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Chemically Sensitive High Throughput Parallel Analysis of Solid Phase Supported Library Members

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This paper introduces Fourier transform infrared imaging as a powerful spectroscopic tool for the parallel identification of members of resin-supported combinatorial libraries. This technique combines the chemical specificity and high sensitivity of FTIR with the ability to rapidly analyze multiple supported resin beads simultaneously. It is shown here that the chemical identity of ligands on a variety of supported resin beads can be identified in a single experiment without destroying or otherwise perturbing the system.

Combinatorial chemistry has emerged over the past few years as one of the most powerful approaches to drug, material, and catalyst discovery. While effective synthetic schemes have been developed to generate millions of compounds, the design of efficient screening and structural characterization methodologies still remains challenging and presents a bottleneck in the overall process. In order to make this process more efficient, truly parallel screening and characterization techniques, which are capable of analyzing many samples simultaneously, must be devised. This paper presents the first results of the application of a parallel analytical method, where infrared spectra can be acquired from many solid phase supported library members at once.

Typical methods for structural elucidation of solid phase supported library members involve using direct methods such as X-ray photoelectron spectroscopy,[1] magic angle spinning NMR spectroscopy,[2] Fourier transform infrared (FTIR) spectroscopy,[3] X-ray diffraction,[4] and mass spectrometry[5] or indirect methods such as deconvolutive procedures,[6,7] readout of chemical tags,[8,9] fluorescence labeling,[10] or radio frequency signals.[11] While each method has its advantages and disadvantages, no single method is capable of noninvasive, high throughput, and real-time spectroscopic study of multiple members of a combinatorial library.

Infrared-based techniques are extremely popular for the analysis of combinatorial libraries because of the benefits of low cost, ease of use, and rapid data collection. One of these techniques is single bead FTIR microspectroscopy,[12,13] which uses an FTIR microscope to acquire IR spectral information from a single bead. While this technique can provide highly detailed chemical information, it has the limitation of being able to examine only one bead at a time. An extension of this technique, FTIR spectroscopic mapping, has also been reported.[14] In this study, a map of approximately 300 different resin beads was collected, and the resulting spectra were used to determine the identity of the beads. While providing chemically specific information, this technique requires a collection time of 5 h for each map.

As a first step toward truly parallel[15] analysis, infrared thermography has been used to select active supported catalysts.[16] While being able to analyze a large number of beads at once, this technique provides no chemically specific information and is therefore of limited utility for the characterization of supported ligands. Recently, it has been shown that a new technique that uses a near-IR imaging spectrometer can be used to simultaneously monitor the progress of reactions on several different supported resins.[17] However, due to the low absorptivity inherent to near-IR spectral bands, this technique requires that the sample contain many supported resin beads in order to generate a measurable signal. Additionally, the near-IR spectral region has an inherently low chemical sensitivity, which complicates spectral interpretation and limits the applicability of this technique.

FTIR imaging in the mid-IR spectral region has recently emerged as a technique for the analysis of spatially heterogeneous chemical and biological systems.[18−22] This technique is derived from the coupling of a focal plane array (FPA) detector with an infrared spectrometer,[23,24] which allows the simultaneous collection of spatial and spectral information. FTIR imaging eliminates the need for the classical step-and-collect mapping procedure[14] for the acquisition of spatially resolved infrared spectral data and is therefore a genuine imaging technique. This improves the image quality and dramatically shortens the data collection time. In this study, we have used FTIR imaging in the mid-IR spectral region to simultaneously acquire spectral information from multiple solid phase supported library members.

The experimental setup used in this study consists of an FTIR spectrometer, CaF_2 objective and condensing lenses, and a 64×64 pixel mercury cadmium telluride (MCT) FPA detector. A detailed description of the instrumentation can be found in a previous publication.[25] This system is capable of collecting a spectral imaging data set consisting of 4096 spatially resolved spectra in the 1360−2720 cm^{-1} spectral range with 8 cm^{-1} spectral resolution in less than 20 s. Depending on the specific optical setup employed, the field

