

Raman Spectroscopy

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ORGANIZATION

The period covered by this document is from the beginning of 1995 through late 1997. The most recent comprehensive review of this topic appeared in 1994 (1) and covered late 1991 through late 1993. Thus, there exists a gap in coverage of just over a year. It is hoped that this review is sufficiently comprehensive to incorporate important results first described in publications that appeared during that time period.

With roughly 11 000 CAS citations to Raman over the last two years, this “awakening giant” (2) has apparently ended its slumber! This number is nearly double the 1994 figure and reflects several important trends. Clearly, the ready availability and relatively low

cost of CCD cameras, notch filters, and compact, rugged lasers has made it easier to construct a Raman apparatus. Likewise, the purchase of packaged Raman spectrometers has been facilitated by the creation of numerous companies selling stand-alone instruments. However, the main factor leading to the upsurge in Raman-related publications has been the worldwide explosion in materials science, with the accompanying need for materials characterization. In several respects, Raman is well-suited for this task. First, the ability to acquire vibrational data for a variety of nontransparent solids—sometimes under extreme conditions—sets it apart from infrared. Second, the development of Raman microscopy has made it possible to look at very small quantities of materials, or even domains within materials, with a resolution of a few square micrometers. Third, Raman is nondestructive. Finally, the use of UV and near-IR excitation sources has become commonplace, extending the window from which Raman scattering can be observed.

This review gives extensive coverage to materials characterization by Raman, to biological applications of Raman, and to surface-enhanced Raman scattering (SERS), because it is felt that these topics are of greatest importance to the analytical community. The review is organized into five parts. The first section covers books, periodicals, and reviews in a fairly comprehensive fashion. This is followed by sections on (i) instrumentation, methods, data analysis, and theory; (ii) materials; (iii) SERS and nonlinear Raman methods; and (iv) biological applications of Raman. None of these sections are comprehensive, focusing rather on research highlights and on examples that illustrate breadth within a particular area.

BOOKS, PERIODICALS, AND REVIEWS

Several useful books were published during this period. These include a new two-volume edition of Nakamoto’s classic book on infrared and Raman spectra of coordination compounds, with one self-contained volume devoted to basic theory and applications to small molecules, and the second on larger molecule applications in coordination, organometallic, and bioinorganic chemistry (3, 4). A four-volume handbook on IR and Raman was published (5), as were the proceedings from the Fourteenth and Fifteenth International Conferences on Raman Spectroscopy (6, 7). Texts on introductory Raman (8), IR and Raman methods (9), Raman and IR in biology and biochemistry (10), modern techniques (11), and Raman microscopy also appeared (12). The latter had chapters on basic aspects of Raman microscopy, as well as chapters on applications in materials science, in earth, planetary, and environmental sciences, in biology, in medicine, and in forensic science. The proceedings of a conference on the spectroscopy of biological molecules was published, with the

majority of the contributions focused on vibrational spectroscopy (13). Most recently, a book on phonon-Raman scattering in semiconductors has become available (14).

Three periodicals have published special issues devoted entirely or largely to aspects of Raman scattering. Topics have included Raman microscopy and imaging (15), spectroscopic studies of superconductors (16), applications of FT-Raman (17), a 70th birthday commemoration for Derek Long (18), and Raman resonances in ultrafast spectroscopy (19). Numerous Raman reviews on a wide variety of topics were published between 1995 and 1997. Following loosely the overall outline of this review, they have been grouped below into the following broadly defined areas: general/theory, instrumentation, materials and molecular systems, biological molecules and systems, and surface Raman.

The most significant review on Raman theory published during this period was that focusing on resonance Raman (rR), describing steady-state spectra in terms of time-dependent quantities (20). Other noteworthy theory reviews included one on vibrational optical activity (21) and one on coherent Raman spectroscopic studies of gases (22).

A number of reviews dealing with basic aspects of Raman have appeared (23–26). Other general reviews cover progress in analytical Raman spectroscopy (27), near-infrared Raman measurements (28), remote Raman for monitoring and control of chemical processes (29), and the history of Raman (30) and of resonance Raman (31). Two well-referenced publications focus on Raman applied to transient systems (i.e., time-resolved experiments) (32, 33). Finally, a review on resonance Raman intensities and charge-transfer reorganization energies has appeared (34).

Reviews on instrumental advances described herein are confined to those that have had a demonstrable impact on the practice of Raman scattering. Thus, instrumental developments relating to X-ray or free-electron lasers, for example, have been omitted. The remaining instrumentation reviews fall into three categories: lasers, detectors, and fiber-optic sampling. Diode and UV lasers have both had significant impact in recent years, described in refs 35 and 36, respectively. Two reviews have appeared on new detector technologies and their impact on Raman, though only one is in English (37). A review focusing on the impact of fiber-optic sampling on analytical Raman spectroscopy has been published (38). Also, a review on data manipulation in spectroscopy describes approaches to baseline correction, spectral data smoothing, signal-to-noise ratio enhancement, and the like (39). A handy little review listing resources and references for interpretation of IR and Raman spectra has been published (40). Pemberton has published two very important papers on frequency/wavelength calibration issues for multichannel Raman spectrometers (41, 42), one of which is a review describing the experimental variables affecting instrument precision. A review describing the use of Raman spectroscopy for process/quality control has been published (43). The paper focuses on recent developments in instrumentation and data analysis as they apply to selected topics such as polymorphism in pharmaceuticals, hard carbon coatings on computer disks, and chemical reactions (e.g., polymerization, hydrogenation, curing). Finally, the report of an IUPAC commission regarding nomenclature, symbols, and units and their usage in Raman scattering processes was recently made available (44).

The use of Raman scattering in materials science has seen an exponential increase, with a concomitant exponential increase in materials-related Raman reviews. A number of reviews provide overviews of Raman spectroscopy and materials science, as practiced in academia and industry (45–52). Nine reviews have appeared that discuss to a greater or lesser extent Raman scattering of fullerenes, fullerides, and carbonaceous materials (53–61). Polymers have been extensively studied by Raman, with reviews covering numerous aspects of polymer chemistry. A good and comprehensive recent review on the use of FT-Raman for analysis of a wide variety of polymeric materials has appeared (62), along with two short general reviews on Raman and polymers (63, 64). In addition, reviews on detailed aspects of polymer structure/function and manipulation have been published. These include polymer deformation (65, 66), dispersion (67), crystallinity (68), orientation (69), tribology (70), strain (71), and polymer/polymer as well as polymer/solid interfaces (72, 73). The use of chemometrics in conjunction with FT-Raman as a means to characterize physicochemical properties of polymers has been reviewed (74). Raman has been used to probe the structure of water in polymer systems, with a focus on how polymer chain chemical properties impact water domain size and hydrogen-bonding properties (75). Normal Raman spectroscopy has been shown to be useful for characterization of synthetic vs natural rubbers (76), and third-order nonlinear optical effects have been employed in the study of conjugated polymers (77).

Several reviews have emerged that focus on semiconductors. One deals with Raman scattering by phonons in structured semiconductors (78); others deal specifically with groups III–V (79) and II–VI (80) or group IV (i.e., Si/Ge) (81) superlattices. A review concerning the use of micro-Raman spectroscopy to study local mechanical stress in Si integrated surfaces is available (82), as is one on femtosecond excited-state dynamics of semiconductors (83). A number of reviews deal more generally with optical characterization of semiconductors, in which Raman is one of several techniques discussed. These focus on monitoring of semiconductor growth by MOCVD and MBE (84), on stresses and strains in heterostructures (85) and in semiconductor devices (86), on in situ optical methods (87), on near-field methods (88), on recent developments (89), and on ex situ characterization approaches (90).

During this time period, five noteworthy reviews concerning Raman spectroscopy and high- T_c superconductors have appeared. Two focus specifically on phonon modes (91, 92), one describes the role of symmetry in Raman spectroscopy of unconventional superconductors (93), one is concerned with structure/spectroscopy relationships, with an emphasis on the oxygen stoichiometry (94), and the last describes the Raman spectra of organic superconductors as well as those based on C_{60} and YBCO thin films (95).

Several reviews on the applications of Raman to environmentally related materials have appeared. A well-referenced general review on spectroscopic measurements of acid/base properties of solids focuses on zeolites, clays, and catalysts (96); other reviews deal specifically with micro-Raman applied to earth sciences (97) and to minerals (98). A 122-reference review on Raman spectroscopy on zeolites was published (99), as were shorter reviews on Raman spectroscopy of pulp and paper samples

(100) and on pyridyls adsorbed on clays (101).

A number of reviews on miscellaneous materials-related topics were published between 1995 and 1997. These include two general reviews on spectroscopic characterization of supported metal oxide heterogeneous catalysts (102, 103), as well as reviews dealing specifically with metals and ceramics (104), colloids and conducting polymers (105), glasses (106), reactions in supercritical solvents (107), surfactants at the solid/liquid interface (108), solvent/solute interactions (109), and laser-based optical characterization of microdroplets (110), including nonlinear methods (111). The second part of a review dealing with stimulated Raman and Rayleigh spectroscopy of optical molasses also appeared (112).

