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# A Comparison of Various FTIR and FT Raman Methods: Applications in the Reaction Optimization Stage of Combinatorial Chemistry

Bing Yan,<sup>\*,†</sup> Hans-Ulrich Gremlich,<sup>‡</sup> Serge Moss,<sup>‡</sup> Gary M. Coppola,<sup>†</sup> Qun Sun,<sup>†</sup> and Lina Liu<sup>†</sup>

Novartis Pharmaceuticals Corporation, 556 Morris Avenue, Summit, New Jersey 07901, and Core Technologies Area, Novartis Pharma AG, CH 4002, Basel, Switzerland

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Four different FTIR methods-single-bead FTIR, beam condenser, macro attenuated total reflection (macro-ATR), and KBr pellet methods-and macro and single-bead FT Raman methods have been investigated, and the relative utility was compared for the analysis of resin-bound organic compounds and the monitoring of solid-phase organic reactions. Furthermore, the comparison includes two additional methods from the literature: diffuse reflectance infrared Fourier transform spectroscopy and photoacoustic spectroscopy. While all of these methods have some utility for solid-phase sample analysis, the relative merits of these methods vary particularly in such areas as the information content, spectral quality, sensitivity, speed, sample requirement, and the instrument cost. Both single-bead FTIR and beam condenser FTIR methods have been found to be superb methods in each of these aspects. In the following way, these methods meet many of the essential requirements for a thin layer chromatography (TLC) equivalent for solid-phase synthesis: (1) Only a single bead or 50-100 beads are needed for analysis so that reaction is not interrupted and is monitored in real-time. (2) A high-quality spectrum can be recorded within a few minutes. (3) No sample preparation is required, making the analysis time even shorter than that for TLC analysis. (4) These two FTIR methods provide qualitative, quantitative (the percentage of conversion), and kinetics information on organic reactions carried out on resin supports. Finally, from the synthetic chemist's point of view, the additional advantages of the beam condenser method, such as the low cost and the ease of operation, make it a more suitable choice as a TLC equivalent for solid-phase organic synthesis applications.

# Introduction

Solid-phase organic synthesis (SPOS)<sup>1</sup> is a powerful technique to assemble compound libraries in high-throughput parallel and combinatorial<sup>2</sup> synthesis. Before these libraries can be made, a time-consuming reaction optimization is always required. In this optimization process, it is difficult to monitor the reaction progress on solid supports in the same manner as thin layer chromatography (TLC) in solution chemistry. To analyze SPOS intermediates and products, chemical cleavage is often required to separate the compounds from the solid support. Indirect information obtained from a solution sample is then used to judge what has occurred on the solid support. A solid-phase reaction often must be run longer than necessary (to ensure a complete reaction) before the cleavage and in-solution analysis. However, to "cleave and analyze" is a time-consuming, expensive, and laborious process. Some synthetic intermediates are not stable enough for the "cleave and analyze" protocol. It is especially a waste of time and sample for

<sup>†</sup> Novartis Pharmaceuticals Corporation.

<sup>‡</sup> Novartis Pharma AG.

characterizing intermediates in multistep synthesis. Photochemical linkers have simplified this procedure, but the types of reactions that can be carried out on such labile linkers are limited. In addition, some intermediates are photolytically unstable and cannot be isolated. It is therefore necessary to obtain chemical information directly on resin support.

Organic reactions selected to assemble a combinatorial library are preferably reliable and well-behaved. Therefore, the structural determination of resin-bound totally unkown compounds is rarely required. For the most part, the analytical task is to confirm the desirable chemistry rather than a full structural elucidation. Since FTIR is a technique sensitive to organic functional group changes, it is well suited for the confirmation of organic transformations. The principle of monitoring reactions by IR is based on the functional group interconversions via chemical reaction or by appearance or disappearance of functional groups carried by building blocks or protecting groups introduced or removed during the reaction. The functional group to be monitored need not be directly involved in the reaction that is monitored. Therefore, for the rehearsal of a library synthesis on solid support, building blocks can even be selected to contain an IR detectable group at a remote site.

<sup>\*</sup> To whom correspondence should be addressed. Phone: 908-277-6023. Fax: 908-277-2785. E-mail: bing.yan@pharma.novartis.com.

In practice, FTIR is becoming a vital technique for monitoring reactions in SPOS while its power in solution chemistry has faded away for decades. On-resin analysis has been obtained on 5–10 mg of resin beads using techniques such as the KBr pellet method,<sup>3</sup> FT Raman,<sup>4</sup> diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS),<sup>5</sup> photoacoustic spectroscopy,<sup>6</sup> and alternatively, by FTIR microspectroscopy on a single-resin bead.<sup>7</sup> Resinbound compound confirmation and characterization have been reported with all of the above-mentioned methods. However, features of these methods such as the sample requirement, the spectral information and quality, the speed of analysis, and the instrument cost vary. To optimize the application of vibrational methods in combinatorial chemistry, a critical comparison of these methods is needed.