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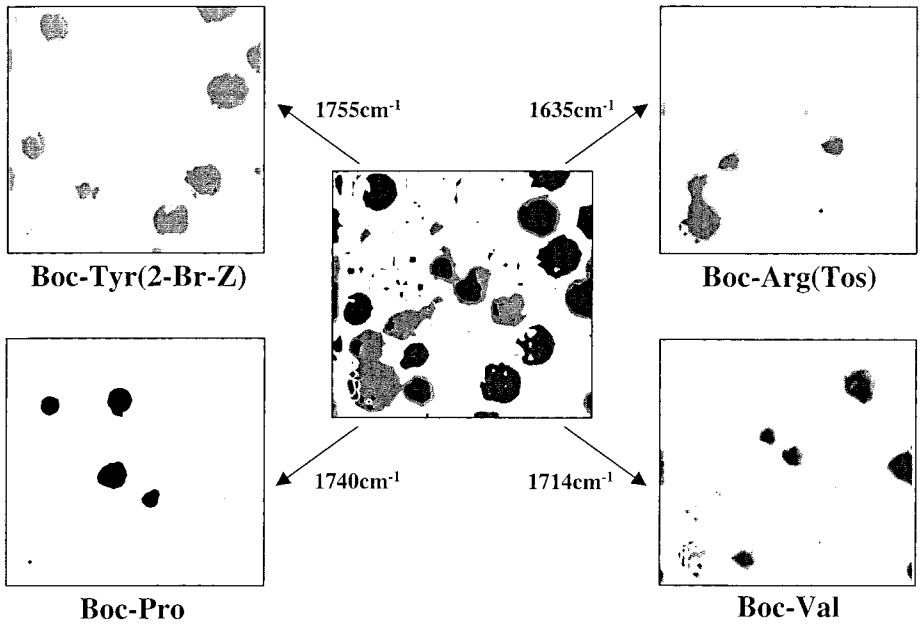

Figure 1. Images of a mixture of beads with supported amino acid ligands. Images were generated by plotting bands specific to Boc-Pro (C═O stretch, 1740 cm^{-1}), Boc-Tyr(2-Br-Z) (C═O stretch, 1755 cm^{-1}), Boc-Arg(Tos) (C═N stretch, 1635 cm^{-1}), and Boc-Val (C═O stretch, 1714 cm^{-1}). The center image is a composite of all of the individual images, showing the location of all beads in the field of view.

of view can be varied from an area of a few hundred square micrometers to several square centimeters. All data processing and manipulation routines were implemented through in-house written software.

Two different sample geometries will be explored here. The first is applicable to the identification of resin-bound compounds from a split-and-pool synthesis, where beads carrying different ligands are mixed together. We identified resin-bound ligands in a mixture of ~25 beads comprised of four natural amino acids.[26] A mixture of these beads was placed onto a polished CaF_2 window and swollen with methylene chloride. The solvent was allowed to evaporate, and another CaF_2 window was placed on top. Slight pressure was maintained in order to flatten the beads, which was necessary to obtain spectra free from saturation effects. This sample was placed in the field of view of the spectrometer, and images were collected. After this analysis, the beads could be recovered without damage and submitted for further analysis by simply re-swelling them in solvent, causing them to resume their spherical shape.

The images from this experiment are shown in Figure 1. By selecting a spectral frequency that is unique for each ligand and plotting the absorbance value for each pixel at that frequency, an image is generated that clearly reveals the location of each type of supported ligand. Representative spectra from each type of bead are shown in Figure 2. Each one of these spectra was acquired from a single pixel of an image, without any coaddition or smoothing. It should be noted that this type of analysis is not limited to ligands that have well-separated spectral bands. More complex types of classification routines, such as principal component analysis,[27] can also be interfaced with this technique to identify ligands either where the spectra are highly convoluted (such as those containing a longer sequence of amino acid residues) or where a minimum of human intervention is desired. This is currently under active investigation in our laboratory.

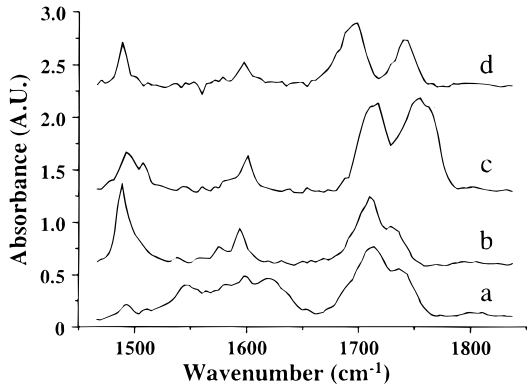

Figure 2. Representative spectra from each type of supported ligand bead shown in Figure 1: (a) Boc-Val, (b) Boc-Tyr(2-Br-Z), (c) Boc-Arg(Tos), (d) Boc-Pro.

