukersite straight chain aliphatic saturated and unsaturated hydrocarbons with carbon number up to C₁₇ as well as rare long alkyl chain homologous 5-alkylresorcinols have been displayed in rapidly generated summary chromatograms. Both flash and programmed pyrolysis express the same regularities in pyrolysate compound composition while the concentrations of similar compounds differ. Also, one can see in the chromatograms abundance of aromatic compounds as alkyl derivatives of benzene, toluene, naphthalene, anthracene and phenanthrene typical not only for Kukersite but for many other oil shales as well. Furthermore, the trace amount of variety of sulfur compounds were also identified using PFPD and mass spectrum.

The potential of such multi-functional Py-GC/MS system includes thorough investigation the compounds formed resulting from Kukersite and Dictyonema flash and programmed pyrolysis, studying the regularities of formation different compounds depending on pyrolysis temperature creating the fundamentals of advanced technologies for oil shale more effective liquefaction in obtaining liquid fuel and chemicals. With this aim the Estonian Kukersite and Dictyonema oil shales are investigated in the frames of international collaboration between Frontier Laboratories Ltd. and Laboratory of Oil Shale and Renewables Research at Tallinn Technical University (Estonia).

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Keywords: Oils Shales, Kukersite and Dictyonema, Multi-functional pyrolyzer, EGA, Heart-cutting GC/MS, simultaneous detection with MS and FPD,

Development of new pyrolysis–GC/MS system incorporated with on-line micro-ultraviolet irradiation for rapid evaluation of photo, thermal, and oxidative degradation of polymers

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Keywords: On-line micro-UV irradiator, Pyrolysis–GC/MS, Evolved gas analysis, Material degradation, Polymer deterioration

Abstract

A new pyrolysis–GC/MS system incorporating with on-line micro-ultraviolet (UV) irradiation was developed to make rapid evaluation of the synergistic material deterioration during UV irradiation (wavelength: 280 - 450 nm) under oxidative atmospheres with appropriate heating. The basic effectiveness of the system was demonstrated by using polystyrene, polypropylene and polycarbonate as the test samples. The volatile products evolved during deterioration of the polymers were analyzed on-line by thermal desorption GC/MS, and then the residual degraded polymers were analyzed by evolved gas analysis (EGA)/MS and/or Py–GC/MS to obtain specific thermograms and pyrograms. Based on these results, the deterioration mechanism of the polymeric materials during irradiation under oxidative atmosphere can be evaluated using a polymer sample on the order of sub-milligram within a relatively short period of time.

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Control of Concentration of Components and Level of Malodorants in Kitchen Scraps

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Summary

In this research, two tests were performed to investigate how temperature and oxygen concentration affect the emission of malodorants as food decays over time. The first test was performed with the focus on temperature and model of kitchen scraps kept at three temperatures: 5°C, 15°C and 25°C. The results showed that the model kept at 25°C produced the highest concentration of malodorant components; however, there was only a small difference in the concentration of malodorant components between the models kept at 5°C and 15°C. The second test was performed to examine how oxygen concentration affects malodorant emission from food scraps at five oxygen concentrations: 0%, 5%, 10%, 15% and 20%. The results showed that the model kept at 5% oxygen concentration had lower malodorant level; however, after about four days, there was no clear difference in malodorant level among all the models.

Key words: kitchen scraps, malodorant, oxygen concentration, putrefaction

1. Introduction

Malodorants are produced from kitchen scraps owing to the growth of microorganisms, which implies that preventing such growth would prevent kitchen scraps from emitting malodorants. To examine this idea, we tried to control environmental factors, such as temperature, oxygen concentration, carbon dioxide concentration and the water content of food. The research was conducted focusing on the effects of temperature and oxygen concentration on malodorant emission.

2. Materials and Methods

Experiment #1:

Models of kitchen scraps were kept at three temperatures: 5°C, 15°C and 25°C for 25 days. To measure the concentration of gases emitted from the models, GC (FPD/FID) and GC/MS were used.

Experiment #2:

The air in the container with the models of kitchen scraps was replaced with a mixture of oxygen and nitrogen with various oxygen concentrations: 0%, 5%, 10%, 15% and 20%. Oxygen concentration was measured with disposable detectors. Malodorant level was determined by sniffing and GC (TCD) was used to measure the concentrations of CO₂ and CH₄ in the container.

3. Results and Discussion

In the first experiment, the results showed that the model kept at 25°C produced the highest concentration of malodorant components; however, there was only a small difference in the concentration of malodorant components between the models kept at 5°C and 15°C. In the second experiment, the model kept at 5% oxygen concentrations had lower malodorant level; however, after about four days, there was no clear difference in malodorant level among all the models. Interestingly, the model of kitchen scraps kept at 0% oxygen concentration showed no change in its appearance after 10 days.

Thus, to prevent malodorant emission from kitchen scraps, it is favorable to keep them in a place at 15°C and for up to four days, with lower oxygen concentrations.

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Validation of a Fast Multiresidue Method for the Determination of Pesticides Residues in Food Using Mini Solid-Phase Extraction and Large Volume Injection-Gas Chromatography

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In recent years, the problem of pesticide residues in food has been raised on a number of occasions and concern about the safety of food has increased. In order to respond to the increase in the number of pesticides subject to inspection according to the adoption of the Positive List system, the simultaneous analysis of more than 300 constituents is required for GC/MS (Gas Chromatography/Mass Spectrometry). However, it is difficult to greatly increase the number of samples inspected, because a pretreatment process of each sample is time consuming using current analysis techniques. Therefore, in order to accelerate the pretreatment process, we examined fast multiresidue analysis method for the determination of pesticide residue in food, which combines reducing sample volume using a large volume GC injection method, and re-concentrating the samples by the solid phase extraction (reversed phase mode). As a result, conventional procedures, such as liquid-liquid distribution by a 1)separatory funnel, and concentration process by an evaporator etc., were able to be omitted. The pretreatment process time by one person after fractioning was 15 minutes for one sample and 60 minutes for ten samples.

Moreover, measurement by the SCAN mode is employed because of the difficulty of setting the ion set etc. in SIM (Selected Ion Monitoring) mode for MS for an increased number of pesticides to be measured. In each of the food, a 50% or higher recovery rate was obtained for more than 280 constituents of pesticides. Almost all compounds were achieved a relative standard deviation of $< 10\%$ (n=5).

