Inelastic Electron Tunneling Spectroscopy

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Summary. Inelastic electron tunneling spectroscopy is a useful technique for the study of vibrational modes of molecules adsorbed on the surface of oxide layers in a metal-insulator-metal tunnel junction. The technique involves studying the effects of adsorbed molecules on the tunneling spectrum of such junctions. The data give useful information about the structure, bonding, and orientation of adsorbed molecules. One of the major advantages of inelastic electron tunneling spectroscopy is its sensitivity. It is capable of detecting on the order of 10^{10} molecules (a fraction of a monolayer) on a 1-square-millimeter junction. It has been successfully used in studies of catalysis, biology, trace impurity detection, and electronic excitations. Because of its high sensitivity, this technique shows great promise in the area of solid-state electronic chemical sensing.

Inelastic electron tunneling spectroscopy (IETS) is a technique which has shown great sensitivity and resolution in studies of the vibrational spectra of molecules adsorbed on the surface of a metal oxide in a metal-insulator-metal tunnel junction (*1*). The physical process underlying this spectroscopy is the inelastic interaction of a tunneling electron with an adsorbed molecule. The spectra can be used to identify the chemical species on the oxide surface and even to determine their orientation. This technique is also becoming a powerful tool for the study of surface and interface phenomena and has been applied in the following areas: (i) surface chemistry and heterogeneous catalysis, (ii) biology and surface physics, (iii) trace impurity detection, (iv) metal-molecule interaction, and (v) adhesion. The recent development of the infusion method, whereby molecular species can be introduced into fabricated tunnel junctions, has opened up the possibilities for new surface chemical studies with inelastic electron tunneling. In addition to its applications in surface studies, electron tunneling has proved extremely valuable for the study of the electronic properties of thin metallic films and tunable light-emitting thin-film devices.

Inelastic electron tunneling spectroscopy is based on the use of a solid-state device called a tunnel junction. This can be regarded as a capacitor which consists of two electronically conducting materials (such as metals or semiconductors) separated by an ultrathin insulating layer (~ 10 to 30 Å). Electron tunneling is a quantum mechanical phenomenon. The wave function of an electron from one metal can extend into the insulating layer and overlap the wave function of an electron from the other metal. Because of the overlap, electrons can tunnel through the insulator from one metal electrode to the other. This occurs either without loss of energy (elastic tunneling) or with loss of energy to elementary excitations of the molecules adsorbed on the insulating layer (inelastic tunneling). Elastic electron tunneling is the definitive technique for determining the energy gap and density of states of a superconductor (*2*). This article concerns the technique and applications of IETS. It is designed to introduce IETS to a broader scientific community and to stimulate new applications of inelastic electron tunneling in the area of chemical sensing. In the past few years several good review articles (*3*, *4*), a conference proceedings (*5*), and an excellent book (*6*) have been published on IETS.

Infrared spectroscopy, Raman spectroscopy, and electron energy loss spectroscopy are a few other techniques for measuring the vibrational spectra of adsorbed species. Infrared spectroscopy has been used most widely. It does not require cryogenic temperatures, and commercial instrumentation is readily available. However, it has lower sensitivity than the other techniques and covers a limited spectral range. Electron energy loss spectroscopy is a relatively new method which essentially involves measuring the energy loss of low-energy electrons (typically ~ 5 eV) to the vibrations of adsorbed molecules. Although it has the advantages that the molecules are studied in situ on metal surfaces and there is no top metal electrode to shift vibrational modes, it has poor resolution and charging effects make it difficult to apply to adsorption on insulating surfaces.

Quantum Mechanical Electron Tunneling

The process of electron tunneling between metallic electrodes when a voltage, V, is applied between them can be visualized in terms of a rather simple physical picture. In an energy level diagram, the two Fermi levels of the metals are separated by an energy eV (Fig. 1a). According to energy conservation, the electrons from metal I are capable of making horizontal transitions into empty states in metal II. This is an elastic tunneling process and the electron does not lose any energy. To a first approximation, the current-voltage characteristic of elastic tunneling is linear.

Alternatively, the tunneling electron from metal I can give some of its energy to a local impurity state or molecular vibrational excitation and can still find empty states in the second metal electrode. This is an inelastic electron tunneling process. It occurs only when $eV > h\nu$ ($h\nu$ is the vibrational energy of an adsorbed molecule) because the tunneling electron from metal I cannot lose an amount of energy $h\nu$ and still tunnel into an empty state of metal II. The threshold voltage for such an inelastic event is equal to $h\nu/e$, where h is Planck's constant, ν is the vibrational frequency of the adsorbed molecules, and e is the electronic charge. There is a different threshold voltage for each vibrational mode of the molecule.

A typical current-voltage characteristic of a metal-insulator-metal junction, when a variable bias is applied across it, is shown in Fig. 1b. The tunneling current has contributions from both elastic

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and inelastic processes. The onset of an inelastic tunneling process causes an increase in the conductance at the threshold voltage. It is observed that only a small fraction of the electrons tunnel inelastically. In the derivative curve (Fig. 1c) there is a small step of the order of 0.1 to 1 percent of the conductance at that point, and thus this effect cannot be easily seen. But when the second derivative of the current with respect to the voltage, d^2I/dV^2, is plotted against the bias voltage (Fig. 2d), sharp peaks appear. That is why, in practice, one always records second derivative curves. The second derivative curves are analogous to the infrared absorption spectra, and hence it is convenient to compare them with infrared data. The peaks in the second derivative curves correspond to the energies associated with the various vibrational levels of the molecular species adsorbed on the oxide layers. The positions of the peaks give information about the structure and bonding of the molecules, and their amplitudes may give useful information about the orientation of the molecules on the oxide surface of the metal-insulator-metal tunnel junctions. Since a typical organic molecule has a large number of vibrational modes, there are a large number of peaks in an actual inelastic tunneling spectrum.

