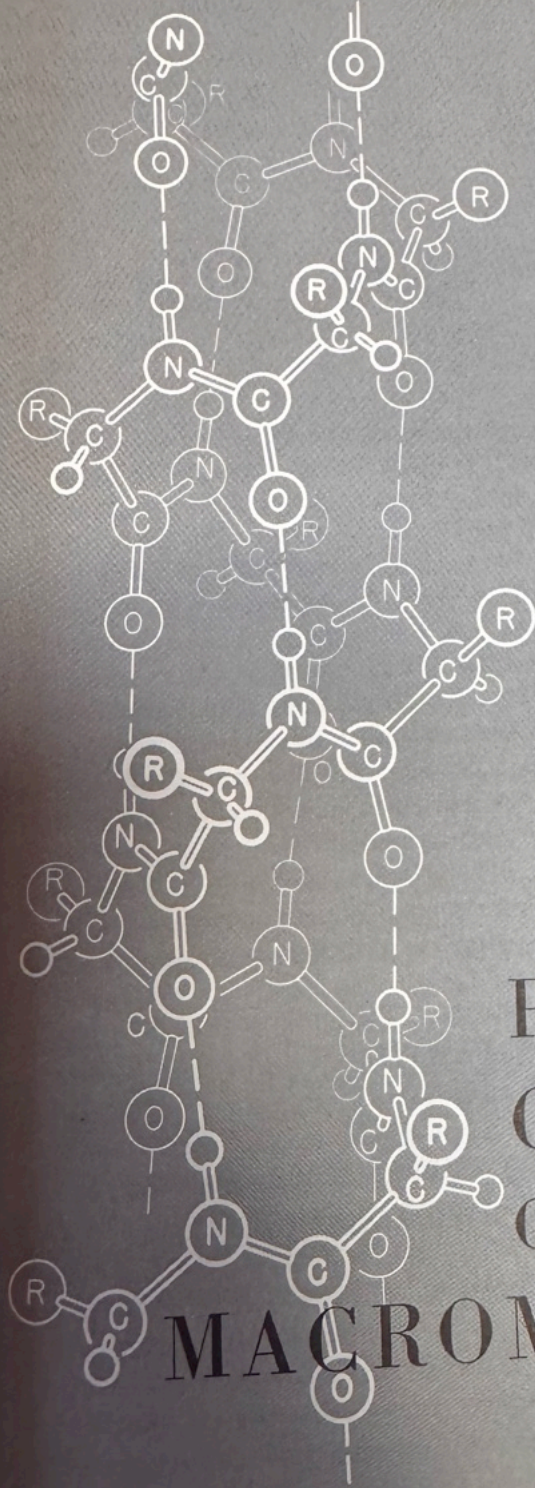




QD
471
T164
C.2

PHYSICAL CHEMISTRY OF MACROMOLECULES

CHARLES TANFORD

Professor of Physical Biochemistry
Duke University

JOHN WILEY & SONS, INC., NEW YORK · LONDON

COPYRIGHT © 1961 BY JOHN WILEY & SONS, INC.

All rights reserved. This book or any part thereof must not be reproduced in any form without the written permission of the publisher.

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 61-11511

PRINTED IN THE UNITED STATES OF AMERICA

CONTENTS

chapter 1. INTRODUCTION

1

1. A brief history, 1
2. Varieties of macromolecules, 2
- References, 13

chapter 2. MOLECULAR STRUCTURE

15

3. The principles of x-ray diffraction, 16
4. Diffraction of x-rays by macromolecular solids, 37
5. Spectroscopy, 73
6. Other methods for structure determination, 100
7. General conclusions, 126
- References, 133

chapter 3. STATISTICS OF LINEAR POLYMERS

138

8. Molecular weight averages and distributions, 138
9. Average dimensions, 150
10. Distribution functions for polymer configuration, 170
- References, 178

chapter 4. THERMODYNAMICS

180

11. Fundamental principles of the thermodynamics of solutions, 180
12. Statistical treatment of the chemical potential in non-electrolyte solutions (especially flexible polymers), 192
13. Osmotic pressure, 210
14. Macro-ions, 221
15. Phase equilibria, 238
16. Sedimentation equilibrium, 254
- References, 272

chapter 5. LIGHT SCATTERING

275

- 17. Light scattering by particles small compared to the wavelength of light, 275
- 18. Light scattering by larger particles, 297
- References, 315

