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**Sequential injection-capillary immunoassay for  
chondroitin sulphate proteoglycans**

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Chondroitin sulfate is a potential biomarker for joint diseases and ovarian cancer [1,2]. Immunoassay is commonly used for quantitative analysis of this biomarker. Batch well immunoassay normally involves many steps of incubation and washing that are tedious, highly time consuming and requires skillful technicians. Automatic immunoassay was carried out with sequential injection analysis to overcome these limitations. A low cost glass capillary is used as a solid support for immobilization of chondroitin sulfate or equivalent proteoglycans. This capillary alternative well suits the flow system and eliminates the back pressure problem that normally occurs with a packed bead column. Competitive immunoassay of chondroitin sulfate proteoglycans was demonstrated using this system.

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**P-105**

**Simple labs on chip approach with time-based detection**

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Simple lab-on - chip approach with time-based detection is proposed. In this presentation , we will discuss how to fabricate a simple platform which is made of acrylic piece with channels in different shapes for sample and reagent(s) introducing using flow manipulation. The detection is to monitor changes with time-based involving migration/diffusion of the reaction zone. The changes may be spectrometric or electrochemical. This led to simple micro -analysis with lab-on-chip approach with visual detection(with a stop-watch).Quantification can be made via a calibration graph which are due to relationship of time and concentration of analyte of interest. Various applications will be demonstrated .They include acid and base determination based on neutralization reactions (with an indicator); ascorbic assay based on redox reaction; iron determination, using thiocyanate reagent and enzymatic reactions.

Kate

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method), so it could be an interesting alternative method for determination of proteins.

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#### S1-PO-4

### Determination of Ammonium in Soil and Water by Flow Injection Gas Diffusion - Conductometric System

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Ammonium and ammonia play important role in agricultural and environmental fields. Their contents in soil and water can be used as an indicator for either plant nutrient or pollution. The determination of them in these samples is quite difficult because the analytes usually present at low level in a variety of interferences. In this work, a flow injection with a sensitive conductometric detection for determination of ammonium was developed. Different designs of conductometric flow cell and signal amplification were investigated in order to improve sensitivity of the system. A standard/sample solution was injected into a stream of 2.0 M sodium hydroxide donor solution, which will convert ammonium to ammonia gas. A gas diffusion unit was employed for on-line separation of ammonia gas from a donor solution by diffusing through a teflon membrane to dissolve into an acceptor stream (water). Conductance of the acceptor stream which was increased linearly with the concentration of ammonium in the injected solution was continuously recorded as a peak. A linear calibration graph plotting between peak height and ammonium concentration was obtained in a range up to 1.2 ppm NH<sub>4</sub><sup>+</sup> with a limit of detection of 0.05 ppm. Precision of the method was 5.2 % for 11 replicates injection of 0.5 ppm NH<sub>4</sub><sup>+</sup> standard solution. A sample throughput of 60 h<sup>-1</sup> was achieved. The proposed method was successfully applied to soil extract and surface water samples validating by spectrophotometric standard method. The system consumed small amounts of cheap chemical and there is no interferences from particulates and colored species.

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#### S1-PO-5

### Green Tea Extract as an Alternative Reagent for Flow Injection Determination of Iron

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The purpose of this work is to develop the system for determination of metal ions that utilizes natural extracts as an alternative reagent. Green tea extract is used for quantification of iron. A simple and low cost flow injection technique was developed for this study. The green tea extract was prepared in acetate buffer pH 4.8 and could be used without further purification. The intensity of purple complex of green tea extract with iron was monitored using a laboratory made detector with LED green light source. The effects of some parameters were investigated. The optimum conditions for Fe (II) determination were flow rate 3.0 ml min<sup>-1</sup>, mixing coil 100 cm and extraction time 20 minutes. A linear calibration graph was obtained in the range of 20-80 mg Fe l<sup>-1</sup>. Some preliminary studies of calibration graph will be presented.

