Advances in Infrared and Raman Spectroscopy

VOLUME 7

Edited by
R.J.H.Clark and R.E.Hester

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R. J. H. CLARK
University College, London

R. E. HESTER
University of York

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LIST OF CONTRIBUTORS

L. ANDREWS, Chemistry Department, The University of Virginia, Charlottesville, Virginia 22901, U.S.A. (p. 59).

P. R. CAREY, Division of Biological Sciences, National Research Council of Canada, Ottawa K1A OR6, Canada (p. 1).

J. HOWARD, Department of Chemistry, The University of Durham, South Road, Durham DH1 3LE, U.K. (p. 86).

T. F. HUNTER, School of Chemical Sciences, The University of East Anglia, Norwich NR4 7TJ, U.K. (p. 283).

G. J. ROSASCO, National Bureau of Standards, Washington D.C. 20237, U.S.A. (p. 223).

V. R. SALARES, Division of Biological Sciences, National Research Council of Canada, Ottawa K1A OR6, Canada (p. 1).

P. C. TURTLE, School of Chemical Sciences, The University of East Anglia, Norwich NR4 7TJ, U.K. (p. 283).

T. C. WADDINGTON, Department of Chemistry, The University of Durham, South Road, Durham DH1 3LE, U.K. (p. 86).

PREFACE TO VOLUME 1

There are few areas of science which have not already benefited from the application of infrared spectroscopic methods, and progress in this field remains vigorous. Closely related information on chemical and biological materials and systems is obtainable from Raman spectroscopy, though there are also many important differences between the types of information yielded and the types of materials and systems best suited to study by each technique. The close relationship between these two sets of spectroscopic techniques is explicitly recognized in this Series. Advances in Infrared and Raman Spectroscopy contains critical review articles, both fundamental and applied, mainly within the title areas; however, we shall extend the coverage into closely related areas by giving some space to such topics as neutron inelastic scattering or vibronic fluorescence spectroscopy. Thus the Series will be firmly technique orientated. Inasmuch as these techniques have such wide ranging applicability throughout science and engineering, however, the coverage in terms of topics will be wide. Already in the first volume we have articles ranging from the fundamental theory of infrared band intensities through the development of computer-controlled spectrometer systems to applications in biology. This integration of theory and practice, and the bringing together of different areas of academic and industrial science and technology, constitute major objectives of the Series.

The reviews will be in those subjects in which most progress is deemed to have been made in recent years, or is expected to be made in the near future. The Series will appeal to research scientists and technologists as well as to graduate students and teachers of advanced courses. The Series is intended to be of wide general interest both within and beyond the fields of chemistry, physics and biology.

The problem of nomenclature in a truly international Series has to be acknowledged. We have adopted a compromise solution of permitting the use of either English or American spelling (depending on the origin of the review article) and have recommended the use of SI Units. A table on the international system of units is given on p. xiii for reference purposes.

R. J. H. CLARK
R. E. HESTER

PREFACE

The present volume continues the policy of the Editors of this Series of commissioning critical review articles in both fundamental and applied aspects of infrared and Raman spectroscopy, as well as in topics closely allied to these. The Volume opens with an authoritative and timely review of Raman and resonance Raman spectroscopy of molecules of biological interest, a subject which is growing rapidly at the present time. The review begins with a consideration of the features of protein structure which are probed effectively by Raman spectroscopy, and then moves on to a discussion of the application of resonance Raman spectroscopy to studies of metalloproteins, photobiological pigments, heme proteins, nucleic acids, RNA and DNA, viruses, lipids and membranes.

The next chapter is devoted to the spectroscopy of molecular ions in noble gas matrices. This relatively new and developing area is bound to be one of increasing interest as the knowledge and practice of matrix-isolation spectroscopy becomes more widely known and appreciated. Furthermore, the vibrational properties of the many new species so isolated open the way for intriguing comparisons with analogous neutral species. In Chapter 3, an expert overview of molecular spectroscopy with neutrons is presented in which both the scope and the limitations of the technique are outlined. The purpose of the article is to discuss the applications of neutron scattering to vibrational spectroscopy, to describe the relevant mathematical relationships, and to give examples of recent work done.