Theoretical Background

The theory of IETS must predict the positions, line widths, and intensities of the vibrational peaks in the tunneling spectra. The peak position, $V = h\nu/e$, is based only on energy conservation and is independent of the details of the electron-molecule interaction. However, the line shape and intensity depend on the electron-molecule interaction and are much more difficult to predict. Soon after the discovery of IETS by Jaklevic and Lambe (*1*), Scalapino and Marcus (*7*) developed a theory which included a dipole potential of the molecule and its image in the tunneling barrier and calculated the excess tunneling current due to this potential for the infrared-active modes. Their theory predicted the orders of magnitude of the peak intensities for the infrared-active vibrational modes. They also predicted that the interaction would be strongly dependent on the orientation of the molecule in the tunnel junction. They found that the tunneling electron couples strongly to the dipole oscillating perpendicular rather than to the dipole oscillating parallel to the oxide layer. Lambe and Jaklevic (*8*) extended the theory of Scalapino and Marcus (*7*) to include the effects of the interaction of the tunneling electron with the induced dipole moment of the molecule through the bond polarizability, and this theory works well for calculating the orders of magnitude of the peak intensities.

Ability to resolve the peaks in the tunneling spectra is an extremely important consideration in IETS, as in any other spectroscopy. For normal metal electrodes, simple theoretical considerations show that the resolving power of IETS has a fundamental limitation of 5.4 kT, where k is Boltzmann's constant and T is absolute temperature (*8*). At room temperature the line width is approximately 0.15 V, and therefore it is almost impossible to observe the tunneling spectra at room temperature because the excitations occur over a range of 0.1 to 0.5 V. At 77 K the lines are easily observed but not well resolved, and at 4.2 K the line width is ~ 2 mV and the natural width of most solid-state molecular lines is seen. The best resolution is obtained with superconducting electrodes at low temperatures (*8*). For a more detailed account of the theoretical developments, the reader is referred to an excellent review by J. Kirtley [in (*5*), p. 80].

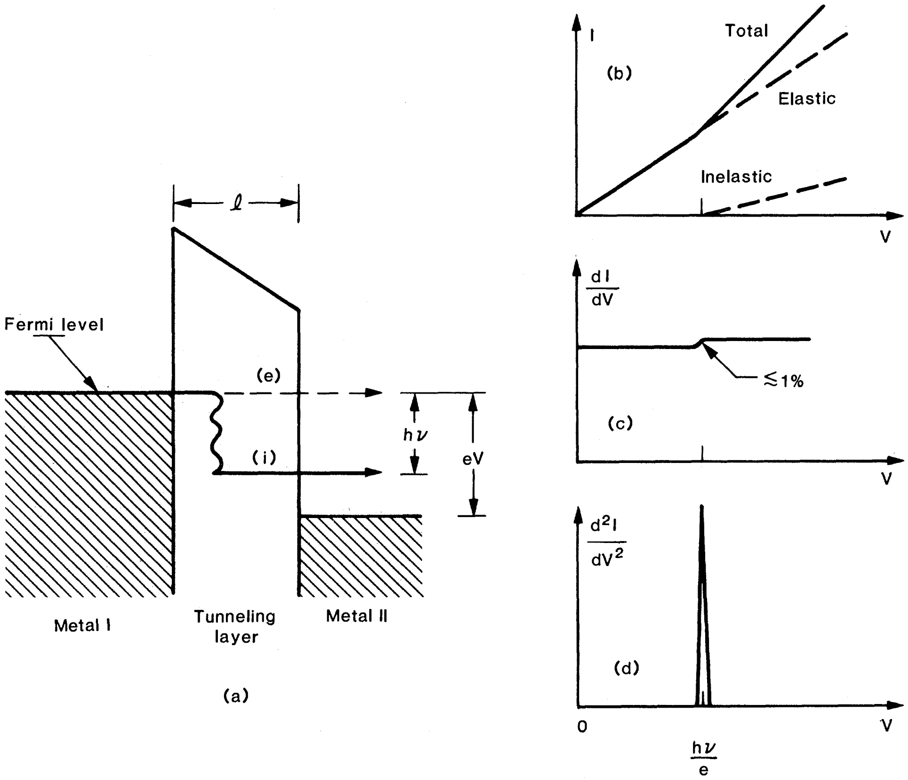


Fig. 1. (a) Schematic of a Fermi level diagram for a metal-insulator-metal tunnel junction illustrating elastic (*e*) and inelastic (*i*) tunneling. (b) The inelastic current has a threshold at $V = h\nu/e$. First derivative (c) and second derivative (d) spectra. The standard tunneling spectrum (d) accentuates the small increase.

Experimental Techniques

The experimental methods used in inelastic electron tunneling are similar to those used in all electron tunneling experiments. The sample is critical in a tunneling experiment, and hence sample preparation will be described here in some detail.

