Direct Monitoring of Combinatorial Chemistry Reactions by Infrared Microspectroscopy

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What is combinatorial chemistry? Combinatorial chemistry is quickly becoming a very popular technique for organic synthesis in the pharmaceutical and biotechnology fields. Basically, combinatorial methods allow one to obtain thousands of derivatives of chemical compounds in a very quick and efficient manner. The method begins when a molecule of interest is attached to resin beads. Once the molecule is attached, a number of reactions can be run on the beads. The beads can be split up into several subsets, and different reactions can be run on each of the subsets. Because the beads are in the solid phase, it is extremely easy to separate the beads from the liquid layer after the reactions have completed.

After the first set of reactions is finished, the subsets can be mixed together and re-divided into a second group of subsets. Now, each subset contains several different reaction products of the molecules attached to the beads. By running different reactions on each of these subsets, one obtains even more reaction products. If this cycle is repeated, it becomes obvious how literally thousands of different derivatives of a single molecule can be combined rather quickly. Hence, this process earned the name of Combinatorial Chemistry.

How can the reaction products be detected and characterized? There are a variety of methods currently being investigated for the analysis of the final reaction products. Some groups cleave the molecule from the bead after the reaction, and detect it by traditional methods. We propose that infrared microspectroscopy could be used to detect and characterize the reaction products of the molecules, directly on a single bead, without having to cleave the molecule from the bead. This direct sampling capability will greatly speed the entire process of combinatorial chemistry.

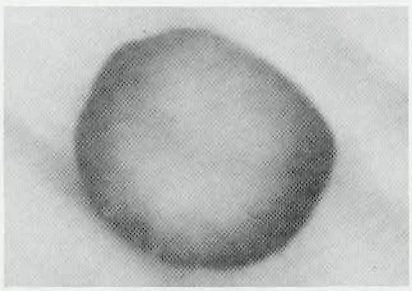
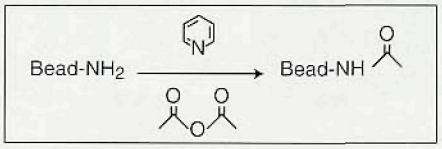


Figure 1: Video image capture of a single unreacted combinatorial bead

In this paper, we present the results from three different bead samples: an unreacted bead (Figure 1) and two reacted beads. We will show that high-quality infrared spectra can be obtained of single beads, and the reaction products can easily be detected and characterized.

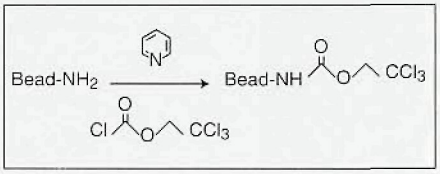
Experimental

The unreacted beads (from Nova Bio Chem) were polystyrene based, with a polyethylene glycol group added on to the polystyrene base. Attached to the polyethylene glycol group was an active amine group. This amine group was the reactive site where core molecules were added. The reactions are detailed below.



Scheme 1

To a mixture of 4.86 mg of beads in CH_2Cl_2 (1 ml) was added 50 µl of pyridine (M.W. = 79.10) and 50 µl of acetic anhydride (M.W. = 102.19). The mixture was stirred at room temperature (25° C) for 24 hours. The mixture was filtered (through Whatman qualitative filter paper cat. 1001 042), washing five times with CH_2Cl_2. The beads and filter paper were put under vacuum to remove volatiles.



Scheme 2

To 4.00 mg of beads was added 2.0 ml of pyridine (M.W. = 79.10) and 100 µl of trichloroethyl chloroformate (M.W. = 211.86). The mixture was stored at room temperature (25° C) for 24 hours. The mixture was filtered, washing five times with CH_2Cl_2. The beads and filter paper were put under vacuum to remove volatiles.

Individual beads were removed from each of the three sets of beads and placed on a Tacky Dot Array glass slide (from SPI Supplies). These glass slides have 40 µm diameter adhesive dots, spaced 500 µm apart. Individual beads were held on to the glass slide by placing them on the adhesive dots.

All samples were run on a Nicolet/Spectra-Tech microscope with an ATR objective with a ZnSe internal reflection element. This ATR objective can be used on any Nicolet/Spectra-Tech microscope. In addition, the InspectIR™, which has a built-in ATR element, can also be used. All spectra were acquired with 8 cm^{-1} spectral resolution with Happ-Genzel apodization, and 128 scans were co-added for each spectrum. The background spectra were obtained through air. The sample size was circular, with a diameter of 42 µm.

