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Chromatography and Related Techniques in China: a Status Report in 1998-1999

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Abstract

A overview of the developments of chromatography and related techniques in China during the period of 1998-1999. Specific topic areas covered include gas chromatography (GC), high-performance liquid chromatography (HPLC), capillary electrophoresis (CE) and capillary electrochromatography (CEC), solid phase extraction (SPE) and solid phase microextraction (SPME).

Keywords: chromatography and related techniques, review, status report.

1. Introduction

The research on and application of chromatography and related techniques is one of the most active areas of analytical chemistry in China. Almost all aspects of chromatography and related techniques currently used in the world are involved. We have reviewed the status of chromatography and related techniques in China [1,2]. In this period, two symposia on chromatography and related techniques were held. They were the 12th National Symposium on Chromatography (NSC XII) and 8th Beijing Conference and Exhibition on Instrumental Analysis (BCEIA VIII). Table 1 lists the distribution of the papers on various separation modes published in international journals, Chinese periodicals and the two national symposia by Chinese scientists during December 1998 through December 1999. It can be seen that liquid chromatography plays the most important role in the separation science in China. The number of papers about CE and CEC has increased obviously in comparing to that in 1996-1997 [2].

2. Theory and Optimization

Geng Xingdu et al. investigated the matching relation between the number of experimental points and that of parameters of retention equations employing two, three and four parameters of the stoichiometric displacement model for the retention (SDM-R) of homologue in liquid chromatography [3]. They have studied the convergent phenomenon of homologues in reversed phase liquid chromatography according to the stoichiomatric displacement model for retention. A general equation was derived for calculating the coordinates of the convergent point. The physical meaning of the coordinates was discussed from the point of view of thermodynamics. The derived equation was compared and found to fit very well [4] with experimental data in the mobile phase.

The retention equation and the relationship between retention parameters and the parameters of molecular structure deduced by Zou Hanfa et al. from statistic thermodynamics in reversed phase liquid chromatography. It has been used to explain the difference in

Table 1. Distribution of the papers in 30 periodicals and two symposia on chromatography and related techniques

Type of Source	GC	LC	CE&CEC	Fundamental and Others	Total
NSC XII	175	196	20	42	433
BCEIA VIII	15	29	25	12	81
30 periodicals	249	612	241	60	1162
Total	439	837	286	114	1676
Percent %	27.3	50.7	15.0	7.0	

selectivity toward a particular species of 16 polycyclic aromatic hydrocarbons (PAHs). They found that each pair of binary solvents methanol/water, acetonitrile/water and isopropanol/water has its own unique selectivity[5].In another paper, they discussed theoretically and experimentally the delay and dispersion of mobile phase in the gradient elution of HPLC [6].

Bian Liuzhan et al. studied the thermodynamic behavior for some proteins and small molecules in hydrophobic interaction chromatography in the temperature range from 21-80. The thermodynamic parameters of these proteins were determined by using van't Hoff relationship (lnk'-1/T). The conformational change of the proteins was judged in chromatographic process by using the obtained standard entropy charge and free energy [7]. Liang Heng established the separation model of CE based on nonequilibrium thermodynamics separation theory. By taking the operating voltage as controllable parameter and the net separation entropy of solute system as objective function, the optimizing control orbit can be obtained by optimal design of the separation system, [8].

Zhang Lefeng presented a new method for the calculation of thermodynamic parameters based on two or more retention times under different temperature conditions in gas chromatography. These thermodynamic parameters can predict retention time under any other temperature conditions [9].

Jiao Qingcai derived a few linear retention equations including the various chromatographic parameters (for example, column temperature, composition of mobile phase, number of carbon atoms of the solute molecules, solvent molecules and alkylligand on bonded phase surface) by using thermodynamic method based on both the displacement adsorption multi-interaction model in reversed-phase liquid chromatography and the Martin equation. Both the linear retention rules and the experimental phenomena could be explained by these equations. The other linear retention rules which were not reported in literatures have also been predicted and verified experimentally [10].

The kinetic origin of the profile was further investigated based on the idea of relaxation theory in the mobile phase by a way of jumping process. The mathematical expression of profile was gained. The form of profile was also discussed digitally with the computer solution and compared with the experimental results [11,12]. Dai Chaozheng studied the dynamic process of electrochromatography and compared with that of HPLC. A general plate height equation has been derived to express the effect of axial dispersion in the CEC process, mass transfer resistance at the mobile phase, kinetic resistance associated with the reversible binding of eluite by the stationary phase and the temperature distribution effect [13].