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### Assay of Chondroitin Sulfate with Time Based Detection in a Simple Macro – Channel Reaction Flow Cell

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The assay of chondroitin sulfate (CHS) using chromatic dyes such as methylene blue (MB) and dimethylmethylene blue (DMMB) has been investigated. A reaction flow cell with time based detection was proposed as a simple lab on-chip approach. Chondroitin sulfate sample and dye reagent were introduced and come into contact in the flow cell. Migration of the sample zone into the reagent line started at the point of contact. The increase of CHS concentration caused the decrease of migration time of the reaction zone. Detection could be done simply by timing the migration with a stop watch without the need of any complicated detection device.

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### A Simple Flow Injection Spectrophotometry for Determination of Chondroitin Sulfate

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A simple flow injection system was developed for determination of chondroitin sulfate (CS) based on the reaction with methylene blue (MB). A standard/ sample solution was injected into the stream of  $2 \times 10^{-5}$  M methylene blue. The solution was mixed and flowed to a spectrophotometric flow cell. The decrease in absorbance of MB was monitored at 664 nm in corresponding to the increasing of CS concentration. A calibration graph in range of 0–8 mg L<sup>-1</sup> was obtained with a detection limit of 0.1 mg L<sup>-1</sup>. The proposed system was applied to assay CS in pharmaceutical and clinical samples.

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### Coupling of Capacitively Coupled Contactless Conductivity Detector with Membraneless Vaporization and Its Application in Determination of Carbonate by Flow Analysis

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This work describes utilization of a flow-through contactless detector namely ‘capacitively coupled contactless conductivity detector’ or C<sup>4</sup>D in flow analysis. Generally, C<sup>4</sup>D is coupled with a chromatographic technique especially capillary electrophoresis. C<sup>4</sup>D is classified as a universal detector and it is therefore seldom used as a stand-alone detector. In order to exploit application of C<sup>4</sup>D in flow analysis, a simple step of separation was adopted to improve the selectivity. In this work, a coupling of our recent gas phase separation, called ‘membraneless vaporization’ (MLV-VP), with C<sup>4</sup>D was carried out. This allowed for selective detection of volatile compound that dissolves in a flowing acceptor stream to be accomplished. Volatilization of analyte was induced inside a close chamber that had an appropriate acceptor flowing through it. Dissolution of the volatile compound that resulted in changes in conductivity of the acceptor solution, can be monitored using the C<sup>4</sup>D. This technique has been successfully applied to quantitative analysis of carbonate in natural waters and cements.

S1-PO-80

**Determination of Enzyme Activity Based on rate of Migration**

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An application of a macro-channel reaction flow cell with visual detection of the rate of migration for determination of enzyme activity will be presented. The intensity of color of the enzyme-substrate product is normally proportional to its concentration. Concentration affects the rate of migration. Therefore, this rate of migration can be used to estimate enzyme activity. This simple reaction flow cell has a potential to be used as an easy detection unit for enzyme based immunoassay or for quantitative analysis of enzyme from various sources.

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S1-PO-81

**Determination of Total Selenium in Selenium Enriched Rice by Electrothermal Atomic Absorption Spectrometry and the Effect of Foliar Application of Selenite and Selenate Fertilizer on the Selenium Content in RD29 Rice Cultivar**

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In the present work, the influence of foliar application of selenite and selenate fertilizer on increasing the total selenium content in RD29 rice product in order to enhance human selenium dietary intake for cancer chemopreventive activity was investigated. Total selenium concentrations in selenium-enriched rice were determined by electrothermal atomic absorption spectrometry. The plot experiment was conducted using Randomized Complete Block Design (RCB) with three replicates and five treatments using RD29 rice cultivar. The foliar spraying with selenite or selenate fertilizer was thoroughly applied on the rice leaves in the heading stage of rice growth at the rate of 0, 80, and 160 of Se ha<sup>-1</sup>. The rice seeds were harvested in the period of 105 days. The harvested rice seeds were dried and processed to polished rice products and subsequently digested with 3 mL concentrated nitric acid and 1 mL of 30% v/v hydrogen peroxide using microwave digestion system. The analytical condition of ETAAS technique was optimized and applied for the determination of total selenium in rice samples using palladium nitrate as a chemical modifier. The optimized condition for the determination of total selenium in rice was at the pyrolysis time of 10 s and the pyrolysis temperature of 900°C. The atomization temperature was 2,400°C. The limit of detection of the method and M<sub>0</sub> (Characteristic mass) were 2.80 µg/L and 29.24 pg, respectively. The precision defined as %RSD was 4.45% (at concentration of Se 25 µg/L, n=5). The selenium contents of rice were significantly increased to 0.338–0.669 mg/kg by foliar application of selenium fertilizer. The selenium content in rice by application of selenite fertilizer was 30% higher than that by a selenate fertilizer, which indicated that selenite fertilizer exhibited greater efficiency on increasing Se content in RD29 rice products. The results indicated that selenium-enriched rice obtained by foliar application of selenium fertilizer to increase the selenium content of rice could be a good selenium source for Thai population and for future production of selenium-enriched rice supplement. The speciation of selenium by gas chromatography mass spectrometry (GC-MS) and <sup>77</sup>Se-NMR techniques is still required since cancer chemopreventive activity and bioavailability of selenium have been found to be species-dependent which is the subject to on-going studies.

