

Surface-Enhanced Infrared Absorption of *p*-Nitrobenzoic Acid on Planar Silver Halide Fiber

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Surface-enhanced infrared absorption (SEIRA), recently discovered for trypsin on a planar silver halide surface, is now demonstrated for the small organic molecule, *p*-nitrobenzoic acid (pNBA). Evaporation of pNBA solutions in acetonitrile yields the acid dimer (confirmed by the unique IR signature of carboxylic acid dimers). The absorption changes for pNBA with quantity exhibit two regimes: “enhanced” and “regular”. A plot of absorbance versus quantity reveals how SEIRA changes with distance from the surface. The surface orientation of pNBA has the long and short axes (unit cell) parallel to the surface (packing density/carbonyl peaks). “Slice” spectra differentiate between surface pNBA and other layers. Most of the numerous previous reports on pNBA actually refer to the *p*-nitrobenzoate anion probably generated through reaction of surface silver oxide with the acid.

The recent discovery of surface-enhanced infrared absorption (SEIRA) in addition to the expected amplification for trypsin on a planar silver halide surface¹ stimulated us to extend the use of our technique to small organic molecules. A substantial fraction of the reports on SEIRA involved the study of the small polar molecule *p*-nitrobenzoic acid (pNBA)^{2–7} because of its use in the paper⁸ reporting the discovery of SEIRA. We were thus encouraged to find out if pNBA displayed SEIRA on planar AgX fibers. Most of the numerous previous studies on pNBA actually refer to the *p*-nitrobenzoate anion generated by “surface ionization” [reaction of the acid with silver to yield hydrogen and the anion,² water-promoted dissociation in the presence of Ag,^{3,4} “chemisorption” on the Ag surface,⁹ or unspecified]. Anion formation probably results from the reaction of silver oxide in the surface^{10,11} with the acid. The anion forms even on gold¹² and lead.¹³ If the pNBA is too far from the surface to react, then the dimer (labeled as the acid) is detected.^{5,14,15} The present study using the planar AgX fiber has led to five important results: (1) The acid (as the dimer) and NOT the anion is readily observed for pNBA, (2) the acid dimer exhibits SEIRA and may be observed in less than monolayer amounts, (3) the absorption changes for pNBA (dimer) with quantity can be divided easily into two regimes (“surface-enhanced” and “regular”), (4) a plot of absorbance versus quantity reveals how SEIRA changes with distance from the surface, and (5) the carbonyl IR bands of the dimer change with quantity. Results showing SEIRA for a small nonpolar molecule, *n*-dodecane, will be reported separately.

Materials and Methods

The fiberoptic IR cell was originally developed^{16,17} to study the spectra of materials deposited on a cylindrical AgX fiber [AgX, usually AgBr:AgCl, 0.6:0.4].^{18,19} The development of a flattened fiber²⁰ promised substantial sensitivity gains, which were realized with trypsin along with the surprising discovery of SEIRA.¹ A schematic of the planar AgX fiber next to the

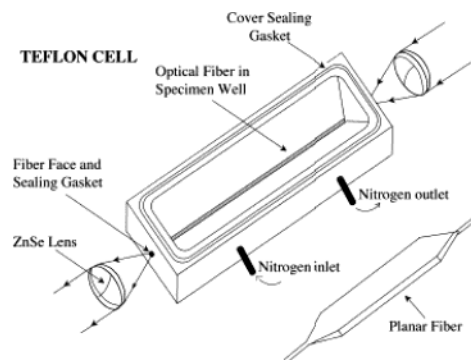


Figure 1. Schematic drawing of the Teflon-lined cell described in the text. A drawing of the planar fiber (not to scale) is shown at the right.

cell into which it is placed is shown in Figure 1. Planar portion: *l* 3.9–4.0 cm, *w* 0.4 cm, *d*, 155–220 μm . Cylindrical portion: *d*, 0.9 mm. In the present work, planar fibers with a thickness of either 175 or 180 μm have been used.

Acetonitrile (HPLC—Spectroquality, BioLabs, Jerusalem) and *p*-nitrobenzoic acid (pNBA) (Sigma-Aldrich) were used without further purification.

