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Review

# Role of Fourier transform infrared spectroscopy in the rehearsal phase of combinatorial chemistry: a thin-layer chromatography equivalent for on-support monitoring of solid-phase organic synthesis

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**Abstract**

The adaptation of diverse organic reactions to solid supports requires significant reaction optimization efforts. A convenient on-support analytical method functionally similar to TLC in solution chemistry is very advantageous. As a TLC-equivalent method, the single bead FTIR is a simple, sensitive, fast, and convenient analytical method to monitor SPOS without stopping the reaction or cleaving the product. As with TLC, single bead FTIR provides a wide range of information such as qualitative assessment, quantitative determination, and reaction kinetics. Studies with the single bead FTIR have not only provided a tool for daily monitoring of the solid-phase reactions, but a way to understand the properties of polymer-bound substrate and the nature of polymer-supported organic reactions. It has assisted in the selection of a wide range of reaction conditions rapidly for SPOS in the rehearsal phase of combinatorial chemistry. Due to its convenience and efficiency, FTIR internal reflection spectroscopy has evolved as a useful analytical methodology for monitoring of combinatorial chemistry reactions directly on polymer surface. 

*Keywords:* Reviews; Organic sythesis; Fourier transform infrared spectroscopy

## Contents



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## 1. Introduction

Combinatorial chemistry [1–12] in solution and on polymer supports has become an important approach for the discovery of novel chemical entities. Synthesis of small molecules with the latter approach i.e. solid-phase organic synthesis (SPOS) [13–21] is of particular relevance to pharmaceutical research, since it provides a way to rapidly synthesize diverse small drug-like organic molecules. Three popular supports used in SPOS are (1) 1%-divinylbenzene (DVB) co-polymerized polystyrene resins (PS resins), (2) polystyrene-based resin extensively grafted with polyethyleneglycol linkers (PS-PEG resins) and (3) the surface-modified polyethylene pins.

Organic reactions on solid supports often require conditions quite different from those used in solution. Before a compound library can be made, a time-consuming solid-phase reaction optimization is often required. This rehearsal phase can take months while the final synthesis of the library would take only weeks.

In the rehearsal phase, optimal reaction conditions need to be evaluated and implemented rapidly. Currently, the products or intermediates are chemically (e.g. using TFA) or photochemically cleaved from the resin and then purified for analysis with classic spectroscopic methods. The indirect information obtained from the compound derived from cleavage is used to judge the intended chemistry on the solid support. However, to ''cleave and analyze'' is a time-consuming, expensive and laborious process. Some synthetic intermediates are not stable enough for this protocol. It is a waste of time and sample for characterizing intermediates in multi-step synthesis by ''cleave and analyze'' method. These chracteristics have consequently stimulated the development of on-support analytical methods.

Recent progresses in analytical methods associated with combinatorial chemistry have been summarized in several recent reviews [22–26]. This article is specifically limited to reviewing the application of FTIR techniques in the monitoring of SPOS and the impact of these methods on the rehearsal phase of combinatorial chemistry. These applications are illustrated with examples in this overview. Experimental details can be found in original papers given in references.

## 2. The monitoring of reactions on polystyrene-based resin

### *2.1. Techniques and backgrounds*

The unavailability of thin-layer chromatography (TLC) in SPOS has caused severe analytical problems in the routine quality control of the solid-phase reactions. Consequently other methods have risen in importance to meet the challenge, e.g. diverse FTIR techniques. Analysis of solid-phase organic reactions has been performed on 5–10 mg of resin beads using various techniques such as the KBr pellet method, [27–29] diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) [30], photoacoustic FTIR spectroscopy (PAS) [31] and, alternatively, by FTIR microspectroscopy on a single resin bead [32–34]. We recently compared some FTIR and Raman techniques in the analysis of solid-bound organic compounds in terms of spectral information and quality, and the sample and time requirement. We have found that the single bead FTIR is one of the best methods for a rapid analysis of the trace amount of samples to get a wide range of information on the reaction.

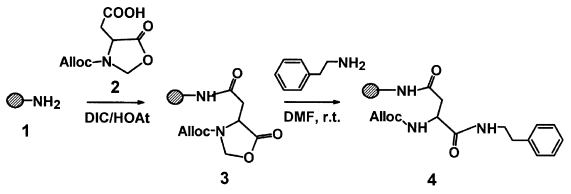
Organic reactions selected to assemble a combinatorial library should be preferably robust and well-behaved. Therefore, the resin-bound structures to be monitored are rarely unknowns. Consequently the major analytical task is to confirm the intended chemistry rather than a full structural elucidation. Since FTIR is a technique sensitive to organic

functional group changes, it is well suited for this purpose. The principle of monitoring reactions by IR is based on the functional group inter-conversions via chemical reaction or by the 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. For the rehearsal of a library synthesis on solid supports, building blocks to be used in solid-phase reaction can even be selected to contain an IR detectable group at a remote site.