**chapter 6. TRANSPORT PROCESSES.
VISCOSITY**

317

- 19. Flow processes in viscous fluids, 317
- 20. Macromolecules as hydrodynamic "particles," 336
- 21. Diffusion, 346
- 22. Sedimentation in the ultracentrifuge, 364
- 23. Viscosity, 390
- 24. Transport in an electric field, 412
- 25. Rotary motion, 432
- References, 451

**chapter 7. ELECTROSTATIC FREE ENERGY
AND POLYELECTROLYTES**

457

- 26. Calculation of the electrostatic potential and free energy, 458
- 27. Flexible linear polyelectrolytes, 489
- 28. Polypeptides, polysoaps, proteins, nucleic acids, 508
- References, 523

chapter 8. MULTIPLE EQUILIBRIA

526

- 29. General theory of multiple equilibria, 526
- 30. Hydrogen ion titration curves, 548
- 31. Competitive and other complex equilibria, 573
- References, 585

**chapter 9. KINETICS OF MACROMOLECULAR
REACTIONS**

587

- 32. Kinetics of polymerization, 588
- 33. Polymer degradation, 611
- 34. Kinetics of protein denaturation, 624
- 35. Kinetics of enzyme action, 639
- 36. Special techniques, 656
- References, 667

APPENDICES

A. Mathematical formulae	671
B. Vector notation	673
C. Optical methods for determining concentration gradients	676

AUTHOR INDEX

681

SUBJECT INDEX

693

LIST OF IMPORTANT SYMBOLS

a	major semi-axis of ellipsoid	I	ionic strength
a_i	thermodynamic activity of component or species i in a solution	J_i	flux of component i across unit cross section per second
B	second virial coefficient	K	equilibrium constant
b	minor semi-axis of ellipsoid	K	constant relating observed displacement in Schlieren optical system to dc/dx
C	concentration, moles/liter or moles/cc.	$\mathcal{K}$	optical constant in light scattering
c	concentration, g/cc.	k	rate constant
$\bar{c}$	velocity of light	k	equilibrium constant for association reactions
D	diffusion coefficient	k	Boltzmann's constant
D	dielectric constant	L	phenomenological coefficient in thermodynamic treatment of flow processes
E	internal energy	l_{av}	average bond length in a polymer chain
$\mathcal{E}$	electric field strength	M	molecular weight
F	Gibbs' free energy	m	concentration, moles/kg. of solvent
f	frictional coefficient	N	concentration, molecules/cc.
G	general symbol for any measurable property	$\mathcal{N}$	Avogadro's number
g_i	grams of component i in a mixture or solution	n_i	number of moles of component or species i in a solution
H	enthalpy	$\tilde{n}$	refractive index
h_{av}	average end-to-end distance of a polymer chain		
h_{ij}	distance from segment i to segment j of a polymer chain		

P, p	pressure	x	degree of polymerization of a polymer chain
q	electrostatic charge in esu	γ_i	activity coefficient (molarity scale) of component or species i in a solution
R	radius	Z, z	electrostatic charge in units of protonic charges
R_G	radius of gyration	α	polarizability
R_θ	experimental quantity in light scattering	α	empirical expansion factor for polymer dimensions
$\mathcal{R}$	gas constant	β	effective bond length in polymer chains
r	symbol for radial distance	γ_i	activity coefficient (molal scale) of component or species i in a solution
S	entropy	ϵ	proton charge in esu
s	sedimentation coefficient	η	viscosity
T	temperature	$[\eta]$	intrinsic viscosity
t	time	λ	wavelength
$\mathbf{u}, u$	flow velocity in a fluid	u_i	chemical potential of component or species i in a solution
V	volume	ρ	density
$\bar{V}_i$	partial molal volume of component or species i in a solution	σ	number of elements in a polymer chain
v_i	partial specific volume of component or species i in a solution		
W	statistical probability		
W_{el}	electrostatic free energy		
X_i	mole fraction of component or species i in a solution		

(where c refers to concentration in grams per cubic centimeter) which in turn is easily obtained from refractive index gradients if each component has the same refractive index increment.

21e. Diffusion coefficients of proteins. Protein molecules in solution bear electrostatic charges. Even at the isoelectric point, where the average net charge is zero, there will be molecules with net charge ± 1 , ± 2 , etc., and even those molecules which actually possess zero net charge are multi-polar ions; i.e., they contain many positive and negative charges, in equal numbers. The diffusion of proteins is therefore undoubtedly influenced by electrostatic forces. It is ordinarily believed that a moderately large concentration of a low-molecular-weight electrolyte, such as KCl, will eliminate any such influence, and this contention is borne out by the fact that diffusion coefficients at moderately high ionic strengths become essentially independent of the net charge (e.g., Creeth⁴²). Introducing an electrolyte of course means that we are no longer dealing with a two-component system. As was pointed out on p. 353, however, this factor is believed to be unimportant if the third component (i.e., the added electrolyte) is initially present at the same concentration everywhere. In any event, proteins dissolved in salt solutions are generally regarded as two-component systems, and D^0 values are obtained for them by extrapolation of apparent diffusion coefficients to zero concentration.

