Application Note: 50861

In-Situ Analysis of Combinatorial Beads by Dispersive Raman Spectroscopy

Key Words

- Combinatorial Chemistry
- Confocal Imaging
- Microscopy
- Pharmaceutical
- Raman
- Reaction Monitoring
- Spectral Mapping
- Spectroscopy

Introduction

The recent development of solid phase combinatorial chemistry as a methodology for the rapid production of chemical diversity has revolutionized the way in which drug discovery is conducted within the pharmaceutical industry. Throughout the evolution of solid phase synthesis, analytical technology has faced the challenge of providing a new range of highly specialized analytical tools tailored to the intricacies of the solid phase environment.

A common implementation of combinatorial synthesis involves the use of functionalized polystyrene-based beads (100-250 µm diameter) as a support medium, onto which various compounds are attached for further reaction. Traditional applications of HPLC, MS, and NMR provide insight into the resin bound chemistry; however, these techniques require the reaction products to be cleaved from the polymer support. Infrared and Raman analyses have proven to be valuable analytical tools for the on-bead, non-destructive analysis of solid phase chemistry.[1-4] Comprehensive characterization of on-bead chemistry consists of not only final product analysis but also the intermediate reaction chemistry and kinetics. Recent emphasis has been on in-situ vibrational analysis, where the molecular structure and reaction kinetics are analyzed in real-time on the polystyrene substrate, throughout a reaction or solvent washing step.

The complementary nature of FT-IR and FT-Raman spectroscopies within the solid phase analysis arena has been well documented.[1-3] In this note, the Thermo Scientific Nicolet™ Almega™ dispersive Raman spectrometer coupled to a prototype Raman flow-cell[1,2] is utilized for real-time, single-bead analysis of diffusion characteristics and reaction kinetics throughout a series of dynamic chemical processes within the bead core. In these analyses, dispersive Raman microscopy, through its accommodation of visible frequency lasers, has been found to further complement the previous Fourier transform (FT) based analysis. Improved ν^4 Raman scattering characteristics of visible lasers coupled with CCD sensitivity reduce the need for high laser power and allow for shorter spectral acquisition times during the investigations. Improved diffraction characteristics of the shorter wavelength lasers also yield enhanced spatial resolution to permit improved spatial mapping of ligand binding throughout the bead cross-section subsequent to a reaction of interest. Finally, both FT and dispersive Raman techniques permit in-situ analysis without sample preparation.

Analyte Diffusion within the Bead Substrate

In order to get a more comprehensive picture of solvent swelling and diffusion processes within the bead, an experiment has been devised which permits direct investigation of analyte and/or solvent concentrations at the bead core.[2-3] In the present adaptation of this experiment, the rate of solvent exchange was investigated by mounting a bead in the flow-cell, N,N-dimethylformamide (DMF) was introduced and the bead was allowed to swell in the DMF reagent stream. Figure 1 presents the spectrum of an aminomethylstyrene bead swollen in DMF. The bands at 866 and 660 cm^{-1} correspond to DMF and are indicative of the degree of swelling or solvent concentration at the bead core.

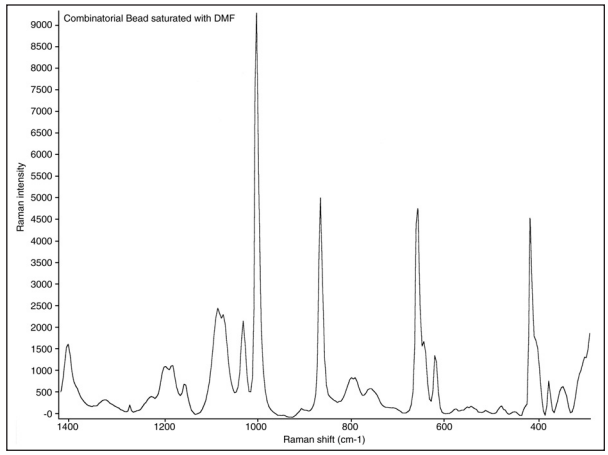

Figure 1: Dispersive Raman spectrum of a single combinatorial bead saturated with DMF

Acquisition of a time-resolved spectral series was initiated using the Nicolet Almega dispersive Raman spectrometer in conjunction with Thermo Scientific OMNIC™ software. After collection of several spectra of a bead in the DMF stream, the solvent stream was switched to a DMF solution containing 25% (w/w) CCl_4. Diffusion of CCl_4 into the bead core was subsequently monitored in real-time. After 34 minutes, the solvent stream was switched back to 100% DMF and elimination of CCl_4 was also examined. Figure 2 shows a spectrum of the polystyrene bead after sufficient time was given for the CCl_4 to equilibrate with the bead. Peaks at 450 and 308 cm^{-1} are indicative of the presence of CCl_4 in the bead.