TLC is a technique that reaches every chemist's bench. A equivalent method would be needed to assist the quality control of SPOS in the rehearsal phase of combinatorial chemistry. If the method is to be as convenient as TLC, it should detect necessary spectral changes reflecting a chemical transformation and the reaction kinetics. It is preferably rapid, sensitive, and easy to operate in the format of openaccess laboratory. Additionally, it should consume as little sample as possible, and it should be of low cost. We carried out a series of investigations of six FTIR and FT Raman techniques [(1) single-bead FTIR, (2) FTIR using a beam condenser, (3) FTIR using a macro attenuated total reflection (macro-ATR) accessory, (4) macro FT Raman technique, (5) single-bead FT Raman, and (6) FTIR by the KBr pellet method] in analyzing solid-bound organic compounds. Two methods (single-bead and beam condenser FTIR methods) were selected to monitor solid-phase organic reactions in this work. Besides these FTIR and FT Raman methods, two previously reported methods [(7) the diffuse reflectance infrared Fourier transform spectroscopy or DRIFT method<sup>5</sup> and (8) the photoacoustic method<sup>6</sup>] were also included in our comparisons. Here we report key results from our studies.

## Background

**Resin Samples.** Solid supports commonly used for assembling compound libraries are polystyrene- (PS) or polystyrene-poly(ethylene glycol)- (PS-PEG) based resin beads. They have a diameter of  $50-120 \,\mu$ m in the dry state and can be enlarged when swollen with suitable solvent. The size distribution of PS resins is generally wider compared to that of PS-PEG resins. The loading is in the range of 0.2–0.4 mmol/g for PS-PEG resins and 0.6–1.2 mmol/g for PS resins. Reactive groups are linked to a benzene ring via short spacers in PS resins and via a ~60 PEG unit long spacer in PS-PEG resins.

**Spectroscopy Methods.** Eight FTIR and FT Raman techniques have been evaluated in this investigation. These methods are briefly described in the following.

**1. FTIR Microspectroscopy.** This method records spectrum from a small sample (such as a single bead) in the transmission mode. A microscope accessory is required for the measurement. A liquid nitrogen cooled mercury–

cadmium-telluride (MCT) detector is used to enhance the sensitivity. Several beads are spread on a diamond window and flattened with a compression cell (SpectraTech, Shelton, CT). The IR beam is focused on a flattened single bead using the view mode of the microscope. The blank area surrounding the bead is cut off using an adjustable aperture. A spectrum is recorded with 32 scans (<1 min). Then, the focus is moved to the nearby blank area of the same size on the diamond window to record the background.

**2. Beam Condenser.** A simple optical accessory is used to reduce the IR radiation from a typical 8 mm beam to 2 mm at the sample plane. This allows the analysis of smallest quantity of pressed resin beads (50-100 beads) without using the KBr dilution. A diamond compression cell is used to flatten beads and in the measurement. The same diamond cell without beads is then used to record a background spectrum.

3. Attenuated Total Reflection (Macro-ATR). Under certain conditions, infrared radiation passing through a prism made of a high refractive index infrared transmitting material (ATR crystal) will be totally internally reflected. If a sample is brought in contact with the totally reflecting surface of the ATR crystal, an evanescent wave in the less dense medium extends beyond the reflecting interface and the evanescent wave will be attenuated in regions of the infrared spectrum where the sample absorbs energy. The intensity of the wave decays exponentially with the distance from the surface of the ATR crystal. The distance, which is on the order of micrometers, makes ATR generally insensitive to sample thickness, allowing for the analysis of thick or strong absorbing samples. Micro-ATR measurement of the partial surface from a single bead has been reported by us<sup>11</sup> before. In this work, macro-ATR measurement was performed on  $\sim 0.2$  mg of beads.

4 and 5. Macro and Single-Bead FT Raman Spectroscopy. FT Raman spectroscopy is based on inelastic light scattering, in which scattered photons exchange energy with the sample. Most commonly, the scattered photon loses energy to a vibrational mode of the sample molecule, leading to a downward frequency shift. This Raman shift is equal in energy to the light absorbed by the same molecule in an IR absorption experiment if the mode is active toward both techniques. Because of the differences in the selection rules, both FTIR and FT Raman spectra must be measured to obtain the complete vibrational characteristics of a totally symmetric molecule. For an asymmetric molecule, a FT Raman spectrum may not be necessary since it provides information similar to that in an FTIR spectrum except the intensity difference.

The factor contributing to the intensity of Raman band is the change of polarizability during the vibration. Therefore strong FT Raman signals can be obtained for functional groups with low polarity and high polarizability such as  $C \equiv$ C, C=C, N=N, S-S, C-H, S-H, C=N, C=N, C=S, and C-S and symmetric vibrations of groups with a high degree of symmetry such as NO<sub>2</sub>, SO<sub>2</sub>, CO<sub>2</sub>, and aromatic rings. Since bands intensity is enhanced by conjugation, FT Raman is also useful for confirming the presence of conjugated groups.

Scheme 1



The excitation light used in FT Raman is in the nearinfrared region. The spectra produced are free of the fluorescence interference that is usually typical for spectra obtained by visible laser excitation. FT Raman spectra can be acquired in macro-mode on 0.2 mg of beads or in micromode such as single-bead FT Raman microspectroscopy.