Sample preparation. Thin-film tunnel junctions (Fig. 2, b and c) are fabricated on an insulating substrate such as glass or glazed ceramic. The junction fabrication involves four steps: (i) deposition of the first metal electrode, (ii) oxidation of the first metal electrode, (iii) doping of the oxidized electrode with the molecules to be studied, and (iv) deposition of the top metal electrode. These steps are described in detail below. The fabrication process is carried out in a high-vacuum evaporator (Fig. 2a). It is extremely important that the evaporator be kept free of organic contaminants. Any type of vacuum system that is contaminant-free can be used for this purpose—for instance, an oil diffusion pump system (~ 10^{-7} torr) with liquid nitrogen trapping and a zeolite trap on the foreline, or an ion pump or cryo pump oil-free system (~ 10^{-9} torr).

The first metal electrode, most commonly aluminum (because it is relatively easy to form uniform, pinhole-free aluminum oxide) about 600 Å thick and 1 mm wide, is evaporated onto the substrate through a metal mask usually made out of aluminum or stainless steel; magnesium has also been used successfully to vary the chemical properties of the adsorbing oxide layer. The thickness of this electrode is not critical, but it is usually kept in the range of 400 to 1000 Å so that the film is smooth and continuous.

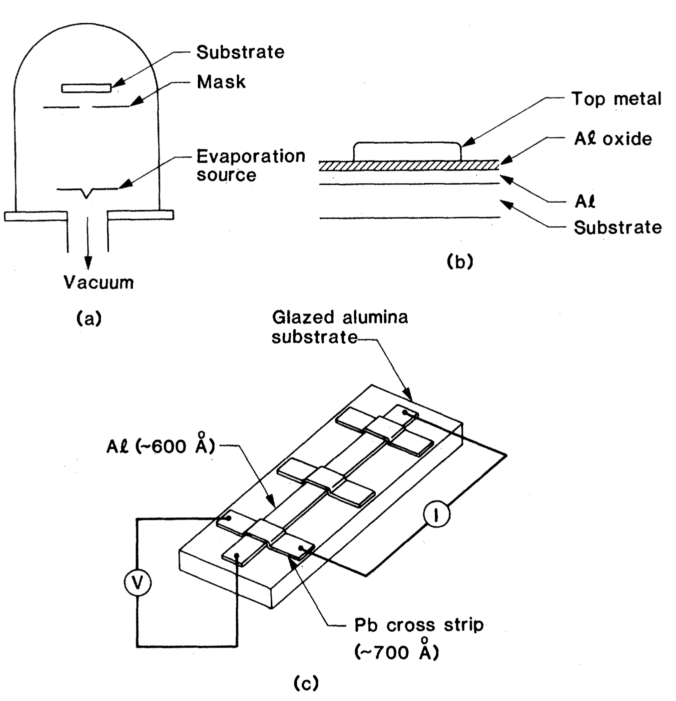


Fig. 2. Schematic showing some experimental details of metal-oxide-metal junction fabrication.

Oxidation. Oxidation of the aluminum film is the most important step in formation of the junction. The resistance of a tunnel junction is proportional to exp (l/l_0) where l is the thickness of the insulating layer and $l_0 \cong 1$ Å. Thus the resistance of the tunnel junction changes by an order of magnitude if the thickness of the insulating layer changes by only a few angstroms. The oxide layer, with a thickness in the range of 10 to 30 Å, has to be extremely uniform, otherwise all the current would flow through any pinhole or thin spot that was present. Two techniques may be used for oxidizing the bottom metal electrode: (i) thermal oxidation and (ii) glow discharge oxidation. In the former technique the bottom metal electrode, say aluminum, is left in air at room temperature for several minutes, which results in a uniform insulating oxide layer about 10 to 20 Å thick. This is a time-honored method and is generally easier than glow discharge oxidation, and of course it requires no equipment. However, the thermal oxidation technique suffers from unavoidable contamination of the junction by organic impurities from the atmosphere. In the glow discharge oxidation technique (*9*) an electrical discharge is set up between an aluminum cathode, typically at -500 V d-c, and the rest of the system, while a pressure of approximately 50 μm of oxygen is maintained. Exposure of the samples to the resulting glow discharge for approximately 20 minutes produces an oxide film 10 to 20 Å thick; a small d-c oxidizing potential (~ 1 V) applied to the aluminum film promotes slightly thicker oxide growth (~ 30 Å). Samples prepared by glow discharge oxidation show no sign of organic contamination.

Recently, Moodera *et al.* (*10*) succeeded in artificially depositing an insulating layer of Al_2O_3 by electron beam evaporation on substrates cooled to 77 K. This new method should allow the fabrication of tunnel junctions on many previously unstudied electrodes.

Molecular additions. Doping the insulating layer with the molecules to be studied is, in practice, the most difficult step in junction fabrication. It is necessary to achieve a stable coverage of up to a few monolayers inside the tunnel junction, and the doping procedure must not cause unwanted chemical changes in the deposit, such as decomposition due to heating. The procedure most commonly used involves liquid or vapor doping of the oxidized aluminum strip as an intermediate step in junction fabrication. The liquid and vapor methods are described below. Both have been used successfully for a variety of molecular types.

