Relationship between SERS Intensity and Both Surface Coverage and Morphology for Pyrazine Adsorbed on a Polycrystalline Gold Electrode

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The dependence of the surface-enhanced Raman scattering (SERS) of pyrazine (pz), adsorbed on polycrystalline gold electrodes ("smooth" and rough), on both the surface coverage and morphology was investigated. The morphology of the rough gold electrode was observed by atomic force microscopy (AFM), and the surface coverage data were obtained from previous electrochemical measurements. The plots between the SERS intensity of the pz ring-breathing mode (ca. 1016 cm^{-1}) and the surface morphology parameters (roughness factor) were obtained. The SERS intensity reached a maximum between 20 and 30 ORCs. Independent AFM measurements indicated that roughness features with an average size of ca. 100 nm are present in the surface which yielded the strongest SERS signal. The dependence of the SERS intensity on the surface morphology agrees well with the electromagnetic calculations of the enhancement factor for gold spheres. The SERS intensities from pz adsorbed on an "unroughened" gold electrode track the surface concentration up to ca. two-thirds of a monolayer. As the amount of pz in the surface approaches the monolayer value, this dependence becomes inverse, due to the interactions between the induced molecular dipoles present on a heavily packed surface. The SERS intensities from a smooth surface (zero ORC) were obtained by extrapolation from the spectrum of pz adsorbed on gold electrodes with different degrees of roughness. The calculated SERS intensities were compared to the surface coverage, and a linear relationship was obtained for a wide range of potentials.

Introduction

The nature and the orientation of molecules adsorbed on certain rough electrode surfaces (mainly Ag, Au, and Cu) can be readily determined by electrochemical surface-enhanced Raman scattering (SERS).[1,2] The million-fold enhancement of the Raman signal makes this technique sensitive even to submonolayer amounts of adsorbate. This surface-enhancement phenomenon, allied to basic characteristics of the Raman scattering, such as the weak water activity, suggests that the SERS effect is potentially a very powerful tool for the investigation of electrochemical problems. However, the magnitude of the enhancement depends on several factors, such as applied potential, excitation wavelength, surface coverage, and morphology.[1,2] The dependence of the electrochemical SERS intensities on the applied potential and on the energy of the incident photon has been widely studied.[3,4] The relationships are well-understood and can be interpreted based on the two most accepted surface-enhancement theories—the charge-transfer (CT)[5] and the electromag-

netic (EM)[6] mechanisms. However, no studies have dealt with the dependence of the SERS intensities on both surface coverage and morphology for molecules adsorbed on gold electrodes.

The relationship between SERS intensity and surface morphology has been addressed for species adsorbed on silver surfaces. Several authors[7–9] have shown that, for silver electrodes, a maximum electrochemical SERS signal was obtained when the size of the surface roughness (determined by SEM) ranged from 80 to 100 nm. An apparatus for the simultaneous measurement of the silver electrode morphology (by scanning probe microscopy) and the SERS signal has been developed.[10] However, no attempt to systematically relate the surface features to the optical enhancement was made.[10] The size distribution of the roughness features on silver electrodes has been analyzed from their inelastic Mie scattering.[11] Unfortunately, the distribution of large surface bumps (>15 nm), which is supposed to yield strong SERS spectra, cannot be determined by this method, because their acoustic bands are merged into the Rayleigh line.[11] Two-dimensional SERS images of pyridine adsorbed on rough silver electrodes have been obtained and compared to the surface's AFM data.[12] The results indicated that surface features from 50 to 400 nm were responsible for the

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(1) Brolo, A. G.; Irish, D. E.; Smith, B. D. *J. Mol. Struct.* **1997**, *405*, 29.
(2) Pettinger, B. In *Adsorption of Molecules at Metal Electrodes*; Lipkowski, J., Ross, P. N., Eds.; VCH: New York, 1992; Chapter 6, p 285.
(3) Guzonas, D.; Irish, D. E. *Langmuir* **1990**, *6*, 1102.
(4) Birke, R. L.; Lu, T.; Lombardi, J. R. In *Techniques for Characterization of Electrodes and Electrochemical Processes*; Varma, R., Selman, J. R., Eds.; Wiley: New York, 1991; Chapter 5, p 211.
(5) Otto, A.; Mrozek, I.; Grabhorn, H.; Akemann, W. *J. Phys. Condens. Matter* **1992**, *4*, 1143.

(6) Moskovits, M. *Rev. Mod. Phys.* **1985**, *57*, 783.
(7) Tuschel, D. D.; Pemberton, J. E.; Cook, J. E. *Langmuir* **1986**, *2*, 380.
(8) Cross, N. A.; Pemberton, J. E. *J. Electroanal. Chem.* **1987**, *217*, 93.
(9) Kruszewski, S. *Adsorpt. Sci. Technol.* **1994**, *1*, 217.
(10) Tian, Z. Q.; Li, W. H.; Ren, B.; Mao, B. W.; Chen, J. G.; Mu, J. Q.; Zhuo, X. D.; Wang, D. *J. Electroanal. Chem.* **1996**, *401*, 247.
(11) Felidj, N.; Aubard, A.; Levi, G. *J. Chem. Phys.* **1996**, *104*, 9735.
(12) Yang, X. M.; Ajito, K.; Tryk, D. A.; Hashimoto, K.; Fujishima, A. *J. Phys. Chem.* **1996**, *100*, 7293.

enhancement of the Raman signal.[12] The differential reflectance spectrum of an electrochemically roughened silver electrode is also dependent on the surface morphology.[13] The enhancement factor of the Raman signal depends on the surface reflectivity. Silver electrodes with roughness bumps ranging between 100 and 300 nm presented a minimum reflectivity and a maximum SERS signal.[13] This range is in good agreement with the SERS intensities obtained from deposited silver in UHV procedures.[14] In this case, a maximum SERS signal was obtained for slowly-deposited silver, which produced silver bumps of 150-nm average size.

Various studies regarding the relationship between enhanced optical properties and the surface morphology, determined by scanning probe microscopy, for Ag films deposited over glass,[15] silica,[16] graphite,[17] mica,[18] Formvar,[19] and suspended polymer particles[20] have been reported. The metallic silver, deposited over different supports, yields distinct SERS-active substrates. The morphology (and the optical response) of these deposits depends on several factors, such as the nature of the substrate[21] and the conditions under which the metallic deposition is made.[22] The sizes of the silver bumps presented in these films are between 5 and 20 nm.[15−22] The SERS response depends also on the film thickness[22] and on the aggregation of the silver particles.[23] Silver films deposited on polymer nanoparticles have also been prepared.[24] SERS enhancement factors as high as 10^7 were obtained from these substrates. The silver-coated nanosphere of ca. 500 nm yielded the largest SERS signal.[24]

A relationship between the SER intensity and the fractal dimension has been proposed for molecules adsorbed on vacuum-deposited silver.[25,26] The silver films formed at low temperature (~100 K) possessed significant fractal characteristics and were SERS-active. Films formed at high temperature (~300 K) possessed few fractal characteristics and gave no SERS activity.

Very few works deal with the relationship between SERS intensities and surface morphology for molecules adsorbed on gold surfaces (when compared to silver surfaces). SERS spectra from rhodamine 6G were obtained for sputtered gold films on Au and Au/Si alloys.[27] SERS activity was correlated to crystallite size in the 10−25-nm range and to roughness larger than 15 nm.[27] The dependence of the SERS response on the surface morphology was also investigated for self-assembled mono-

layers of ferrocenylazobenzenes adsorbed on a Au thin film deposited on mica.[28] Enhancement factors of the order of 10^3 were observed from these rough surfaces containing gold spheroidal particles with diameters in the 50−100-nm range.[28] Silver-coated gold colloids have been used as SERS substrates.[29,30] A glass slide covered with these silver-modified gold colloid particles of various sizes was prepared using a combinatorial approach.[31] The two-dimensional SERS spectra obtained from this substrate showed a maximum response from particles of ca. 20-nm size.[31]

The relationship between electrochemical SERS intensities and surface coverage has been investigated for halides and pseudo-halides adsorbed on silver electrodes.[32,33] A linear dependence between SERS intensity and surface concentration has been found for 2-amino-5-nitropyridine adsorbed on a rough silver electrode.[34] The study of the relationship between SERS intensity and surface coverage for molecules adsorbed on gold electrodes has been limited by the lack of independent electrochemical measurements of the surface concentration of the organic molecules. Recently, these electrochemical data are being produced in our center.[35]

A previous study of the dependence of the SERS intensity of the pyridine (py) ring-breathing mode on surface coverage, determined independently by electrochemical means, has been conducted in our laboratory.[36] For pyridine adsorbed on an "unroughened" gold electrode, the SERS intensities showed a direct proportionality to the surface concentration at low coverage (≤ two-thirds monolayer). As the surface coverage value approached one monolayer, the SERS intensity decreased, and the relationship became inverse.[36] This deviation of the SERS intensities at high coverage was attributed to the depolarizing effects which the adsorbed molecules have on the local electromagnetic field.[36] The effect of the surface roughness was also investigated. Both the SERS intensities and their dependence on the applied potential were dependent on the degree of roughness of the surface. The SERS intensities obtained from such rough surfaces were significantly larger than from the unroughened surface but did not present any simple relationship with surface coverage.[36]

It is essential to have intensity/coverage data for a number of adsorbates prior to formulating a general hypothesis. Here we extend the work to the nonpolar pyrazine molecule; because of its higher symmetry, vibrational modes which are normally Raman-inactive can provide information about surface orientation. Its surface concentration−electrode potential profile is known.[37] We report data for pyrazine (pz) adsorbed on unroughened polycrystalline gold electrodes and on elec-

(13) Bryant, M. A.; Pemberton, J. E. *Langmuir* **1990**, *6*, 751.