Zhao et al. investigated the molecular structure and feature selection for 76 kinds of homologues (1 to 8 chlorine substituent) for 3, 4, 5 and 6 chlorine substituted compounds including more than ten kinds of isomerides of polychlorinated dioxin (PCDD), Artificial neutral network (ANN) was used to relate the molecular structure and retention time of PCDD [14]. Lin Leming et al. predicted the retention values in thin layer chromatography by using back-propagation artificial neutral networks (ANNs) and the multi-parameter regression analysis with topological indexes. The rather good correlation for 15 amino acids and 24 phenols and aniline derivatives were obtained [15]. Tang Wanying [16] and Zhang Suping [17] have predicted the chromatographic retention value by gray model parameters. Wang Lianshen published two papers concerning the relationship between the retention values and the molecular structure of pollutants such as indole compounds and heterocyclic nitrogen compounds in liquid chromatography [18,19]. The QSAR in TLC has been reviewed by Wang Q. et al. [20].

Wang Hong established the systematic optimization for the MEKC separation of PTC-amino acids by using a dynamic scouting optimization method-controlled weighted centroid variable size simplex algorithm [21]. Wang Jingqing [22] and Lin Xuan [23] applied pattern recognition method and artificial neural network to solve the optimization of operating conditions in HPLC. Zhang Weibing theoretically discussed the relationship of retention of compounds in multiple injection. The results showed that the capacity factors of two solutes in a sample and the interval between the two injections were the main factors affecting the separation [24].

In comparing with that of 1996-1997, the number of papers about resolution of overlapping chromatographic peaks has increased obviously. These methods included the window factor analysis technique [25], wavelet transform [26], orthogonal projections [27] and fast Fourier transform [28]. Wang Lishi compared the results of wavelet smoothing and wavelet denoising for processing CE signals. They found that the signal peaks are broadened and shortened by using wavelet smoothing, however, the peaks are well described by using wavelet denoising [26]. Chen Dizhao et al. developed the heuristic evolving latent projections (HELP) and annihilation of rank and resolution by projection (AR-RP). The approach have been successfully applied to the qualitative and quantitative analysis of complete real multicomponent system with phenols and PAHs [29].

3. Gas Chromatography

Wu Caiying's group has published several papers on the preparation and characterization of new stationary phases for capillary gas chromatography such as crown ether capped cyclodextrine, fulleropyrrolidine functionalized polysiloxane, derivatives of calixarene and mixed stationary phases [30-34]. The polarity, chemical stability and thermostability of the stationary phase have been

evaluated.

Zhou Liangmo have extensively studied the retention mechanism in the separation of chiral compounds on the bonded cyclodextrine stationary phase in GC [35,36]. Yuan Liming et al. separated ethylbenzene/benzene, o-xylene/benzene, styrene/benzene and styrene/toluene on the mixed stationary phases composed of perpentylated bata-cyclodextrin and $AgNO_3$, perpentylated beta-cyclodextrin and $TlNO_3$, Benton-34 and $AgNO_3$ in GC. The positive or negative synergistic effects was investigated extensively [37-39]. A mesoporous molecular sieve of the HMS type was prepared and its applications as adsorbent for gas-solid chromatography were evaluated by probing system as C 1-C 15 paraffins mixtures, aromatic hydrocarbons, alcoholes, halohydrocarbons and ketones. The results indicated the mesoporous molecular sieve adsorbent have unique features in comparing to the conventional micro-molecular sieve and porous polymer adsorbents [40].

Most of the GC papers focused on the applications of GC to environmental, medical and natural plant analyses. Xu Guowang et al. monitored aldehydes and ketones pollutants in the gas of fixed pollution source [41], Zhang Xiangming et al. determined the emission amount of aromatics in exhaust gases from paint industry [42]. Li et al. analyzed the medicine by using GC [43].

