Infrared Spectroscopy for Chemically Specific Sensing in Silicon-Based Microreactors

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Fourier transform infrared (FT-IR) spectroscopy in a multiple internal reflection (MIR) geometry is integrated with silicon-based microreactors to allow detection of a wide range of chemical species while taking advantage of inexpensive batch fabrication techniques applicable to silicon substrates. The microreactors are fabricated in silicon and glass using standard microfabrication and selective etching techniques. The small (\sim 1 cm side) reactor size provides access to nearly the full mid-IR frequency region with MIR-FT-IR, allowing us to probe both solution-phase and surface-bound chemical transformations. The wide applicability of this approach is demonstrated with two representative test cases: kinetics of acid-catalyzed ethyl acetate hydrolysis and amidization of surface-tethered amine groups.

The detection of chemical species in micro total analysis systems (μ TAS) presents both familiar and new challenges,^{1,2} including the ability to sample drastically reduced volumes and the compatibility of detection techniques with materials amenable to device fabrication. Although fluorescence is the most widely used detection method for μ TAS,¹ it suffers from the need to chemically modify most analytes to induce fluorescence. Electrochemical detection, which has gained popularity owing to the ease of conversion from the detected quantity to an electrical signal, is limited to electroactive analytes.

Although absorption spectroscopic methods for detection are attractive because of their near universality, the sensitivity of these techniques is often a limitation in the short path length configurations dictated by μ TAS. Some progress has been made in

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traditional absorption spectroscopy by optimizing the optical configuration for the sensitivity demands of μ TAS,^{3–5} while alternative approaches have focused on detecting more sensitive photothermal⁶ and refractive index changes.^{7,8} The chemical specificity of NMR spectroscopy has been exploited in an on-chip NMR probe,⁹ but without the spectral resolution that makes NMR such a powerful analytical technique for complex macroscopic samples.

Fourier transform infrared (FT-IR) spectroscopy is in many ways an ideal detection technique, since virtually all molecules of interest absorb IR radiation. In contrast to UV/vis absorption, FT-IR is a fingerprinting method. Characteristic functional groups are apparent by quick inspection of most IR spectra, and the unique IR signature of many molecules allows identification of an unknown analyte by comparison with spectral libraries. Several difficulties arise, however, in the effort to integrate FT-IR with μ TAS. First, the strong IR absorption of many solventsparticularly water-limits the quantity of analyte solution through which the incident IR radiation can travel. The materials of the microreactor themselves must also allow coupling of the IR light to the sample. Traditional IR-transparent materials (e.g., CaF₂, ZnSe) are not compatible with the usual techniques used for the fabrication of micro total analysis systems, whereas many traditional µTAS substrates (e.g. glass, polymers) are strong IR absorbers.

These incompatibilities between μ TAS substrate materials and the optical requirements for FT-IR have led researchers in most previous studies to couple FT-IR to the μ TAS via an analysis chamber that is separate from the microreactor itself.^{10–13} In one

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case, on-chip FT-IR detection was achieved by deep reactive ion etching to form a thin transmission cell within a Si substrate,¹⁴ but this approach does not provide the surface sensitivity required for sensor-type applications in which solution- or gas-phase analytes react with a substrate-bound reagent. The ideal optical configuration to combine solution-phase and surface sensitivity is multiple internal reflection (MIR) FT-IR, which samples both the substrate surface and any solution in contact with it via IR light propagated from inside the substrate.

Silicon (Si) is a natural choice for the fabrication of microreactor substrates¹⁵ and a material that is compatible with MIR-FT-IR, albeit subject to the limitations outlined below. The central role of Si in microelectronics and in microelectromechanical systems (MEMS) means that methods for processing it are welldeveloped, including parallel processing techniques to enable the fabrication of many microreactor devices on a single silicon wafer. The use of silicon microfabrication techniques also offers the possibility of producing structures with fully integrated electrical circuits. Furthermore, surface chemistry for well-defined functionalization of Si surfaces is also well-established, making Si substrates an ideal choice for sensor-type applications.