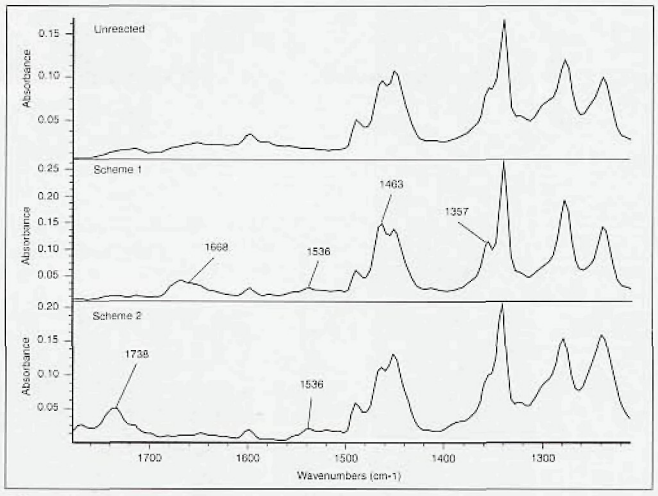


Figure 2: Infrared ATR spectra of top) unrected bead, middle) Scheme 1 reacted bead, and bottom) Scheme 2 reacted bead

Results and Discussion

Scheme 1: The reaction product can be characterized from the infrared spectrum (middle spectrum, Figure 2). Spectral bands from the bead substrate can be seen (top spectrum, Figure 2), but new features have appeared in the spectrum of the reacted product. The appearance of a band at 1668 cm^{-1} is indicative of the amide I stretch. The band at 1536 cm^{-1} represents the amide II vibration. In addition, the growth of the bands at 1463 cm^{-1} and at 1357 cm^{-1} show an increase in the amount of CH_3 present.

Scheme 2: The reaction product spectrum is the bottom spectrum shown in Figure 2. Again, spectral bands from the bead substrate are visible, but there are new bands present. The carbonyl stretch apparent at 1738 cm^{-1} is indicative of the carbamate product. In addition, the appearance of a band at 1536 cm^{-1} is due to the CNH group.

Conclusions - Continued on Page 24

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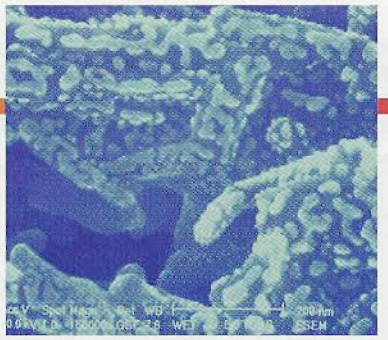

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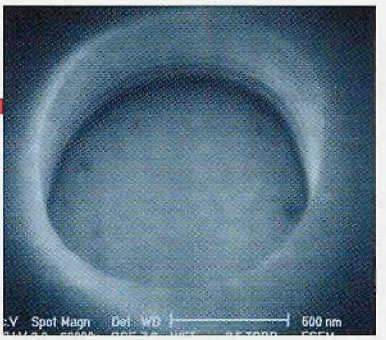

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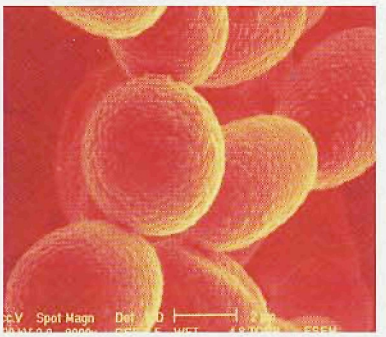

