

## Contents

Foreword . . . . .	XVII
Preface . . . . .	XIX

### THEORETICAL ASPECTS OF LIQUID CHROMATOGRAPHY

Fundamental concepts ( <b>J. Novák</b> and <b>J. Janák</b> ) . . . . .	3
Introduction . . . . .	3
Principle of chromatography . . . . .	4
Chromatographic systems . . . . .	5
Chromatographic techniques . . . . .	7
Basic chromatographic quantities . . . . .	8
Basic processes in chromatography ( <b>J. Novák</b> , <b>J. Janák</b> and <b>S. Wičar</b> ). . . . .	11
Flow of mobile phase through a packed column. . . . .	11
Diffusion of solute within the phases . . . . .	15
Equilibration of solute between the phases . . . . .	17
General description of the chromatographic process ( <b>J. Novák</b> , <b>J. Janík</b> and <b>S. Wičar</b> ). . . . .	25
Solute mass balance in the chromatographic system . . . . .	25
Concept of ideal linear chromatography . . . . .	31
Concept of the theoretical plate . . . . .	33
Dynamics of zone spreading . . . . .	35
Chromatographic resolution . . . . .	40
Physico-chemical basis of chromatographic retention in liquid–liquid and liquid–solid systems ( <b>J. Novák</b> ) . . . . .	45
Interaction of solute with the phases . . . . .	45
Thermodynamics of sorption equilibrium . . . . .	48
Gel permeation chromatography ( <b>M. Kubín</b> ). . . . .	57
Introduction . . . . .	57
Principles of gel permeation chromatography . . . . .	57
Physical basis of the separation process . . . . .	59
Fundamentals of ion-exchange chromatography ( <b>O. Mikeš</b> ) . . . . .	69
Principles and terminology . . . . .	69
Characterization of ion exchangers . . . . .	73
Reactions, affinity and selectivity in ion exchange . . . . .	75
Ion-exchange equilibria and kinetics . . . . .	77
Column operation and ion-exchange chromatography . . . . .	80
Ion exclusion, ion retardation, the ion-sieve process and partition chromatography on ion exchangers . . . . .	83
Ligand-exchange chromatography . . . . .	85
Ion exchange in non-aqueous solutions . . . . .	85
Affinity chromatography ( <b>J. Turková</b> ) . . . . .	89
Principles of affinity chromatography . . . . .	89

Choice of bound affinant . . . . .	92
General aspects of the <b>affinant</b> – <b>sorbent</b> bond . . . . .	94

## TECHNIQUES OF LIQUID CHROMATOGRAPHY

Instrumentation for liquid chromatography ( <b>M. Krejčí, Z. Pečan and Z. Deyl</b> ). . . . .	101
Classical instrumentation for liquid chromatography . . . . .	102
Introduction . . . . .	102
Columns and accessories . . . . .	103
Column preparation and introduction of sample . . . . .	110
Techniques of elution . . . . .	112
Analysis of effluent . . . . .	115
Preparative and industrial liquid chromatography . . . . .	120
How to learn the technique . . . . .	122
Techniques of high-efficiency liquid chromatography . . . . .	123
Principal differences between classical and high-efficiency liquid chromatography . . . . .	123
The function of a liquid chromatograph . . . . .	127
Mobile phase reservoirs . . . . .	128
Gradient-forming devices . . . . .	129
Manipulation. . . . .	132
Pumping systems . . . . .	133
Pressure <b>pulse-damping</b> device . . . . .	137
Sample introduction devices . . . . .	139
Columns. . . . .	143
Thermostats . . . . .	145
Detectors. . . . .	146
Evaluation of different detectors . . . . .	162
Counter-current chromatography . . . . .	162
Sorbents ( <b>J. Janak, J. Čoupek, M. Krejčí, O. Mikeš and J. Turková</b> ) . . . . .	169
Rational classification . . . . .	170
Sorbents for liquid–solid chromatography . . . . .	174
Supports and stationary phases for <b>liquid–liquid</b> chromatography . . . . .	182
Column <b>packings</b> for gel chromatography . . . . .	187
Ion-exchange materials . . . . .	202
Sorbents for affinity chromatography . . . . .	215
Mobile phases ( <b>O. Mikeš and R. Vespalec</b> ) . . . . .	233
Mobile phases for <b>liquid–liquid</b> chromatography . . . . .	234
Mobile phases for <b>liquid–solid</b> chromatography. . . . .	248
Mobile phases for ion-exchange chromatography . . . . .	261
Calculation of gradients . . . . .	270