4. High Performance Liquid Chromatography

Preparation of liquid chromatographic columns has attracted attention from several groups in China. Zhou Liangmo prepared the highly efficient rod-shape poly (methacrylic acid-co-styrene-co-divinylbenzene) column by an in-situ free-radical polymerization in column. Aromatic acids and polycyclic aromatic hydrocarbons were separated [44]. Geng Xingdu prepared poly (acrylamide-co-butyl methacrylate-co-N, N'-methylenebisacrylamide) hydrophobic interaction chromatographic continuous rod column in situ by direct polymerization of monomer mixture and porogenic solvents in stainless tube. This column could be used in separation and in the study of retention mechanism of proteins [45].

Wang Jingfang et al. prepared molecular imprinting polymers (MIPs) against Cbz-L-Ser, Cbz-L-Ala and Cbz-L-Pro utilizing acrylamide plus 2-vinylpyridine as combined basic functional monomers and evaluated in HPLC mode. The polymer spherical stationary phase for HPLC have been prepared by different groups [46-48]. Cheng Liren et al. have prepared the chiral stationary phase coated cellulose derivatives, polymer coated silica and cross-linked polymer HPLC stationary phase [49-51].

Da Shilu et al. have prepared some mixed functional HPLC stationary phases containing aza crown and beta-cyclodextrin, 8-quinolinol derivatized beta-cyclodextrin bonded silica via continuous solid-liquid reaction method. The results showed that the separation ability of the new mixed functional stationary phase for disubstituted benzene isomers was better than that on each of the functional bonded silica stationary phase. The 3- (Aza-18-crown-6) propylsilyl and calixa [4] rene bonded silica stationary phases were synthesized also [52-54]. Zhang Qinghe et al. prepared porous zirconia, silica-zirconia and magnesia-zirconia particles by sol-gel process. The physical and chemical properties and chromatographic character were studied [55,56].

Zhou Liangmo et al. described the recent advances of membrane chromatography in liquid chromatography [57]. The group of Zou Hanfa has prepared several membrane medium with different functional groups. The column efficiency and the column pressure drop [58,59] have been studied. Shang Zhenghua prepared an affinity material for endotoxin removal from nylon 66 microfiltration membrane. The results of immobilization of histidine ligand on the membrane with different activation agents were compared and evaluated [60].

Most of the HPLC papers focused on the applications of HPLC analysis to medicine and clinic. New development and its applications on biochemical research of affinity and hydrophobic interaction chromatography (HIC) have been reviewed [61,62]. The number of papers about HPLC in medical analysis is about 60% of the published in the Journal of Yaowu Fenxi Zazhi during 1998-1999.

The applications of ion chromatography have many reports in the past two years [63-65].

5. Capillary Electrophoresis and Electrochromatography

Several reviews summarized the current state of the theory and practice of capillary electrophoresis, e.g. the electroosmosis [66], temperature effect and temperature gradient technique [69], affinity [68] and non-aqueous capillary electrophoresis [69]. The applications of capillary electrophoresis have summarized in several reviews on environmental analysis [70], Chinese traditional medicine [71], polysaccharides [72], alkaloid [73], toxin [74], DNA [75], speciation analysis [76] and chiral enantiomers [77].

Lin Bingcheng et al. developed a novel buffer based on low molecular weight hydroxypropylmethylcellulose to separation DNA fragments. The influence of additives such as urea and mannitol was investigated. The possible mechanism for the separation of DNA fragments was also discussed [78]. Chen Yi et al. separated basic protein using a homemade electric field modulated capillary electrophoresis, which can offer both radial and axial electric fields with only one high power supply. The adsorption of protein molecules onto the tubing wall can be regulated by the application of a radial voltage : as the band broadening decreases with the increase of the radial voltage. This implied that it may be possible to dynamically control the adsorption of protein molecules on silica surface [79,80]. Chen Jieke et al. reported the use of home-made CE with amperometric detection system to the analysis of neuro-

transmitters in a single sympathetic nerve cell of rat. Isoproterenol was selected to be the internal stand and for the determination of norepinephrine and epinephrine in a single sympathetic nerve cell [81]. In another paper, they developed a sensitive, rapid and a accurate method to analyze of the amine acid neurotransmitters in dorsal root ganglion of art by using CE with a laser-induced fluorescence-charge coupled device and fluorescein isothiocyanate [82]. A capillary electrophoresis-laser induced fluorescence-intensified charged coupled device system was used for the high-sensitivity analysis of two important nature amino sugar, glucosamine, galactosamine and glucosaminic acid [83]. Che Fa-Yun et al. analyzed 8-aminonaphthalene-1, 3, 6-trisulfonate derivatized oligosaccharides by CE electrospray ionization quadrupole ion trap MS, and they have studied the distribution of ovalbumin glycoforms by CE [84]. The applications of CE in medicine and natural plant, some example are listed in Table 2.

