CHIMIA 52 (1998) Nr. 6 (Juni)

COLUMNA ANALYTICA

Column Editor: Prof. Renato Zenobi, ETH Zürich

Chimia 52 (1998) 284–287

ISSN 0009–4293

News and Trends in Analytical Chemistry PITTCON'98 Report: New Orleans, March 1–5, 1998

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Abstract. A personal view of some challenging and remarkable news in general analytical chemistry shown at Pittcon'98 in New Orleans is presented. Mergers of companies, which are involved in the development of instrumentation, have changed the face of the exhibition. Developments forced by the requirements of high efficiency and high throughput in the drug-discovery area are described in the second and third section. Medals awarded by journalists for the most outstanding products are presented in the forth section. Last, but not least, advancements in a more scientific area such as electrochromatography are discussed.

1. The Effect of Mergers

Unlike other years, the Pittsburgh conference 1998 opened on Sunday, March 1, at the Morial Convention Center in New Orleans, LA, and closed on Thursday evening. Surprisingly, the last symposium on Thursday on electrochromatography was well-attended and continued for over an hour after the official closing time. The exhibition opened on Monday morning and closed on Thursday 3 p.m. The face of Pittcon's exhibition has changed recently owing to considerable changes in the ownership structure of instrumental companies. There is no doubt that 1997 and the first months of 1998 involved more changes in the ownership of these companies than at any other time in their recent history. The 'Analytical Instrument Industry Report' (February 25, 1998) lists more than 250 M&A (Mergers and Acquisitions) transactions over the past five years, including more than 85 in 1997. Well-established companies have made acquisitions or entered into alliances which have given them access to technologies and products for the high-throughput screening (HTPS) and drug-discovery markets. Examples are: *Beckman*'s acquisitions of robotics pioneer *Sagian* and *Coulter*, the recapitalization of *Fisher Scientific*, *P&E*'s purchase of *PerSeptive Biosystems* plus a controlling share in *Tecan*, *Waters*' addition of *Micromass*, *HP*'s purchase of *ATI*, and *HP*'s investment in the drug-discovery start-up *IRORI*. Other companies have made acquisitions or entered into alliances in order to be able to offer products which enable them to keep up with the trends and developments in portable instrumentation. An example is *HP*'s purchase of *ATI*, thus acquiring portable, miniaturized GCs. Further, *Perkin Elmer* acquired exclusive rights in *HTK sensors* (quartz microbalances).

ThermoQuest Corporation acquired *Carlo Erba Instruments*, and *Horiba* purchased *ISA* taking it into the Raman sector. This last event was interpreted as possibly signalling a renewed interest in expanding into overseas markets. In addition to these technology-driven acquisitions, other driving forces have been globalization and geographic expansion, increased shareholder values and profits, and enhancing competiveness by achieving or surpassing a critical mass. The outcome of these developments is obvious: A few extremely large booths, such as those of *Perkin Elmer*, *ThermoQuest*, *Hewlett Packard*, *Waters*, *Beckman*, and *Shimadzu*, dominated the exhibition, which also included many medium, small, and very small booths. This, despite the fact that small- and medium-sized companies are supposed to be the driving forces for releasing innovative new technologies onto the market.

The exhibition covered in one single hall an area of $7 \cdot 10^5$ square feet or 65 000 m^2 (*ca.* 570 × 115 m) and hosted 1212 exhibitors. The total of 83 meeting rooms of the convention center occupied 12 654 m^2 and hosted the conference with its 21 parallel sessions. To pass by each booth just once, would have meant hiking over 7 miles or 11 km. Each year, between 30 000 and 33 000 visitors are expected. A Swiss delegation of Messe Basel and the New Swiss Chemical Society (NSCS)/Section of Analytical Chemistry (SACh), represented by *H. Burkhard* and *U.E. Spichiger*, met with the president of the organizing committee of Pittcon'98, *Sarah L. Shockey*, in order to discuss common interests.

2. Instrumentation for Analysis in Drug Discovery: Pieces of a Puzzle?

In the light of the technical acquisitions mentioned above, one technological domain developing rapidly is that of instrumentation which is driven by the needs of the pharmaceutical industry in drug development and high-throughput screening (HTPS). At Comtech'98 in Montreux, *Brian H. Warrington* of *SmithKline Beecham* printed a vivid picture of the situation in the drug-discovery area. Influenced by regulatory restrictions, R&D costs rose and accounted for up to 15% of sales in the 90s, compared to 5–8% in the 80s. A strongly competitive market leads to price restraints, and the fast modification of diseases as well as socioeconomic pressures means that quick development of

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new generics involving novel targets, increased productivity, and reduced cycle times is required. He claimed that the current situation in drug discovery is similar to a puzzle: Innovative and helpful novel techniques are presented and released to the market. However, the links between the pieces of equipment, which would allow full automation of the synthesis including consecutive analytical and biological screening, decision making and feedback control, are missing.

