CHROMATOGRAPHY IN FOOD SCIENCE AND TECHNOLOGY

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To our children
Éva Cserhádi and András and Gergely Forgács

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PREFACE

Chromatography has been developed as a powerful and rapid technique for separation compounds with highly similar molecular characteristics even from complicated matrices. Because of excellent separation characteristics and versatility, chromatographic methods have found growing acceptance and application in food science and technology for the separation and quantitative determination of a wide variety of compounds such as food ingredients, additives, vitamins, environmental pollutants, and more. The objectives of this monograph are compilation and concise evaluation of the newest results in this rapidly developing domain of chromatography, brief enumeration of the methods applied, and critical discussion of the results. The book is meant to be self-sufficient in terms of the needs of the average professional who intends to work in this interesting field. We are confident that the book will be found useful as a valuable reference book for researchers and serious students interested in the topics covered.

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LIST OF ABBREVIATIONS

AA	ascorbic acid
ACN	acetonitrile
AE	anion exchange
AG	agmatine
A-LAGI	geometrical isomer of α -linolenic acid
AMF	anhydrous milk fat
ANN	artificial neural network
AOAC	Association of Official Analytical Chemists
APCI	atmospheric pressure chemical ionization
AQC	6-aminoquinolyl- <i>N</i> -hydroxy succinimidyl carbamate
ASTED	automated sequential trace enrichment of dialysate
BITC	butylisothiocyanate
BMIS	beet medium invert syrup
BO	borage oil
BSA	bovine serum albumin
BSTFA/TMS	bis (trimethylsilyl) trifluoroacetamide trimethylchlorosilane
BTC	butylthiocarbamyl
CA	cadaverine
β -CDS	β -cyclodextrin-bonded silica
CE	capillary electrophoresis
CGA	chlorogenic acid
CGE	capillary gel electrophoresis
CIEF	capillary isoelectric focusing
CITP	capillary isotachopheresis
CLND	chemiluminescent nitrogen detection

CMP	caseinomacropeptide
CN	carbon number
CPP	caseinophosphopeptide
CV	coefficient of variation
CZE	capillary zone electrophoresis
d.b.	dry basis
DDT	dithiotreitol
DECTP	diethylchlorothiophosphate
DG	diacyl glycerol
DH	degree of hydrolysis
DHAA	dehydroascorbic acid
DHIAA	dehydroisoascorbic acid
DI	direct injection
DM	dry material
DMA	dimethyl amine
DMF	dimethylformamide
DMS	dimethyl sulphide
DNPH	2,4-dinitrophenylhydrazine
DNPU	dinitrophenylurethane
Dns-AA	dansylamino acid
DTNB	5,5'-dithio-bis (2-nitrobenzoic acid)
ECN	equivalent carbon number
ED	electrochemical detection
ECD	electrochemical detector
EDTA	ethylenediametetraacetic
EI	electron impact
ELSD	evaporative light scattering detector
EOF	electroosmotic flow
EPO	Evening Primrose oil
EPT	endpoint temperature
ES	electrical stimulation
ESI-MS	electron spray ionization mass spectrometry
ETA	ethanol amine
F	furaldehyde
FAB-MS	fast atom bombardment mass spectrometry
FAME	methylated fatty acid
FBD	fermented bread dough
FD	flavor dilution
FDAA	(1-fluoro-2, 4-dinitrophenyl)-5-L-alanine amide
FFAP	free fatty acid column