(14) Dawson, P.; Haas, J. W., III; Alexander, K. B.; Thompson, J.; Ferrell, T. L. *Surf. Sci. Lett.* **1991**, *250*, L383.

(15) Roak, S. E.; Rowlen, K. L. *Chem. Phys. Lett.* **1993**, *212*, 50.

(16) Lacy, W. B.; Williams, J. M.; Wenzier, L. A.; Beebe, T. P.; Harris, J. M. *Anal. Chem.* **1996**, *68*, 1003.

(17) Zoval, J. V.; Biernacki, P. R.; Penner, R. M. *Anal. Chem.* **1996**, *68*, 1585.

(18) Semin, D. J.; Lo, A.; Roak, S. E.; Skodje, R. T.; Rowlen, K. L. *J. Chem. Phys.* **1996**, *105*, 5542.

(19) Roark, S. E.; Semin, D. J.; Rowlen, K. L. *Anal. Chem.* **1996**, *68*, 473.

(20) Schueler, P. A.; Ives, J. T.; DeLaCroix, F.; Lacy, W. B.; Becker, P. A.; Li, J.; Caldwell, K. D.; Drake, B.; Harris, J. M. *Anal. Chem.* **1993**, *65*, 3177.

(21) Roark, S. E.; Rowlen, K. L. *Anal. Chem.* **1994**, *66*, 261.

(22) Semin, D. J.; Rowlen, K. L. *Anal. Chem.* **1994**, *66*, 4324.

(23) Chen, M. C.; Tsai, S. D.; Chen, M. R.; Ou, S. Y.; Li, W-H.; Lee, K. C. *Phys. Rev. B* **1995**, *51*, 4507.

(24) Van Duyne, R. P.; Hulteen, J. C.; Treichel, D. A. *J. Chem. Phys.* **1993**, *99*, 2101.

(25) Douketis, C.; Hasslett, T. L.; Wang, Z.; Moskovits, M.; Ianotta, S. *Prog. Surf. Sci.* **1995**, *50*, 187.

(26) Douketis, C.; Wang, Z.; Hasslett, T. L.; Moskovits, M. *Phys. Rev. B* **1995**, *51*, 11022.

(27) Maya, L.; Vallet, C. E.; Lee, Y. H. *J. Vac. Sci. Technol. A* **1997**, *15*, 238.

(28) Caldwell, W. B.; Chen, K.; Herr, B. R.; Mirkin, C. A.; Hulteen, J. C.; Van Duyne, R. P. *Langmuir* **1994**, *10*, 4109.

(29) Bright, R. M.; Walter, D. G.; Musik, M. D.; Jackson, M. A.; Allison, K. J.; Natan, M. J. *Langmuir* **1996**, *12*, 810.

(30) Freeman, R. G.; Hommer, M. B.; Grabar, K. C.; Jackson, M. A.; Natan, M. J. *J. Phys. Chem.* **1996**, *100*, 718.

(31) Baker, B. E.; Kline, N. J.; Treado, P. J.; Natan, M. J. *J. Am. Chem. Soc.* **1996**, *118*, 8721.

(32) Weaver, M. J.; Hupp, J. T.; Barz, F.; Gordon, J. G., II; Philpott, M. R. *J. Electroanal. Chem.* **1984**, *160*, 321.

(33) Wetzel, H.; Gerischer, H.; Pettinger, B. *Chem. Phys. Lett.* **1983**, *78*, 5324.

(34) Foresti, M. L.; Funtikov, A. M.; Guidelli, R.; Muniz-Miranda, M. *J. Electroanal. Chem.* **1994**, *367*, 223.

(35) Lipkowski, J.; Stolberg, L. In *Adsorption of Molecules at Metal Electrodes*; Lipkowski, J., Ross, P. N., Eds.; VCH: New York, 1992, Chapter 4, p 171.

(36) Stolberg, L.; Lipkowski, J.; Irish, D. E. *J. Electroanal. Chem.* **1991**, *300*, 563.

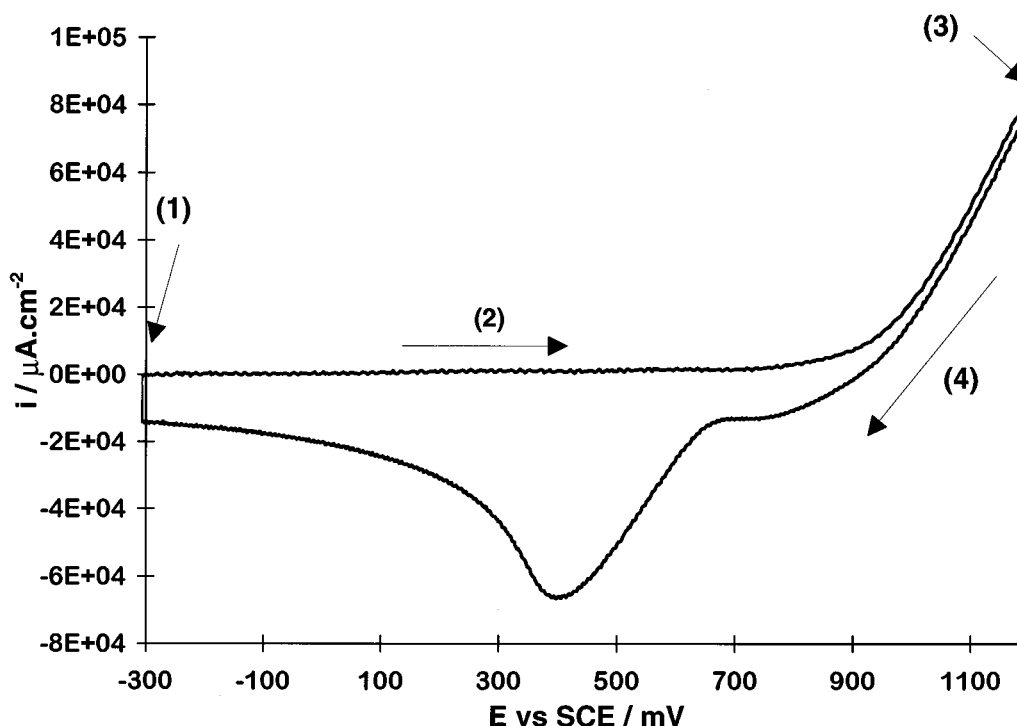(37) Iannelli, A.; Richer, J.; Lipkowski, J. *Langmuir* **1989**, *5*, 466.

Figure 1. Cyclic voltammogram of a polycrystalline gold electrode in 0.1 M KCl. A complete ORC consists of the following steps:[40] (1) −300 mV was applied for 30 s; (2) anodic scan at 1 V/s; (3) +1.2 V was applied for 1.2 s; (4) cathodic scan at 0.5 V/s.

trodes roughened to different degrees, as monitored by AFM. It is shown that by extrapolation of intensity data measured for the roughened surfaces, one can obtain intensities for a "smooth" electrode which track the surface coverage over a wide range of electrode potentials. Gold surfaces are more reproducible (i.e., less contaminated by oxide/hydroxide species) than silver and hence are the electrode material of choice.

Experimental Section

Solutions. All solutions were prepared from Milli-Q water. All SERS experiments were performed in 0.1 M KClO$_4$ (BDH). The KClO$_4$ was purified by calcinating at 300 °C, recrystallizing twice from Milli-Q water, and then drying. KCl (0.1 M, BDH) was used for the preparation of the rough gold electrode. Pyrazine (99+%, Aldrich) was used without further purification. The solutions were purged with nitrogen for at least $^1/_2$ h before taking measurements, and a gentle N$_2$ stream was kept over the top of the solution during the experiments.

Electrodes. The working electrode was a polycrystalline 4-mm-diameter gold rod from Johnson Matthey (99.99% pure) mounted in a Teflon holder. The gold electrode was polished to a mirror finish with progressively finer grades of alumina, ending with the 0.3-μm grade. The electrode was then rinsed with copious amounts of Milli-Q water and treated in an ultrasonic bath to remove any excess of alumina. Finally, the electrode was cleaned by flaming and quenching with Milli-Q water[38] and transferred to an electrochemical cell for the activation procedure.

The counter electrode was a platinum wire, separated from the working electrode compartment by a porous glass frit. A saturated calomel electrode (SCE) was used as the reference electrode. Therefore, all potentials reported in this work are relative to the SCE. A Luggin capillary was used to minimize the IR drop.