## PRACTICE OF LIQUID CHROMATOGRAPHY

Operation of a modern liquid chromatograph ( <b>R. Vespalec and M. Krejčí</b> ) . . . . .	283
Preparation of the apparatus . . . . .	283
Sorting of sorbents according to particle size . . . . .	285
Determination of the activity of alumina by <b>thin-layer</b> chromatography . . . . .	290
Column preparation. . . . .	291
<b>Sample</b> preparation and application . . . . .	295
General comments . . . . .	297

Practice of gel chromatography (J. Čoupek, M. Kubín and Z. Deyl)	301
Choice of gel packing	301
Choice of solvent and operating temperature	303
Apparatus for gel chromatography	304
Special gel chromatographic techniques	311
Evaluation of gel permeation chromatographic data	312
Determination of molecular weights of naturally occurring macromolecular compounds by molecular sieve chromatography	317
Practice of ion-exchange chromatography (O. Mikeš)	325
Introduction	325
Choice of suitable ion exchangers	325
Methods for the fractionation of ion exchangers	353
Decantation and cycling of ion exchangers	354
Buffering of ion exchangers	355
Deaeration of ion exchangers and filling of chromatographic columns	356
Application of samples	360
Methods of elution	363
Calculation of flow-rates	364
Evaluation of fractions	366
Regeneration and storage of ion exchangers	366
Practice of affinity chromatography (J. Turková)	369
Preparation of the solid support with a bound affinant	369
Sorption conditions	370
Conditions for elution	372
Preservation of solid sorbents with a bound affinant	375
Analytical utilization of chromatograms (J. Novák, J. Janák and S. Wičar)	377
Identification	377
Quantitation	386
Radiochromatographic techniques (I.M. Hais and J. Dršata)	403
Introduction	403
Detectors	404
Detection modes	408

## APPLICATIONS

Hydrocarbons (J. Churáček)	417
Introduction and general techniques	417
Chromatography on adsorbents	417
Chromatography on gels	421
Other methods of chromatography of hydrocarbons	423
Alcohols and polyols (J. Churáček)	431
Introduction and general techniques	431
High-speed liquid and gel permeation chromatography of free alcohols	431
Chromatography of alcohols on ion exchangers	435
Chromatography of derivatives of alcohols and glycols	437
Separation of polyols and polymeric diols	438

