Inamuddin
Mohammad Luqman *Editors*

Ion Exchange Technology II

Applications

Springer

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Inamuddin • Mohammad Luqman
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Preface

Ion exchange is a process of exchanging ions between stationary and mobile phases. It is a natural process that has been in practice for ages. Since commercial development took place in the last century, both academic and industrial research have been improving technology to find new applications.

This edition covers applications of ion exchange materials and technology in various fields, including fuel cells, catalysis, wastewater treatment and water purification, selective separation, concentration and recovery of toxic metal cations and anions, chemical and biochemical separations, pharmaceuticals, therapeutic applications, and electronics.

Chapter 1 covers the basic principles and modern applications of ion exchange technology in the separation of amino acids, peptides, and proteins, which are very useful biomaterials. Chapter 2 deals with whey disposal, which presents a huge obstacle for the dairy industry, as it is an expensive and problematic process. Chapter 3 presents the application of ion exchangers in the speciation and fractionation of elements in food and beverages. The main method used in this area is solid-phase extraction, which includes procedures with single sorbents or two coupled sorbents. Chapter 4 covers the applications of ion exchangers in the alcohol manufacturing industry. Ion exchangers contribute to remove potassium and to stabilize wine against the sediments of potassium bitartrate crystals in the acid cycle. Furthermore they help treating wine for cation and removing anion from grape in grape sugar production. The use of ion exchange resins in continuous sugar process industry is reviewed in Chap. 5. a particular focus is given to chromatographic methods and, specifically to continuous annular chromatography and simulated moving bed (SMB) apparatus.

Chapter 6 deals with the study of the application of ion exchange resins as catalysts in the synthesis of isobutyl acetate, one of the industrially important reactions. The ability of ion exchange resins to bind to drugs, which helps in masking the bitter taste of drugs, drug release, and drug stabilization, has made it a popular choice with pharmaceutical companies. Chapter 7 focuses on the therapeutic applications of ion exchange resins. Chapter 8 adds to chapter 7 by reviewing the application of ion exchange resins in kidney dialysis. The development and use

of synthetic ion exchange resins for kidney dialysis is a relatively recent achievement.

An overview of zeolites as inorganic ion exchangers for environmental applications is presented in Chap. 9. The presence of fluoride, pesticides, radionuclides, organic wastes, and heavy toxic metal ions in the aquatic environment has been of great concern to engineers, environmentalists, and scientists because of their increased discharge, toxic nature, and adverse effects on receiving waters. Chapter 10 addresses these points using ion exchange materials and technology. Chapter 11 presents an overview of the ion exchange of metal ions, focusing on their recovery, separation, and pre-concentration. In Chap. 12, a study for a chelating resin containing iminodiacetic acid group (Diaion CR11) is presented to separate trivalent chromium, copper, and iron from synthetic and industrial effluents. In Chap. 13, a similar study to determine and model the effect of cadmium and zinc ions in solution for the removal of Cr(VI) via ion exchange with hydrotalcite, a clay mineral media, is discussed. Chapter 14 presents an overview of the role of the absorbing materials, especially phenol-based resins, for "3d" and "4f" metals ions from the discharge of the related industries.

Planar chromatography, i.e., paper and thin-layer chromatography, is a simple and fast method for the separation of various classes of organic and inorganic compounds. An overview related to the usage of inorganic ion exchangers in planar chromatographic separations is presented in Chap. 15. Chapter 16 deals with a similar topic, i.e., an overview of cation-exchanged silica gel–based thin layer chromatography of organic and inorganic compounds. Finally, Chap. 17 discusses different ion exchange materials used for the removal of various anions (nitrate, fluoride, perchlorate, arsenate, chromate, phosphate, thiocynate, etc.) from water.

These chapters provide an in-depth practical knowledge of ion exchange materials suitable for postgraduate students, researchers, and R&D specialists in the chemical and biochemical industries.

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Mohammad Luqman
Editors

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We are most indebted to the grace of the Almighty "One Universal Being," who inspires entire Humanity to knowledge, and who blessed us with the needed favor to complete this work.

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Editors' Bios

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List of Abbreviations

z	Axial coordinate (m)
D_b	Axial dispersion (m^2/s)
d	Diameter (m)
q^{eq}	Equilibrium adsorbed concentration (kg/m_{solid}^3)
u	Interstitial velocity (m/s)
L	Length (m)
	Liquid flow rate (m^3/s)
C	Liquid phase concentration (kg/m^3)
	Mass transfer coefficient (s−1)
α_B^A	Separation factor
	Solid flow rate (m^3/s)
u_S	Solid velocity (m/s)
t_s	Switching time (s)
t	Time variable (s)
$(C_2H_5)_2O$	Diethyl ether
12MR	Twelve-membered ring
1M2P	1-methoxy-2-propanol
2M1P	2-methoxy-1-propanol
4MR	Four-membered ring
4-VP	4-Vinylpyridine
6MR	Six-membered ring
8MR	Eight-membered ring
abAz, aaBs	Activities
ABEC	Aqueous biphasic extraction chromatographic
AC	Activated carbon
AcOH	Acetic acid
AE	Aminoethyl
$AgNO_3$	Silver nitrate
Al(III)	Aluminum(III)
$AlCl_3$	Aluminium chloride
Amb200CT	Amberlite-type cation exchange resin

APAS	Aminophosphonic acid silica
As(III)	Trivalent arsenite
As(V)	Pentavalent arsenate
ASV	Anodic stripping voltammetry
B.C.E	Before the common era
BAT	Best available technology
BEA	Zeolite beta
BS	Banana stem
BT	Breakthrough
BuOAc	Butyl acetate
BuOH	Butanol
BV	Bed volume
C	Cholic acid
C.E	Common Era
C_6H_{14}	Hexane
$C_6H_5CH_3$	Toluene
CBUs	Composite building units
CCl_4	Carbon tetra chloride
CCP	Colloidal calcium phosphate
CD	Cyclodextrin
CDC	Chenodeoxycholic acid
$CdCl_2$	Cadmium chloride
CE	Cation-exchange, cation-exchanger
Ce(III)	Cerium(III)
CEC	Cation-exchange capacity
CER	Ceralite IRA 400
CF	Feed concentration, mg/L
CH_2Cl_2	Dichloromethane
$CHCl_3$	Chloroform
Clin-Fe	Clinoptilolite-iron
ClO_4^-	Perchlorate
CM	Carboxymethyl
CMC	Ceramic matrix composites
CMX	Cation exchange membranes
COD	Chemical oxygen demand
CP	Coconut coir pith
CPE	Carbon paste electrode
CRM	Certified reference material
CSV	Cathodic stripping voltammetry
$CuSO_4$	Copper sulfate
CV	Crystal violet
Cyt *c*	Cytochrome *c*
D	Diffusion coefficient
D4R	Double four-membered ring
D6R	Double six-membered ring

D_{app}	Apparent diffusion coefficient
Dax	Axial dispersion coefficient, cm^2/s
DC	Defluoridation capacity
DD-MSWV	Double differential multiple square wave voltammetry
DDT	Dichlorodiphenyltrichloroethane
DE	Decolorization efficiency
DEA	Diethylamine
DEAE	Diethylaminoethyl
DEAPA	Diethylaminopropylamine
DETA	Diethylenetriamine
DFs	Decontamination factors
DF-STEM	Dark field scanning transmission electron microscopy
DL	Detection limit
Dm	Molecular diffusivity, cm^2/s
DMAHP	Dimethylaminohydroxypropyl
DMAPA	Dimethylaminopropylamine
DMFC	Direct methanol fuel cell
DMG	Dimethylglyoxime
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
Dp	Intraparticular diffusion coefficient, cm^2/s
dp	Particle diameter, cm
DP-ASV	Differential pulse anodic stripping voltammetry
DPCSG	1,5-diphenylcarbazide doped sol–gel silica
DPGME	Di-propylglycol-methylether
DPV	Differential pulse voltammetry
D-R	Dubinin-Radushkevich
DR	Dubinin-Raduskevish isotherm
DS	Degree of sulfonation
D_s	Diffusion coefficient in the solution phase
DVB	Di-vinyl benzene
E(OC)	Open circuit electrochemical potential
$E(OC)_{CE}$	Open circuit electrochemical potential
$E_{1/2}$	Half-wave potential
E_{appl}	Applied potential
ECL	Electrochemiluminescence
ECTEOLA	The product of reaction of epichlorohydrin, triethanolamine, and alkali cellulose
ED	Electrodialysis
EDA	Ethylenediamine
EDR	Electrodialysis reversal
EDS	Energy dispersive X-ray spectroscopy
EDTA	Ethylenediaminetetraacetate
EDX	Energy dispersive X-ray spectroscopy
E_i	Initial potential