FID	flame ionization detector
FMOC	fluoromethyl chloroformate
FPD	flame photometric detection
FPLC	fast protein liquid chromatography
FSD	fermented sourdough
FT-IR	Fourier transform infrared spectroscopy
GC	gas chromatography
GC-MS	gas chromatography–mass spectrometry
GCO	gas chromatography combined with olfactometry
GCO-H	gas chromatography–olfactometry of headspace
GL	galactolipid
GLC	gas-liquid chromatography
GPC	gel permeation chromatography
GSC	gas-solid chromatography
HBPF	hydrophobic bitter peptide fraction
HDM	hand-deboned meat
HFCS	high-fructose corn syrup
HI	histamine
HMF	5-hydroxymethyl-2-furaldehyde
HP-FFAP	Hewlett-Packard free fatty acid column
HPGFC	high-performance gel filtration chromatography
HPLC	high-performance liquid chromatography
HPSEC	high-performance size exclusion chromatographic
HPTLC	high-performance thin-layer chromatography
HTCGC	high-temperature capillary gas chromatography
IAA	isoascorbic acid
IBLC	<i>N</i> -isobutyryl-L-cysteine
ICP-MS	inductive-coupled plasma–mass spectrometry
ID	isotope dilution
I.D.	inner diameter
IEC	ion-exchange chromatography
IGC	inverse gas chromatography
IMAC	immobilized metal ion affinity chromatography
IR	infrared
IRMS	isotope ratio mass spectrometry
IS	internal standard
isoBOC	isobutyloxycarbonylated
ISSPH	isoelectric soluble soybean protein hydrolysate
IV	iodine value

LC	liquid chromatography
LC-MS	liquid chromatography–mass spectrometry
LD	limit of detection
LLE	liquid–liquid extraction
LLE-EC	liquid–liquid extraction with external concentration
LLE-IC	liquid–liquid extraction with internal concentration
LQ	limit of quantification
MDGC	multidimensional gas chromatography
ME	mercaptoethanol
MECC	micellar electrokinetic capillary chromatography
MEEKC	microemulsion electrokinetic chromatography
MF	milk fat
MG	monoacylglycerol
MRM	mechanically recovered meat
MS	mass spectrometry
MSG	monosodium glutamate
na	not analyzed
NANA	<i>N</i> -acetyl neuraminic acid
NBD	<i>N</i> -chloro-7-nitrobenzofurazan
NC	not calculable
nd	not determined
ND	not detected
NDMA	<i>N</i> -nitrosodimethylamine
NL	neutral lipid
NMR	nuclear magnetic resonance
NQ	not quantified
ODS	octadecylsilica
OPA	<i>o</i> -phthalaldehyde
OW	optothermal window
PAAGE	polyacrylamide agarose gel electrophoresis
PAD	pulsed amperometric detector
PAGE	polyacrylamide gel electrophoresis
PCR	principal component regression
PGA	pteroylmonoglutamic acid
PHE	phenyl ethylamine
PHVO	partially hydrogenated vegetable oil
PITC	phenyl isothiocyanate
PL	phospholipid
PP	1,3-dipalmitoyl-glycerol

PrCN	propionitrile
PTC	phenylthiocarbamyl
PTV	programmed temperature vaporizer
PU	putrescine
PUFA-TG	triglyceride fraction containing polyunsaturated fatty acids
Py-GC-MS	pyrolysis–gas chromatography–mass spectrometry
PYR	pyrrolidine
RI	refractive index
RMT	relative migration times
RP	reversed phase
RPBA	radioprotein-binding assay
RP-HPLC	reversed-phase high-performance liquid chromatography
RP-TLC	reversed-phase thin-layer chromatography
RSD	relative standard deviation
RT	retention time
SD	standard deviation
SDEC	simultaneous distillation–extraction with concentration
SDENC	simultaneous distillation–extraction without concentration
SDS	sodium dodecylsulfate
SE	serotonin
SEC	size exclusion chromatography
SE-HPLC	size exclusion high-performance liquid chromatography
SFC	supercritical fluid chromatography
SFE	supercritical fluid extraction
SI	silica
SIM	selected-ion monitoring
SLL	Syrian Local Large
SM	spermine
SMM	<i>S</i> -methylmethionine
SP	spermidine
SPE	solid-phase extraction
SPME	solid-phase microextraction
STP	sodium tripolyphosphate
TAA	total amino acid
TAG	triacylglycerol
TBDMS	<i>tert</i> -butylmethylsilylate
TCA	trichloroacetic acid
TEA	triethylamine
TEMED	<i>N,N,N',N'</i> -tetramethyl-ethyleme-diamine
TFA	trifluoroacetic acid

TG	triglyceride
THF	tetrahydrofolate
TIC	total ion chromatogram
TLC	thin-layer chromatography
tr	trace
TR	tryptamine
TY	tyramine
UBD	unfermented bread dough
UHT	ultra heat treatment
UV-VIS	ultraviolet-visible
VC	variation coefficient
VOME	vegetable oil methyl ester
WP	whey powder
WPC	whey protein concentrate