6. KBr Pellet Method. This is a widely used sampling technique for solid samples. The method consists of mixing finely ground (0.5  $\mu$ m average particle size) sample with a pure, dry spectroscopic grade of KBr powder, usually at a concentration of about 1% sample in KBr, and transferring the mixture to a die where it is pressed until the KBr particles coalesce into a clear disk. The disk is measured using the regular FTIR instrument in which the diameter of the IR beam is 8 mm. For resin samples, although polystyrene–PEG resins can be ground into small particles, polystyrene resin beads cannot. The sample particle size in the resin KBr pellet is ~80  $\mu$ m rather than the required 0.5  $\mu$ m. This causes problems such as light scattering when the KBr pellet is used (see Results and Discussion below).

7. Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT). When infrared radiation is directed onto the surface of a solid sample, two types of energy reflectance can occur. One is specular reflectance and the other is diffuse reflectance. The specular component is the radiation which reflects directly off the sample surface (it is the energy that is not absorbed by the sample). Diffuse reflectance is the radiation which penetrates into the sample and then emerges. A diffuse reflectance accessory is designed so that the diffuse reflected energy is optimized and the specular component is minimized. The optics collect the scattered radiation and direct it to the infrared detector.

**8.** Photoacoustic Spectroscopy. When modulated infrared radiation is absorbed by a sample, the substance heats and cools in response to modulated infrared energy impinging on it. The heating and cooling is converted into a pressure wave that can be communicated to a surrounding gas and so detected by an acoustic detector (essentially a sensitive microphone in the enclosed sample chamber). In such measurements, the acoustic detector replaces the infrared detector of the spectrometer.

Resin beads contain 99% of functional groups in the bead interior. A transmission measurement is a method predominantly measuring the bead interior. Among the methods compared in this work, 1, 2, and 6 are transmission analysis methods for the study of whole beads while 3, 7, and 8 are based on surface analysis only. Methods 4 and 5 are based on the analysis of light scattering from the bead exterior.

#### **Results and Discussion**

In this comparison study, we carried out two series of experiments. First, an array of resin-bound compounds (Scheme 1) carrying diverse organic functional groups were analyzed by various methods in order to compare the capability of each method in detecting the characteristic bands and the resulting spectral quality. Then we monitored two series of solid-phase organic reactions using the selected methods to demonstrate their ability in TLC-like reaction monitoring. Finally, we compare merits of various methods based on these results and the additional criteria such as sample consumption, the simplicity of the instrument, the operation, and the cost of instrumentation.

Analysis of 10 Resin-Bound Compounds by Single-Bead FTIR, Beam Condenser, Macro-ATR, Macro and Single-Bead FT Raman, and KBr Pellet Methods. 1. 1% Divinylbenzene (DVB) Copolymerized Polystyrene Resin. Figure 1a–j shows the spectra of resins 1–10 obtained by five different methods. Figure 1a shows the spectra of 1% DVB copolymerized polystyrene. All techniques seem to provide reasonably good data. Among them,



3500 3000 2500 2000 1500 1000 3500 3000 2500 2000 1500 1000

### Wavelength (nm)

**Figure 1.** Spectra of resin-bound organic compounds listed in Scheme 1 obtained by various FTIR and FT Raman methods. Resins are (1) polystyrene resin, (2) Merrifield resin, (3) Wang resin, (4) formylpolystyrene, (5) carboxylpolystyrene, (6) brominated Wang resin, (7) oxime resin, (8) Fmoc-Cys(*t*-Buthio)-Wang resin, (9) NovaSyn TG aldehyde resin, (10) NovaSyn TG thiol resin.

single-bead IR and the beam condenser technique produced high-quality spectra. The spectrum recorded on a macro-ATR shows a more overlapping fingerprint region and water interference in the region above 3200 cm<sup>-1</sup>. The macro FT Raman spectrum of the polystyrene backbone, dominated by the strong trigonal ring breathing vibration of the monosubstituted benzene rings at 1000 cm<sup>-1</sup>, is of good quality. The complementary selection rules are reflected in the complementary peak intensity in the spectrum compared with those obtained by FTIR. The spectrum obtained by the KBr pellet method is a little more noiser and also shows moisture interference. The noise in the spectrum may be due to the fact that the 8 mm sampling area of the pellet is only partially filled with whole resin beads ( $\sim 80 \ \mu m$ particles), decreasing the sampling efficiency. The inclusion of more bead interfacial surfaces and KBr itself are both sources of moisture. The baseline is often tilted in a KBr pellet spectra, owing to the light scattering problem originated from the heterogeneity in the pellet.

2. Merrifield Resin. Figure 1b shows spectra of Merrifield resin obtained by various methods. Spectral quality is of the same order as in Figure 1a. The typical CH<sub>2</sub>-Cl wagging vibration at 1263 cm<sup>-1</sup> is rather weak in spectra obtained by ATR and FT Raman methods. A very weak band for C-Cl stretching was observed in the Raman spectrum at  $\sim$ 700 cm<sup>-1</sup> (not shown). The baseline is tilted in the KBr pellet spectrum.

