1. INTRODUCTION

Immunoassays are widely used in the molecular biology field, providing the molecular basis for many clinical applications (refs). Currently bead immunoassay is one of the most topics widely used. Bead immunoassay, in which antigen or antibody are supported on a solid phase are of interested. A flow-based with bead immunoassay

Up to date, there are some reports on bead injection with sequential injection method (refs). They included sequential injection (SI) with a jet ring cell in the early works (ref) and a lab-on-valve technique in the recently works (ref). We report a new method for automated bead-based immunoassay using lab-at valve (LAV) (refs). It is based on the sequential injection (SI) providing the required microfluidically manipulated ability to handle the microparticles/beads, sample and reagents to perform an immunoassay. The flow-based immunoassay offers an automatic assay carrying out rapidly in an integrated unit. The beads/microparticles were discarded after use, thus avoiding regeneration step. The fresh particles were introduced.

Chondroitin 6-sulfate assay (C6S) which is important for diagnosis of cartilage disease was chosen to be a model compound for developing the system based on competitive immunoassay.

Chondroitin sulfate is one of cartilage proteoglycan which is a major constituent in various connective tissues (ref). There were several works reported that chondroitin sulfate and epitopes on chondroitin sulfate chains are important biomarker of cartilage destruction (ref).

The purpose of the present work is to develop the novel SI-LAV bead based immunoassay system for chondroitin 6-sulfate (C6C) and epitopes on chondroitin sulfate chains from Shark A1 (refs). The system was simply composed of the auxiallry apparatus to facilitate the development of higher throughput for more automated immunoassay. The detection system used was the spectrometric measurement which commonly used in ELISA. The model compounds of chondroitin 6-sulfate (C6C) and C6S epitopes based on competitive immunoassay are therefore chosen for developing the system. The assays are important for diagnosis of cartilage disease. It is a promising performance to use the system for disease screening.

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2. EXPERIMENTAL

2.1 Chemical and Regents

Chondroitin 6-Sulfate sodium salt from shark cartilage (Product No. C4384) was a chemical from Sigma-Aldrich (Saint Louis, MO).

Shark cartilage proteoglycan (A1 fraction); (Shark A1): The shark A1 (Sh-A1) is a highly associated form shark proteoglycan molecules from which separated other small one by CsCl gradient centrifugation. Al fraction was the largest major component in cartilage containing the bottom two fifths of the extractant after centrifugation. The detail for the method of preparation was described previously (ref. 2 MS thesis). The A1 fraction was purchased from Assoc. Prof. P. Kongtaweelert, Department of Biochemistry, Faculty of Medicine, Chiang Mai University, according to the general procedure reported previously (ref.thesis MS).

Monoclonal Antibody WF6; (mAb WF6): The specific monoclonal antibody WF6 (IgM) was synthesized by immunization of female Balb/c mice with the embryonic shark PG-

A1 fraction by using a standard hybridoma technique. The monoclonal antibody WF6 was gifted from Assoc. Prof. P. Kongtaweelert, according to the method description reported previously (ref.thesis MS). Dilution of the WF6 antibody used was 1 mg/mL.

Goat Anti-Mouse IgM Horseradish Peroxidase Conjugate (A8786), IgM-HRP, was purchased from Sigma-Aldrich (Saint Louis, MO). The working dilution used was 1:2,000.

The TMB Peroxidase substrate, TMB-H_2O_2 (3,3',5,5'-tetra-methylbenzidine in an acidic buffer) was purchased from SureBlue™ TMB Microwell Peroxidase Substrate (1-Component) (Cat. No. 52-00-02), KPL (Gaithersburg, MD). The TMB-H_2O_2 solution is ready to use.

Carrier used is phosphate buffer saline (PBS, 10 mM, pH 7.4), 1 L of PBS contain 0.26 g KH_2PO_4, 2.17 g $Na_2HPO_4{\cdot}2H_20$ 1.44 g, 8.71 g NaCl, 0.5 mL of TWEEN 20. Dilution of WF6 antibody and Anti IgM-HRP were made with PBS.

Working dilution of standard C6S and Sh-A1 was spiked in 6 g/L bovine serum *albumin* (in 10 mM PBS) to imitate a real biological fluid which had a total protein at around 6 g/L.

2.2 Pre-coupling C6S /Sh-A1 on beads

Sepharose 4B™ was purchased from Amersham Biosiecnes (Uppsala, Sweden). Beads were washed following the manufacurer suggested procedure. The Carbodiimide method was used as coupling procedure. The target antigen (C6S or Sh-A1) was dissolved in coupling solution, deionized water pH 4.5 adjusted, to obtaind 2.5 mg/mL

and 5 mg/mL for C6S and Sh-A1, respectively. The antigen solution (10 mL) was added to the beads, 5.0 g (wet weight), and followed by adding carbodiimide power 0.1951 g. The mixture was end-over-end rotated for 18 hrs at 4 ^{o}C. The pH of the mixture was measured by pH paper during the first hour and adjusted to pH 4.5. Acetic acid (1.0 M) was used as a blocking solution by rotation with beads for 4 hrs at 4 ^{o}C. The coupled bead was washed throughly with three cycles of 0.1 M acetate buffer, pH 4.0 containing 0.5 M NaCl followed by a wash with 0.1 M Tris-HCl buffer pH 8.0 containing 0.5 M NaCl. The C6S/Sh-A1 coupling beads was kept in 10 mM PBS at 4 ^{o}C until use.

