

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

Supada Khonyoung^a, Jaroon Jakmunee^a, Prachya Kongtawelert^b, Kate Grudpan^a,
Supaporn Kradtap Hartwell^a

^a*Department of Chemistry, Faculty of Science and Institute for Science and Technology Research and
Development, Chiang Mai University, Chiang Mai 50200, Thailand*

^b*Thailand Excellence Center for Tissue Engineering, Department of Biochemistry, Faculty of medicine,
Chiang Mai University, Chiang Mai 50200, Thailand.*

E-mail: kradtas@yahoo.com

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.

References:

- [1] P. Pothacharoen, S. Siriaunkgul, S. Ong-Chai, J. Supabandhu, P. Kumja, C. Wanaphirak, K. Sugahara, T. Hardingham, P. Kongtawelert, J. Biochem. 140 (2006) 517.
- [2] C. Belcher, R. Yaqub, F. Fawthrop, M. Bayliss, M. Doherty, Ann. Rheum. Dis. 56 (1997) 299.

K.Grudpan^{a,*}, J. Jakmunee^a, W. Jangbai^a, K. Jitmanee^a, J. Junsomboon^a, T. Kanyanee^a,
S. Kradtap Hartwell^a, W. Kumutanat^a, A. Laokuldilok^a, S. Lapanantnoppakhun^a,
P. Nuntaboon^a, S. Pencharee^{a,b}, K. Ponghong^a, W. Siriangkhawut^a, W. Sriporaya^a,
S. Suphareok^a, S. Tontrong^a, K. Watla-iad^{a,c} and W. Wongwilai^a

^a *Department of Chemistry and Research Laboratory for Excellence in Development for Miniaturization
in Flow-based Analysis, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand*

^b *Department of Physics, Faculty of Science, Ubon Rajathanee University, Ubon Rajathanee 34190,
Thailand*

^c *Present address: Department of Chemistry, Faculty of Science, Mae Fah Luang University, Chiang
Rai 57100,
Thailand*

E-mail: kate@chiangmai.ac.th, kgrudpan@gmail.com

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.

Kato

PACCON

PURE AND APPLIED CHEMISTRY
INTERNATIONAL CONFERENCE 2009

Abstracts

JANUARY 14-16, 2009
NARESUAN UNIVERSITY, PHITSANULOK, THAILAND

PACCON 2009 ABSTRACTS

**PURE AND APPLIED CHEMISTRY
INTERNATIONAL CONFERENCE**

Organized by
**Sustainable Development in Chemistry Based
on Indigenous Knowledge**



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

REFERENCES

1. Association of Official Analytical Chemists: AOAC Official Method 991.20.
2. Association of Official Analytical Chemists: AOAC Official Method 992.15.
3. Junsomboon, J.; Jakmunee, J. *Anal. Chim. Acta*, **2008**, 627, 232-238.

S1-PO-4

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

Jaroon Jakmunee^{1,2*}, Jaroon Junsomboon¹

¹Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200 Thailand

²Science and Technology Research Institute, Chiang Mai University, Chiang Mai, 50200 Thailand

*E-mail: scijjkmn@chiangmai.ac.th, Tel :+ 66-53-941909

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_4^+ 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_4^+ standard solution. A sample throughput of 60 h⁻¹ 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.

REFERENCES

1. P.R. Hesse. *A Textbook of Soil Chemical Analysis*, William Clowes and Sons Limited, London, 1971.
2. Junsomboon, J.; Jakmunee, J. *Anal. Chim. Acta*, **2008**, 627, 232-238.

S1-PO-5

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

Pivanut Pinyou¹, Jaroon Jakmunee^{1,2}, Somichai Lapanatnoppakhun^{1,2}, Kate Grudpan^{1,2}, Supaporn Kradtap Hartwell^{1,2*}

¹Department of Chemistry and Research Laboratory for Excellent in Development for Miniaturization in Flow-based Analysis, Faculty of science, Chiang Mai University, Chiang Mai, 50200 Thailand

²Research Laboratory for Development of Analytical Instrumentation, Center for Instrumentation and Process Technology, Institute for Science and Technology Research and Development, Chiang Mai University, Chiang Mai, 50200 Thailand

*E-mail: kradtas@yahoo.com, Tel: +66-53-941910

The purpose of this work is to develop the system for determination of metal ions that utilizes natural extracts 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⁻¹, mixing coil 100 cm and extraction time 20 minutes. A linear calibration graph was obtained in the range of 20-80 mg Fe l⁻¹. Some preliminary studies of calibration graph will be presented.

REFERENCES

1. Settheeworrit, T.; Hartwell, S. K.; Lapanatnoppakhun, S.; Jakmunee, J.; Christian, G. D.; Grudpan, K. *Talanta*. **2005**, 68, 262-267.
2. Udnan, Y.; Jakmunee, J.; Jayasavati, S.; Christian, G. D.; Synovec, R. E.; Grudpan, K. *Talanta*. **2004**, 64, 1237-1240.
3. Grudpan, K. *Talanta*. **2004**, 64, 1084-1090.