EIS	Electrochemical impedance spectroscopy
ENM	Electrospin nano-fiber membrane
EPA	Environmental protection agency
Ep_b	Backward peak potential
EPBI(DMG)	Epoxidized polybenzimidazole(Dimethylglyoxime)
Ep_f	Forward peak potential
ETSS	Ethyl styrene sulfonate
EU	European union
EW	Equivalent weight
F	Faraday constant
F-AAS	Flame atomic absorption spectrometry
FAU	Faujasite
Fc	Ferrocene
Fc^+	Ferricinium cation
FCC	Fluid catalytic cracking
Fe(III)	Iron(III)
$FeCl_3$	Ferric chloride
$FeCl_4^-$	Tetrachloroferrate
Fe-LLT	$(La,Fe)TiO_3$
$FeSO_4$	Ferrous sulfate
FS	Full scale
FTIR	Fourier transform infrared
FVA	Fibrous anion exchanger
GC	Gas chromatography
GCE	Glassy carbon electrode
GDC	Glycodeoxycholic acid
GF-AAS	Graphite furnace atomic absorption spectrometry
GIS	Gismondine
GLC	Glycolithocholic acid
GMA/MBA	Glycidyl methacrylate/N,N´-methylene bis-acrylamide
GME	Gmelinite
$H_2AsO_4^-$	Dihydrogen arsenate
H_3AsO_3	Arsenious acid
H_3Cyt	Cytric acid
HA	Humic acid
HAIX-F	Hybrid anion exchange fiber
HASB	Hard soft acid base
$HAsO_4^{2-}$	Hydrogen arsenate
HA-Zr-PILC	Humic acid-immobilized zirconium-pillared clay
HBPEI	Hyperbranched polyethylenimine
HFO	Hydrated Fe(III) oxide
HIX	Hybrid ion exchange
HIX-NF	Hybrid ion exchange-nanofiltration
HMW	High molecular weight
HPA	Hydrated tungstophosphoric acid

HPCIC	High performance chelation ion chromatography
HPLC	High performance liquid chromatography
HPO_4^{2-}	Mono hydrogen phosphate
HPTLC	High performance thin layer chromatography
HREM	High resolution electron microscopy
hRF	RF ×100
HTC	Hydrotalcite
HZO	Hydrous zirconium oxide
IAEA	International atomic energy agency
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
IDA	Iminodiacetic acid
IE fiber	Ion exchange fiber
IE	Ion exchange
IEC	Ion exchange capacity
IEV	Ion exchange voltammetry
IND	Indion FR 10
IO	Iontosorb oxin
Ip_p	Peak current for analytes in the polymer phase
Ip_s	Peak current for analytes in the solution phase
IR	Infrared
IS	Iontosorb salicyl
ITO	Indium tin oxide
IZA	International zeolite association
K_2CrO_4	Potassium dichromate
Ka	Equilibrium constant
KBr	Potassium bromide
k_D	Distribution coefficient
Kd	Distribution coefficient
k_{ex}	Site to site electron exchange rate constant
kf	Film mass transfer coefficient, cm/s
KFI	KFI framework type zeolite
KI	Potassium iodide
kL	Langmuir parameter, L/mg
K_X^M	Selectivity coefficient
Ks	Selectivity coefficient
L	Bed height, cm
La(III)	Lanthanu(III)m
La_2O_3	Lantanum oxide
LB	Langmuir-Blodgett
LBL	Layer-by-layer
LC	Lithocholic acid
LDH	Layered double hydroxides
LDPE	Low density polyethylene
L-His	L-Histidine

LiBr	Lithium bromide
LMW	Low molecular weight
LOD	Limit of detection
LOQ	Limit of quantitation
L-Phe	L-Phenylalanine
L-Pro	L-Proline
LRW	Liquid radioactive waste
LS	Lab scale
LTA	Linde type A framework type zeolite
L-Trp	L-Trptophan
MAP	Maximum aluminium P, GIS framework type zeolite
MAS	Magic angle spinning
MAs, MBs	Molarities
mAz, mBz	Molalities
MB	Methylene blue
MCM-41	Mobil mesoporous material
MEL	Maximum exchange level
MELs	Maximum exchange levels
MFI	ZSM-5 (five)
MG	Malachite green
MHL	Metal proton ligand
MMC	Metal matrix composites
MMT	Montmorillonite
MMW	Moderate molecular weight
$MnSO_4$	Manganese sulfate
MOR	Mordenite
MS	Mass spectrometry
MSWV	Multiple square wave voltammetry
MTA	Methylthriamyl ether
MTB	Methylthributhyl ether
MV	Methyl viologen
MW	Molecular weight
MWW	MCM-22 (twenty-two)
N	Noise
n	Number of columns (−)
N,N-Me2-L-Phe	N,N-dimthyl-phenylalanine
NaOH	Sodium hydroxide
ND	Number of mass transfer units by pore diffusion (= $\tau\varepsilon$P DP /R2)
N-DC	N, N′ di(caroxymethyl)dithiocarbamate
Nf	Number of film mass transfer units (= 3 (1 – ε) kf τ/(ε R))
NH_3	Ammonia
NH_4Cl	Ammonium chloride
$NiSO_4$	Nickel sulfate
nm	Nanometer
NMP	N-methyl-2-pyrrolidinon

NMR	Nuclear magnetic resonance
NORM	Naturally occurring radioactive materials
NP	Nernst-Planck
OIV	International organisation of vine and wine
P	Phosphate
PA	Polyamide
PAB	4-Aminobenzyl
PAMAM	Polyamidoamine
PAN	Polyacrylonitrile
PAn/SD	Polyaniline/sawdust
PANF	Polyacrylonitrile fiber
PANI	Polyaniline resin
PANI/HA	Polyaniline/humic acid nanocomposite
PBI	Poly(benzimidazole)
PC	Polycarbonate
PC	Paper chromatography
PDDMACl	Poly(diallyldimethylammonium chloride)
PDDPCl	Poly(1,1-dimethyl-3,5-dimethylenepiperidinium chloride)
Pe	Axial Peclet number (= uL/εDax)
Pe	Peclet number (−)
PE	Polyethylene
PEEK	Poly(ether ether ketone)
PEG	Polyethylene glycol
PEI	Poly(ether imine)
PEIMPA	Polyethylenimine methylenephosphonic acid
PEK	Poly ether ketone
PEKEKK	Poly(ether ketone ether ketone ketone)
PEM	Poly(ethylene mercaptoacetimide)
PEMFC	Polymer electrolyte membrane fuel cell
PES	Polyether sulphone
PET	Poly(ethyleneterphthalate)
PFSA	Perfluorosulfonic acid
PGCP	Coconut coir pith grafted with polyhydroxyethylmethacrylate
PGHyFeO–COOH	Carboxylate functional group into polyacrylamide-grafted hydrous iron(III) oxide
PI	Polyimides
PILC	Pillared clay
PLE	Polymeric ligand exchanger
PLE's	Polymeric ligand exchangers
PMA	Poly mtharcylate
PMC	Polymer matrix composites
PMeT	Poly(3-methylthiophene)
POPs	Persistent organochlorine pollutants
PP	Polypropylene
PPO	Poly(phenylene oxide)

