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Developments in Protein Assays with Flow Injection/Sequential Injection Techniques

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Abstract

The development of flow-based techniques, including flow injection and sequential injection analysis, offers an excellent on-line alternative with respect to conventional wet chemical assays with the merits of promoted automation, reduced sample/reagent consumption, minimized sample contamination, improved accuracy and analysis throughput with programmable fluidic manipulations. The superb compatibility to external equipments, designable flow manifolds and enclosed analytical environment make flow-based technique a promising potential platform for the bioassays. In the present mini-review, the up-to-date applications of flow injection/sequential injection approaches in protein assays including automated quantification, on-line qualitative separation, adsorption and extraction, activity investigations and immobilization studies are summarized, and future perspectives are discussed.

Keywords flow injection, sequential injection, protein analysis, bioassays

1 Introduction

Flow injection analysis (FIA), which obtains analytical data from dynamic processes taking place in a flow manifold, has been developed into a versatile and powerful platform for wet chemistry analysis with the merit of automated fluidic manipulations, reduced sample/reagent consumption, minimized sample contamination, improved analysis throughput, and the increasing applications of FIA in quantitative/qualitative assays are evident from the recent literatures [1,2].

Sequential injection analysis (SIA), the updated FIA technique proposed in 1990, further broaden the application fields of flow-based techniques for its ability to accomplish various analytical assignments with a similar physical configuration [3]. The bead injection (BI) concept based on SIA protocol even offers a more flexible way for microbeads manipulation in flow-based manifolds and the automated transportation of solid materials-adsorbents within the flow system well facilitates their renewal whenever necessary and provides a high degree of repeatability when metering, packing and perfusion of beads with samples and reagents [4]. The recently proposed lab-on-valve (LOV) system on the basis of sequential injection and bead injection brings to flow-based analysis the third generation, which significantly facilitates integration of various analytical units into the valve and provides great potential for miniaturization of the entire system[5].

In an automatic mode, the FI/SI systems play very important roles as replacements for the labor-intensive manual procedures. The characteristics of simple instrumentation, ease of operation, reliability, low running cost and performed in an enclosed environment make FI/SI techniques among the most suitable approach for bio-assays, especially when expensive and rare sample and reagent are processed. The proliferous applications exploiting FI/SI techniques in protein assays, including automated quantification, on-line separation, adsorption and extraction, activity investigations and immobilization studies well demonstrates the vast potential of these on-line protocols and the up-to-date progresses in these fields are reviewed as detailed in the following.

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2 Automated Quantitative Assays

Various automatic FI/SI approaches coupling with different detection techniques including chemiluminescence, fluorescence, resonance light scattering, spectrophotometry, amperometry and mass spectrometry have been adopted for the quantification of proteins in various sample matrices. As a comparison to the conventional manually operated analytical procedures, the above mentioned on-line protocols provide much faster assays with economic consumption of total protein species as well as reagents.

2.1 Chemiluminescent detection

Chemiluminescence is a very sensitive detection technique most suitable for hyphenating with flow-based experimental designs and various chemiluminescent reactions have been exploited for on-line detection of proteins [6-10]. The most important key point to the success of achieving satisfactory selectivity in chemiluminescence detection is the choice of appropriate reaction system and the manipulation of the chemical conditions in order to yield a response from only the interested species. Costin *et al* proposed a FI chemiluminescence protocol for the selective detection of six amino acids in the presence of other co-existing species [11]. High selectivity was achieved by the application of different chemiluminescence reaction systems in addition to the manipulation of the reaction conditions/experimental parameters where a certain amino acid gives rise to a response only to a particular reaction. This approach offers significant advantages over conventional methods as in such a case it is not necessary to perform derivatization, separation or extraction of the analytes, which dramatically reduced the analysis time and improved the accuracy of the assays.

2.2 Fluorimetric detection

A sensitive procedure for the quantification of total protein species in human serum was presented with sequential injection sampling and fluorometric detection, based on the rapid reaction between fluorescamine and primary amino acid [12]. A few microliters of sample and fluorescamine solutions were employed to facilitate the reaction of protein with fluorescamine by giving rise to a blue-green–fluorescent derivative, which was afterwards excited by a 400 nm radiation, and the emitted fluorescence was monitored at 470 nm. By loading a 5.0 μl of sample solution and a 4.0 μl of fluorescamine solution (0.075%, m/v), a linear calibration graph was obtained within the range of 0.3-12.5 μg ml^{-1}, along with a much improved detection limit of 0.1 μg ml^{-1} as compared to 10.0 μg ml^{-1} achieved by using a conventional manually operated procedure based on the same reaction system.