Activation Procedures. *Preparation of a Rough Gold Electrode.* The "activation procedure" is the process of creation of the necessary roughness, which allows the electrode surface to support the SERS effect. A clean gold electrode was transferred to a typical electrochemical cell containing 0.1 M KCl solution for activation (roughening). The hanging electrode technique

was used to make contact between the gold and the electrolyte solution.[39] Several methods for activating the gold electrode have been described in the literature.[40−42] The method developed by Gao et al.[40] yields a stable and reproducible surface and was used in this work. One complete oxidation−reduction cycle (ORC) from this method is illustrated in Figure 1. The anion [AuCl$_4$]$^-$ is the main product from the electrochemical dissolution of metallic gold in chloride medium.[43] The formation of this anion is controlled by the diffusion of chloride to the gold surface. When the potential scan is reversed to the negative direction, the concentration of gold chloride species close to the surface is very high. The deposition of metallic gold is very fast (the scan rate was 500 mV/s), creating patches of gold clusters on the surface. As the number of ORCs increased, the size of these roughness features also increased. The cathodic and the anodic charges for each ORC were calculated by integrating the area under the voltammogram. Distinctly different rough surfaces were prepared by varying the number of ORCs performed. Figure 2 shows that the cathodic charge increases linearly with the number of ORCs, and the ratio between the cathodic and the anodic charge remains constant for different numbers of ORCs within 10%. Typically, 60% of the anodic charge is recovered in the reverse scan, indicating that some of the oxidation product diffused away from the electrode into the bulk solution. Following the last cycle, the activated electrode was rinsed with Milli-Q water and then transferred to the SERS cell, described elsewhere.[1]

Preparation of an "Unroughened" Gold Electrode. The SERS spectra of pz from an unroughened gold electrode were also obtained. The unroughened gold electrode was prepared using the technique developed in our previous work.[36] A cleaned gold electrode was placed in the SERS cell containing 0.1 M KClO$_4$ and the desired concentration of pz. The electrode was submitted to continuous cleaning cycles between the limits of −0.8 and 1.1

(38) Clavilier, J. *J. Electroanal. Chem.* **1980**, *107*, 211.

(39) Dickertmann, D.; Schultze, J. W.; Koppitz, F. D. *Electrochim. Acta* **1976**, *21*, 967.

(40) Gao, P.; Gosztola, D.; Leung, L.-W. H.; Weaver, M. J. *J. Electroanal. Chem.* **1987**, *233*, 211.

(41) Busby, C. C.; Creighton, J. A. *J. Electroanal. Chem.* **1982**, *140*, 379.

(42) Baltruschat, H.; Staud, N.; Heitbaum, J. *J. Electroanal. Chem.* **1988**, *239*, 361.

(43) Schmid, G. M.; Curley-Fiorino, M. E. In *Encyclopedia of Electrochemistry of the Elements*; Bard, A. J., Ed.; Dekker: New York, 1975; Vol. IV, Chapter 3, p 87.
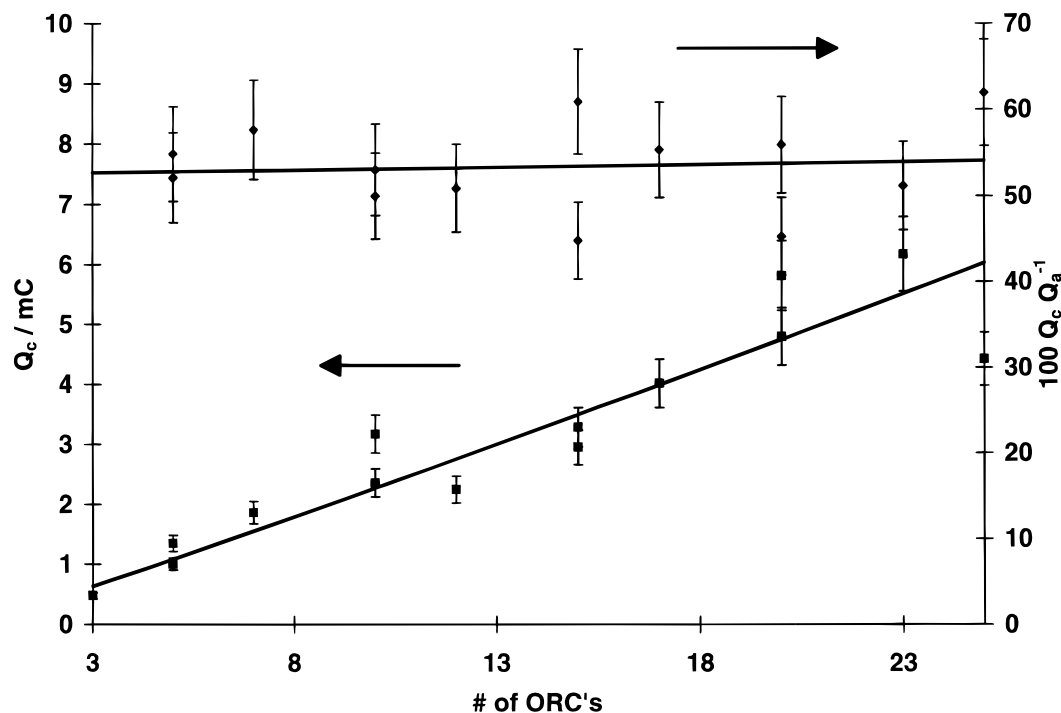
Figure 2. Dependence of both the cathodic charge (Q_c) and the ratio between the cathodic and the anodic charge (Q_c/Q_a) on the number of ORCs. Q_c and Q_a were calculated from the area under the cathodic and anodic curves on the cyclic voltammograms.

V at 20 mV/s. This cycling persisted until a cyclic voltammogram reproduced itself from one cycle to the next and displayed the features of a clean gold electrode.

Instrumentation. *Spectroelectrochemical Measurements.* A Dilor OMARS-89 spectrometer equipped with an OMA was used in the SERS experiments. The OMARS-89 was operated using the 1800 grooves/mm gratings with 2 gratings operating in subtractive mode and a third grating dispersing the output onto the 512 diode array detector. The spectrometer was interfaced to an IBM AT computer. The spectra were transferred to a 486 computer where the programs for baseline correction and bandfitting were available. A potentiostat PAR Model 273 was used in the electrochemical and SERS experiments. The cyclic voltammograms were recorded using a Kipp & Zonen *XYY* plotter.

A Coherent 599 dye laser, pumped with a Coherent Innova 70 argon ion laser operating in the all-lines mode, provided the laser excitation. The laser dye was DCM (Lambda Physik), and all spectra were excited by a 650-nm laser line.

For all SERS experiments, the mechanical slit widths were 150 μm, which give a spectral slit of, approximately, 3 cm^{-1}. The laser power was set to 50 mW. An accumulation time of 1 s and 10 acquisitions were used to obtain the spectrum of pz adsorbed on a rough gold electrode. For unroughened gold electrodes, an accumulation time of 2 s and 100 accumulations were used.

Atomic Force Microscopy Measurements. The microscope was a Nanoscope E (Digital Instruments). Standard silicon nitride tips were mounted on gold-plated 200-μm V-shaped cantilevers (spring constant 0.12 N/m), and a 15-μm piezo scanner was used. The roughened gold substrate was activated (as described above) and dried before each measurement. All AFM images were obtained in air in the contact mode (i.e., at constant force). Roughness factors (R) were calculated from each image (image dimensions of 1 μm × 1 μm) obtained from differing numbers of ORCs. The nanoscope software was used in these calculations. The roughness factors were directly proportional to the number of ORCs (R increases as the number of ORCs increases). Consequently, according to Figure 2, the roughness factor is also linearly dependent on the cathodic charge.