Theory and Practice of Chromatography

1.1 GAS CHROMATOGRAPHY

The term *gas chromatography* (GC) summarizes chromatographic methods when the mobile phase is gas and the stationary phase is solid or liquid (gas–solid chromatography = GSC or gas–liquid chromatography = GLC). GC has proven to be a powerful technique for the qualitative and quantitative analysis of a wide range of materials in such diverse areas as environmental, clinical and food analysis, and petroleum and chemical production. The analysis of flavor and aroma components, the types and amounts of fatty acids, and the possible presence of adulterants are some applications of gas chromatography in the food industry. There are serious limitations to the types of compounds suitable for GC analysis. The most decisive physical property of a compound is its volatility. The compound needs to have appreciable vapor pressure at a temperature below 350–400° C. Another important characteristic is a compound's ability to vaporize rapidly without decomposing or reacting. Instability at high temperatures usually renders a compound unsuitable for GC analysis.

Common gas chromatographic equipment consists of a carrier gas system, injector, gas chromatographic column, detector, and data processing unit. Carrier gas is generally a permanent gas with low or negligible adsorption capacity, such as hydrogen, helium, or nitrogen. The nature of carrier gas may influence the separation characteristics of the GLC systems and can modify the sensitivity of the detection. Use of hydrogen as a carrier gas is hampered by its high flammability and by the strict safety regulations concerning its application. Helium is an excellent choice, but the elevated price of helium considerably reduces its use. Nitrogen is the most frequently used carrier gas in laboratory practice; it is not expensive, and many separations can be successfully

carried out with nitrogen carrier gas. Some other gases have also been used for the solution of special separation problems or for the elucidation of theoretical principles.

The stability and reproducibility of the carrier gas flow rate is a prerequisite of a successful gas chromatographic analysis. Many injector types have been developed with the objective to deliver the vaporized sample to the head of the GC column with a minimum initial bandwidth. Sample inlets, referred to as injectors or injection ports, can be classified into two major groups: vaporization and on-column injectors. Vaporization injectors utilize high temperatures to vaporize a liquid sample rapidly. Usually a syringe is used to introduce the sample into the heated injector. In this case the sample rapidly vaporizes, mixes with the carrier gas, and is transported into the column. Any portion of the sample that does not rapidly vaporize either remains in the injector or at the front of the column. In either case, these residues will eventually contaminate the GC apparatus and make cleaning necessary. On-column injectors deposit the sample directly into the column without relying upon vaporization of the sample and its subsequent transport into the column.

Headspace sampling involves placing a liquid or solid sample in a vial sealed with a septum cap. The sample is thermostatted, and portions of the volatile components move into the space above the sample. The amount of each compound in the headspace is dependent on the volatility of the compound and its concentration in the sample. The procedure requires sufficient time for the sample components to equilibrate between the headspace and the sample at the setpoint temperature before a measured portion of the headspace is removed using a needle inserted into the vial headspace. The sampled portion is transported through a heated transfer line to the column using carrier gas. Headspace sampling is well suited for analysis of the volatile components in a liquid or solid sample.

The gas chromatographic column has to be thermostatted at constant temperature (isocratic separation mode) or according to a predetermined temperature program (temperature gradient). Since the column temperature is one of the most decisive parameters in GC analysis, its exact regulation is of paramount importance. The stationary liquid phase of GC columns has to comply with the following requirements: low vapor pressure, high chemical stability, and relatively low viscosity at the temperature of analysis; selectivity for the sample components under investigation; and good wetting capacity both for the surface of the inert support or for the possibly inert wall of the column. The length of a packed column is limited to about 3 m because of the high pressures that are required to maintain the carrier gas flow rates or velocities necessary for optimal performance. Solid supports wetted by the stationary liquid phase are characterized by low adsorption capacity and by large surface area. Inert supports improve peak symmetry and do not interfere with the partition

of analytes between the gas and the liquid stationary phases. Both the absence of adsorption centers on the support surface and the large surface area contribute to the even distribution of the wetting agent, which is a prerequisite of high separation performance. Higher column efficiency is obtained with small support diameters that vary over a narrow range. Common solid support diameters are 80–120 mesh (125–177 μm). The amount of the stationary phase coated on a support is generally between 1% and 10%, with 30% being the highest normally encountered. In addition to column packings that consist of supports coated with a stationary phase, porous polymer packings have also found application. These packing materials are frequently used for the separation of highly volatile compounds.