Although the use of MIR-FT-IR with Si substrates is a common technique for increasing sensitivity to Si surface species,¹⁶ silicon has not found widespread use as a more universal MIR substrate, because multiphonon bands effectively absorb all the incident IR radiation below 1500 cm⁻¹. However, it was recently shown that shortening the length of the Si substrate parallel to the incident IR beam can extend the frequency cutoff well below 1000 cm⁻¹,¹⁷ providing access to a large portion of the so-called IR "fingerprint" region, even in studies of the Si/liquid interface.^{18,19} The remaining obstacle to incorporating Si MIR substrates into μ TAS is the economical fabrication of these substrates, since MIR crystals are traditionally produced by individually polishing the substrates, which makes them both expensive to manufacture and difficult to integrate with other microreactor features. Anisotropic etching of silicon has been used to create beveled Si surfaces that are suitably polished for use in optical systems,^{20,21} providing the potential for parallel production of multiple Si crystals for MIR-FT-IR applications.

In the present work, we incorporate anisotropic etching into the parallel fabrication of Si-based microreactors with MIR-FT-IR

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detection, as described briefly in a preliminary publication.²² We demonstrate the utility of this approach both for analysis of solution-phase reactions and for detection of the reaction of a solution-phase analyte with a surface-bound functional group in situ, the first example of an on-chip FT-IR detection scheme suited to both of these general applications.

EXPERIMENTAL SECTION

Microreactor Fabrication. Microreactors were fabricated by anodically bonding MIR crystals produced on silicon wafers and fluidic channels created in Pyrex glass wafers. Figure 1a shows a schematic of the fabrication sequence for these devices. To produce suitably polished optical surfaces by anisotropic etching of Si, alignment of a mask to the wafer flat is not sufficient. We therefore used a two-step method described by Ensell²¹ for improving alignment. This technique is based on the formation of square pyramidal etch pits during potassium hydroxide (KOH) etching of Si(100); these features form with their square cross sections aligned to the $\langle 110 \rangle$ axes that the wafer flat(s) approximate. Production of two series of test pits on opposite sides of one wafer face allows identification of an exact $\langle 110\rangle$ axis by connecting the "best-aligned" pits on either side of the wafer. The reader is referred to ref 21 for a detailed explanation of this method, summarized as follows.

A layer of low-pressure chemical vapor deposition (LPCVD) silicon nitride (~1 to 2 μ m) was first deposited onto the Si wafer (FZ Si(100), 1901–3680 Ω cm, double-side-polished, ~500 μ m thick). A first mask with a series of alignment marks (Figure 1b) was used to pattern a layer of photoresist (OCG-825, OCG Microelectronic Materials, Inc.); these alignment marks consisted of two sets of 41 circles (71 μ m diameter). Each circle was created with its center along an arc of radius 45 mm, and the circles in each set were arranged relative to one another with an angle of 0.1° between then. The two sets of marks were positioned on opposite sides of the wafer and were a mirror image of one another. This pattern was transferred into the nitride using reactive ion etching, and the layer of photoresist was removed using acetone.

To create the test etch pits, the wafer was blown dry, and the exposed Si was etched for ~30 min in a 20% KOH bath at ~80 °C. Although the origin of this etchant anisotropy is very complicated,²³ it is well-known that aqueous bases etch Si(111) faces much more slowly than other faces. In the case of KOH etching of Si(100), if an etch mask exposes a square of silicon whose sides align perfectly to the $\langle 110 \rangle$ axes, the result is a square pyramidal pit with the faces of the pit defined by $\{111\}$ planes. In our case, where the etch mask exposes a circular area of silicon, the KOH bath will still create the same axis-aligned etch pit, in this case with its size determined by the diameter of the circle.²⁴ While the circular openings in the nitride layer are evenly displaced relative to one another, the relative displacements of the KOH-etched test pits depend on their alignment to the $\langle 110 \rangle$

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Figure 1. (a) Schematic of microreactor fabrication process (not drawn to scale). A single path for IR light propagation is shown in the last picture in the sequence; in fact, IR light will enter the reactor from multiple positions along the bevel of the MIR substrate. (b) Top view of a portion of the mask used to etch alignment marks into the silicon nitride. (c) Top view of KOH-etched alignment marks (square cross section) in Si exposed by alignment marks (circular) etched in the nitride.

axes (Figure 1b). The smaller the lateral displacement between two adjacent etch pits, the better aligned to the (110) axis those pits and their corresponding partners on the opposite side of the wafer. Examination of the etch pits under an optical microscope enabled identification of the set of alignment marks most closely aligned to the $\langle 110 \rangle$ axes (e.g., those with the smallest displacement relative to their two nearest neighbors); this set of alignment marks was then used to orient subsequent masks.