A SI renewable surface heterogeneous fluorescence immunoassay system with a chip-based micro-flow-through cell had been developed for the determination of human IgG in serum by Zhu *et al* [13]. The immobilized antibody was prepared by conjugation of sheep anti-human IgG antibody to protein-A coated Sepharose-4B beads. Fluorescein isothiocyanate labeled anti-human IgG antibody was then used as the second antibody. The immobilized antibody beads, serum and the second antibody were sequentially injected into the chip-based micro-flow-through cell where a sandwiched antibody-antigen conjugate with fluorescence probe was formed and the fluorescence intensity was monitored in the cell via optical fibers. After the measurement, the used beads were discharged and the cell was ready for the next operation cycle. In this system, a detection limit of 0.1 mg l^{-1} IgG was achieved at a sample-throughput of 11 per hour. RSDs of 1.7% and 5.2% were observed for intra-day and inter-day determinations of serum samples containing 3.9 mg l^{-1} IgG.

2.3 Resonance light scattering

Resonance light scattering (RLS) technique has been developed to be a very useful way for the quantification of analytes by measuring the intensity of light scattering with a common spectrofluorometer. However, the reproducibility of general RLS method is not satisfactory for the unstable or un-equilibrated dye-staining systems. The flow-based analytical techniques, which achieve analytical data from the dynamic processes, provide a satisfactory resolution to the above mentioned dilemma and therefore a series of on-line RLS procedures have been developed for the determination of proteins [14-17].

Based on the enhancement of the RLS intensity of Eriochrome Black T (EBT) in the presence of protein in an acidic medium, Li *et al* reported an on-line FIA-RLS procedure for protein determination [18]. Human serum albumin (HSA) and bovine serum albumin could be quantified in the concentration ranges of 7-36 μg ml^{-1} and 8-44 μg ml^{-1} respectively. Most importantly, the reproducibility of the system was greatly improved attributed to the accurate fluidic manipulations of FIA. For instance, the average RSD value of general RLS procedure was 4.82% for 20 μg ml^{-1} EBT-HSA system, whereas the average RSD value of FIA-RLS was only 0.76% for the same reaction system. This illustrated that the FIA-based RLS approaches was more stable than the general RLS method.

2.4 Spectrophotometric detection

As the third generation of flow injection analysis, the commencement of the lab-on-valve (LOV) concept provides a potential miniaturized protocol for the bioassays attributed to its unique characteristics for the design of flow manifold. The permanently incorporated multi-purpose flow cell in the LOV system allows a series of miniaturized fluidic operations to be performed conveniently. Furthermore, in-valve spectroscopic measurements could be readily conducted in order to monitor the reactions taking place inside the flow cell by the communication of optical fibers and the optical length in the flow cell could be readily adjusted by manipulating the relative positions of the optical fibers in order to control the sensitivity for different species [19].

A mesofluidic LOV system had been adopted by Chen *et al* for the spectrophotometric determination of protein contents in human serum, urine, milk, and yoghourt [20]. By using 20 μl sample and 4.0 μl reagent solutions, a linear calibration curve was obtained within the range of 12.5-200 μg ml^{-1} of protein along with a sampling frequency of 60 per hour. This LOV procedure for the quantification of protein not only significantly reduced the sample and reagent consumption, but also offered an improved analytical frequency and minimized the risk of sample cross contamination, at the same time provided an automatic, rapid, and accurate protocol for protein assays.

The accurate manipulations of fluidic flow and microcarrier beads in LOV system also well facilitate the in-situ monitoring of spectroscopy change on the surface of the microbeads. Two different detection modes, namely micro-affinity chromatography (μ-AC) and micro-bead injection spectroscopy (μ-BIS), had been applied to the determination of protein by using a same LOV system with IgG as a model analyte [21]. As illustrated in Figure 1, the beads are retained up-stream in the flow cell of the LOV system. The absorbance of the eluted analyte solution was then monitored post-column in the μ-AC mode, while the spectral changes of the bead surface was detected in the μ-BIS mode. When employing a longer light path by monitoring the absence of light scattering in the μ-AC, a higher sensitivity is achieved as compared to that obtained in the μ-BIS, that is, the limit of detection of the μ-AC technique is determined to be 5 ng μl^{-1} IgG, and that of the μ-BIS technique was 50 ng μl^{-1}.