Owing to their higher separation, capacity capillary (also called open tubular) columns have also been extensively used in food analysis. The inner surface of a capillary column is coated with a thin layer of the stationary phase. Most of the stationary phases are cross-linked and covalently bonded to the fused-silica surface. The amount of stationary phase in a capillary column is denoted by the film thickness, which is typically 0.1–5 μm . Compound retention is proportional to film thickness in capillary columns; retention increases as the film thickness increases, and it decreases as the film thickness decreases.

Detectors interact with the solute molecules as they exit the column. This interaction is converted into an electrical signal that is sent to a recording or data storage device. A chromatogram is then created, which is a plot of the intensity of the signal versus elapsed time. The primary differences between detectors are in the lowest amount of a compound that is detectable (sensitivity) and in which compounds produce the strongest detector response (selectivity). Many different detectors (flame ionization, nitrogen–phosphorus, flame photometric, electron capture, thermal conductivity, atomic emission, electrolytic conductivity, chemiluminescence, etc.) and data handling devices have been developed for the sensitive and selective detection and quantitation of sample components. The newest developments are presented in the prospects of the firms producing gas chromatographs.

1.1.1 Fundamentals

The distribution of solute molecules between the stationary and mobile phases (carrier gas) is defined by the distribution constant (K_D), which is the ratio of the concentration of the solute molecules in the stationary phase to that in the mobile phase:

$$K_D = \frac{\text{compound concentration of stationary phase/compound}}{\text{concentration of mobile phase}} \quad (1.1)$$

The relationship between retention, column temperature, and solute properties is described by

$$\ln K_D = -\Delta G^0/RT \quad (1.2)$$

where K_D is the distribution constant, ΔG^0 is the change in Gibbs free energy for the evaporation of compound from the stationary phase, T is the column temperature (Kelvin degree), and R is the ideal gas constant. Equation (2) shows that the differences in the Gibbs free energy for the evaporation of the solutes from the stationary phase result in different distribution of the solutes. The amount of the time that the compound spent in the column is called the retention time (t_R). The partition ratio, also known as the capacity factor (k'), is the amount of time that a compound spends in the stationary phase relative to the mobile phase.

$$k' = (t_R - t_0)/t_0 \quad (1.3)$$

The retention index (I) method introduced by Kováts [1] highly increased the reproducibility and reliability of the determination of the retention characteristics. The basis of the method is the comparison of the retention time of a given analyte, with the retention times of normal hydrocarbons eluting before and after the analyte. The retention index I can be calculated by the following equation:

$$I = 100c + 100(\log V_{N_x} - \log V_{N_c})/(\log V_{N_{c+1}} - \log V_{N_c}) \quad (1.4)$$

where x refers to the analyte, c refers to the number of carbon atoms of the n -hydrocarbon eluting before the analyte, and $c + 1$ refers to the number of carbon atoms on the n -hydrocarbon eluting after the analyte. V_N is the net retention volume. In practice the net retention time is usually obtained by measuring the distance on the recorder chart between the peaks of nonsorbed and sorbed solutes.

The separation factor (*alpha*) characterizing the separation of two molecules can be calculated by the following equation:

$$\alpha = k_2/k_1 \quad (1.5)$$

where k_1 is the partition ratio of the earlier eluting peak and k_2 is the partition ratio of the later eluting peak.

Resolution (R) is a measure of peak separation that takes peak widths into account:

$$R = 1.18 (t_{R2} - t_{R1})/(w_{h1} + w_{h2}) \quad (1.6)$$

$$R = 2(t_{R2} - t_{R1})/(w_{b1} + w_{b2}) \quad (1.7)$$

where t_{R1} and t_{R2} are the retention times of peaks 1 and 2, w_{h1} and w_{h2} are the peak widths at half height of peaks 1 and 2, w_{b1} and w_{b2} are the peak widths at the base of peaks 1 and 2, respectively.

