2009 年度　フローインジェクション分析研究懇談会　各賞受賞者

2009 年度 JAFIA の各賞受賞者が下記の通り決定し，2009 年 11 月 27 日（金）に大阪府立大学で開催の第 48 回フローインジェクション分析講演会において表彰されました。

受賞者の方々の栄誉を称え，ますますのご健勝とご研究のご発展をお祈りいたします。

（１）FIA 学術栄誉賞

Prof. Bo Karlberg (Stockholm University, Sweden)

Prof. Ari Ivaska (Åbo Akademi University, Finland)

（２）FIA 学術賞

戸田　敬　氏（熊本大学大学院自然科学研究科　教授）
業績「新規なフロー分析デバイスの開発と環境解析への実応用」

Prof. Petr Solich（Faculty of Pharmacy, Charles University, Czech Republic）
業績「Flow Injection Analysis and Sequential Injection Analysis and Their Applications in Pharmaceutical and Environmental Area」

Assist. Prof. Duangjai Nacapricha（Faculty of Science, Mahidol University, Thailand）
業績「Selective Detection and Determination Methods for Gaseous Substances in Flow-Based Analysis」

（３）FIA 進歩賞

椎木　弘　氏（大阪府立大産学官連携機構　准教授）
業績「流れ系分析における電気的および電気化学的手法を用いた検出器の開発に関する研究」

（フローインジェクション分析褒賞委員会）

2009 年度　　日本分析化学会フローインジェクション研究懇談会
フローインジェクション分析学術賞

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【業　績】　新規なフロー分析デバイスの開発と環境解析への実応用

当初，接触反応を利用したフッ化物イオンの FIA [1]や流れ分析による会合定数の決定・反応機構の解明[2,4]に取り組んだのをきっかけに，以来，流れ分析デバイスの小型化，流れ分析による微量ガス成分の自動測定，流れ分析の環境解析への実応用に従事してきた。それぞれについて概要を紹介する。

1. 流れ分析デバイスの小型化：μTAS が提唱される以前 1988 年にフローインジェクション分析システムを 2 cm 角のシリコンチップに集積化を行った。以降シリコンやガラスチップ上へのフロー系や検出器の集積化や小型化をはかり，新たな機能の創出や特性の飛躍的な向上をはかってきた[9,11]。例えば，リング・ディスク電極を厚み 5 μm の溶液層に配置したガラスチップのフロースルー検出器を考案し，リング電極生成物の 100%をディスク電極で検出することに成功した[6]。反応熱を検出するため熱容量の小さなフロースルー型熱量センサを開発し，酸・塩基量の定量に応用した[3]。ニードル型のイムノアッセイも手掛け，IgG 3 pg の検出限界を得た[9,11]。また，フロー分析に必要な周辺技術の開発も多く，マイクロチャネル内の溶液微小流量を測定するフローセンサや流量制御デバイスの開発を行っている[23]。

2. 微量ガス成分の自動分析：FIA の応用分野として環境分析は大きな柱である。その中でも大気[8,16-18,30]や呼気[19,26,27,31]などガス成分の分析を得意とし，極低濃度のガス成分の測定に取り組んできた。ガスをフロー系の溶液内に取り込む種々のスクラバーを検討し，マイクロチャネル化することでガスの吸収特性を大幅に向上させた。ハニカム型のパターンにマイクロチャネルをアレンジした捕集デバイスはその代表格であり，従来のインピンジャーの 2 万倍の捕集濃縮効率が得られ，ppbv レベルのガスの連続測定を可能にした[22,33]。本ハニカムデバイスは標準ガスの発生にも応用することができる[29]。また，送液部や検出部も小型化したマイクロガス分析システム（μGAS）を提唱した[16]。μGAS は，連続分析だけでなくモバイル分析が可能で，都市部の汚染物質の濃度分布のマッピングを行うことができる。さらにこの気体成分高感度分析法に分析成分の気化を組み合わせて，天然水中微量溶存物質の現場型高感度分析システムを構築した[18,20,25]。本法により, ダム湖の sub-ppb レベルのヒ素を酸化状態別に深さ方向分布の測定に成功した。