Validation of a Post-column Reaction GC/FID As an Accurate Assignment Method of the Specific Values for Organic Compounds

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Summary

For the determination of accurate specific values for organic compounds, an analytical method using post-column reaction GC/FID system was developed. The system was equipped with two reactors between a chromatographic column and a detector. The separated analytes from the separation column were converted to carbon dioxide, then, to methane by two reactors, and finally detected by FID as methane. The analytical method was validated with an internal standard addition method and an absolute calibration method using the phthalate esters solution of SI-traceable certified reference material. Obtained results have shown that the analytical method using the post-column reaction GC/FID system was an accurate assignment method of the specific value for organic compounds.

Key word: Post-column reaction; GC/FID; reference material; phthalate ester.

1 Introduction

An accurate assignment method of specific values is very important. An analytical method using post-column reaction GC/FID system was developed for the precise determination of organic compounds more simply and quickly. The system was equipped with two reactors between a chromatographic column and a detector. The separated analytes from the separation column were converted to carbon dioxide, then, to methane by two reactors, and finally detected by FID as methane. For showing its effectiveness as an accurate assignment method, the analytical method was validated with an internal standard addition method and an absolute calibration method using the phthalate esters solution of SI-traceable certified reference material.

2 Materials and Methods

The certified reference material CERI-jcss-0619 (CRM), eight ester phthalates mixture standard solution, was purchased from Chemical Evaluation and Research Institute (CERI; Tokyo, Japan). *n*-Hexane was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI, USA). *n*-Eicosane was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). *n*-Eicosane was diluted with *n*-hexane, and this mixture was used as an internal standard solution.

3 Result and Discussion

The internal standard solution was added to the CRM, and this mixture was measured with the post-column reaction GC/FID system by an internal standard addition method with *n*-eicosane as an internal standard. Obtained values of phthalate esters were compared with the reference values of phthalate esters, respectively. The obtained values and the reference values were in good agreement with one another. Concentrations of an unknown phthalate esters mixture was measured with two measurements methods. One of the methods was the post-column reaction GC/FID system and the other was an absolute calibration method using the CRM. The results were in good agreement with each other. These results have shown that the analytical method using the post-column reaction GC/FID system was an accurate assignment method of the specific values for organic compounds. We will report the design concept for the validation / calibration of standard mixtures.

Determination of Trace Amount of Formaldehyde Based on the Spectrophotometric Measurement of Excess Sulfite for Formation of Formaldehyde-Sulfite Compound

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Summary

Optimal conditions of reaction of sulfite with formaldehyde and measurement of an excess sulfite for the reaction were established. A spectrophotometric measurement of an excess sulfite for formation of sulfite-formaldehyde adduct was carried out by using cobalt (II)-azide complex reagent. Formaldehyde could be determined from an consumption of sulfite. The proposed method is successfully applied to determination of formaldehyde at 10^{-5} M levels in aqueous solution.

Key word:

Formaldehyde, sulfite, cobalt (II)-azide complex, cobalt (III)-azide complex, spectrophotometry

1 Introduction

We found that sulfite give a catalytic effect on an air-oxidation of cobalt (II) into cobalt (III) in an aqueous solution containing large amounts of azide, in which cobalt (II)-azide complex indicates purple and cobalt (III)-azide yellow. A spectrophotometric measurement of sulfite at 10^{-5} M levels based on the catalytic reaction was already investigated and presented. Otherwise, it is well known that sulfite produce a stable adduct compound *via* its reaction with formaldehyde in an aqueous solution. Therefore, if a known amount of sulfite were allowed to react with formaldehyde to form sulfite-formaldehyde adduct and then a residual sulfite were measured by the spectrophotometric method using cobalt (II)-azide complex reagent, determination of formaldehyde can be expected from an consumption of sulfite *via* its reaction with formaldehyde.

2 Method

Sample solution (10 ml) containing formaldehyde was mixed with sulfite solution (8 ml) adjusted to pH 6.0 by adding acetate-acetic acid buffer solution (2 ml), and the mixture was allowed to stand for 20 min. A 10.0 ml of the obtained mixture was added to a reaction solution (pH 5.6) comprised of acetic acid, cobalt (II) chloride and sodium azide and the reaction solution was allowed to stand for 20 min. After diluting it to 25 ml with water, absorbance of the solution was measured at 370 nm.

3 Result and Discussion

Under the optimal conditions, calibration plots of formaldehyde showed a linear line in the range 0–160 μ M with negative slope, because increasing of formaldehyde concentration caused the decrease of the residual sulfite and also the decrease of absorbance for yellow cobalt (III)-azide compound, owing to use of a known excess amount of sulfite on the reaction with formaldehyde. The proposed method can be successfully applicable to determination of trace amount of formaldehyde in aqueous solution.

Effect of alcohol addition on determination of thiosulfate, sulfite, sulfide and thiocyanate based on its reaction with cerium(IV) and the fluorescence detection of cerium(III)

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Summary

The fluorescence intensity for cerium(III) was extremely increased when thiosulfate, cerium(IV) and ethanol were coexisted in the reaction solution compared with the case in absence of ethanol. Similar increase of fluorescence intensity by addition of ethanol was observed on the determination of sulfite, sulfide and thiocyanate with this method. This method gave much higher sensitivity for the thiosulfate, sulfite, sulfate and thiocyanate measurement compared with a method based on the reaction of cerium(IV) with these reducing reagents without using methanol.

Key word: fluorescence detection; cerium; thiosulfate; alcohol addition

1 Introduction

An analytical method for the determination of reducing reagents such as thiosulfate, L-ascorbic acid and nitrite using their reactions with cerium(IV) and the fluorescence detection of cerium(III) has been reported as a postcolumn fluorescence detection previously [1]. In the determination of thiosulfate by this method, it was observed that the intensity of cerium(III) fluorescence was extremely enhanced in the presence of ethanol. In this work, effect of addition of various alcohols on the determination of thiosulfate and application of this method to the determination of sulfite, sulfide and thiocyanate have been investigated.

2 Materials and Methods

To a 25 mL of volumetric flask, 18 mL of alcohol aqueous solution, 5 mL of sample solution, 1 mL of cerium(IV) sulfate - sulfuric acid were added. After dilution with water to a mark of the flask, fluorescence intensity was measured at regular intervals. Fluorescence of cerium(III) was monitored using excitation and emission wavelengths of 256 nm and 354 nm, respectively.