2.3 Serum sample

Serum samples were obtained from the volunteer students, age 22-26 years (Flow-based analysis group, Chiang Mai University). A sample of fastingmorning serum was collected from a voluteer in the laboratory. Serums were kepted at -20 ^{o}C freezer before analysis required.

2.4 Instrumentation

2.4.1 SI-LAV apparatus

The syringe pump (Cavro XL3000) was purchased from FIAlab Instruments Inc. (Bellevue, WA). The ten-port selection valve (C25-3180EMH) was a product from VICI Valco Instruments Co. Inc. (Houston, TX). The spectrometric detection system used a model LS-1 light source, a USB2000 spectrometer and two 400-μm (FIA-P400-SR ($^{1}/_{16}$ inch-o.d. or 1.587 mm diameter)) from Ocean Optics, Inc. (Dunedin, FL). The laboratory designed flow cell was connected with a PEEK cross 0.020 inch thru-hole (P-729) (Upchurch Sciencetific, Oak Harbor, WA). The three-way isolation solenoid valve (A-0136772) was purchased from Cole Parmer International, USA. All connecting $^{1}/_{16}$

inch-o.d. tubing were FEP Teflon and PEEK (Upchurch Sciencetific, Oak Harbor, WA). The instrument was controlled by the FIAlab for windows 5.0 (version 5.9.158) software. The signal out put was recorded by the LabView software (v.7). The data evaluated and graph generated was made by OriginLab v.7 (Northampton, MA).

The automated instrument for bead immunoassay was developed using the LAV apparatus for spectrophotometric measurement. The instrument (Figure 1) consists of a syringe pump (syringe volume 1000 μL), a holding coil, ten-port selection valves, a three-way solenoid valve, a lab-at-valve flow cell, a bead reservoir (plastic syringe 1 mL), a PEEK cross flow cell with a fiber optics light source and a detector. Sample and bead introduction are carried out by combinations of reversible flow pump motion, valves port-position selected and solenoid valve switching direction. The LAV flow cell schematic is shown in Figure 2. All connecting tubing was shortened as much as possible to minimize dilution and reagent consumption.

2.4.2 Operation sequences

The SI-LAV operating sequences was designed and modified from a typical ELISA batch-well competitive immunoassay for chondroitin 6-sulfate and Sh-A1 (ref.). The beads and the reagents were aspirated via the selection valves by a controlled-flow motion of the syringe pump. The certain beads amount was achieved by the LAV flow-cell cavity. Solenoid valve was needed in order to retain the microparticles within the LAV flow-cell and to bypass the flowing reagents to carry out an immunoassay. The operation sequences were drawn in Table 1.

3. RESULTS AND DISCUSSIONS

3.1 Developed LAV flow-cell

A LAV flow-cell for bead immunoassay with spectrometric measurement was designed. It was made of Perspex and comprised a cylindrical cavity (1.5 mm i.d., 4.5 mm long) with three flow connections. One connection, the frit 5 μm was placed to retain the bead in the cavity. Bead inlet, bead outlet and beads blocking connections were made by using the same connection via MV2. Beads were introduced into the cavity of the LAV cell from MV2 (beads reservoir). Beads were retained in the cell by changing valve position MV2 (plug), to block the beads in the cell and by switching 3-way solenoid valve to by pass the flowing reagents. Bead discarding was done by selection of MV2 (bead outlet) and stopper of the solenoid valve. The whole cavity of the flow cell was filled with beads, approximately 8 μL.

3.2 Assay condition

Assay condition was investigation to perform bead-based SI-LAV immunoassay as following.

Incubation: A slow flow rate (1 μL/min) incorporating a pulsed flow was performed in the incubation steps (Table 1, steps 10, 15). These flow patterns prolonged the contact time of the sample zone, thus would lead the more effective binding between beads and reagents. Air segments were aspirated to prevent dilution of the sample and reagent zones (Table 1, steps 3, 7, 13, 18).

Reagent concentration: The amounts of mAb WF6 (0.25, 0.5, 1, 2 mg/mL) were varied while amounts of the others were fixed (beads 8 μL, 100 μg/mL C6S). The suitable concentration was found to be 1 mg/mL.

Fresh bead introduction: Used beads were discarded and the fresh beads were introduced into the LAV flow-cell after each run. Thus, there is no need to regenerate the beads. However, the used beads can be collected for further refreshing.