After cleaning the wafers twice in a piranha cleaning bath (sulfuric acid/hydrogen peroxide, 3:1; Caution: piranha solution reacts violently with organics and should be handled with extreme care), the steps to pattern the nitride layer were repeated using a second mask that defined the edges of the silicon crystal and fluidic ports to introduce solutions into the devices. Anisotropic

etching of the wafer for several hours etched through the wafer. Removal of the nitride mask using reactive ion etching produced a wafer of polished silicon MIR crystals.

The Pyrex glass wafer was coated with a layer of image-reversal photoresist (AZ-5214E, Hoechst Celanese, Somerville NJ) that was then patterned using a mask with fluidic channels. After a brief exposure to an oxygen plasma to remove debris in areas where the photoresist had been removed, a layer of chromium (~200 nm) was deposited onto the wafers using an electron-beam evaporator. Metal deposited onto the photoresist was lifted off as the photoresist dissolved in an acetone bath, and then the wafer was cleaned. Channels (\sim 50 μ m deep) were transferred into the wafer by etching in dilute hydrofluoric acid (HF) using the chromium metal as an etch stop. The chromium layers were removed in a standard chromium etch, and then the silicon and Pyrex wafers were cleaned in piranha solution. The silicon and Pyrex wafers were anodically bonded²⁵ by placing the wafers (aligned to one another) in contact, heating them to ${\sim}450$ °C, applying a DC voltage (~700 V) across the wafers for approximately 5 min, then slowly cooling the wafer stack to room temperature. This anodic bonding step produced sealed devices. Dicing the wafer stack produced individual microreactors with integrated MIR crystals.

Ports for the introduction of fluid into the microreactor were incorporated into the silicon MIR crystal. We attached microtubing to these ports using black poly(dimethylsiloxane) (PDMS, Sylgard 170, Dow Corning, Midland MI) and epoxy.²⁶ The use of black PDMS effectively blocks out any stray light from the IR beam in the lateral direction. Maintaining a maximum beam size in the vertical direction is, in fact, advantageous: because the height of the microreactor substrate is greater than the beam size, this approach yields maximum beam/sample interaction with no possibility of stray light reaching the detector. Our ability to use the maximum aperture diameter for the IR beam avoids the limitation previously put forth¹² that the FT-IR spot size must be reduced significantly for use with μ TAS. When mounting the device in the sample holder, we also used a piece of black PDMS on the glass side of the device to block out stray light on that side.

Ethyl Acetate Hydrolysis. In all experiments, ethyl acetate (Fisher, A.C.S. grade) was dissolved in deionized H₂O (Millipore, 18.2 MΩ) to yield a 0.409 M solution. After \sim 30 min of vigorous stirring, the solution was adjusted with 6.05 M HCl to the desired HCl concentration, with the resulting solutions all falling in the range 0.3-0.4 M in ethyl acetate. Immediately after addition of the acid, the first reaction aliquot was removed for analysis in the microreactor. (This choice of experimental protocolmaintaining vigorous stirring of the reaction mixture during the reaction progress—is discussed below in Results and Discussion.) Subsequent aliquots were transferred to the microreactor as soon as the spectrum of the previous sample was finished, ~ 9 min apart. An excess of the total volume needed to fill the microreactor and inflow and outflow tubing (~ 1 mL) was flowed through the microreactor each time to ensure optimal mixing; however, as evidenced by the experiment described above, any reaction

⁽²⁵⁾ For a thorough explanation of anodic bonding (in this reference, called "field assisted bonding"), see: Madou, M. J. Fundamentals of Microfabrication; CRC Press: Boca Raton, FL, 2002, pp 384-389.

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mixture left over from the previous aliquot is not expected to alter the data from the next sample. Because the spectrum of each aliquot is an average of the reaction progress over a ~6-min time period, as detailed below, the resulting spectral changes represent the average ethyl acetate concentration over this interval. For kinetic analysis, the important parameter is Δt between the first and subsequent aliquots, which we obtain from the time stamp recorded at the start of each spectrum. Because the reaction progresses during transfer of the aliquot from the reaction vessel to the microreactor, this time measurement is more accurate than the recorded time interval for aliquot removal.