3 Result and Discussion

To investigate effect of kind of alcohol on the determination of thiosulfate using cerium(III) detection, 2.4 mol/L of various alcohol (methanol, ethanol, 1-propanol and 2-propanol) was added. The fluorescence intensity of cerium(III) for the same concentration of thiosulfate was increased by addition of every alcohol used in this study compared with the case in absence of alcohol. During these alcohol, ethanol exhibited highest increase of the fluorescence intensity, followed by 1-propanol, methanol, 2-propanol. The fluorescence intensity measured at reaction time for 2 minutes was related to the concentration of thiosulfate. Such enhancement of the fluorescence intensity by addition of alcohol might be attributed that thiosulfate accelerate reduction rate of cerium(IV) by alcohol; the reduction rate of cerium(IV) by alcohol is low.

Sulfite, sulfide and thiocyanate instead of thiosulfate were determined on this method with 2.4 mol/L of ethanol addition. Like in the case of determination of thiosulfate, the increase of fluorescence intensity of cerium(III) by addition of ethanol were observed in the case of determination of sulfite, sulfide and thiocyanate.

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Stationary Phases for Ion Chromatographic Determination of Inorganic Anions in Seawater

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Summary

Stationary phases for determination of inorganic anions in seawater have been developed in ion chromatography. Stationary phases, which allow us to use high-concentration eluents, have been examined in order to avoid effects of matrix ions contained in seawater. Retention of analyte anions increased with increasing eluent concentration for poly (ethylene glycol) stationary phases with molecular mass of 10,000 – 20,000, which was advantageous to determine trace iodide and thiocyanate in seawater samples. Quaternary ammonium surfactants could be directly introduced onto silica gel *via* ion exchange followed by hydrophobic interaction. These stationary phases also allowed us to use high-concentration eluents for determination of nitrate, bromide and nitrate in seawater samples. Monolithic capillary columns, packed capillary columns and conventional-size columns have been prepared and evaluated. Use of conventional-size columns with 4.6 mm I.D. improved concentration sensitivity and achieved detection limits at ng/mL levels.

Key word:

Ion chromatography; stationary phases; inorganic anions; seawater

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Analysis of Raw Water and Drinking Water by High-throughput Ion Chromatography

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Summary

We present here a technique of ion chromatography analysis which is developed for the high-throughput measurement of common anions or cations within 5 minutes. The separation was performed with small particle resin packing, the short column and particular chromatograph system, which are optimized for rapid resolution. As a result of having applied this method in the analysis of the water samples such as raw water or drinking water, 7 kinds of anions or 6 kinds of cations were separated excellently and measured without any interference.

Key words: Ion chromatography, high-throughput measurement, raw water, drinking water

1 Introduction

Ion chromatography(IC) is an efficient analytical method for determining ionic species in several waters, which has been used one of the main water quality monitoring method. Recently, the trend of HPLC is high-throughput and high-pressure analysis. Analysis time of high-throughput HPLC is equal to or less than 3 minutes. On the other hand, in conventional IC analysis, it needs more than 10 minutes for common ionic species analysis. We developed a high-throughput IC system (IC-2010) and columns (TSK-GEL SuperIC HS series) for analysis time of less than 5 minutes. In order to realize high-throughput analysis, small particle rigid resin was packed in short column. And packing method and IC system were optimized for high-pressure analysis. We introduce these techniques of system and packing materials in this presentation.

2 Materials and Methods

The chromatographic analyses are performed by ion chromatograph, Tosoh IC-2010 constituted by a dual pump, an on-line degasser, a column oven, an automatic sample injector, a conductmetric detector, a gel-change-able suppressor module and an auto-dilutor. The data analysis and system control was done by Tosoh IC-WS processing system. The separation of anions was performed on TSKgel SuperIC-Anion HS (4.6mmI.D. x 10cm) and TSKguardcolumn SuperIC-A HS (4.6mmI.D. x 1cm) using 3.8mM NaHCO₃ + 3.0mM Na₂CO₃ as eluent. The separation of cations was performed on TSKgel SuperIC-Cation HS (4.6mmI.D. x 10cm) and TSKguardcolumn SuperIC-C HS (4.6mmI.D. x 1cm) using 3.0mM MSA + 0.4mM 12-crown-6-ether + 0.2mM histidine as eluent. All the reagents used in this study were guaranteed grade purchased from Wako Pure Chemical Inc.

3 Result and Discussion

Excellent separation of common anions or cations within 5 minutes was obtained and sensitivity was improved with this IC system and columns. As the result of having applied this method in the analysis of raw water such as rain, river water or spring water and drinking water, no interference peaks showed and common ionic species were measured. Sensitivity and repeatability were satisfied for the measurement of common ionic species in raw or drinking water. The results showed that this method was useful for the high-throughput measurement of common ions in raw water and drinking water.

Simultaneous Determination of Halogen Compounds and Sulfur Oxides in Flue Gas by Ion Chromatography

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Ion chromatography (IC) is a suitable analytical method for the determination of anions. As an analytical method for the halogenated compounds in flue gas, bromine compound, fluorine compound, chlorine and hydrogen chloride (HCl) are listed in JIS. However, IC has not been adopted in JIS except for HCl. It is because that these components are absorbed in a 0.1 M sodium hydroxide solution, it is interfered with the measurement of F^- and Cl^- ions.

This paper describes the development of the pretreatment equipment for the flue gas analysis by IC, and applied it to real flue gas analysis. The F^- , Cl^- , Br^- and SO_4^{2-} in the absorbing solution could be clearly separated by IC using pretreatment equipment. The halogenated compounds and sulfur oxides in flue gas could be simultaneously determined by IC.

Determination of Halogens and Sulfur by Combustion-Ion chromatography

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Summary

It's examination of the total halogen and sulfur analysis using the fully automated combustion- ion chromatography system. We have a good result, 95-103% recovery on standard sample, fluorite, analysis. It also can be quick and easy measurement for the sulfur and halogen in organic and inorganic materials.

Key word: Halogen; Combustion; Ion chromatography; Halogen Free;

1. Introduction

Total Halogen and sulfur analysis become important in quality control and monitoring of the environmental pollution. Therefore, easy method for Halogen and sulfur analysis is required. Combustion is a very superior method for decomposing Halogen in materials and absorbing it to solution. In addition, ion chromatography is a method to measure the halogenide ions and sulfate ion in the solutions quickly with high sensitivity. It is very effective method in many fields for Total Halogen and sulfur analysis in materials that combining two technologies, combustion and ion chromatography (CIC method). We report it as an introduction of fully-automated CIC system and measurement examples.