Chapter 4 consists of a timely review of the application of Raman spectroscopy to microanalysis, both qualitative and quantitative aspects being discussed. Technical aspects of Raman microprobes and microscopes are detailed, and applications in the fields of biology/pathology, mineralogy/geology, environmental analysis and industrial quality control all are considered. The final chapter consists of a review of optoacoustic and thermo-optic detection in infrared spectroscopy and in the study of relaxation processes. It is just 100 years since Alexander Graham Bell discovered the optoacoustic effect, and hence it is fitting to consider now the progress which has been made in the application of this phenomenon to various spectroscopic fields.

As with earlier volumes, we have favoured IUPAC nomenclature and the use of SI units. A table of SI units, and conversion factors from the units of other systems to SI units, is included on pp. xiii–xv.

R. J. H. Clark
R. E. Hester

January 1980

THE INTERNATIONAL SYSTEM OF UNITS (SI)

Physical quantity	*Name of unit*	*Symbol for unit*
SI Base Units		
length	metre	m
mass	kilogram	kg
time	second	s
electric current	ampere	A
temperature	kelvin	K
amount of substance	mole	mol
SI Supplementary Units		
plane angle	radian	rad
solid angle	steradian	sr
SI Derived Units having Special Names and Symbols		
energy	joule	$J = m^2 kg s^{-2}$
force	newton	$N = m kg s^{-2} = J m^{-1}$
pressure	pascal	$Pa = m^{-1} kg s^{-2} = N m^{-2} = J m^{-3}$
power	watt	$W = m^2 kg s^{-3} = J s^{-1}$
electric charge	coulomb	$C = s A$
electric potential difference	volt	$V = m^2 kg s^{-3} A^{-1} = J A^{-1} s^{-1}$
electric resistance	ohm	$\Omega = m^2 kg s^{-3} A^{-2} = V A^{-1}$
electric conductance	siemens	$S = m^{-2} kg^{-1} s^3 A^2 = \Omega^{-1}$
electric capacitance	farad	$F = m^{-2} kg^{-1} s^4 A^2 = C V^{-1}$
magnetic flux	weber	$Wb = m^2 kg s^{-2} A^{-1} = V s$
inductance	henry	$H = m^2 kg s^{-2} A^{-2} = V s A^{-1}$
magnetic flux density	tesla	$T = kg s^{-2} A^{-1} = V s m^{-2}$
frequency	hertz	$Hz = s^{-1}$
activity (radioactive)	becquerel	$Bq = s^{-1}$
absorbed dose (radiation)	gray	$Gy = J kg^{-1}$

SOME NON-SI UNITS

Physical quantity	*Name of unit*	*Symbol and definition*
Decimal Multiples of SI Units, Some having Special Names and Symbols		
length	ångström	$Å = 10^{-10}\,m = 0.1\,nm = 100\,pm$
length	micron	$\mu m = 10^{-6}\,m$
area	are	$a = 100\,m^2$
area	barn	$b = 10^{-28}\,m^2$
volume	litre	$l = 10^{-3}\,m^3 = dm^3 = 10^3\,cm^3$
energy	erg	$erg = 10^{-7}\,J$
force	dyne	$dyn = 10^{-5}\,N$
force constant	dyne per centimetre	$dyn\,cm^{-1} = 10^{-3}\,N\,m^{-1}$
force constant	millidyne per ångström	$mdyn\,Å^{-1} = 10^{2}\,N\,m^{-1}$
force constant	attojoule per ångström squared	$aJ\,Å^{-2} = 10^{2}\,N\,m^{-1}$
pressure	bar	$bar = 10^{5}\,Pa$
concentration	—	$M = 10^{3}\,mol\,m^{-3} = mol\,dm^{-3}$
Units Defined Exactly in Terms of SI Units		
length	inch	$in = 0.0254\,m$
mass	pound	$lb = 0.453\,592\,27\,kg$
force	kilogram-force	$kgf = 9.806\,65\,N$
pressure	standard atmosphere	$atm = 101\,325\,Pa$
pressure	torr	$Torr = 1\,mmHg = (101\,325/760)\,Pa$
energy	kilowatt hour	$kW\,h = 3.6 \times 10^{6}\,J$
energy	thermochemical calorie	$cal_{th} = 4.184\,J$
thermodynamic temperature	degree Celsius[a]	$°C = K$

