Advances of Chromatography in Analysis of Complex Samples

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The separation and identification of complex samples is an urgent task that analytical chemistry has to face. Chromatography as an important analysis technique has been widely applied to many fields such as life science\ environment\ medicine\ food and petrochemical engineering. Recently with the progress of science and technology higher and higher requirements have been put forward for the analysis of practical samples. Accordingly chromatography has simultaneously undergone rapid development. Here we would like to make a brief summary on the newest research results of National Chromatographic R. & A. Center and hopefully it could reflect the advances of chromatography in the analysis of complex samples.

1 High resolution separation

1.1 Rapid optimization of separation conditions

Accompanying more applications of high performance liquid chromatography\ HPLC\ optimization of the separation conditions becomes more and more important. The development of an optimization method experiences a process from the “black box” method the empirical method to the theoretical method. In our recent work a new method for the precise prediction of retention time of solutes under linear gradient elution by simultaneously calibrating the effect of delay time of instrument and the distribution of mobile phase in the column on the migration of solutes has been proposed and further validated by the prediction of retention times of amino acids. In addition a systematic strategy for the quick optimization of binary and ternary gradient elution conditions has been brought forward based on which a software package that can be used for the fast optimization of multivariate gradient elution conditions stepwise linear and mixed gradient has been developed and applied to the analysis of complex samples [1][2].

1.2 Multidimensional chromatography based on separation techniques

Highly efficient separation techniques such as gas chromatography GC\ HPLC and capillary electrophoresis CE have been powerful tools for the analysis of complex samples. However the resolution and peak capacity of one dimensional separation are very limited therefore the development of multidimensional separation which can offer high efficiency\ high resolution and high peak capacity is indispensable.

For comprehensive two-dimensional GC\ GC\ a model has been recently developed for predicting the retention of components in two-dimensional 2D separation. Factor analysis has been applied to estimate the orthogonality of the system. In addition GC\ GC and time-of-flight mass spectrometry TOF MS have been hyphenated to obtain more information about unknown compounds. All these methods have been successfully applied to the analysis of complex samples such as cigarette smoke and over 2 200 compounds have been identified [3][4].

Through an automatically controlled eight-port valve a 2D-HPLC system has been set up and applied to the analysis of traditional Chinese medicine\ TCM [5][6]. Various combinations of separation modes have been achieved such as
strong cation exchange[]= SCX[]= and reversed-phase[]= RP[]= columns[]= RP and normal phase columns[]= and affinity and RP columns[]= which could be respectively applied to the profiling and target analysis of complex samples.

Now two-dimensional electrophoresis[]= 2DE[]= has firmly held a central position in proteomics because of its unparalleled resolving power with up to 3 000 – 10 000 proteins visualized in a standard 2D gel format. However[]= its unavoidable drawbacks have undermined the prospects for 2DE as a dominant separation technique in proteomics[]= and stimulated the development of alternative technologies. Recently[]= our research focus is put on the development of the 2D-CE system[^70]. With a microdialysis hollow fiber membrane as the interface[]= several 2D-CE modes were proposed[]= including 2D-capillary isoelectric focusing electrophoresis[]= CIEF[]= - capillary gel electrophoresis[]= CGE[]= 2D-CIEF-capillary non-gel electrophoresis[]= and 2D-CIEF-capillary zone electrophoresis[]= CZE[]. The hollow fiber interface was proved easy to fabricate[]= fast for mass transmission[]= free of complex valves[]= and efficient to transfer the fractions from the first capillary to the second one without loss of samples. With such a platform[]= standard protein mixtures as well as complex proteins extracted from liver cancer cells were separated. Further work on the development of new kinds of interface for 2D-CE is being carried out[^96].

2 High throughput analysis

2.1 Capillary electrophromatography

Capillary electorochromatography[]= CEC[]= is a relatively new micro-separation technique that combines the advantages of HPLC and CE with high resolution[]= high efficiency[]= short analysis time[]= low consumption of both samples and solvents[]= and so on. Therefore[]= it has been seen a rapid development in the past years.

In the fundamental study[]= the retention of both neutral and ionic compounds in CEC has been studied systematically. Equations to describe the effect of operation parameters on the migration of solutes have been obtained[]= and further validated by experimental results[^10][^11]. In addition[]= various new kinds of CEC columns have been prepared[]= such as chiral columns with cellulose-based stationary phase[^12][]= packed columns with hydrophilic interaction stationary phase[^13]= and monolithic columns made by in situ polymerization[^14][^15]. All these columns have shown great advantages in the analysis of drugs[]= peptides[]= nucleotides[]= humic degradation compounds and so forth.