Phenols (J. Churáček and J. Čoupek) . . . . .	441
Introduction . . . . .	441
Gel chromatography . . . . .	441
Adsorption chromatography . . . . .	442
Ion-exchange chromatography . . . . .	445
Ethers and peroxides (J. Churáček) . . . . .	451
Oxo compounds (J. Churáček) . . . . .	455
Introduction . . . . .	455
Aliphatic and cyclic aldehydes and ketones . . . . .	456
Quinones . . . . .	459
Applications in lignin chemistry . . . . .	461
Carbohydrates (K. Čapek and J. Staněk, Jr.) . . . . .	465
Introduction . . . . .	466
General techniques . . . . .	467
Liquid–solid chromatography . . . . .	467
Liquid–liquid chromatography . . . . .	469
Gel chromatography . . . . .	472
Ion-exchange chromatography . . . . .	473
Automated detection methods . . . . .	475
Mono-, oligo- and deoxy saccharides . . . . .	483
Chromatography on charcoal–Celite . . . . .	483
Chromatography on cellulose . . . . .	486
Chromatography on ion-exchange resins . . . . .	487
Chromatography on molecular sieves . . . . .	493
Amino sugars . . . . .	496
Free amino sugars . . . . .	496
Mutual separation of amino sugars and amino acids . . . . .	499
Derivatives of amino sugars and chromatographic methods used in the synthesis of amino sugars . . . . .	500
Sugar derivatives . . . . .	501
Alditols . . . . .	501
Glycosides . . . . .	504
Ethers and acetals . . . . .	506
Esters . . . . .	507
Sugar acids . . . . .	507
Sugar phosphates . . . . .	515
Polysaccharides (K. Čapek and J. Staněk, Jr.) . . . . .	523
Introduction . . . . .	523
Ion-exchange chromatography . . . . .	524
Gel permeation chromatography . . . . .	525
Polysaccharide–protein complexes (M. Juřicová and Z. Deyl) . . . . .	529
Glycosaminoglycans (mucopolysaccharides) . . . . .	529
Glycoproteins and glycopeptides . . . . .	538
Lower carboxylic acids (J. Churáček and P. Jandera) . . . . .	543
Introduction . . . . .	543
General techniques . . . . .	543
Separation of carboxylic acids on the basis of molecular sorption, using aqueous and non-aqueous organic solvents . . . . .	545

Ion-exchange chromatography of carboxylic acids in various aqueous acids or buffered solvent systems . . . . .	551
High-speed ion-exchange chromatography of carboxylic acids with anion exchangers of controlled surface porosity . . . . .	565
Other separation techniques for carboxylic acids . . . . .	567
Higher carboxylic acids (J. Pokorný) . . . . .	575
Introduction and general remarks . . . . .	575
Separation as fatty acid derivatives . . . . .	575
Chromatography on adsorbents in general use . . . . .	576
Chromatography on specific adsorbents . . . . .	577
Gel and ion-exchange chromatography . . . . .	578
Lipids (J. Pokorný) . . . . .	581
Introduction and general remarks . . . . .	581
Separation of lipids into classes . . . . .	581
Separation of glycerol esters and other neutral lipids . . . . .	585
Separation of phospholipids and other polar lipids . . . . .	588
Steroids (Ž. Procházka) . . . . .	593
Introduction . . . . .	593
General techniques . . . . .	594
Introductory and theoretical considerations . . . . .	594
Sample preparation and application . . . . .	595
Liquid–solid chromatography . . . . .	595
Liquid–liquid chromatography . . . . .	597
Gel chromatography . . . . .	601
Ion-exchange chromatography . . . . .	602
Detection . . . . .	603
Applications . . . . .	604
Sterols . . . . .	604
Androgens . . . . .	604
Estrogens . . . . .	605
Gestagens (progestins) . . . . .	613
Corticosteroids . . . . .	614
Bile acids and other steroid acids . . . . .	617
Steroidal glycosides . . . . .	618
Steroidal insect hormones . . . . .	619
Terpenes (O. Motl) . . . . .	623
Introduction . . . . .	623
Hydrocarbons . . . . .	624
Ethers, epoxides and furans . . . . .	629
Esters . . . . .	630
Aldehydes and ketones . . . . .	631
Lactones . . . . .	632
Alcohols . . . . .	633
Acids . . . . .	633
Amines (Z. Deyl) . . . . .	637
Introduction . . . . .	637
Aliphatic mono-, di- and polyamines . . . . .	637
Aromatic amines . . . . .	643
Aromatic amines and aliphatic polyamines in mixtures . . . . .	645