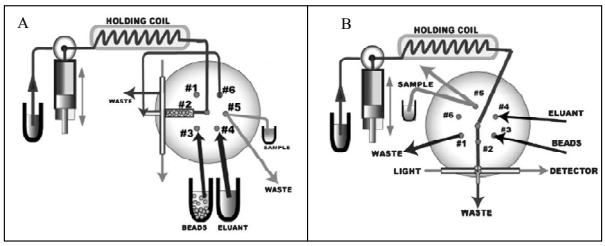

Figure 1. The schematics of μ-AC(A) and μ-BIS(B) systems. Both μ-AC and μ-BIS were based on a micro-sequential injection device composed of a syringe pump, a two-way valve, a holding coil, and a lab-on-valve (LOV) manifold mounted on a six-port multi-position valve. [From Ref.21, Y. Gutzman et al, Analyst, 2006, 131, 809-815, by permission of the Royal Society of Chemistry]

2.5 Amperometric detection

Based on the electrocatalytic oxidation of cysteine at a pretreated platinum electrode, an electrochemical detector was established for the selective determination of cysteine in amino acid mixtures and human urine samples with detection by bi-amperometry in a simple flow injection manifold. A sampling frequency of 180 h^{-1} and quantification of cysteine in some real-world samples without any sample pretreatment procedure were achieved [22]. The assay of cysteine has also been performed by employing a FI amperometric detection system based on the reaction of amino acids with chloramine-T [23]. A linear calibration graph was obtained with a cysteine concentration up to 10 μg ml^{-1}, along with a sampling frequency of 220 h^{-1}.

Nanjo *et al* proposed a FI system with an enzyme reactor for the measurement of fructosyl amino acids and fructosyl peptides in protease-digested blood samples with an inert support as enzyme reactor with a hydrogen peroxide electrode. The proposed FIA system gave rise to a linear response to the concentration of the fructosyl valine over the dynamic range of 7.8×10^{-6} to 5.8×10^{-4} mol l^{-1} [24].

2.6 Mass spectrometric detection

The performances for FIA on-line sample pretreatment techniques followed by detection with electrospray ionization mass spectrometry (ESI-MS), tandem mass spectrometry and electrospray ionization high-field asymmetric waveform ion mobility mass spectrometry (ESI-FAIMS) had been investigated for the determination of underivatized amino acids [25]. The experimental results showed that ESI-FAIMS-MS gave rise to somewhat improved sensitivity and significantly better signal-to-noise (S/N) ratio when compared to those achieved by conventional ESI-MS, this was mainly due to the elimination of background noise and being able to partially or completely resolve all the potential isobaric overlaps arising from amino acids, which implies that ESI-FAIMS-MS being the preferred method for the quantitative analysis of proteinogenic amino acids in real world sample matrices.

Ogata *et al* reported a fully automated affinity chromatography system using a LOV apparatus coupled to an ESI-MS for the simultaneous measurements of multiple ligand affinities to proteins [26]. The LOV apparatus used in this study achieved fully automated, precise, and repetitive reloading of beads with immobilized protein at a well-designed column capacity, infusion of a precise sample volume, and delivery of the eluted solution into the mass spectrometer. In this automated LOV mode, 1.0 mg of protein was sufficient for 35 repetitive analyses and equilibrium dissociation constants (K_d) could be determined in the range of 10^5-10^7 mol l^{-1}, which offered a very useful platform for screening compounds in the low-micromolar range and has extensive versatility to adjust the dynamic range for other measurements.

As a summary, the detailed applications of some flow-based techniques in the automated quantification of protein are highlighted in Table 1.

3 On-line Qualitative Separation

3.1 Capillary electrophoretic separation

Since the development of FIA, quite a few investigations have been directed to the hyphenation of flow injection analysis with capillary electrophoresis (CE) by adopting a suitable interface [27], and this hyphenation approaches have greatly enhanced both sample injection and separation efficiency. In addition, the reduced consumption of sample and reagent as well as their ability to the separation and analysis of small molecules in complex sample matrices opened a promising avenue for the applications in biochemistry.