[a]Celsius or 'Centigrade' temperature θ_C is defined in terms of the thermodynamic temperature T by the relation $\theta_C/°C = T/K - 273.15$.

OTHER RELATIONS

1. The physical quantity, the wavenumber (units cm^{-1}), is related to frequency as follows:

$$cm^{-1} \approx (2.998 \times 10^{10})^{-1} s^{-1}$$

2. The physical quantity, the molar decadic absorption coefficient (symbol ε) has the SI units $m^2 mol^{-1}$. The relation between the usual non-SI and SI units is as follows:

$$M^{-1} cm^{-1} = 1 mol^{-1} cm^{-1} = 10^{-1} m^2 mol^{-1}$$

3. It appears that for many years to come a knowledge of the 'electromagnetic CGS' unit system will be a necessity for workers in various fields of spectroscopy, but for practical purposes it is usually sufficient to note that for magnetic flux density, 1 gauss (G) corresponds to 10^{-4} T and for electric dipole moment, 1 debye (D) corresponds to approximately 3.3356×10^{-30} C m.

The SI Prefixes

Fraction	*Prefix*	*Symbol*	*Multiple*	*Prefix*	*Symbol*
10^{-1}	deci	d	10^{1}	deca	da
10^{-2}	centi	c	10^{2}	hecto	h
10^{-3}	milli	m	10^{3}	kilo	k
10^{-6}	micro	μ	10^{6}	mega	M
10^{-9}	nano	n	10^{9}	giga	G
10^{-12}	pico	p	10^{12}	tera	T
10^{-15}	femto	f	10^{15}	peta	P
10^{-18}	atto	a	10^{18}	exa	E

CONTENTS

Chapter 1

RAMAN AND RESONANCE RAMAN STUDIES OF BIOLOGICAL SYSTEMS†

P. R. Carey and V. R. Salares

Division of Biological Sciences, National Research Council of Canada, Ottawa, K1A OR6, Canada.

1 INTRODUCTION

The application of Raman spectroscopy to biochemical problems is one area which truly underwent a revolution with the advent of the laser. Pioneers such as Edsall clearly recognized the potential of Raman studies of aqueous solutions of biological molecules in the 1930s[1] but, as a result of the difficulties associated with lamp excitation, the long photographic exposures necessary, and the large volumes of optically pure sample needed, the application lay dormant. Recent and continuing technical advances in spectrometers, detection systems and especially lasers as excitation sources, are opening up the entire area of biochemistry for Raman studies. A measure of success is that vibrational spectroscopists can now look biochemists straight in the eye and ask 'What is a problem of major biochemical importance?' and then proceed to supply some answers that the *biochemists* find interesting. Universally recognized subjects such as DNA, viruses, photosynthesis and vision have already felt the impact of Raman spectroscopy.