3. 流れ分析の環境解析への実応用：　単に分析手法の開発だけにとどまらず，独自の分析法を環境解析に生かしてきた。他のグループで困難であった対象の環境分析を遂行し，オリジナリティのある環境解析に生かしてきた。大気中の微量成分の動態や[14,24,28]，土壌から発生する成分に関する知見[21]などをいくつか明らかにしている。微量ガス成分の現場における連続分析は，大気環境の把握に大きなインパクトがあり，環境分野における国外との共同研究にもつながっている。

（フローインジェクション分析褒賞委員会）

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Research interest: Flow methods (Flow Injection Analysis, Sequential Injection Analysis, Sequential injection chromatography, HPLC, UPLC) in pharmaceutical and environmental analysis; Automation of analytical procedures; Automation of dissolution and permeation tests; Development and validation of novel HPLC methods for determination of impurities in pharmaceuticals; Application of novel trends in stationary phases in HPLC (monolithic columns, sub-2-micron columns); Development of HPLC methods for analysis of markers in biological materials; Environmental analysis of pharmaceuticals in soil and waste water.

Flow Injection Analysis and Sequential Injection Analysis and Their Applications in Pharmaceutical and Environmental Area

Last twenty years more and more strict regulations related to the quality control of pharmaceuticals led to increasing demands on the automation of analytical assays carried out in appropriate control laboratories. At the same time, during more than thirty years of its existence, the FIA technique became a versatile instrumental tool that contributed substantially to the development of automation in pharmaceutical analysis. This can be well documented by a number of reviews on the use of FIA in the analysis of drugs. This pioneer period has been also characterized by construction of FIA analysers (FIA-20) in Czech Republic, later in development of novel methods for their applications for agricultural laboratories and pharmaceutical area.

In the beginning of the 21th century activities on the field of flow methods has turned to the application of SIA system in different areas, again mainly in pharmaceutical area for example for automation of dissolution and permeation tests used in pharmaceutical industry.

In 2003 a new method called Sequential injection chromatography has been firstly described, giving the possibility to analyze several compounds in on run similar as chromatographic techniques, keeping all advantages of low–pressure SIA system available as flexibility and modularity. The method using monolithic columns as a separation modul brings a new possibilities for separation of analytes. New generation of sequential injection analysis has already been consolidated as a good alternative of high performance liquid chromatography for analysis of simple samples. Implementation of short monolithic chromatography column into flow system brings new area of use – on-line chromatographic separation of multi-compound sample in low-pressure flow system.

Application of SIC to the area of pharmaceutical analysis can be divided according to the type of pharmaceutical formulation. For the determination of compounds in liquid pharmaceutical mixtures it is usually not necessary to do sample pretreatment because of the absence of interferents. The ionic compound present usually do not interfere with the chromatographic measurement. Several SIC methods for the analysis of such types of samples have been reported. Ambroxol, methylparaben and benzoic acid were determined simultaneously in various pharmaceutical syrups and drops with salicylic acid as the internal standard. Naphazoline nitras and the preservative methylparaben were determined in eye drops using

ethylparaben as the internal standard. This method involves the simultaneous use of two UV wavelengths to increase the selectivity of analysis. Topical solution containing salicylic acid and triamcinolone acetonide was analyzed with with propylparaben as the internal standard. In the case of betamethasone and chloramphenicol determination in eye drops with propylparaben as the internal standard, a simple sample pretreatment procedure involving extraction into methanol with diluted H_3PO_4 was used.

SIC was also used for chromatographic determination of ambroxol hydrochloride and doxycycline in pharmaceutical capsules with ethylparaben as the internal standard and for the simultaneous determination of paracetamol, caffeine and acetylsalicylic acid in common antipyretic and antiflogistic tablets with benzoic acid as the internal standard. In both methods a simple and fast pretreatment step involving extraction into an organic solvent was required.

Due to interferences in the matrix it is necessary to carry out simple and fast pre-treatment of topical semisolid formulations. Extraction with organic solvent had to be done before the determination of triamcinolon acetonid and two conservants (methylparaben and propylparaben) in a topical cream. Ketoprofen was used as the internal standard, Extraction also preceded the determination of salicylic acid and its ester methylsalicylate in topical pharmaceutical preparations and sodium diclofenac and the conservants methylparaben and propylparaben in a topical emulgel.