In a typical experiment (aliquot to contain 25 ng), a sample (1 mg) of pNBA is weighed and dissolved in 20 mL of acetonitrile. The solution (3 mL) was mixed with 21 mL of acetonitrile. From the latter solution, an aliquot of 4 μL is distributed (11–12 drops) with an Eppendorf pipet as uniformly as possible on the planar portion of the fiber. After the cell is closed, the sample is exposed to a slow flow of dry N_2 (1 mL/min) that has been passed through a purification train of calcium chloride, dried molecular sieves 4A, and silica gel containing cobalt indicator. The flow is metered by a rotaflow valve. Each addition required that the cell be briefly opened, the sample added, and the cell closed again.

The FTIR spectrometer is an Bruker Equinox 55/S equipped with two sources [Globar, mid-IR (MIR); tungsten, near IR (NIR)], two beam splitters [KBr, MIR; CaF_2 , NIR] and two detectors [DTGS, MIR; TeInAs, Peltier-cooled, NIR]. Only the MIR system was used in the present research. Infrared spectra

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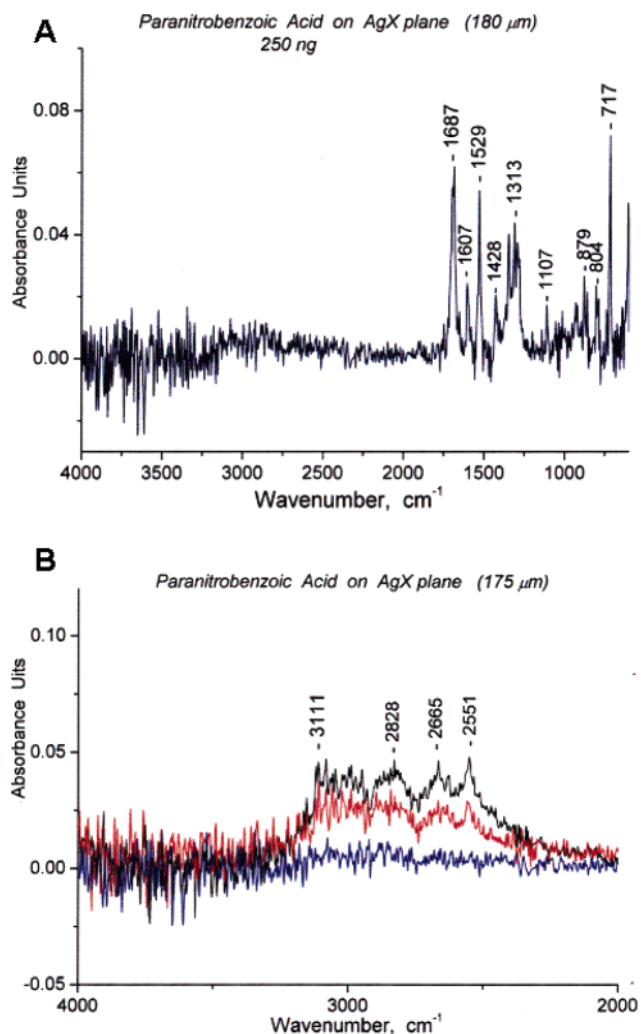


Figure 2. (A) IR spectrum of 250 ng of pNBA dimer deposited from an acetonitrile solution. (B) IR spectrum of the dimer in the OH region, characteristic of carboxylic acid dimers, for 250 ng, 1 μ g, and 10 μ g.

are recorded (300 scans) continuously over the range from 7000 to 600 cm^{-1} at a resolution of 4 cm^{-1} . The removal of the acetonitrile is followed in the MIR and deemed to be complete when further changes in the spectrum are not observed over 10 min (ca. 20–30 min). After baseline smoothing, the data were transferred to the Origin 6.1 program for analysis, spectral displays, and plotting.

Results

We have succeeded, apparently for the first time, in observing a monolayer or less of the acid (as the dimer). The simple procedure of depositing pNBA through evaporation of acetonitrile solutions on the planar silver halide element leads to the acid dimer, using amounts between 25 ng and 15 μ g. The maximum for the carbonyl band is found at 1687 cm^{-1} , a position clearly distinct from that of a monomeric aromatic carboxylic acid near 1740 cm^{-1} . Although dimerization of carboxylic acids is a well-known process,²¹ the differences in the position of the monomer and dimer maxima are unfamiliar to many chemists. Irrefutable evidence for the carboxylic acid dimer comes from the broad OH stretching band region (2500–3500 cm^{-1}) characteristic of such dimers.^{22–24} The IR spectrum of 250 nanograms of pNBA deposited from an acetonitrile solution is shown in Figure 2A. The spectrum covers the wavelength range between 4000 and 600 cm^{-1} , a range

