Instrumental Analysis in the Biological Sciences

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Preface

Instrumental techniques of analysis have now moved from the confines of the chemistry laboratory to form an indispensable part of the analytical armoury of many workers involved in the biological sciences. It is now quite out of the question to considcr a laboratory dealing with the analysis of biological materials that is not equipped with an extensive range of instrumentation. Recent years have also seen a dramatic improvement in the ease with which such instruments can be used, and the quality and quantity of the analytical data that they can produce. This is due in no small part to the ubiquitous use of microprocessors and computers for instrumental control. However, under these circumstances there is a real danger of the analyst adopting a 'black box' mentality and not treating the analytical data produced in accordance with the limitations that may be inherent in the method used. Such a problem can only be overcome if the operator is fully aware of both the theoretical and instrumental constraints relevant to the technique in question. As the complexity and sheer volume of material in undergraduate courses increases, there is a tendency to reduce the amount of fundamental material that is taught prior to embarking on the more applied aspects. This is nowhere more apparent than in the teaching of instrumental techniques of analysis. Clearly there is not sufficient time to study the molecular basis of all the techniques in depth, but unless the fundamental principles are understood, the analyst will never be able to develop a critical approach to an analytical problem, nor will he/she be able to evaluate the significance of the data produced. There are many excellent texts describing the various instrumental techniques, some of which are cited in this volume. However, they would appear to be more suitable for chemistry undergraduates rather than for students working in the applied biological sciences. The present volume attempts to provide such students with an adequate theoretical background of the techniques most relevant to the biological sciences and to show how the techniques may be applied to a wide range of analytical problems.

The techniques discussed may be simply divided into separative (liquid and gas chromatography, electrophoresis) and non-separative (various spectrometric techniques, flame techniques and electrochemical methods). In all of the chapters the theoretical basis has been discussed as far as is necessary to understand the technique in question. This naturally leads to a somewhat uneven treatment from chapter to chapter, for example the theoretical aspects of nuclear magnetic resonance are conceptually far more complex than those involved in chromatographic separations. No attempt has been made to cover all the possible analytical techniques which find application in the biological sciences, as this would have resulted either in a very superficial coverage of the techniques or in an excessively long book. The book has rather concentrated on those techniques considered to be the most important from a qualitative and quantitative analytical point of view.

It is hoped that the book will find a wide audience amongst those students studying a wide range of biologically based subjects, including biochemistry, medicine, nutrition, agricultural chemistry, pharmaceutical chemistry, environmental science and food science. It may well also prove useful to those scientists embarking on research in the biological sciences without a grounding in instrumental techniques, including those working in industry and other scientific establishments.

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Contents

Abbreviations and symbols

A	Absorbance
AAS	Atomic absorption spectrometry
AFS	Atomic fluorescence spectrometry
α	Degree of ionization (alpha)
ATP	Adenosine triphosphate
ATR	Attenuated total reflectance
AUFS	Absorbance units full scale
B, B_0, B_1	Applied magnetic field
β	Phase ratio (high-performance liquid chromatography); Bohr magneton (ESR) (beta)
BOD	Biochemical oxygen demand
c	Mass transfer term
c	Velocity of light in a vacuum
CIE	Commission Internationale de l'Eclairage
CM	Carboxymethyl
CT	Computed tomography
CW	Continuous wave
d	Separation of teeth in a diffraction grating
D_g	Diffusion coefficient in the gas phase
D_l	Diffusion coefficient in the liquid phase
δ	Chemical shift (delta)
DEAE	Diethylamino-ethyl
e	Electronic charge
E	Energy of atomic or molecular state; kinetic energy (mass spectrometry)
E_i	Energy of ith energy level
ΔE	Difference in energy between states
ECD	Electron capture detector (gas chromatography); electrochemical detector (electrochemistry)
ELDOR	Electron double resonance
ENDOR	Electron nuclear double resonance
EPR	Electron paramagnetic resonance
ε	Molar absorptivity (absorption coefficient) (epsilon)
ε^0	Solvent strength based on heat of adsorption
ESR	Electron spin resonance
eV	Electron volt
η	Viscosity (eta)
F	Force
[F]	Concentration of ground state fluorescent molecule
[F*]	Concentration of first excited singlet
FES	Flame emission spectrometry
FID	Flame ionization detector (gas chromatography); free induction decay (nuclear magnetic resonance spectroscopy)
FPD	Flame photometric detector
FTIR	Fourier transform infrared
g	Landé g-factor, spectroscopic splitting factor (electron spin resonance)
G	Free energy
γ	Magnetogyric ratio (gamma)