3. Wang Resin. The structural difference of Wang resin (Figure 1c) from the polystyrene resin is that the former contains a benzylic hydroxyl group. A sharp band at 3573 cm<sup>-1</sup> and a broad band at 3449 cm<sup>-1</sup> observed by all methods with the exception of the FT Raman method are attributed to the free and intraresin hydrogen-bonded hydroxyl groups.8 A strong  $CH_2$ -OH bending vibration at 1232 cm<sup>-1</sup> is observed by all methods except FT Raman. The FT Raman spectrum in this figure is almost identical to that of the polystyrene resin as shown in Figure 1a except small differences in 800-900 cm<sup>-1</sup> region. This is due to the different selection rule in Raman scattering, and vibrations of polar groups are not easily observable. Although FT Raman signals of some of these polar groups can be better observed when a UV-vis laser is used for excitation, fluorescence is often a problem.

**4. Formylpolystyrene Resin.** The spectrum of formylpolystyrene resin is characterized by a band at  $2713 \text{ cm}^{-1}$  attributed to the aldehyde (O=)C-H stretch and a band at  $1702 \text{ cm}^{-1}$  attributed to the carbonyl group (Figure 1d). All FTIR methods can detect these signals except for interference and S/N ratio problems in cases of ATR and KBr pellet. In FT Raman spectrum, these signals are relatively weaker.

**5.** Carboxylpolystyrene Resin. Spectra of carboxylpolystyrene resin are shown in Figure 1e. In the liquid or in solution at concentrations over 0.01 M, carboxylic acids exist as dimers due to strong hydrogen bonding. Because of the strong bonding, a free hydroxyl stretching vibration near 3500 cm<sup>-1</sup> is observed only in very dilute solution in nonpolar solvents in which a mixture of monomer and dimer is observed. In the spectrum of resin-bound carboxylic acid,

the O-H shows a broad band from 3300 to 2500  $cm^{-1}$ , a feature of hydrogen-bonded carboxyl groups. A sharp band at 3450 cm<sup>-1</sup> is also observed in spectra recorded by singlebead, beam condenser, and KBr pellet methods. This band is an indication of the presence of a portion of free carboxyl groups. This "site isolation" effect is further corroborated by the frequencies of the carbonyl group. The band at 1722 cm<sup>-1</sup> was attributed to the free carboxyl groups and the band at 1690 cm<sup>-1</sup> to the associated carboxyl groups.<sup>9</sup> The origin of the band at  $1662 \text{ cm}^{-1}$  is not so clear. Since this band remains after esterification of the resin, it may come from the manufacturing intermediate which is a ketoester or an amide formed with an amine impurity during the resin preparation process. In addition to O-H and the carbonyl groups, the -OH in-plane bending at 1423 cm<sup>-1</sup>, the C-Ostretching at 1280 cm<sup>-1</sup>, and the O–H out-of-plane bending at 940 cm<sup>-1</sup> are all observed. The carbonyl vibrations were weakly observed by the FT Raman method. In addition, the above-mentioned vibrations are not observed except the band at  $1280 \text{ cm}^{-1}$ . The strong intensity of the benzene ring stretching band at 1608 cm<sup>-1</sup> is an indication of a highly conjugated system.

6. Brominated Wang Resin. Brominated Wang resin showed characteristic peaks at 1685 cm<sup>-1</sup> for the ketone carbonyl group and a strong band at 1242 cm<sup>-1</sup> for the  $\Phi$ -C(=O) stretching and bending vibrations (Figure 1f) besides other features. Spectra from the single-bead and beam condenser methods clearly display these signals. ATR and KBr methods also show these features except the interference these methods are usually subjected to. The macro FT Raman method showed a weak band for the benzene-conjugated carbonyl at 1685 cm<sup>-1</sup> and a C-Br vibration below 600 cm<sup>-1</sup> (not shown), but failed to clearly detect the  $\Phi$ -C(=O) stretching and bending vibration at 1242 cm<sup>-1</sup>.

**7.** Oxime Resin. The spectrum of oxime resin (Figure 1g) contains several interesting features: a sharp hydroxyl band at 3503 cm<sup>-1</sup>, a weak carbon—nitrogen double-bond stretch at 1660 cm<sup>-1</sup>, and asymmetric and symmetric stretching vibrations of the nitro group at 1523 and 1345 cm<sup>-1</sup>. All of these signals have been identified by FTIR methods except the usual interference and the overlapping fingerprint region bands in the ATR method and usual interference and baseline tilting in the KBr pellet method. FT Raman spectroscopy failed to unambiguously detect bands at 3503 and 1523 cm<sup>-1</sup>. However, the N=O symmetric stretching of the nitro group at 1345 cm<sup>-1</sup> is stronger in the FT Raman spectrum than in the IR spectrum.

**8. Fmoc-Cys**(*t*-**Buthio**)-**Wang Resin.** Figure 1h shows the spectra of Fmoc-Cys(*t*-Buthio)-Wang resin obtained by various methods. Characteristic features in this compound are an ester carbonyl, a Fmoc carbonyl, an amide NH, and a disulfide bond. The two carbonyl bands overlap at 1727 cm<sup>-1</sup>, and NH stretch is at 3420 and 3350 cm<sup>-1</sup> as a doublet. These features are easily observed by FTIR methods, but not by FT Raman. The vibration of S–S is between 450 and 550 cm<sup>-1</sup>, which is outside the range of regular FTIR instrument optics. Therefore, this signal was not observed

by FTIR methods. The vibration of the S-S bond was detected by FT Raman method (Figure 1h inset).