To interpret diffusion data so obtained the protein molecules or ions are regarded as particles in the sense discussed in section 20. Equation 20-6 (or, where necessary, equation 20-7) gives the volume of such a particle in terms of an empirical degree of solvation, δ_1 . The radius of a sphere with this volume ($v_h = 4\pi R_0^3/3$) would be

$$R_0 = \left[\frac{3M}{4\pi\mathcal{N}} (\bar{v}_2 + \delta_1 v_1^0) \right]^{1/3}$$

and the frictional coefficient of this hypothetical sphere is given by equation 19-13 as $f_0 = 6\pi\eta R_0$. The actual frictional coefficient of the particle may be expressed in terms of f_0 by means of the ratio f/f_0 ; i.e., $f = (f/f_0)6\pi\eta R_0$. The ratio f/f_0 will be unity if the particle actually is a sphere but, in general, will be greater than unity.

Combining these relations with equation 21-9 we can now write for the diffusion coefficient extrapolated to zero concentration

$$f = \frac{kT}{D^0} = 6\pi\eta \frac{f}{f_0} \left[\frac{3M(\bar{v}_2 + \delta_1 v_1^0)}{4\pi\mathcal{N}} \right]^{1/3} \quad (21-26)$$

It is seen by equation 21-26 that the diffusion coefficient depends on the molecular weight and partial specific volume of the protein, both of which

are measurable quantities. It depends also, however, on *two* unknown factors: ff_0 , representing the deviation of the shape of the protein hydrodynamic particle from a sphere, and δ_1 , representing the solvation. We clearly cannot determine both these factors from a single measurement of the diffusion coefficient. Thus there is an ambiguity in the interpretation of diffusion coefficients, as the discussion of the data below will show.

To obtain a function containing only these unknown parameters we calculate the *minimum* possible frictional coefficient, $f_{\min}$, or the corresponding maximum diffusion coefficient, $D_{\max}^0$, compatible with the known molecular weight and $\bar{v}_2$. This is obtained by setting $ff_0 = 1$ and $\delta_1 = 0$ in equation 21-26, giving

$$f_{\min} = \frac{kT}{D_{\max}^0} = 6\pi\eta \left(\frac{3M\bar{v}_2}{4\pi N} \right)^{1/3} \quad (21-27)$$

The ratio of the observed diffusion coefficient to this calculated value is then the desired quantity,

$$\frac{f}{f_{\min}} = \frac{D_{\max}^0}{D^0} = \frac{f}{f_0} \left(\frac{\bar{v}_2 + \delta_1 v_1^0}{\bar{v}_2} \right)^{1/3} \quad (21-28)$$

(In many literature compilations this ratio is referred to as ff_0 . We have introduced the new symbol $ff_{\min}$ since we have used ff_0 to represent the effect of shape alone on the frictional coefficient.)

It should be noted at this point that diffusion coefficients of proteins are conventionally corrected to "standard conditions," the standard solute being pure water at 20° C. The values of D^0 so corrected are those which the protein solutions in pure water at 20° C would have if the molecular parameters M , $\bar{v}_2$, ff_0 , and δ_1 were all to remain constant, independent of the solvent. If this were so, then, with D^0 representing the diffusion coefficient measured at temperature T in a solvent (e.g., a salt solution) of viscosity η and T_s representing the "standard" temperature and η_s the viscosity of the "standard" solvent at that temperature, the "standard" diffusion coefficient, D_s^0 , is given by equation 21-26 as

$$D_s^0 = D^0 T_s \eta / T \eta_s \quad (21-29)$$

With $T_s = 293.16^\circ \text{K}$ and $\eta_s = 0.01002$ poise we get the usual standard conditions, the corresponding value of D^0 being often designated $D_{20,w}^0$. It should be noted that this correction has no effect on the experimental ratio $ff_{\min}$ given by equation 21-28.

It is important to observe that the computation of $D_{20,w}^0$ by equation 21-29 is purely an algebraic procedure. The experimental value of D^0 which would actually be obtained in pure water at 20° C would often be quite different from $D_{20,w}^0$ for the molecular parameters such as M and ff_0 often change with even minor alterations in temperature or electrolyte concentration.