1) Vapor phase doping: The oxidized aluminum surface is exposed to the vapor of the molecules of interest simply by admitting a small amount of the vapor directly into the vacuum chamber. Under the right conditions, a monolayer is deposited. This technique is well suited for molecules that have a reasonable vapor pressure and form strong chemical bonds to the surface of the oxide layer; examples of such molecules are formic acid and acetic acid. For molecules with a low vapor pressure, deposition can be accomplished by evaporating them from a heated source in a manner similar to deposition of metal films. The molecules must be sufficiently stable to withstand the necessary heating. For molecules that have a high vapor pressure and form weak bonds with the oxide, such as gases, solvents, and alcohols, the substrate must be cooled below room temperature in order to freeze the molecules in place.

2) Liquid phase doping: The oxidized aluminum film is either dipped directly into a solution, or a drop of a weak (~ 0.1 percent) solution of the dopant is applied to the substrate; and the excess is removed by spinning, leaving a very thin layer. When the solvent evaporates away, a uniform molecular layer is left behind. This method is convenient for molecules with a low vapor pressure or those that bond strongly to the surface. The thickness can be controlled by regulating the solution concentration. This technique is also well suited for doping of large molecules, such as those of biological interest, which tend to decompose if high-temperature evaporation is attempted. It also has the advantage that it does not contaminate the vacuum chamber.

Completion of the tunnel junction. The last step in fabricating the tunnel junction is the deposition of a top metal electrode. This is deposited in the form of a cross strip by evaporation to produce a junction typically 1 mm^2 in area. Although a number of metals have been successfully employed as the top electrode, lead is most widely used because of its chemical inertness. Lead also has the advantage of being superconducting at 4.2 K, which improves the resolution.

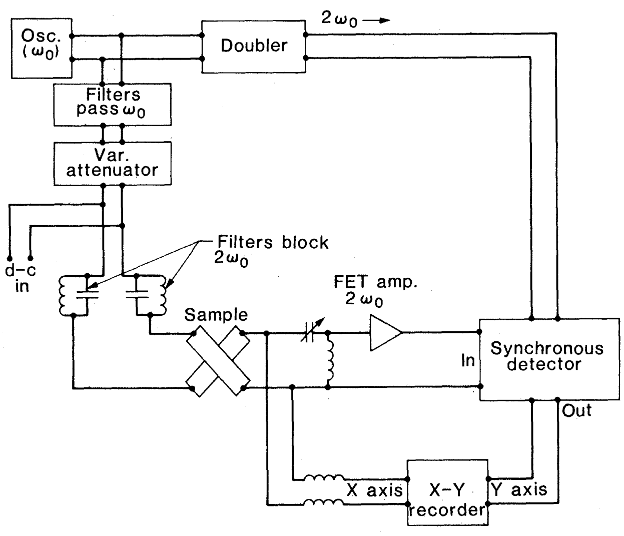
Infusion method. So far, we have described the doping technique before deposition of the top electrode film. In the recently developed infusion method (*11*), the organic molecules are introduced into already fabricated junctions through the top electrode. This technique is easier and more flexible than other doping methods and has been used successfully with molecules that are difficult to use with other methods. It also allows simultaneous and sequential doping, which can be used to study chemical reactions inside the junction. Although only polar molecules have been used so far, there is no reason to believe that this method cannot be used with nonpolar species.

Infusion is carried out by exposing the complete tunnel junction to an atmosphere with a relative humidity in the range of 50 to 95 percent. Organic molecules with a sufficient vapor pressure may be mixed with a small amount of water, which acts as a carrier for the dopant molecules. The presence of water promotes the mobility of the organic molecule through the metal. The infusion time is 1 minute to 1 hour for a coverage of about one monolayer.

The molecules can also be first applied to the external surface of the top metal electrode of the tunnel junction and then later infused by exposing the junction to humid gas. This technique is useful for strong adsorbers such as acids or high-molecular weight species of low vapor pressure.

The conditions that determine whether an organic layer is formed include the mobility of the organic molecules in water and their relative concentration.

Measurement technique. The measurements involve a careful study of the current-voltage characteristics of a tunnel junction. In practice, one measures the second derivative (d^2I/dV^2 or d^2V/dI^2) versus V because of the appearance of sharp peaks in the second derivative curves. The derivatives d^2I/dV^2 and d^2V/dI^2 are equivalent and are related by $d^2V/dI^2 = -(dV/dI)^3\, d^2I/dV^2$, where dV/dI is relatively constant over the voltage range of interest, at least for aluminum oxide and magnesium oxide junctions. Most important, dV/dI has no large sharp structure in the voltage range of interest. The useful range of most tunneling spectra is 0 to 0.5 V. In essence, the measurement technique detects changes in conductivity by detecting second harmonic generation. The a-c technique allows the observation of small structures in the presence of a large background, and with modulation these observations become direct and sensitive. The method essentially involves the application of a small a-c modulation signal in addition to the