Raman reviews covering molecules and/or molecular systems encompass the following areas: transition element compounds (113), main group elements (114), excited states of inorganic compounds (115), Grignard reagents (116), coordinated ligands (117), pericyclic ring-opening reactions (118), gas-phase molecular complexes and clusters (119), molecules in disordered systems (studied by linear and nonlinear optical methods) (120), and supramolecular chemistry (121). In addition, reviews have appeared that discuss IR and Raman spectroscopy applied to conformational equilibria (122) and to determination of effective bond charges (123).

Reviews published in this period encompassing biologically related Raman measurements can be classified into three categories: those focused on particular application or instrumentation areas, those focused on particular classes of biomolecules, and those focused on more general topics. Thus, reviews on applications of FT-Raman in the pharmaceutical industry (124), on Raman monitoring of drug penetration (125), on histochemical analysis of biological tissue (126), on dermatological applications (127), and on urinary calculi (128) have appeared.

A comprehensive and well-referenced review on the application of Raman spectroscopy to (photoreactive) retinal proteins appeared (129), along with one on resonance Raman studies of photosynthetic molecules and on the reaction center itself (130, 131), one on heme-enzyme oxygen intermediates (132), one on quinoproteins (133), one on copper-sulfur proteins (134), one on metalloproteins (135), and one on applications of resonance Raman scattering to heme protein-bound nitric oxide (136). A review describing EPR, XANES, and resonance Raman studies of cucumber ascorbate oxidase and fungal laccase (both copper proteins) was published (137). A detailed account of UV resonance Raman as a probe of local protein structure appeared (138).

More general biological Raman reviews focus on pharmaceutical research (139), on vibrational Raman optical activity (140-142), on biomedical applications (143, 144), and on the use of Raman to study proteins and other biomolecules (145, 146). A recent review describes applications of a suite of spectroscopic approaches, including Raman, to biomolecular processes (147).

Reviews on surface Raman can be neatly divided into two categories: those dealing with significant chemical or electromagnetic enhancement at noble metal surfaces (i.e., SERS) and those not dealing with the subject. The former category includes a general review (148) and reviews on the application of SERS to contraband detection (149), to the orientations and conformations of flexible molecules at metal interfaces (150), to chemical sensors (151),

to metal/adsorbate interactions (152), and to environmental analysis (153). The use of surface plasmon polaritons to increase the sensitivity of Raman measurements has been discussed from both theoretical and experimental perspectives (154). Unenhanced vibrational microspectroscopy of surfaces and of particles on surfaces is discussed in a 30-reference review (155); more general reviews on Raman (156) and resonance Raman (157) of surfaces have also appeared.

INSTRUMENTATION AND DATA ANALYSIS

The ever-increasing range of chemistries to which Raman spectroscopy is applied has generated a constant flux of papers describing instrumental advances. Broadly classified, these papers either describe improvements to traditional Raman instrumentation or present new sampling or detection formats for spectroscopic interrogation. For the purpose of this review, the body of work will be divided into the following categories: general instrumentation, remote sensing, microscopy/imaging, and applications.

General Instrumentation. Many of the efforts in instrumental optimization have dealt with increasing the sensitivity, resolution, and precision of traditional Raman spectrometers. Two particularly important papers described the calibration of multichannel spectrometers in terms of both instrumental factors and the use of calibration standards (158, 159). Mathematical standardization of wavelength-shifted Raman spectra has been discussed (160). Advances in multichannel detection for low-light-level Raman (161) and Raman difference spectroscopy (162) have also been described. By increasing the efficiency of a Raman spectrometer and using a low-noise CCD, scattering has been observed from monolayer quantities of material without the benefit of surface enhancement (163).

Numerous methods for eliminating background radiation and fluorescence have been developed, including differential and Fourier transform techniques (164), synchronous scanning of an optical parametric oscillator (OPO) (165), UV-opaque liquid filters (166), and dielectric band-pass filters (167). The available spectral region over which a coherent anti-Stokes Raman spectrum may be acquired has also been increased through synchronous scanning of an OPO (168). Coupling a Ti-Al₂O₃ laser with a Rb vapor filter has resulted in the measurement of FT-Raman spectra within as close as 3 cm⁻¹ of the Rayleigh line (169). Two-dimensional correlation spectroscopy, which correlates Raman and IR spectra, has been demonstrated (170). Other instrumental advances included the conversion of a double monochromator into a multipurpose dual spectrometer (171), development of a non-dispersive Raman detector for quantitative analysis of biological samples (172), and evaluation of stability and reproducibility of tunable external cavity-diode lasers for spectroscopy (173). Various laser sources have also been evaluated for use in FT-Raman spectroscopy; it was determined that sources will be unacceptable if they cause greater fluorescence than that observed from Nd-YAG lasers (174). The multiplex disadvantage of NIR FT-Raman has been overcome through the use of interference filters that transmit only the selected weak bands that were obscured during collection of a wide spectral window (175). Through elimination of thermal background interference (176), modulated FT-Raman using a pulsed Nd-YAG laser has been demonstrated to exhibit

a factor of 4 increase in signal-to-noise ratio over a continuous-wave experiment. A high-resolution, large-beam (160 mm) rotational/vibrational FT-Raman instrument with 0.02–0.03-cm⁻¹ resolution has also been described (177).

Remote Sensing. Advances in low-cost fiber optics and miniaturized detectors have led to a rapid increase in the use of Raman spectroscopy for remote sensing applications. Recent work in this area has focused on both the optimization of fiber-optic design and the tailoring of probes for specific applications. A general overview of the needs for improved data handling in remote sensing has been presented (178). On the instrumental side, Raman probe designs have been modeled (179) and evaluated (180–182) in terms of optimal tip geometry. Other studies have focused on coupling efficiency, damage threshold, and sensitivity for UV Raman fiber probes in the presence of adsorbing materials (183). Background discrimination, sample volume, and probe sensitivity has been investigated as a function of laser source and fiber design (184). A method for the measurement of the Raman gain spectrum of a fiber has been described (185). The silica background in fiber probes has been studied in terms of tip geometry, numerical aperture, cladding material, and silica type (186).

Remote microscopy has been achieved through the development of fiber-optic confocal microscopes (microprobes) (187, 188), thereby providing high-resolution, three-dimensional imaging capabilities in a portable package. Remote microimaging has also been accomplished using a coherent optical microfiber array as a probe (189). Integration of a quartz capillary into a dual-fiber probe has resulted in a 70-fold enhancement of probe sensitivity (190). A 15-fold enhancement over bare-fiber probes has been reported for a Teflon-clad fiber designed for real-time monitoring of polymer curing (191). This particular probe is exceedingly versatile as it is suitable for both bulk and thin-film measurements. Another fiber design utilized chemical modification of an array of sensor tips to induce spatially defined analyte adsorption at the probe surface (192). A probe has also been developed to withstand high-intensity γ radiation for remote sensing in radioactive sites (193). Fiber-optic cone penetrometers have been utilized for aggressive environments such as the Hanford waste site (194). The octane number of commercial gasoline has been determined using a fiber-optic Raman probe (195). Fiber-optic Raman has also been used to determine the amount of organic vapor that partitions into a solid-phase extraction medium (196). Other types of probes have been developed for high temperatures (197, 198), environmental sampling (199), biological systems (200, 201) and rugged, repetitive on-line sampling (202).

Microscopy/Imaging. Another area of current interest is Raman imaging. This technique allows for spatial determination of the composition of solid and semisolid materials. Overviews of the relevant techniques and various applications have been presented, including a special issue of the *Journal of Raman Spectroscopy* (203) and a discussion of new directions for the technique (204). New instrumental designs based on acoustooptic tunable filters (205–207) or liquid-crystal tunable filters (208) produce high wavelength and spatial resolution without a monochromator or spectrometer for wavelength selection. Raman imaging systems have been designed for interrogation of heterogeneous polymer blends (209, 210) while UV-Raman imaging has

been demonstrated in both biological and materials applications (211). Principle component analysis has been accomplished using microscopic Raman line imaging (212).

A drive for higher spatial resolution in Raman microscopy has led to two new types of instrument. The first is the confocal Raman microscope, which relies on a pair of confocal pinholes to provide a high degree of spatial resolution along with axial sectioning of semitransparent materials (213, 214). A variation of this instrument has been described where the pinhole is replaced by a fiber-optic bundle, thereby allowing for an increase in imaging depth without sacrificing spectral resolution (215). The utility of this technique was demonstrated through the imaging of the complex interfacial processes caused by entrapment of immiscible liquids in a porous material (216). Three-dimensional imaging through a turbid medium that strongly approximates biological tissue has also been demonstrated using a confocal Raman microscope (217).

A number of papers have appeared on near-field Raman imaging in which the spatial resolution of the instrument is smaller than the diffraction limit experienced by traditional or confocal microscopes. Two general instrumental design papers describe the construction and operation of a typical instrument (218, 219). The technique has also been applied to the imaging of solids (KTP) (220, 221) and liquids (CCl₄) (222).