PP-ST-DVB	Polypropylene with grafted polystyrene with divinylbenzene
PPy	Polypyrrole
PPy/SD	Polypyrrole/sawdust
PrO	Propylene oxide
PrOH	Propanol
PSDC	PS-DVB copolymer
PS-DVB	Polystyrene divinylbenzene
PSS	Poly(4-styrene sulfonate)
PSSH	Poly(styrenesulfonic acid)
PSSNa	Poly(sodium styrenesulfonate)
PSU	Polysulphone Udel
PSU-NH_2	Aminated polysulfone Udel
PTFE	Poly(tetrafluoroethylene)
PV	Pervaporation
PVA	Poly (vinyl alcohol)
PVA/EDTA	Poly(vinyl alcohol)/EDTA
PVA/P4VP	Poly(vinyl alcohol)/poly(4-vinylpyridine)
PVC	Poly (vinyl chloride)
PVI/PVP	Polyvinylimidazole / Polyvinylpyrrolidone copolymers
PVP	Polyvinyl pyrollidone
PVPP	Polyvinylpyrrolidone
Q	Adsorbed concentration in equilibrium conditions, mg/g wet resin
QA	Quality assurance
QC	Quality control
QCS	Quaternary chitosan salt
qmax	Maximum adsorption capacity (Langmuir parameter), mg/g wet resin
R	Particle radius, cm
R2	Coefficient of determination
Re	Reynolds νυμβερ (= υ ρ dπ /ε μ)
REC	Real exchange level
RF	Retardation factor
RO	Reverse osmosis
ROS	Reactive oxygen species
RP	Reversed-phase
RP-18 silica gel	Octadecyl silica gel
RPA	Amino pentamine resin
RQA	Quaternary amine resin
Rv	Product of ionic radius of cation and its valence
S	Signal
SAED	Selected area electron diffraction
SBA	Strong basic anion-exchange
SDB	Styrene-divinylbenzene
SEC	Size exclusion chromatography

SEE	Standard error of estimate
SEM	Scanning electron microscopy
s-IPNs	Semi-interpenetrating polymer networks
SIR	Solvent impregnated resins
SMM	Surface modifying macromolecules
SPE	Screen printed electrode
SPEEK	Sulfonated poly(ether ether ketone)
SPI	Sulfonated polyimide
SPME	Solid phase microextraction
SPSU	Ortho-sulfonesulfonated poly (ethersulfone)
$SrCl_2$	Strontium chloride
ST	Polystyrene
ST-DVB	Styrene–divinylbenzene
STEM	Scanning transmission electron microscopy
STPP	Sodium tripolyphosphate
SWV	Square wave voltammetry
T	Temperature
t	Time
TAG	Triacylglycerol
TCB	Phenol-trichlorobenzene
TDS	Total dissolved solids
TEC	Theoretical exchange level
TEM	Transmission electron microscopy
TETA	Triethylenetetraamine
Tg	Glass transition temperature
THF	Tetrahydrofuran
TiOx(OH)y	Ti-oxohydroxide
TLC	Thin layer chromatography
TMFE	Thin mercury film electrode
TPA	Tripropylamine
TPABr	Tetrapropylammonium bromide
TPIR	Total polyphenol index removed.
U	Bed superficial velocity, cm/s
u*	Normalized radial coordinate (r/R)
UF	Ultra filtration
UHT	Ultra high temperature
USEPA	United States environmental protect agency
UV	Ultraviolet light
V	Scan rate
Vc	Estimated volume occupied by cations in FAU supercavity
VOCs	Volatile organic chemicals
WBA	Weak base anion
WHO	World health organization
WNF	Waste nuclear fuel
WWTG	World wine trade group

x	Dimensionless axial coordinate (–)
X	Dimensionless solute concentration in the bulk liquid phase (C/CE)
XAD	Commercial polystrene divinylbenzene resin
Xp	Dimensionless solute concentration in the liquid inside pores (Cp/CE)
Y(III)	Yttrium(III)
Z	Bed axial coordinate, cm
z*	Normalized axial coordinate (z/L)
ZA, ZB, SA, SB	Equivalent fractions
ZK-5	KFI framework type zeolite
Zn-LLT	$(La,Zn)TiO_3$
ZP	Zirconium phosphate
ZrP	Zirconium phosphate
ZSM-5 MFI	Framework type zeolite
ΔG0	Free energy change
ΔH	Enthalpy change
ΔS	Entropy change
δ	Thickness of the diffusion layer
η	Interaction term
μ	Viscosity of the fluid, g/cm.s
Φ	Thickness of the polymer layer
ε	Bed porosity
ε_p	Particle porosity
θ	Normalized time (t/τ)
ρ	Density of the fluid, g/cm^3
ρ_h	Wet density of the adsorbent, g/cm^3
τ	Space time, s

Subscripts and Superscripts

E	Eluent stream
X	Extract stream
k	Number of column (k =1,2,…12)
j	Number of section (j =I,II,III,IV)
E	Raffinate stream
i	Species in binary mixture
*	Operating conditions in SMB
AER	Anion-exchange resin
AEX	Anion-exchange
ALA	Alpha-lactalbumin
API	Active pharmaceutical ingredient
ARF	Acute renal failure

b	Bulk
BET	Surface area and porosity measurement equipment
BLG	Beta-lactoglobulin
BSA	Bovine serum albumin
c	Column
CAC	Continuous annular chromatography
CEC	Capillary electrochromatography
CER	Cation-exchange resin
CKD	Chronic kidney disease
CM	Carboxymethyl
CMP	Caseinomacropeptide
CsEBS	Cesium salts of ethylbenzenesulfoacid
CSS	Cyclic steady state
DB-WAX	GC column
DEAE	Diethylaminoethyl
DVB	Divinylbenzene
ED	Electrodialysis
ESRD	End stage renal disease
ETBE	Ethyl *tert*-butyl ether
EW	Egg white
F	Feed stream
FDA's	Food and drug administration's
FID	Flame ionization detector
GFR	Glomerular filtration rate
GIT	Gastrointestinal tract
Hb	Haemoglobin
HD	Hemodialysis
HGMF	High-gradient magnetic fishing
HPLC	High performance liquid chromatography
HSA	Human serum albumin
IE	Ion exchange
IEC	Ion exchange chromatography
IER	Ion exchange resin
Ig	Immunoglobulin
IO	Integrated-optic
It	Intermediary (Pseudo-SMB process) stream
LDF	Linear driving force
LDL	Low density lipoprotein
LDL-C	Low density lipoprotein cholestrol
LF	Lactoferrin
LP	Lactoperoxidase
Lys	Lysosyme
m- DVB	meta-Divinylbenzene
MA	Membrane adsorber

MAC	Membrane adsorption chromatography
MANAE	Monoaminoethyl-n-aminoethyl
MCGs	Medicated chewing gums
MCLC	Membrane convective liquid chromatography
MDDS	Mobile drug delivary system
MINI, MIDI(d), 3-21G*	Basis sets for non-empirical calculations
MMM	Mixed matrix membrane
MP2/3-21G* and MP2/MIDI(d)	Level of theory of non-empirical calculations with using basis sets 3-21G* and MIDI(d) and with accounting for electronic correlation in the frame of the second order Moeller-Plesset perturbation theory
MS	Mass spectrometry
MTBE	Methyl *tert*-butyl ether
NCBI	National center for biotechnology information
p	Particle
PAD	Pulsed amperometric detection
PD	Peritoneal dialysis
PU	Purity
QPI	Quaternized polyethyleneimine
RE	Recovery
RESD	Renal end stage disease
RHF	Restricted Hartree-Fock method for closed shalls
Rnase	Ribonuclease
ROHF	Restricted open shall Hartree-Fock method
RPC	Reverse phase chromatography
SCF MO LCAO	Model, in which a molecular orbital (MO) is represented as a linear combination of atomic orbitals (LCAO), are examined in light of ab initio self-consistent field (SCF) computations with bases of various sizes
SCX	Strong cation-exchange
SMB	Simulated moving bed
SMCGs	Synthetic medicated chewing gums
SPS	Sodium polystyrene sulfonate
ST-DVB Matrix	Styrene – divinylbenzene matrix
TAME	*Tert*-amyl-methyl ether
TMB	True moving bed
UDCA	Ursodeoxycholic acid
UF	Ultrafiltration
WKB method	Wentzel-Kramers-Brillouin method
WPC	Whey protein concentrate
WPI	Whey protein isolate