To measure the free concentration of verapamil (a basic drug) enantiomers in the binding system of human serum albumin (HSA), Lin Bingchen et al. established a capillary electrophoresis method, liquid precolumn, and the method was examined systematically. The method was used to determine the binding constants of the basic racemic drug, verapamil to human serum albumin under drug-HAS binding equilibrium [110,111]. Luo Guoan determined the binding constant between fluorescein isothiocyanate labeled bovine serum albumin and its monoclonal antibody using the ligand separation mode of affinity capillary electrophoresis with laser induced fluorescenece detection [112]. On-line concentration technique of capillary electrophoresis was studied by Ou Qingyu [113] and Sun Jingzheng [114], separately.

Capillary electrochromatography, which represents a hydrid of chromatographic and electrophoretic techniques, has experienced dramatic growth in the past two years. Many papers have been published during the covered period dealing with the theory and practice of CEC. The separation mechanism of CEC have been studied by several groups [115,116]. Fu Ruonong et al. discussed the effect of double layer overlap on the performance and electroosmotic velocity in the packed capillary CEC. The theory analysis on the double layer overlap in the packed CEC was described. The results showed that the packing structure and the homogeneity of the packed bed were the internal factor that resulted in the emergence of the double layer overlap in the packed CEC [117]. In the another paper, they studied the chromatographic behavior pressurized flow-driven electrochromatography. The experimental results were discussed in relation to theoretical considerations [118]. Zhang Lihua et al. proposed a new method for shorten the retention time of samples in CEC by a commercial CE instrument with the ability to change the mobile phase vials automatically. The method was proved to have quite good reproducibility [119].

Zou Hanfa et al. summarized the current state of open tubular capillary electrophoresis [120]. The methods for preparation of the coated capillary used in CE have been reviewed [121]. Fu Ruonong et al. prepared the open tubular capillary electrochromatography column by using sol-gel process [122]. Liu Xiaoda et al. prepared a cross-linked polyacrylamide coated capillary by radiation-induced with basic buffers [123]. High performance linear polyacrylamide capillary gel electrophoresis columns were prepared and characterized by Shi Wei et al. [124].

Liu Zheng et al. studied the relationship between retention value in micellar electrokinetic chromatography and LogP. The effect of organic modifier on the capacity factors of solutes have been examined too [125,126]. Zhang Lihua found the main factors contributing to the retention of solutes in reversed phased capillary electrochromatography were the solute size and the ability of solute to accept hydrogen bond [127].

The detection of capillary electrophoresis was another hot topic. Fang Yuzhi analyzed theoretically the factors affecting zone broadening in capillary electrophoresis-amperometric detection at disk-shaped electrode. A model equation about it was given. The effect of injection time of sample, separation voltage and dead volume of the detector on zone broadening was experimentally examined, and the model equation derived for the zone broadening was verified [128]. Wang Erkang et al. described a new electrochemical cell assembly possessing advantages of easier construction and operation, and good reproducibility. The construction and operation was demonstrated to be reliable and precise by examining the reproduciblity, the concentration range of linearity and detection limit for phenols and catecholamines with the cell [129]. Wang Lishi et al. described the development of a scanning voltammetric detector for CE. The scanning electrode potential waveform generation and the current signal acquisition were controlled by a microcomputer interfaced with a home-made card witch is equipped with 12 bit digital-analog converter (D/A) and a 12 bit analog-digital converter (A/D). The methods of potential control and signal acquisition are discussed [130]. Chen Yi et al. established a novel CE system which offered several ways to control electroosmosis by electric field. The main feature of the system was a specially fabricated capillary-cartridge unit, with which they can achieve CE separation and electroosmosis control using flexible silica capillary and only one high-voltage power supply [131]. Lin Bingchen et al. complied a computer program to work out the ray tracing of UV detector of HPCE. Based on results obtained by calculation, some practical methods for adjusting the distance between lens and capillary were provided to make the UV detector to work on optimal conditions [132].