In general, biochemical systems can be grouped into four classes: proteins, nucleic acids, lipids and membranes, and carbohydrates. For an introduction to the basic chemistry of these molecules the reader is referred to one of the excellent standard books on biochemistry, for example, those by Lehninger,[2] Stryer[3] or Metzler.[4] Partly because of their poor light-scattering properties and the difficulties of sample handling, carbohydrates have not yet been extensively studied by Raman spectroscopy. A good introduction to this area has been given recently by Vergoten *et al.*[5] in Vol. 4 of this series and carbohydrates will be omitted from the present review. The dividing lines between resonance, preresonance and normal Raman spectra are not always clear. However, Section 3, on protein sites, is almost exclusively concerned with resonance Raman (r.R.) studies of visible chromophores in a variety of

†NRCC Number 17496.

protein systems, although recent advances in the u.v.-excited r.R. spectra of proteins are also dealt with. Section 2, on protein structure, and Sections 4 and 5 on nucleic acids and membranes, respectively, are concerned mainly with normal Raman spectroscopy. However, the first r.R. studies on nucleic acids and membranes are now appearing and are reviewed at the end of the relevant sections. Several reviews on various aspects of Raman spectroscopy in biochemistry have appeared recently.[5–14] Some of these are in early volumes of this series of advances[5–8] and provide excellent background material for the present article. By providing a synoptic view and only giving emphasis to the most recent developments we have attempted to minimize duplication wherever possible. One active and fascinating application, that of resonance Raman spectroscopy to the visual process, is the subject of a separate review in a subsequent volume. It will not be dealt with here.

The problem of background luminescence has been encountered by so many people attempting to start work in the bio-Raman area that some comments may be useful on this topic. There are two sources of luminescence, that which is intrinsically associated with a chromophore in the sample and/or that from an unwanted impurity. In many cases impurities are solely responsible for 'background' problems. They can often be eliminated by carefully purifying the sample by standard biochemical techniques. These techniques are not, in general, difficult and can quickly be acquired. If the sample has an intrinsic fluorescent or phosphorescent component, a change of the wavelength of excitation often produces improvement in the ratio of Raman to luminescence photons in the Raman spectral region, and this may also be tried for samples with fluorescent contaminants. Sometimes the addition of a fluorescent quencher, such as KI, also helps, but care must be taken that the quencher does not chemically perturb the sample. It is often merely a question of persistence; only for those samples that have an appreciable quantum yield of fluorescence from an intrinsic chromophore is the situation hopeless. For such samples the chances of getting a r.R. spectrum rest on technological innovations. These have been outlined recently[9] and include the time discrimination of Raman against fluorescence photons,[15] modulation of the excitation wavelength,[16] and coherent anti-Stokes Raman spectroscopy (CARS).[17] However, these approaches require considerable technical sophistication and it may take some time for their application to become routine.

2 PROTEIN STRUCTURE PROBED BY RAMAN SPECTROSCOPY

Proteins can have very different roles in biochemistry. Thus, some proteins exist simply as passive structural building blocks in complex biological assemblies while many others actively participate in time-dependent biological processes. The keratins, the structural units in, for example, hair and nails, typify the 'passive' class, while enzymes, which catalyze chemical reactions with inimitable selectivity and speed, are prime examples of active 'worker' proteins.

To arrive at a proper understanding of proteins, structure has to be determined in every case and, in addition, for those proteins possessing an active working role, a means must be devised for finding out how they work or, in biochemical terms, how they function.

To a reasonable approximation the Raman and r.R. studies on proteins break down along structure and function lines, respectively. Essentially, the normal Raman spectrum of a protein is a *structure* probe while the r.R. spectrum, from a specific protein site, often enables one to focus on the *function* of the protein. Thus, the normal and r.R. studies are, in a sense, complementary. Normal Raman studies will be dealt with here, leaving r.R. topics for the following section. In view of the articles already available on the Raman spectra of proteins, and especially Frushour and Koenig's earlier contribution in this series,[7] the present section will be synoptic and will focus only on areas which have been particularly active in the past two or three years.

2.1 Secondary structure from peptide backbone vibrations

The observed normal Raman spectra of proteins consist of various modes of the amino-acid sidechains, together with modes originating from the peptide backbone. Among the latter the amide I and amide III modes are the most sensitive to conformational changes in the protein. These modes are due predominantly to C=O stretching and to C—N stretching admixed with N—H in-plane bending, respectively.