Advantages of automated flow methods can be efficiently utilized in monitoring of pharmaceutical process. Franz diffusion cell is often used as a standard vessel for controlling the liberation of active compounds from topical preparations. It consists of two parts—donor part and acceptor part—that are separated by a membrane. The donor compartment holds the drug preparation and the acceptor compartment the receiving medium. For release experiments, normally artificial membranes are used to separate the donor and receptor compartment physically. However, the membrane should allow the active ingredient readily to diffuse receiving the medium as it is “released” from the dosage form and not be rate limiting for the diffusion. Connection of a SIC manifold to Franz diffusion cell has enabled to create fully automated system for the in vitro release testing of composed semisolid dosage forms. This system was used for the determination of two active substances in topical pharmaceutical formulation composed of lidocaine and prilocaine with trimecaine as the internal standard. Samples were taken automatically in 10 min intervals during a 4 h release test.

Latest activities in the development of flow methods have been devoted to the area of sample preparation and manipulation using SIA technique. This promising area of novel instrumentation application of SIA can bring a substantial improvement for automation of sample preparation, thus saving time and cost.

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Selective Detection and Determination Methods for Gaseous Substances in Flow-Based Analysis

Interferences present in samples often contribute severe errors in quantitative analysis. In order to prevent the interferences, samples are usually treated with some specific protocols to separate the analyte from the interfering matrix. For laborious protocols, an on-line sample treatment coupling directly to the measurement manifold or instrument are preferable than the conventional off-line treatment. In regard to this inconvenience, on-line treatment techniques, contemporary used in the flow-based techniques such as in flow injection (FIA) and in sequential injection (SIA) [1], should be considered.

On-line separation of volatile analytes (e.g., NH_3, SO_2, CO_2 etc.) from the sample matrix can be carried out using membrane-based techniques such as gas-diffusion (GD) [2-6], pervaporation (PV) [7-8]. In these membrane techniques, a device is specifically design for separating the gaseous analyte from the matrix. GD and PV devices are both furnished with hydrophobic membrane (usually PTFE) to separate between two aqueous streams (donor and acceptor). Only volatile compounds can diffuse from one side to the other side through the membrane pores, and this provides selectivity in the subsequent detection. Comprehensive reviews of these techniques, including some other membrane-based separations, can be found in references 2 and 3.

Our group has been working on analysis of iodine species, with utilization of GD [9-10] and PV techniques [11]. Use of the membrane also has some drawbacks such as low mass transfer efficiency, short life-time of membrane and therefore not cost-effective. We therefore introduced a new technique with a membraneless device for on-line separation of volatile substance from sample interferences. The technique no longer uses the porous membrane in the separation [12]. This short article contains a very brief tour of the two membrane-based techniques (GD and PV) as well as the latest membraneless technique for on-line sample treatment in flow-based analysis.

1. Membrane-based technique for on-line separation

For a GD device, both the donor and the acceptor streams are touching the membrane at all time. Dirty samples can cause blockage of the pores and reduction in life-time of the membrane. The GD device is not suitable for slurry samples. Taking these problems into consideration, a PV device was therefore introduced by M.D.L. de Castro in 1995 [7]. In the configuration of a PV device, there is always an air-gap to prevent the contact between the membrane surface and the sample [7-8]. This could notably prolong the life-time of the membrane.

2. The recent membraneless technique

Although the PV device seems to function well and it is applicable to direct analysis of slurry samples, the sensitivity is remarkably reduced. The mass transfer from the donor side to the acceptor side is poor for the PV technique due to the thickness of membrane. The membrane sheet must provide sufficient rigidity to stand the pressure from the acceptor to avoid noisy baseline due to vibration of the membrane [13]. We therefore introduced the membraneless vaporization (MBL-VP) technique with some membraneless devices [12] that serve the same purposes to the GD and the PV devices in providing on-line separation of volatile compounds from the matrix.