9. PS-PEG Aldehvde Resin. The detection of PS-PEG-bound organic functional groups and the monitoring of reactions on PS-PEG resins are not as easy as that on PS resins. This is because (1) the bead cannot be flattened due to the poor mechanical stability of PS-PEG resins, (2) the loading is usually 3-6-fold lower on PS-PEG resins compared to that on PS resins, (3) there is always a huge band at  $\sim 2900 \text{ cm}^{-1}$  originating from the CH<sub>2</sub> stretching and a huge band at 1125-1055 cm<sup>-1</sup> from the ether bonds in PEG spacer, and (4) it is impossible to completely remove moisture from PS-PEG resins. Even though there are difficulties with PS-PEG resins, the kinetics of seven different organic reactions on PS-PEG resins has been studied and compared with that on polystyrene resins using the single-bead FTIR method.<sup>10</sup> Using several milligrams of beads, spectra of PS-PEG resins with the quality comparable to data in Figure 1i,j were obtained by the DRIFT method. In the present study, two PS-PEG resins were examined.

Spectra of TentaGel S–CHO were recorded by various methods and are shown in Figure 1i. This aldehyde resin is linked to the PEG spacer by an amide linkage. The quality of the spectra is generally reduced for all methods compared to the data for PS resins. Single-bead and beam condenser methods can detect the aldehyde carbonyl group at 1720 cm<sup>-1</sup> and the amide carbonyl band at 1670 cm<sup>-1</sup>. Other methods do not provide useful information in this case.

10. TG Thiol Resin. Spectra of NovaSyn TG thiol resin are shown in Figure 1j. The only characteristic feature is the stretching of S-H at 2515 cm<sup>-1</sup>. This weak signal was not detected by any method except single-bead IR. Spectra obtained by beam condenser, ATR, and FT Raman show better resolution in the fingerprint region. However, no compound signal was detected due to the intrinsic low intensity of the S-H vibration and the low loading of the substrate on resin. Although the single-bead IR spectrum is oversaturated by resin background, it seems that only the overloading effect in the single-bead FTIR (i.e., without flattening the bead) allows the detection of the S-H stretching signal.

11. Macro and Single-Bead FT Raman Methods. All resins were also measured by the single-bead FT Raman method. The spectrum obtained with one bead by means of the FT Raman microscope, although noiser, is nearly comparable to that obtained with 0.2 mg in the "macro" mode (Figure 2). However longer acquisition time (10-20 min) is always required with the microscope method and the high laser irradiance (1000 mW) that increases the chances for photolysis or photodecomposition.

**Comparison of Techniques.** We have compared FTIR and FT Raman techniques presented in Figure 1 along with the published data on DRIFT<sup>5</sup> and photoacoustic<sup>6</sup> techniques in terms of information content and quality such as signal/ noise ratio, resolution and artifact, sample requirement, speed, ease of operation, and the instrument cost (Table 1).

**1. Information Content and Quality.** In general, all FTIR methods provide useful information on the solid-phase



Figure 2. Comparison of macro FT Raman spectra and singlebead FT Raman spectra. (A) Single-bead FT Raman spectra of resins 1, 3, 5, 7, and 9. (B) Macro FT Raman spectra of resins 1, 3, 5, 7, and 9.

Table 1.
Comparison of Various FTIR and FT Raman

Techniques<sup>a</sup>
Provide the second se

	info content	rapid analysis	less sample	spectral quality	low cost	total
single-bead	5	5	5	5	2	22
beam condenser	5	5	3	5	4	22
ATR	5	5	3	3	4	20
macro FT	3	4	3	4	1	15
Raman single-bead FT Raman	3	2	5	3	1	14
KBr	5	2	1	2	5	15
DRIFT	5	5	1	3	4	18
photoacoustic	5	5	1	3	3	17

<sup>*a*</sup> Note: Various methods were compared for features listed in these entries. Their relative rankings in these aspects were scored. The highest score is 5 and the lowest is 1.

reactions, but the quality and the extent of the revealed information vary. From Figure 1, it is clear that single-bead FTIR and the beam condenser methods are subject to less artifact interferences and therefore offer more observable information on the resin-bound compounds. Spectra obtained by the ATR method exhibit somewhat lower resolution in the fingerprint region compared with those obtained by single-bead and beam condenser methods. Moisture interference at 3100–3500 cm<sup>-1</sup> is significant in ATR spectra. The KBr method is the only destructive method among all methods in this investigation. It is not possible to grind the beads. The whole beads in the pellet cause light scattering and thus baseline tilting. Moisture interference is also prominent. Because of the different selection rules for Raman spectroscopy, many important vibrations are not detectable by the FT Raman method such as OH, NH, aliphatic C=O, C=N, and C-O vibrations, only considering the limited examples studied in this work. However, some IR-inactive or weak vibrational modes can be detected by FT Raman such as the stretching of S-S (Figure 1h). Thus FT Raman can be considered as a complementary technique to FTIR methods in the analysis of solid-bound organic molecules.