### *2.2. Yes-and-no information on solid-phase organic reactions*

Similar to the role of TLC in solution organic synthesis, FTIR provides quick answer to the most important question any chemist would ask first: is my reaction working? An example of the successful monitoring of a multi-step indazole synthesis has been presented before [35]. Another example is the monitoring of the solid-phase synthesis via 5-oxazolidinones [36]. As shown in Scheme 1, starting from the *N*-carbamate protected aspartic acid, we first prepared in high yield (>90%) and purity by known procedures (paraformaldehyde, *p*-TsOH, azeotropic removal of water) the oxazolidinone **2**. We used the Alloc protected oxazolidinones **2** due to the facile removal of the Alloc group by $Pd^0$. In the next step, the oxazolidinones **2** was attached to Knorr resin **1** using standard DIC/HOAt coupling methods. The analysis of the polymer-bound oxazolidinones **3** by single 'flattened' bead FTIR microspectroscopy showed the characteristic carbonyl IR band at 1800 $cm^{-1}$ (Fig. 1). This unique band served as a useful and distinct indicator for product identification in a series of parallel synthesis reactions.

Absorbance saturation is observed for spectra in this figure and some of the following figures in the fingerprint region. Care should be taken to make sure the band that is used for analysis not be saturated. The cause for spectral saturation is the high local concentration in the bead. Bead flattening can effectively reduce the saturation effect and improve the spectrum [33]. When resin is treated with methanol (as in Figs. 1 and 2) or other unfriendly solvents, the




Scheme 1.

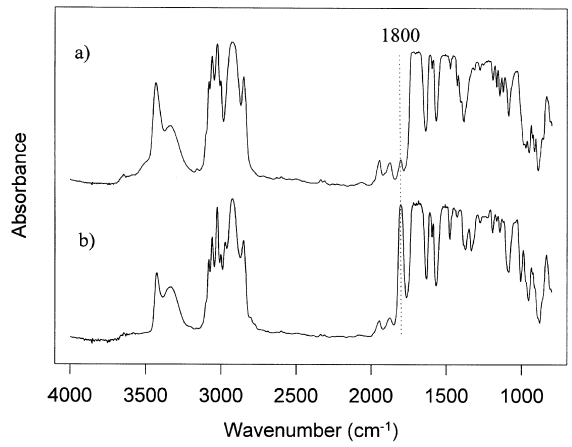



Fig. 1. Single bead IR spectra were taken as previously described. (a) The IR spectrum of **1** and (b) The IR spectrum of **3**.

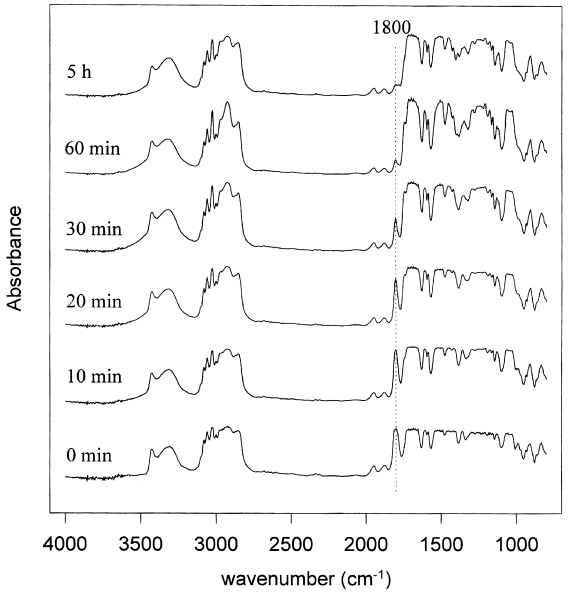



Fig. 2. Single bead IR spectra at various times during the synthesis of **4**. The starting resin (at 0 min) is **3**.

bead is hardened and not easily flattened. PS-PEG resins can not be flattened due to their poor elasticity.