Reaction of Surface Amine Groups. Amine-functionalized surfaces were prepared by reaction of oxidized Si with (aminopropyl)triethoxysilane (APTES, Aldrich, 99%). Prior to reaction with the APTES, Si surfaces were rigorously cleaned according to the protocol described by Fadeev and McCarthy²⁷ in a solution of \sim 70% sulfuric acid in which 6 g of sodium dichromate was dissolved and \sim 30% hydrogen peroxide (30% aqueous). This cleaning step is required both to rid the surface of hydrocarbon contamination and to fully hydroxylate the overlying silica to allow siloxane bond formation between APTES molecules and surface silanol groups. The reactive cleaning solution was incompatible with organic components of the microreactor (PDMS or epoxy), however. The in situ amidization experiments were therefore carried out on beveled Si pieces (FZ Si(100), 10-100 Ω cm, double-side-polished) with the same length dimension (\sim 1 cm) as the substrates for the microreactors. In place of the microreactor, the Si was placed in a Teflon-based liquid cell with a liquid/ Si contact area per side equivalent to the contact area for the trough microreactors. This reusable cell, which is described in detail elsewhere,¹⁸ relies on a spring-loaded seal between the Teflon reservoir, a polished graphite insert, and the polished Si sample. Because the design of the liquid cell allows contact with both sides of the Si substrate, this experiment provides twice the sensitivity of the microreactors, a difference addressed below in Results and Discussion.

The cleaned silicon substrates were reacted with 1-2 mmol APTES in dry toluene for 24 h at room temperature. The samples were held vertically in the reaction mixture in a custom Teflon holder to allow reaction with both sides of the polished substrates. After removal from the reaction solution, the substrates were thoroughly rinsed sequentially in toluene, ethanol/toluene (1:1) and ethanol, then dried with compressed, filtered air.

To identify IR peaks characteristic of amide bond formation on the surface amine groups, we carried out ex situ reaction and transmission IR characterization (described below) of analogous, unbeveled Si substrates. In these experiments, the APTESfunctionalized substrates (made of the same Si as the beveled substrates) were reacted with 0.1 M acetic anhydride in methylene chloride overnight at room temperature. After removal from the reaction solution, samples were rinsed twice in methylene chloride and dried with compressed air.

For the in situ experiments, a beveled APTES-functionalized substrate was inserted into the Teflon liquid cell. The cell was first flushed with CCl_4 (used instead of CH_2Cl_2 to limit absorbance in the mid-IR region) for acquisition of background spectra. A solution of acetic anhydride (0.1 M in CCl_4) was then introduced

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into the liquid cell via syringe and was flowed through continuously during spectral acquisition, using a syringe pump at a rate of 4 mL/h.

Spectral Acquisition. All FT-IR data were obtained with a Nicolet Nexus 670 spectrometer operating at 4 cm⁻¹ resolution. The incident IR beam was parallel to the faces of the microreactor, with no additional optics added to the spectrometer sample compartment, resulting in an incidence angle of 35.3° from the surface normal of the beveled edge. This geometry gives rise to \sim 12 internal reflections before exiting the microreactor, with an incidence angle of 64.4° from the surface normal at the microreactor/liquid interface. Spectra for in situ experiments were acquired with a liquid nitrogen-cooled MCT-high D* detector. For the ethyl acetate hydrolysis experiment, each spectrum consisted of 500 scans (approximately 6.2-minute collection time); each spectrum for the in situ amidization experiment consisted of 100 scans (\sim 1.2-min collection time).

Data for the ex situ amidization experiment were acquired in an external transmission geometry, with the incident IR beam at 60° to the surface normal of the Si sample. Spectra for this experiment were collected with a thermoelectrically cooled DTGS detector and consisted of 1000 scans each for sample and background.

RESULTS AND DISCUSSION

Reactor Characteristics. Lithography and anisotropic etching enable the parallel fabrication of many Si MIR crystals. We fabricated 16 device substrates from one 100-mm Si wafer, with a total processing time of 12 h, or <1 h per device. This compares favorably with the time to polish each substrate individually, a process that takes at least 3 h per substrate. This advantage to our approach would be increased dramatically by simultaneous processing of multiple wafers, since alignment of the mask for the final KOH etch step is the only nonparallel step in the process. Furthermore, fabrication of MIR substrates by the technique detailed above can be combined with other microfabrication techniques to integrate the simple microreactors used in this study with more elaborate functionality—for instance, a separation module or electronics for signal conversion—from one single Si substrate.