3.3 Monitoring Signal

Signal due to the bead immunoassay was monitored in real-time by an optical fiber spectrometer. The response was monitored for the entire spectra at peak maximum (Figure 4 (a)). The sensitivity of the measurement in the UV at 375 nm, and the Visible at 675 is the highest, however the signal at 375 nm could suffer from low light intensity, the response in the visible 675 nm is therefore monitored as a detected wavelength. The SI profiles of the various standards C6S competed with the packed bead-C6S (Figure 4 (b)). During the runs, a well reproducible level of zeroed baseline was obtained.

3.4 Competitive binding curve

The competitive binding curve was typical generated using the expression B / B_0, which is the unitless ratio *vs.* log [Analyte]. B is the signal from the amount of the label bound to the beads when the sample analyte is present. B_0 is the signal from the amount of the label bound to the beads when the sample analyte is absent.

The C6S/Sh-A1 analyte was mixed with a fixed amount of WF6 specific antibody, and the analyte in the sample competed with the coating C6S/Sh-A1 on beads for the binding with specific antibody.

The analyte C6S/Sh-A1 binds to the WF6 specific antibody thus preventing the antibody from binding with the C6S/Sh-A1 coating on the bead surface. Therefore, an increase in amount of C6S/Sh-A1 in sample solution resulted in a decrease of the signal.

The two model of competitive binding immunoassay of C6S and Sh-A1 were investigated. The same conditions were used for the two models. The competitive binding curves are shown in Figure 4. Concentrations of C6S standards over range from 100-3200 μg/mL (curve detail…), the Sh-A1 range from 500-200 000 ng/mL were obtained. Sh-A1 gave the better sensitivity of the assay. This could be due to the larger size for Sh-A1 than C6S, which could make it possible to have a high binding property with mAb WF6. Therefore Sh-A1 could be used as a potential standard antigen as a relative equivalent of chondroitin sulfate epitopes in a serum (ref).

3.5 Comparison of the operation steps of the convenentioal ELISA and the developing system

3.6 Preliminary analysis of Cs epitopes in human serums

Sh-A1 was used as a potential standard antigen for the assay of a relative equivalent of chondroitin sulfate epitopes in human serum. The data for chondroitin sulfate epitopes in human serum by the developed SI-LAV method is given in Table 2.

4. CONCLUSION

A microfluidic flow-microparticles based immunoassay has been developed. It is rapid, automatic and can be used for real time analysis. Apart from C6S epitope assay, applications to other analytes should be possible using this instrument.

These results demonstrate the potential of the developed system for assay where the C6S epitope levels are lower for normal case. The high levels from patient serums are currently under investigation.

REFERENCES

ACKNOWLEGEMENTS

This project was supported by grants from the Thailand Research Fund. Department of Chemistry, Faculty of Science, Chiang Mai University was thanked for the laboratory facility needed. Thanks are due to Dr. Siripat Suteerapataranon for helping in the early designed of the flow cell, Mr. Lucksagoon Ganranoo for assisting with solenoid valve assembly.

List of Tables

Table 1 Operation sequences

Step	Port (MV1)	Port (MV2)	Volume*/ μL	Flow rate/ μL/sec	3-way Solenoid valve	Description
1	Beads	Beads resevior	~ 8	NA	On	Manual fill beads inlet to LAV cell
2	Beads (PBS)	Plug	-100	1	On	Flush beads with PBS
3	MC2	Plug	+20	20	On	Load air segment-A1
4	WF6 antibody	Plug	+25	20	On	Get WF6 antibody
5	Standard solution	Plug	+50	20	On	Get a compettive standard
6	WF6 antibody	Plug	+25	20	On	Get WF6 antibody
7	MC2	Plug	+20	20	On	Load air segment-A2
8	MC2	Plug	-/+100	20	On	Third times reversible flow for mixing
9	Waste	Plug	-25	20	On	Discard air-A2
10	Beads	Plug	-80	1	On	Inject the mixture to packed beads
11	Waste	Plug	-50	20	On	Discard air-A1
12	Bead (PBS)	Plug	-300	1	On	Flush PBS carrier to beads
13	MC2	Plug	+20	20	On	Load air segment-B1
14	Anti-IgM HRP	Plug	+60	1	On	Get Anti-IgM HRP
15	Beads	Plug	-50	1	On	Inject Anti-IgM HRP to beads
16	Waste	Plug	-50	20	On	Discard air-B1
17	Bead (PBS)	Plug	-300	1	On	Flush PBS carrier to beads
18	MC2	Plug	+20	20	On	Load air segment-C1
19	TMB-H_2O_2	Plug	+60	1	On	Get TMB-H_2O_2
20	Beads	Plug	-50	1	On	Inject TMB-H_2O_2 to beads
21	Waste	Plug	-50	20	On	Discard air-C1
22	Bead (PBS)	Plug	-650	1	On	Flush PBS carrier to beads
23	Bead (PBS)	Waste	-1000	100	Off	Clear packed bead in LAV cell

* Postitive (+) means syringe aspiration, negative (-) means syringe dipensing

3-way solenoid valve ‘On’; in order to bypass the flowing reagents, ‘Off’; in order to retain the beads with in the cell.