**2.** Sample Requirement. KBr pellet, DRIFT, and photoacoustic methods generally require 5-10 mg of beads. Among these methods, DRIFT and photoacoustic method

are not destructive to the sample. The sample can be reused for synthesis after the analysis. This is clearly an advantage. However, under regular SPOS scale, the reaction has to be interrupted by analysis with these methods due to the demanded sample size. Macro FT Raman needs about 0.2 mg of resin beads for a 5 min analysis (laser power = 350mW). Single-bead FT Raman can be performed using FT Raman microspectroscopy in 10-20 min and a 1000 mW laser output. The results obtained with both FT Raman methods are comparable (Figure 2). ATR and the beam condenser methods only require enough beads to cover the area of the ATR crystal ( $\Phi = 0.5$  mm) and the diamond window ( $\Phi = 1.5$  mm) of the compression cell. Although oversample can ensure the coverage, 50-100 beads is the minimum amount of sample to be used. The most sensitive method is the single-bead FTIR method. We have demonstrated in the past<sup>11</sup> that the detection limit of the method is about 125 fmol of sample which is about 1/3840 of a singlebead loading. The amount of sample required by singlebead FTIR, single-bead FT Raman, beam condenser, and ATR analysis is so small that the reaction can be monitored without the need to interrupt the reaction (real-time reaction monitoring). This is clearly the advantage similar to what TLC offers in the monitoring of the solution synthesis.

**3.** Speed of Analysis. No sample preparation is required for all methods except the KBr pellet method. All FTIR methods exhibit a high speed of analysis (1-2 min) except that the KBr pellet method requires the making of pellet (~10 min). At a moderate laser power (~350 mW), the accumulation of a FT Raman spectrum from a 0.2 mg sample requires ~5 min. An increase in the laser power can reduce the accumulation time. However, it may cause photoreactions or photodecompositions of the sample. Single-bead FT Raman analysis requires 10-20 min and at least 1000 mW of laser power. However, the measuring time highly depends on the Raman activity of the sample.

Automation. The single-bead FTIR and single-bead FT Raman methods can be automated by using a computercontrolled motorized stage (SpectraTech, Shelton, CT). Automated analysis using DRIFT and KBr methods can be obtained commercially (Pike Technologies, 2919 Commerce Park Drive, Madison, WI 53719; Spectros Instruments, Ins., 175 North Street, Shrewsbury, MA 01545). Macro FT Raman can be automated by a rotating wheel assembly. Automation of ATR and of photoacoustic spectroscopy are not known to our knowledge.

**4. Instrumentation Cost.** FTIR is the most popular analytical instrument in organic chemistry laboratories. To use the above-mentioned techniques for analyzing polymerbound organic molecules, accessories to a regular FTIR are required. They can be arranged in order of increasing cost as follows: KBr, beam condenser, ATR, DRIFT, photoacoustic, single-bead FTIR, macro FT Raman, and single-bead FT Raman.

**5. DRIFT and Photoacoustic Methods.** We did not carry out studies with these two techniques. The comparison with these two methods was on the basis of the refs 5 and 6, assuming that authors had optimized their methods. In DRIFT measurement, several milligrams of resin was used.





The analysis time was similar to other FTIR methods. The spectral quality can be seen from ref 5. In general, the fingerprint region is subject to signal overlapping in DRIFT methods. The cost of the accessory is, however, low (similar to ATR).

In photoacoustic measurement, less than 10 mg of resin was used. The measuring time is about a few minutes (500 scans), that is, fast enough for routine applications. The spectral quality is similar to DRIFT and ATR spectra. Signal overlapping in the fingerprint region is also observed. The cost of this accessory is higher than DRIFT, ATR, and beam condenser.

The above comparisons are summarized in Table 1 in terms of numeric scores from 1 to 5. Scores based on relative comparisons in each category are summed, and the final scores are compared. In general, the most appropriate technique for a laboratory is the technique that is conveniently available. However, the value/cost ratio of these methods varies. For those chemists who are searching for a method to monitor SPOS, the comparisons offered by Table 1 are very informative. Single-bead FTIR is an impressive method. It provides the highest quality spectrum, fast analysis, and compatibility particularly with one-bead-onecompound libraries. The beam condenser method offers the similar quality spectrum with a little more beads (50-100)beads). With the same speed of analysis, the operation is much simpler. Furthermore, the cost of a beam condenser is much lower than an IR microscope. The ratings of other methods are also listed.

**Monitoring of Polymer-Supported Organic Reactions.** Next we intend to compare these methods as a routine tool for the monitoring of solid-phase organic reactions. By monitoring reactions shown in Schemes 2 and 3, the qualititative and quantitative aspects and kinetics capability of these methods are evaluated. Because of the large sample consumption and the whole bead scattering problem, the KBr pellet method is not suitable for noninterrupting analysis of these reactions. FT Raman cannot detect hydroxyl stretching vibrations and is therefore not used here. Because of the moisture interference in the hydroxyl stretching region in ATR method, it is not appropriate for the analysis of these reactions. Since impressive results have been obtained by the single-bead FTIR and beam condenser FTIR methods,



Figure 3. IR spectra of resin-bound compounds 3, 11, and 12 (Scheme 2) obtained by (A) single-bead FTIR and (B) beam condenser FTIR.