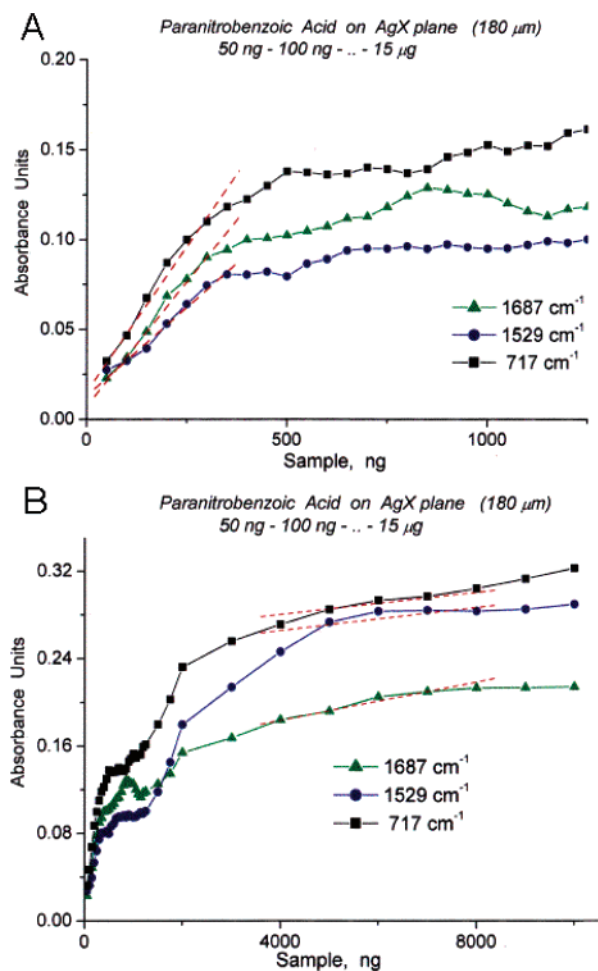


Figure 3. (A) Absorbances (A) for quantities (Q) (50–1250 ng) versus quantity. The curves are smoothed with adjacent averaging smoothing. The points for the “enhanced” range between 50 and 300 ng are correlated by lines with the parameters $A_{1687} = 0.00721 + 2.76929 \times 10^{-4}Q$, $A_{1529} = 0.01301 + 2.197 \times 10^{-4}Q$, and $A_{717} = 0.01516 + 3.23929 \times 10^{-4}Q$. (B) Absorbances (A) for quantities (Q) between 50 and 10000 ng versus quantity. The points from Figure 2A are included to illustrate the relationship to those from microgram amounts. The points for the “regular” range between 4000 and 8000 ng are correlated by lines with the parameters $A_{1687} = 0.1484 + 8.8 \times 10^{-6}Q$, $A_{1529} = 0.2446 + 5.3 \times 10^{-6}Q$, and $A_{717} = 0.26 + 5.1 \times 10^{-6}Q$.

substantially greater than most previous reports. The unmistakable IR signature of a carboxylic acid dimer in the OH stretching region is shown for 250 ng, 1 μ g and 10 μ g in Figure 2B.

Four series of pNBA measurements were made, as follows: (1) initial 25 ng, increment 25 ng, final 700 ng; (2) initial 50 ng, increment 50 ng, final 1400 ng; (3) initial 50 ng, increments (a) 50 ng (to 1250 ng), (b) 250 ng (1250 to 2000 ng); (c) 1000 ng (2000 to 15000 ng); (4) initial 1 μ g, increment 1 μ g, final 12 μ g. Results from (3) are illustrated with plots of the absorbance at three different wavelengths versus quantity (50–1250 ng, Figure 3A; 0–10000 ng, with focus on the range from 1500 to 10000 ng, Figure 3B). Plots were smoothed by adjacent averaging smoothing. The wavelengths were selected on two grounds, first because of their chemical significance (C=O group), and second, because the peaks were reasonably strong and well defined. The plots in Figure 3A show a strong rise with increasing quantity up to 300 ng and must be due to the effects of surface enhancement. In contrast, the absorbance changes for quantities “far” from the surface (4000–8000 ng) are much smaller. Reasonably good lines can be drawn through the points for these ranges. The ratio (R) of the slopes for the