Table 2 Comparison of ELISA method and SI-LAV method

Operation steps*	**ELISA method (ref)**	**SI-LAV method**
1. Coupling antigen	Onto well-plate ELISA (>18 hr)	Onto Sepharose bead[a] (~4 hr)
2. Introducing C6S/Sh-A1 and WF6 antibody mixture	100 μL mixture/well (1 hr, 4 ^{o}C and 37 ^{o}C, manual)	50 μL+50 μL of C6S/Sh-A1 and WF6 (100 s, R_T, air automatic flow with air segmented)
3. Conjugation of Anti-IgM HRP	100 μL (1:4000)/well (7 hr, 37 ^{o}C, manual)	50 μL (1:4000) (50 s, R_T, automatic flow with air segmented)
4. Loading substrate TMB-H_2O_2	100 μL (1:4000)/well (7 hr, 37 ^{o}C, manual)	50 μL (1:4000) (50 s, R_T, automatic flow with air segmented)
5. Stop reaction	100 μL (2 M H_2SO_4)/well (20 min, 37 ^{o}C, manual)	Not required

Table 3 C6S epitopes in human serum samples

Sample no.	Pre added Sh-A1 (ng/mL)	Sh-A1 added (ng/mL)	Sh-A1 found (ng/mL)	% Recovery
S1	571	2500	2994	97
S2	1129	2500	3038	84
S3	1095	2500	3110	87

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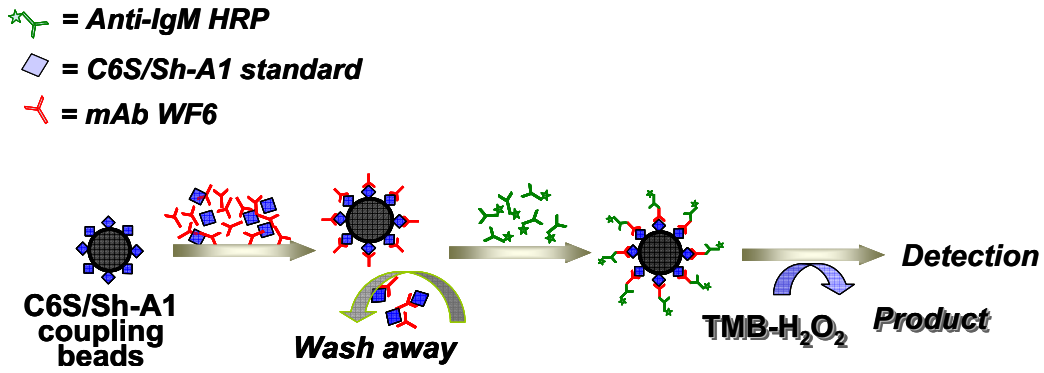

Figure 1 Competitive formate for C6S/Sh-A1 using identical C6S/Sh-A1 coupling beads