2.2 Microfluidic chip

Miniaturization of analytical and bioanalytical instruments has developed rapidly in the past ten years. Up to now[]= different kinds of micro total analysis systems[]= µTAS[]= have been presented for different extents[]= and applied to practical analyses. Among them[]= microchip electrophoresis has been regarded as an emerging new technology that promises to lead the next revolution in chemical analysis because of its prominent advantages[]= such as high efficiency[]= high throughput[]= easy operation[]= and low consumption of samples and reagents. Recently[]= with it we have obtained the high throughput analysis of genes[]= oligosaccharides and proteins[]= [^16][^17].

2.3 Matrix assisted laser ionization time-of-flight mass spectrometry

Matrix assisted laser ionization time-of-flight mass spectrometry[]= MALDI-TOF MS[]= has advantages of high throughput[]= low detection limit[]= mixture analysis ability[]= and good tolerance to sample contamination[]= making it a powerful tool for biomolecule analysis.

In our study[]= it has been proved that the mixture of surfactant with organic matrix could suppress the matrix signal so that small molecule could be easily detected by MALDI-TOF MS. Another effective way is to take porous silica as the matrix[]= and the micropore structure of silica has a great effect on the ionization of small molecules. In addition[]= through the electrochemical etching of silica[]= and bonding of proteins on the surface[]= a new method to study the interaction between drugs and proteins has been developed[^18]. All these results have extended the analysis and detection range of samples by MALDI-TOF MS[]= and make the fast sieving and characterization of the substrates and products of molecular interaction and biological catalytic reaction in a wider molecular weight range possible.
3 Highly sensitive detection

3.1 Microextraction technique

A universal sample preparation technique and its relevant apparatus-solid phase microextraction adsorption bar and thermal desorption device have been developed by which over 104 times concentration could be obtained which is quite useful to enrich semi-volatile and non-volatile components from all hydrolysable and liposoluble samples and has been applied to the analysis of environmental samples foods and sera of animals.

3.2 On-line concentration

In CEC limited by the short detection path isotachophoresis field amplification stacking effect and sweeping technique have been successfully applied to improve the detection sensitivity. However in CEC because of the existence of stationary phase more parameters might be considered to reach such a goal. In our previous work samples could be dissolved in a solution with lower salt and organic modifier concentrations compared to that in the mobile phase and injected for a relatively long time. During the separation field amplification stacking effect and chromatographic zone sharpening effect will co-work to decrease the detection limit. By this way in packed and monolithic CEC columns the detection sensitivity of basic drugs could be improved respectively by 17 000 and 24 000 times.

3.3 Development of novel fluorescent derivatization reagents

The enthusiastic adoption of the HPLC method has had a great impact on chemical analysis not only in chemistry and biochemistry but also in pharmacology toxicology clinical sciences genetics forensic science environmental science quality control in industry and many other fields. Currently fluorescence detection is still recognized to be one of the most sensitive detection technologies. However to detect many nonfluorophore analytes the derivatization of the interest analytes with an appropriate fluorophore must be accomplished. Therefore derivatization of the analytes with fluorescent reagents has been adopted which can alter the chemical physical properties of target compounds to make them easier to be analyzed. Recently we have successfully designed and synthesized several novel fluorescence reagents with different chromophores such as ACD 2-acridone oxyethylcarbonylimidazole AOC 2-hydroxyethyl-carbazole 2-hydroxyethylacridone HEA 9-carbazole ethyl chloroformate CEOC ethyl chloroformate AEOC which can offer high efficiency for the derivatization of fatty acids bile acids and amino compounds.

3.4 Highly sensitive detector

The laser-induced fluorescence LIF detector is one of the most sensitive detectors and is commonly used in the detection of trace environmental and biological samples. However its wide application is generally limited by its bulky size high cost and complicated optical structure. Therefore it is urgent to develop a cheap compact and portable LIF detector for HPLC CE and even microfluidic systems. Recently we have developed such detectors with laser diode laser double-pumped sol state laser and light emitting diode as the excitation sources whose performances are comparable to the commercial detectors while their size and the price are decreased obviously.