2. Principle

AQF100 (Mitsubishi Chemical Analytech combustion system) is used for combustion.

The sulfur and halogen in samples are reacted to sulfur oxide and hydrogen halogenide by combustion. The combustion gas is absorbed by absorbent containing hydrogen peroxide and it is sampled as a sulfate ion and a halogenide ion. These ions are measured by ion chromatography, and concentration in sample is determined.

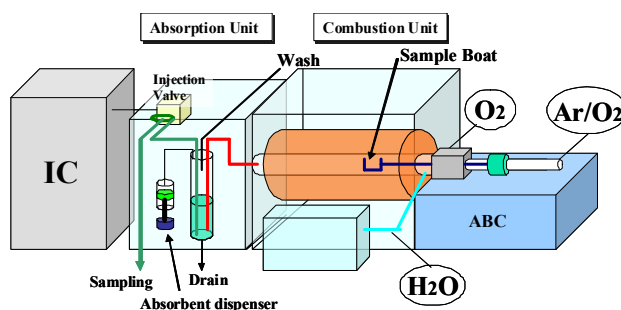


Fig 1 AQF100 combustion system

3. Result

a) Polyethylene standard sample (ERM EC-680K)

	1	2	3	Average(ppm)	RSD,%	Recovery,%
Cl	104	105	106	105	0.95	102.6
Br	96.9	95.5	97.4	96.6	1	100.6
S	73.8	72.8	75.5	74	1.8	97.4

b) Chloride in Glass

	1	2	3	Average(ppm)	RSD,%	Recovery,%
OPAL Glass	138	143	151	144	4.55	103
Borosilicate	332	330	334	332	0.60	94.9
Lead Glass	516	479	490	495	3.84	99.0

Reference material of sulfur blank solvent for the liquid fuels

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Summary

The sulfur blank solvent, NMIJ RM 4216-a, for the determination of sulfur in the liquid fuels was developed by National Metrology Institute of Japan (NMIJ), National Institute of Advanced Industrial Science and Technology (AIST). Reference value was determined by the combustion followed by the ultraviolet fluorescence method using the standard addition method. Trap and release unit was used, and the signal appeared without sample injection was subtracted as the background for the determination of trace sulfur in organic solvent.

Key word: trace sulfur, standard addition method, ultraviolet fluorescence detection, background, reference material, fuel

1 Introduction

Sulfur content is one of the important properties to evaluate the quality of the fuel. In petroleum industries, accurate analysis of sulfur becomes important for the quality control. Moreover, sulfur at sub mg kg⁻¹ to µg kg⁻¹ is well known as the catalyst poison at reformer in fuel cell system. For the determination of low level sulfur, microcoulometry, X-ray fluorescence method, ion chromatography and ultraviolet fluorescence (UV-FL) method are common techniques. These methods require calibration standards. In the preparation of sulfur standard solutions sub mg kg⁻¹ to µg kg⁻¹, high purity sulfur compound or sulfur solution with the higher concentration is diluted with organic solvents. In this case, sulfur in solvent causes analytical bias. Thus, it is important to estimate the sulfur content in organic solvent. In our poster, it is described the measurement method of the trace sulfur in organic solvent, and the development procedure of the RM 4216-a.

2 Materials and Methods

Toluene was chosen as the candidate for RM 4216-a. CRM 4215-a was used as the high concentration sulfur standard solution. A total sulfur analyzer TS-100V (Mitsubishi Chemical Analytech Co., Ltd., Kanagawa, Japan) was used. For the determination of trace sulfur, a trap and release unit TRU-100 (Mitsubishi Chemical Analytech Co., Ltd., Kanagawa, Japan) was connected after the combustion tube to accumulate the sulfur dioxide (SO₂) that was formed during combustion of the sample.

3 Results and Discussion

Toluene, the candidate for RM 4216-a was dispensed to 30 mL portion in 350 amber glass ampoules. The between-bottle homogeneity of RM 4216-a was evaluated by determining 10 amples selected from the lot of 350 amples.

All gravimetric standard addition samples were prepared by spiking RM 4216-a with CRM 4215-a. The 30 mL alumi-seal vials with mininert valves (GL Sciences Inc., Tokyo, Japan) were used for the preparation of standard addition samples to avoid the change of concentration due to evaporation of organic solution.

Using the trap and release unit, a signal without sample injection was appeared. It is considered that, when the condition of the combustion tube temperature is not high enough, impurities containing sulfur in the carrier gases can not be oxidized to SO₂, and no appeared the background signal. Therefore, the signal without sample injection was subtracted as a background in the determination of trace sulfur in organic solvent using the standard addition method.

Evaluation of error sources in a gravimetric technique for preparation of a reference gas mixture (a case of carbon dioxide in synthetic air)

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Summary

Gravimetric blending method is one method of preparing a primary reference gas mixture. The uncertainty of for the mass measurements is necessary to be equal to or less than a few mg, for preparation of the reference gas mixtures, under current conditions of our facilities, equipments and materials. There are many sources of errors in this method. In this study, several sources of errors were re-evaluated on our preparation process of carbon dioxide in synthetic air. As a consequence of the re-evaluations, it was found that the difference of readings on balance between reference cylinder and sample cylinder were significant for gravimetric concentrations of the gas mixtures under the certain conditions of our facilities.

Key word: Gas analysis, Gas metrology, Preparation of standard gas, International comparison, Electronic mass comparator

Carbon dioxide (CO₂) is one of the most important greenhouse gases. Its concentration in the atmosphere has been monitored using non-dispersive infrared gas analyzers (ND-IR). High-accuracy and stable reference gas mixtures in high-pressure gas cylinders are required for the calibration of ND-IR. Measurements of CO₂ in the atmosphere require the uncertainty in the concentration of reference gas mixtures be lower than 0.1 μmol/mol.