2.1.1 Polypeptide models

Extensive use has been made of polypeptides to set up structure–Raman-spectra correlations. The wavenumbers of the different bands in the amide I and III regions for α-helical, antiparallel β-pleated sheet, and random-coiled conformations for polypeptides of known structure are summarized in Table 1. Intense bands in the 900–1000 cm^{-1} region, attributed to C_α—C—N stretching, have also been found to be conformationally sensitive. From Table 1, the amide I band wavenumbers for α-helical structures range from 1645–1656 cm^{-1}, while those of antiparallel β-pleated sheets are distinctly higher at 1666–1679 cm^{-1}. In solution studies, the intense band near 1645 cm^{-1} due to the water bending mode often obscures the amide I band. Spectra from D_2O solutions permit the separation of the water band from the amide I band.

In the amide III region, antiparallel β-pleated sheet structure gives rise to an intense band at 1229–1240 cm^{-1} and a weaker feature at 1289–1295 cm^{-1}. Problems arise in the amide III region from overlap of bands from amino-acid sidechains, and from the lack of a well-defined, agreed upon, amide III feature associated with the α-helical structure. The assigned amide III band wavenumbers of the α-helices given in Table 1 show marked irregularity. Nevertheless, it has been suggested that the *absence* of spectral intensity at

TABLE 1.
Peptide backbone raman-active vibrations of polypeptides of known structures

Polypeptide	Wavenumber[||]/cm^{-1} Amide I	Amide III	Skeletal	Structure analysis
α-Helix (left-handed)				
Poly-L-alanine, solid[a,b]	1655	1265, 1275, 1283	909	X-ray[c]
Poly-γ-benzyl-L-glutamate, solid[a,d]	1650	1294	934	X-ray[e]
Poly-β-benzyl-L-aspartate, solid[f]	1663		890	i.r., X-ray[g]
Poly-L-glutamic acid, solid[h]	1656	1246 w	926	i.r. dichroism[i]
Poly-L-leucine, solid[b,d]	1653	1294, 1261	931	c.d.
Poly-L-lysine				
·· HCl, solid at 50% humidity[a,k]	1655	1295		X-ray[l]
·· HCl, film at 92% humidity[m]	1647	1256, 1218	945	i.r.[n]
·· HPO_4, single crystal[m]	1645	1246, 1210		X-ray and electron diffraction[o]
Aq. soln, pH 11.8, 4 °C[p,q,r]	1645	1311 s, 1200–1300 vw[‡]	945	c.d.[s]
Antiparallel β-pleated sheet				
Polyglycine I, solid[a,t]	1674	1295, 1234		X-ray[u]
Poly-L-valine, solid[a,b,d]	1666	1289, 1229		X-ray[v]
Poly-L-serine, solid[4]	1668	1235		i.r., X-ray[w]
Poly-L-alanine, mechanically deformed solid[b]	1669	1243, §1231		X-ray[x]
Poly-L-lysine, aq. soln, pH 12, 52 °C[p,q]	1670	1240	1002 w	c.d.[s]
Poly-β-benzyl-L-aspartate, heat-treated solid[f]	1679	1237		X-ray[y]
Random coil				
Poly-L-lysine, aq. soln, pH 4[a,p,r]	1665 br	1243–1248	958	o.r.d.[s] X-ray[l]
Sodium poly-L-glutamate, solid[h]	1649	1247	938	i.r.[h]
Poly-L-glutamic acid, aq. soln, pH 11[h,q]	1665	1249	949	c.d.

Table 1 continued

‡ Considerable difficulty in assigning the amide III vibration of poly-L-lysine arises from overlapping side-chain vibrations. Low intensity between 1200 and 1300 cm^{-1} has been assigned to the amide III mode[m,p,q] and the strong 1311 cm^{-1} band to lysine side-chain vibration.[m] On the other hand, Chen and Lord,[a] and Lippert *et al.*[r] assign the 1311 cm^{-1} to predominantly amide III vibration.

§ Assigned to disordered poly-L-alanine. ‖ w = weak, vw = very weak, s = strong, br = broad.