Nomenclature

a	Minimum approximation distance between ions
A	External particle surface area
A_γ	Debye-Huckel constant
$A_{ij}^{'}, A_{ji}^{'}$	Margules parameters
A^{z_A}, B^{z_B}, C^{z_C}	Counter ions with valences z_A, z_B, z_C
$\overline{A}^{z_A}, \overline{B}^{z_B}$	Counter ions with valences z_A, z_B inside the exchanger
a_i	Activity of species i in solution
$\overline{a}_i$	Activity of species i in exchanger
$A_i^{z_i}$	Generic counter ion i with valence z_i
a_p	External surface area per unit particle volume
B	Second Virial coefficient
$B_{j,i}$	Langmuir constant
C_b	Solute concentration at breakthrough time
$C_{ef,i}$	Concentration of sorbate in the effluent
$C_{F,i}$	Concentration of species i in the feed
C_i	Molar concentration of species i in solution
C_i^*	Molar concentration of species i at the exchanger/film interface
$C_{N,i}$	Normality of species i
$C_{N,t}$	Total normality of solution
$C_{p,i}$	Molar concentration of species i inside the pores
$\overline{C}_{p,i}$	Average concentration of species i inside the pores
C_{sat}	Saturation concentration
C_t	Total molar concentration of ionic species in solution
d	Particle diameter
D_A, D_B	Self-diffusion coefficients of species A and B
D_{AB}	Interdiffusion coefficient
$D_{eff,p,i}$	Effective diffusion coefficient of species i in macropores
$D_{eff,s,i}$	Effective diffusion coefficient of species i in micropores
D_f	Diffusion coefficient in the film
D_i	Diffusion coefficient of species i
$Đ_{ij}$	MS surface diffusivity of the pair $i-j$
$Đ_{is}$	MS surface diffusivity corresponding to the interaction between i and the fixed ionic charges
D_L	Axial dispersion coefficient
e	Electron charge
$E_{i,j}$	Energy of adsorption of ion i on site j
$\overline{E}_i$	Average adsorption of ion i
F	Faraday constant
F_i	Fractional attainment of equilibrium of species i
g_{ij}	Energy parameter characteristic of the $i-j$ interaction
I	Ionic strength

J_{i}	Diffusion flux of species i
k	Boltzmann's constant
k_{1}	Rate constant of the first order sorption
k_{2}	Rate constant of the second order sorption
$K_{\mathrm{aB}}^{\mathrm{A}}$	Corrected selectivity coefficient
k_{AB}	Bohart and Adams rate constant
$K_{\mathrm{B}}^{\mathrm{A}}$	Thermodynamic (equilibrium) constant
K_{C}	Selectivity coefficient
K_{D}	Distribution coefficient
k_{f}	Convective mass transfer coefficient
K_{LDF}	Linear driving force coefficient
$K_{\mathrm{S}}^{\mathrm{M_x M_m}}$	Stability constant
k_{Th}	Thomas rate constant
k_{YN}	Yoon-Nelson rate constant
L	Column length
$\mathrm{M}^{\mathrm{m+}}$	Cation
m_{i}	Molality of species i
m_{t}	Total molality of ionic species
n	Freundlich constant, number of ionic species in solution
N_{0}	Avogadro's constant
n_{c}	Number of counter ions
n_{f}	Number of functional groups
N_{i}	Molar flux of species i
$N_{\mathrm{p,i}}$	Diffusion fluxes of species i through the macropores
$N_{\mathrm{s,i}}$	Diffusion fluxes of species i through the micropores
n_{w}	Number of water molecules in the zeolite
$n_{\mathrm{x}} + n_{\mathrm{y}}$	Total number of tetrahedral in the unit cell of zeolite
q_{i}	Molar concentration of ionic species i in exchanger
p	Parameter in binomial distribution
p_{j}	Equivalent fraction of exchanger site of type j
$\overline{q}_{i}$	Average loading of ionic species i in exchanger
q_{i}^{*}	Resinate concentration in equilibrium with the fluid concentration
Q_{i}	Equivalent ionic concentration of species i in exchanger
Q_{i}^{e}	Surface excess of ion i
$Q_{\mathrm{j,i}}$	Equivalent ionic concentration of species i on exchanger site j
q_{M}	Kusik-Meissner parameter
q_{max}	Maximum sorbate concentration in the solid phase
q_{s}	Molar concentration of ionic fixed groups in exchanger
q_{t}	Total molar concentration of ionic species in exchanger
Q_{t}	Ion exchange capacity (in equivalents)
r	Radial position
R	Particle radius
$\Re$	Universal gas constant
t	Time
T	Absolute temperature

$t_{1/2}$	Time required for 50% sorbate breakthrough; stoichiometric time
t_b	Breakthrough time
U_0	Superficial velocity
u_i	Electrochemical mobility of species i, velocity of diffusing species i
V_{ef}	Volume of effluent
V_L	Volume of fluid phase
V_s	Volume of solid phase
V_{ZLC}	Volume of the ZLC column
W_{exch}	Mass of exchanger
W_{ij}	Weighting factor
x_i	Ionic fraction of species i in solution
X_i	Equivalent ionic fraction of species i in solution
X^{x-}	Anion
y_i	Ionic fraction of species i in exchanger
Y_i	Equivalent ionic fraction of species i in exchanger
y_s	Mole fraction of ionic fixed groups in exchanger
z_i	Valence of ionic species i

Subscripts

e	Equilibrium
f	Free
0	Initial condition
s	Solid, fixed ionic groups of the exchanger
t	Total
intra	Intraparticle

Greek Letters

$\overline{\alpha}^{i}_{j}$	Average separation factor
δ	Film thickness
α^{A}_{B}	Separation factor
$\overline{\gamma}_i$	Activity coefficient of species i in exchanger
γ_i	Activity coefficient of species i in solution
ε_b	Bed porosity
μ_i	Chemical potential of species i in solution
ρ_w	Density of pure solvent
ε	Dielectric constant
θ	Dimensionless time (–)
λ_i	Distribution coefficient of species i
φ	Electric potential
ε	External porosity (–)
$\tau_{d,m}$	Maximum value of τ_d
$\tau_{d,i}$	Minimum value of τ_d

ν_a	Number of anions per electrolyte
ν_c	Number of cations per electrolyte
ε_p	Particle porosity
ν_i	Pure-component molar volume
γ	Ratio of fluid and solid velocity in section j (–)
Γ	Reduced activity coefficient of Meissner and Kusik
σ_i	Standard deviation of energy distribution
$\overline{\mu}_i$	Surface chemical potential of species i
Γ_{ij}	Thermodynamic factor
τ_d	Time constant for intraparticle diffusion
ν	Volumetric flow rate, number of site types
$\Lambda_{ij}, \Lambda_{ji}$	Wilson parameters

Chapter 1
Separation of Amino Acids, Peptides, and Proteins by Ion Exchange Chromatography

Tanja Cirkovic Velickovic, Jana Ognjenovic, and Luka Mihajlovic

Abstract Separation of amino acids, peptides, and proteins (bioanalytes) via ion exchange (IE) has widespread usage because it is usually very simple to design and it has high capacity and easily achievable control of the separation process. Amino acids, as principal constituents of proteins and having a plethora of biological functions of their own, are always in focus when developing novel methods. Separation and quantification of amino acids is essential in food science, medicine, agricultural science, etc. Peptides exist in nature and have diverse functions. Digestion of proteins by enzymes also gives complex mixtures of peptides and IE finds its application in peptide separation. There are lots of reasons for the popularity of IE in protein isolation and purification. It is used in research, analysis, and large-scale purification of proteins. Ion exchange is ideal for the initial capture of proteins because of its high capacity, relatively low cost, and its ability to survive rigorous cleaning regimes. This chapter covers basic principles and modern applications of IE in separation of amino acids, peptides, and proteins.