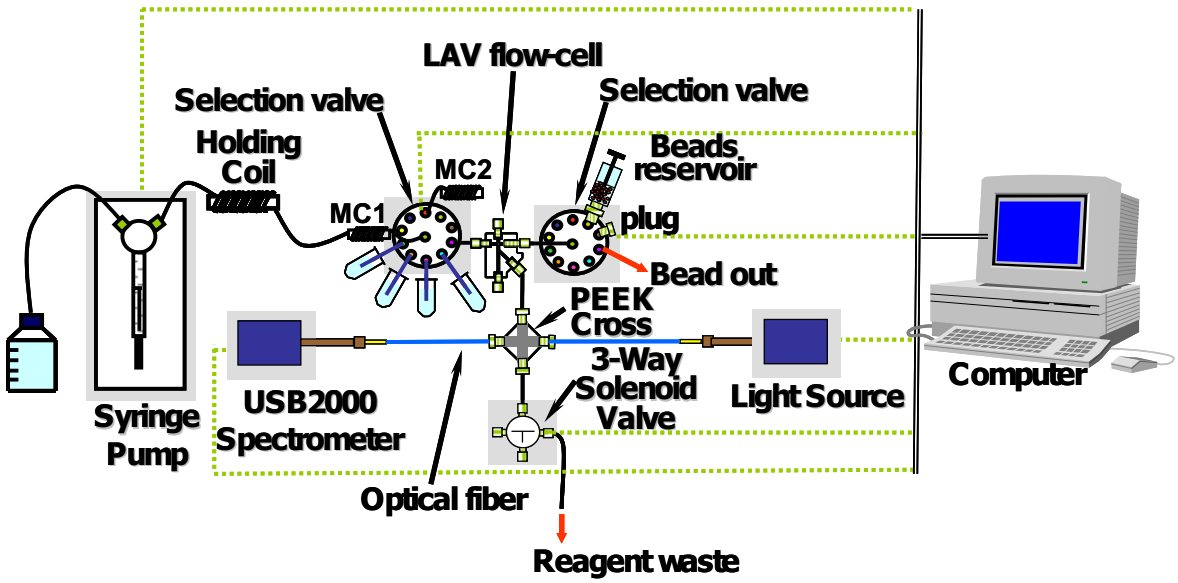

Figure 2 Schematic diagram of the SI-LAV system

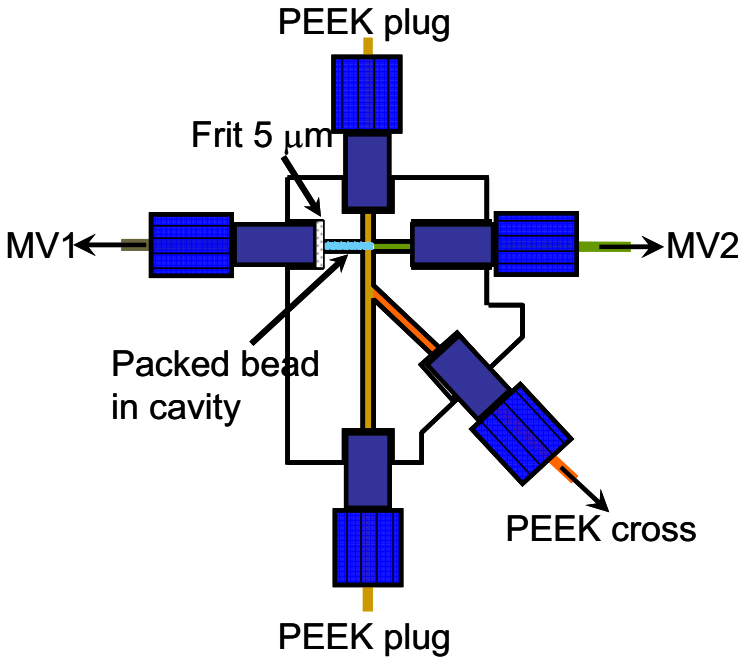

Figure 3 LAV-flow cell

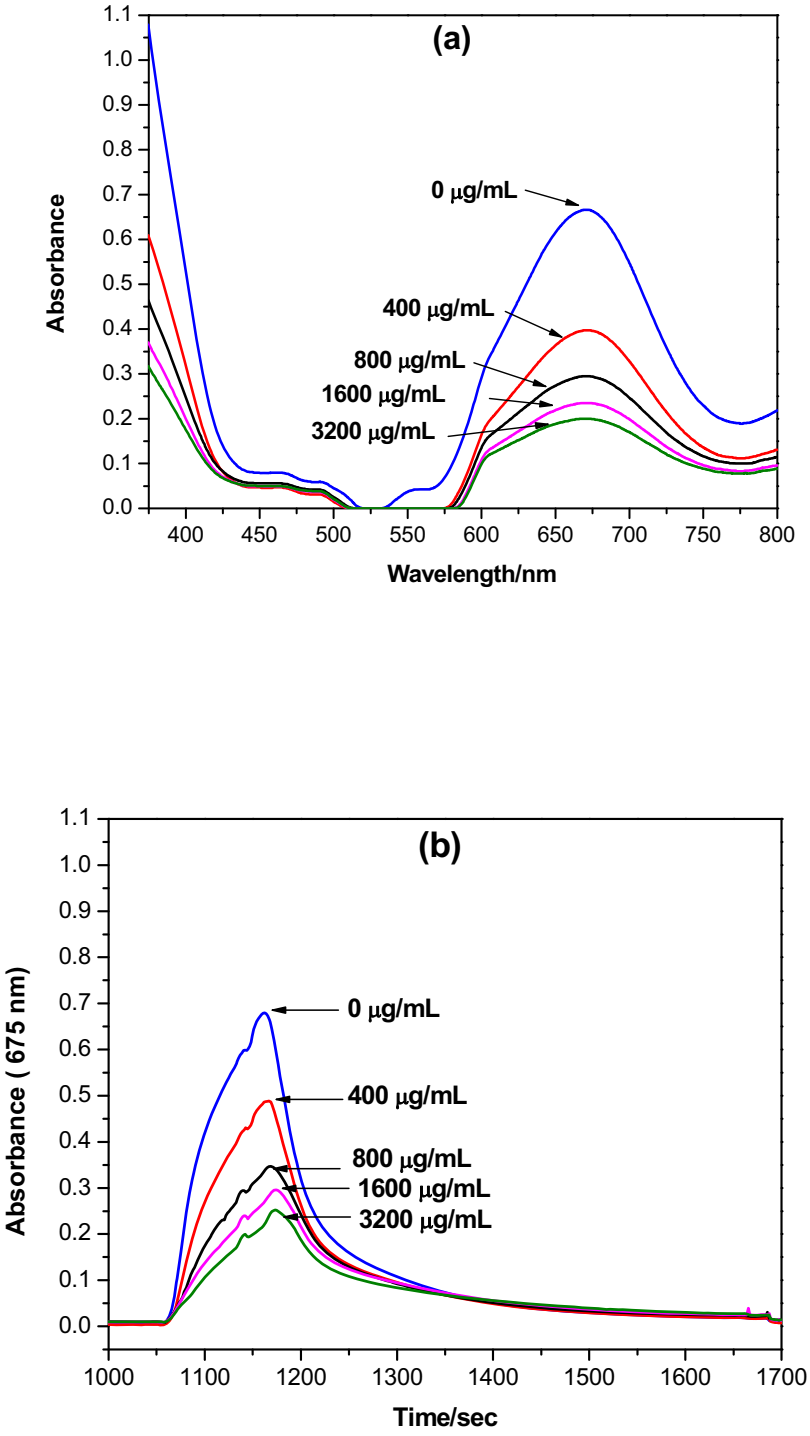

Figure 4 (a) Spectra at peak maximum from different C6S standards competed, (b) SI profiles

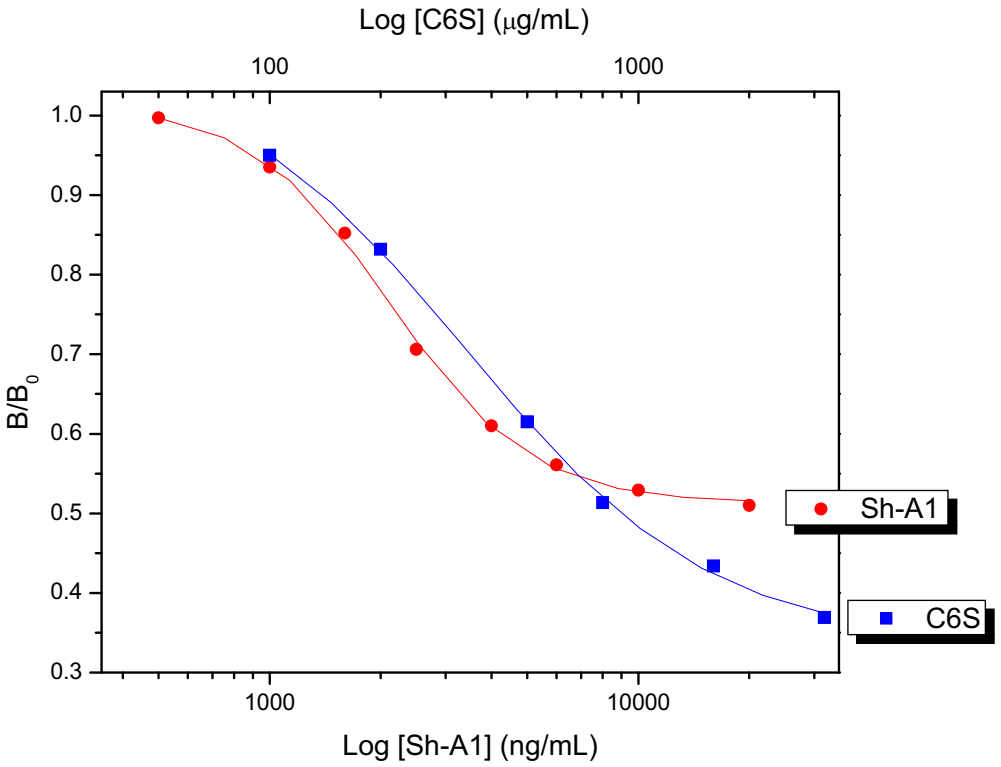

Figure 5 Competitive binding curve for Shark A1 (Sh-A1) and chondroitin 6-sulfate (C6S) standards

THE FLOW-MICROPARTICLES BASED IMMUNOASSAY SYSTEM

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Keywords: Microparticles; Immunoassay; Flow system

This presentation will be discussed on utilizing microparticles as mobile solid-phases for immunoassay. The larger surface area/volume of such microparticles as compared to a microtiter well-plate makes immunoassay ease of miniaturization and mobility with high sensitivity and rapidity. Investigation on some flow systems will be discussed. Antibody against specific proteoglycans is chosen to be a model for flow-enzyme liked microparticles based immunoassay system. Some selected microparticles, specific antigen, specific antibody, peroxidase-conjugated antibody and peroxidase substrate were employed for flow systems with spectrophotometric detection. Performance of flow-microparticles based immunoassay systems and advantages will be discussed.

(1) E. Diamandis, T. Christopoulos (Eds.), Immunoassay, Academic Press, San Diego, 1996.

Development of Sequential Injection Lab-at-Valve-Bead Immunoassay System for Chondroitin 6-Sulfate

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This work introduces a development of a sequential injection-lab-at-valve (SI-LAV) immunoassay system. The SI-LAV system provides microfluidic handling ability to manipulate the bead, sample and reagents required to perform an immunoassay. Chondroitin 6-sulfate (C6S) assay is important for diagnosis of cartilage disease. The C6S analyte is mixed with a fixed amount of C6S specific antibody. The analyte in a sample competes with the C6S coating on beads for the binding with the specific antibody. The C6S coating beads and the reagents are aspirated and traped in a specially designed device attached to a selection valve. Flow manipulation is made using a syringe pump. The investigation on assay conditions will be discussed.