Scheme 3



these two methods are the better choices for monitoring the reactions in Schemes 2 and 3.

A brominated ether **11** was synthesized by the reaction of 4-bromophenol with Wang resin under Mitsunobu conditions (Scheme 2). The reaction is almost quantitative as seen by the nearly complete disappearance of the hydroxyl stretching at 3577 and 3458 cm<sup>-1</sup> (spectra 1 and 2 in Figure 3). The integration of the band areas at 3577 and 3458 cm<sup>-1</sup> indicates a conversion yield of ~95%. The lithium—halogen exchange followed by reaction with 4-chlorobenzaldehyde produced secondary alcohol **12**. A clear indication is the formation of hydroxyl group stretching signals (spectrum 3 in Figure 3). Results obtained by the beam condenser method are comparable with those obtained by single-bead FTIR method.

Single-bead FTIR has been extensively used for kinetics studies in the past.<sup>10,11,14</sup> To establish the kinetics capability



**Figure 4.** IR spectra of the reaction product at various times during the reaction in Scheme 3 obtained by (A) single-bead FTIR and (B) beam condenser FTIR.

of the beam condenser method, we studied the kinetics of the reaction in Scheme 3. The reaction of succinimidyl 6-(*N*-(7-nitrobenz-2-oxa-1,3-diazo-4-yl)amino)hexanoate with Wang resin produced compound **13** (Figure 4). The disappearance of the starting material bands at 3577 and 3458 cm<sup>-1</sup> with time coincides with the formation of bands at 1732, 1580, and 3352 cm<sup>-1</sup> that are all attributable to the product. The completion of the reaction can be confirmed with confidence by the disappearance of the hydroxyl bands at 3577 and 3458 cm<sup>-1</sup> (Figure 4). The area integrations of the emerging band at 1732 cm<sup>-1</sup> were plotted against time, and the time course was fitted to a pseudo-first-order rate equation with a rate constant of  $3.8 \times 10^{-4}$  s<sup>-1</sup>.

# **Concluding Remarks**

For the purpose of confirming the desired chemistry on solid supports, FTIR is a fast, sensitive, and convenient method. In principle, all FTIR and FT Raman methods are suitable for such analysis. Although the methods can be selected through comparisons, the best method for a specific laboratory is still the method that is conveniently available. Because differences exist among various methods in terms of sensitivity, speed, sample requirement, and the instrument cost, a comparison of merits of various FTIR and FT Raman methods in the context of resin bead analysis and the monitoring of solid-phase organic reactions was conducted in this investigation. Both single-bead FTIR and beam condenser FTIR methods are shown to be superb methods for the purpose of solid-phase reaction monitoring. The beam condenser method requires a little more beads (50-100 beads) compared to the single-bead FTIR method, but this disadvantage is well balanced by the low cost of the beam condenser and the ease of operation.

The single-bead and beam condenser FTIR methods meet several requirements as a TLC equivalent for solid-phase synthesis: (1) Only 50–100 beads are needed for analysis. The removal and examination of this tiny amount of beads from the reaction suspension practically does not perturb the reaction so that the reaction is virtually monitored in realtime like TLC. (2) A high-quality spectrum can be recorded within a few minutes. (3) No sample preparation is required, reducing the whole analysis time to even shorter than a TLC analysis. (4) These two FTIR methods provide qualitative, quantitative (the percentage of conversion), and kinetics information on organic reactions carried out on solid supports. (5) From the synthetic chemist's point of view, the low cost and the ease of operation are key factors to make the beam condenser method the better method as a TLC equivalent in SPOS.

The ATR method, although not the best method for resin analysis, is the method of choice for the surface grafted solid supports such as multi-pins<sup>12</sup> and "MicroTubes" (IRORI, San Diego, CA). In such analysis, it may be superior to other surface FTIR analysis methods. The FT Raman method scored low in our comparison due to the less information content and the higher cost. However, it provides complementary information to FTIR, especially for resin-bound compounds containing symmetric vibrations of organic functional groups such as NO<sub>2</sub> and bands below 600 cm<sup>-1</sup> such as S–S and C–Cl stretching vibrations.

# **Experimental Section**

**Materials.** All the resins used in this study were purchased from NovaBiochem (San Diego, CA) except TG S-CHO resin which was purchased from Rapp Polymere GmbH (Tubingen, Germany). All polystyrene resins are based on 1% cross-linked divinylbenzene-styrene copolymer. They are 100–200 mesh with a loading of 0.6–1.2 mmol/g. PS–PEG resins are based on 1% DVB polystyrene grafted with poly(ethylene glycol) linkers that are functionalized. All reagents, if not specified, were purchased from Aldrich (Milwaukee, WI).

The Single-Bead FTIR Method. All spectra were collected on a Nicolet Magna 550 FTIR spectrophotometer coupled with a NicPlan microscope. The microscope is equipped with a  $15 \times$  Cassegrain objective and liquid nitrogen cooled MCT detector. The general procedure for IR measurement is as in ref 7. Flattened bead was used throughout experiments.<sup>7b</sup>

**Data Treatment and Analysis.** IR spectra were normalized by making the intensities of a polystyrene band at 1947  $cm^{-1}$  equal. The areas under the typical bands of the starting material or the product were integrated. The values of integration were then plotted against time. These data points were fitted to a pseudo-first-order rate equation by using a nonlinear regression program—SigmaPlot for windows (Jandel Scientific, San Rafael, CA) on a personal computer.