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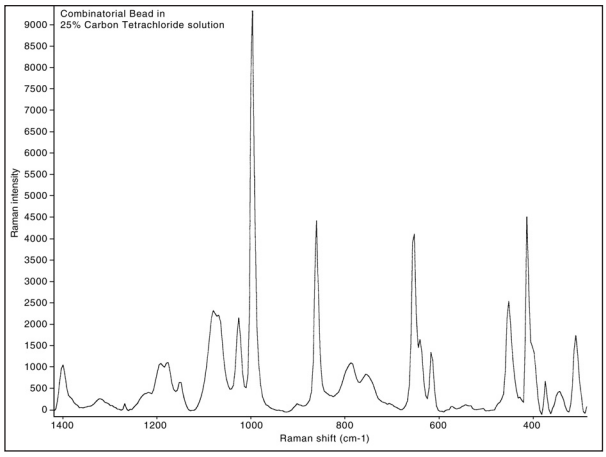

Figure 2: Dispersive Raman spectrum of a single combinatorial bead saturated with a solvent mixture of 0.25% CCl_4 in DMF

Figure 3 illustrates the comprehensive spectral series obtained from the bead core throughout the diffusion process of the CCl_4 pulse in the flow experiment. There is a rapid increase in the peaks at 450 and 308 cm^{-1} which indicates rapid uptake and equilibration of CCl_4 in the bead core. Upon equilibration, no change was noted until time t = 34 minutes, when the flowing solvent was switched back to pure DMF. A decrease in the 450 and 308 cm^{-1} peaks resulted from displacement of CCl_4 with pure DMF. In this series, Raman bands corresponding to CCl_4 are viewed against the complex spectral background that arises from the presence of the bead itself and from the DMF carrier solvent. Only bands at 450 and 308 correspond to CCl_4.

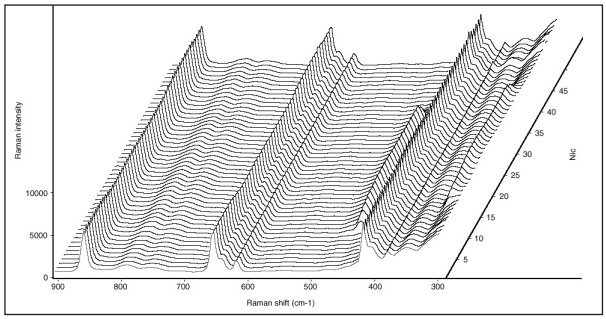

Figure 3: Waterfall spectral plot of a combinatorial bead in the flow cell over time as the flowing solvent is switched from pure DMF to a mixture of 25% CCl_4 in DMF and then switched back to pure DMF

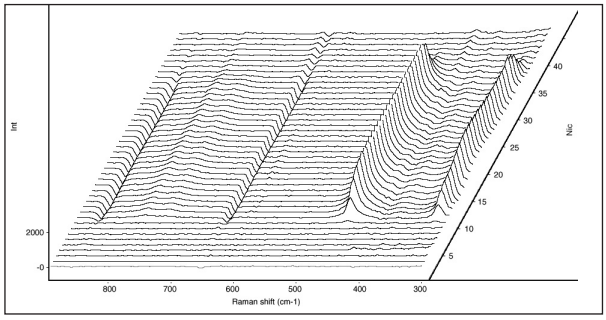

Figure 4: Waterfall plot of the data presented in Figure 3 with the background subtracted. The initial spectrum of a DMF saturated bead was subtracted from all spectra in the series to eliminate all spectral features that did not change during the experiment.

Figure 4 illustrates a recalculation of the Figure 3 spectral series in which the initial (t_0) spectrum of the series has been subtracted from each subsequent spectrum in the series. Figure 5 is an expansion of the data around the CCl_4 peaks of interest.