S1-PO-6

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

Worathip Sriporaya¹, Somchai Lapanantnoppakhun^{1,2}, Kate Grudpan^{1,2}, Supaporn Kradtap Hartwell^{1,2,*}

¹Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand 50200

²Science and Technology Research Institute, Chiang Mai University, Chiang Mai, Thailand 50200

*E-mail: kradas@yahoo.com. Tel/Fax: +66-53-941910

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.

REFERENCES

1. Zhang, L.; Lu, N.; Zhao, F.; Li, K. *Anal. Sci.* **2004**, 20, 445–450.
2. Pospíchal, R.; Nesměrál, K.; Rychlovský, P.; Němcová, I. *Anal. Lett.* **2007**, 40, 1167–1175.
3. Zhou, S. G.; Jiao, Q. C.; Chen, L.; Liu, Q. *Spectro. Lett.* **2002**, 35(1), 21–29.
4. Templeton, D. M. *Int. J. Biol. Macromol.* **1988**, 10, 131–136.

S1-PO-7

A Simple Flow Injection Spectrophotometry for Determination of Chondroitin Sulfate

Supada Khonyoung¹, Jaroon Jakmunee^{1,2}, Kate Grudpan^{1,2}, Supaporn Kradtap Hartwell^{1,2,*}

¹Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200 Thailand

²Science and Technology Research Institute, Chiang Mai University, Chiang Mai 50200 Thailand

*E-mail:kradas@yahoo.com, Tel/ Fax: +66-53-941910

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 2×10^{-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⁻¹ was obtained with a detection limit of 0.1 mg L⁻¹. The proposed system was applied to assay CS in pharmaceutical and clinical samples.

REFERENCES

1. Ohkuma, S.; Furuhashi, T. *Proc. Japan Acad.* **1970**, 46, 974–978.
2. Zhou, S. G.; Jiao, Q. C.; Chen, L.; Liu, Q. *Spectro. Lett.* **2002**, 35, 21–29.
3. Zhang, L.; Li, N.; Zhao, F.; Li, K. *Anal. Sci.* **2004**, 20, 445–450.
4. Pospíchal, R.; Nesměrál, K.; Rychlovský, P.; Němcová, I. *Anal. Lett.* **2007**, 40, 1167–1175.

S1-PO-8

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

Kamonthip Sereenonchai^{1,2}, Phoonthawee Sactear^{1,2}, Nathawut Choengchan^{1,3}, Sasithorn Muncharoen^{1,2}, Natchanon Amornthammarong^{1,4}, Duangjai Nacapricha^{1,2,*}

¹Flow Innovation-Research for Science and Technology Laboratories (First Labs)

²Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Mahidol University, Bangkok, 10400 Thailand

³Department of Chemistry, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, 10520 Thailand

⁴Atlantic Oceanographic & Meteorological Laboratory, National Oceanic & Atmospheric Administration (NOAA), Ocean Chemistry Division/AOML, Florida, 33149 USA

*E-mail: scdnc@mahidol.ac.th, Tel: +66-2-2015127

This work describes utilization of a flow-through contactless detector namely 'capacitively coupled contactless conductivity detector' or C⁴D in flow analysis. Generally, C⁴D is coupled with a chromatographic technique especially capillary electrophoresis. C⁴D is classified as a universal detector and it is therefore seldom used as a stand-alone detector. In order to exploit application of C⁴D 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' (MBL-VP), with C⁴D 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 C⁴D. 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

Kraingkrai Ponghong¹, Worathip Sriporaya¹, Supaporn Kradtap Hartwell^{1,2}, Kate Grudpan^{1,2,*}

¹ Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand 50200

² Science and Technology Research Institute, Chiang Mai University, Chiang Mai, Thailand 50200

* E-mail: kate@chiangmai.ac.th, Tel/Fax: +66-53-941910

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.

REFERENCES

1. Gabbianelli, S.; Falcioni, G.; Nasuti, C.; Cantalamessa, F. *Toxicology*. **2002**, 175, 91 – 101.
2. Passos, M.L.C.; Sarava, M.L. M.F.S.; Lima, J. L.F.C. *Talanta*. **2008**, 77, 484 – 489.

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

Wipharat Chuachud^{1*}, Ratana Sananmuang¹, Yuthapong Udnan¹, Nivat Nabheerong², Warangkana Pimon¹, Kunyaporn Pumduang¹

¹ Department of Chemistry, Faculty of Science, Naresuan University, Phitsanulok, 65000 Thailand

² Phitsanulok Rice Research Center, A. Wang-thong, Phitsanulok, 65130 Thailand

* E-mail: wipharatc@nu.ac.th, Tel: + 66-55-261000 ext 3427

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⁻¹. 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 M₀ (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 ⁷⁷Se-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.