Results and Discussion

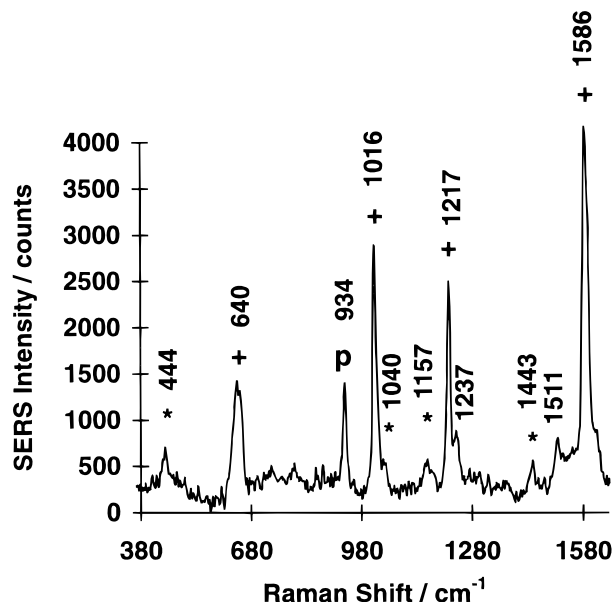**Dependence of SERS Intensities of Pyrazine Adsorbed on a Polycrystalline Gold Electrode on**

Figure 3. SERS spectrum of pz adsorbed on a rough gold electrode (five ORCs). $E_{appl} = -50$ mV. (*) Forbidden bands in the NRS of pz; (+) A_g modes (most enhanced); (p) perchlorate band. The vibrational frequencies, in cm^{-1}, are indicated. A 650-nm excitation line with laser power of 50 mW was used. The acquisition time was 1 s, and 10 accumulations were made. A complete analysis of the pz spectrum (adsorbed and in solution) can be found in refs 44−46.

the Surface Morphology. Figure 3 shows the SERS spectrum of pz adsorbed on a rough gold electrode (produced after five activation ORCs). The system is Au/ KClO$_4$, 1 mM pz; no halide is present. The normal Raman spectra (NRS) of aqueous solutions of pyrazine and its protonated forms have been studied recently.[44] The vibrational frequencies from the pz bands observed in Figure 3 are in good agreement with the frequencies

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(44) Brolo, A. G.; Irish, D. E. *Z. Naturforsch. Teil A* **1995**, *50*, 274.
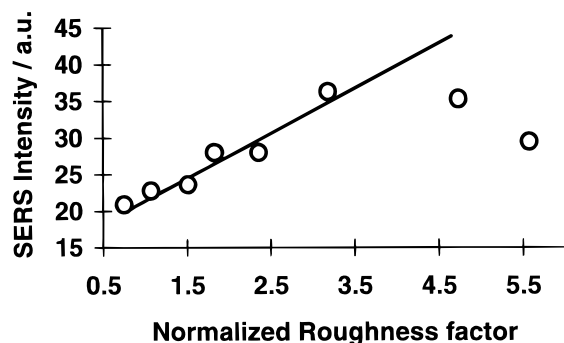
Figure 4. Dependence of the ring-breathing mode SERS intensity of pz on the normalized roughness factor (R), obtained from the AFM images.

observed in the NRS of aqueous pz.[44] The small shifts of the pz bands on adsorption suggest a weak interaction between pz and the gold surface. An extensive study of pz adsorbed from aqueous halide solutions on a silver electrode[45,46] showed that pz binds to the electrode surface via the N lone pair (end-on configuration). The relative intensities for the pz bands observed in the SERS spectrum presented in Figure 3 are similar to those observed for pz adsorbed on silver electrodes; therefore, we can conclude that pz also adsorbs on gold with the same orientation as it adsorbs on silver.

Figure 4 shows the dependence of the SERS intensity of pyrazine's ring-breathing mode, 1016 cm^{-1}, on the roughness factor (obtained for a rough gold electrode). The roughness factor was normalized by setting the value of a surface not subjected to any ORC to zero. The SERS intensities presented in Figure 4 and subsequent Figures have been normalized using the 934-cm^{-1} perchlorate stretch mode (from 0.1 M ClO$_4^-$) as an internal standard. This procedure avoids intensity changes due to laser power fluctuations. The SERS intensity increased with the roughness factor, reaching a maximum for a roughness factor value between 3 and 4 (which corresponds to a number of ORCs between 20 and 30), and decreased after the value of the normalized roughness factor reached ca. 4.5 (around 35 ORCs) (Figure 4). A similar result was obtained by Gao et al. for the adsorption of benzonitrile and thiocyanate on gold electrodes.[40] The dependence of the SERS intensity on the roughness factor must be related to the morphology of the rough gold substrate.

Figure 5a–c presents atomic force microscopy (AFM) images of the gold surface with different roughness factors. In this case, the gold electrode was roughened in 0.1 M KCl solution with the appropriate number of ORCs and transferred to the AFM apparatus. The morphology of these surfaces (observed in the air) is considered to be similar to a rough electrode immersed in aqueous solution (used in the SERS experiments). Rough gold surfaces are very stable SERS substrates,[40] which suggests high morphological stability. Therefore, we believe that it is valid to compare the SERS results (obtained using a rough gold electrode immersed in aqueous solution) to the AFM data (obtained *ex situ*).

The gold electrode with a normalized roughness factor equal to ca. 0.7 (subjected to only five ORCs) (Figure 5a) presented roughness features of the order of 40 nm. As the number of ORCs increased, the small bumps seemed

to coalesce to create bigger features. Figure 5b shows the AFM image for a gold surface after 20 ORCs (normalized roughness factor of ca. 3). The sizes of the roughness features are now of the order of 100 nm. This surface yields a strong SERS signal. The optimum 100-nm size found here for the electrochemically produced roughness is markedly larger than the 10−25-nm range obtained from UHV studies of sputtered gold films.[27] As the number of ORCs further increased, the average size of the roughness features increased. After 60 ORCs (normalized roughness factor of ca. 10), the average size of the roughness was ca. 400 nm, and the conical features were similar in size and shape. This kind of surface is not expected to be a good substrate for SERS. In fact, a very weak SERS spectrum was obtained from this substrate.

Two models are generally accepted to explain the SERS effect: the charge-transfer (CT)[5] and the electromagnetic (EM)[6] mechanisms. The CT model attributes the enhancement to the formation of a molecule−metallic adatom complex. A charge-transfer band, which has an energy similar to the photon energy of the incident light, between the molecule and the metal would increase the Raman cross section as in the resonance Raman effect.[5] The EM mechanism attributes the enhancement to the excitation of the metallic surface plasmons polaritons by the incident and the scattered photons.[6] The response of the rough metallic surface to an external electromagnetic field has been successfully described theoretically, and this EM mechanism can quantitatively predict several of the SERS features.[6]

Several pieces of experimental evidence indicate that the contribution from the CT mechanism to the SERS of pz adsorbed on a silver electrode cannot be discarded.[45−49] However, it has recently been proposed, based on the calculation of the bond polarizabilities of adsorbed pz, that the CT is not as important for pz adsorbed on gold electrodes (under 1.06-μm excitation) as it is for pz adsorbed on silver electrodes.[50] The CT mechanism does not predict a dependence of the SERS intensity on the surface morphology; however, the variation of the SERS signal with the surface roughness can be interpreted as being due to changes in the density of adatoms and, consequently, a variation in the number of adatom−molecule complexes. Unfortunately, it is very difficult to track the number density of adatoms in very rough surfaces such as the ones presented in Figure 5. Therefore, it is not possible with these data to infer the real contribution of the CT to the SERS signal.

The dependence of SERS intensity on surface morphology is predicted by the electromagnetic enhancement mechanism.[6] The enhancement factor seems to be strongly dependent on the shape of the roughness features. In fact, theorists have calculated the enhancement factor for several metallic particle morphologies.[6] These calculations have been made using various formalisms and considering both isolated and grouped spherical (or spheroidal) particles.[51−57] Several of the classical papers

(45) Brolo, A. G.; Irish, D. E. *J. Electroanal. Chem.* **1996**, *414*, 183.

(46) Brolo, A. G.; Irish, D. E. In *Solid−Liquid Electrochemical Interfaces*; Jerkiewicz, G., Soriaga, M. P., Uosaki, K., Wieckowski, A., Eds.; ACS Symposium Series No. 656; American Chemical Society: Washington DC, 1997, Chapter 22, p 310.
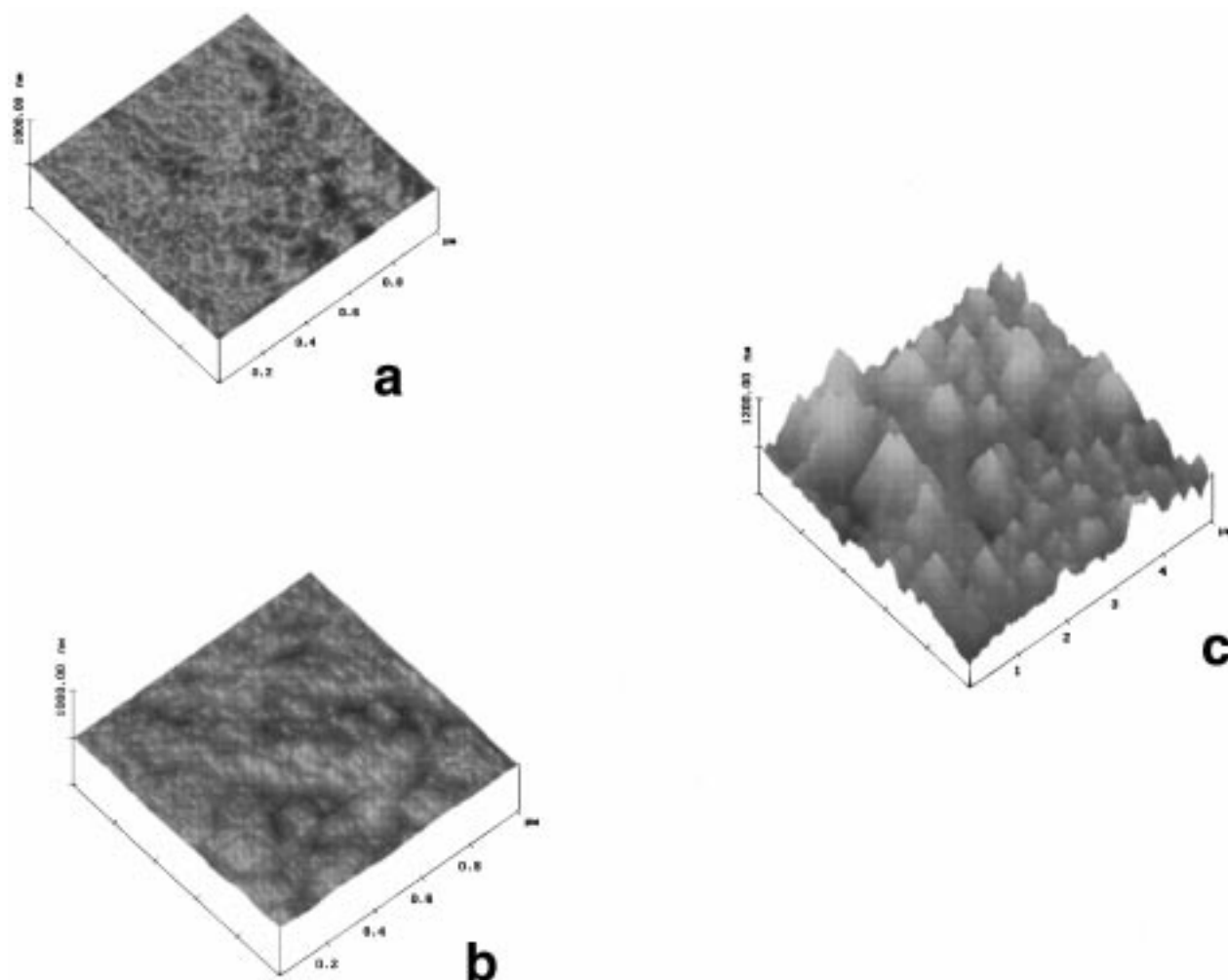
(47) Arenas, J. F.; Woolley, M. S.; Otero, J. C.; Marcos, J. I. *J. Phys. Chem.* **1996**, *100*, 3199.