The second geometry explored here is amenable to the analysis of supported ligands, where each type of bead is separated from the others, for example, in a parallel reactor. Additionally, beads isolated from a reaction mixture at different times during the course of a reaction can be analyzed simultaneously, greatly reducing the time required to collect kinetics data. The example demonstrated here is the oxidation of a primary alcohol to an aldehyde, which has been described previously in the literature.[28−30] The reaction was carried out,[31] and beads removed from the reaction mixture were placed onto a polished CaF_2 window. The beads removed at different times were arranged into groups that were well separated from each other, with each group containing only one type of bead. As in the previous example, another window was placed on top, and images were collected.

The data from this experiment are shown in Figure 3. The image at the top was generated using the raw output from the FPA, which is averaged over all collected wavelengths. Spectra from each group of beads in the spectral imaging data set were then used to quantify the kinetics of the

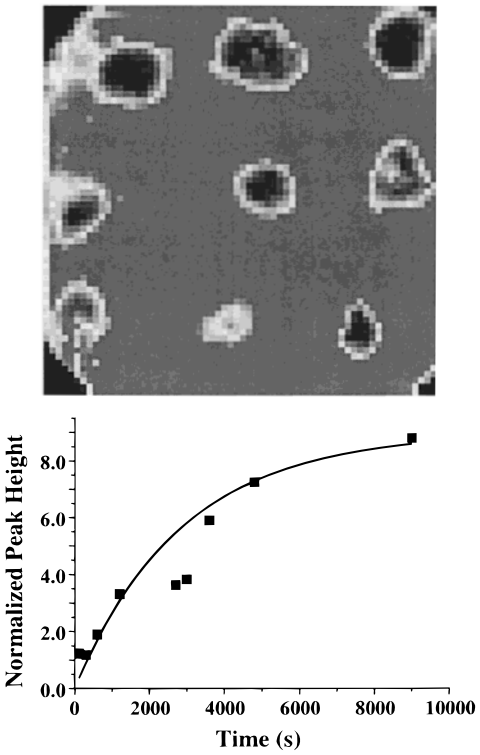

Figure 3. (top) Image of nine groups of beads, each removed at different times during the reaction described in the text. Each small group consists of approximately 10−20 beads. (bottom) Plot of the thickness corrected 1688 cm^{-1} C═O stretching band from spectra corresponding to each collection of beads in the above image. A fit to a single exponential expression yielded a rate constant of $k = 3.5 \pm 0.6 \times 10^{-4}$ s^{-1}. The error bars, determined from the peak-to-peak noise of the absorbance spectra, are small enough to be contained within the data points.

reaction. This was accomplished by plotting the integrated area of the 1688 cm^{-1} C═O stretching band of the spectrum from each group of beads versus the time corresponding to when the bead was removed from the reaction mixture. Thickness correction was performed by dividing the absorbance intensity of the 1688 cm^{-1} band by the absorbance intensity of the aromatic combination band around 1945 cm^{-1}, which corresponds to the polystyrene support. The resulting data are shown at the bottom of Figure 3. These data were fitted to a single exponential function.[32] The resulting rate constant is $k = 3.5 \pm 0.6 \times 10^{-4}$ s^{-1}, which agrees within the experimental error to values determined in previous studies.[28−30]

In summary, FTIR imaging is nondestructive, is highly chemically sensitive, and provides a solution for the rapid characterization of resin-supported library members without the need for using chemical encoding methods or invasive analytical techniques. Because of its rapidity in generating chemically specific images, this technique is particularly amenable to the study of in situ reaction kinetics of an entire combinatorial library.