A FI-based split-flow sample introduction system was developed and coupled to a CE system through a falling-drop interface [28], as illustrated in Figure 2. Sequential introduction of a series of sample solutions of 3.3 μl gave rise to a carryover of 2.5% at a sampling throughput of 48 h^{-1} with samples containing a mixture of fluorescein isothiocyanate (FITC)-labeled amino acids.

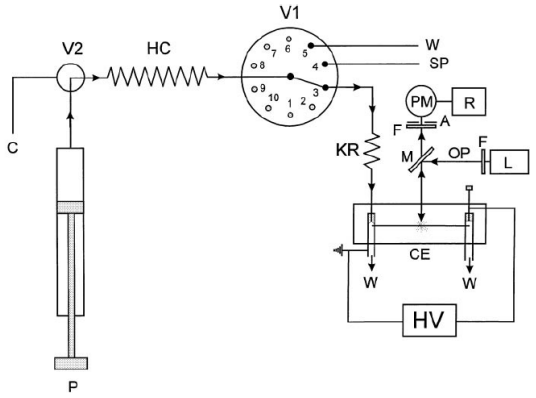

Figure 2. Schematic diagram of the SI-sample introduction microfluidic CE system. [From Ref.28, Q. Fang et al, Anal. Chim. Acta, 1999, 390, 27-37, by permission of the Elsevier Science Publishers]

A FI micro-chip based capillary electrophoresis system for the separation of amino acids was afterwards developed by the same group of authors by using a similar split-flow sampling interfacing unit integrated onto a micro-chip [29]. A sampling throughput of up to 144 samples per hour was achieved, along with a 2% carryover and a precision of 3.2% RSD by continuously introducing a series of 30 μl of sample solutions containing the mixture of FITC-labeled amino acids.

An on-column polymer-imbedded graphite inlet electrode for capillary electrophoresis coupled on-line with a flow injection system by using a poly(dimethylsiloxane) interface was described with a newly designed electrode providing high voltage to the electrophoretic separation [30]. The electrode consisted of a conductive polyimide/graphite imbedded coating immobilized onto the capillary electrophoresis column inlet. This integrated electrode gave the same separation performance as a commonly used platinum electrode. The on-line FIA-CE system was used with electrospray ionization(ESI)-time of flight(TOF)-mass spectrometric detection. The authors validated this hyphenation technique by successful separation of three peptides(methionine-enkephalin, neurotensin, and substance P) in an electrolyte consisting of 50% formic acid/ammonia and 50% acetonitrile.

The on-line coupling of SI and CE via an in-line injection valve for automated derivatization of amino acids and peptides had recently been described [31]. Dichlorotriazinylamino -fluorescein serves as the derivatization agent, enabling sensitive laser-induced fluorescence detection of the derivatives. When using des-Tyr(1)-[Met]-enkephalinamide as the model analyte, on-line electrophoretic analysis was achieved. Glycine has been selected as the internal standard in order to correct for variations in the reaction time and filling of the injection loop. For enkephalin, good reproducibility, linearity and a favorable limit of detection of 30 ng ml^{-1} were achieved.

Table 1 Summary of flow-based techniques for automated quantification of proteins