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S1-PO-82

**Simple Analytical Method for the Determination of Mercury, Lead, and Cadmium in Rice and Rice Products for the Development of Simple Screening Heavy Metal Test Kits**

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A simple analytical method for the determination of mercury, lead, and cadmium in rice and rice products was developed in this work using dithizone extraction method with spectrophotometric detection at the wavelength of 487, 504, and 484 nm, respectively. The uses of different types of acid and oxidizing reagents for the extraction of heavy metals from rice samples were investigated. The optimized condition for the extraction of mercury, lead, and cadmium from rice and rice products, i.e., extraction time, extraction temperature, concentration of acid, and concentration of oxidizing reagent were determined. By using the mixtures of suitable masking agents, the extracted samples at the volume of 20 mL was adjusted



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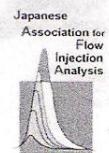
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**On-line Capillary Based Competitive Enzyme-linked Immunosorbent Assay  
for Detection of Zeatin Riboside**

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**Abstract**

Zeatin riboside (ZR) is in cytokinins type a plant hormone that plays an essential role in regulating plant growth and development. The level of ZR presented in plant tissues is a good indicator of the resistance of plants to abiotic environmental stresses and to necrotic pathogens. Highly specific and sensitive technique usually used for determination of ZR is immunoassay. However, conventional 96-well plate format has the drawback in term of highly time consuming and tedious operation steps. Therefore, sequential injection (SI) combined with enzyme-linked immunosorbent assay (ELISA) was developed to obtain an on-line automation and rapid immunoassay system. The zeatin-bovine serum albumin (ZR-BSA) was used as the antigen to immobilized onto the wall of glass capillary. Immobilized ZR-BSA competed with ZR in sample for a limited amount of monoclonal antibody against ZR (MAb-ZR). Upon removing of the unbound species, anti-mouse IgG conjugated with horseradish peroxidase (HRP) enzyme and 3,3',5,5'-tetramethylbenzidine (TMB) substrate were introduced. The colored product was monitored at 650 nm. Various parameters such as concentration of reagents and incubation time were optimized.

*Keywords:* Sequential injection; Zeatin riboside; Plant hormone; Immunoassay

### The Use of Red Rose Extract as Natural Indicator for Ascorbic Assay

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#### Abstract

Green chemistry principles involve the reduction or elimination of the use of hazardous substances. Employing natural reagents in place of synthetic reagents is one way to perform green chemistry. Natural reagents are environmentally friendly and low cost as compared to the synthetic reagents. In this work, the use of the red rose (*Rosa hybrids*) extract as an indicator in flow injection acid-base titration was explored. Ascorbic acid was used as a model analyte to evaluate the performance of the red rose indicator. Normal flow (n-FI) and reverse flow (r-FI) were compared. The color of red rose extract is changed with pH. The change in absorbance of the mixture was detected at 520 nm. A calibration graph in the range of 0-8 mM ascorbic acid was obtained with  $R^2 = 0.9915$ . The proposed system was applied to determine ascorbic acid in vitamin C tablet and injection samples.

**Keywords:** Natural reagents, Green chemistry, Flow injection

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รางวัลช่างทองคำ<sup>๙</sup>  
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มหาวิทยาลัยเชียงใหม่ประจำปี 2552

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