Applications. The ability of Raman spectroscopy to deliver specific chemical identification, coupled with the wide range of instrumental and sampling methodologies available, has led to its continuing use in both new and old venues. Applications to reaction monitoring, chromatographic detection, environmental monitoring, and materials chemistry have all been described. The following is an abbreviated listing of the papers of general interest to the analytical chemist.

Perhaps the most vigorous area involves the use of Raman in on-line monitoring of reactions. Azo dye synthesis has been monitored in a novel circulating sample cell that allows real-time determination of the reaction progress (223). FT-Raman has been coupled with a new sampling cell to follow copolymerization reactions (224). Despite poor correlation between Raman and NMR monitoring, Raman was identified as the potentially more useful technique due to its rapid sampling capabilities. Using a TiO₂ Raman tracer, off-line monitoring of polymer extrusion reactions has allowed for determination of residence time distributions for high-density polyethylene (225). Semiconductor processing has been controlled through the monitoring of Raman spectra of cleaning agents used in the system (226). A high-pressure optical cell has been used in the Raman monitoring of organic peroxide decomposition, an important reaction in the high-pressure polymerization of olefins (227). Laser Raman has also been used to monitor a very different type of synthetic process: the evolution of stack gas pollutants from coal-based power plants (228).

Hyphenated analytical techniques often involve the coupling of a separation method with a spectroscopic detector. Accordingly, Raman has been used as a detector in chromatographic instruments. A detector for high-performance liquid chromatography has been demonstrated to have a detection limit of 480 mg/L for toluene while also being able to distinguish between coeluting analytes (229). Surface-enhanced Raman has been used

as a detector for liquid chromatography by flowing a silver hydrosol (a SERS substrate) through the chromatography column eluant in a windowless flow cell (230). The system was optimized with respect to integration time, eluant flow rate, and hydrosol flow rate, and the detection limits for five eluants were determined. A universal Raman detector for microchromatography has been described and its utility demonstrated for the detection of nitro compounds (231). Raman spectroscopy of RNA that had undergone preconcentration via capillary isotachopheresis has also been demonstrated (232). In a very different application, Raman scattering from the contents of a capillary has been used to optimize the alignment of that tube in a laser beam for spectroscopic detection (233). A combination of Raman and scanning tunneling microscopy has also been used to characterize electrochemical interfaces, allowing compositional, topographic, and electronic structure information to be obtained simultaneously (234).

Excitation of Raman scattering by evanescent waves and other surface waves has been described in a number of papers. An integrated optics waveguide has been modified with Ag particles to produce a surface-enhanced Raman waveguide instrument (235). A new method for coupling into a cryostat-encased waveguide has been described, allowing for variable-temperature waveguide Raman spectroscopy (236). Stimulated Raman scattering in waveguides has been shown to produce a 5 order of magnitude gain over normal Raman scattering (237). Evanescent scattering by phonons in crystal lattices has been proposed as a method for vibrational interrogation of an entire Brillouin zone (238). Grating excitation of surface plasmon modes has produced stimulated surface-enhanced Raman scattering from copper phthalocyanine films (239). Surface plasmons generated by grating coupling have also been used to enhance signal in Raman microscopy (240).

A wide variety of other applications have been developed. FT- and surface-enhanced Raman have been described as methods suitable for corrosion studies (241). A liquid-crystal light valve-gated CCD has been used in Stokes/anti-Stokes temperature measurements in flames (242). Coherent Raman measurements of nanoshocks in solids has been proposed as a method for understanding shock-induced deformation in materials (243). A thorough evaluation of Raman optical activity as an analytical tool for the determination of chiral purity was presented with claims of precision to within 1% in enantiomeric mixtures (244). Automated phase modulation allowed rapid polarization-modulated Raman measurements of liquid-crystal orientation (245). This technique provides for multiple polarizations to be interrogated in a single measurement, thereby decreasing the number of measurements required to determine absolute orientation. A thermostated Raman/impedance cell was used for the simultaneous determination of electrical, structural, and chemical changes that occur in response to thermal effects (246). Enzymatic reaction processes were monitored in a new microcirculating system designed to allow the monitoring of reaction intermediates (247). Finally, the detection of Semtex plastic explosive has been realized in normal and micro-Raman using a new spectroscopic detector that employs a narrow band-pass filter to isolate specific Raman bands (248).

Theory. In the past, a great deal of emphasis has been placed on gaining a greater understanding of normal and resonance- and surface-enhanced Raman spectroscopy. However, with many of the current theories being viewed as fairly satisfactory, most recent efforts have focused on generalization of current calculation methods or analysis of more specific aspects of Raman scattering. Specifically, Raman optical activity has been investigated for the case where incident radiation is resonant with an electronic state of the molecule (249). The results were compared to resonance Raman results, and deviations from the theory were explained in terms of contributions from multiple electronic states. In another report, the reflection principle of continuum resonance Raman scattering was described using a time-dependent formalism in a manner similar to previous methodologies (250). A new method for calculation of the Raman amplitude from excitation profiles has also been reported (251).

The prediction of surface-based Raman spectra from metal films is typically complicated by surface enhancement effects. Several papers appeared on surface-enhanced Raman theory during this period, two of which are discussed here. The first describes a model where surface roughness is treated as metal cylinders embedded in a metal surface (252). This treatment allowed the authors to evaluate a wide range of surface feature sizes, as well as a range of interparticle spacings, including the case where surface features are in close contact. A second paper describes the enhancement effect near the percolation threshold for a discontinuous silver film (253). A theory for a somewhat different surface spectroscopy, stimulated Raman scattering, was described in terms of an imaging experiment where the micrometer-scale surface morphology may contribute to variations in the signal intensity (254). This morphology-dependent stimulated scattering process is then proposed as a method for spatial imaging of chemical and structural moieties in microstructures.

Two noteworthy papers have appeared on the use of density functional theory (DFT) to predict Raman spectra. Static DFT was compared to Hartree–Fock (HF) and experimental results for a series of small molecules (255). It was found that both gradient-corrected DFT and HF were superior to local DFT in their predictive power. Time-dependent DFT was utilized in a subsequent paper to more correctly model the frequency dependence of the polarizability (256). This treatment was found to improve upon previous HF and static DFT results in terms of the scattering cross sections that were obtained. Other numerical methods have also been used to calculate resonance Raman spectra (257). Another aspect of any analytical method is the issue of data handling and uncertainty in measurements. The measurement of temperatures using Stokes/anti-Stokes ratios and the uncertainty associated with this technique have been addressed (258). The difficulty of obtaining quantitative data from compact Raman spectrometers has been discussed, and mathematical procedures for peak interpolation and wavelength calibration are presented (259).

MATERIALS

Over the past two years, use of Raman scattering in materials science has been widespread. For the purposes of this review, the literature will be divided into the following broadly defined categories: superconductors, semiconductors, carbonaceous ma-

terials including fullerenes, catalysts, oxides/gels/glasses/clays, molecules and molecular systems, environmental materials, archeological materials, biological materials, polymers, and particles/droplets. Papers included in this section represent a small fraction of those published between 1995 and 1997.

Superconductors. Superconducting materials have been widely studied by Raman, and a detailed discussion of research findings is beyond the scope (and relevance) of this review. Instead, the results of a few key papers are highlighted. Probing the chemistry of thin organic films on superconductors has proven difficult. A breakthrough in this area has come from Zhu et al., who reported the first Raman spectrum of an organic monolayer on a high- T_c superconductor ($\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$) (260). Their approach involved covalent derivatization of the oxide surface with colloidal Au nanoparticles; near-IR excitation provided direct evidence for a chemical interaction between the NH_2 group of 4-aminopyridine with the superconductor surface.

A variety of studies focus on the superconductor itself. Raman spectra of films of $\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$ as thin as 12 nm (~ 10 unit cells) have been described (261). The data were acquired by subtracting out the spectrum of the host substrate [$\text{LaSrAlO}_4(001)$]. Micro-Raman was used to locate the interface between superconducting $\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$ and nonsuperconducting YBaCu_2O_5 in fast-melt-processed samples (262). The influence of superconductor stoichiometry on frequencies and line widths for the five Raman-active A_g modes in the superconductor $\text{YBa}_{2-x}\text{La}_x\text{Cu}_3\text{O}_7$ has been reported (263). The authors observe a structural transition from orthorhombic to tetragonal at $x = 0.4$. Temperature-dependent, polarized Raman spectra of $\text{HgBa}_2\text{Ca}_2\text{Cu}_3\text{O}_{8+x}$ single crystals indicate abrupt changes in frequencies and line widths for the A_{1g} modes across T_c , but not for the 245-cm^{-1} phonon mode of B_{1g} symmetry (264). Raman has also proven useful in characterization of $\text{YBa}_2\text{Cu}_3\text{O}_x$ films prepared by laser writing (265). The oxygen content (which controls the superconductivity) can be directly measured as a function of writing conditions, illustrating how Raman comprises a nondestructive, fast, sensitive, and spatially precise (i.e., a few micrometers resolution) method to monitor superconductor processing.