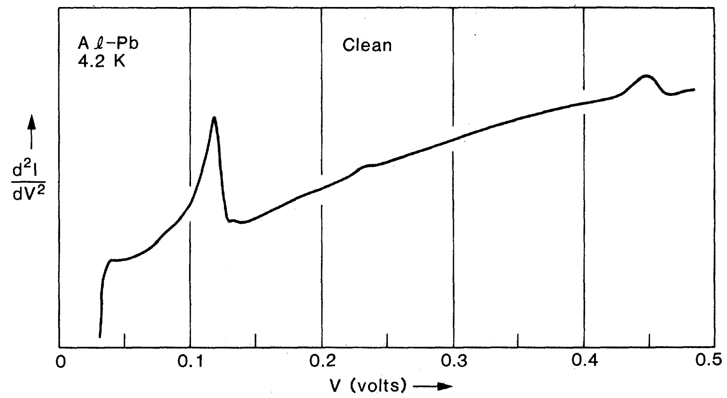
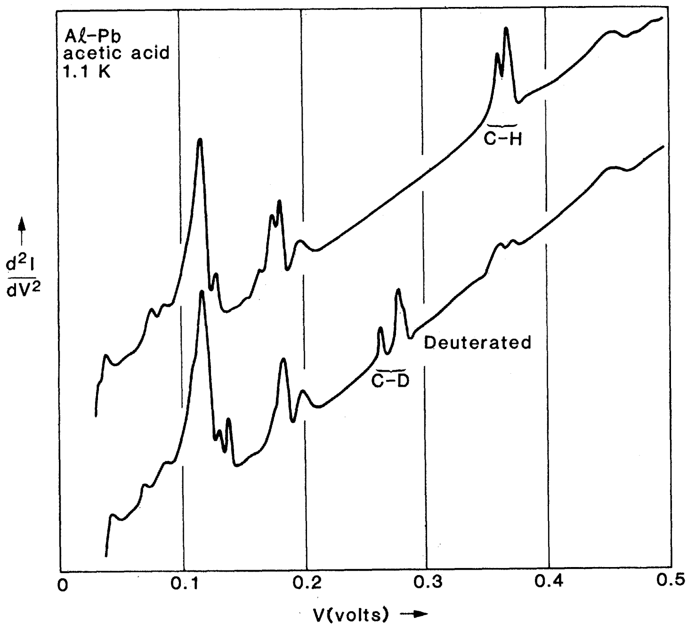


Fig. 3 (left). Schematic diagram of a tunneling spectrometer. Fig. 4 (right). Tunneling spectrum of a clean Al-Al_2O_3-Pb tunnel junction. The peak near 0.12 V is predominantly due to aluminum oxide phonons and the shoulder on the low-voltage side is due to an O–H bending mode. [From Lambe and Jaklevic (*8*)]



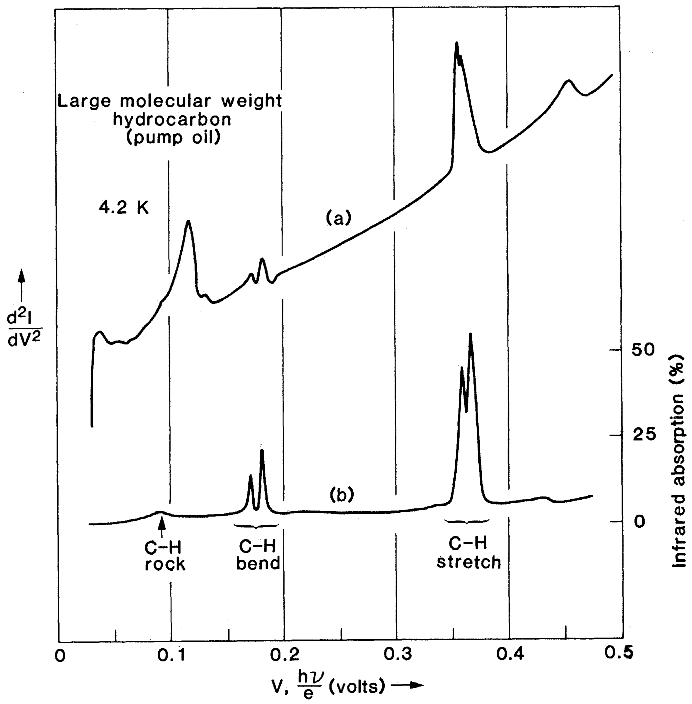


Fig. 5 (left). Tunneling spectra of acetic acid (CH_3COOH) and deuterated acetic acid (CD_3COOD) evaporated onto the oxide of an Al-Al_2O_3-Pb junction. [From Lambe and Jaklevic (*8*)] Fig. 6 (right). (a) Tunneling spectrum of a large molecular weight hydrocarbon evaporated onto the oxide of an Al-Al_2O_3-Pb junction. (b) Assigned infrared absorption spectrum of the same material for comparison. [From Lambe and Jaklevic (*8*)] d-c bias voltage. A primary frequency of about 50 kHz is applied to the junction and an output signal is detected at 100 kHz. A schematic of the experimental setup is shown in Fig. 3; the experimental details are described in (*8*). The amount of 50-kHz modulation that can be used is determined by the line width one is attempting to resolve. Typical modulation levels are between 1 and 5 mV. Smaller modulation results in a smaller signal but is necessary to ensure optimum resolution in some cases. Sample resistance plays an important role in determining the overall sensitivity of the measurement system; the lower the sample resistance, the greater the available signal power. An impedance of ~ 100 ohms for a 1-mm^2 junction is acceptable. Most tunneling spectra are recorded at 4.2 K; the lower the temperature, the better the resolution. Resolution is further improved if one or both of the metal films are superconducting. IETS is capable of detecting a fraction of a monolayer, which corresponds to about 10^{10} molecules for a 1-mm^2 junction. For more details of sample preparation and measurement techniques, the reader is referred to (*3, 8, 12, 13*).

