



## High Throughput On-Bead Monitoring of Solid Phase Reactions by Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS)

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**Abstract:** Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) provides a method for high throughput on-bead monitoring of solid phase reactions. DRIFTS spectra can be acquired with a few milligrams of resin sample and sample preparation is not required. Analysis time is short and an automated DRIFTS accessory is commercially available.

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In the last few years, combinatorial synthesis<sup>1</sup> has evolved from a novel synthetic strategy into the key component incorporated in almost every drug discovery program. Solid phase reactions are much more frequently applied in combinatorial synthesis than solution phase reactions since excess reagents can be used and workup does not require more than filtration. In addition, when multistep synthesis is involved, only solid phase reactions can provide a practical route without tedious purification between each step. In multi-step parallel solid phase combinatorial synthesis where diverse building blocks with very different reactivities are employed, the combinatorial library can be constructed most efficiently when each solid phase reaction is monitored such that incomplete reactions can be resubjected to reaction conditions and unreactive components can be eliminated from the library at each step. However, this strategy requires a high-throughput non-destructive on-bead method for monitoring solid phase reactions. The two most practical technologies for on-bead monitoring of solid phase reactions are FT-NMR<sup>2-4</sup> and FT-IR<sup>5-8</sup> spectroscopy. However, lengthy sample preparation and instrument initialization are obstacles to applying any NMR technology to high throughput monitoring of solid phase synthesis. KBr pellet FT-IR requires laborious sample preparation, and it is difficult to recover the resin samples. FT-IR microscopy is an impressive technique to study solid phase reactions on a single bead. Nonetheless, there is no account of automated FT-IR microscopy. Therefore, KBr pellet FT-IR and FT-IR microscopy are not suitable for high throughput monitoring of solid phase reactions. The application of other techniques for monitoring solid phase reactions such as internal reflection FT-IR spectroscopy<sup>7</sup> and photoacoustic FT-IR spectroscopy<sup>8</sup> are plagued by possible sample contamination problems, inaccessibility of the instrumentation to organic chemists, and the lack of automated devices.

In our combinatorial synthesis program, solid phase reduction of resin-bound azido monosaccharides into the corresponding resin-bound amino monosaccharides was conducted in the presence of trimethylphosphine. It was necessary to monitor the completeness of individual reduction reactions in order to avoid complications in subsequent solid phase reactions resulting from truncated products caused by

unreacted azido compounds. Since IR spectroscopy could provide the most information in this reaction, we were interested in developing a high throughput IR method for analyzing resin samples.

The desired method should satisfy the following requirements: no sample preparation, short analysis time, and simple automation. There are three sampling techniques for IR spectroscopy: transmission, reflectance, and photoacoustic.<sup>9</sup> We recognized that Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS)<sup>9</sup> is the only candidate which can fulfill all the above requirements. DRIFTS has been used to obtain IR spectra of milligram quantities of undiluted powder samples or solid samples diluted with KBr powder. The advantage of DRIFTS is that it does not require sample preparation. The solid sample is held in a metal cup and placed at the focal point of the diffuse reflectance accessory. Hence after analysis solid samples can be put back into reaction vessels for further reactions. Moreover, automated diffuse reflectance systems are already commercially available.<sup>10</sup>

We found that IR spectra of polystyrene based resins could be obtained with DRIFTS<sup>11</sup> in similar manner to regular powder samples. The essential discovery was that DRIFTS spectra of polystyrene based resins could be acquired without dilution with KBr. Hence, the time and material consuming sample preparation step was eliminated. The IR spectrum of a few milligrams of resin sample was obtained in less than 30 seconds (16 scans). By using an automated diffuse reflectance system,<sup>10b</sup> 60 samples can be analyzed in a single batch. The IR spectra (absorbance, 4000-450  $\text{cm}^{-1}$ ) of two blank resins (NovaBiochem HL and NovaBiochem TG resin)<sup>12</sup> acquired separately by DRIFTS and by KBr pellet method are shown in Figure 1a-d. It was noted that the overall profiles of the corresponding DRIFTS (Figure 1a, 1c) and KBr pellet (Figure 1b, 1d) IR spectra of the two resins were very similar. In general, the most useful IR region for organic chemists, 2700-1600  $\text{cm}^{-1}$  (including absorption region of carbonyl, azido and cyano functional groups), was free from severe interference from the polymer backbone absorption in both methods. However, the KBr pellet spectra suffered from drifting baseline due to pellet preparation. It was also found that the weaker absorption peaks in the KBr pellet spectra were intensified in the DRIFTS spectra. This observation was due to a concentration effect since the DRIFTS spectra could be acquired at the highest concentration without dilution with KBr while it was necessary to dilute the sample in the preparation of KBr pellets.

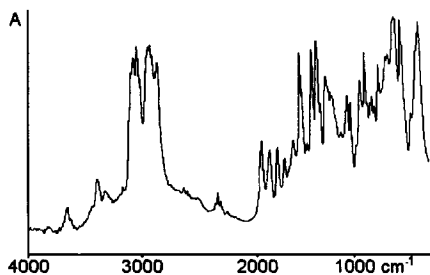


Figure 1a. NovaBiochem HL resins DRIFTS spectrum.