Tryptophan metabolites . . . . .	645
Quaternary ammonium compounds and amino alcohols . . . . .	649
Biogenic amines . . . . .	650
Other non-heterocyclic nitrogen compounds (J. Churáček) . . . . .	657
Introduction . . . . .	657
Nitro compounds . . . . .	657
Amides . . . . .	659
Guanidine and urea derivatives . . . . .	661
Amino acids (Z.J. Zmrhal, J.G. Heathcote and R.J. Washington) . . . . .	665
Analytical chromatography . . . . .	666
Ion-exchange chromatography . . . . .	668
Amino acid analyzers . . . . .	675
Packings for chromatographic columns . . . . .	688
Preparation of eluents and reagents . . . . .	692
Chromatographic elution systems . . . . .	697
Preparation of sample . . . . .	704
Calculation of the elution curve . . . . .	705
Preparative chromatography . . . . .	708
Amino acid derivatives (Z. Deyl and M. Juřicová) . . . . .	713
Introduction . . . . .	713
2,4-Dinitrophenyl (DNP) amino acid derivatives . . . . .	714
5-Dimethylaminonaphthalene-1-sulphonyl (Dns) amino acids . . . . .	726
Hydantoins and substituted hydantoins . . . . .	731
Miscellaneous derivatives . . . . .	736
Peptides (I. Kluh) . . . . .	741
Introduction . . . . .	742
Methods for the separation of peptides . . . . .	742
Analysis of the effluent from the chromatographic column . . . . .	744
Gel permeation chromatography . . . . .	749
Ion-exchange chromatography . . . . .	756
Affinity chromatography . . . . .	768
Partition chromatography . . . . .	770
Proteins (Z. Prusík) . . . . .	773
Introduction . . . . .	773
General rules for the separation of proteins . . . . .	774
Gel permeation chromatography . . . . .	778
Chromatography on glass with controlled pore size . . . . .	781
Ion-exchange chromatography . . . . .	782
Chromatography on hydroxyapatite and on calcium phosphate . . . . .	788
Solubility chromatography . . . . .	789
Technique of gel permeation chromatography in a detergent gradient . . . . .	798
Affinity chromatography . . . . .	799
Detection of proteins in the effluent . . . . .	800
Enzymes (O. Mikeš) . . . . .	807
Special requirements for the chromatography of enzymes . . . . .	807
Techniques and automated analyses . . . . .	809
Oxidoreductases . . . . .	813

Transferases . . . . .	816
Hydrolases . . . . .	818
Lyases . . . . .	823
Isomerases . . . . .	825
Ligases . . . . .	826
Low-molecular-weight constituents of nucleic acids. Nucleosides, nucleotides and their analogues (S. Zadražil) . . . . .	831
Introduction . . . . .	831
General techniques in the separation of low-molecular-weight components of nucleic acids . . . . .	832
Automated procedures for the analysis of nucleic acid components . . . . .	836
Individual types of nucleic acid constituents . . . . .	839
Nucleic acids (S. Zadražil) . . . . .	859
Introduction and general techniques in nucleic acid separations . . . . .	859
Deoxyribonucleic acids . . . . .	862
Ribonucleic acids . . . . .	873
Polynucleotides and large oligonucleotides . . . . .	878
Automated procedures and polynucleotide sequence analysis . . . . .	880
Alkaloids (K. Macek) . . . . .	887
Introduction . . . . .	887
Preparation of samples . . . . .	888
Techniques . . . . .	888
Applications . . . . .	894
Other heterocyclic compounds (J. Davídek, M. Janda and I. Stibor) . . . . .	895
Introduction . . . . .	895
Derivatives of $\gamma$ -pyrone . . . . .	896
Anthocyanins . . . . .	909
Aflatoxins and mycotoxins . . . . .	912
Other compounds containing heterocyclic oxygen . . . . .	915
Porphyrins and related compounds . . . . .	917
Indoles . . . . .	919
Pyridine and related compounds . . . . .	920
Polynuclear aza-heterocyclics and complex mixtures of heterocyclic compounds . . . . .	921
Organic sulphur compounds (J. Churáček) . . . . .	927
Introduction . . . . .	927
Sulphonic acids . . . . .	927
Other sulphur compounds . . . . .	932
High-speed liquid chromatography . . . . .	934
Organic phosphorus compounds (J. Zabranský) . . . . .	939
Application of column chromatography . . . . .	939
Boron compounds (S. Heřmánek) . . . . .	945
General techniques . . . . .	945
Boranes and substituted boranes . . . . .	947
Carboranes . . . . .	950
Ligand derivatives of boranes and carboranes . . . . .	950
Metallocarboranes . . . . .	950