International comparisons on CO₂ in synthetic air at atmospheric level were performed under the direction of the Consultative Committee for the amount of substance (CCQM). Nine national metrology institutes (NMIs) participated in these comparisons. However, the concentrations of primary gas mixtures (PRMs) by each NMI were not consistent within 0.1 μmol/mol. The final report of the internal comparison concluded that there are uncertainty factors missing in the uncertainty budget and thus, more work is needed to identify the origins of the observed discrepancies. In the comparison, all PRMs were prepared using the gravimetric (blending) method. However, each participant used different equipments, cylinders, gases, method, and, procedures. It is important to find out the missing error sources.

There are many error sources in this method, as described in annex E of ISO6142:2001. In the gravimetric blending method, the mole fraction of the gas mixture is calculated based on the mass filled into a sample gas cylinder, the molar mass, and, the composition (the purity and impurities) of parent gases. The mass of each component gas filled in a sample cylinder is measured as the difference in the masses of the sample cylinder between before and after the filling of the gas. Accuracy of the mass measurements depends on mass measurement techniques, gas-filling techniques, and, the quality of the cylinders.

In this presentation, we have re-evaluated the following sources of error;

(1) Difference of mass between reference cylinder and sample cylinder (An error on reading of electronic mass comparator), (2) Cooling of a gas cylinder due to the filling of high-purity liquefied CO₂, and warming of a gas cylinder due to filling with dilution gas, (3) Leakage through valve of the high-pressure gas cylinder, (4) Adsorption/desorption of moisture on external cylinder surface, (5) Stable isotope ratio of ¹³C/¹²C in CO₂ parent gas, (6) Impurity of the vapor phase in the high-purity liquefied CO₂ gas cylinder at various temperatures.

As the result of re-evaluation, it was found that the source (1) was significant [1].

Reference

[1] N. Matsumoto, T. Shimosaka, T. Watanabe, K. Kato, *Analytical and Bioanalytical Chemistry*, **391**, 2061-2069 (2008).

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Study of State of Alkyl Chains for Reversed-Phase: Collapse or Brush up

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Summary

The state of alkyl chains are evaluated by solvation of organic solvent on the stationary phase in reversed-phase chromatography. Solvation volume of organic solvent in a mixture of organic solvent and water as a mobile phase to alkyl chain as a stationary phase was measured directly by retention of organic solvent under lower concentration of organic solvent in a mobile phase, furthermore, indirectly by change of stationary phase volume obtained from measurement of elution volume of uracil as a non-retained substance under higher concentration of organic solvent in a mobile phase. THF solvated 6 times more than methanol on the C18 stationary phase, while acetonitrile solvated 2.5 times. THF solvated in the whole alkyl chain and alkyl chain brushed up while methanol solvated on the surface of agglomerating C18 phase.

Introduction

It had commonly been said that ligand collapse made retention decrease under 100% aqueous conditions for reversed-phase liquid chromatography. However Nagae et al. reported at 2000 that reducing retention under 100% aqueous conditions had nothing to do with ligand collapse and its cause was that a mobile phase like water expelled from the pore of the packing materials by capillarity. In this study, solvation volume of organic solvent was measured, then the state of alkyl chains was considered.

Materials and Methods

Sunniest C18, Sunniest RP-AQUA (C28) and Sunniest C8 manufactured by ChromaNik Technologies Inc were used to measure retention time of organic solvents and unracil as a nonretained sample. Methanol, Acetonitrile and tetrahydrofuran were used as organic solvent.

Result and Discussion

Solvation volume of tetrahydrofuran (THF), acetonitrile and methanol were measured as shown in Fig.1. THF solvated 6 times more than methanol on the C18 stationary phase, while acetonitrile solvated 2.5 times. Solubility of octadecan (C₁₈H₃₈) to not only water but also methanol is almost zero, so that C18 alkyl chain is considered to agglomerate on the surface even if 100% methanol is used as a mobile phase. These results suggested that THF solvated in the whole alkyl chain and alkyl chain brushed up while methanol solvated on the surface of agglomerating C18 phase.

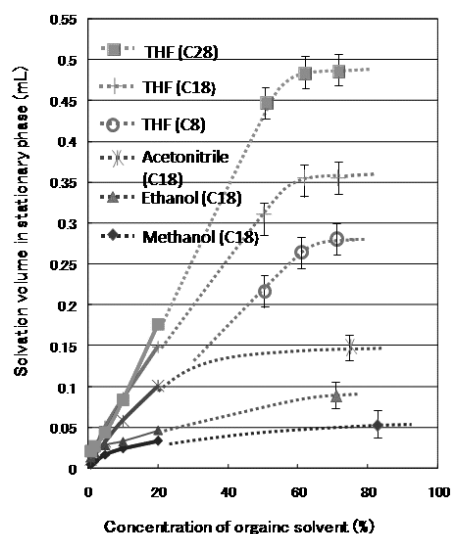


Fig.1. Relationship between concentration of organic solvent and solvation volume
Column: C8, C18, C28, 150 x 4.6 mm

Generic Approach to Analysis of Pharmaceutical Salts Including Inorganic and Organic Counter-ions with Corona CAD

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Summary

The characterization of pharmaceutical salts is critical to the drug development process, for example, in salt selection, confirmation of stoichiometry and detection of impurities. In general, multiple analytical techniques are required to address the diverse nature of analytes (inorganic, organic, anionic, cationic, absorbivity, hydrophobicity, etc.). The objective of this study was to investigate the use of HPLC with Charged Aerosol Detection (CAD) as a generic approach to this broad range of analytes. A zwitterionic stationary phase was operated in HILIC mode using an acetonitrile-water mobile phase pH buffered with ammonium acetate. CAD response was obtained for all analytes, with or without a chromophore. Our initial studies demonstrated the ability to analyze commonly used inorganic positive and negative counter-ions simultaneously along with several active pharmaceutical ingredients (APIs). Limits of detection were in the low ng range with linear response over at least 3 orders of magnitude and intra-assay precision <3% RSD. These data demonstrate the capability to quantify a wide range of analytes that are relevant to pharmaceutical formulation development and characterization.

Keywords: Charged Aerosol Detection ; CAD ; HILIC ; counter-ion ; APIs

1 Introduction

The need to analyze inorganic cations and anions stretches across many fields, ranging from pharmaceutical formulations and product characterization, to environmental analysis. Ion chromatograph with the electric conductivity detector (ICCD) is used mostly for inorganic ion analysis today. Ion chromatography techniques by their very design do not permit the simultaneous analysis of anions and cations in a single run.