Detection technique	Analyte	matrix	Linear range	Detection limit	Analytical frequency	Refs.
Chemiluminescence	Glycine and Arginine	pharmaceutical formulations	1-30 mg l^{-1}	0.20 mg l^{-1} for Glycine 0.25 mg l^{-1} for Arginine	115 h^{-1}	6
	Tryptophan	pharmaceutical preparations and human serums	6.0×10^{-7}-3.0×10^{-5} mol l^{-1}	1.8×10^{-7} mol l^{-1}	50 h^{-1}	7
	L-cysteine	human urines	0.2-80 μg l^{-1}	0.1 μg l^{-1}	60 h^{-1}	8
	Albumin	human serum and urines	1.02-12 mg l^{-1}	0.38 mg l^{-1}		9
	HSA	Human urines	0.05-24.0 μg ml^{-1}	0.03 μg ml^{-1}		10
	Proline, Tyrosine, Histidine, Arginine, Phenylalanine and Trytophan	mixture of 20 naturally occurring amino acids	1×10^{-8}-1×10^{-5} mol l^{-1}	4×10^{-9} mol l^{-1} for Proline 1×10^{-8} mol l^{-1} for Tyrosine 4×10^{-7} mol l^{-1} for Histidine 1×10^{-7} mol l^{-1} for Arginine 7×10^{-6} mol l^{-1} for Phenylalanine 2×10^{-6} mol l^{-1} for Trytophan		11
Fluorometry	Total protein	human serums	0.3-12.5 μg ml^{-1}	0.1 μg ml^{-1}	40 h^{-1}	12
	IgG	human serums	0. 3-7. 0 mg l^{-1}	0.1 mg l^{-1}	11 h^{-1}	13
Rayleigh light scattering	Total protein	human urine and serums	7.0-70.0 μg ml^{-1}	3.75 μg ml^{-1}	26 h^{-1}	14
	Total protein	human serums	0.50-32.00 μg ml^{-1} for HSA 2.00-36.00 μg ml^{-1} for BSA	0.11 μg ml^{-1} for HSA 0.85 μg ml^{-1} for BSA	90 h^{-1}	15
	Total protein	human serums	0.005-18 μg ml^{-1} for HSA 0.008-16 μg ml^{-1} for BSA	5 ng ml^{-1} for HSA 7.8 ng ml^{-1} for BSA		16
	Total protein	human serums	7-36 μg ml^{-1} for HSA 8-44 μg ml^{-1} for BSA	0.882 μg ml^{-1} for HSA 2.507 μg ml^{-1} for BSA	90 h^{-1}	18
Spectrophotometry	Total protein	human serums, urine, milk and yoghourt	12.5-200 μg ml^{-1}	5.6 μg ml^{-1}	60 h^{-1}	20
	IgG		0.1-1.0 μg μl^{-1}	5 μg ml^{-1} (μAC)		21
			0.1-0.4 μg μl^{-1}	50 μg ml^{-1} (μBIS)		21
Amperometry	L-cysteine	amino acid mixture and urines	4×10^{-7}-4×10^{-5} mol l^{-1}	1×10^{-7} mol l^{-1}	180 h^{-1}	22
	Cysteine and Methionine	pharmaceutical and veterinary samples	0.2-10 μg ml^{-1} 0.6-30 μg ml^{-1}	0.06 μg ml^{-1} for Cysteine 0.10 μg ml^{-1} for Methionine	220 h^{-1}	23
	Valine	protease-digested blood sample	7.8×10^{-6}-5.8×10^{-4} mol l^{-1}			24
Mass spectrometry	Proline	yeast samples		2.0 ng ml^{-1}		25

Wu *et al* reported the successful hyphenation of μSI-LOV with a CE system, where LOV is treated as the sampling "front end" for the CE set-up [32], and this integrated μSI-LOV-CE system is adopted for *in situ* protein derivatization [33]. All the necessary micro-fluidic manipulations such as sampling, fluorogenic labeling, and CE capillary regeneration are automatically performed by the μSI-LOV unit. On-line fluorogenic derivatization of Islet proteins (insulin, proinsulin, and c-peptide) are carried out with fluorescamine and then successfully separated by the CE system with fluorometric detection. A precision of 1.3% RSD for peak area, 0.5% RSD for electro-migration time, and 2.8% RSD for peak height were achieved with 3.45 mmol l^{-1} insulin.

3.2 Sequential chromatographic separation

The use of programmable flow for chromatographic separation was originally proposed by Satinsky *et al* to the assay of methylparaben, propylparaben, and butylparaben in a pharmaceutical preparation [34]. Later on, the sequential affinity chromatography miniaturized within a LOV system was exploited by Erxleben and Ruzicka for the separation of mouse IgG, chicken IgG and bovine serum albumin [35]. Instead of the 1.0 ml columns used in the traditional analytical affinity chromatography, a 10 μl renewable column was adopted in this miniaturized LOV chromatography system. Downscaling and integrating a column within the LOV module offered prominent improvement in the sensitivity, i.e., a 60-fold increase in the detection limit of mouse IgG was obtained with this miniaturized renewable column compared to that of conventional 1.0 ml column (Hi-Trap ProteinA) mounted on the external fitting of the LOV flow cell, in which extensive dilution of the analyte was generally inevitable by the volume eluted from external column. At the same time, shortened analytical time and reduced consumption such as an assay cycle of less than 2 min and a reduced waste volume of 2 ml were achieved. In contrast, traditional affinity chromatography carried on 1.0 ml column consumed 30 ml of mobile phase and took 30 min to complete.