(48) Huang, Y.; Wu, G. *Spectrochim. Acta* **1990**, *46A*, 377.

(49) Thietke, J.; Billmann, J.; Otto, A. In *Dynamics on Surfaces−Proceedings of the XVII Jerusalem Symposium on Quantum Chemistry and Biochemistry*; Pullman, B., Jortner, J., Nitzan, A., Gerber, B., Eds.; Reidel: Jerusalem, 1984; Vol. 17, p 345.

(50) Zhong, F.; Wu, G. *J. Mol. Struct.* **1994**, *324*, 234.

(51) Kerker, M.; Wang, D.-S.; Chew, H. *Appl. Opt.* **1980**, *19*, 4159.

(52) Takemori, T.; Inoue, M.; Ohtaka, K. *J. Phys. Soc. Jpn.* **1987**, *56*, 1587.

(53) Zeman, E. J.; Schatz, G. *J. Phys. Chem.* **1987**, *91*, 634.

(54) Aravind, P. K.; Nitzan, A.; Metiu, H. *Surf. Sci.* **1981**, *110*, 189.

(55) Xu, M.; Dignam, M. J. *J. Chem. Phys.* **1993**, *99*, 2307.

Figure 5. AFM images of rough gold electrodes. (a) $R = 0.7$ (five ORCs); (b) $R = 3.1$ (20 ORCs); (c) $R = 10.5$ (60 ORCs).

in SERS, including the earliest theoretical works, can be found elsewhere.[58] Accurate calculations have been recently performed using the discrete dipole approximation (DDA) method to determined Raman intensities from small metal particles of arbitrary shape.[59] It also has been shown that the distance between the surface features is an important factor (together with their size and geometry), since contributions from surface plasmon modes localized between the particles is a major factor for the overall effect.[60] A million times enhancement in the Raman signal was calculated for the py ring-breathing signal from a "small" (radius $<<$ incident light) silver sphere.[51] A peaked excitation profile (enhancement factor vs excitation wavelength plot) was obtained for such a small particle (radius $\approx$ 5 nm) due to a dipolar response from the metallic sphere. The superposition of multipolar fields broadens the excitation profile as the radius of the metallic particle increases.[51] The contribution of these high-order dipoles also decreases the magnitude of the maximum delocalized Raman enhancement factor by several orders of magnitude. The dependence of the enhancement factor on the radius of the spherical particle is not as large for gold as it is for silver.[61] The enhancement

factor from a 1010-cm^{-1} vibration (close to the pz ring-breathing frequency at ca. 1016 cm^{-1}) of a molecule adsorbed on a spherical gold particle is also dependent on the radius of the metallic particle.[61] Considering an excitation wavelength equal to 650 nm (the same excitation wavelength used in this work), the calculated enhancement factor would be approximately 100 for 5-nm-radius gold spheres.[61] This enhancement factor increases to almost 1000 as the radius increases to 50 nm.[61] However, 500-nm gold spheres give an enhancement factor of only 10, approximately.[61] These calculated results agree well with the experimental data presented in Figures 4 and 5. Hence, even these simple EM calculations, considering just the delocalized resonances, are enough to explain the qualitative trend of the dependence of the SERS intensity on surface morphology.

Dependence of the SERS Intensities of pz Adsorbed to an Unroughened Polycrystalline Gold Electrode on Surface Coverage. Figure 6 shows the dependence of the relative integrated intensity of the ring-breathing SERS band of pyrazine, 1016 cm^{-1}, adsorbed on an unroughened gold electrode, on the surface coverage (determined independently by electrochemical methods[37]), for several pyrazine concentrations. A reasonably linear relationship between SERS intensity and surface coverage was obtained for the most dilute pz solution ([pz] = 5.50 $\times$ 10^{-5} M). The SERS intensities presented in Figure 6 for other pz concentrations (concentrations of pz equal to

(56) Barber, P. W.; Chang, R. K.; Massoudi, H. *Phys. Rev. B* **1983**, *27*, 7251.

(57) Wang, D.-S.; Kerker, M. *Phys. Rev. B* **1981**, *24*, 1771.

(58) *Selected Papers on Surface-Enhanced Raman Scattering*; Kerker, M., Ed.; SPIE Milestone Series; SPIE: 1990; Vol. MS10.

(59) Yang, W-H.; Schatz, G. C.; Van Duyne, R. P. *J. Chem. Phys.* **1995**, *103*, 869.

(60) Garcia-Vidal, F. J.; Pendry, J. B. *Phys. Rev. Lett.* **1996**, *77*, 1163.
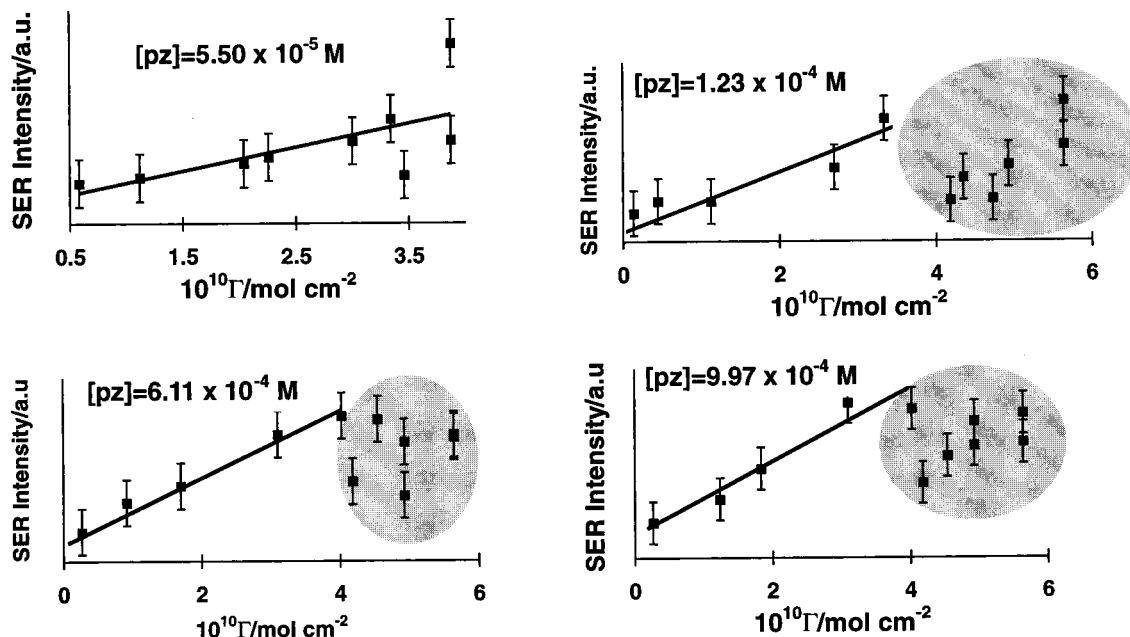
(61) Kerker, M. *J. Opt. Soc. Am. B* **1985**, *2*, 1327.