Theoretical plate number (N) characterizes the separation capacity of a chromatographic column. There are no plates in a GC column; however, this concept, borrowed from the distillation theory, was successfully used to characterize column performance. The number of theoretical plates is calculated by

$$N = 5.545(t_R/w_h)^2 \quad (1.8)$$

The theoretical aspects and practical applications of various gas chromatographic methods are discussed in more detail in References [2–5].

1.2 LIQUID CHROMATOGRAPHY

The term *liquid chromatography* (LC) summarizes chromatographic methods in which the mobile phase is liquid and the stationary phase is an inorganic or organic solid. According to the shape of the solid-phase matrix, liquid chromatographic methods can be roughly divided into planar and column chromatography. According to the relative polarity of the stationary and mobile phases, LC methods are classified as normal (adsorption) and reversed-phase separation mode. When the LC system consists of a nonpolar mobile phase and a polar stationary phase, it is called normal-phase chromatography. In contrast to the normal-phase separation mode, the reversed-phase mode requires the combination of a polar mobile phase and a nonpolar stationary phase.

1.2.1 Thin-Layer Chromatography

Thin-layer chromatography (TLC) is a planar chromatographic technique extensively used as a rapid and easy to carry out analytical tool. TLC has proved to be suitable for the semiquantitative determination of various organic and inorganic molecules in complicated mixtures. The principle of the traditional TLC is very simple: the mixture of solutes to be separated is spotted on the solid surface and moved with an adequate mobile phase system.

The different strength of interaction of solute molecules with both the stationary and mobile phase results in different mobility and separation. A wide variety of inorganic and organic sorbents have been tried as a stationary phase for TLC; however, mainly silica and silica with covalently bonded ligands on the surface are the most frequently used supports. The polarity order of the various silica and bonded silica plates is approximately the following: silica > amino silica > cyano silica > octadecyl (C_{18}) silica. Other sorbents such as alumina, diatomaceous earth (*Kieselguhr*), celluloses, and polyamides have found only limited application. The performance of a TLC layer depends on the physicochemical characteristics of the sorbent, such as specific surface area and pore volume, mean pore diameter and pore size distribution, and particle distribution and size. The average particle size of TLC sorbents is between 10 μm and 50 μm . A smaller particle size and narrower particle size distribution

improve the resolution, decrease analysis time, and enhance the sensitivity of detection (high-performance TLC = HPTLC). The solvent strength (elution capacity) of a solvent is determined by its capacity to move a solute. Higher solute mobility indicates higher elution strength. The order of elution strength on commonly used silica is: *n*-heptane > *n*-hexane > *n*-pentane > cyclohexane > carbon tetrachloride > toluene > dichloromethane > diethylether > acetonitrile > 1-butanol > 2-propanol > acetone > ethanol > dioxane > tetrahydrofuran > methanol > pyridine > water. The elution order is nearly opposite in reversed-phase TLC (RP-TLC).

1.2.1.1 FUNDAMENTALS

The retention of a solute in TLC and in RP-TLC is characterized by the R_f value: the distance from the origin to the center of the separated zone divided by the distance from the origin to the solvent front. The capacity factor k can be defined by the ratio of retention times of a solute in the stationary (t_s) and mobile phase (t_m), respectively:

$$k = t_s / t_m \quad (1.9)$$

The relationship between capacity factor and R_f is

$$R_f = t_m / (t_m + t_s) = 1 / (k + 1) \quad (1.10)$$

$$k = (1 - R_f) / R_f = 1 / R_f - 1 \quad (1.11)$$

Because the relationship between the R_f value of the solutes and the concentration of the stronger component in the eluent (C) is logarithmic, the R_M value was introduced to describe the correlation more easily:

$$R_M = \log (1 / R_f - 1) \quad (1.12)$$

$$R_M = R_{M0} + b \cdot C \quad (1.13)$$

where R_{M0} is the theoretical R_M value of a solute extrapolated to zero concentration of the stronger component in the mobile phase and b is the change of the R_M value caused by unit change of the concentration of the stronger component in the mobile phase (C).