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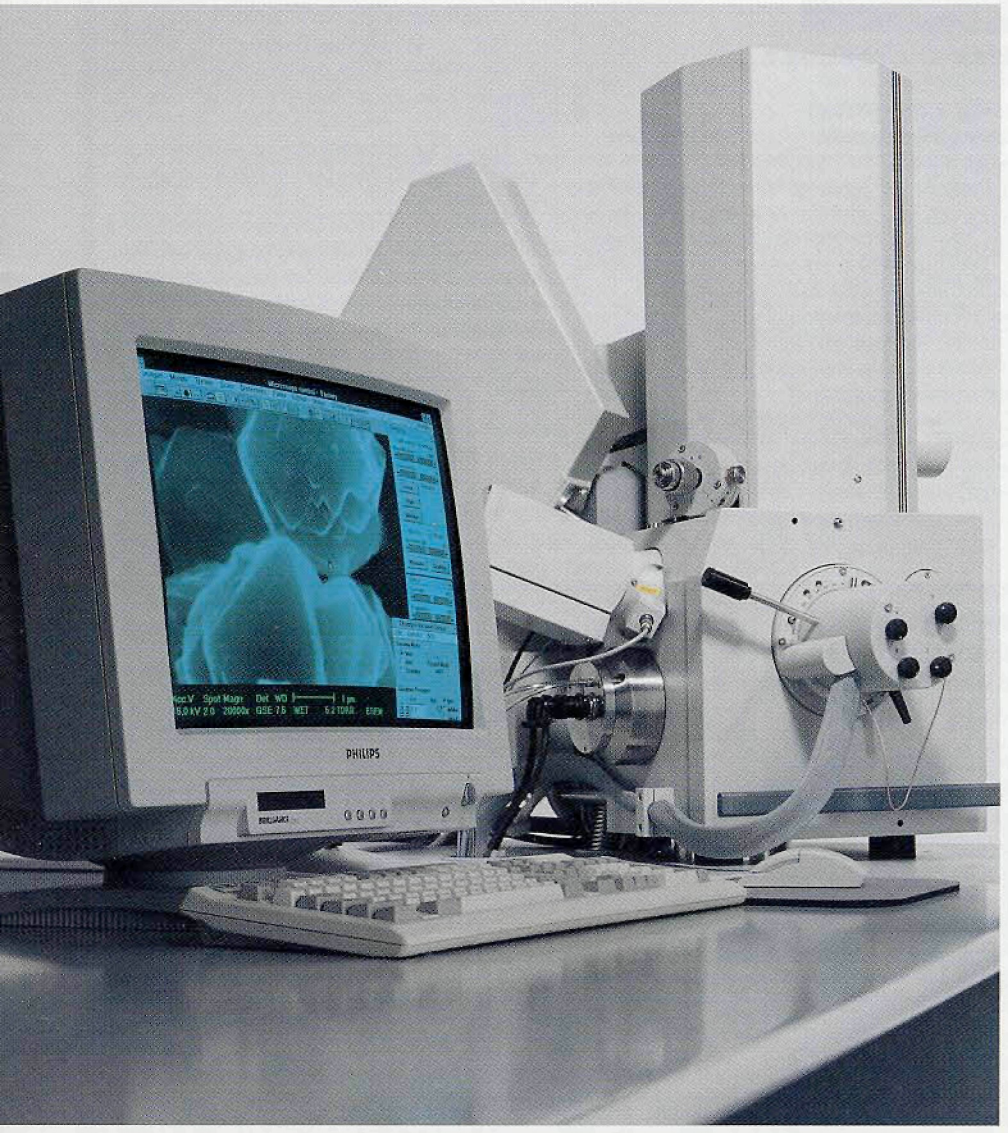

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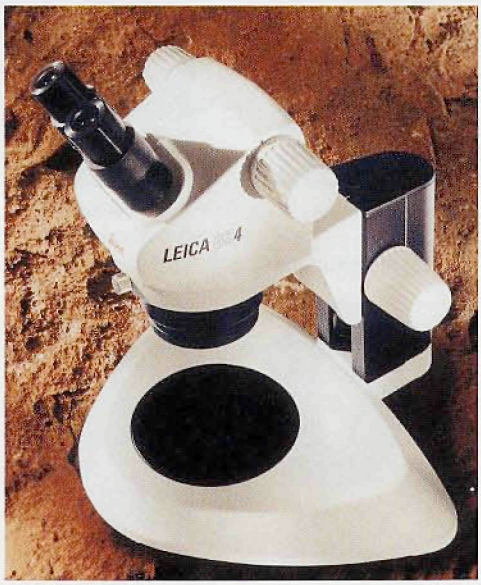

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Direct Monitoring of Combinatorial Chemistry Reactions by Infrared Microspectroscopy - Continued from Page 22

Conclusion

Infrared microspectroscopy was used to detect and characterize reaction products present on resin bead substrates. ATR microspectroscopy was chosen because it requires only a single bead for analysis, and the technique is non-destructive to the sample. A bead is simply placed on a glass slide, contact is made between the bead and the ATR crystal, and a spectrum is obtained. Clearly, infrared microspectroscopy can enable combinatorial chemists to monitor their reactions at each step of the synthesis. Because there is no sample preparation involved, the entire process increases the overall efficiency of this new and exciting technique for organic synthesis.

1. "Special Report: Combinatorial Chemistry", Chemical & Engineering News, February 12, 1996, pages 28-73.
2. "Combinatorial Chemistry Speeds New Product Discoveryby Michael Sullivan, Today's Chemist at Work, November 1995, pages 14-18.
3. "Infrared Spectrum of a Single Resin Bead for Real-Time Monitoring of Solid-Phase Reaction", by B. Yan *et al*, Journal of Organic Chemistry, Vol 60, 1995, pges 5736-5738

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