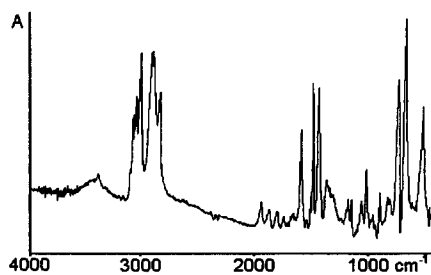


Figure 1b. NovaBiochem HL resins KBr spectrum

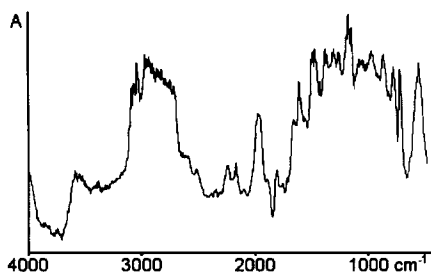


Figure 1c. NovaBiochem TG resin DRIFTS spectrum.

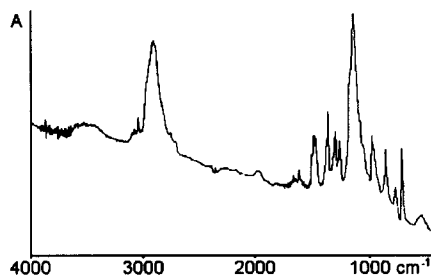
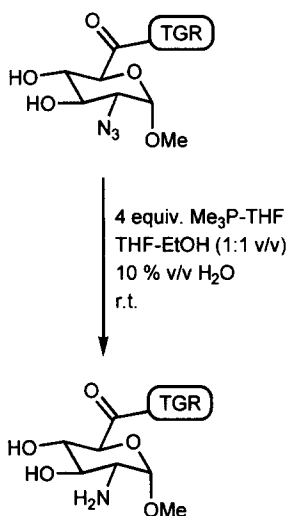


Figure 1d. NovaBiochem TG resin KBr spectrum

DRIFTS was subsequently applied in monitoring the solid phase reduction reaction of azido monosaccharides bound to NovaBiochem TGR resins<sup>13</sup> as illustrated in Scheme 1. The resin-bound azido monosaccharide was treated with trimethylphosphine in aqueous THF-EtOH. The DRIFTS spectra of the product resins acquired at various times during the course of the solid phase reduction reaction are overlaid as shown in Figure 2. The progress of the solid phase reduction reaction was monitored by the disappearance of the azide absorption band at 2108  $\text{cm}^{-1}$  (marked with  $\downarrow$ ).



Scheme 1

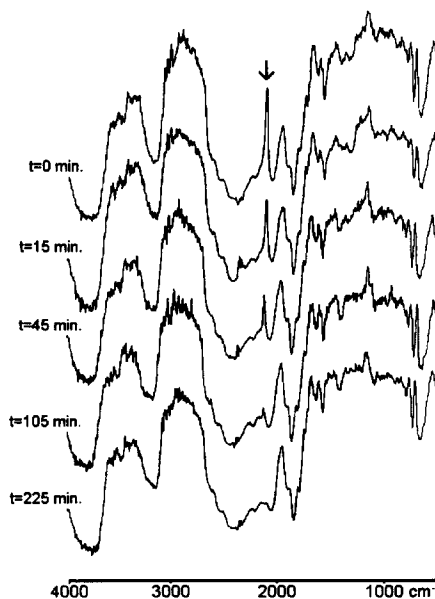


Figure 2. Time resolved DRIFTS spectra of solid phase reduction

In conclusion, we have demonstrated that DRIFTS provides a sensitive and convenient method for high throughput on-bead monitoring of solid phase reactions. Sample preparation is not required for widely used polystyrene based resins. Milligram amounts of resin are sufficient for analysis. The DRIFTS accessory is easy

to operate, and analysis of each sample can be completed in less than 30 seconds. Finally, an automated DRIFTS accessory is available for high throughput analysis.<sup>10</sup>

## References and Notes

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11. A few milligrams of resin was spread as a thin layer in a 4 mm DRIFTS sample cup. It was not necessary to cover the entire sample cup with resin. IR spectra were acquired in absorbance mode ( $4000-450\text{ cm}^{-1}$ ) with 16 scans at a resolution of  $4\text{ cm}^{-1}$  and were smoothed with a second degree six point Savitsky-Golay algorithm on a Midac Prospect FT-IR spectrophotometer equipped with a Spectros Instruments Diffuse Reflectance Accessory. Alignment and background were obtained once at the beginning with an empty sample cup.
12. NovaBiochem HL is a polystyrene resin with amino terminals. NovaBiochem TG is a polyethylene glycol grafted polystyrene resin with amino terminals
13. NovaBiochem TGR is a polyethylene glycol grafted polystyrene resin modified with a Rink linker.

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