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References and Notes

(1) Yoo, S.-E.; Gong, Y.-D.; Seo, J.-S.; Sung, M. M.; Lee, S. S.; Kim, Y. *J. Comb. Chem.* **1999**, *1*, 177.
(2) Warass, R.; Wieruszeski, J.-M.; Lippens, G. *J. Am. Chem. Soc.* **1999**, *121*, 3787.
(3) Yan, B.; Gremlich, H.-U.; Moss, S.; Coppola, G. M.; Sun, Q.; Liu, L. *J. Comb. Chem.* **1999**, *1*, 46.
(4) Klein, J.; Lehmann, C. W.; Schmidt, H.-W.; Maier, W. F. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 3369.
(5) Sussmuth, R. D.; Jung, G. *J. Chromatogr. B* **1999**, *725*, 49.
(6) Nefzi, A.; Ostresh, J. M.; Houghten, R. A. *Chem. Rev.* **1997**, *97*, 449.
(7) Boger, D. L.; Chai, W. Y.; Jin, Q. *J. Am. Chem. Soc.* **1998**, *120*, 7220.
(8) Still, W. C. *Acc. Chem. Res.* **1996**, *29*, 155.
(9) Lam, K. S.; Lebl, M.; Krchňák, V. *Chem. Rev.* **1997**, *97*, 411.
(10) Yan, B.; Martin, P. C.; Lee, L. *J. Comb. Chem.* **1999**, *1*, 78.
(11) Xiao, X.-Y.; Zhao, C.; Potash, H.; Nova, M. P. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 780.
(12) Shapiro, M. J.; Lin, M.; Yan, B. *On-Resin Analysis in Combinatorial Chemistry*; Czarnik, A. W., DeWitt, S. H., Eds.; American Chemical Society: Washington, DC, 1997; pp 123−151.
(13) Pivonka, D. E.; Simpson, T. R. *Anal. Chem.* **1997**, *69*, 3851.
(14) Haap, W. J.; Walk, T. B.; Jung, G. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 3311.
(15) Here, the term "truly parallel" refers to the ability of the analytical technique to simultaneously collect information from multiple samples, as opposed to methods such as mapping, in which the final data set contains information from all members but was actually collected in a sequential manner.
(16) Taylor, S. J.; Morken, J. P. *Science* **1998**, *280*, 267.
(17) Fischer, M.; Tran, C. D. *Anal. Chem.* **1999**, *71*, 2255.
(18) Lewis, E. N.; Gorbach, A. M.; Marcott, C.; Levin, I. W. *Appl. Spectrosc.* **1996**, *50*, 263.
(19) Kidder, L. H.; Kalasinsky, V. F.; Luke, J. L.; Levin, I. W.; Lewis, E. N. *Nature Med.* **1997**, *3*, 235.
(20) Bhargava, R.; Wang, S.-Q.; Koenig, J. L. *Macromolecules* **1999**, *32*, 2748.
(21) Snively, C. M.; Koenig, J. L. *Macromolecules* **1998**, *31*, 3753.
(22) Snively, C. M.; Koenig, J. L. *J. Polym. Sci. B* **1999**, *37*, 2261.
(23) Lewis, E. N.; Treado, P. J.; Reeder, R. G.; Story, G. M.; Dowrey, A. E.; Marcott, C.; Levin, I. W. *Anal. Chem.* **1995**, *67*, 3377.
(24) Lewis, E. N.; Levin, I. W. *Appl. Spectrosc.* **1995**, *49*, 672.
(25) Snively, C. M.; Katzenberger, S.; Oskarsdottir, G.; Lauterbach, J. *Opt. Lett.* **1999**, *24*, 1841.
(26) The beads were obtained from Advanced ChemTech and were all composed of 100−200 mesh Merrifield resin (1% DVB cross-linked) with a loading of 0.5−0.9 mmol/g. The supported ligands were as follows: Boc-Tyr(2-Br-Z), Boc-Val, Boc-Arg(Tos), and Boc-Pro.
(27) Koenig, J. L. *Spectroscopy of Polymers*; American Chemical Society: Washington, DC, 1992.
(28) Yan, B.; Sun, Q.; Wareing, J. R.; Jewell, C. F. *J. Org. Chem.* **1996**, *61*, 8765.
(29) Li, W.; Yan, B. *J. Org. Chem.* **1998**, *63*, 4092.
(30) Yan, B. *Acc. Chem. Res.* **1998**, *31*, 621.
(31) The materials and reaction stoichiometry were identical to those in ref 28. The reaction was carried out at room temperature under continuous stirring. At several times during the reaction, a small portion of the beads was removed, washed with dimethyl formamide then methylene chloride, and dried under ambient conditions.
(32) Yan, B.; Fell, J. B.; Kumaravel, G. *J. Org. Chem.* **1996**, *61*, 7467.

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