Semiconductors. From 1995 to 1997, Raman spectroscopy was used extensively to characterize semiconductor surfaces as well as the physical and chemical processes occurring there. For the purposes of this review, semiconductor systems will be grouped into the following topics: Ga-, Si-, Zn-, and Cd-based semiconductors.

The most thoroughly studied Ga semiconductor continues to be GaAs. Raman spectroscopy was used to probe several aspects of Ga semiconductor behavior. Electronic-state mixing, due to intervalley electron scattering, is observed for GaAs/AlAs superlattices (266). Molecular hydrogen and deuterium were isolated and detected on GaAs by Raman spectroscopy (267). Changes in surface structure after UV laser ablation were observed with a micro-Raman probe (268). Quantum wells in GaAs surfaces have been examined in relation to longitudinal-optical phonons, standing acoustic waves, and Raman efficiency in a planar microcavity (269–271). Photoluminescence studies were carried out at the interface of GaN and wurtzite layers (272). Characterization of GaN thin films on sapphire, a rapidly developing material for blue optoelectronic devices, was accomplished by applying a right-angle

incident laser beam or by focusing through a microscope lens, thereby avoiding the strong sapphire Raman bands (273). The structural properties of GaN films on sapphire were also reported (274).

Si semiconductors remain an industrially important material to characterize. Stress profile analysis for P-line defects and stress gradients were examined at the Si/ SiO_2 surface interface (275, 276). Examination of porous Si semiconductors with an Ar^+ laser (514.5 nm) revealed a frequency shift dependence with illumination time. Increasing illumination time led to more narrow line widths and a shift of the $500\text{--}520\text{-cm}^{-1}$ peak to higher energy (277). Nanostructures in porous Si surfaces have been investigated by Raman and have been used to explain quantum size effects (278).

High-pressure (up to 36.0 GPa) studies of ZnSe semiconductors were used to observe changes in the LO and TO phonon peaks (279). Spin-flip Raman scattering was employed to study compensation on p-type ZnSe semiconductors doped with N (280).

Resonance Raman is an excellent probe for Cd semiconductor surfaces. Nanocrystals of CdSe have been assigned to the C_{3v} point group (281). Thin films of CdS have been deposited on Au and resonance Raman data have been used to elucidate the film's optical properties (282). Surface reconstruction of CdS monolayers on Au has been described (283). Electrodeposition has been used to prepare arrays of semiconductor molecular wires, whose optical properties are dependent on wire diameter (284).

Carbonaceous Materials Including Fullerenes. Even more pervasive than papers describing Raman characterization of high- T_c superconductors are those describing carbonaceous materials, especially those that are diamond-like. Indeed, from the volume of citations, it would appear that Raman spectra are a prerequisite to publication! The main information that can be gleaned from Raman spectra of diamond-like materials is the presence (and amount) of impurities in the carbon hybridization (i.e., sp^2 vs sp^3). The telltale Raman signature for the cubic structure of diamond (and diamond-like phases) is the sharp Raman band at 1332 cm^{-1} (285). Also, natural diamond exhibits a narrower line width than synthetic material. Thus, decreases in band line width and increases in band intensity upon heating to temperatures up to 1200 K are taken as evidence of film stress reduction via impurity oxidation (286). The near-IR Raman spectrum of CVD-deposited diamond films was reported to be a sensitive probe of in-plane thermal conductivity (which, in turn, correlated inversely with the amount of nondiamond carbon phase) (287). Cross-sectional Raman microscopy was used to study defect structures created in diamond by megaelectronvolt ion implantation (288). By collecting Raman spectra in a direction perpendicular to irradiation, the extent of damage could be determined as a function of depth from the irradiated surface.

Other carbonaceous materials are equally amenable to investigation by Raman, with carbon nanotubes comprising a prominent recent example: the Raman spectra of single-walled carbon nanotubes depends on diameter (289, 290) as well as on preparation method (291, 292). Likewise, fullerene structure in crystals and polymers has been determined by Raman spectroscopy. Various methods for growing fullerene shells (293) have been outlined, with a special emphasis on polymerization reactions (294–296). Resonance Raman scattering and solvent interactions

have been reported for fullerene/solvent interfaces (297, 298). Evaluation of the pentagonal-pinch mode by cross-sectional Raman analysis shows agreement with current ab initio calculations (299).

Catalysts. Characterization of heterogeneous catalysts is an important endeavor that has made use of every spectroscopic tool available. Raman scattering has been particularly invaluable in three aspects: determination of catalyst oxidation state, evaluation of the role of the support, and identification of adsorbed intermediates. All three types of measurements can be carried out in situ. For instance, in situ Raman studies of V_2O_5 catalysts supported on TiO_2 or SnO_2 showed that, during the oxidation of methane to CO and CO_2 , the V^{5+} sites were partially reduced under steady-state conditions (300). Likewise, in situ Raman was used to obtain insight into the mechanism of O_2 reduction at iridium octaethylporphyrin- and iridium tetraphenylporphyrin-coated Au and carbon electrodes (301). UV resonance Raman has been applied by Li and Stair to a variety of catalytically relevant surfaces, including zeolites and sulfated zirconia (302–304). A description of the instrumentation they used and a comparison of ultraviolet and visible excitation have appeared (305). Using a confocal Raman microscope, reactive surface oxygen species on cerium oxide catalysts have been detected, and their reactivities with CH_4 and C_2H_6 have been determined as a function of temperature (306). Finally, the oxidation of ethanol on MoO_3 has been studied using a high-throughput Raman spectrometer; both catalytically active (Mo–O) and spectator (Mo–O–Mo) intermediates were observed (307).

Oxides, Glasses, Gels, and Clays. Traditionally, the stationary phases of greatest interest to analytical chemists have been chromatographic supports, and using the increased sensitivity available with modern Raman instrumentation, they can now be directly investigated. For example, Pemberton has characterized the chain conformations of octadecylsilane stationary phases in commercially available silica-based packing materials (308). Likewise, a Raman characterization of polyamines adsorbed onto silica gel has been reported (309). Resonance Raman has been used to probe chromophores interacting with acidic sites on untreated and calcined SiO_2 –alumina (310), allowing the density of Brønsted sites to be ascertained as a function of temperature. A variety of oxides (e.g., TeO_2 , RuO_2 , TiO_2 , $PbTiO_3$, and SiO_2) have been studied as glasses, sol–gels, xerogels, and aerogels using Raman (311–320). These studies have been useful not only to monitor phase transitions but also to detect defects and changes in metal oxidation state. Similar types of measurements have been carried out on molecular sieves, zeolites, and clays, with additional interest in the detection of species present in pores or cavities (321–329). These species can be of supramolecular dimensions, as in the detection of nanometer-sized Ag particles in glasses using waveguide Raman spectroscopy (330), or can be solvent, as in a Raman spectroscopic study of the water in gels and sols (331).

Molecules and Molecular Systems. Though H_2O is a weak Raman scatterer, the cross section for the 3400-cm^{-1} mode has been measured for excitation wavelengths between 215 and 550 nm (332). The structure of supercritical H_2O has been studied by Raman (333). Dissolved gases in H_2O have been detected using near-IR Raman spectroscopy (334), and the H_2O associated with Nafion membranes has been observed by Raman (335). In

the latter study, differences in the level of intermolecular hydrogen bonding were detected as a function of Nafion molecular weight. Raman spectroscopy has been widely used to study high-energy materials, both in the steady state, such as in the detection of contraband explosives and bombs (336–339), and in shocked solids or liquids, such as propellant formulations (340–343). For the former, the focus of the work is on rapid detection and/or classification, while for the latter, the interest is in time-resolved measurements during rapid changes in sample temperature and/or pressure.

Environmental Materials. Raman spectroscopy has been utilized as a noninvasive probe for a multitude of environmental chemistries. Spectroscopic monitoring has been performed on inorganic species in water (344), sulfates in brackish water (345), humic substances in various media (346), lignin in hardwood pulp samples (347), diesel particulate matter in coal mines (348), and gaseous pollutants in the atmosphere (349). Identification of minerals in lunar rocks (350) has been demonstrated with an in situ Raman probe. Screening has been carried out on pollutants in stack gases (351), organophosphorus pesticides in various media (352), mixed wastes in drums (353), and asbestos, transite, and concrete in hazardous waste sites (354). FT-Raman has been used to identify and quantify oxygenates in sealed gasoline ampules (355).

Archeological Materials. Analysis of archeological objects by Raman spectroscopy is a rapidly developing field. Characteristic Raman bands are used to identify various materials and detect degradation products of pigments and dyes. The noninvasive and nondestructive nature of Raman spectroscopy makes it an ideal probe for the composition of rare or invaluable objects.