The general theory, fundamentals, and practical application of TLC have been described in other excellent books [6–8] and reviews [9,10].

1.2.2 High-Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) is the most frequently used column chromatographic technique. Its popularity is caused by the high separation power, excellent selectivity, and the high diversity of solutes that

can be separated with this method. Similar to TLC, the majority of measurements are carried out either in normal or in reversed-phase separation mode using silica and modified silica supports. However, in contrast to TLC, reversed-phase HPLC (RP-HPLC) is a method of preference for the analysis of the majority of solutes. RP-HPLC uses less toxic solvents (water and an organic modifier miscible with water), causing less environmental pollution; the equilibrium time of RP-HPLC supports is considerably lower than the normal-phase ones; and the retention of solutes can be more or less predicted as the retention is mainly governed by the hydrophobicity of solutes, which can be calculated with reasonable accuracy. In addition to normal- and reversed-phase separation modes, HPLC offers a unique possibility to employ supports with different separation mechanisms, such as ion-exchange, size exclusion, and gel permeation chromatography.

Separation in ion-exchange HPLC is based on the interaction of the charged solute with the oppositely charged surface of the stationary phase. The retention of the electrostatically bonded solutes can be easily influenced by adding salts to the mobile phase or by modifying its pH value. Size exclusion chromatography (SEC) is a technique for separating molecules according to their effective shape and size in the aqueous mobile phase. Gel permeation chromatography (GPC) is similar to SEC, but it generally uses organic solvents for the separation of water-insoluble macromolecules. The stationary phases used in these separation modes are porous particles with closely controlled pore size. The molecules of solute can enter the pores of the support according to their size and shape. The choice of a mobile phase for this separation mode is simpler than other HPLC modes since only one solvent is required.

A common HPLC system consists of an injection device, a column, the mobile phase delivery system, a detector, and a signal output device. Injector is an important part of the HPLC system because it has to inject a precise volume of solute solution into the mobile phase under high pressure. The dimensions of HPLC columns and the character of supports vary considerably according to the retention behavior of the analytes. Information about the use of various columns and HPLC supports in food analysis is included in the corresponding chapters of the book. The mobile-phase delivery system includes a pump with filter, a degasser, and transfer tubing. It transfers the mobile-phase components into the separation system with a constant, precise, and reproducible flow rate. When the composition of the mobile phase is held constant during the separation process, the method is called isocratic elution. In order to increase the efficiency of the separation or eluting solutes with highly different physicochemical characteristics in one run, the composition of the mobile phase can be continuously changed in a predetermined manner (gradient elution). A wide variety of detectors are used in HPLC, applying different physicochemical principles or exploiting different molecular characteristics of solutes. The most commonly used detectors are ultraviolet-visible (UV-VIS), fluorescence,

refractive index (RI) detector), and various electrochemical detectors (ECD). Recently, hyphenated techniques (a combination of HPLC with a detector suitable for the identification of the solutes) have gained growing acceptance and application in HPLC. Thus, HPLC was successfully combined with other analytical spectroscopic methods such as mass spectrometry (MS), infrared spectrometry (IR), and Fourier transform infrared spectrometry.

1.2.2.1 FUNDAMENTALS

The capacity ratio (k') can be defined in both adsorption and reversed-phase chromatography by

$$k' = (t_R - t_0)/t_0 \quad (1.14)$$

where the t_R is the retention time (the time passed from the injection to the appearance of the solute peak maximum) and t_0 is the dead time. The relationship between the logarithm of the capacity ratio ($\log k'$) and the volume fraction of the stronger component in a mobile phase composed of two solvents can be described by

$$\log k' = \log k'_0 + b \cdot C \quad (1.15)$$

where the $\log k'_0$ value is the $\log k'$ value of the solute extrapolated to zero concentration of the stronger component in the mobile phase (C); the b (slope) value shows the sensitivity of the change in the retention of a given solute caused by unit change in the concentration of the stronger component in the mobile phase.

The characteristic chromatographic parameters (separation factor, resolution, theoretical plate number) in HPLC can be calculated similarly like in gas chromatography [see equations (5)–(8)]. The theoretical aspects [11] and practical applications of HPLC [12,13], the support most frequently used for the various HPLC separations [14], and the application of HPLC for sample preparation [15] have been described earlier and discussed in more detail.