In the first design, the MBL-VP device had two parallel grooves for donor and acceptor flows. These two grooves are covered with a flat-top acrylic. There is an air-gap present above the two streams so that vapor of the volatile compound can react with the color forming reagent in the acceptor flow. The air above the two liquid streams is virtually a semi-permeable membrane. We first demonstrated the applicability of this concept with direct analysis of ethanol in liquor samples [12], following by analysis of ethanol for the quality control of gasohol fuel [14]. Later we presented a cylindrical shape MBL-VP device for accommodating direct analysis of solid samples such as calcium supplement tablets [15]. Apart from our group, there has been another group working independently on development of a device called 'thin layer distillation', which also gives the separation of the volatile species from sample matrix [16].

Conclusions

The recent MBL-VP technique can be an alternative technique to GD and PV techniques for on-line and selective separation of volatile compounds from sample matrix.

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フローインジェクション進歩賞

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【業　績】流れ系分析における電気的および電気化学的手法を用いた検出器の開発に関する研究

椎木弘君は，分子サイズや形に起因するナノ空間を創製し，分子認識に適用することで高感度な電気的あるいは電気化学的検出法の開発を行っている。その成果は，原著論文 53 編，総説と解説 13 編，著書 5 編にまとめられている。以下，研究概要を示す。

1．金ナノ粒子を用いたナノ空間の構築と高感度電気抵抗型検出器の開発

物質構造をナノスケールで制御または空間的に配置することにより，新しい機能を発現する。同君は，金とチオール分子が化学吸着することに着目し，チオール分子を用いて金ナノ粒子を絶縁基板上に固定化する手法[1)]を確立した。隣り合うナノ粒子はチオール分子により架橋されており，そのサイズにより粒子間ギャップを任意に調節し，二次元膜の電気伝導性を絶縁～導電体まで制御した[2)]。また，粒子間に構築されたナノ空間を利用し，ナノギャップ電極として機能させた。ナノギャップにプローブ DNA を配置し，二重鎖形成に伴う微小な電気抵抗変化を直接取り出すことに成功した[3)]。この現象を利用して電気抵抗型 DNA 検出法[4-6)]を開発し，ラベルフリー，迅速かつ簡便な検出を可能にした。また，金属ナノ粒子と生体分子の相互作用によるナノ構造体の構築[7-10)]においても成果を挙げている。

2．分子鋳型による異性体認識能を利用した高感度電気化学検出器の開発

分子鋳型法の歴史は古く，目的分子と相補する分子鋳型を高分子材料に刷込み分子認識に利用してきた。しかしながら，目的分子自身が有する特異的な形、性質を有効利用していないため，微量な目的分子の高感度センシングは困難であった。同君は，分子鋳型を有する過酸化ポリピロール膜の形成過程を水晶振動子法により追跡し，過酸化処理による脱ドープと同時に膜が硬化され，それにより形成された分子鋳型が高選択的に分子認識することを明らかにした。これを利用し，光学異性体[11)]，構造異性体[12)]，胆汁酸類[13)]，ダイオキシン擬似物質[14)]などについて高感度な電気化学的検出を可能にした。

3．分子鋳型の分子認識と非侵襲計測への適用

生活習慣病に含まれる高コレステロール血症は動脈硬化や狭心症の危険因子であり，合併症のリスクから生体濃度の適正レベルの維持はゆとりある日常生活を送るための必要条件となっている。血中と皮膚に存在するコレステロール量に相関があることが報告されたが，酵素法では低感度であるため皮膚での定量には至らなかった。同君は，アルキルチオールが金電極上に高配向性の自己集合単分子膜を形成することに着目し，コレステロールの分子鋳型を形成した。コレステロール認識に伴う電流応答を読み取ることにより，高感度検出が可能になった[15)]。皮膚コレステロールに着目した新しい非侵襲計測の可能性を示しただけでなく，酵素法に代わる新しい医療計測の概念を確立した。

このほかにも導電性高分子を用いた複合体電極を作製し，神経伝達物質[16)]や ATP[17)]を対象とした高感度・高選択的電気化学式検出器の開発に成功した。

以上，椎木弘君は独自の手法でナノ空間を構築し，分子認識へ適用することで，これまで計測が困難であった物質の高感度計測を可能にした。これらの成果を基に今後，新たな分子認識 FIA の発展に大きく貢献することが期待される。

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