4 Preparative chromatography

Although much effort has been put on the analysis by chromatography recently preparative chromatography has attracted more and more attention. In a sense the level of the technology of preparative chromatography determines the development of medicine food and fine chemical industry of a country. In our center with the comprehensive consideration on the yields purity and time separation conditions for preparative chromatography has been optimized. Separation and purification of active components and elimination of toxic components in traditional Chinese medicine have been achieved. In addition preparative chromatography has also been applied to the isomer separation of chemically synthesized medicines.

5 Applications

5.1 Clinical diagnostics of serious diseases

Malignant tumor is a major curse to human
health. At present, the key to saving the patient’s life lies in an early diagnosis and operation. Recently, we have developed the CE or HPLC based methods for cancer diagnosis by analyzing the nucleosides in patient’s urine. Furthermore, the principal component analysis (PCA) or artificial neuron network (ANN) has been combined for data processing to improve the accuracy of our analysis [26][37].

5.2 Screening and analysis of bioactive compounds in TCM by chromatography

As we know, TCM is a complex mixture but only a few compounds are responsible for the pharmaceutical and/or toxic effects. Chromatography is one of the main techniques applied in the field because of its powerful separation efficiency and sensitive detection. Separation of TCM by conventional chromatography such as GC or RP HPLC and normal-phase HPLC is generally of no correlation between their retention and bioactivities. However, affinity chromatography is based on the biological interactions between biologically active compounds and immobilized proteins or enzymes and antibodies. We introduced the novel strategy for screening and analysis of the biologically active components in Angelica sinensis Olivi. Diels with immobilized HSA on silica as the stationary phase [26]. Ten major peaks were obtained from the methanol extract of Angelica sinensis and two principal peaks identified as ferulic acid and ligustilide are the principal biological active components.

5.3 Analysis of environmental samples and food

Environment and health theme is one global focus being paid more attention than before. The requirement for the analysis of complex unknown samples such as in the fields of environment, food and pharmaceutics is rising. To develop suitable analytical method rapidly and conveniently orient to different objects in application and research is the demand not only for chromatography but also for diverse coupling analysis with chromatography. A general approach to build an analytical method depending on experiences and simple trial tests costs a great deal of manpower and material resources. However, a suitable analytical method can be developed quickly and delibera-ately with the computer aided programmable method. Therefore, we developed a complex sample analytical system for condition optimization assistant qualification and accurate quantification rapidly based on target optimization strategy [29].

As shown in the above-mentioned aspects, we are confident that with the continuous development of the theories and techniques, chromatography will play a more and more important role in the future.

References

Biographical Sketch

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Prof. ZHANG Yukui obtained his bachelor degree of science in 1965 from Nankai University, China. Then he began his research career in Dalian Institute of Chemical Physics, DICP, Chinese Academy of Sciences, CAS. In 1983 he worked in The University of Tubingen, Germany as a visiting scholar. In 1989 he was promoted to be a research professor of DICP and Director of National Chromatographic R. & A. Center. In 1992 he visited Research Triangle Park US-EPA for cooperative research. From 1994 to 1998 he was the Deputy Director of DICP. In 2003 he was selected as the Member of Chinese Academy of Sciences.  

In the past years, Prof. ZHANG has been working on the research of fundamental theory and the development of new techniques of chromatography, including gas chromatography, GC, high performance liquid chromatography, HPLC, membrane chromatography and capillary electrophoresis, CE. Many research results such as the development of HPLC and membrane chromatography columns, multi-variance separation theory of chromatography and intelligent HPLC have won several prizes from CAS. From 1990s his research is focused on the high efficient separation and high sensitive detection of biomolecules. Devoting himself to multidimensional separation, including 2D-GC, 2D-HPLC, 2D-CE, and their hybridization with mass spectrometry. The development of on-line concentration techniques and synthesis of new fluorescent dyes have been carried out as well. In addition, he also works on the gene expression and purification of tissue kallikrein, construction of cDNA phage display library of liver cancer cell and kits for the fast diagnosis of apoplexy.  

Now Prof. ZHANG is the member of Degree Committee of CAS, Director of Chromatography Specific Interest Committee of Chinese Chemistry Society, President of Chinese Chromatography Society, Editor-in-Chief of Chinese Journal of Chromatography and the member of Editorial Board of Journal of Chromatography A. He has undertaken many national projects. Up till now he has published over 400 papers, 7 books and applied more than ten patents.