Discrimination between blue textile dyes by Raman spectroscopy has been demonstrated (356). FT-Raman studies can differentiate between cotton fabric at different stages of production (357). Likewise, substances responsible for degrading ancient Egyptian linens have been determined by Raman spectroscopy (358). Confirmation of the authenticity of amber artifacts is possible through a comparison of their Raman spectra with those of modern pine resins (359). Identification of glaze materials in ancient Egyptian faience objects (360) and 13th century Italian pottery (361) has also been reported. Several studies have examined the pigments of medieval wall paintings (362–364), while FT-Raman has been used to study resins in American Indian artifacts (365) and “Dragon’s Blood” from Socotra island (366).

Numerous papers have been published that describe manuscript pigment structure and degradation. Identification of cinnabar in Chinese manuscripts (367) and vermilion, azurite, malachite, white lead, and lead tin yellow type I in medieval Latin and German manuscripts is reported (368, 369). Light-induced degradation of realgar (a soft, orange-red arsenic ore used as a pigment) to pararealgar has been detected in a Italian Renaissance masterpiece by Tintoretto (370).

Biological Materials. Raman is becoming an increasingly popular technique for biomedical applications such as the identification or characterization of biological materials. Applications concerning almost every part of human and animal anatomy have appeared. A confocal Raman microprobe study has revealed structural differences between the outer and inner regions of human hair (371). A study comparing untreated with bleached

human hair has shown that spectral changes occur upon cysteine oxidation to cysteic acid (372). Oxidation and reduction of protein disulfide bonds in hair has been studied by FT-Raman to monitor photobleaching processes (373). The molecular composition of human teeth has been mapped and identified along the enamel/dentin interface (374, 375). A study of 700-year-old and modern human teeth by FT-Raman spectroscopy compared collagen and dentin (376). The chemical interaction between 4-methacryloxyethyltrimellitic acid and enamel was investigated with a Raman microprobe (377).

Human and animal bones have been widely studied with Raman spectroscopy. The presence of heme-containing compounds in dinosaur bone was confirmed by resonance Raman analysis (378). FT-Raman studies have been used to date human bones (379) as well as to discriminate between genuine and imitation scrimshaw (380) and ivory (381, 382). Another FT-Raman study compared bull horns, cat claws, bird feather quills, and pheasant beaks with keratinous tissue, human nails, and callus (383). A comparison between sheep and human bone with synthetic hydroxyapatite showed little correlation in Raman spectra (384).

FT-Raman studies have elaborated differences in skin samples between modern humans and those obtained from a ca. 6000-year-old corpse preserved by ice encapsulation (385, 386) and from a 15th century mummy (387). Penetration of the human stratum corneum was examined by FT-Raman spectroscopy (388). Raman spectroscopy has also been used to study tissues from human breast (389, 390), human and bovine eyes (391, 392), brain (393), and hearts (394). Characterization of debris from wear on polyethylene knee replacement joints has been examined by Raman investigations of synovial fluid (395). A second study has identified a strong β -carotene signal associated with all polyethylene debris (396).

Polymers. Raman spectroscopy is an essential tool for characterization of the structure, environment, and dynamics of polymeric materials. The method's high sensitivity to polymer primary and secondary structure, as well as to solvation state, has allowed for detailed information to be obtained on a wide variety of "soft" materials. Furthermore, the portability of the technique allows for its use in on-line process monitoring.

Raman spectroscopy has great utility in the characterization of polymeric fibers because of low fluorescence background and the ability to obtain both second- and fourth-order orientation functions from polarized data (397). Thus, Raman spectroscopy was used to monitor the effects of firing temperature and polymer drawing during manufacturing (398). Cis/trans ratios in polymeric composites have been studied as a function of temperature (399). Polyethylene production has been monitored for amorphous-phase orientation and deformation in uniaxially drawn material (400, 401) and when peroxide-oxidized precursors are used (402). Similarly, the stress in a composite multifiber has been observed and related to manufacturing flaws, ultimately leading to design improvements (403). On-line Raman spectroscopy of crystallization has allowed for corrections to be made during polymer fabrication (404).

Use of Raman spectra to determine polymer conformation has been the subject of several publications. Raman has been used to observe conformational disorder in waxes (405), solvent

reorientation in polyacrylamide gels (406), and strain inhomogeneities in highly oriented gel-spun polyethylene (407) and for monitoring curing in divinyl ethers and epoxies (408–411). A wide range of side products in the formation of PMDA-ODA polyimide has been observed by FT-Raman with a near-IR detector (412). Iodine doping of natural rubber and pseudointerpenetrating polymer lead to fingerprint resonance Raman spectra of $>1000\text{ cm}^{-1}$ (413).

Several papers have explored polymer-based electrolytes. Changes in crystallinity were observed in polydioxane/LiClO₄ and NaClO₄ electrolytes (414). Chain conformational changes have been described for dilute polyacrylic electrolytes (415). Positional monitoring of Li ions in polyacrylonitrile electrolyte solutions has been achieved with Raman spectroscopy (416). Likewise, total ion association was examined in three polyether-based electrolyte systems (417).

Particles and Droplets. Size differences in crystalline samples and nanoclusters lead to significant shifts in Raman spectral intensity. In 1996, Lehnert and co-workers reported that Raman intensity increased with decreasing particle size (418). Corresponding results are obtained with PbTiO₃ ultrafine powders (419). Both theoretical and experimental examinations of Si nanocrystals relating particle size and Raman peak intensity have appeared (420, 421). Detection and size determination of Ag nanoclusters in soda-lime glass has been determined using waveguided Raman (422). Polarized Raman has been used to study the orientation of metallophthalocyanine monolayers on carbon surfaces (423) and of mordenite crystals in one-dimensional nanochannels (424).

Several groups have acquired Raman spectra from optically levitated single micrometer-sized droplets (425–431). This new experimental method can be used to monitor mass and energy transport within single particles (425). In these experiments, visible laser light is used for both optical levitation and Raman excitation. When the size of the drop is a multiple of the excitation wavelength, the incident field is said to be in resonance with the cavity (morphology-dependent resonances, MDRs), leading to (detectable) anomalies in scattering efficiencies (426). In comparison to bulk solvents, a dramatic enhancement in Raman scattering relative to fluorescence has been observed (427). Raman spectra were used to analyze the composition and determine the temperature of single aerosol particles containing environmentally significant species such as sulfate and nitrate (428). Using the Stokes/anti-Stokes ratios of the inert tracer CaF₂, temperatures of aqueous droplets could be measured down to $\sim 200\text{ K}$. Observation of MDRs both in Raman spectra and for elastically scattered light was used to monitor a phase transition in microdroplets containing glycerol, water, and ammonium sulfate (429). Two groups have studied photoinitiated polymerization within the droplet, using either visible (430) or ultraviolet (431) light to initiate the reaction, and Raman scattering to monitor its progress. Based on these promising results, we anticipate a significant increase in the use of levitated microdroplets in analytical chemistry.

RAMAN SCATTERING AT SURFACES

Surface Raman scattering studies can be divided into two general categories: those that involve significant ($= 10^3$) surface

enhancement from a roughened noble metal substrate and those that do not. This section will discuss the former first, as they are more numerous.

Surface-enhanced Raman scattering, like resonance Raman, provides a means of significantly improving signal-to-noise ratios in Raman experiments. However, because the SERS effect is dependent on the close proximity of analyte molecules to roughened noble metal surfaces, and because the active sites, enhancement factors, and exact enhancement mechanism(s) in any given experiment cannot always be predicted beforehand, this technique has not yet become industrially important or commercially viable. While the majority of SERS publications still deal with mechanism elucidation or substrate development, an increasing number describe analytical applications. For example, recent reports of single-molecule SERS allow the technique to begin to challenge fluorescence methods for low detection limit applications, particularly since SERS has the added capability to provide vibrational information. Although SERS remains less reliable than fluorescence for the present, it appears that advances in substrate reproducibility should soon overcome this obstacle.

SERS Substrate Development/Characterization. SERS substrate development remains an active area of research, with many publications devoted to descriptions of novel substrates as well as to investigations of more conventional substrates. The goal of most SERS substrate development work has been production of inexpensive, rugged, reproducible surfaces for analytical applications. Harris and co-workers have prepared Ag island films coated with 50–65-Å SiO₂ and shown them to be both SERS-active and good models for adsorption studies at SiO₂ (432). Maya et al. have prepared sputtered Au and Au/SiO₂ films as simple, rugged SERS substrates (433). Another approach to SERS has been adsorption of analyte onto AgCl, which is reduced to Ag particles for NIR-SERS detection of analyte (434). SERS-active microelectrode arrays have been prepared by electroplating of Ag onto Ir or Pt microelectrodes (435, 436). Polymeric substrates prepared by layering Ag particles onto etched poly(ethylene terephthalate) were shown to yield enhancement factors of 10⁴–10⁵, with <20% variability (437). Laser ablation has been used to prepare adsorbate-free Ag sols from Ag foils in H₂O (438). High-temperature annealing of thick Ag films has been shown to produce stable films with oriented ellipsoidal surface features, giving rise to angle-dependent absorbance and good electromagnetic enhancements (439). Other novel substrates include Ag₂O colloids (440), alkali metal colloids (441), Ag films formed by a plasma oxidation/reduction process (442), and novel electrode treatments (443, 444).