Figure 6. Dependence of the SERS intensity of pz adsorbed on an unroughened gold electrode on the surface concentration (Γ). The concentration of pz in solution is indicated on each plot. A fairly linear relationship between SERS intensity and surface coverage is observed for low surface coverage. The shadowed areas indicate the region where the linearity breaks down and the points become scattered. The error bars indicate the uncertainty of the area of the bands determined by the band-fitting procedure. These errors range from ca. 20% of the intensity value (for the weak peaks, at low surface coverage) to 5% (for the strong peaks, at high surface concentration of pz). The SERS spectra were obtained using a 650-nm excitation line with 50 mW of power. The spectral slit was ca. 3 cm^{-1}. The integration time was 2 s, and 100 accumulations were made.

1.23×10^{-4}, 6.11×10^{-4}, and 9.97×10^{-4} M) fail to track the surface coverage at surface concentrations higher than ca. 4×10^{-10} mol/cm^2 (inside the shadowed area in Figure 6). The same behavior has been observed for pyridine adsorbed on polycrystalline gold electrodes[36] and other systems such as cyanide adsorbed on silver island films.[62] The decrease of the SERS intensity as the adsorbed molecular dipoles approach each other (high surface coverage) can be explained using electromagnetic arguments.[63–65] As the surface coverage approaches the monolayer value, each adsorbed molecule is subjected to depolarizing fields from its oscillating neighbors.[66] This depolarizing effect decreases the magnitude of the local electromagnetic field responsible for the enhancement. Figure 6 also shows that, despite the linearity between SERS intensity and surface coverage observed for all pz concentrations (up to two-thirds of a monolayer), the slopes of the SERS intensity vs surface coverage plots increase marginally as the solution is made more concentrated in pz. The linearity was also observed for pyridine adsorbed on polycrystalline gold,[36] indicating that the SERS intensity increases with the increase of the number of molecules on the electrode surface, but the rate of this increase depends on both the pz concentration and the activation procedures.

Figure 7a–d shows the dependence of both the SERS intensity of pz adsorbed on an unroughened polycrystalline gold electrode and the surface coverage (from ref 37) on the concentration of pz in solution, for four selected potentials. The surface concentration (Γ) of pz adsorbed on gold becomes independent of the amount of pz in solution. On the other hand, the SERS intensities increase with the solution concentration of pz for all potentials

(Figure 7). The "cleaning" ORCs, used to prepare the unroughened gold electrode, are not expected to cause any drastic change in the surface morphology. In fact, only a few tenths of nanometers of the gold surface is removed during the positive sweep.[67] Therefore, the SERS intensity increases with the solution concentration (at these relatively high concentrations) when there is no change in the surface concentration for these unroughened surfaces.

For several systems, it has been observed that the SERS intensities obtained after activation ORCs performed in the presence of the organic adsorbate (or halides) are higher than when the electrode is activated in the absence of adsorbates.[68] However, the activation in the presence of the adsorbate may lead to erroneous results.[68,69] Based on these works, a tentative explanation for the increase of the SERS intensity with the solution concentration for this unroughened substrate is given in Figure 8. During the cleaning ORC's positive sweep, in the presence of the organic compound, a complex may be formed between the metal's oxidation products and the organic substrate (Figure 8a and 8b). A higher number of these complexes will be formed in a solution more concentrated in the organic substrate (Figure 8e and 8f). The complex (metal cation–organic molecule) may be reduced during the cathodic part of the sweep, together with the "free" cation (Figure 8c and 8g). The metal–organic molecule system, formed on the surface during the electrochemical reduction process, decreases the mobility of the adatoms, creating "stable" active sites. Thus, the incorporation of the metallic adatoms into the metallic lattice is hindered.[70] The metallic deposit, which is not further stabilized by

(62) Murray, C. A.; Bodoff, S. *Phys. Rev. B* **1985**, *32*, 671.
(63) Chew, H.; Wang, D.-S.; Kerker, M. *Phys. Rev. B* **1983**, *28*, 4169.
(64) Arunkumar, H. A.; Bradley, E. B. *J. Chem. Phys.* **1983**, *78*, 2882.
(65) Xu, M.; Dignam, M. *J. Chem. Phys.* **1994**, *100*, 197.
(66) Murray, C. A.; Bodoff, S. *Phys. Rev. Lett.* **1984**, *52*, 2273.

(67) Dickertmann, D.; Schultze, J. W.; Vetter, K. W. *J. Electroanal. Chem.* **1974**, *55*, 429.
(68) Beer, K. D.; Tanner, W.; Garrell, R. L. *J. Electroanal. Chem.* **1989**, *258*, 313.
(69) Brolo, A. G.; Irish, D. E. *J. Chem. Soc., Faraday Trans.* **1997**, *93*, 419.
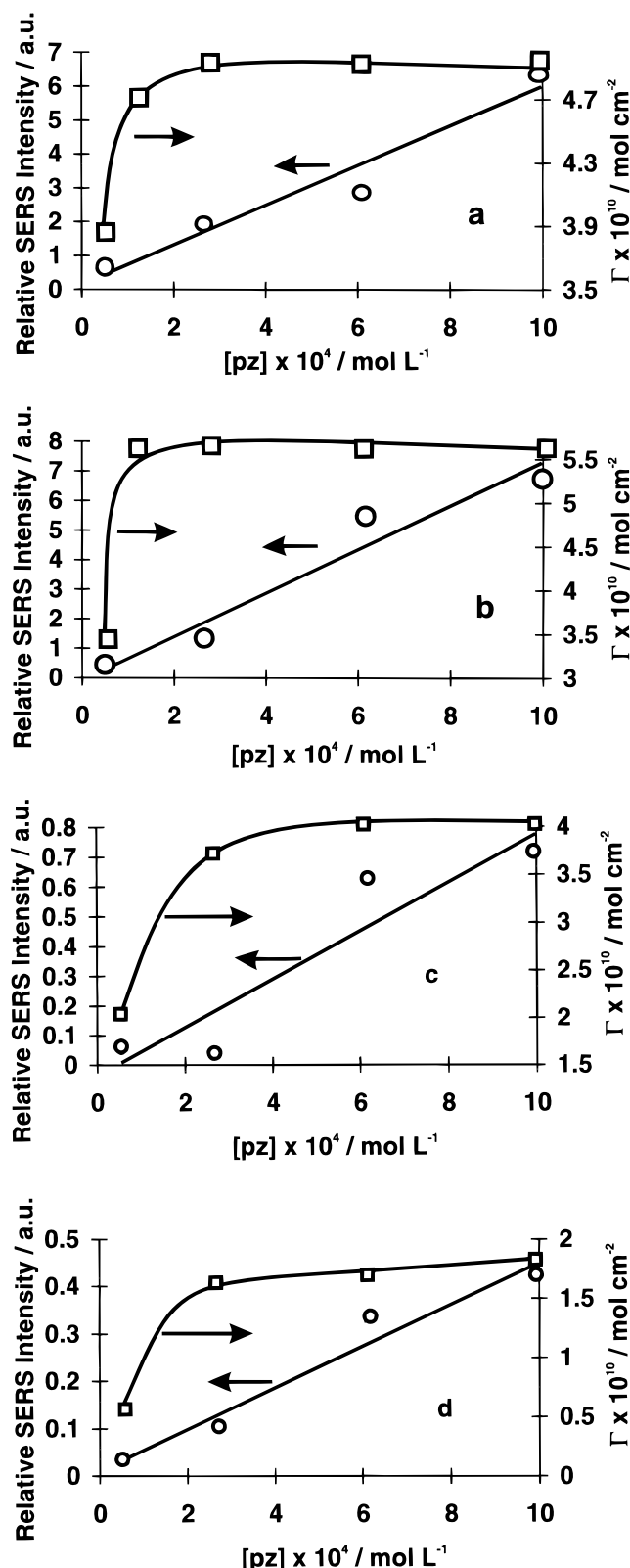(70) Sobocinski, R. L.; Pemberton, J. E. *Langmuir* **1988**, *4*, 836.

Figure 7. Dependence of both the SERS intensity (from an unroughened gold electrode) and the surface coverage on the solution concentration of pyrazine. (a) +350 mV; (b) +150 mV; (c) −150 mV; (d) −450 mV.

the organic molecule, is "unstable" and will be incorporated into the lattice. When the equilibrium between the amount of adsorbate in solution and on the metal is established, the same amount of adsorbate is present on the surface for both concentrations, because, as was

demonstrated by Figure 7, the surface concentration is independent of the solution concentration at all potentials, for the studied solutions. However, the most concentrated solution may have more organic molecules adsorbed on stable active sites (Figure 8h) than the diluted solution (Figure 8d), and, therefore, the SERS spectrum obtained for molecules adsorbed from more concentrated solutions is expected to be more intense. This model implies a distinction between adatom sites (i.e., SERS-active sites) and the total surface area available for electrochemical pz adsorption.

Attempts to obtain the SERS spectra by electrochemically cleaning the electrode in aqueous perchlorate solution and posterior adding pz (*ex situ* activation procedure) were unsuccessful. This fact indicates that the active sites formed by the mechanism presented in Figure 8 are essential to produce the Raman scattering enhancement from an unroughened gold surface.