### 2.3. Reaction kinetics on resin support

There is a serious lack of kinetic studies for polymer supported reactions. There are two reasons for this. First, the general lack of analytical tools for on-resin analysis in the past has limited our capability to pursue such studies. Second, most available methods (such as gel-phase NMR and most FTIR methods, such as KBr pellet method) would require too much sample (>10 mg) for a single time point. This is impractical for general use in kinetic studies considering the small-scale synthesis and the cost of resins. In contrast, the single bead FTIR provides a convenient tool for kinetics studies [32,37–42].

To determine the reaction kinetics of the second step in Scheme 1, the progress of the reaction of resin **3** with an ten-fold excess of phenethylamine in DMF (Scheme 1) was monitored by taking spectrum of a single bead at various times during the course of this reaction and results are shown in Fig. 2. The intensity of the band at 1800 $cm^{-1}$ decreases with time indicating a rapid formation of the resin-bound product. Integrations of areas under the oxazolidinone carbonyl band at 1800 $cm^{-1}$ were plotted against time in Fig. 3. The reaction time course appears to be single exponential suggesting the rate only depends on the available reactive sites on the resin when the other reagent is in excess. Several solid-phase reactions have been fitted with a pseudo-first order reaction rate equation [39]. Data were analyzed by curve-fitting to a first-order rate equation. The best fit is shown in Fig. 3 as the solid line. The pseudo-first order rate constant is $1.23\times 10^{-3}$ 1/s for the ring-opening reaction. The lack of IR peaks of side products and the fit to a first order reaction rate equation strongly suggest there are no side reactions. This was further confirmed by analyzing the cleaved product (TFA:$H_2O$, 95:5) by HPLC, MS, and NMR. The product **4** was clearly identified and obtained in a 'crude' yield of 80% in a purity of >90% by RP-HPLC.

Using the combination of FTIR microspectroscopy and a flow-through cell, organic reactions on a single flattened bead can be monitored in situ in the selected IR-friendly solvent (such as $CHCl_3$ or $CH_2Cl_2$). This instrument set-up has been used to monitor a benzoylation reaction of the aminomethylstyrene resin and a series of coupling reactions to the polystyrene tritylchloride resin [41]. In a separate report, a time-dependent photocleavage reaction of a nitrile-containing compound was monitored [42].

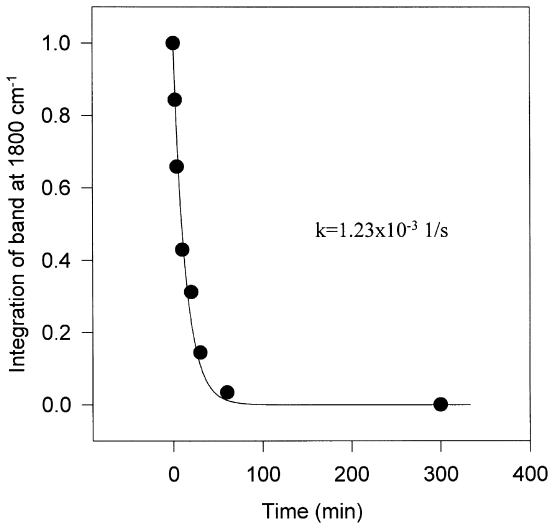


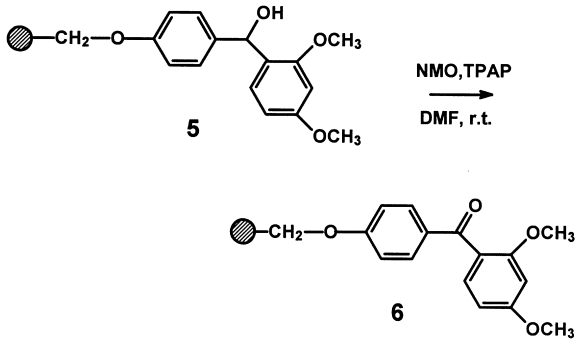

Fig. 3. Time course of the reaction in the second step (ring opening) in Scheme 1. All spectra were normalized by making the intensity of the polystyrene band at 1945 $cm^{-1}$ equal. The area integrations for the IR band at 1800 $cm^{-1}$ were plotted against time. Lines were calculated from the best fit to a first order reaction equation with a rate constant shown.

### 2.4. Quantitative estimation of chemical conversions (%) on resin

In a typical TLC experiment, if the spot of a starting material disappears and the product spot is formed, the chemical transformation can be estimated as completed. In a similar fashion, chemical conversions (%) can be conveniently estimated by single bead FTIR analysis by observing the disappearance of the starting material band quantitatively by peak area integration (see Figs. 2 and 3).