In this study, we used Merck (SeQuant) polymeric Zwitterionic column (ZIC-pHILIC) and binary gradient system and separated inorganic cations and anions, organic acids and bases, APIs and Counter-ions and detected them by Corona CAD, UV, and LCMS system. All the data presented here were obtained without changing mobile phase composition or column.

2 Materials and Methods

Column: Merck (SeQuant) ZIC-pHILIC; 4.6 x 150 mm, 5 µm (Merck)

Column temperature: 30°C

Mobile phase A: 15 % 100 mM ammonium acetate, pH 4.68, 5 % methanol, 20 % IPA, 60 % acetonitrile

Mobile phase B: 50 % 30 mM ammonium acetate, pH 4.68, 5 % methanol, 20 % IPA, 25 % acetonitrile

Gradient: T=0 min 20 %B, T=3 min 20 %B, T=24 min 70% B, T=26 min 70 %B, T=32min 15 %B, T=34 min 20 %B,
T=40min 20 %B

Flow rate: 0.5 mL/min, Injection volume: 10 µL, Sample vial: polypropylene

3 Results and Discussion

In this study, we showed that the quantitative analysis of an inorganic and organic counter-ions, API salts and the impurities of the w/w level of 0.1% was possible by a single chromatographic method. The Corona CAD approach is able to measure both anions and cations simultaneously, on a single platform. It offers both time and cost savings when compared to conventional ICCD approaches. The Corona CAD method uses conditions compatible with LC-MS.

The HPLC-CAD-UV-MS platform can be used to more fully characterize a sample.

Simultaneous Cation and Anion Analysis with Charged Aerosol Detection

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Summary

Quantitative analysis of anions and cations is an important aspect of drug development and quality control. This includes confirmation of stoichiometry, counter-ion identity and completeness of pharmaceutical salt formation as well as detection of ionic impurities. Ion chromatography is often used for these analyses but a variety of specialized columns, ion suppressors and modes of operation are required to address the range of analytes of interest. In this study, hydrophilic interaction chromatography (HILIC) was used in combination with charged aerosol detection (CAD). A polymeric zwitterionic stationary phase operated in HILIC mode allowed separation of anions and cations in a single chromatographic run.

Keywords : HPLC Universal Detection ; Charged Aerosol Detection ; anions and cations
Ion chromatography

1 Introduction

Ion chromatography with conductivity detection (ICCD), is the most common approach for the measurement of ions. ICCD typically uses a weak ionic resin for its stationary phase and a high ionic strength mobile phase. An additional neutralizing suppressor column is often required to remove background eluent ions. ICCD cannot measure both anions and cations simultaneously, so either the operator must wait for a considerable period when switching chemistries or multiple instruments must be used. Due to the amount of time to recondition the column and detector, gradient methods are typically not implemented with ICCD. In this study, it was shown that the mobile phase conditions of the pH, buffer strength and organic modifier concentration could be manipulated to effect a given separation. The results demonstrate that the present method can readily measure the ionic impurities and the counter ions, etc. and can become a powerful substitution for conventional ion chromatography.

2 Materials and Methods

The use of a binary gradient HPLC system in this study allows for the analysis of a range of anions and cations (ex. chloride, nitrate, sulfate, phosphate, sodium, potassium).

Column : Sequant ZIC-pHILIC; 4.6 x 150mm, 5µm(Merck)

Column temperature : 30°C

Mobile phase A : 15% 100mM Ammonium Acetate, pH 4.68, 5% Methanol, 20% IPA, 60% Acetonitrile

Mobile phase B : 50% 30mM Ammonium Acetate, pH 4.68, 5% Methanol, 20% IPA, 25% Acetonitrile

Flow rate : 0.5 mL/min

Detector : CoronaCAD (ESA Bioscience inc.)

3 Results and Discussion

The method, without sample extractions, can determine ion concentrations in the high ppb range, with sufficient accuracy and precision to meet pharmaceutical testing requirements. Ion analyses were shown to be quantifiable over a wide range of concentrations, covering 800ppb to 100ppm. LOD (limits of detection) and LOQ (limits of quantification) of each ion were 1.3~7.0 ng and 4.0~12.0 ng as mass on column. The amount of variance at the 12.5-ppm points was found to be less than 5 % RSD over a 7 day period, indicating that the method is also precise. Minor adjustments to the gradient allowed marked improvement in ion resolution without the need for changing mobile phase composition, illustrating the chromatographic power of this approach.

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P24

Feature of new organic synthesis chiral polymer as HPLC stationary phase.

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Summary Polymaleimide derivative spiral structure has great ability of chiral separation. We applied this polymer as HPLC stationary phase. It is suitable for the chiral separation of a wide range of substrates including carbonyl (ketones, esters, carboxylic acids, amino acid derivatives), alcohols and others. We think polymaleimide derivative nitrogen atom concern chiral recognition of carbonyl groups. This stationary phase works not only normal phase but also reversed phase mode.

Key word: HPLC, chiral separation, polymer phase, carbonyl compound

Introduction In many cases, HPLC chiral separation columns apply for analysis enantiomer excess of chiral compounds. It has some kinds of stationary phase, for example, polysaccharide, cyclodextrin, crown ether and small molecule optical compounds. Those columns have a good performance to certain compounds but some stationary phases do not work wide range compounds. We used polymaleimide derivatives as HPLC stationary phase and it worked wide range chiral compounds especially carbonyl compounds. We introduce performance of this new stationary phase as HPLC column.

Materials and Methods 76 kinds of Carbonyls (ketones, esters, carboxylic acids, amino acid derivatives), alcohols and other chiral compounds used as test analyte of chiral separation. Analytical mode is normal phase (Hexane/IPA) and reversed phase (Acetonitrile/Water).

Result and Discussion 76 chiral compounds analysis by this new type HPLC columns. It includes many kinds of carbonyl compounds. The most successful separation is ketones and esters. This polymaleimide derivatives chiral stationary phase has nitrogen atom in spiral structure. Carbonyl compounds come close to nitrogen area of polymaleimide derivatives spiral. One of racemic form is easy to come close spiral area and other racemic form is difficult to come close. So, this spiral polymer has ability of chiral recognition of carbonyl compounds, we think.