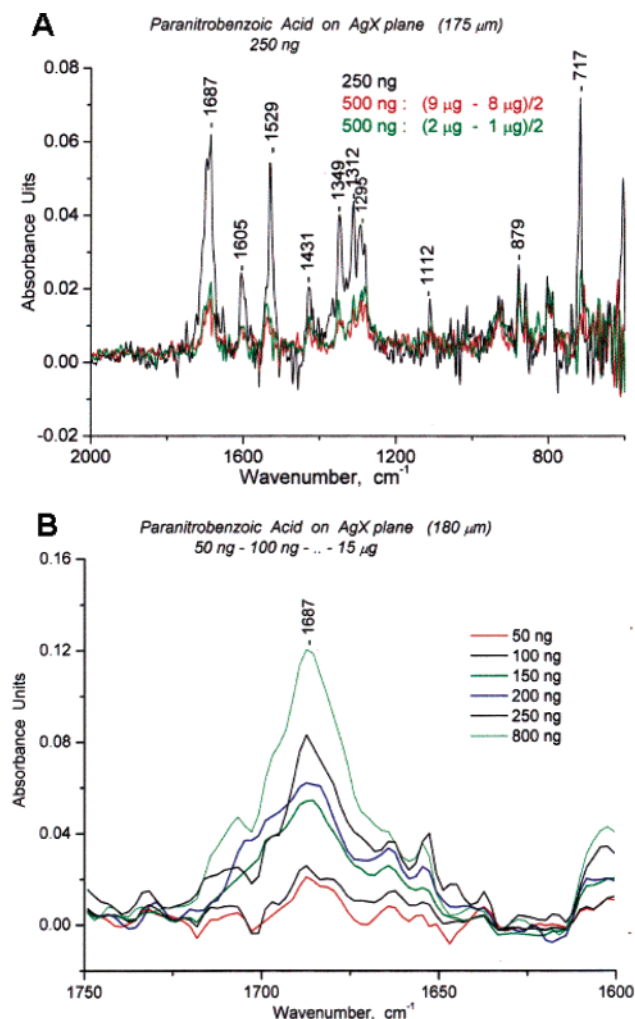


Figure 4. (A) Spectra of “slices” of surface 250 ng and two of 500 ng removed from the surface. The substantial difference between the spectrum for the “slice” on the surface and those at a distance from the surface is due to SEIRA, because all of the sample would be affected by the amplification over that for a cylindrical fiber (see ref 1) due to the increased number of internal reflections in the planar fiber. (B) Absorption region between 1650 and 1750 cm^{-1} having multiple maxima, with carbonyl peaks probably occurring between 1679 and 1715 cm^{-1} . For 50 ng there are two of similar magnitude at 1679 and 1687 cm^{-1} . The former grows until about 150 ng, whereas the 1687 cm^{-1} band grows as a combination of two or three peaks until 800 ng. A band near 1655 cm^{-1} grows steadily with amount.

small and large quantity lines is a measure of the enhancements and are $R_{1687} = 31.5$, $R_{1529} = 37.2$, and $R_{717} = 63.5$. Averages of all of the slopes for the different series lead to $R_{\text{av}1687} = 42.9$, $R_{\text{av}1529} = 28.7$, and $R_{\text{av}717} = 6.2$. It appears that the intensity of the 717 cm^{-1} peak may be more sensitive than the others to some external influence. Because there are no obvious wavelength effects on the spectra, we cannot conclude at this time that such effects do or do not exist. It seems clear that the enhancements are in the range 30–40, as has been found for SEIRA in other cases. For trypsin¹, the same procedure affords $R_{1638} = 8.35$ and $R_{1540} = 10.5$. Preliminary data for dodecane (Kosower, Markovich, and Borz, unpublished results) suggest that $R_{2924} = 25.7$.

The enhancements can be exhibited in a dramatic way by “slice” spectra, in which we compare the spectrum of a surface layer (250 ng is close to a full monolayer on our planar element) to the spectra of small amounts removed from the surface. Such spectra are shown in Figure 4A for a series in which the

enhancements were somewhat smaller; even so, it was necessary to use 500 ng “slices” to make the spectra readily visible. The “slices” were generated by subtracting the spectrum for 8 μg from that for 9 μg and that for 1 μg from that for 2 μg , then dividing the differences by 2. It is clear that most, but not all, of the SEIRA effect is gone at the level of 2 μg . A beautiful example of “slice” spectra for methanol at 124 K has been reported.²⁵

Discussion

The initial paper for SEIRA⁸ reported a spectrum for pNBA, but this was later found to be hydrocarbon, probably from pump oil.² a conclusion confirmed by others.^{5,6} Some of the important results noted above are discussed below.