Another example is the oxidation of a secondary alcohol to a ketone. In solution, the catalytic oxidation of a secondary alcohol to a ketone by tetra-*n*-

propylammonium perruthenate (TPAP) is generally slower than the oxidation of a primary alcohol suggesting the catalyst is sterically demanding. The sterically hindered secondary alcohol **5** in Scheme 2 would be expected to be further hindered by the attachment to the polystyrene resin. We expect that the rate of this reaction will provide a lower limit estimate for the oxidation rate of a secondary alcohol by TPAP on solid support. The starting material Rink Acid was treated with 0.2 eq. TPAP in the presence of *N*-methylmorpholine *N*-oxide (NMO). IR spectra taken on a single bead from the reaction mixture at various time intervals after the initiation of the reaction are shown in Fig. 4. The disappearance of a band at 3568 $cm^{-1}$ attributed to the hydroxyl group and the increasing band at 1654 $cm^{-1}$ attributed to the ketone carbonyl are evident. Areas under these two peaks, when plotted against time, fit with a first order rate equation (Fig. 5). The average rate constant is $1.9\times10^{-4}$ $s^{-1}$. The same rate for the disappearance of the hydroxyl group and the appearance of the ketone carbonyl indicates that only one reaction, i.e. the oxidation of the alcohol **5** to the ketone **6**, is occurring. Based on the change in area integrations under the 3568 $cm^{-1}$ band, a 97% conversion of **5** to **6** takes about 4 h. More examples on the quantitative aspect of the single bead FTIR can be found in [37,38,40]. Note that the estimation of the reaction yield with single bead IR was obtained without the need to stop or interrupt the reaction or cleave the product from the support. By resorting to the isotopic synthesis and a separately predetermined standard calibration curve, reactions




Scheme 2.

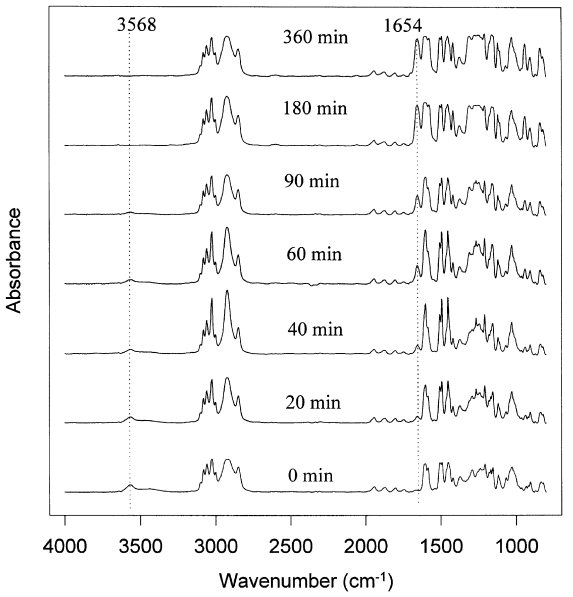



Fig. 4. IR spectra taken from a single bead at various times in the course of the Scheme 2. Spectra were taken from a single flattened bead at 0, 20, 40, 60, 90, 180 and 360 min after the initiation of the oxidation reaction. The hydrogen-bonded and unbonded hydroxyl stretch at 3420 and 3568 $cm^{-1}$ disappears as the ketone carbonyl group at 1654 $cm^{-1}$ increase.

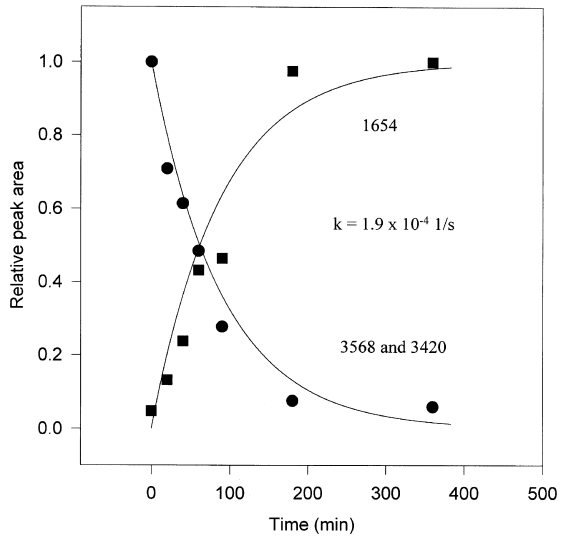



Fig. 5. The time course of Scheme 2. All spectra were normalized as described in Fig. 3. The integrated areas for the hydroxyl band from 3181 to 3637 $cm^{-1}$ (●) and the ketone carbonyl band from 1625 to 1715 $cm^{-1}$ (■) for spectra at various times were plotted against time. Lines were calculated from the best fit to a first order reaction equation with a rate constant shown.

using isotope labeled protecting groups or substrates have been quantitated [34].