The MIR prisms we fabricated were successfully integrated with microfluidic channels to produce microreactors with detection capability. For any such applications, the material for the channels must be able to bond to silicon and must be compatible with the solutions with which it is in contact. We used Pyrex glass for the channels here, but we have also produced reactors from PDMS using soft lithographic techniques.¹⁴

For most of the experiments performed here, the channel design was very simple, either a single, trough-like channel ~0.75 cm wide or a serpentine channel ~100 μ m wide (Figure 2a). These designs result in a microreactor volume of ~3 to 4 μ L in contact with the Si substrate. We chose this straightforward design of a single reaction chamber to maximize surface area in contact with the MIR crystal and to minimize detection time. Ultimately, the optimal microchannel design would be a compromise between the greater detection volume afforded by a single chamber and the need to mix multiple reactant streams via microfluidic channels. The design used in this study, however, could be



Figure 2. (a) Photograph of a microreactor fabricated with glass channels. (b) IR throughput of a microreactor with a glass trough filled with H_2O .

modified (by modifying the mask for the Pyrex wafer) to include more complex channel designs.

As predicted, the 1-cm length of the Si substrate for the microreactor allows transmission of significant IR intensity throughout the mid-IR range, as shown in Figure 2b. In this singlebeam spectrum from a water-filled microreactor, the low-frequency cutoff (\sim 850 cm⁻¹) arises from a combination of the intrinsic cutoff of the MCT detector and Si phonon bands. Absorption is evident both from components of the microreactor (PDMS and glass, as labeled in the figure) and from stretching (\sim 3100–3800 cm⁻¹) and bending (\sim 1640 cm⁻¹) bands of H₂O. The relatively small interaction region between the water and the Si allows significant IR throughput, even in the regions of H₂O absorption, a distinct advantage for analyzing bands, such as ν (C=O) modes that often overlap with $\delta(H_2O)$ in aqueous systems. It is also important to note that the selective etching used to generate bevels for the IR prism creates a surface of high enough optical quality to couple sufficient light into the Si prism without excessive scattering.

Ethyl Acetate Hydrolysis. Although the acid-catalyzed hydrolysis of ethyl acetate is an extremely simple reaction, it provides an excellent test case for the use of IR to detect or characterize organic reactions. One of the biggest challenges in following the reactions of organic species is the conservation of characteristic functional groups in reactants and products. This challenge is exemplified by ester hydrolysis, since carbonyl stretches for esters and the corresponding carboxylic acids may well overlap, as they do in this case for ethyl acetate and acetic acid (Figure 3). The aqueous medium for this reaction provides a further challenge, since hydrogen bonding makes it difficult to quantify growth in ν (OH) modes from the alcohol and acid products.

As the spectra of reactants and products in Figure 3 demonstrate, the ability to monitor the fingerprint region below 1500 cm^{-1} is critical in this case. Although there are no significant peaks



Figure 3. Infrared spectra (100 scans) of reactants and products of ethyl acetate hydrolysis, acquired in aqueous solution (5 mL/100 mL H₂O) in a microreactor: ethyl acetate (solid line), ethanol (dotted line), and acetic acid (dashed line). All spectra are ratioed to a background of pure H₂O in the microreactor.

Table 1. Rate Constants for the HCI-Catalyzed Hydrolysis of Ethyl Acetate^a

[ethyl acetate] ₀ , M	[HCl], M	k (× 10 ⁻² min ⁻¹)
0.375	0.5	$0.23 \\ 0.23^{b}$
0.341	1.0	0.40
0.307	1.5	0.71
0.3629	1.714	${1.17^c} \ {1.12^d}$
0.274	2.0	1.21
0.1157	2.435	1.66^{c} 1.570^{d}

^{*a*} Unless otherwise noted, the values were obtained in this study. ^{*b*} Value from experiment carried out in microreactor with no stirring (e.g., data from Figure 4). ^{*c*} Value from ref 29. ^{*d*} Value from ref 30.

for any of the three species that do *not* overlap appreciably with those from the remaining species, the sharp peak from ethyl acetate at 1379 cm⁻¹ (derived mainly from the symmetric deformation of the methoxy group)²⁸ is easily distinguished from the relatively broad band of acetic acid that it overlaps. Monitoring the depletion of this sharp band concomitant with the growth of a broad band around it does not require any spectral deconvolution, only straightforward integration of the depletion peak, as demonstrated below. It is important to note that this spectral region is only accessible with a Si substrate when using a shortsample geometry. Although there are, no doubt, organic reactions for which finding a unique spectral feature will prove impossible, the ability to monitor the fingerprint region typically provides access to a multitude of discrete peaks, thereby increasing the chances of finding a suitable tag for reactant or product.