4 On-line Adsorption and Extraction

By incorporating accessory reactors into the flow lines of a flow injection/sequential injection system or at the external multi-position valve ports of a SI system to accommodate and handle solid materials as an integral requirement for the chemical assay provide a potential protocol for the automation and miniaturization of solid-phase based assays, which have been well demonstrated in the on-line adsorption and/or extraction of proteins of interest.

Zacharis *et al* reported a sequential injection manifold incorporated with a monolithic strong anion-exchanger disk for on-line drug-protein interaction studies [36]. The strong retention of the protein on the monolithic strong anion exchanger and the release of the free form of the drug was achieved easily by the manipulation of the programmable fluidic flow freely in the low pressure SIA system.

On-line solid-phase extraction is the predominant sample-processing method that has been growing rapidly as a consequence of its straightforward operation and high separation and preconcentration capabilities [37]. A sequential injection system incorporating a multiwalled carbon nanotubes (MWCNTs) microcolumn had been exploited successfully by Du *et al* to facilitate the online selective extraction of hemoglobin and cytochrome-c from human whole blood [38], as illustrated in Figure 3. Enrichment factors of 11 and 15 for hemoglobin and cytochrome c were achieved under a loading sample volume of 2.0 ml with this on-line extraction system, and the practical applicability of this system was well demonstrated by processing of human whole blood for the selective isolation of hemoglobin. A similar SI manifold has been used for the selective adsorption/extraction of acidic proteins by the same group of authors, incorporating a thin layer of the composite of poly-diallyldimethylammonium chloride and multiwalled carbon nanotubes (PDDA-MWNT) as the adsorption/extraction module. An enrichment factor of 17 for the isolation of human serum albumin from human whole blood was achieved [39].

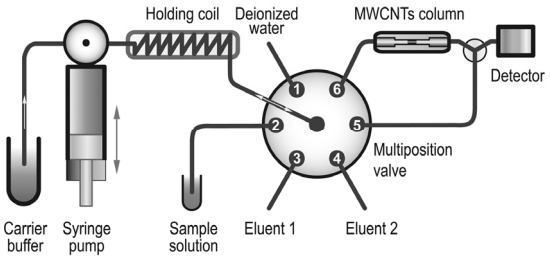

Figure 3. Flow manifold of the sequential injection multiwalled carbon nanotubes (MWCNTs) microcolumn-based solid-phase extraction system for the isolation of basic protein species. [From Ref.38, Z. Du et al, Chem. Eur. J., 2007, 13, 9679-9685, by permission of the John Wiley & Sons, Inc.]

5 Activity Investigations

The transit feature of the signal recorded under thermodynamically non-equilibrated conditions in a flow injection/sequential injection system is most suitable for the measurement of activity-based properties of certain biological species or compounds/reagents. At this point, Itoh *et al* first developed a simple and convenient method for monitoring the activity of a recombinant human matrix metalloproteinase-7 with FI technique [40]. The experimental results indicated that the FIA method was not only simple and fast, but also sensitive enough for screening and analyzing the inhibitory activities of large numbers of test compounds.

A rapid assay protocol by employing FI technique to perform electrochemical oxdization, fluorogenic derivatization, and fluorescence detection for the determination of the activity of the purified catechol-*O*-methyltransferase from porcine liver was described by Aoyama *et al* [41]. It was demonstrated that the kinetic parameters obtained by using this FIA procedure are similar to those derived from a HPLC system, but a much higher sampling throughput was achieved by adopting the flow injection scheme.

Recently, Staggemeier *et al* developed a FIA procedure by coupling with a linear pH gradient system and a dynamic surface tension detection unit (DSTD) for protein surface activity measurement. This system has been established as a high-throughput sampling method for screening the protein surface activity at the air/liquid interface as a function of pH. This method not only provided an innovative approach for probing the pH-induced conformational changes of proteins by exploring surface tension measurements, but also presented a suitable complement for the conventional methodologies based on spectroscopic measurements [42]. This research group also developed a multi-dimensional DSTD system, in a parallel

configuration with a UV-Vis diode array absorbance detector, which was incorporated into the FIA manifold. The system was used specifically for studying the effects of chemical denaturants, such as urea, guanidinium hydrochloride, and guanidinium thyocyanate, on the surface activity of globular proteins at the liquid-air interface [43].