This presentation serves several purposes. First it eliminates all static spectral components such as the bead and most of the DMF carrier solvent from the profile to yield a much cleaner illustration of spectral activity. In this figure, presence of the CCl_4 pulse and the corresponding reduction in DMF concentration at the bead core are dramatically highlighted. Upon introduction of the CCl_4 containing solution, the peaks at 450 and 308 cm^{-1} increase dramatically, then level off until the solvent is switched back to pure DMF. Interestingly, the negative peaks at 866 and 660 cm^{-1} indicate a relative decrease in the amount of DMF in the bead, thus showing DMF displacement as well as CCl_4 uptake.

It is also noteworthy that the very clean baseline of the Figure 5 spectral series highlights the outstanding spectral subtraction capabilities not generally attributed to dispersive spectrometer systems. Finally, since Raman spectroscopy is based in molecular emission, as opposed to the infrared molecular absorption experiment, anomalies due to strong or totally absorbing solvent/bead bands have little impact on Raman based spectral subtraction results. The ability of Raman spectroscopy to produce high quality subtractions for spectra containing strong yet undesired spectral features opens the door to high quality analyses of a virtually unlimited range of solvents and beads relative to the infrared experiment.

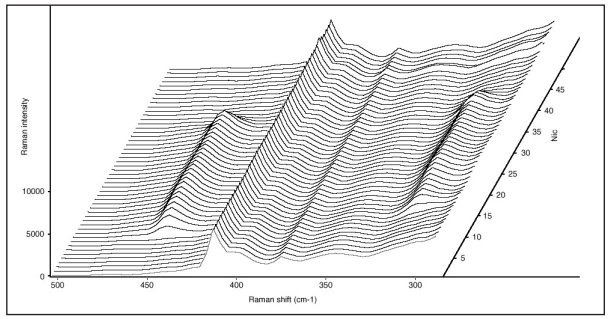

Figure 5: Expansion of Figure 4 around the CCl_4 peaks of interest

Homogeneity of Reaction

Another analysis of merit is the determination of the distribution of compound loading, or the location of reacted sites, within the functionalized bead. Loading analysis was obtained from a 10-µm thick sliced cross-section of a reacted bead using the Raman microscope's mapping capability. Figure 6 illustrates the chemical distribution profile extracted from an area map of the bead obtained at moderate spatial resolution using the Nicolet Almega dispersive Raman spectrometer. The bead is easily distinguished from the surrounding epoxy material, however, the relative peak intensities and positions are uniform across the entire bead, indicating that the reactant product is evenly dispersed across the bead and suggesting the reaction sites within the bead are homogeneously distributed.

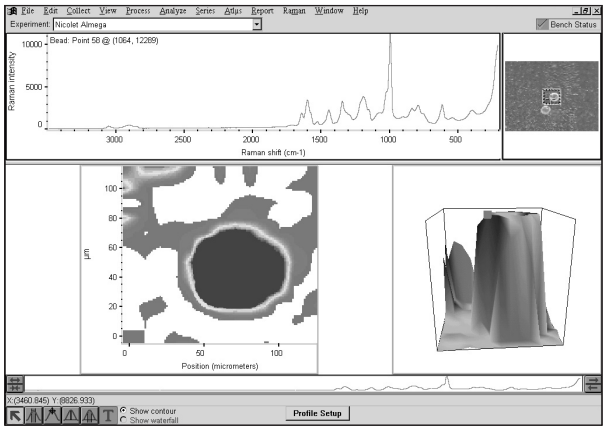

Figure 6: OMNIC Atlµs™ software chemical image extracted from a microscope map of the reacted bead. The image represents the distribution of reacted sites within the core of the bead.

Conclusion

Raman spectroscopy is capable of real-time monitoring of solvation and reactions within the polystyrene beads used for combinatorial synthesis without the need to cleave the samples. The combination of ease of sampling and abundant chemical information make it possible to determine such aspects as reagent diffusion, reaction kinetics and loading homogeneity within a single bead.

References

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3. Don E. Pivonka, Donald L. Palmer and Thomas W. Gero, *Applied Spect.*, 53(9), 1027, 1999.
4. Application Note, "Direct Monitoring of Combinatorial Chemistry Reactions by Infrared Microspectroscopy" (Spectra-Tech. Inc., Shelton, Connecticut, 1995).

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