EXPLOITING SIZE-BASED ELEMENT SPECIATION BY GRAVITATIONAL FIELD-FLOW FRACTIONATION COUPLED WITH ICP-MS. **Rattikan Chantiwas**, Institute for Science and Technology Research and Development, Chiang Mai University, Muang, Chiang Mai 50200 Thailand, Siripat Suteerapataranon, School of Science, Mae Fah Luang University, Muang, Chiang Rai 57100 Thailand, Horst Geckeis, Institut für Nukleare Entsorgung, Postfach 3640, D-76021 Karlsruhe, Germany, Ronald Beckett, Water Studies Centre, Department of Chemistry, Monash University, Victoria 3800 Australia, Kate Grudpan, Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200 Thailand; rattikan@chiangmai.ac.th

Gravitational field-flow fractionation coupling with ICP-MS for size-based element speciation of clay mineral particles has been investigated. The mass concentration of particles was monitored by using UV detector. The eluent from the UV detector was merged with Rh standard and then introduced directly into the ICP-MS nebulizer. Mass and elemental based particle size distribution can be estimated under some certain assumptions. The results showed that decrease in efficiency of ICP-MS signal was observed for particles larger than 10 μm. This could be due to either loss of larger particles in the nebulizer or incomplete atomization and ionization of elements in the micronsize- particles larger than 10 μm. In additions, it was observed that band broadening of the element GrFFF-ICP-MS fractograms was apparently greater than that of flow FFF-ICP-MS of nanosize humic substances.

ภาคผนวก ข4

A NOVEL BEAD IMMUNOASSAY-SEQUENTIAL INJECTION SYSTEM FOR AN IMPORTANT PROTEOGLYCAN

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This work introduces a development of a novel sequential flow based-immunoassay system. The sequential injection (SI) system provides microfluidic handling ability to manipulate the beads, sample and reagents to perform an immunoassay. Bead-based immunoassay can be made by a flow manipulated of a required reagent through the beads using the SI system with a simple lab-designed flow-cell. A specific proteoglycan assay is important for diagnosis of cartilage disease. The competitive immunoassay for an important proteoglycan was demonstrated. A proteoglycan analyte was mixed with a fixed amount of a specific antibody, and the analyte in a sample competed with the proteoglycan coating on beads for the binding with the specific antibody. The investigation on assay conditions for developing the system will be discussed.

การพัฒนาระบบบีดอินเจคชันสำหรับการหาปริมาณคอนดรอยติน 6-ซัลเฟต

DEVELOPMENT OF BEAD INJECTION SYSTEM FOR CHONDROITIN 6-SULFATE ASSAY

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บทคัดย่อ: งานวิจัยนี้ได้พัฒนาระบบบีดอินเจคชันสำหรับการหาปริมาณคอนดรอยติน 6-ซัลเฟต (ซีเอสซี) โดยใช้บีดเป็นเฟสของแข็งเคลื่อนที่ในระบบที่ใช้การไหลแบบซีเควนเชียล โดยการหาปริมาณปริมาณคอนดรอยตินซัลเฟต มีความสำคัญในการวินิจฉัยผู้ที่เป็นโรคกระดูก งานวิจัยนี้ได้นำบีดที่เคลือบด้วยซีเอสซีเข้าสู่โฟลเซลล์ จากนั้นฉีดสารผสมของสารซีเอสซีมาตรฐานกับแอนติบอดีที่จำเพาะต่อซีเอสซี แอนติบอดีคู่คอนจูเกตของเปอร์ออกซิเดส และซับสเตรทเปอร์ออกซิเดส ในระดับไมโครลิตรเข้าสู่โฟลเซลล์ และผลิตภัณฑ์ที่เกิดขึ้นบนบีดจะถูกตรวจวัดที่ความยาวคลื่น 630 นาโนเมตร ระบบซีเควนเชียลโฟลนั้น ประกอบด้วย ปั๊มแบบซิรินจ์ ซีเล็กชันวาล์ว สวิทชิงวาล์ว และออพติคอลดีเทคเตอร์ ระบบบีดอินเจคชันที่พัฒนาขึ้นนี้เป็นระบบกึ่งอัตโนมัติ จากผลการทดลองเบื้องต้น พบว่าได้ช่วงความเข้มข้นของสารซีเอสซีมาตรฐาน 500-6000 ไมโครกรัมต่อมิลลิลิตร (ค่าสมการถดถอยเชิงเส้น 0.99) และความสามารถในการทำซ้ำ (ที่ความเข้มข้นของซีเอสซี 1000 ไมโครกรัมต่อมิลลิลิตร, จำนวน 4 ครั้ง) ซึ่งแสดงโดยค่าเปอร์เซ็นต์ของความเบี่ยงเบนมาตรฐานสัมพัทธ์เป็น 5 เปอร์เซ็นต์