6 Protein Immobilization Studies

Immobilization chemistry is a key component for the successful production of selective supports and the immobilization reactions of six proteins (albumin, ovalbumin, lysozyme, human IgG, ribonuclease A and cytochrome C) on the surface of agarose beads have been studied by using a lab-on-valve-bead injection (LOV-BI) manifold by Ruzicka *et al* [44], by measuring the rate and yield of the coupling reactions. By exploiting the BI technique, the currently recommended protocols for the reductive amination was shortened from several hours to only a few minutes and leakage of immobilized ligands was measured by *in situ* direct spectroscopic monitoring of the captured beads, which suggested that BI spectrophotometry is a useful tool for the quality control of agarose-based chromatographic supports, as well as for the optimization of a wide variety of immobilization chemistries, as used for synthesis of chromatographic supports, immobilization of enzymes, and derivation of bio-sensing surfaces. The real-time monitoring of protein immobilization by using this protocol resulted in the surprising finding that currently used immobilization protocols are far from optimized.

The hyphenation of FIA technique with quartz crystal microbalance (QCM) system offers great potential in proteomics to study affinity among biomarcomolecules. The effect of four protein immobilization approaches including physical adsorption, thioamine thiolation methods, oxidized dextran spacer methods and thiol–gold chemisorption-based self-assembled monolayer (SAM) method on QCM-FIA immunoassay of anti-HSA were investigated by Liu eat al[45]. At a low analyte concentration, the SAM using 11-mercaptoundecanoic acid as QCM surface activating agent generated a larger frequency shift than other immobilization methods, implying that the use of thiolated long-chain fatty acid constructed as SAM may thereby potentially be a useful protein immobilization method in QCM-FIA application.

Conclusions

The flow-based techniques have been well accepted as the indispensable components for automation of the analytical procedure with lower sample/reagent consumption and higher analytical frequency. The programmable fluidic manipulations have clear advantages over the labor-tensive conventional analysis and thus significantly improved the analysis precision. The miniaturized consumption offers an excellent resolution to the dilemma usually encountered in bioassays, such as limited sample sources and expensive bio-chemical reagents.

FI/SI has opened a promise avenue for the automation and miniaturization of solution-based assays and prominent accomplishments have been achieved during the last decades. Nevertheless, the potentials of these on-line techniques are worthy of further exploitation, especially for the processing of biologically interested sample matrices. The on-line sample pretreatment capabilities of the flow-based systems by the integration of various separation modules would provide vast potentials for matrix removal and protein isolation and/or preconcentration, which are most demanded in proteomic investigations.

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References

[1] E. H. Hansen, Flow injection bibliography. http://www.flowinjection.com/

[2] E. H. Hansen, M. Miro, Trend. Anal. Chem., **26**, 18 (2006)

[3] C. E. Lenehan, N. W. Barnett, S. W. Lewis, Analyst, **127**, 997 (2002).

[4] J. Ruzicka, L. Scampavia, Anal. Chem., **71**, 257A (1999)

[5] J. Ruzicka, Analyst, **125**, 1053 (2000)

[6] J. Michalowski, A. Kojlo, Talanta, **54**, 107 (2001)

[7] Y. D. Liang, J. F. Song, J. Pharmaceut. Biomed. Anal., **38**, 100 (2005)

[8] B. X. Li, Z. J. Zhang, M. L. Liu, C. L. Xu. Anal. Bioanal. Chem., **377**, 1212 (2003)

[9] L. Gamiz-Gracia, A. M. Garcia-Campana, F. A. Barrero, L. C. Rodriguez, Anal. Bioanal. Chem., **377**, 281 (2003)

[10] C. B. Huang, K. Zhang, X. L. Liu, S. F. Wang, Luminescence, **22**, 393 (2007)

[11] J. W. Costin, P. S. Francis, S. W. Lewis, Anal. Chim. Acta, **480**, 67 (2003)

[12] X. W. Chen, J. H. Wang, Talanta, **69**, 681 (2006)

[13] H. L. Zhu, H. W. Chen, Z. L. Zhou, Chin. J. Anal. Chem., **32**, 841 (2004)

[14] E. Vidal, M. E. Palomeque, A. G. Lista, B. S. F. Band, Anal. Bioanal. Chem., **376**, 38 (2003)

[15] Y. Li, L. J. Dong, Y. H. Zhang, Z. D. Hu, X. G. Chen, Talanta, **71**, 109 (2007)