Experimental Results and Applications

Much of the earlier experimental work was focused on understanding the characteristics of IETS. It was first necessary to investigate the background spectrum of an undoped clean tunnel junction to make sure that no molecules were observed. Figure 4 shows a typical spectrum of an undoped clean Al-Al_2O_3-Pb junction. It was found that OH groups were inevitably present (see structure at ~ 0.1 V), but that the junction was free of other contamination. The role of the surface OH group has been the subject of considerable study, since it is very important to the nature of catalytic surfaces.

Figure 5 shows a vibrational spectrum of acetic acid evaporated onto the oxide of an Al-oxide-Pb junction. The positions of the peaks are directly related to the frequency of oscillation of the molecule, ν, by $eV = h\nu$. It was essential to prove beyond doubt that the vibrational modes of acetic acid molecules were being observed. The spectrum obtained in a deuteration experiment, in which deuterated acetic acid was evaporated onto the oxide, is also shown in Fig. 5. Note that the C–D stretch mode shifted down by roughly $1/\sqrt{2}$ from the C–H stretch mode. This experiment showed that the isotope effect plays a useful role in tunneling and proved that the vibrational modes of the molecule were being observed. The final identification of the spectra was made by comparison with infrared and Raman data; just as in infrared and Raman spectroscopy, the characteristic vibrations of groups can be used to identify the molecule.

The next important question was whether one could really deposit molecules onto an aluminum oxide surface and subsequently deposit a lead film over this, and expect the molecules to survive this rather traumatic experience. It was instructive to compare infrared spectra with the IETS spectra. Figure 6 shows the tunneling spectrum for an Al-oxide-Pb junction doped with a large molecular weight hydrocarbon evaporated from a tantalum boat. Note that, in addition to the peaks observed in undoped junctions, there are peaks near 175 and 360 mV. Figure 6 also shows an infrared spectrum of the same material for comparison. The two curves agree quite well, and the comparison suggests

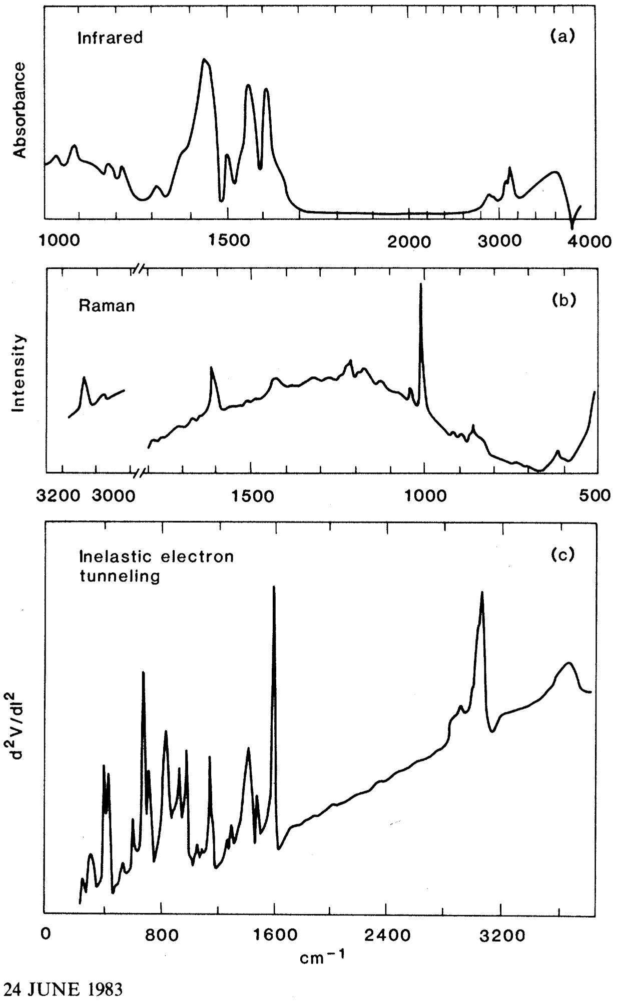
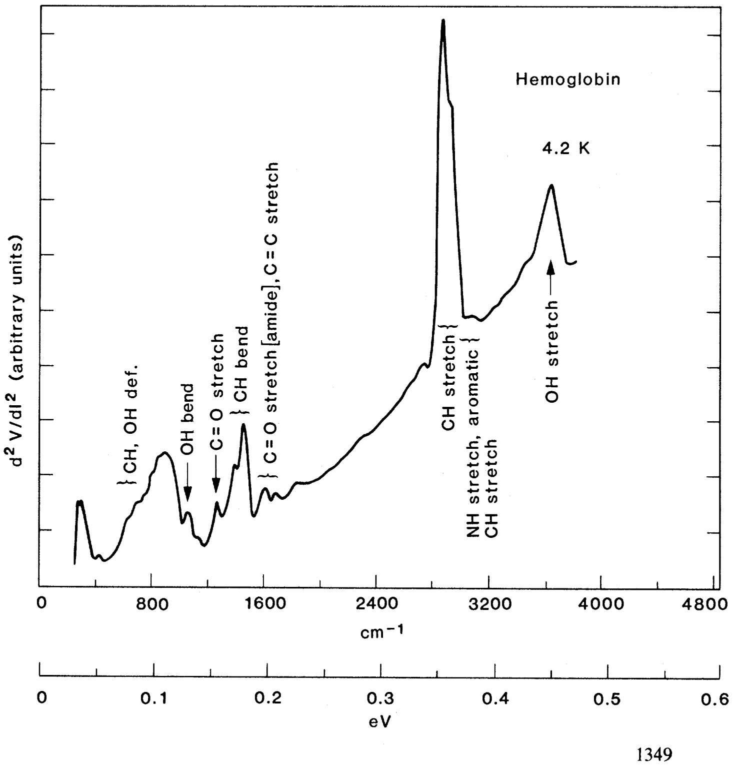