Recently, SERS substrates having a very narrow distribution of roughness feature size have been described. Highly ordered periodic arrays of Ag features have been prepared by nanosphere lithography, in which Ag is evaporated onto preformed arrays of nanospheres which are then removed, leaving behind the Ag particles formed in the interstices (445). SERS substrates have also been prepared by self-assembly of monodisperse colloidal Au and colloidal Ag particles from solution onto functionalized glass or metal substrates (446–450). Kinetic control of interparticle spacing for Au colloid monolayers (451) and combinatorial optimization of Ag-coated Au substrates (452) have been described. Advantages of colloid monolayer SERS substrates are

high reproducibility, ease of preparation, and low cost (453). In addition, this solution-based approach allows preparation of SERS substrates in any geometry (454).

Ag coatings have been chemically reduced onto Au colloidal particles in solution (455) and on Au colloid monolayers on glass substrates (456) to increase the SERS enhancement of the more reproducible yet less-enhancing colloidal Au substrates. Ag has also been photoreduced onto TiO₂ surfaces, to produce SERS-active films with low background signal (457), and electrochemically reduced onto nonferrous metals and alloys (458).

Matrixes for SERS. Several recent reports discuss the modification of thin-layer chromatography (TLC) plates for use as SERS substrates (459–464). Szabo and Winefordner have compared commercially available Ag-coated TLC plates with plates coated by chemical reduction and have performed a series of experiments to evaluate substrate performance for separation and detection (460). Matejka et al. have compared SERS activity of TLC plates upon which Ag particles were grown under a variety of conditions (459). Raman microscopy has been used in conjunction with Ag colloid-treated TLC plates for the detection of impurities in certified dye standards (461). Multichannel SERS on TLC plates has been coupled to liquid chromatography to identify separated dyes at the tens of nanograms per milliliter level (463). The ease of preparation of these substrates, coupled with the ability to do separation on the same substrate, make them attractive to analytical chemists; however, their relatively low enhancements and high variability (10–30%) (460) are substantial concerns.

Several methods have been introduced to improve SERS substrate stability. The effectiveness of various organothioli monolayers for improving the stability of Ag SERS substrates has been investigated, where minimal degradation was observed for surfaces stored in water for a month (465). Alumina-based substrates have been coated with poly(vinylpyrrolidone), which provides protection to the substrate as well as selectivity for those molecules that can permeate the polymer film (466). Murphy et al. have described two sol-gel methods to increase substrate mechanical stability (467). In the first, colloidal Au is encapsulated within a porous thin film; the second involves coating Au atop a surface composed of aluminum oxide particles (467). In a separate study, Au particles have been encapsulated in a porous glass matrix and characterized for use as SERS substrates (468).

Characterization of SERS Substrates. Although colloidal Ag particles and Ag films have been employed as substrates for SERS for over a decade, much attention remains devoted to understanding and improving the nanoscopic optical properties and morphology of these substrates. Rowlen and co-workers have compared the sensitivity, reproducibility, stability, and ease of preparation for five SERS substrates (469). This group has also conducted a quantitative study of Ag film morphology using transmission electron microscopy and atomic force microscopy (470). Munro et al. have published a careful study of Ag colloid optimization for use in resonant-SERS studies of negatively charged dye molecules (471). Similar colloid preparations were used in studies of submonolayer reactants at the Ag surface (472). Activation of colloidal Ag sols by various anions has been studied by optical spectroscopy, TEM, and wavelength-dependent SERS, leading to the optimization of this system for near-IR excitation

(473). Morphological studies of Ag electrodes (474) and adsorbate–Ag colloid films (475) have also been carried out, as has a study of absorbance/reflectivity of SERS-active Ag electrodes (476). Carbon contamination of Ag electrodes caused by cleaning the electrodes has been investigated (477). Gadenne and co-workers have used SERS to identify the percolation transition in studies of thin Ag films (478). Zhu et al. used SERS mapping of azobenzene monolayers to compare differently prepared Au substrates (479).

The combination of SERS with various microscopic techniques has led to simultaneous optical and morphological characterization of substrates. Moskovits and co-workers have used micro-Raman to study single adsorbate-coated aggregates of colloidal Ag as a function of cluster size and excitation wavelength (480, 481). This group has also investigated Ag films at higher resolution by coupling SERS with scanning tunneling microscopy (STM) (482). Sun and co-workers have used STM/SERS to measure scattering from single Ag features created with the STM tip on flat Au(111) (483). Near-field optical microscopy (NSOM) has been used to image analytes on Ag substrates (484). SERS of analytes adsorbed to single colloidal Ag nanoparticles on a glass substrate has been observed by Emory and Nie using near-field SERS; they found that a small fraction of the particles in a polydisperse sample were responsible for nearly all of the enhancement (485). These authors have also reported SERS of single rhodamine 6G (R6G) molecules and claimed enhancement factors of 10^{14} – 10^{15} at individual highly enhancing Ag nanoparticles (486).

SERS Methods. Several methods of deposition of Ag particles onto materials that are not enhancing for SERS have been used to acquire SERS of molecules adsorbed to the underlying material. Penner and co-workers have electrochemically deposited Ag nanocrystallites onto etched graphite surfaces to enhance scattering from graphite defect modes (487). Ag has been evaporated onto Si, GaAs, and InP surfaces for investigation of adsorbates on these semiconductors (488, 489). Evaporation of Ag onto diamond films has been performed in situ in order to investigate films formed by hot-filament assisted chemical vapor deposition of CH_4 and H_2 (490). Single-molecule detection via SERS has been reported for R6G and crystal violet adsorbed to Ag sols (491, 492). In these studies, extremely low analyte concentrations combined with a small spot size allowed for detection of an average of 0.6 molecule per unit sampling volume.

Several papers focused on combining SERS with other analytical techniques such as gas chromatography (493), thin-layer chromatography (464), and flow injection analysis (494) have appeared. Carron and co-workers described a new gas chromatography detector composed of SERS substrates coated with 1-propanethiol; this layer served to concentrate organic analytes at the surface (493). Horvath and co-workers developed a method for drug analysis by separating a mixture via TLC, followed by SERS detection from the chromatography plate (464). The analysis of organic pollutants in water using flow injection analysis coupled with SERS has also been proposed (494).

Futamata has reported a method for Raman band intensity enhancement through prism-coupled surface plasmon polariton excitation (495). An increased enhancement from weak Raman signals of adsorbates on Au, Cu, and Ag was observed (496). Futamata has also described SERS from roughened Ag surfaces

where scattering is excited by prism coupling into the surface plasmon mode (497). Other advances in SERS methodology include a NIR/FT-SERS microspectrophotometer with micrometer-scale resolution (498) potential-averaged SERS for studies at extreme electrode potentials (499) and a new cell for in situ spectroelectrochemistry (500).

Applications of SERS. Sensors utilizing SERS detection have been developed for a wide range of analytes, including organic molecules in aqueous solutions (501), nerve agents (502), and molecules of environmental importance (467, 503, 504). A SERS-based enzyme-linked sandwich immunoassay has also been developed (505), whereby the reaction of a peroxidase with *o*-phenyldiamine and hydrogen peroxide yielded azoaniline which was then detected at a SERS substrate. Subpicogram quantities of the explosive TNT have been detected by SERS at colloidal Ag and Au (506). In a forensic science application, dye-modified cotton fibers have also been studied by SERS (507).

Various organized monolayers and multilayers on metal substrates have been studied by Raman. Incorporation of anthracyclines into lipid mono- and bilayers has been investigated by Bolard and co-workers (508, 509). SERS has also been proposed as a method for investigation of drug transport through model or biological membranes by measurement of arrival times at the surface (510). Chamberlain and Pemberton have electrochemically grown colloidal Ag underneath lipid monolayers at the air/water interface to enable SERS investigation of the monolayers (511), and SERS has been used to study submonolayers of polyaromatic hydrocarbons (512).

Ag was evaporated onto a thin film of 4-(*N,N*-dimethylamino)-3-acetamidonitrobenzene to facilitate acquisition of Raman data (513). SERS was used in the study of carbon steel corrosion occurring in CO_2 -saturated brine solutions (514) and to monitor the properties of thin iron oxide films (515). The structure of a polymer/metal interface has been characterized to improve understanding of polymer adhesion (516). Carron and co-workers have used SERS to investigate UV photooxidation of alkanethiols on Ag surfaces (517), while Yang et al. have performed SERS imaging on a UV-photopatterned *p*-nitrophenol SAM (518) and a SAM monolayer patterned by microcontact printing (519).