<b>Vitamins (J. Davídek)</b> . . . . .	953
Introduction . . . . .	954
<b>Fat-soluble vitamins</b> . . . . .	955
<b>Vitamin A group</b> . . . . .	955
Calciferols . . . . .	957
Tocopherols . . . . .	960
<b>Vitamin K group</b> . . . . .	961
<b>Water-soluble vitamins</b> . . . . .	962
Thiamine . . . . .	962
Riboflavin and other flavins . . . . .	965
Nicotinic acid and its derivatives . . . . .	967
Pyridoxine group . . . . .	968
Biotin . . . . .	970
Pantothenic acid and coenzyme A . . . . .	971
Folic acid and other pteridine derivatives . . . . .	972
Corrinoids . . . . .	973
L-Ascorbic and L-dehydroascorbic acids . . . . .	975
<b>Antibiotics (V. Betina)</b> . . . . .	979
Introduction . . . . .	979
Penicillins and cephalosporins . . . . .	980
Carbohydrate antibiotics . . . . .	985
Macrocyclic antibiotics . . . . .	994
Tetracyclines and related antibiotics . . . . .	996
Nucleoside antibiotics including polyoxins . . . . .	999
Peptides and related antibiotics . . . . .	1000
Miscellaneous antibiotics . . . . .	1003
<b>Pesticides (J. Zabranský and J. Churáček)</b> . . . . .	1009
Introduction and general techniques . . . . .	1009
Chlorinated pesticides and their metabolites . . . . .	1014
Phosphorus pesticides . . . . .	1018
Carbamate pesticides and their metabolites . . . . .	1024
Pyrethrins . . . . .	1029
<b>Synthetic dyes (J. Churáček and J. Gasparič)</b> . . . . .	1033
Introduction . . . . .	1033
General techniques . . . . .	1033
Chromatography on adsorbents . . . . .	1034
Chromatography on hydrophilic gels . . . . .	1035
<b>Pigments of plastids and photosynthetic chromatophores (Z. Šesták)</b> . . . . .	1039
Introduction . . . . .	1039
Sample preparation . . . . .	1040
Chromatographic procedures . . . . .	1041
Detection . . . . .	1048
<b>Macromolecular substances and plastics (M. Kubín and J. Čoupek)</b> . . . . .	1051
Introduction . . . . .	1051
Vinyl polymers . . . . .	1053
Rubbers . . . . .	1056
Polyolefins . . . . .	1057
Polycondensates . . . . .	1062



Copolymers . . . . .	1063
Miscellaneous polymers . . . . .	1064
Oligomers . . . . .	1066
<b>Cells and subcellular particles (M. Juřicová and Z. Deyl)</b> . . . . .	<b>1075</b>
Introduction . . . . .	1075
Ribosomes . . . . .	1076
Viruses . . . . .	1077
Bacteriophages . . . . .	1081
Blood cells . . . . .	1082
Cells from the spleen . . . . .	1083
Bone marrow cells . . . . .	1084
<b>Inorganic, coordination and organometallic compounds (F. Jursík)</b> . . . . .	<b>1087</b>
Introduction . . . . .	1087
Simple inorganic compounds . . . . .	1088
Cations . . . . .	1088
Anions . . . . .	1096
Coordination and organometallic compounds . . . . .	1099
General survey . . . . .	1099
Geometrical isomers . . . . .	1100
Optical isomers and diastereoisomers . . . . .	1101
Relationship between chromatographic behaviour and configuration of optical isomers . . . . .	1103
Ferrocenes . . . . .	1108
Metallocenes . . . . .	1109
<b>Isotopes and radioactive compounds (J. Dršata and I.M. Hais)</b> . . . . .	<b>1115</b>
Isotopic effects in liquid column chromatography . . . . .	1115
Separation of radioactive substances . . . . .	1125
<b>Subject index</b> . . . . .	<b>1127</b>
<b>List of compounds chromatographed</b> . . . . .	<b>1141</b>