Development of a single-step ELISA capillary by enzyme proximity-dependent reaction

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Summary

A single-step enzyme-linked immunosorbent assay (ELISA) capillary for capillary-assembled microchip (CAs-CHIP) is developed. This ELISA capillary involves dual immobilization strategies of antibodies and enzyme proximity-dependent reaction. Inside the capillary, first, glucose oxidase (GOD) and 1st antibody (Ab) were covalently immobilized, then peroxidase (POD)-labeled 2nd Ab was non-covalently immobilized. When the sample solution containing antigen, glucose, amplex red and ascorbic acid enters into the capillary via capillary force, the POD-labeled 2nd Ab releases from the wall of the capillary, which ultimately trapped on the surface of the capillary as a sandwich immunocomplex. Then, H₂O₂ released by enzyme reaction of glucose/GOD smoothly react with amplex red/POD and generate resorufin having red fluorescence. When the antigen is not contained in the sample solution, POD-labeled 2nd Ab in bulk is inhibited by ascorbic acid. Therefore the conversion of amplex red to resorufin is reduced. Here preliminary experiment to demonstrate this concept was carried out.

Key word: capillary, capillary-assembled microchip, ELISA, microchip, sensor

1 Introduction

ELISA is one of the most efficient and effective clinical diagnostic tools for the determination of various biomolecules related to certain disease. Its miniaturization may lead to minimal reagents/sample consumption and faster immunoreaction in a microspace. Generally, microfluidic immunosorbent assay involve washing steps and incubations with antigen/antibody. Although sensitive and selective, the complicated fluid handling inherent to such bioanalytical assay may be time-consuming. In this study, we developed a new single-step ELISA capillary allowing simple analysis of proteins.

2 Materials and Methods

A single-step ELISA capillary(square, I.D.100 μ m \times 100 μ m) was prepared by covalently immobilizing GOD and anti-human IgG. Then HRP-labeled anti-human IgG was immobilized non-covalently with the aid of poly(ethylene)glycol serving as a scaffold. The sample solution contains sample (human IgG), glucose, amplex red and ascorbic acid, and introduced into single-step ELISA capillary via capillary force. All single-step capillary characterizations were done using a fluorescence microscope.

3 Result and Discussion

Before single-step ELISA experiments, preliminary experiments to demonstrate enzyme proximity-dependent reaction were carried out. When the capillary immobilizing GOD and anti-human IgG was treated with the sample solution(human IgG),2nd Ab solution, then the reagent mixture solution(glucose, amplex red and ascorbic acid), step by step, increase of fluorescence intensity was observed. On the other hand, when the reagent mixture solution containing 1000 times higher concentration of 2nd Ab was directly introduced into the same capillary, fluorescence intensity was weaker than that obtained in the former experiment, although the 2nd Ab concentration was much higher. These results suggest that the enzyme proximity-dependent effect was involved in reaction mechanism. Furthermore, when our initial concept of single-step ELISA was carried out, it was confirmed the quantitative measurement for the different human IgG concentration in the ng/mL range was possible.

COMBINABLE-PDMS CAPILLARY (CPC) SENSOR ARRAY

TOWARDS ALL SINGLE-STEP ASSAY OF

MULTIPLE ANALYTES ON A SINGLE MICROFLUIDIC DEVICE

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Summary

In this study, we fabricated the Combinable-PDMS Capillary (CPC) sensor allowing single step assay by simply introducing a sample solution by capillary force. CPC is composed of a convex-shaped PDMS plate immobilizing undissolvable reagents and a concave-shaped PDMS plate immobilizing dissolvable reagents. Since the concave-shaped PDMS has a deeper channel size compared to that of convex structure, combining these PDMS plates allowed easy fabrication of capillary structure possessing different reagents inside the same capillary. This method is useful for immobilizing two different reagents which reacts each other. As applications, we carried out an enzyme inhibition assay and a competitive immunoassay using the CPCs.

Key word: combinable-PDMS capillary(CPC), immunoassay, enzyme / antigen / antibody immobilization

1 Introduction

In order to make the biochemical assay “single-step” using a capillary, immobilization of both biological reagents and organic/inorganic small molecule reagents are often required, however, in some cases, it is technically very difficult since these species react each other during the immobilization procedure (i.e., antigen-antibody, enzyme-substrate....etc). To solve this problem, we propose the use of CPC that assembles two independent PDMS structures immobilizing specific reagents to form the capillary. This capillary-type sensor can be easily arrayed to fabricate multiplexed sensor array. In order to demonstrate the usefulness of CPCs, an enzyme inhibition assay and a competitive immunoassay were chosen as examples.

2 Materials and Methods

Fabrication of PDMS capillary was demonstrated in Figure 1A. Two different PDMS molds varying in dimensions were used to create convex-shaped and concave-shaped PDMS plates. For enzyme inhibition assay, fluorescent substrates and trypsin, and for competitive immunoassay, HRP-labeled antibody and antigen, GOD, were immobilized on concave-shaped PDMS and convex-shaped PDMS, respectively. Combining these two PDMS plates formed the PDMS capillary.

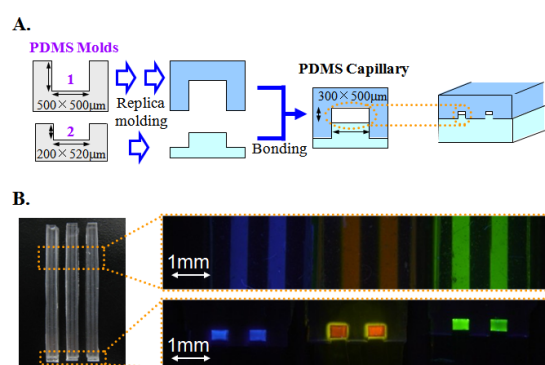


Figure 1. A. Fabrication of PDMS capillary.
B. Actual picture of PDMS capillary array.

3 Result and Discussion

Figure 1. shows the typical fabrication procedure and resulting CPC array containing different fluorescent solutions. CPC sensors for different analytes can be easily arrayed to fabricate multiple-sensing array. For enzyme inhibition assay, principle of operation was confirmed by difference of fluorescence response to sample solutions with and without inhibitor. For competitive immunoassay, principle was confirmed in a similar manner using antibody sample solutions. Here, other preliminary results will be presented.

In situ monitoring of fast chemical processes under the hydrothermal conditions

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Summary

Recently, we have established the capillary flow hydrothermal reactor system for the UV-vis absorption spectrophotometer (CHUS) for monitoring reactions within 0.002 – 200 s at temperatures up to 400 °C. To investigate the scope of CHUS, we observed the oxidation of benzyl alcohol (PhCH₂OH) to benzaldehyde (PhCHO) and benzoic acid (PhCOOH) and the heat stability of DNA. Using CHUS, the present study demonstrates the capability and usefulness of CHUS for hydrothermal applications in practical and fundamental areas.