Fig. 7 (left). Comparison of (a) infrared, (b) Raman, and (c) tunneling spectra of the same system. [From Hansma (*3*)] Fig. 8 (right). Tunneling spectrum of protein hemoglobin liquid-doped onto the oxide of an Al-Al_2O_3-Pb junction. [From Simonsen *et al.* (*16*)]



that the peaks near 175 and 360 mV are associated, respectively, with C–H bending and C–H stretching of the hydrocarbon. This is a simple example of how almost all vibrational mode identification has been made in IETS.

A variety of organic molecules, including acids, bases, and ring compounds, have been studied with this technique. The recent introduction of molecular infusion (*11*) alleviates much of the concern about "damage" from counter electrode evaporation, since the molecules are infused after the complete junction is fabricated.

Catalysis and surface chemistry. IETS has found application in this area because of its great sensitivity in determining the vibrational structure of surface-adsorbed species and their chemical products. Much of the pioneering work in this field has been carried out by Hansma (*3*) and Weinberg (*4*).

Alumina and magnesia represent important model surfaces for the study of catalysis. Figure 7, a and b, shows the infrared and Raman spectra of benzaldehyde adsorbed on alumina. Good resolution is observed in the infrared spectrum between about 1300 and 1700 cm^{-1}, but the resolution and sensitivity deteriorate outside this limited region. The Raman spectrum in Fig. 7b shows a few more modes. Figure 7c shows the tunneling spectrum of benzaldehyde on the oxidized aluminum strip. Note that in this spectrum we have d^2V/dI^2 as the ordinate instead of d^2I/dV^2; as mentioned earlier, the two are equivalent. The spectrum in Fig. 7c has a resolution comparable to the best optical resolution in the region 1300 to 1700 cm^{-1}. The most interesting feature of this tunneling spectrum is that it has the same resolution throughout the entire range from 200 to 4000 cm^{-1}. This example clearly demonstrates the advantage of IETS in sensitivity and resolution over infrared and Raman spectroscopy. Both the infrared-active and Raman-active vibrational modes are observed in the tunneling spectra with comparable magnitudes. Although much of IETS surface chemistry is focused on organic groups, inorganic surface chemistry has also been studied. This is illustrated by the work of Hipps *et al.* (*14*) on $\mathrm{Fe(^{12}K^{15}N)_6}^{4-}$ and $\mathrm{Fe(^{12}C^{14}N)_6}^{4-}$ adsorbed on oxidized aluminum.

Biology. IETS has been applied to a wide range of biological compounds, and in many cases a well-resolved spectrum has been obtained. Most of the work on the identification of biochemicals with IETS has been performed by Coleman and his collaborators (*15*). Work on biological compounds has been largely on small monomers such as amino acids, nucleotides, and lipids. IETS has been used successfully to distinguish among slightly different adenine derivatives. Figure 8 shows the tunneling spectrum of protein hemoglobin (*16*). The resolution in this case is not sufficient to resolve any more than the envelope of the huge number of adjacent modes for this large molecule.

Adhesion. Since aluminum is an important structural material, its surface interactions are of great technological interest. Suppose we have two pieces of aluminum bonded by an adhesive. The adhesive is usually in contact with aluminum oxide rather than with the metal, since an oxide quickly forms on the surface of aluminum when it is exposed to air or cleaned with etching solutions prior to bonding. The chemical nature of the interface is very important for the integrity and life of the bond. It is difficult to investigate the detailed nature of the bond between the oxide layer and the first adhesive monolayer by conventional infrared techniques. H. W. White, L. M. Godwin, and T. Wolfram [in (*5*), p. 70] used IETS to study the adhesion of epoxy to aluminum. They observed aging effects in the epoxy, possibly due to chemisorption or polymerization. This area seems quite promising for future research.

Electronic excitations. The observation of electronic excitations in electron tunneling stems from early work by Tsui (*17*) on semiconductor junctions. Tsui showed that one can detect the excitation of semiconductor plasmons, which are the vibrational modes of the electron gas in the tunneling electrodes. The surface plasmons have the interesting property that they can be coupled out of the electrodes and produce electromagnetic radiation. Lambe and McCarthy (*18*) showed that inelastic tunneling excitation of surface plasmon modes can result in light emission when electrodes are properly prepared. They used rough and porous silver as the top electrode and developed etching processes for coupling out the light. One can actually see light being emitted from the junction. The light emission establishes a fundamental quantum relation between the maximum optical frequency and the applied voltage and shows an unusual quantum effect; that is, a continuum of radiation is produced which has a cutoff frequency $h\nu_{\mathrm{max}} = eV$, where V is the applied voltage. Thus this is a kind of voltage-tunable light source. At present, it is not very efficient. At best, about one photon is produced externally for every 10^5 electrons passed through the junction. Because several fundamental questions are involved, a number of investigations are in progress in this area.