Surface Enhanced Infrared Absorption of *p*-Nitrobenzoic Acid Dimer. The acid (as the dimer) and NOT the anion is readily observed for pNBA in contrast to most previous reports. The surface-enhanced infrared absorption spectrum of *p*-nitrobenzoic acid on planar AgX is reasonable in quality considering that only 250 ng (approximately a monolayer) of sample is present. Enhancement is established by the three criteria introduced previously:¹ (a) absorbance greater than that expected for pNBA (Figure 3A) on the basis of measurements made for samples “far” from the surface (Figure 3B); (b) an analysis of the increase in absorbance with quantity that show the enhanced absorbance is maximized for about one monolayer of sample (300 ng) (Figure 3A); (c) absence of a strong wavelength dependence in the spectrum.

Spectra of *p*-Nitrobenzoic Acid Dimer Observed in Less Than Monolayer Amounts. Many spectra within the four series of measurements were taken using less than a monolayer of dimer. As an example, the region from 1650 to 1750 cm^{-1} is shown in Figure 4B.

Two Spectroscopic Regimes for pNBA on AgX. The plots of absorbance versus quantity suggest that two regimes of absorbance change may be distinguished by the slopes of linear correlations, “enhanced” and “regular” (Figure 3A,B). The plots constitute an experimental measurement of how the SEIRA effect on AgX changes with distance from the surface.

The crystal structure of pNBA [unit cell ($12.97 \times 5.07 \times 21.43$ Å, $Z = 8$)]^{26,27} shows two parallel stacks of dimers at right angles to one another. The pNBA dimer molecule will deposit on the surface as scattered molecules, shifting to organized (crystalline) as the monolayer grows. The initial orientation of the dimer to the surface may present the “face” or the “side” of the dimer, but probably not the “end” (the nitro group). To be organized as a monolayer, “side” interaction with the surface allows stacking as in the crystal structure. One can estimate that about 227 ng would fill a monolayer with this orientation on a perfectly planar surface; AFM measurements¹ indicate some roughness, so filling a monolayer and defining its orientation is approximate. Still, our data suggest that around 300 ng constitutes a monolayer, so we may surmise that a good proportion of the pNBA dimer in the monolayer lies with benzene rings perpendicular to the surface, with the long and short axes of a dimer stack parallel to the surface. The changes in several bands around 1687 cm^{-1} with increasing quantity are shown in Figure 4B. Deconvolution of the 1687 cm^{-1} region suggests that there are 5–6 carbonyl bands (1676–1715 cm^{-1}) that change in relative importance with increasing quantity (not shown). The penetration limit for pNBA is about 10 μg , corresponding to about 31 layers (12.97 Å/layer) or a total of about 0.04 μm thickness. One should note the apparent lack of a wavelength effect in the SEIRA spectra.

It is interesting to compare the spectrum of the initial monolayer with spectra of material located “far” from the AgX surface, a comparison facilitated by examination of expanded “slices” such as those illustrated in Figure 4A. For a few bands, there are small changes in the position of a band, or in the relative heights of particular peaks. The carbonyl absorptions at 1687 and 1696 cm^{-1} for 250 ng (about one monolayer) develop into four peaks for a “slice” at 9 μg at 1676, 1687, 1696, and 1704 cm^{-1} . (The decreases in intensities have already been noted.) A symmetric NO_2 peak at 1349 cm^{-1} in the monolayer becomes two peaks at 1349 and 1339 cm^{-1} at 9 μg . A peak at 717 cm^{-1} in the monolayer decreases and one at 706 cm^{-1} appears at 9 μg . In sum, the interactions with the surface dominate the strongly enhanced monolayer spectra whereas additional environments influence the peak positions for the “bulk”; the acid dimer may be ordered or disordered. In all cases, the basic character of the spectra as those of the dimer of *p*-nitrobenzoic acid are retained.

As stated previously, our working hypothesis at this time is that an image dipole induced at the surface by the molecular dipole of a proximate molecule can be invoked to explain the electromagnetic enhancement. We shall consider these matters further in a subsequent communication.

Conclusions

The study of *p*-nitrobenzoic acid dimer has suggested (1) an orientation of the organized molecule on the AgX surface, (2) the filling of monolayers along with a measure for the extent of the SEIRA effect, (3) spectral measurement for the pNBA dimer molecule (and thus for other suitable carboxylic acids),²² and (4) the inference of structural arrangements of molecules on the surface that would be difficult to obtain by other means.

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