Table 2. The applications of capillary electrophoresis and capillary electrochromatography in analysis of drugs nature plants

sample	components	reference	sample	components	reference
Tianma injection	Gastrodin	85	Anisodamine	Enantiomer	98
	Sulfamethoxzolum	86	Basic drugs	Ofloxacin	99
Sulphonamide	trimethoprimum			Terbutaline	
medicines	sulphaguanidine			Chlorpheniramine	
	sulfadimidinum			Norephedrine	
Compound	Teophyllinum	87		Indole-3-acetic acid,	100
theophyllinum	Phenobarbitalum		Plant hormones	Indole-3-butyric acid	
Tablet	Phenocetin			Gibberellin	
				6-benzyl amine purine	
Cortex magnoliae	Magnolol and	88	Human Urine	Chlorpheniramine	101
officinalis	konokiol				
Human urine and	6-Mercaptopurine	89	paeonia	paeonol	102
bovine serum			suffruficosa andr	paeoniflorin	
albumin			Paeonia albiflora		
			pall		
Dihydropyridine		90		Cycllic adenosine	103
calcium channel				monophosphate	
blockers				Cycllic guanosine	
				monophosphate	
Aminophenylline	Theophylline	91	Human urine	Creatinine	104
and timina tablets	Phenobarbital			Uric acid	
Danggui	Ferulic acid	92	ATP medicament	ATP, ADP, AMP	105
Vitaphkol eye drops	niacinamide	93	Radix salviae	B -3,4-dihydroxyphenyl	106
	adenosing		miltiorrhizae	lactic acid	
	thimerosal			Protocatechuic acid	
				Protocatechuic aldehyde	
	Chlopheniramine	94	Laithyrus satius	B -N-oxalyl- α β -	107
Cold madicine Pa-	Paracetamol			diaminopronic acid	
er-ke	Phenylpropanolami			homoarginine	
	ne				
	Dextromethophane				
Enalapril maleate	Enalapril maleate	95	Chinse Medicines	Tetradrine	108
compound tablet	Hydrochlorothiazide			Fargchinoline	
Tetracycline	Impuritices	96	Gastrodia Eleta	Active compounds	109
antibiotics			BL		
swellfish	Tetrodotoxin	97			

6. Others

Wang Zhenyu prepared the solid-phase coating with hydroxy-terminated poly (dimethylsiloxane) in SPME. The coating possessed higher thermal stability shorter extraction and desorption time and the stronger affinity to both polar and nonpolar compounds. The ideal results was obtained for the enviromental samples [133]. Zhang Daoning et al. have compared the SPME and SPE to enrich organochloride compounds in water samples and

analyzed by GC with electron capture detector. The results showed that SPME was a faster and more effective method in sample preparation. Several commercial solid phases in extraction of moderately polar organic compounds were compared and the results indicated that polyacrylated was the best [134]. Wang Yiru et al. analyzed naphthalene, 1-nitronaphthalene and 2-nitronaphthalene by SPME with 100 μm polydimethylsiloxane and GC-ITD detection and the limits of the three compounds were 0.25, 0.30 and 0.20 μg. L-1, respectively [135]. Zou Hanfa et al. determinated the aromatic amines in the waste water by SPE and HPLC [136].

You Jing et al. reviewed the applications of supercritical fluid extraction (SFE) on environmental sample analysis. Li Hua reviewed the development and application of SFC in enantiomers separation. The separation modes and choices of experimental condition were discussed also [137]. Du Qizhen et al. determined the retention percentages of the stationary phase at various flow rates of fifteen two-phase solvent systems in three types of countercurrent chromatographic apparatus equipped with small middle and large-bore colied columns [138]. Cao Xueli et al. separated and purified the isoflavones from Pueraria Lobata by high-speed countercurrent chromatography [139]. Wang Hong reviewed the recent development in principle and applications of optical chromatography, and they theoretically studied the relationship between the particle radius with the intensity of the applied laser radiation force using a ray-optics model by analyzing the force-status of the separated particles in the mobile phase [140]. Guan Yafen analyzed the heavy oils from petrochemical industry by an on-line coupled packed capillary HPLC and high temperature capillary gas chromatography [141,142].

In conclusion, chromatographers of China have obtained great achievements in chromatography and related techniques, especially, capillary electrophoresis, which have been studied extensively and used to separate various samples. We believe we can make greater progress through the efforts of chromatography researchers.

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