Abstract: Bead injection system for determination of chondroitin 6-sulfate (CsC) was developed by utilizing bead as a mobile solid-phase in a sequential flow method. Chondroitin sulfate assay in body fluids is important for diagnosis of cartilage diseases. CsC coated bead was injected to the laboratory-designed flow cell. Then microfluid amounts of a mixture of

CsC standard, CsC specific antibody, peroxidase-conjugated antibody and peroxidase substrate were sequentially introduced. The product of colored beads was monitored in real time at 630 nm. The sequential flow system composed of a syringe pump, a selection valve, a switching valve and an optical detector. The developed bead injection system was operated semi-automatically. Calibration range of 500-6000 µg/mL CsC (R, correlation coefficeint = 0.99) and reproducibility (1000 µg/mL CsC, n=4) of 5 %RSD were obtained.

Methodology:

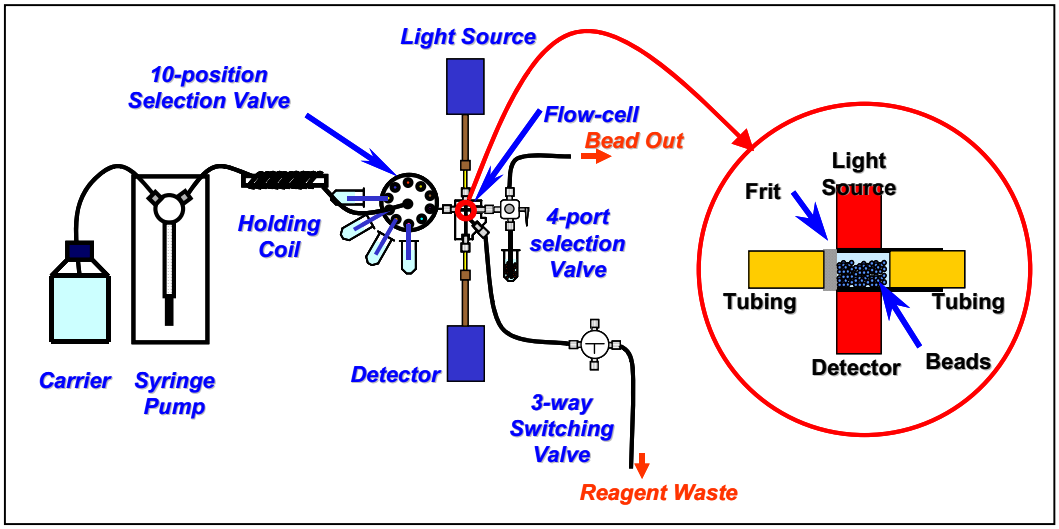

Figure1. Scheme diagram of the developed bead injection system.

Results, Discussion and Conclusion: Bead injection system based on competitive immunoassay was developed for the determination of CsC. Calibration for CsC is demonstrated in Figure 2. Reproducibility (1000 µg/mL CsC, n=4) of 5 %RSD were obtained. The system will be further developed for fully-automatic operation and will be optimized to improve sensitivity.

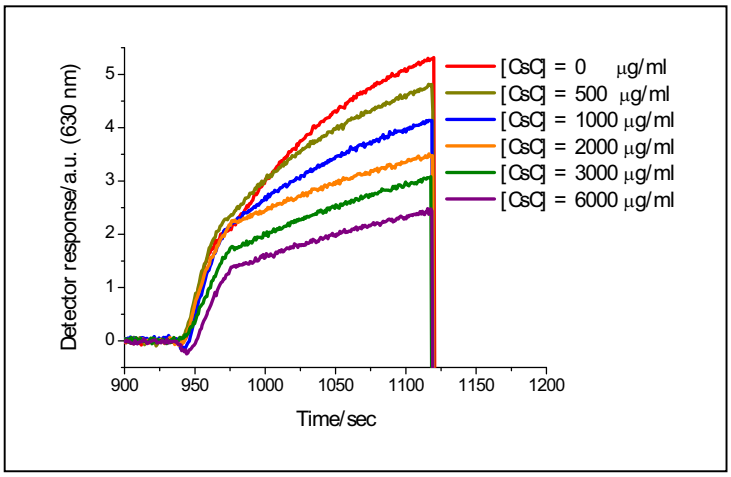

Figure 2 Profiles of bead injection for different chondroitin 6-sulfate concentrations

Acknowledgement: The Post-doc Research Grant to RC and the Senior Research Scholar to KG from the Thailand Research Fund (TRF) are acknowledged for financial support, the Department of Chemistry, Faculty of Science, Chiang Mai University is thanked for laboratory facilities.

References: [1] E. Diamandis, T. Christopoulos (Eds.), Immunoassay, Academic Press, San Diego, 1996.

[2] A.D. Carroll , L. Scampavia , D. Luo , Åke Lernmark and J. Ruzicka, *Analyst*, 2003, 128 (9), 1157 - 1162

Keywords: Bead Injection, Sequential Flow System, Chondroitin 6-Sulfate