[16] K. J. Tan, Y. F. Li, C. Z. Huang, Luminescence, **20**, 176 (2005)

[17] 17 L. J. Dong, Y. Li, Y. H. Zhang, X. G. Chen, Z.D Hu, Microchim Acta, **159**, 49 (2007)

[18] Y. Li, L. J. Dong, W. P. Wang, Z. Hu, X. G. Chen, Anal. Biochem., **354**, 64 (2006)

[19] X. W. Chen, J. H. Wang, Anal. Chim. Acta, **602**, 173 (2007)

[20] X. W. Chen, J. Jiao, J. H. Wang, Chin. J. Anal. Chem., **36**, 1601 (2008)

[21] Y. Gutzman, A. D. Carroll, J. Ruzicka, Analyst, **131**, 809 (2006)

[22] C. Zhao, J. C. Zhang, J. F. Song, Anal. Biochem., **297**, 170 (2001)

[23] M. C. Icardo, O. A. Estrela, M. Sajewicz, J. V. G. Mateo, J. M. Calatayud, Anal. Chim. Acta, **438**, 281 (2001)

[24] Y. Nanjo, R. Hayashi, T. Yao, Anal. Sci., **22**, 1139 (2006)

[25] M. McCooeye, Z. Mester, Rapid. Commun. Mass Sp., **20**, 1801 (2006)

[26] Y. Ogata, L. Scampavia, T. L. Carter, E. K. Fan, F. Turebek, Anal. Biochem., **331**, 161 (2004)

[27] P. Kuban, B. Karlberg, Trend. Anal. Chem., **17**, 34 (1998)

[28] Q. Fang, F. R. Wang, S. L. Wang, S. S. Liu, S. K. Xu, Z. L. Fang, Anal. Chim. Acta, **390**, 27 (1999)

[29] S. L. Wang, X. J. Huang, Z. L. Fang, P. K. Dasgupta, Anal. Chem., **73**, 4545 (2001)

[30] J. Samskog, S. K. Bergstrom, M. Jonsson, O. Klett, M. Wetterhall, K. E. Markides, Electrophoresis, **24**, 1723 (2003)
[31] C. K. Zacharis, F. W. A. Tempels, G. A. Theodoridis, A. N. Voulgaropoulos, W. J. M. Underberg, G. W. Somsen, G. J. de Jong, J. Chromatogr. A, **1132**, 297 (2006)
[32] C. H. Wu, L. Scampavia, J. Ruzicka, Analyst, **127**, 898 (2002)
[33] C. H. Wu, L. Scampavia, J. Ruzicka, Analyst, **128**, 1123 (2003)
[34] D. Satinsky, J. Huclova, P. Solich, R. Karlicek, J. Chromatogr. A, **1015**, 239 (2003)
[35] H. Erxleben, J. Ruzicka, Analyst, **130**, 469 (2005)
[36] C. K. Zacharis, G. A. Theodoridis, A. Podgornik, A. N. Voulgaropoulos, J. Chromatogr. A, **1121**, 46 (2006)
[37] M. Miro, E. H. Hansen, Trend. Anal. Chem., **25**, 267 (2006)
[38] Z. Du, Y. L. Yu, X. W. Chen, J. H. Wang, Chem. Eur. J., **13**, 9679 (2007)
[39] Z. Du, Y. L. Yu, J. H. Wang, Anal. Bioanal. Chem., **392**, 937 (2008)
[40] M. Itoh, M. Osaki, T. Chiba, K. Masuda,T. Akizawa, M. Yoshioka, M. Seiki, J. Pharmaceut. Biomed. Anal., **15**, 417 (1997)
[41] N. Aoyama, M. Tsunoda, K. Nakagomi, K. Imai, Biomed. Chromatogr., 16, 255 (2002)
[42] B. A. Staggemeier, E. Bramanti, C. Allegrini, K.J. Skogerboe, R.E. Synovec, Anal. Chem., **77**, 250 (2005)
[43] E. Bramanti, C. Allegrini, M. Onor, G. Raspi, K.J. Skogerboe, R. E. Synovec, Anal. Biochem., **351**, 100 (2006)
[44] J. Ruzicka, A. D. Carroll, I. Lahdesmaki, Analyst, **131**, 799 (2006)
[45] Y. C. Liu, C. M. Wang, K. P. Hsiung, Anal. Biochem., **299**, 130 (2001)

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