Finally, the results demonstrate that, for the unroughened gold surfaces, the SERS intensities are indeed proportional to both the surface coverage (up to two-thirds of a monolayer) (Figure 6) and the solution concentration of the organic adsorbate (Figure 7). These results suggest that SERS can be applied as an analytical tool to determine the concentration of organic compounds in dilute solution;[71] however, one must be aware that different results may be obtained for different preparations of the substrate.[72]

Dependence of the SERS Intensities of pz Adsorbed on a "Smooth" Polycrystalline Gold Electrode on Surface Concentration. A poor relationship between SERS intensity and surface coverage was found in our previous work[36] for py adsorbed on a rough polycrystalline gold surface (prepared by activating the gold in KCl solution—see Experimental Section). The potential profiles were dependent on the number of ORCs and, consequently, on the surface morphology. We found, qualitatively, the same problems for the adsorption of pz on rough gold electrodes. Possibly, the lack of relationship occurs because we are comparing data obtained from a rough surface (SERS intensities) with data obtained from a smooth surface (surface coverage). In order to minimize this problem, we developed a procedure to calculate the SERS intensity of a smooth surface from the data obtained for a rough surface. The procedure consists of the following steps:

• The SERS spectrum was obtained for different numbers of ORCs.

• The area under the pyrazine ring-breathing mode (ca. 1016 cm^{-1}) was calculated for different applied potentials and normalized using the 934-cm^{-1} band from the aqueous perchlorate as an internal standard.

• As we observed in Figure 4, there is a linear relationship between the SERS intensity and the roughness factor (or cathodic charge). Therefore, plots were made of the SERS intensity vs the cathodic charge (the cathodic charge is proportional to both the number of ORCs and the normalized roughness factor (Figures 2 and 5)) for different potentials (Figure 9).

• The SERS intensity vs roughness plots for each potential (Figure 9) were extrapolated to zero roughness, and thus, the SERS intensity from a gold electrode not subjected to any ORC was graphically evaluated from the intercept.

Figure 10 shows the extrapolated SERS intensity plotted against the electrode potential. The surface coverage of

(71) Om-Apinyan, S.; Rajmankina, T.; De La Cruz, C.; Jayasooriya, U. A. *Spectrochim. Acta A* **1997**, *53*, 877.

(72) Xue, G.; Lu, Y.; Gao, J. *Appl. Surf. Sci.* **1994**, *78*, 11.
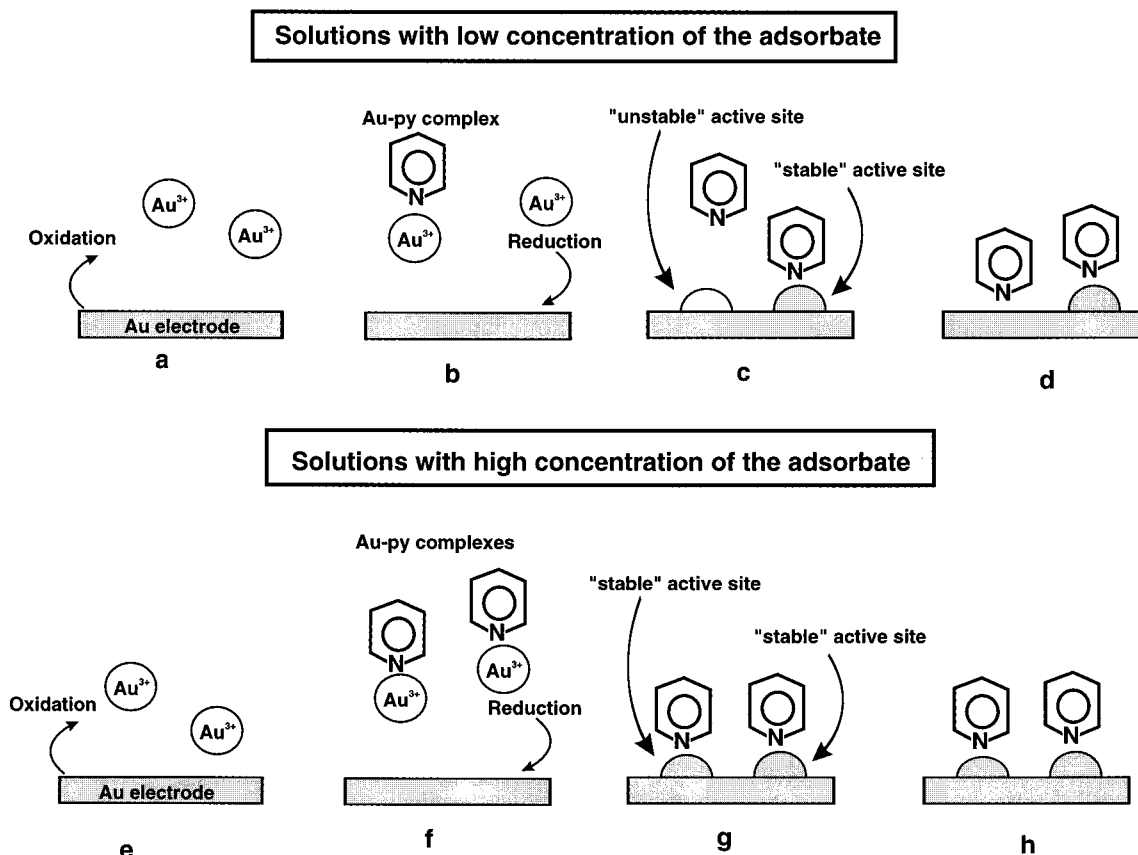
Figure 8. Effect of the solution concentration of the adsorbate (pyridine is used as an example; the same principle is valid for other organic molecules) on the SERS intensity. For solutions with low concentration of py: (a) Gold ions are formed during the oxidation process. (b) The gold ion in solution forms a complex with py. However, the solution concentration of py is small, and some gold remains free. (c) Adatoms are formed on the reduction process. The adatom formed from the complex is stable and has no mobility. The adatom formed from the free cation is unstable and can be incorporated into the lattice. (d) Consequently, few molecules are adsorbed on the SERS-active sites. For solutions with high concentrations of py: (e) The gold ions are formed. (f) Higher amounts of Au–py complexes are formed. (g) The number of stable active sites formed during the reduction process is also higher. (h) The same surface concentration as in d should yield a higher SERS signal, because more molecules are now adsorbed on stable active sites.
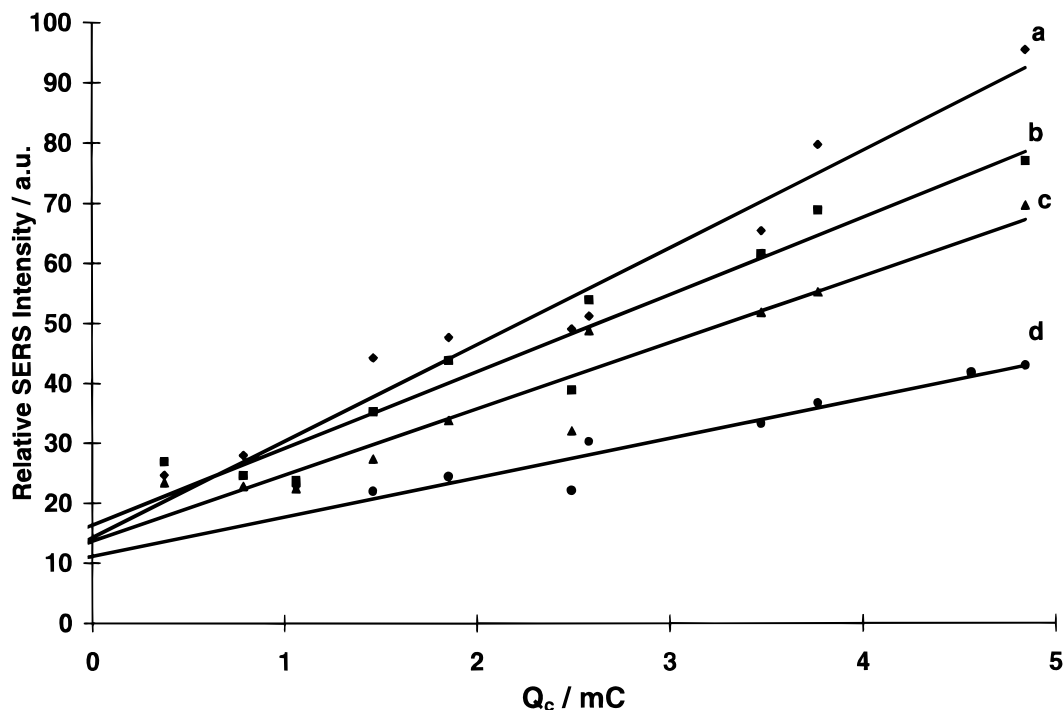