Key word: Hydrothermal reaction; *in situ* UV-vis spectrophotometry; Alcohol oxidation; Stability of DNA

1 Introduction

Hydrothermal reactions are becoming important both in fundamental and practical areas. For the industrial and environmental applications of these reactions, the reduction of resources and energy consumptions are desirable. In addition, these processes are interested in fundamental studies regarding the origin of life and geochemical problems. Here, we show the capillary flow hydrothermal reactor system for the UV-vis absorption spectrophotometer (CHUS), which enables to monitoring reactions within 0.002 – 200 s up to 400 °C.¹⁾ By using CHUS, we have successfully monitored the reaction behaviors of peptides and nucleic acids. In the present study, we successfully applied CHUS for the oxidation of benzyl alcohol.

2 Materials and Methods

The CHUS system consists of a high-pressure pump, a high temperature reactor, a temperature controller, a pressure regulator, a UV-vis absorption spectrophotometer, and a cooling bath. A fused-silica capillary, of which a detection window was made for *in situ* UV-vis observation, is settled in the reactor. The behaviors of nucleic acids were observed within 5 - 150 s at 150 – 330 °C. The reactions of the oxidation of benzyl alcohol were monitored within 0.64 – 61.6 s at 300 °C and below using an aqueous solution containing PhCH₂OH, Na₂WO₄ and H₂O₂.

3 Result and Discussion

<Behaviors of nucleic acids> It was observed that double-stranded DNA was converted to single-stranded DNA (ssDNA) at temperatures over 100 °C, ssDNA became insoluble and then degraded monomers at over 225 °C.²⁾

<Oxidation of benzyl alcohol> The aqueous solution, mentioned above, prepared 11 % benzaldehyde (PhCHO) and 0.3 % benzoic acid (PhCOOH) at 300 °C in 0.64 s, and 4.5 % PhCHO and 0.1 % PhCOOH at 200 °C in 61.6 s. In the reaction at 300 °C, an *in situ* monitoring of the formation of PhCHO from PhCH₂OH was succeeded at the wavelength of maximum absorption for PhCHO (250 nm).

1) Patent 3378936

2) K. Kawamura and H. Nagayoshi, *Thermochim. Acta*, 466, 63 (2007).

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Introduction of high selective and high sensitive Reduction Gas Detector (RGD) for GC

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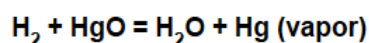
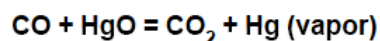
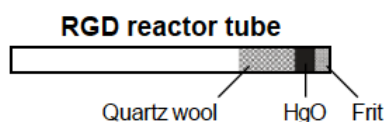
Summary

There are some detectors that measure reducing gases, the SRI Reduction Gas Detector (RGD) is the most sensitive detector available for hydrogen, carbon monoxide, and other reducing gases. Low ppb detection limits are achievable for hydrogen. Detection limits in the ppt level are achievable for carbon monoxide.

The carbon monoxide and hydrogen cause HgO with the reductive reaction, and mercury vapor is generated. The mercury vapor is measured with the UV lamp.

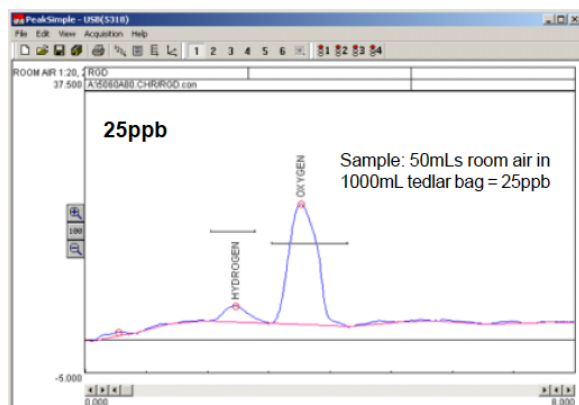
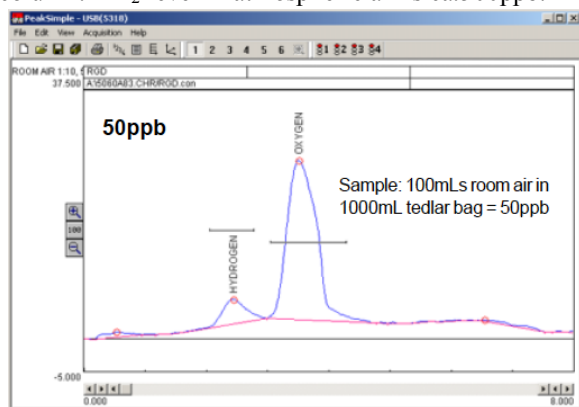
Materials and Methods

The RGD detector uses a mercuric oxide reaction tube and a mercury lamp in a heated UV detector cell. The reaction tube is heated to 260-300°C. Located immediately downstream of the reaction tube, the UV detector cell is heated to 170°C. The UV detector cell is equipped with a mercury lamp and a UV photodiode. The gaseous mercury absorbs the UV light from the mercury lamp as it flows through the cell.



Result and Discussion

The following two chromatograms were produced with an SRI RGD equipped GC. Room air samples were diluted with nitrogen in tedlar bags, then injected by gas sampling valve onto the Molecular Sieve packed column. H₂ level in atmospheric air is ca.500ppb. This system can be detected around 25 ppb H₂ in air.



Analytical Parameters:

Valve injection; 1 mL sample loop

Column: Molecular Sieve 13X

1/8 in.o.d., 2 meter long,

Carrier gas, helium at 5 mL/min.

RGD gain: High

Reactor temperature: 290°C

Detector temperature: 170°C

Valve temperature: 60°C

Concentration at 50 ppb, Results: upper,

Component	Ret.time(min.)	Area (count)
Hydrogen	2.433	100.8250
Oxygen	3.500	796.4090

Hydrogen	2.433	100.8250
Oxygen	3.500	796.4090

Concentration at 25 ppb, Results: lower

Component	Ret.time (min.)	Area (count)
Hydrogen	2.450	55.2755
Oxygen	3.516	571.6580

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Oxygen	3.516	571.6580