The current drug-screening process consists of combinatorial and parallel synthesis of compounds, consecutive characterization, separation, and purification by liquid chromatography-mass spectrometry (LC-MS), where the fraction collector is controlled according to the mass spectrum and subsequent activity screening. A range of companies committed themselves to the drug-discovery domain and to finding the missing pieces of the puzzle! For automated solid-phase synthesis, *IRORI* (La Jolla, CA) offers radio-frequency-labelled microreactors including a high-speed sorting system which reads the radio-frequency labels and displays the composition of the products on the PC monitor. Previously, it was clinical chemistry that influenced robotics and the miniaturization of equipment. Now the drug-discovery area is exerting a comparable influence. Miniaturization of titer plates has consequences for dosing systems in the context of solvent evaporation and volume accuracy. *Zymark* showed a standalone robotic system of *ca.* 12 m length which is fully protected from the environment and offers pCO_2, humidity- and temperature-controlled conditions. The system makes it possible to work with different types of microwell plate formats involving 96, 385, or 1536 wells, where the last one initializes a technique using 10^{-9} l volumes known as ultra-high-throughput screening (UHTS).

The processes of separation, identification, purification, isolation, and characterization routinely involve **LC-MS** coupling and **on-line proton nuclear magnetic resonance** (^{1}H-NMR) as an emerging technique. Spectra have to be acquired from a small share of the sample volume in the domain of 10^{-6} to 10^{-9} l with high resolution in order to be able to distinguish the target from educts and by-products. Different companies, *e.g., Perkin Elmer/ Perseptive Biosystems*, respond to this demand by modifying existing mass-detecting instruments. The facilities to make a tryptic digest of peptides and to take spectra from the microwell directly by matrix-assisted laser-induced mass spectrometry (MALDI-MS) are now available. The software supports can access the Swiss protein database for the identification of amino acids and sequences.

A new challenge for instrumental analysis has emerged from the on-line coupling of ^{1}H-NMR to liquid chromatography, which then provides more detailed information about the structure of compounds. This is used in particular to identify isomeric compounds with identical mass. The resolution and the mode of operation depend primarily on the amount of substance in the 60 or 120 µl continuous flow cell (the geometry of this cell is a top secret among competing companies), the strength of the magnetic field (300–600 MHz magnets), the homogeneity of the field lines in the microenvironment, the orientation of the solenoid relative to the external magnetic field, and the suppression of baseline signals by using an appropriate software. Currently, the use of 20–30% deuterated solvent mixed with undeuterated one is recommended. On-line ^{1}H-NMR is operated in the stop-flow or continuous flow mode. The continuous flow mode was said to be possible if the amount of substance is >1 mg per cell volume (*H. Senn, F. Hoffmann-La Roche Ltd.*). The major players and competitors in this analytical technique are *Varian* and *Bruker*. An excellent overview on miniaturized ^{1}H-NMR is given by *Dean L. Olson et al.* [1].

3. The Affinity Chromatogram

There is a bottleneck in the automation of the drug-discovery process when it comes to high-throughput screening of the biological activity in order to identify novel leads and novel targets. Referring

again to *Brian H. Warrington* of *Smith-Kline Beecham*, he says that the success rate of investigations of the Structure-Activity Relationship (SAR) is high for receptor-agonist and -antagonist studies as well as for enzyme inhibitors (>85%). It is modest (<15%) in the area of interactions between targets and cytokines, ion channels, and nuclear receptors, as well as for protein-protein interactions. Lead identification is rarely successful with RNA- and DNA-protein interactions or DNA-DNA interactions. To speed up the information yield and to decrease the time it takes to get a product on the market, SAR studies are going to be addressed by high-throughput screening and parallel monitoring using miniaturized analytical techniques.

Assays with labeled compounds are compared with those using no labels. Capillary electrophoresis (CE), miniaturized liquid chromatography (LC), various techniques in mass spectrometry, hybridized and coupled analytical techniques, as well as genomic assays have had an increasing impact on SAR studies. The chromatography forum of the *Delaware Valley Dal Nogare Award*, where *James W. Jorgenson* was awarded a price for 'Exploring the Limits of Resolution in Liquid Chromatography and Capillary Electrophoresis', was dominated by interaction studies. *E.S. Yeung*, Iowa State University, presented studies on single cells using CE as both a separation and temporal monitoring device. *J.M. Ramsey*, Oak Ridge National Laboratories, TN, showed *on-chip* high-speed separations in order to explore the kinetics of interaction processes in the millisecond domain, and also described a concept of implementing and multiplexing PCR on a chip. Optical detection was performed using a focused laser beam and laser-induced fluorescence (LIF).