Figure 9. Dependence of the SERS intensity of pz on the cathodic charge (proportional to the surface roughness). (a) +450 mV; (b) +150 mV; (c) −150 mV; (d) −550 mV. The SERS spectra from a rough surface were obtained using a 650-nm excitation line at 50 mW. The integration time was 1 s, and 10 accumulations were made. The spectral slit width was ca. 3 cm^{-1}.
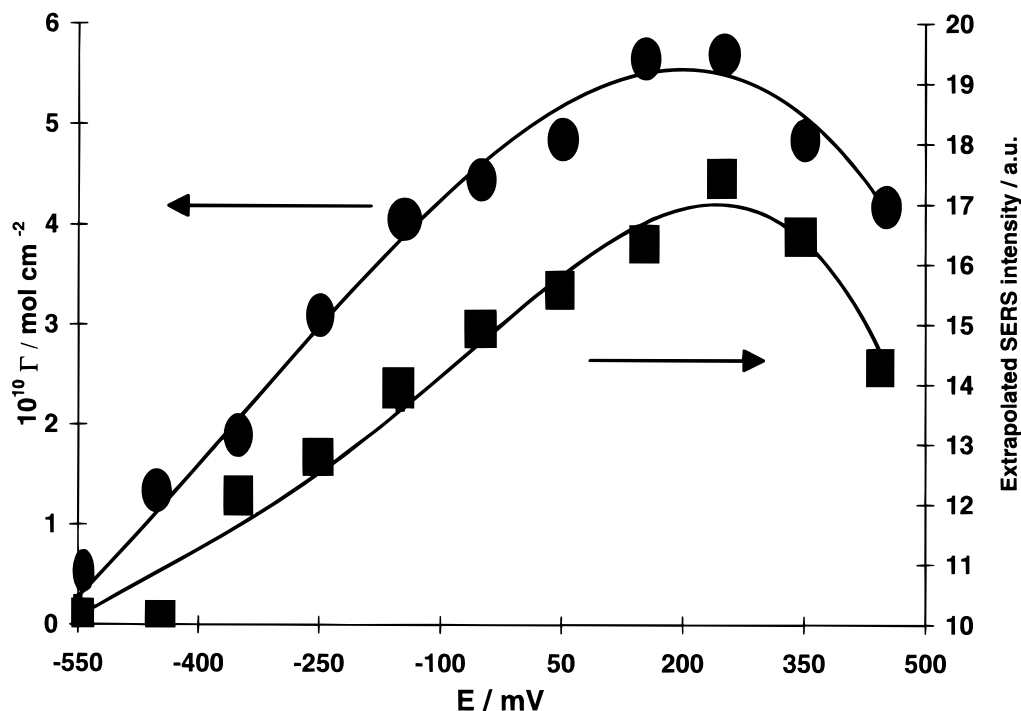
Figure 10. Surface concentration of pz vs potential (from ref 37) and extrapolated SERS intensity of the pz ring-breathing mode (SERS intensity at zero ORC, calculated from Figure 9) vs potential.
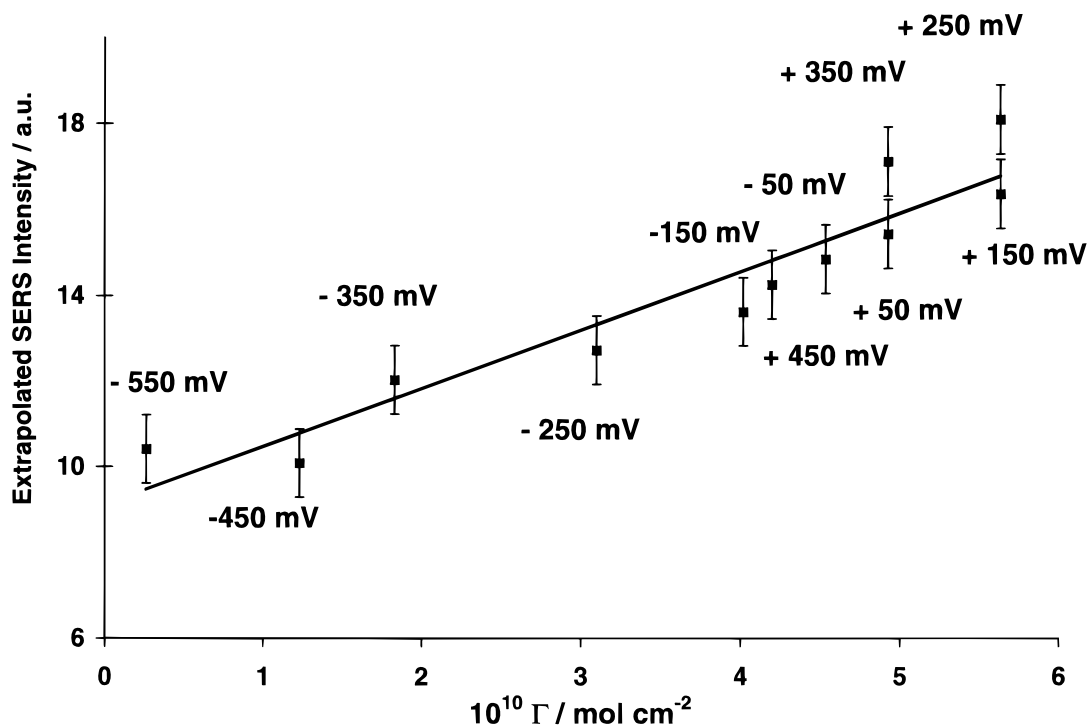

Figure 11. Dependence of the extrapolated SERS intensity of the ring-breathing mode of pz on the surface coverage. The applied potentials are indicated on the graph.

pyrazine adsorbed on a gold electrode, obtained from ref 37, is also shown in Figure 10. The striking resemblance between the two curves presented in Figure 10 is confirmed in Figure 11, where the extrapolated SERS intensity is plotted against the surface coverage for each potential. The extrapolated SERS intensity tracks the surface coverage over all the range of potentials.

One may be surprised by the observation that the extrapolated SERS intensities (for a smooth surface) are only 1 order of magnitude lower than the optimal SERS signal obtained from a rough surface (Figure 9). This

result raises questions about the physical significance of our procedure and requires comments.

It is not possible to excite the surface plasmons directly from a smooth surface due to a momentum mismatch between the incident photon and the surface resonances;[2] therefore, a direct verification of the magnitude of these extrapolated SERS intensities cannot be obtained. However, it has been demonstrated, from the observation of SERS spectra using silver-deposited films over polymer nanospheres,[24] for instance, that the electromagnetic enhancement factor can be partitioned into different

contributions from localized and delocalized surface plasmon excitations. Considering an overall enhancement of 10^7 from these silver-coated particles, it was proposed that a factor of ca. 10^2 was dependent on the size of the nanospheres.[24] The roles of these localized and delocalized surface plasmon contributions have been further confirmed recently.[73] The SERS spectra from single silver particles (ca. 1-μm diameter) were observed.[73] Additional enhancement was obtained from a cluster of such particles, indicating that the interactions between the surface features are important factors for the overall enhancement. The dependence of the SERS intensities on the surface roughness (presented in Figure 9) indicates that even a mild rough gold surface presents a large enhancement factor (only ca. 1 order of magnitude smaller than the "optimal" value). This enhancement may contain large contributions due to random substructure roughness which supports localized surface plasmon resonances. As the sizes of the surface roughness increase (and the SERS intensities also increase), the contribution from the delocalized surface plamons polaritons becomes more significant. This additional enhancement, due to delocalized resonances, is ca. 10^2 for silver;[24] hence, it is not unreasonable to observe that its contribution in a rough gold electrode is 1 order of magnitude.

Conclusions

We have presented the dependence of the SERS intensities of pz adsorbed on a polycrystalline gold electrode on both the surface coverage and on the surface morphology.

The SERS intensities from rough gold electrodes increase with the surface roughness, reaching a maximum at ca. 25 ORCs. AFM pictures showed that the size of the surface roughness features increases with the number of ORCs, and surface features of ca. 100 nm yielded a strong SERS signal. A weak SERS response was observed from surfaces with roughness features of ca. 400 nm. These results are consistent with the expected enhancement factor calculated for gold spheres with different sizes.

The SERS technique proved to be useful for the determination of the amount of pz adsorbed on a low coverage surface (surface concentrations smaller than 4×10^{-10} mol cm^{-2}) of unroughened polycrystalline gold electrodes. As the surface concentration approaches one monolayer, the SERS signal decreases, due to the depolarizing effects on the local electromagnetic field from neighboring dipoles. The cleaning procedure (ORCs in the presence of the organic molecule), used in the preparation of the unroughened gold electrode, may increase the contribution from the adatom−molecule complex to the overall enhancement. The amount of these complexes is dependent on the solution concentration of the organic adsorbate. This effect can be useful in the determination of the amount of analyte in solution using SERS.

The dependence of the potential profile of the SERS intensities on the surface roughness has also been investigated. The extrapolated SERS intensity (for no ORCs) is directly proportional to the surface coverage for a wide range of potentials.

(73) Xiao, T.; Ye, Q.; Sun, L. *J. Phys. Chem.* **1997**, *101*, 632.