REFERENCES

1. Chen, L.; Yang, F.; Xu, J.; Hu, Y.; Hu, Q.; Zhang, Y.; Pan, G. *J. Agric. Food Chem.* **2002**, 50, 5128-5130.
2. Hu, Q.; Chen, L.; Xu, J.; Zhang, Y.; Pan, G. *J. Sci. Food Agric.* **2002**, 82, 869-872.

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

Wipharat Chuachud^{*}, Ratana Sananmuang, Yuthapong Udnan, Auamporn Wohan, Natinee Kaewvichian

Department of Chemistry, Faculty of Science, Naresuan University, Phitsanulok, 65000 Thailand

* E-mail: wipharatc@nu.ac.th, Tel: + 66-55-261000 ext 3427

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



2010 PATTAYA THAILAND

นายจรณ ใจมูม
Jaroon Jakmoo

**The 16th International Conference on Flow Injection Analysis,
Including Related Techniques**

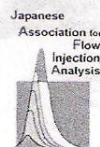
**25 - 30 April 2010
Pattaya, Thailand**



**MAHIDOL
UNIVERSITY**
Wisdom of the Land



PERCH-CIC



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

Supada Khonyoung,¹ Kanokwan Sringam,² Jaron Jakmune,¹
Kate Grudpan,¹ Suporn Kradtap Hartwell^{1*}

¹Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

²Central Laboratory, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand
e-mail: kradtas@yahoo.com

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

Preeyaporn Reanpang, Jaroon Jakmunee, Kate Grudpan, Supaporn Kradtap Hartwell*

Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
e-mail: kradtas@yahoo.com

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

References

- [1] S. Armenta, S. Garrigues, M. de la Guardia, Trends Anal. Chem., 27 (2008) 497-511.
- [2] T. Pojanagaroon, S. Watanesk, V. Rattanaphani, S Liawrungrath, Talanta, 58 (2002) 1293- 1300.

รางวัลช่างทองคำ
มหาวิทยาลัยเชียงใหม่

รางวัลมหาวิทยาลัยเชียงใหม่ “ช้างทองคำ” ประจำปี 2552



ข่าวสารมหาวิทยาลัยเชียงใหม่

พิธีทำบุญวันคล้ายวันสถาปนา พิธีเปิดป้ายมหาวิทยาลัย และพิธีมอบรางวัล
มหาวิทยาลัยเชียงใหม่ประจำปี 2552

มหาวิทยาลัยเชียงใหม่จัดพิธีทำบุญวันคล้ายวันสถาปนา พิธีเปิดป้ายมหาวิทยาลัย และพิธีมอบรางวัลมหาวิทยาลัยเชียงใหม่ประจำปี 2552 แก่ คณาจารย์ และบุคลากรที่มีผลงานดีเด่นประจำปีการศึกษา 2552 โดยมี ศาสตราจารย์เกียรติคุณ นพ.อาวุธ ศรีศุกรี อุปนายกสภามหาวิทยาลัย อดีตอธิการบดี มช. เป็นประธาน นายอมรพันธุ์ นิมนันท์ ผู้ว่าราชการจังหวัดเชียงใหม่ ศาสตราจารย์ ดร.พงษ์ศักดิ์ อังกลสิทธิ์ อธิการบดีและผู้บริหาร คณาจารย์ ข้าราชการ เจ้าหน้าที่และพนักงานมหาวิทยาลัยเชียงใหม่ร่วมในพิธี พร้อมกันนี้มหาวิทยาลัยเชียงใหม่ ได้จัดให้มีพิธีมอบรางวัลมหาวิทยาลัยเชียงใหม่ประจำปี 2552 "ช้างทองคำ" และ "โล่ประกาศเกียรติคุณ" แก่ คณาจารย์ และบุคลากรที่มีผลงานดีเด่นประจำปีการศึกษา 2552 เพื่อเป็นการยกย่องเชิดชูเกียรติ แก่บุคลากรในด้านการเรียนการสอน-ด้านการวิจัย ด้านกิจการนักศึกษาและด้านการบริหารงานทั่วไป ผู้ที่เกี่ยวข้องในการปฏิบัติงานได้สร้างสรรค์ผลงานด้านวิชาการ ด้านการวิจัยและด้านปฏิบัติงานบริหารจัดการสนับสนุนงานมหาวิทยาลัยไปสู่ความเป็นมหาวิทยาลัยชั้นนำ เมื่อวันอาทิตย์ที่ 24 มกราคม 2553 ณ ศาลาธรรม มหาวิทยาลัยเชียงใหม่

[post:24 มกราคม 2553 14:37:34] (view: 650)