The kinetics of ethyl acetate hydrolysis were monitored over a range of HCl concentrations. First-order rate constants from this study are presented in Table 1 and compared to literature values in the same HCl concentration range derived via interferometry²⁹ and titration.³⁰ As outlined briefly above, the reaction was monitored by following the depletion of the ethyl acetate peak at 1379 cm⁻¹. Because the primary focus of the current study is to

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Figure 4. Sample kinetic data from ethyl acetate hydrolysis in 0.5 M HCl. Spectra in (a) show the growth of the ethyl acetate depletion peak as a function of time from top to bottom (from 6 to 112 min). All spectra are ratioed to the first spectrum acquired after introduction of the reaction mixture into the microreactor. Spectra are offset for clarity. The plot of integrated peak area vs time in (b) is fit to eq 1 to yield the rate constant in Table 1.

demonstrate the efficacy of IR detection in our microreactors, the acid-catalyzed hydrolysis of ethyl acetate was in most cases monitored by carrying out the reaction with vigorous stirring in a macroscopic sample and removing aliquots at timed intervals for analysis in the microreactor. This approach was taken to ensure adequate mixing of the sample as the reaction progressed, since initial experiments showed some phase separation in unstirred ethyl acetate solutions in the concentration range used in this study.

One data set was obtained by directly injecting the initial reaction solution into the microreactor and following this solution as a function of time; the spectra acquired from this experiment, carried out with 0.5 M HCl in a microreactor with a trough-like channel, are presented in Figure 4a. The resulting plot of peak area vs time, shown in Figure 4b, is fit to the expression

$$\operatorname{area} = C(1 - e^{-\kappa t}) \tag{1}$$

where *C* is a constant and *t* is the time elapsed since analysis of the first aliquot. The comparison in Table 1 demonstrates that our results are consistent with the earlier, more detailed studies of this system that employed macroscopic sampling methods, demonstrating the efficacy of our microreactor analysis. Furthermore, within our limits of precision, the experiment carried out entirely within the microreactor (e.g., without vigorous external mixing) is identical to an analogous experiment at 0.5 M HCl with more complete mixing. To reliably ensure adequate mixing, however, would require modification of our simple microreactor design—in this case, optimized for detection only—to mix one or more reactant streams.

The sensitivity of our detection scheme can be estimated from the data in Figure 4a, in which the smallest quantifiable peak shown (second spectrum from the top) corresponds to a change of 7 mM ethyl acetate. While the 1379 cm⁻¹ peak was chosen for optimal selective quantification of ethyl acetate in this particular reaction mixture, it is obviously (Figure 3) not the most intense peak available for analysis. For example, ν (C=O) at 1707 cm⁻¹ has an integrated absorptivity ~4.7 times greater than the peak analyzed in this work. It is also important to note that sensitivity in this experiment is tied to the sampling interval, since smaller signals could in some cases be resolved with a greater number of scans. (Conversely, for monitoring kinetics of a faster reaction than ethyl acetate hydrolysis, the need for shorter scan times would result in a lower sensitivity). Taking into account these considerations, it is reasonable to expect our detection scheme to yield sensitivity in the 1–10 mM range for a wide range of reaction systems.

Quantitative analysis of solution-phase reactions using MIR-IR may be hindered in the case of preferential adsorption of reactants or products to the microreactor substrate, in this case silica. Although the penetration depth in this experiment is ~ 0.77 μ m at the frequency of our peak analysis,³¹ the exponential decay of the evanescent IR wave causes surface-associated species to be weighted more heavily in sampling than their solution-phase counterparts. However, although ethyl acetate is known to adsorb to silica from nonpolar solvents,32,33 it is unlikely that such adsorption takes place from aqueous solution. Evidence of any such adsorption taking place in our system would be obscured by the peak shifts arising from hydrogen bonding between ethyl acetate and water, which shifts both ν (C=O) and ν (C-C-O) peaks of the ethyl acetate as much or more as does interaction of adsorbed ethyl acetate with surface silanol groups in a nonpolar solvent.32 In any case, the good agreement between our rate constants and those derived from more macroscopic sampling techniques suggests that such adsorption effects do not play a significant role in analysis of the aqueous ethyl acetate system. For systems in which such effects are found to dominate (e.g., analysis of polar molecules in nonpolar solvents), functionalization of the Si substrate to yield a more hydrophobic surface may be advantageous.