**Beam Condenser Method.** About 50-100 beads were put on a SpectraTech 4X BC sample plan diamond window. Beads were pressed between two such windows using a compression cell. A spectrum was taken using the beam condenser unit (SpectraTech) that was mounted in a Nicolet Magna 550 FTIR spectrometer. The sample was scanned 128 times with a 4 cm<sup>-1</sup> resolution. The background was the clean sandwiched diamond cells.

**ATR Method.** The spectral data were obtained in the  $4000-650 \text{ cm}^{-1}$  range with 32 scans at 4 cm<sup>-1</sup> resolution

by means of a Bruker IFS 28 spectrophotometer equipped with a SplitPea accessory (SPA). The SPA internal reflection microsampling accessory is described by Harrick et al.<sup>13</sup> For all measurements, a MCT detector was used. The background spectra were scanned with the clean silicon internal reflectance element as the reference. To produce the good contact needed for reproducible spectra, the specially configured pressure plate of the SPA permits application of known clamping pressures between the sample and the optical element. No sample preparation is required.

FT Raman Method. FT Raman spectra were recorded on a Bruker RFS 100 FT Raman spectrophotometer equipped with a liquid nitrogen cooled germanium detector. The resolution was 4 cm<sup>-1</sup>. About 0.2 mg of resin beads was slightly pressed into a small conical hole of an aluminum holder and then placed in the focus of the laser beam (laser spot diameter  $\sim 100 \ \mu m$ ) for illuminating the sample and analyzing the scattered radiation. The 125 scans ( $\sim 5 \text{ min}$ ) were accumulated to obtain a spectrum with good signal/ noise ratio. The laser output is around 350 mW. All the spectra were corrected for instrumental response. By using the Raman microscope, a single bead was also investigated. The coupling of the microscope to our stand-alone FT Raman spectrometer RFS 100 is performed by NIR-fiber optics. The size of the laser spot on the sample is defined by the selected objective ( $40 \times = 15 \ \mu m$ ). A CCD video camera allows one to align the sample easily. Automated measurements can be performed by means of a computer-controlled x-ytranslation stage. One bead deposited onto a metallic plate (stainless steel or gold) with a highly reflecting surface was focused and excited under the microscope objective.

The laser wavelength was 1064 nm generated with a Nd: YAG laser. The fluorescence problem was completely avoided in this study.

**KBr Pellet Method.** Approximately 5 mg of resin beads and 300 mg of KBr (Sigma IR grade potassium bromide) were ground in a stainless steel vial with a stainless steel ball using a Crescent Wig-L-Bug for 20 s. A KBr pellet was pressed using SPEX 3624B X-Press at 12 tons of pressure under house vacuum. IR spectrum was recorded using a Nicolet Magna 550 FTIR with 128 scans for the sample and the background which was a plain KBr pellet made from 300 mg of KBr. The resolution was 4 cm<sup>-1</sup>.

Synthesis of Resin-Bound 11. Wang resin (1.0 g, 0.82 mmol/g) was swelled with benzene and then drained. To the moist resin was added 20 mL of benzene followed by 4-bromophenol (1.42 g, 8.2 mmol) and triphenylphosphine (2.15 g, 8.2 mmol). The mixture was shaken for 5 min until the reagents dissolved, and then diethyl azodicarboxylate (1.43 g, 8.2 mmol) was added. Shaking was continued for 5 min. The solvent was drained, and the resin beads were washed with benzene (2x), methylene chloride (2×), methanol (2×), and methylene chloride (3×).

Synthesis of Resin-Bound 12. To a mixture of 11 (105 mg, 0.082 mmol) in 1.5 mL of benzene (under argon) was added 0.4 mL of a 1.6 M solution of *n*-butyllithium in hexane (0.4 mmol). The mixture was then stirred at 60 °C for 3.5 h. After cooling, the liquid phase was removed and 2 mL of dry benzene was added. The mixture was stirred for 2

min, and then the benzene was removed. To the resin was added 1 mL of benzene followed by a solution of 4-chlorobenzaldehyde (57 mg, 0.41 mmol) in 0.5 mL of benzene. After the mixture was stirred for 3 h., the solvent was removed and the resin was washed with benzene  $(1\times)$ , methanol  $(2\times)$ , *N*,*N*-dimethylformamide (DMF)  $(2\times)$ , and methylene chloride  $(3\times)$ .

**Synthesis of Resin-Bound 13.** Wang resin (60 mg, 1.0 mmol/g) was washed with 4 mL of DMF for 15 min and then drained. Diisopropylcarbodiimide (89 mg, 0.3 mmol) was added to a solution of succinimidyl 6-(N-(7-nitrobenz-2-oxa-1,3-diazo-4-yl)amino)hexanoate (38 mg, 0.3 mmol) in 1 mL of dry DMF. The mixture was added to the resin after stirred for 5 min. Then 4-(N,N-dimethylamino)pyridine (7.3 mg, 0.06 mmol) was added to the resin suspension. The reaction then was stirred in an orbit shaker.

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