1.1 Introduction

The technique of ion exchange (IE) is based on interactions between charged moieties. Amino acids, peptides, and proteins (bioanalytes) are water-soluble, charged amphoteric molecules and as such, able to bind via Coulomb's interactions to oppositely charged moieties covalently linked to an insoluble carrier (matrix, or a stationary phase). Stationary phases for ion exchange separations are characterized by the nature and strength of the acidic or basic functions on their surfaces and the

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types of ions that they attract. Cation exchangers retain and separate positively charged ions (analytes) on a negative surface, while anion exchangers retain and separate negatively charged ions on a positive surface. There are at least two general approaches for separation and elution with each type of ion exchanger [1]. Strength of the interaction between stationary phase and the analyte can be controlled by changing the ionic strength of the solution. Also, ionic properties of ionizable functional groups by pH titration of the stationary phase or the analyte can be controlled, allowing great variability in the design of IE experiments.

Strong ion exchangers bear functional groups that are always ionized, over a wide range of pH values. They are typically used to retain and separate weak acids or bases. These weak ions may be eluted by displacement with ions that are more strongly attracted to the stationary phase exchange sites (so-called salt elution). Alternately, weak ions may be retained on the column and subsequently neutralized by changing the pH of the mobile phase, causing them to elute (so-called pH elution). Combined salt/pH elution is possible, as well as isocratic elution.

Weak ion exchangers bear functional groups that above or below a certain pH value may lose their ability to retain ions by charge, i.e., those groups are titrable over a narrower pH range comparing to the strong ion exchanger. When charged, they are used to retain and separate strong ions. Strong ions are ionized over a wide pH range and often bind strongly to the exchange sites of a matrix. It is often difficult to elute strong ions by displacement; therefore, the matrix exchange sites may be neutralized by permitting elution of the charged analytes with a mobile phase [1, 2].

Separation of bioanalytes via ion exchange has widespread usage because it is usually very simple to design and it has high capacity and easily achievable control of the separation process. Both strong and weak ion exchangers found application in the separation of amino acids, peptides, and proteins [3–6]. The binding of proteins to the IE stationary phase predominantly occur via charged amino acid residues at the surface of the protein, although certain posttranslational modifications may contribute to the binding (such as sialyl, sialoglycosyl, phosphoryl groups) [7]. Majority of amino acids, peptides, and proteins are weak ions. Sometimes, it is more convenient to apply methods for separation of strong ions by neutralizing the stationary phase in order to preserve the biological function of the molecules. Although the principles behind separation of amino acids, peptides, and proteins are the same, separation of large molecules, such as proteins by IE, requires matrices of high porosity and moderate density of ionizable groups [8]. Original matrices, developed for separation of small molecules, have been modified and a great variety of stationary phases suitable for separation of proteins is available today [9].

In most cases, maintenance of the functional integrity of a protein during separation is necessary. Mild conditions and short separation time of the ion exchange separation help proteins retain their conformation and thereby the biological function, which is often necessary for many downstream applications.

1.2 General Principles of Ion Exchange Chromatography of Bioanalytes

1.2.1 Theory

For amphoteric compounds, such as amino acids, peptides, and proteins, *the isoelectric point (pI, the pH at which the net charge is zero) of the compound and its stability* at various pH values determine the separation strategy.

At a pH above its pI, the bioanalyte of interest will be negatively charged, and at a pH below its pI, the compound will be positively charged. If the compound is stable at a pH above its pI, an anion exchanger is used (Table 1.1). If the compound is stable at a pH below its pI, a cation exchanger is used (Table 1.2). The operating pH also determines the type of exchanger to use (Tables 1.1 and 1.2).

It should be kept in mind that the pKa value of a residue represents a pH at which ionized and nonionized forms of a functional group are at equilibrium. For a group to be more than 90% titrated, usually 0.5 pH value above the pKa is necessary. To assure an essentially neutral, or a fully charged, analyte or particle surface, the pH must be adjusted to a value at least 2 units beyond the pKa. It should be kept in mind

Table 1.1 Short guidelines for ion exchange of bioanalytes using anionic exchangers

Strength of the ion exchanger	Weak anionic, pKa around 9	Strong anionic
Net charge of the bioanalyte	Negative at pH above its pI	Negative at pH above its Pi
Charge of exchanger	Positive below pH 9	Positive
Running conditions	0.5–1.5 units below the pKa of the exchanger	0.5–1.5 units above the pI of the bioanalyte
Bioanalytes separation	Separation possible for bioanalytes of pI 7.5 and below	Separation of weakly basic and acidic bioanalytes, because of usually better pH stability outside extreme pH values (below 3 and above 10)

Table 1.2 Short guidelines for ion exchange of bioanalytes using cationic exchangers

Strength of the ion exchanger	Weak cationic, pKa around 5	Strong cationic
Net charge of the bioanalyte	Positive at pH below its pI	Positive at pH below its pI
Charge of exchanger	Negative above pH 5	Negative
Running conditions	0.5–1.5 units above the pKa of the exchanger	0.5–1.5 Units below the pI of the bioanalyte
Bioanalytes separation	Separation possible for bioanalytes of pI 6.5 and above	Separation of weakly acidic and basic bioanalytes, because of usually better pH stability outside extreme pH values (below 3 and above 10)

that the pH value and additives of the mobile phase can alter the surface properties of a protein such as the charge density and accessibility or relative location of charged residues, making IE separation of proteins sometimes unpredictable and proving the superiority of practice over theory in IE of bioanalytes. Therefore, the guidelines presented in Tables 1.1 and 1.2 should be regarded as suggestions when optimizing IE separation of bioanalytes.

When using a strong cation exchanger for separation of strong bases, both remain charged, making the base difficult to elute. It can only be removed by a competing base that binds even stronger and displaces the compound of interest at the exchange sites. This approach is not very practical as very strong acids and bases may be corrosive to materials used in IE and may cause structural instability of bioanalytes. A more practical approach is to use a weak cation exchanger for separation of strong bases and neutralize the surface of the exchanger by lowering the pH of the mobile phase.

The basis for IE is competition between ions of interest and other ions for oppositely charged groups on an ion exchanger. The interaction of a protein and an ion exchanger depends not only on the net charge and the ionic strength but also on the surface charge of a protein. It is clear that the more highly charged a protein is, the more strongly it will bind to a given, oppositely charged ion exchanger. Similarly, more highly charged ion exchanger (i.e., those with a higher degree of substitution with charged groups) usually bind proteins more effectively than weakly charged ones. Conditions, for example, pH, that alter the charge on either the protein or ion exchanger will affect their interactions and may be used to influence the ion exchange process [10].

The pH is one of the most important parameters in determining protein binding as it determines the charge on both the protein and the ion exchanger. Although proteins are complex ampholytes that have both positive and negative charges over a wide pH range, as a rule, binding to an ion exchanger occurs only when there is a net charge on the protein of opposite sign to that found on the ion exchanger. The isoelectric point of a bioanalyte depends on ionizable amino acid residues in its structure. Positive charges are usually provided by histidines, arginines, and lysines, depending on the pH of the surrounding buffer. Any free N-terminal amine will also contribute with a positive charge below pH 8. Negative charges are principally provided by aspartate and glutamate residues and the C-terminal carboxyl group. Virtually all these residues are ionized above pH 6. At higher pH values (above 8), cysteines may become ionized too. The charged groups nearly always reside on the protein surface. Exceptions are mainly metalloproteins, where an internal metal ion is often coordinated by charged residues. Posttranslational modifications can also influence charge properties of side residues, and those often contribute to the so-called microheterogeneity often noticed in IE separation of proteins.