Adsorption of molecules onto electrode surfaces has been studied by SERS, taking advantage of simultaneous spectral acquisition and potential control. Hatchett and co-workers have investigated the electrochemical adsorption of ethanethiolate on Ag electrodes (520), while others have used SERS to study the adsorption of Ru and Os poly(vinylpyridine) (521). SERS has also been used to characterize thin films of 1,8-diaminonaphthalene on Ag, Au, and Cu electrodes (522). The oxidation of aryl sulfonates and aryl sulfonates (523), films of $\{fac\text{-Re}(2,2'\text{-bipyridine})\text{-}(\text{CO})_3(\text{NC}_5\text{H}_4)\text{CH}_2\text{NHCO}(\text{CH}_2)_2\text{S-}\}_2$ (524), and ω -functionalized self-assembled diacetylenic and polydiacetylenic monolayers has also been studied by electrochemical SERS (525).

In the field of catalysis, SERS has been used in conjunction with mass spectrometry to study the reduction of NO by CO on Pt and Pd substrates (526). Adsorbed CO and thiocyanate at activated Pt surfaces have been investigated by confocal Raman microscopy (527). A ternary catalyst ($\text{Cu}/\text{ZnO}/\text{Al}_2\text{O}_3$) has been studied with respect to structure and gas adsorption (528). Several papers focused on rhodium as the catalytic substrate for

the study of NO reduction (529), adsorption and hydrogenation of CO (530), and methanol oxidation (531).

There have been some recent SERS studies of humic substances adsorbed to noble metal substrates. Francioso and co-workers studied different fractions of Irish peat (532). A variety of SERS substrates have been proposed for studies of humic acid, including Ag sols (533, 534), Au electrodes (535), and silver-nitrate modified Fe–C–Cr–Ni surfaces (536). A SERS-active sample has also been prepared in the form of a pellet prepared from an aqueous mixture of humic acid and silver powder (537).

Cytochrome *c* continues to be a popular protein for resonant SERS (SERRS) investigations due to its intense heme absorbance and good adsorption characteristics. The orientation of this protein at colloidal Ag surfaces has been investigated by Macdonald and Smith under a variety of conditions (538). Maeda et al. have studied the effect of chain length upon SERRS of cytochrome *c* separated from the Ag surface by monolayers of carboxy-terminated alkanethiols (539). Halide-modified Ag sols prepared by laser ablation were found to reduce adsorbed cytochrome *c* (540). Vilker and co-workers have reported the first nonresonant SERS spectrum for cytochrome *c* at an Ag electrode (541). The same group has also reported on charge transfer between putidaredoxin and an Ag electrode (542). Photochemical charge transfer between flavin mononucleotide and an Ag electrode has been investigated by time-resolved SERS (543).

Resonant SERS at Ag substrates has also been used to investigate the metal coordination sphere of cobalamins (544) and binding of NO and inhibitors/fatty acids to cytochrome P-450 (545, 546) and to identify chlorophyll *a* allomers (547). SERRS and resonance Raman have been used to examine the structure and redox states of cytochrome *c*₃ adsorbed to Ag substrates (548).

SERS is becoming an increasingly popular method for investigation of drug/ nucleic acid interactions. Manfait and co-workers have studied binding of acridine drugs to DNA and topoisomerase II (549). This group has also applied SERS microscopy to the study of antitumor drug action within living cancer cells (550). SERS has been used to identify covalent adducts between another antitumor drug [*N*(2)-methyl-9-hydroxyellipticinium] and amino acids and nucleic acids (551). Based on SERS investigations, Sanchez-Cortes et al. have proposed a model of binding between the antiretroviral drug hypericin and DNA (552). SERS studies of other antitumor drugs and antimicrobial agents have also been reported (553–557). In addition, the structure of adsorbed RNA triple helices has been investigated (558).

SERS Theory and Experimental Tests of Theory. SERS theory has continued to be an active research area in recent years, but is largely beyond the scope of this review; thus, only a few representative papers will be noted here. A discrete dipole approximation has been used to calculate the optical properties of small particles with arbitrary shapes (559). Linear chains of Ag spheres have been modeled with varying interparticle spacing and chain length (560). The effect of temperature on SERS has also been investigated by theory (561). In experimental tests of SERS electromagnetic enhancement theory, Sun and co-workers used Raman probes attached to alkanethiol SAMs to investigate the effect of analyte–surface separation upon enhancement factor; varying the chain length of the SAM molecule, they found

measured enhancement factors varied exponentially with distance from the metal surface (562, 563).

Chemically Enhanced and Unenhanced Raman. Campion has reported SERS from the first surface in which chemical enhancement exceeded electromagnetic enhancement. The molecules (pyromellitic dianhydride) dissociatively chemisorbed onto the Cu(111) surface in ultrahigh vacuum and exhibited an electronic absorbance feature which could be resonantly excited (564). Related papers by this group discuss the role of charge-transfer resonances in unenhanced surface Raman scattering (565) and the mechanism of chemical and electromagnetic SERS effects for molecules at single-crystal metal surfaces (566, 567). Chemical enhancement and charge-transfer effects in SERS have also been investigated for analytes at Au(210) surfaces (568) and at roughened Au and Ag electrodes (569, 570). The chemical enhancement for pyridine SERS on colloidal Ag afforded by coadsorption of several inorganic anions has been reported (571). Liu and McCreery have used Raman spectroscopy to study reactions of nitrophenyl groups covalently bound to carbon electrodes (572). Unexpectedly intense Raman scattering from azobenzenes bound to carbon electrodes have been observed by Liu and McCreery and have been attributed to resonant excitation of a surface–analyte chromophore (573).

NONLINEAR RAMAN TECHNIQUES

For the most part, the growing number of nonlinear Raman techniques remain in the realm of physical chemists or chemical physicists. However, some methods, and in particular coherent anti-Stokes Raman (CARS), are making progress as analytical tools for real-world chemistry problems.

CARS has been used in investigations of jet diffusion flames (574, 575) and to gain evidence for the wall association of radicals in expanding thermal arc plasmas (576). Baxter et al. have described a novel optical paramagnetic oscillator source for CARS of combustion processes (577). CARS has been used to acquire time-resolved vibrational spectra of shocked polycrystalline materials (578). Schmitt et al. have applied femtosecond CARS to ground- and excited-state dynamics of iodine vapor (579). Picosecond time-resolved resonance CARS has been used in studies of the bacteriorhodopsin and bathorhodopsin photocycles (580, 581). Resonance CARS has also been applied to aggregation and linker studies in biliproteins (582).

Kanger et al. have measured CARS spectra for analytes adsorbed to waveguides and determined the third-order polarizabilities of the waveguides (583). Electronically excited sodium atoms have been investigated by a combination of CARS, resonant CARS, and degenerative four-wave mixing (584). Secondary structure determination of bovine albumin by polarization-sensitive multiplex CARS has been performed and compares well with conventional Raman and circular dichroism estimates (585).

A number of other specialized techniques have been developed. A nonlinear version of vibrational optical activity has been described (586). Resonance hyper-Raman scattering and second harmonic scattering have been applied to studies of CdS and CuBr quantum dots (587–589). Characterization of micrometer-scale droplets has been performed using stimulated Raman scattering (SRS) (590). Quasi-continuous-wave SRS has been used to determine methane concentrations at atmospheric pressure (591).

Kneipp et al. have observed excited vibrational state pumping by spontaneous surface-enhanced Raman scattering (592). A 2-D Raman spectroscopic method using nonresonant fifth-order Raman has also been developed and applied to the study of the intermolecular modes of CS₂ (593).

BIOLOGICAL APPLICATIONS OF RAMAN

Raman spectroscopy continues to be used widely as a probe of structure and function in biochemical systems and as a quantitative technique for biomarkers. Motivation for its use is driven by difficulties associated with acquisition of infrared vibrational data for water-soluble species, by enhanced availability of ultraviolet and near-infrared excitation sources (where fluorescence interference is minimized), and by the increased realization that unique, fingerprint-like spectra can be obtained for species as small as low-molecular-weight metabolites and as large as living organisms. This section is divided into four parts. Three of the four parts focus on structure/function issues for proteins, nucleic acids, and lipids, respectively, and the final part is devoted to bioanalytical and/or diagnostic applications. It should be noted that the full gamut of Raman experimental methodologies has been utilized by the biological community, including Raman microscopy, Raman difference spectroscopy, ultraviolet-, visible-, and near-infrared-resonance Raman, surface-enhanced Raman scattering (see above), polarized Raman, and vibrational Raman optical activity.

Proteins. Several different approaches have been used to probe protein secondary, tertiary, and quaternary structure. A common feature in these experiments is the presence of conformationally and/or environmentally sensitive Raman bands. For example, amides exhibit five conformationally sensitive vibrational bands; good agreement is found between experimental Raman and IR spectra of L,D-dipeptides and calculated normal modes (594). Likewise, it was shown that binding of the drug camptothecin to human serum albumin induced local structural modifications involving changes in the configuration of two disulfide bonds and movement of a tryptophan residue into a hydrophilic environment (595). The water accessibility to each of the four tryptophan (Trp) indole N-H sites in the gramicidin A transmembrane channel was determined by Raman analysis of polypeptides with selectively deuterated Trp residues (596). Binding of avidin to biotin leads to a intensity increase in the Trp 1360-cm⁻¹ mode, due to an increased hydrophobicity, suggesting that Trp interacts directly with biotin (597). Tryptophan environments and orientations were also used to understand interactions between α and β subunits of a telomere-binding protein (598). Likewise, a study of interactions between lysozyme and whey proteins indicated the presence of hydrophobic interactions, as evidenced by intensification of spectral bands assigned to CH and CH₂ bending vibrations, by changes in disulfide vibrational frequency, and through a lowering in α -helix and β -sheet content (599). UV resonance Raman has been used to probe the solution conformation of the extracellular domain of human tumor necrosis factor (600) and to follow isomerization dynamics of amides and peptides (601).