1.3 CAPILLARY ELECTROPHORESIS (CE)

Separation by electrophoresis is obtained by differential migration of solutes in an electric field. In CE, separation is performed in narrow-bore capillaries, typically 25–75 μm inner diameter (I.D.), usually filled with a buffer. Use of the capillary has numerous advantages, particularly with respect to the detrimental effects of Joule heating. The high electrical resistance of the capillary enables the application of very high electrical fields (100–500 V/cm) with minimal heat generation. Moreover, the large surface area to volume ratio

of capillary efficiently dissipates the generated heat. The use of the high electrical fields results in short analysis time, high efficiency, and resolution. Peak efficiency, often in excess of 10^5 theoretical plates number, is caused by the plug profile of the electroosmotic flow, an electrophoretic phenomenon that generates the bulk flow of solution within the capillary. This flow also enables the simultaneous analysis of all solutes, regardless of charge. Minimal sample volume requirements (1–10 nL), on-capillary detection, and the potential for quantitative analysis and automation make CE a rapidly developing separation technique. CE is characterized by its simple experimental setup. Both ends of a filled capillary are dipped into two buffer reservoirs maintained at the same level. A high voltage is then applied to this capillary. Generally, the sample is injected at the anodic end, and the detector is placed near the cathodic end.

Various modes of capillary electrophoresis can be performed using the same instrument, including capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), micellar electrokinetic capillary chromatography (MECC), capillary isoelectric focusing (CIEF), and capillary isotachopheresis (CITP). In MECC, surfactant above its critical micellar concentration is added to the background electrolyte. Solute with varying degrees of hydrophobicities are partitioned between the hydrophobic core of the micelles and aqueous buffer [16].

1.3.1 Fundamentals

Principal processes governing separation in CE are the electroosmotic flow (μ_{EOF}) and electrophoretic flow (μ_{ep}). Electroosmotic flow is the bulk flow of solvent in the capillary under an applied potential. Electrophoretic flow is the flow of ions owing to charge. Electroosmotic flow can be expressed by

$$\mu_{\text{EOF}} = (\epsilon \cdot \zeta / \eta) \quad (1.16)$$

where ϵ = dielectric constant, ζ = zeta potential, and η = bulk viscosity.

Equation (16) indicates that the mobility is independent on the applied electric field. The zeta potential is essentially determined by surface charge on the capillary wall. Since this charge is strongly pH dependent, the magnitude of the electroosmotic flow (EOF) varies with pH. Depending on the specific conditions, the EOF can vary by more than an order of magnitude between pH 2 and 12. The zeta potential is also dependent on the ionic strength of the buffer, as described by double-layer theory. Increased ionic strength results in double-layer compression, decreased zeta potential, and reduced EOF. The analytical parameters for capillary electrophoresis can be described in similar terms to those for column chromatography. The time required for a solute to migrate to the point of detection is called the migration time and is given by the quotient

of migration and velocity. The migration time and other experimental parameters can be used to calculate the apparent solute mobility:

$$\mu_a = l/t \cdot E = L \cdot l/t \cdot V \quad (1.17)$$

and

$$\mu_a = \mu_e + \mu_{\text{EOF}} \quad (1.18)$$

where l is the effective capillary length, L is the total length, t is the migration time, E is the electric field, and V is the applied voltage. In the presence of EOF, the measured mobility is called the apparent mobility (μ_a). The effective mobility (μ_e) can be determined by extracting from the apparent mobility by independently measuring the EOF using a neutral marker that moves at a velocity equal to the EOF. The separation of neutral solutes in MEKC can be described using by modified chromatographic relationships. The capacity factor k' is given by

$$k' = (t_r - t_0)/t_0(1 - t_r/t_m) = K(V_s/V_m) \quad (1.19)$$

where t_r is the retention time of the solute, t_0 is retention time of unretained solute moving at the EOF rate, t_m is the micelle retention time, K is the partition coefficient, V_s is the volume of the micellar phase, V_m is the volume of the mobile phase.

Various capillary electrophoretic methods are discussed more profoundly in references [16–21].

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