GC	Gas chromatography
GLC	Gas–liquid chromatography
GSC	Gas–solid chromatography
H	Height equivalent to a theoretical plate
ΔH	Enthalpy change
ΔH_x	Enthalpy of solvation of component x
h	Peak height (high-performance liquid chromatography, gas chromatography); Planck's constant
h'	Height of triangle through chromatographic peak
HK	Hexokinase
HPLC	High-performance liquid chromatography
I	Intensity of transmitted radiation; current (electrochemistry); nuclear spin quantum number (nuclear magnetic resonance spectroscopy)
I_0	Intensity of incident radiation
ΔI	McReynold's constant
i.d.	Internal diameter
IE	Ion-exchange
IEF	Isoelectric focusing
IR	Infrared
ISE	Ion-selective electrode
J	Coupling constant
k	Partition ratio (gas chromatography, high-performance liquid chromatography); Boltzmann constant
k'	Capacity factor
K	Partition coefficient
K_a	Acid dissociation constant
K_w	Dissociation constant for water
l	Pathlength
λ	Wavelength (lambda)
λ_b	Blaze wavelength
λ_{max}	Wavelength of maximum absorption
Λ	Molar conductivity (lambda)
LC	Liquid chromatography
m	Mass (mass spectrometry); magnetic quantum number (nuclear magnetic resonance spectrometry) order (ultraviolet)
M	Molar concentration
M_x	Magnetization along the x-axis
M^+	Molecular ion
M_D	Mass of daughter ion
M_F	Mass of charged fragment
M_S	Angular momentum quantum number
μ	Nuclear magnetic moment (mu)
MS	Mass spectrometry
n	Non-bonding (ultraviolet); theoretical plate number (gas chromatography, high-performance liquid chromatography)
N	Normality
n_{eff}	Effective number of theoretical plates
$NADP^+$	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
NMR	Nuclear magnetic resonance spectroscopy
ν	Frequency (nu)
ν_R	Resonance frequency for reference
ν_S	Resonance frequency for sample
$\bar{\nu}$	Wave number
ODS	Octadecylsilyl
pH	$-\log_{10} a_{H^+}$
pI	Isoelectric point
π^*	Pi antibonding

PGB	Prostaglandin B
pK_a	$-\log_{10}K_a$
PMR	Proton magnetic resonance
ppm	Parts per million
p.s.i.	Pounds per square inch
Q	Molecular charge
r	Internal radius (gas chromatography, high-performance liquid chromatography); radius of arc of deflection (mass spectrometry)
R	Resistance (electrochemistry); gas constant
R_F	Retardation factor
rf	Radiofrequency
R_s	Resolution
RI	Refractive index
rmm	Relative molecular mass
RP	Reversed phase
S_l	Magnetization signal of liquid
S_s	Magnetization signal of solid
SCE	Saturated calomel electrode
SCOT	Surface-coated open tubular
SDS	Sodium dodecylsulphate
SEC	Size-exclusion chromatography
SHE	Standard hydrogen electrode
σ	Shielding constant (nuclear magnetic resonance spectroscopy) (sigma)
σ^*	Sigma antibonding
SIM	Selected ion monitoring
STP	Standard temperature and pressure
T	Absolute temperature
T_1	Spin–lattice relaxation time constant
T_2	Spin–spin relaxation time constant
t_p	Time of pulse
t_0	Retention time for non-retained component
t'	Adjusted retention time
t_r	Retention time
τ	Fluorescence lifetime (tau)
TD	Thermionic detector
TLC	Thin-layer chromatography
u	Velocity
$\bar{u}$	Mean linear velocity
UV	Ultraviolet
v	Velocity of ion
V	Voltage
V_0	Interstitial volume
V_p	Pore volume
V_r	Retention volume
w_b	Peak width at base
W_b	Width of triangle at base
$w_{1/2}$	Peak width at half-height
WCOT	Wall-coated open tubular
x', y', z'	Axes in rotating coordinate system
z	Charge

SI units

SI units have been used throughout this book, except for a few instances where other units are still in common use. The following tables are provided to assist the reader in the use of SI units.

Table 1 Basic SI units

Physical Quantity	*Unit*	*Symbol*
Length	metre	m
Mass	kilogram	kg
Time	second	s
Electric current	ampere	A
Temperature	kelvin	K
Amount of substance	mole	mol

Table 2 Derived SI units

Physical Quantity	*Unit*	*Symbol*	*Definition of unit*
Energy	joule	J	$kg\,m^2\,s^{-2}$
Force	newton	N	$kg\,m\,s^{-2} = J\,m^{-1}$
Power	watt	W	$kg\,m^2\,s^{-3} = J\,s^{-1}$
Electric charge	coulomb	C	$A\,s$
Potential difference	volt	V	$kg\,m^2\,s^{-3}\,A^{-1} = W\,A^{-1}$
Resistance	ohm	Ω	$kg\,m^2\,s^{-3}\,A^{-2} = V\,A^{-1}$
Magnetic flux	weber	Wb	$kg\,m^2\,s^{-2}\,A^{-1} = V\,s$
Magnetic flux density	tesla	T	$kg\,s^{-2}\,A^{-1} = Wb\,m^{-2}$
Frequency	hertz	Hz	s^{-1}
Pressure	pascal	Pa	$kg\,m^{-1}\,s^{-2} = N\,m^{-2}$
Conductance	siemens	S	$A^2\,s^3\,kg^{-1}\,m^{-2} = \Omega^{-1}$

Table 3 Non-SI units used in this book

Physical quantity	*Unit*	*Symbol*	*Conversion to SI unit*
Pressure	bar	bar	1×10^5 Pa
	pound per square inch	p.s.i.	$703.07\,kg\,m^{-2}$
	torr	Torr	101 325/760 Pa
Length	foot	ft	3.048×10^{-1} m
Magnetic induction	gauss	G	1×10^{-4} T
Electrical energy	electron volt	eV	$\sim 1.602 \times 10^{-19}$ J