Techniques such as vibrational Raman optical activity (ROA) (602, 603) and polarized Raman also furnish valuable information about protein structure. ROA studies of poly(L-lysine) in H₂O and

D₂O have provided insight into conformational elements present (e.g., rigid loop, α helix, β strand, and left-hand helix) (604). Accordingly, ROA can be used to follow protein folding and/or unfolding (605, 606). Polarized Raman microspectroscopy has provided information about subunit orientation in filamentous viruses (607), as well as data on Trp orientations within subunits (608).

Two approaches are widely used to generate information about catalytically active sites within proteins: Raman difference spectroscopy and, when a chromophore is present, resonance Raman spectroscopy. In the former, careful subtraction of spectra obtained under different conditions yields information about protein residues undergoing structural or environmental changes. For example, pH-dependent conformational changes in *Escherichia coli* dihydrofolate reductase (609) and acylated intermediates of selenosubtilisin (610) have been detected by Raman difference spectroscopy. The latter technique, resonance Raman, is so widely used by biological chemists that discussion in any detail is beyond the scope of this review. It should suffice to point out that rR allows selective interrogation of chromophores within (or associated with) biological macromolecules. Since the typical chromophores in proteins—coordinated metal ions, flavins, porphyrins, etc.—are quite often involved in protein function, be it transport, catalysis, or redox chemistry, rR is an indispensable tool. For the sake of brevity, we cite only a few recent rR studies of metalloproteins (611–617) and of proteins containing other cofactors (618, 619).

Nucleic Acids. Nucleic acid structure and function has been studied by Raman with increasing frequency, from both fundamental and applied perspectives. For instance, it has been shown that Raman depolarization ratios for synthetic DNA and RNA oligonucleotides are sensitive to structure. Specifically, the depolarization ratios for adenine vibrations in poly(dA) and poly(dA)poly(dT) are different, indicating different degrees of sugar pucker (620). A recent paper describes the first use of ROA as a sensitive structural probe for both single-stranded and double-stranded polyribonucleotides (621). Raman tensors for localized base vibrations have been measured for oriented fibers of double-stranded RNA, enabling the use of polarized Raman spectroscopy to determine purine and pyrimidine base residue orientations in ribonucleoprotein assemblies (622). This work may ultimately lead to the use of Raman as a probe of base pairing and base stacking.

Raman spectroscopy has been used to directly observe structural features of a single-stranded helix (623); the use of UV-resonant excitation allowed dA and dG residues to be selectively interrogated. Another unusual structure, a four-stranded cytosine intercalation motif, gives a unique Raman signature that allows the extent of cytosine quadruplex formation to be quantitated (624). A final representative fundamental study involves a combination of differential scanning calorimetry and Raman spectroscopy and has allowed identification of features in the Raman spectrum of B-form double-stranded DNA that are reliable quantitative indicators of DNA melting (625).

More applied work involving nucleic acids has included the use of Raman as a probe of drug/DNA interactions [e.g., diethylstilbestrol (626), vitamin C (627), aspirin (628), camptothecins (629), and cinchonine (630)], using UV, visible, and near-

IR excitation. Likewise, the interactions between dipyridophenazine (dppz) complexes of Ru²⁺ and Os²⁺ [specifically, Ru(phen)₂(dppz)]²⁺ and DNA have been studied by resonance Raman (631, 632). A band that could be uniquely ascribed to intercalated complexes was identified (631). A final set of applications involves work by the Thomas group on protein–nucleic acid complexes (633, 634). In these papers, specific Raman markers were identified either for complexed DNA or complexed proteins. Specific and nonspecific nucleic acid recognition sites were distinguishable, as were amino acids involved in viral assembly.

Lipids. Most of the Raman papers involving lipids have focused on other molecules (e.g., proteins and peptides) located within lipid assemblies. Fewer publications have dealt with spectroscopy of the lipid molecules themselves; several representative articles are mentioned here. Raman microspectroscopy has been used for in situ characterization of cholesterol crystals in artery endothelial cells (635). The structure of lipid–cholesterol vesicles as a function of pressure has been investigated using Raman frequencies, band shapes, and splittings to characterize conformer population, reorientational fluctuations, acyl chain interactions, and phase transitions in the lipid bilayers (636). Carotenoid probe molecules have been added to model membranes of soybean galactolipids to report on the lipid environment (637). Lipid Raman bands from cytotoxic granules in living human killer T lymphocytes were shown to be much stronger than protein bands, suggesting that lipid was a principle component of the granules (638). To aid in the analysis of granule Raman spectra, Takai et al. have examined the Raman spectra for triacylglycerols having different degrees of acyl chain; this information was used to determine that the lipids found in cytotoxic granules differed substantially in unsaturation from those comprising lymphocyte plasma membranes (638).

Applications/Diagnostics. There appears to have been an exponential increase in medically related (i.e., diagnostic) applications of Raman. The rapidity with which data can be acquired and then analyzed with “trained” software, coupled with the ability to manufacture extremely small probes suitable for in vivo work, appears to be a powerful combination for disease detection and identification. Preliminary work toward development of a fiber-optic pulsed UV-resonance Raman probe for real-time monitoring of neurotransmitters in the brain has appeared; this probe has been used to measure Raman spectra from neurotransmitters in vitro (639). A related study describes use of such a probe to measure UV Raman spectra for sex steroids, analogues, and components of human urine (640). The neurotoxin domoic acid has been detected at concentrations below the range commonly found in toxic phytoplankton cells (641). To avoid interference from fluorescence, Dou et al. have used anti-Stokes Raman to determine the concentration of glucose, acetone, and urea in urine samples (642). Longer excitation wavelengths are important for characterization of analytes in biological matrixes such as skin or blood, which absorb much of the visible spectrum but are transparent in the near-infrared. Accordingly, Feld and co-workers have determined concentrations of glucose, lactic acid, and creatinine in aqueous solutions using near-IR Raman (643). This group has gone on to apply near-IR Raman to measure glucose and other blood constituents in human whole blood (644, 645).

The feasibility of using Raman to distinguish between several species of fungi based on differences in their cell wall structures has been shown (646). Raman microspectroscopy has been utilized in studies comparing lymphocyte carotenoid levels in healthy individuals and cancer patients (647).

Near-IR Raman scattering has been used to characterize human coronary arteries and shows promise as a rapid intravascular diagnostic tool for arterial disease (648). Micro-Raman imaging of filipin and phenylalanine distribution in human eye lenses has been described (649). Filipin, which binds specifically to cholesterol, has also been used as an extrinsic Raman probe molecule for determination of cholesterol distributions within rat eye lenses (650).

Several research efforts have been aimed at developing Raman as a noninvasive method for detection of abnormal cells or tissue. For example, Raman imaging of breast tissue has been coupled with multivariate image analysis as a first step toward noninvasive detection and classification of breast cancers (651). Frank et al. were able to distinguish normal, benign, and malignant breast biopsy samples based upon Raman spectra (652). Near-IR FT-Raman spectra of various skin lesions (including benign and malignant cancers) have been compared; although related lesions gave similar spectra, reproducible and unique spectra were associated with each lesion type (653). In a similar study, NIR/FT-Raman was used to study basal cell carcinoma, the most common variety of skin cancer. Neural network analysis was carried out on the resulting spectra, which principally comprised protein-based amides I and III, $\nu(\text{C}-\text{C})$ stretching bands, and characteristic lipid bands (CH_2 scissoring and in-plane twist vibrations) (654). In each of these investigations of abnormal tissue, Raman has been shown to be a promising diagnostic tool.

PROSPECTUS

Several factors are combining to make the future of Raman extraordinarily promising. (i) Recent advances in instrumentation (e.g., compact diode lasers) have been coupled with previous innovations (e.g., CCD detectors) to make low-cost and portable instruments a reality. (ii) Recent publication of extensive Raman spectral databases will raise Raman closer to the status of IR for characterization of organic compounds. (iii) As real-world applications of new materials appear, the use of Raman as a characterization tool will become pervasive. (iv) New types of substrates for SERS appear poised to surmount the barrier to large-scale, reproducible fabrication, opening the door to industrial applications. (v) New data demonstrating single-molecule detection by SERS have offered the first real challenge to fluorescence in bioanalytical chemistry. Finally (vi), the use of Raman in medical diagnostics, while still in its infancy, has the potential to move this technique out of laboratory and into the clinic and the hospital. Future reviews of this topic will reveal the extent to which the promise of Raman spectroscopy has been fulfilled.

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