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[l] U. Shmueli and W. Traub, *J. Mol. Biol.* **12,** 205 (1965).

[m] T.-J. Yu and W. L. Peticolas, *Peptides, Polypeptides and Proteins, Proc. Rehovot. Symp. on Polyamino Acids, Polypeptides and Proteins and their Biological Implications*, Wiley, New York, 1974, p. 370.

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[r] Ref.19.

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[v] R. D. B. Fraser, B. S. Harrap, T. P. MacRae, F. H. C. Stewart and E. Suzuki, *J. Mol. Biol.* **12,** 482 (1965).

[w] Z. Bohak and E. Katchalski, *Biochemistry* **2,** 228 (1963).

[x] S. Arnott, S. D. Dover and A. Elliott, *J. Mol. Biol.* **30,** 201 (1967).

[y] E. M. Bradbury, L. Brown, A. R. Downie, A. Elliott, R. D. B. Fraser, W. E. Hanby and T. R. R. McDonald, *J. Mol. Biol.* **2,** 276 (1960).

[z] G. D. Fasman, C. Lindblow and E. Bodenheimer, *Biochemistry* **3,** 155 (1964).

1235–1240 cm^{-1} is diagnostic of an α-helix.[18] An additional identification band for α-helical structure is a strong skeletal vibration at 909–945 cm^{-1} whose intensity markedly decreases following the transformation of the α-helix to the β-sheet. Unfortunately, this band does not uniquely identify an α-helix, since random-coiled polypeptides also have a band with appreciable intensity near 950 cm^{-1}.

For random-coiled polypeptides in solution the amide I and III bands occur near 1665 and 1248 cm^{-1}, respectively, and are broader than those of α-helix and β-sheet structures. It is questionable, however, whether the band wavenumbers found for the random-coiled polypeptides can be applied to disordered proteins.[13,19] Characteristic frequencies cannot be expected for unordered polypeptide chains or unordered proteins which consist of a large number of different conformations. A further point to consider in using polypeptides as models for protein structure is the presence of an additional symmetry, in the form of repeating side chains in polypeptides, which is absent from proteins.[20]

2.1.2 The transfer of polypeptide correlations to proteins

To assess the suitability of the wavenumber–structure correlations developed for the homopolypeptides for determining protein structure, the band wavenumbers found for proteins of known structure are given in Table 2. The amide I band assigned to the α-helical fraction of crystalline glucagon (1658 cm^{-1}) and tropomyosin (1655 cm^{-1}), two highly helical proteins, occurs close to the observed wavenumber of the helical polypeptides (1645–1656 cm^{-1}), but in insulin it is higher (1662 cm^{-1}). Wide variation is found in the wavenumber of the amide I band due to the random fraction of glucagon and insulin (1685 cm^{-1}), feather keratin (1654 cm^{-1}) and α-casein (1668 cm^{-1}). The amide I bands assigned to the β-fractions of the proteins are similar to those found for the polypeptides but bands due to α- or disordered fractions are usually not resolved, thus limiting the usefulness of the amide I region. The amide III region appears to be more sensitive to heterogeneous secondary structure. While the amide I regions of lysozyme, basic pancreatic trypsin inhibitor, myosin (Table 2), ribonuclease A (Fig. 1) and several histone proteins[21] show a single band, the amide III region consists of two or more components. In ribonuclease A the band at 1239 cm^{-1} is assigned by common consent to the β-fraction but, as in the case of polypeptides, the assignment of an amide III contribution from the α-helical fraction is not agreed upon. There is an appreciable variation in the amide III bands assigned to disordered protein (1235–1270 cm^{-1}), reinforcing the suggestion that there are no characteristic bands for unordered regions of proteins. It is also difficult to separate the amide III bands of β-sheet from those of disordered fractions due to overlap of bands.

In summary, it is apparent that, keeping in mind the limitations discussed above, the amide I and amide III bands in a Raman spectrum are useful