Reaction of Surface-Tethered Functional Groups. The reaction of APTES with hydrated silica to form surface amine (NH₂) groups was chosen for this work to present a high density of NH₂ for subsequent reaction with solution-phase species. Since solution deposition of APTES is known to deposit polymeric multilayers on hydrated silica,³⁴ this route was chosen rather than reaction of a monoalkoxide silane to form a covalently bound monolayer²⁷ with a lower density of surface NH₂ groups.

To identify the IR peaks indicative of the reaction of surface NH_2 groups with solution-phase acetic anhydride, we carried out an ex situ reaction characterized with external transmission IR as described above. As outlined below, analysis of IR spectra of the dry surface is helpful in deciphering the complex spectra arising from the presence of both surface-bound and solution-phase species in the MIR configuration. A transmission spectrum

⁽³¹⁾ Penetration depth was calculated 40 assuming reflection at the $\rm Si/SiO_2$ interface.

⁽³²⁾ Poston, P. E.; Rivera, D.; Uibel, R.; Harris, J. M. Appl. Spectrosc. 1998, 52, 1391–1398.

⁽³³⁾ Scott, R. P. W.; Kucera, P. J. Chromatogr. 1978, 149, 93-110.

⁽³⁴⁾ Kallury, K. M. R.; Macdonald, P. M.; Thompson, M. Langmuir 1994, 10, 492–499.



Figure 5. Transmission infrared spectra of ex situ reaction of acetic anhydride with surface-tethered amine groups in an APTES-derived layer. The spectrum in (a) shows the APTES layer prior to acetic anhydride exposure, whereas the spectrum in (b) shows growth of characteristic amide bands after reaction of amine groups with acetic anhydride. Both spectra are ratioed to a clean, native oxide-coated silicon reference (the surface just prior to functionalization with APTES).



Figure 6. Infrared spectra acquired in situ during reaction of APTESfunctionalized silicon with acetic anhydride in CCl₄. Peaks marked with an asterisk correspond to acetic anhydride, whereas the peaks at 1204 and 1657 cm⁻¹ arise from growth of surface-bound amide species. Growth of the amide I band is highlighted on an expanded scale in the inset. All spectra are ratioed to a spectrum of the initial, APTES-functionalized surface in CCl₄ prior to acetic anhydride introduction.

of the APTES-functionalized surface is presented in Figure 5a. The most intense peaks, from 1000 to 1200 cm⁻¹, arise from ν (Si–O) modes of siloxane bridges between the silica surface and APTES and between APTES molecules in the polymerized overlayer. A weaker, broad δ (NH₂) mode appears at ~1572 cm⁻¹.

Reaction of the surface-bound NH₂ groups with acetic anhydride is marked by the appearance of strong amide I and amide II bands at 1655 and 1559 cm⁻¹, respectively, as shown in Figure 5b. Conversion of surface amines to amide groups is also accompanied by a sharp increase of ν (NH) intensity centered at 3293 cm⁻¹ (data not shown).

Monitoring this amidization reaction in situ reveals detectable reaction progress on a relatively fast time scale. A series of three spectra, acquired at 2.5-min intervals following the introduction of acetic anhydride solution into the liquid cell, is presented in Figure 6. Peaks marked with an asterisk (*) arise from acetic anhydride in solution, identified by comparison both with literature spectra³⁵ and with peaks from the single-beam spectrum in our own experiment. Increased intensity in acetic anhydride peaks

arises from gradual displacement in the near-surface region of the CCl₄ solvent that was used to equilibrate the cell and to acquire background spectra. Growth of surface amide species is marked by increased intensity in the amide I band (centered at 1654 cm⁻¹), as highlighted in the inset to Figure 6. This assignment is supported by concomitant growth both of the 1204 cm⁻¹ peak (seen as a shoulder on an acetic anhydride peak) and of ν (NH) centered at 3300 cm⁻¹ (data not shown). Growth in the amide II band (at 1559 cm⁻¹ in the ex situ experiment) is obscured in this experiment by depletion of the δ (NH₂) mode from the reactant amine, which gives rise to a negative peak centered at ~1584 cm⁻¹.³⁶ However, the near-exact correspondence between the in situ and ex situ bands (within 1 cm⁻¹), as well as the lack of any reactant or solvent bands at those frequencies, allows definitive identification of amide formation.