In addition, influences from neighboring groups and the position in the tertiary structure will affect the pKa for the side-chain groups [11]. The combined influence of all of the charged side chains will give the protein a varying net charge depending on the pH of the solute. Therefore, it is possible to separate proteins using either

fixed positive charges on the stationary phase, anion exchanger, or fixed negative charges, cation exchanger.

A protein must displace the counterions to become attached, and consequently, the net charge on the protein will be the same as that of the counterions displaced, thereof the term "ion exchange." At pH values far away from pI, proteins bind strongly and, in practice, do not desorb at low ionic strength. Near its pI, the net charge of a protein is decreased, and consequently, it binds less strongly. Because the charges on the protein surface are distributed asymmetrically, binding can also occur close to the pI even when overall charge is the same as that on the ion exchanger. Due to Donnan effects, protons are attracted and hydroxyl ions are expelled from the microenvironment of cation-exchange groups. The pH in the matrix is therefore usually one unit lower than in the surrounding buffer. Quite similarly, for anion exchangers, an increase in pH of about one unit occurs. For instance, if a protein is adsorbed at pH 5, it will be exposed to pH 4, which may lead to denaturation if the stability of the protein is poor.

1.2.2 The Matrix

The matrix may be based on inorganic compounds, synthetic resins, or polysaccharides and can be porous and nonporous. The standard supports are beads of polystyrene, silica, or agarose. The matrix characteristics are important for chromatographic properties such as efficiency, capacity, recovery, and chemical and mechanical stability. A wide range of support matrices are utilized in the manufacture of ion exchange media.

The porosity of a stationary phase refers to the total pore volume within the matrix of the support. A very porous support may have either many small pores or a few large pores. The largest compound able to enter the pores under a given set of conditions determines the exclusion limit of a support. Porous media with high exclusion limits are recommended for high molecular weight compounds such as protein and other biomolecules. Low- or high-porosity media with low exclusion limits are recommended for the separation of low molecular weight compounds such as inorganic ions, organic acids, and amino acids.

The first ion exchangers were based on synthetic resins of high hydrophobicity and low porosity, suitable for exchange of small molecules, such as inorganic ions, organic acids, and amino acids. Due to their hydrophobicity and high charge density, these matrices were not suitable for separation of biological samples.

Ionic interactions as the basis for separation and purification of proteins by IEC (ion exchange chromatography) have been applied successfully since the late 1940s. Originally limited to the separation of basic proteins [12], with the introduction of cellulose-based matrices by Sober and Petersen (e.g., carboxymethyl [CM] and

diethylaminoethyl [DEAE] derivatives of cellulose), the utility of IEC was extended to include a wide range of proteins. The ideal matrix should be inert, highly porous, and structurally rigid. Rigidity is essential for scale-up and high-flow-rate processes [13]. Particle diameter and porosity of the matrices greatly influence the medium's capacity and resolution. A medium composed of small-diameter particles reaches equilibrium faster (due to short distances for proteins to diffuse) and results in higher resolution than larger-diameter particles. Porosity can influence the binding capacity because particles with larger pores allow larger proteins to enter the pores, thus increasing a number of available sites for protein to bind. The charged moiety bound to the matrix determines the useful pH range and the type of ion exchanger. There is much accumulated experience on this type of chromatography documented in the literature.

Ion exchangers based on dextran (Sephadex) and agarose (Sepharose CL-6B) and cross-linked cellulose (DEAE Sephacel) were the first ion exchange matrices that combined a spherical form with high porosity. The result was an improvement in flow properties and capacity for biological macromolecules.

Ion exchange chromatography matrices are available as dry, granular material or as preswollen loose beads, but prepacked columns are nowadays common, especially for small-scale analytical work [14, 15]. Although the method is essentially the same, now IE can also be carried out on monolithic columns, on IE membranes [16], and on ion exchange high-performance liquid chromatography (HPLC) columns [17].

Besides porosity, particle size is of particular importance in ion exchange chromatography of bioanalytes. Particle size is measured in micrometers, with dry mesh or wet mesh designations. Smaller particle sizes provide higher resolution and typically require lower operational flow rates; larger particle sizes yield lower resolution but can be operated at higher flow rates.

1.2.3 Charged Groups

The charged moiety bound to the matrix determines the useful pH range and the type of ion exchanger. Their total number and availability determines the capacity of the ion exchanger.

The ion exchangers are usually classified as weak and strong. The names refer to the pKa values of their charged groups, and it does not say anything about the strength with which they bind proteins [18]. Strong ion exchangers are chemically sulfonate and quaternary amino groups (Table 1.3). Most commonly applied ion exchangers are those with immobilized DEAE and CM groups (weak ion exchangers). Phospho-group-immobilized matrices can be used both as ion exchangers and as affinity media for separation of enzymes recognizing phosphoryl esters. The synthesis of an ion exchanger usually takes place by one-step chemical reaction in which a charged molecule containing a reactive group (often a halogenide) is allowed to react with the hydroxyl group–containing matrix (such as a polysaccharides) under strongly alkaline conditions. Most DEAE and CM ion exchangers are produced this way [1].

Table 1.3 Most commonly used charged groups of ion exchange stationary phases

Ion exchanger	Chemical structure of the functional group	Abbreviation
Cation, weak	Carboxymethyl-	CM
Cation, strong	Sulfopropyl-	SP
Cation, strong	Sulfoethyl-	SE
Cation, intermediate	Phospho-	P
Anion, weak	Diethylaminoethyl-	DEAE
Anion, weak	Dimethylaminoethyl-	DMAE
Anion, weak	Polyethyleneimine	PI
Anion, strong	Trimethylaminoethyl-	TMAE
Anion, strong	Trimethylaminomethyl-	Q
Anion, strong	Trimethylaminohydroxypropyl-	QA
Anion, strong	Diethyl-(2-hydroxypropyl)-aminoethyl	QAE
Anion, strong	Quaternized polyethyleneimine	QPI

1.2.4 Commercially Available Exchangers

A great range of commercially available exchangers is available today, having their characteristics clearly described. The capacity of the exchanger is usually defined toward a standard protein, such as HSA (human serum albumin) or lysozyme. As porosity and particle size are of great importance in separation of bioanalytes, the proper support has to be chosen depending on:

- Size of bioanalyte: The higher the size of the analyte, the greater the porosity of the support.
- Its pI value: anion versus cation exchangers, weak versus strong, depending on the separation strategy.
- Separation requirements: analytical, semipreparative, or preparative scale separation/purification of a bioanalyte of interest.

Great range of commercially available exchangers is shown in Table 1.4.

1.2.5 Significance of Buffers in Ion Exchange

As pH value is one of important parameters in IE separation of peptides and proteins, the pH value has to be controlled by buffers. Buffering components used in the IE are presented in the Table 1.5 and arranged according to their pKa values. Volatile buffer systems are especially useful if concentration of the sample by evaporation is to be performed after the IE separation. Combination of volatile components will result in a volatile buffer system. That is why acetic acid/pyridine or bicarbonate/ammonium buffer systems are appropriate for separation of proteins prior to evaporation.