H. Irth, *ScreenTec BV*, Leiden, NL, showed for the first time an affinity spectrum which is monitored in parallel with the UV spectrum from the HPLC detector and the mass spectrum monitored with an API-quadrupole spectrometer. The affinity spectrum is the product of an *on-line* LC-affinity assay based on competitive antigen-antibody reactions, where either the antigen or the antibody is labeled mostly by fluorescein. Parent compounds and their metabolites in plasma samples were clearly distinguished by reversed-phase HPLC and subsequent post-column addition of the labeled affinity protein reducing the concentration of the organic modifier to 10% after separation. Concentrations down to $2 \cdot 10^{-10}$ M, *e.g.*, in the case of digoxin, were detected [2].

4. Pittcon Awards and Separation Techniques

Based on the votes of journalists spezialized on publications from industry, three products were singled out as outstanding among those exhibited throughout the Pittsburgh Conference and Exhibition:

- Gold: *TA Instruments* and *TopoMetrix* for their microthermal analysis on an SPM probe,
- Silver: *Dionex Corp.* for its *Just add Water EG40* technology,
- Bronze: *Thermedic Detection* for its EZ-Flash 'Fast GC' retrofit package for conventional gas chromatography.

M. Reading, Loughborough University, UK, presented his latest invention, the modulated DSC (Differential Scanning Calorimeter), which was awarded the gold medal, in a talk entitled 'Thermal Analysis for the 21st Century – *μTA*™'. The head of an atomic force microscope (AFM) is fitted with a microtemperature probe which not only provides the heat source, but also measures a response providing information similar to that obtained through traditional thermal analysis – on the microscale. Topography, thermal conductivity and thermal diffusivity images are obtained simultaneously. Depth profiling is obtained by varying the vibration frequency of the cantilever. With a lateral resolution of *ca.* 1 μm, any point on the image can be selected for studying the calorimetric and mechanical properties.

Dionex Corporation's new technology and product involves improved performance of ion chromatography. The *EG40* generates an eluent of high purity *on-line* using only deionized water! The *EluGen*™ cartridge generates pure KOH eluent for anion chromatography and pure MSA (methanesulfonic-acid) eluent for cation separations. The KOH as well as the MSA eluent are generated electrochemically. The cartridge is connected to a bottle containing, *e.g.*, 2 M KH_2PO_4 solution in the case of anion separations which provides potassium cations for KOH production. The operation period is said to be *ca.* 1000 h. The eluent is typically free of carbonate which minimizes baseline shifts and variations in the retention time. The result is improved gradient accuracy, lower detection limits, highly reproducible separations, and improved resolution. The *EG40* eluent generator, which consists of an additional bench-top module (50 cm high, 18 cm wide, 43 cm deep), was presented combined with the *DX-500* system hosting an improved continuous and automated self-regenerating suppressor system.

L.S. Ettre, Yale University, New Haven CT, focused on a so-far 'often neglected parameter in programmed-temperature gas chromatography', namely the rate of the temperature program. He showed that this rate is described by a rather complex function, related among other parameters to the carrier-gas flow rate, the column length and radius, and the film thickness of the stationary phase. He concluded that the reduction in analysis time by increasing the rate of the temperature program is much more significant than the reduction in peak resolution. This means that faster analysis can be done with longer columns at the same peak resolution.

Improvements in gas chromatography have mainly addressed digital inlet and on-column temperature and pressure control of the set points combined with an appropriate software. 'With EZ-Flash, every GC can be a Flash-GC'. EZ-Flash is basically a software where an interface card has to be integrated into the oven of the existing GC. The EZ-Flash module combines this software with a column surrounded by a copper tube or coil. The column is heated up with $30° \, s^{-1}$, from 25 to 350° within 11 s. The columns currently have to be bought from *Thermedic*.

Achieving digital temperature and pressure control allowed *Hewlett-Packard* to make headlines with *Retention-Time Locking* in GC measurements. Retention-time locking addresses the high accuracy and reproducibility of retention times between runs and between similarly configured GC instruments. The gold standard in GC, the *Carlo Erba GC Instrument*, now *CE Instruments*, also makes use of digital pressure and temperature control based on physical sensors in the *TRACE*™ *GC 2000*. The measured parameters are adjusted to the set points by software corrections, which results in highly reproducible retention times with a variation of $s_t = 0.001$ s (1SD). The instrument offers, in addition, a large variety of detectors and injectors.