Detecting the reaction of substrate-tethered functional groups in a microreactor is analogous to monitoring chemistry of surface layers in larger samples. Infrared spectroscopy has traditionally been one of a battery of techniques used for characterization of self-assembled monolayers and of any subsequent chemistry carried out on terminal groups of those layers. Most of this analysis is ex situ; for example, reflectance IR is often used to follow reaction of terminal groups of thiol-based SAMs on gold. Traditional (long-sample) MIR-IR has been used previously to characterize the reaction of an amine-terminated siloxane monolayer on Si, but only ex situ.³⁷ To the best of our knowledge, in situ MIR-IR of solution-phase reaction of a siloxane monolayer has been limited to studies of the protonation of terminal carboxylate groups.^{38,39} One of these studies³⁸ employed a traditional MIR (Ge) substrate, while the other used a prism cut from a Si wafer,³⁹ since the ν (C=O) modes of interest are all above 1500 cm^{-1} .

The complexity of the spectra in Figure 5 highlights the difficulty of detecting changes in surface species in the presence of the solution reaction mixture, even for a solution containing only one reactant and no other major components, since MIR-IR detects both surface-bound and solution-phase species simultaneously. While the (solution-phase) acetic anhydride features that dominate these spectra could be removed either by allowing more time for equilibration or by flushing the microreactor with solvent after the surface groups have reacted, either of these approaches increases the time between introduction of the analyte and its detection. The present case, therefore, demonstrates the efficacy of IR detection of surface-bound reaction of a solution-phase analyte in a more demanding environment than may be required for many applications. The ability to access most of the mid-IR fingerprint region provides a large number of IR peaks to aid in deconvolution of spectra of even more complex mixtures than the surface/solution mixture seen in this case and would be essential for application of such a system in chemometrics.

- (39) Gershevitz, O.; Sukenik, C. N. J. Am. Chem. Soc. 2004, 126, 482-483.
- (40) Harrick, N. J. Internal Reflection Spectroscopy, 3rd ed.; Harrick Scientific Corporation: Ossining, New York, 1987.

⁽³⁵⁾ SDBSWeb: http://www.aist.go.jp/RIODB/SDBS/ (accessed March 2004).

⁽³⁶⁾ The apparent maximum for the $\delta(NH_2)$ peak is shifted somewhat from the ex situ spectrum simply by overlap of this mode with the positive-going amide II peak that grows in during depletion of this mode.

⁽³⁷⁾ Lee, M.-T.; Ferguson, G. S. Langmuir 2001, 17, 762-767.

⁽³⁸⁾ Cheng, S. S.; Scherson, D. A.; Sukenik, C. N. Langmuir 1995, 11, 1190– 1195.

Even taking into account the 2-fold increase in sensitivity generated by use of a two-sided reaction cell instead of the prefabricated microreactor for this experiment, the system used in this study yields an easily detectable level of signal (absorbance change of $\sim 10^{-2}$ at the amide I frequency in 5 min for a 1-sided reactor). Obviously, this sensitivity is highly dependent on the system under study and includes the surface density (and accessibility) of reactive sites for the analyte, the oscillator strength of the resulting chemical species formed, and the uniqueness of the IR signature of the product. Quantification of sensitivity in the present experiment as a function of the two-dimensional concentration of surface amine groups is complicated by the multilayer structure of the APTES-derived overlayer; this could potentially be addressed using the more well-defined (but less amine-dense) monoalkoxy-derived monolayer. The issue of a unique IR signature for product identification is ameliorated to some extent by the ability to monitor several frequencies at once: for instance, in the present case monitoring absorbance at 1204 and 1657 cm⁻¹ and in the ν (NH) region. Again, our ability to access the lower-frequency fingerprint region for organic species provides a significant advantage in identifying multiple spectroscopic handles for a given system.

Finally, although the in situ experiment described above demonstrates the utility of our Si-based system for detecting reaction of surface-bound organic groups, further development of this approach is required to incorporate in a well-defined way varied organic surface functionality into the actual microreactors via premodification of the beveled Si platforms. As noted briefly above, the ability to modify the silicon native oxide that serves as the de facto microreactor substrate may be advantageous for inhibiting unwanted adsorption of solution-phase species, as well as for tailoring the microreactors for detection of different (and possibly multiple) analytes via reaction with surface functional groups.

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