Table 1.4 Commercially available exchangers. Data have been compiled from the manufacturer's booklets. The protein in capacity measurements were bovine serum albumin (BSA), human serum albumin (HSA), lysozyme (Lys), bovine hemoglobin (Hb), and ribonuclease (RNase)

Name	Matrix	Functional group	Degree of substitution (μmol/mL)	Available capacity (mg/mL)	Company
DE 23	Fibrous cellulose	DEAE	150	60 BSA	Whatman
CM 23	Fibrous cellulose	CM	80	85 Lys	Whatman
DE 52	Microgranular cellulose	DEAE	190	130 BSA	Whatman
CM 52	Microgranular cellulose	CM	190	210 Lys	Whatman
DE 53	Microgranular cellulose	DEAE	400	150 BSA	Whatman
CN32	Microgranular cellulose	CM	180	200 Lys	Whatman
DEAE Sephacel	Beaded cellulose	DEAE	170	160 BSA	GE Healthcare
DEAE Sephadex A-25	Dextran, Sephadex G-25	DEAE	500	70 Hb	GE Healthcare
QAE Sephadex A-25	Dextran, Sephadex G-25	QAE	500	50 Hb	GE Healthcare
CM Sephadex C-25	Dextran, Sephadex G-25	CM	560	50 Hb	GE Healthcare
SP Sephadex C-25	Dextran, Sephadex G-25	SP	300	30 Hb	GE Healthcare
DEAE Sephadex A-50	Dextran, Sephadex G-50	DEAE	175	250 Hb	GE Healthcare
QAE Sephadex A-50	Dextran, Sephadex G-50	QAE	100	200 Hb	GE Healthcare
CM Sephadex C-50	Dextran, Sephadex G-50	CM	170	350 Hb	GE Healthcare
SP Sephadex C-25	Dextran, Sephadex G-50	SP	90	270 Hb	GE Healthcare
DEAE Sepharose CL-6B	Agarose, 6% cross-linked	DEAE	150	100 Hb	GE Healthcare
CM Sepharose CL-6B	Agarose, 6% cross-linked	CM	120	100 Hb	GE Healthcare
DEAE Bio-Gel A	Agarose	DEAE	20	45 Hb	Bio-Rad
CM Bio-Gel A	Agarose	CM	20	45 Hb	Bio-Rad
DEAE-trisacryl M	Trisacrylate polymer	DEAE	300	90 Hb	Sepracor
CM-trisacryl M	Trisacrylate polymer	CM	200	100 Hb	Sepracor
SP-trisacryl M	Trisacrylate polymer	SP	230	150 BSA	Sepracor
Fractogel TMAE 650 (S)	Synthetic organic polymer	TMAE	–	100 BSA	Merck
Fractogel DEAE 650 (S)	Synthetic organic polymer	DEAE	–	100 BSA	Merck
Fractogel DMAE 650 (S)	Synthetic organic polymer	DMAE	–	100 BSA	Merck

(continued)

Table 1.4 (continued)

Name	Matrix	Functional group	Degree of substitution (μmol/mL)	Available capacity (mg/mL)	Company
Fractogel SO_3 650 (S)	Synthetic organic polymer	S	–	100 Lys	Merck
Fractogel COO 650 (S)	Synthetic organic polymer	COO–	–	100 Hb	Merck
High-performance media					
Mono Q	Synthetic organic polymer	Q	270–370	65 HAS	GE Healthcare
Mono S	Synthetic organic polymer	S	140–180	75 RNase	GE Healthcare
SOURCE 15Q	Synthetic organic polymer	Q	–	25	GE Healthcare
SOURCE 15 S	Synthetic organic polymer	S	–	25	GE Healthcare
SOURCE 30Q	Synthetic organic polymer	Q	–	40 BSA	GE Healthcare
SOURCE 30 S	Synthetic organic polymer	S	–	80 Lys	GE Healthcare
Q Sepharose high performance	Cross-linked agarose	Q	200	70 BSA	GE Healthcare
SP Sepharose high performance	Cross-linked agarose	SP	200	70 RNase	GE Healthcare
DEAE-5-PW	Synthetic organic polymer	DEAE	–	1.5–3	Bio-Rad
SP-5-PW	Synthetic organic polymer	SP	–	1.5–3	Bio-Rad
Mini S	Nonporous synthetic polymer	S	20	4–6	GE Healthcare
Mini Q	Nonporous synthetic polymer	Q	75	4–6	GE Healthcare
HRLC MA7P	Nonporous synthetic polymer	PEI	–	0.6–2	Bio-Rad
HRLC MA7C	Nonporous synthetic polymer	CM	–	0.6–2	Bio-Rad
DEAE-3SW	Silica	DEAE	–	120 BSA	Toso Haas
CM-3SW	Silica	CM	–	45 Hb	Toso Haas

When choosing a suitable buffer system, one has to have in mind that the chosen buffer's pKa should be close to the working pH, and there should not be any interference with mobile phase additives or subsequent screening assays [7]. In addition, the chemical nature of the used ions has considerable influence on separation of ions in ion exchange chromatography.

Many ion exchange media are available in several ionic forms and may be converted from one form to another. The ionic form of a support refers to the counterion presently adsorbed to the support's functional group. Counterions will exhibit

Table 1.5 Examples of the most commonly used zwitterionic and nonzwitterionic buffers (Data compiled from references [7, 21–24])

Buffer component	Titrate with	pKa (20°C)	pH range	Zwitterionic
Glycine	HCl	2.4 (carboxylate)	2.0–3.5	No
Citrate	Phosphoric acid	3.14	3–8	No
		4.77		
		6.39		
Acetate	NaOH	4.75	3.7–5.8	No
Maleate	NaOH		5.5–7.2	No
Phosphate	HCl, sodium hydrogen phosphate	2.12	6.2–8.2	No
		7.21		
		12.67		
Tris (N-Tris-(hydroxymethyl)aminomethane)	HCl	8.3	7.3–9.3	No
Glycine	NaOH	9.5 (amino group)	8.5–10.5	No
Mes (2-(N-morpholino)ethanesulfonic acid)	NaOH	6.15	5.5–7	Yes
Mops (3-morpholinopropanesulfonic acid)	NaOH	7.2	6.5–8.2	Yes
Tricine (N-[Tris-(hydroxymethyl)methyl]glycine)	NaOH	8.15	7–9	Yes
Ches (2-(cyclohexylamino)-ethansulfonic acid)	NaOH	9.55	9–10	Yes
Caps (3-(cyclohexylamino)-1-propanesulfonic acid)	NaOH	10.4	9.8–11	Yes

specific selectivity for each support. The lower the selectivity of a counterion toward the support, the more readily it is exchanged for another ion of like charge. Consequently, the appropriate ionic form will depend on the relative selectivity of the sample ion to be adsorbed.

> In general, the ionic form should have a lower selectivity for the functional group than the sample ion so that the sample ion will displace the counterion and be adsorbed to the support. The sample ion can then be eluted by a second counterion with a higher selectivity for the support.

The affinities of anions to anion-exchange supports follow the Hofmeister series of ions, arranged by their effects on water structure. Chloride anion, one of the most widely used anions in elution from anion exchangers, has a moderate water structure-making ability, being in the middle of Hofmeister series of anions. It should also be noted that Goods' buffers (a groups of zwitterionic organic compounds, primarily sulfonates and tertiary amines) show excellent compatibility with biological systems [19, 20].

1.3 Separation of Amino Acids: Examples and Applications

Amino acids, as principal constituents of proteins and having a plethora of biological functions of their own, are always in focus when developing novel methods. Separation and quantification of amino acids is essential in food science, medicine, agricultural science, etc.

Although fully automated separation methods for amino acids have been developed long time ago [25, 26], more and more new methods have been described to be suitable for wide range of applications [27, 28]. The amino acid composition of proteins was determined by separation and quantification of constituent amino acids, following acid hydrolysis of a given protein [29]. Recently, analyzing amino acids in food stuffs and various biological samples is of growing importance [27, 30, 31]. Specialized applications exist for detection of nonprotein amino acids, but in most cases, samples of amino acids for further separation and quantification are prepared by sample hydrolysis with hydrochloric acid. The complexity of the environment does not allow the separation of amino acid to become a routine practice.