5. Capillary Electrochromatography (CEC)

Capillary electrochromatography has been the subject of discussions for more than ten years. Nevertheless, during the last three years, the interest in this analytical technique has peaked. In 1997, 44 citations on capillary electrochromatography were traced. At Pittcon'98, a symposium organized by *John G. Dorsey* (Florida State University) was dedicated to CEC instrumentation and separation techniques and entitled 'The Future of Liquid

Chromatography'. CEC is expected to complement high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE), and to provide a means of separating species which are not easily soluble in water and are not resolved by HPLC with high resolution and high speed. The most significant hurdle is thought to be the availability of dedicated instruments and capillaries (for overviews and discussions see [2][3]). However, by combining HPLC and CE, not only are the advantages of each technique combined, but also their problems and drawbacks. It seems that the CEC separation technique, when investigated in detail, is rather complex. From the presentations, it became apparent that a more fundamental understanding of the retention mechanism is needed in order to determine the most relevant influencing parameters, and also to identify significant applications and relevant target compounds to be separated.

In CEC, an electric field (0.5–1.5 kV cm^{-1}) is applied along a packed capillary with an inner diameter between 50–200 μm. The particles of the stationary phase are confined by a frit, *e.g.* from fused wetted silica gel. Sampling is preferably done electrokinetically, which shows relatively poor reproducibility. On the other hand, electrokinetic injection was shown by *C. Rimmer* (Florida State University) to improve the reproducibility of the sample volume. A UV detector (210 or 260 nm) is part of the standard equipment. Coupling to a mass detector seems advantageous since the mobile phase is predominately organic and the column effluent flow rate is smaller than in LC (see [3a]). The advantages of CEC are that capillaries packed with particles sized between 0.5 and 5 μm allow separations within minutes, showing plate numbers of 10^5 per column. *C. Horváth* showed that an interparticulate electroosmotically driven flow (EOF) with its typical flat profile must be assumed in order to explain the high resolution combined with quick separation and relatively short retention times [4]. The flow profiles were discussed by *D.J. Rakestraw* (Sandia Natl. Lab) under the catchy title 'CEC: Is It a Mule, a Donkey, or a Workhorse?'. Relatively short columns of 5–25 cm length packed with particles from classical reversed-phase materials are used for the stationary phase (see LC-GC). Dedicated materials are provided by a few companies such as *Hewlett-Packard* [5]. The stationary phase means that a higher peak capacity than in CE can be achieved, and the selectivity can be modified. A modification referring to molecular-imprinted-polymer stationary phases was reported by *V.T. Remcho* (West Virginia University). Generally, the particle size and the homogeneity of the stationary phase do not have the same impact as in HPLC, which indicates that the influence of the eddy diffusion (A-term of the *Van Deemter* equation) and the mass-transfer resistance (C-term) are reduced [6]. This again suggests a separation principle closer to that of capillary electrophoresis.

The fact that solvent bubbles are generated at the boundaries of the frit, which indicates that Joule heat is produced, was discussed. In order to avoid using a frit, which introduces considerable uncertainty into the basic performance of CEC, *in situ* polymerized stationary phases have been considered. Bubble formation is prevented by applying high pressure to both boundaries of the packed column which are bridged to the sample inlet and the detector by a piece of empty capillary. *C. Horváth* also discussed pressure-assisted CEC to enhance and control the linear velocity of the mobile phase. It is used, he mentioned, 'by people who are afraid that EOF cannot be controlled'. Due to the open segments of the capillary, the net flow is, in fact, a mixed hydrodynamic and electroosmotic flow (EOF). The competition between electroosmotic and pressure-driven flow was discussed by *D.M. Lubman et al.* [7].

In CEC, a mixture of pH buffer and organic solvents (modifiers) are used as the mobile phase. Isokratic and gradient elution separations are feasible. *Horváth* showed that the electroosmotic flow is influenced by the permittivity of the mobile phase rather than by its ionic strength. These claims are supported by *W. Thormann*'s models of the electroosmotic flow in capillary electrophoresis [8]. Even if working with C_{18}-silica capillaries, surface charges brought about by dissociated silanol groups are a prerequisite for generating an electroosmotic flow, which is the driving force behind separation in CE.

Clearly, an analytical technique along the lines of CE, which involves a variety of degrees of freedom, would be desirable. Hopefully, this promising technique will not share the fate of Supercitical Fluid Chromatography (SFC) which turned out to be complex, and to have only a few relevant applications.

It has only been possible to describe a few highlights from Pittcon'98, and a personal impression of trends. Despite the large variety of other conferences and exhibitions, from my point of view, Pittcon remains one of the most attractive events in general analytical chemistry. Looking forward to meeting you personally next year in Orlando, Florida, March 7–11, 1999.

Received: April 21, 1998

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