Since molecular electronic excitations have much greater oscillator strength than vibrational excitations, one would think it should be easier to observe electronic transitions. Early attempts to observe electronic excitations failed because of limitations and difficulties which are discussed by deCheveigne *et al.* (*19*). However, deCheveigne and coworkers were able to observe weak electronic transitions in a tunnel junction that was vapor-doped with copper phthalocyanine. Electronic IETS spectroscopy has yet not proved very fruitful, but it remains an interesting area for investigation.

Electronic chemical sensors. So far, we have discussed the spectroscopy of tunneling in terms of the analytical properties that can be used. There are also promising future applications of tunnel junctions in the area of electronic chemical sensing. In such applications an electronic device is used as an in situ sensor to detect the presence of various chemicals. For example, chemically sensitive field effect transistors (FET's), called chem-FET's, are devices which depend on the fact that chemicals can influence the number of charge carriers present in the surface conduction channels of semiconductors (*20*). It should be possible to use electron tunneling as a probe for chemical sensing because chemicals added to the tunnel barrier will affect the transport of current through the barrier. Some of the initial experiments on metal-insulator-metal tunnel junctions exposed to chemical species such as iodine showed large signals in the tunneling spectrum around 2 V, which is characteristic of the chemical species. Because of the extreme sensitivity of electron tunneling, this kind of chemical sensor should be quite useful. We are investigating this concept at Jet Propulsion Laboratory.

Conclusions

IETS is a versatile and useful technique for the study of vibrational modes of molecules adsorbed on the surface of the oxide layer of metal-oxide-metal junctions. One of the major advantages of the technique is its great sensitivity and resolution. It has already been used in studies of catalysis, biology, trace impurity detection, and electronic excitations. The last application is important because of the possibility of using tunnel junctions as practical light sources. Desirable areas for future development include more reliable insulating barriers and tunnel junction devices that could withstand more rigorous chemical and temperature treatments. IETS also shows great promise in the study of corrosion and in chemical sensing because of its extreme sensitivity.

Finally, we wish to mention a fascinating microscopy based on tunneling which was recently described by Bining *et al.* (*21*). Scanning tunneling microscopy is capable of showing three-dimensional surface topography with a resolution orders of magnitude better than that of scanning electron microscopy. This microscopy is based on elastic tunneling. It is possible, however, that the technique could be extended to IETS.

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RESEARCH ARTICLE

RNA Splice Site Selection: Evidence for a 5′→3′ Scanning Model

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Abstract. *Human G_γ-globin genes containing tandem duplications of the donor (5′) or acceptor (3′) RNA splice sites of the second intervening sequence were constructed in order to ascertain the directionality of RNA splice site selection. These genes were introduced into cultured monkey cells, and their transcripts were analyzed. Transcripts of these duplication variants were spliced only at the proximal copy of the duplicated splice sites. These data are consistent with a 5′→3′ model of splice site selection.*

Intervening sequences (IVS) are a feature of a large number of eukaryotic genes (*1*). These sequences are included in the initial nuclear RNA transcripts, but are excised during maturation into cytoplasmic RNA, a process referred to schematically as splicing. RNA splicing is a precise process with highly accurate recognition of appropriate coding-intervening sequence boundaries. However, relatively little specific sequence information appears required to define a splice site (*2, 3*), and the factors that influence the selection of appropriate splice sites are largely unknown. Many messenger RNA (mRNA) precursor molecules are complex, with multiple intervening sequences and large numbers of potential splice sites. Therefore, it is likely that selection of splice junctions is a highly ordered process.

Several models for the appropriate selection of splice sites have been proposed (*4*), the simplest of which is that splice site selection occurs by a directional "scanning" process. In such a model the recognition of specific sequences on a nucleic acid macromolecule by a protein is facilitated by "linear diffusion," the "sliding" of the protein along the length of the nucleic acid chain (*5*). This type of process probably mediates the recognition of the *Escherichia coli* lac operator sequence by the repressor protein (*6*). A lateral diffusion process might either begin at one end of a linear macromolecule or it might initiate internally. If internal, the initial binding of protein and nucleic acid might either be site-specific (that is, mediated by a defined sequence or structure) or nonspecific (random). Futhermore, a scanning process of splice site recognition and IVS excision might either be processive (the splicing protein remaining on the RNA after a splice event and sliding to the next available splice sites) or distributive (the protein dissociating from the mRNA precursor after each splice event and reassociating at random with the same or another molecule). Therefore, a directional component to the splice site selection process does not imply that excision of intervening sequences from a multi-IVS precursor occurs in an obligate or even preferred order. In addition, a directional process of splice site selection could be complicated by a number of modifying factors. These types of models describe an essentially passive process of splice site selection. In some biological systems apparent alternative pathways of RNA splicing and transcript termination or polyadenylation (*7, 8*) might reflect some form of active regulation that modifies or even supersedes any passive mechanisms of splice site selection.

To determine the role of directionality in selection among potential splice junctions, we used the second IVS (IVS-2) of the human G_γ-globin gene as a test system since there is no evidence that alternative splicing of G_γ-globin transcripts ever occurs in normal individuals. Variant G_γ-globin genes containing small tandem duplications which included either the donor (5′) or acceptor (3′) splice sites of IVS-2 were constructed and introduced into cultured mammalian cells with the use of prokaryote-eukaryote "shuttle vectors" capable of replication and expression of cloned eukaryotic genes in monkey cells (*9*). Transcripts of the normal human G_γ-globin gene are appropriately processed in this system.

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