Amino acids are widely used in biotechnology applications. Since amino acids are natural compounds, they can be safely used in pharmaceutical applications, for example, as a solvent additive for protein purification and as an excipient for protein formulations. At high concentrations, certain amino acids are found to raise intracellular osmotic pressure and adjust to the high salt concentrations of the surrounding medium. They are called "compatible solutes" since they do not affect macromolecular function. Not only are they needed to increase the osmotic pressure, they are known to increase the stability of the proteins. Sucrose, glycerol, and certain amino acids are used to enhance the stability of unstable proteins after isolation from natural environments.

The mechanism of the action of these protein-stabilizing amino acids is relatively well understood. A recent review covers various biotechnology applications of amino acids, in particular arginine [32].

1.3.1 Use of Tandem Mass Spectrometry for Amino Acid Quantification

Analysis of amino acids has been commonly performed by precolumn derivatization with orthophthaldialdehyde and/or 9-fluorenylmethylchloroformate followed by reversed-phase high-performance liquid chromatography (RP-HPLC) and fluorescence detection. These methods impose certain limitations, which are overcome by use of mass spectrometry as the detection system. In a paper by Thiele and coworkers [33], ion exchange chromatography on a strong cation-exchange matrix was used for amino acid separation of barley shoots hydrolysates, which were subsequently quantified by tandem MS. Strong cation matrix was selected due to the fact that at pH 3, all amino acids in the sample are in their ammonium ion form. All 20 amino acids were detected and quantified.

Although superior in its precision and accuracy, analysis of MS results can pose problems. Also, mass spectrometry is inherently more costly than other methods of detection. Because of these reasons, several methods for separation and quantification of amino acids have been developed during the last decade.

1.3.2 Pulsed Amperometric Detection Integrated with Anion-Exchange Chromatography for Separation and Detection of Amino Acids

Pulsed amperometric detection (PAD) integrated with anion-exchange chromatography has recently gained attention as the method of choice for separation and detection of amino acids. The method was pioneered by Jandik et al. [34] and is based on pulsed amperometric detection with optimized waveforms for amino acid detection. Separation is achieved on a strong cation-exchange matrix, and the detection method enables accurate quantification of all amino acids. The method employs AEX (anion-exchange chromatography) for separation of sugars from amino acids in order to simplify detection.

This methodological setup has been used for a wide variety of applications. In a paper by Rombouts et al., wheat gluten protein hydrolysate was analyzed in order to quantify its amino acid composition. In contrast to most conventional methods, the analysis requires neither pre- or postcolumn derivatization nor oxidation of the sample. Samples of wheat gluten hydrolysate were separated with AminoPac PA10

analytical column. The chosen detection method, namely, integrated PAD, makes a derivatization step unnecessary [35]. Genzel et al. managed to quantify amino acids in mammalian cell culture media containing serum and high concentrations of glucose using similar methodology [36].

In a similar paper, tryptophan concentrations in proteins, animal feed, cell cultures, and fermentation broths were directly determined. Tryptophan content of a protein or peptide is difficult to determine due to its decomposition during the acid hydrolysis used to release the protein's other amino acids. In this method, tryptophan is separated from common amino acids by anion-exchange chromatography in 12 min and directly detected by integrated pulsed amperometry. The estimated lower detection limit for this method is 1 pmol [37], with negligible interference with glucose in the sample.

Of course, due to matrix effects and sample complexity, in certain applications, special care must be taken with preparation of the samples [38]. Thiele et al. developed a sample preparation method based on water/methanol extraction. Sample preparation was evaluated with four different food samples, sourdough, skim milk, lemon juice, and potato, and proved useful for quantitative analysis of amino acid and carbohydrate contents of food samples.

1.3.3 Simultaneous Separation of Amino Acids and Carbohydrates: AminoPac Columns

For many years, the standard practice for amino acid analysis was based upon cation-exchange chromatography with a step gradient of eluent concentration and pH, combined with ninhydrin postcolumn reaction [1]. Amino acids and carbohydrates are often found in the same samples and are frequently simultaneously quantified after column separation by amperometric or similar setups.

Several specialized columns have been developed for this application, most notably AminoPac series of columns, with polymeric pellicular anion-exchange resin. Capabilities of amino acid separation on an AminoPac PA10 column are shown in Fig. 1.1 and show superb resolution. Two detection modes are shown, with clear advantage of detection mode I.

Simultaneous detection is enabled by integrated pulsed electrochemical detection [28]. Samples of glycoprotein hydrolysate were analyzed, with use of several monosaccharides as standards and with superb resolution. A similar approach directed at pulsed electrochemical detection (PED) following liquid chromatographic separation has been applied to the indirect determination of amino acids and proteins. Limits of detection of amino acids were found to be 2–30 pmol using optimized potential–time waveforms at an Au electrode. Indirect PED provided much greater detection sensitivity toward amino acids than direct PED.

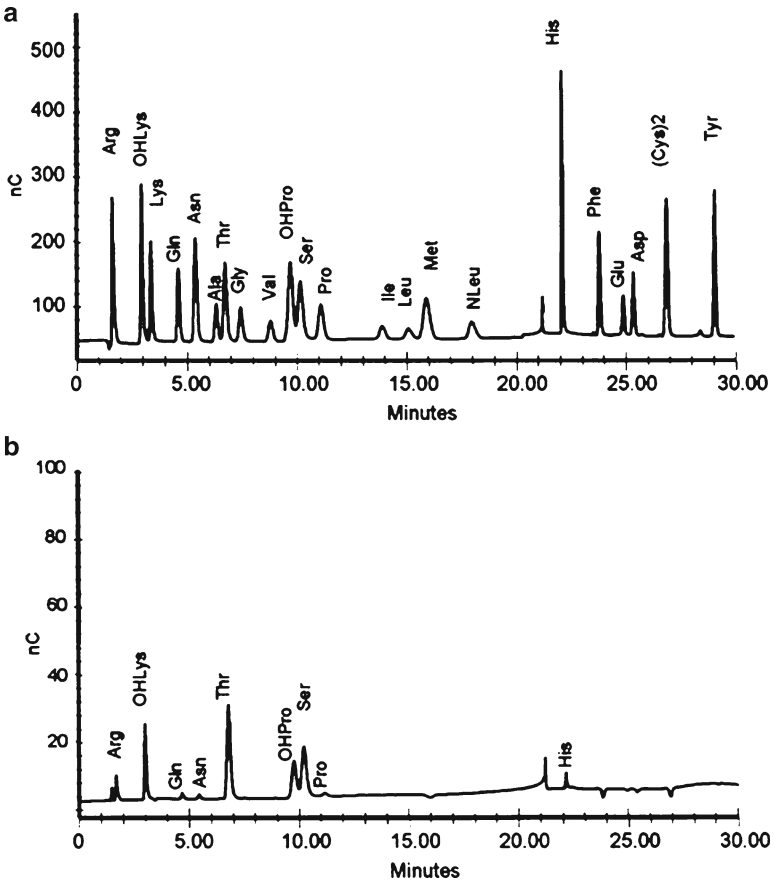

Fig. 1.1 Separation of amino acids on an Aminopac AP10 column. All components were at 200 pmol/dm^3 (Nleu at 313 pmol/dm^3). (**a**) Mode II detection, waveform with E2 = −50 mV. (**b**) Mode I detection, waveform with E2 = 50 mV (Reproduced from Ref. [28] with kind permission of © Elsevier Ltd. (2004))

1.3.4 Reactive IonExchange Chromatography

An interesting approach is that pioneered by Zammouri and further expanded by Harscoat et al. [39, 40]. Reactive ion exchange chromatography takes advantage of the amphoteric character of the amino acids and peptides. Reactions between acidic and basic compounds in the liquid phase allow displacement of the ion exchange equilibria to fix or elute species. Therefore, the chromatographic effect is obtained by the dissociation equilibria in solution rather than ion exchange equilibria of ionic species on the resin. In the papers in question, reactive exchange principles were employed to separate peptides and amino acids from different sources with great