### *2.5. The selection of optimal reaction conditions.*

Because of the speed, sensitivity and convenience of the single bead FTIR method, the optimal solvent, resin, building block, linker, catalyst, mixing method and other conditions have been routinely selected rapidly with the aid of this method. As an example, the selection of mixing method is shown below.

SPOS offers several advantages over solution phase techniques for achieving high throughput synthesis. At the same time, SPOS also poses problems that do not exist in solution synthesis. One of the problems is that one has to select the most efficient reaction mixing method to maximize encounters between the solid-bound reactant and the soluble reagent. Diverse mixing techniques are currently used in various laboratories without a clear understanding of their relative efficiency. Bead suspensions are usually mixed by shaking the tubes, which is fixed at a 45° angle, horizontally by an orbit shaker, by angular shaking (20–30°) on a wrist shaker, by rotating tubes at an angle of 180° or 360° (whole turn) rotations at a certain rpm, by bubbling $N_2$ gas from the bottom of the reactor, and by the conventional magnetic stirring.

In order to compare the mixing efficiency, the formation of a resin-bound dansylhydrazone through the reaction of formylpolystyrene resin (NovaBiochem, 0.58 mmol $g^{-1}$, 1% DVB, 100–200 mesh) and the dansylhydrazine was studied using six different mixing methods. [43] Single bead IR and the fluorescence quantitation method [37] were used to monitor the reaction completion. Fig. 6 shows IR spectra from 40 and 30 individual beads after a 30-min reaction (2-fold excess dansylhydrazine in DMF) shaking with a wrist or orbit shakers. For the reaction studied, the 360° rotation and nitrogen bubbling methods provided highest mixing efficiency under mild conditions. Wrist shaking also showed high mixing efficiency with the maximum setting (vigorous shaking). Magnetic stirring showed the same high mixing efficiency, but this method often breaks beads. The method with 180° rotation mixing required longer reaction time, and may not be preferable for slow reactions. Mixing with an orbit shaker did not give satisfactory reaction yields as compared with the other techniques. A large excess of reagent and a prolonged reaction time, however, can drive this reaction to completion.

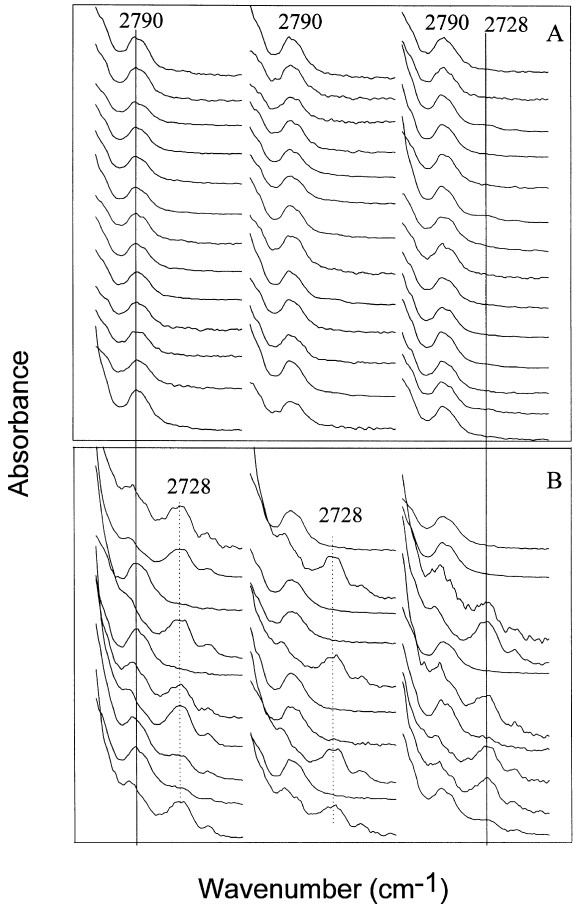



Fig. 6. IR spectra taken from individual formylpolystyrene beads after a 30-min reaction with a two-fold excess of dansylhydrazine in DMF mixed with: A – a wrist shaker and B – an orbit shaker with the reaction tube fixed at a 45° angle.

## 3. The monitoring of reactions on PS-PEG resins: comparing the reaction rate on PS and PS-PEG resins.

### *3.1. Background.*

IR spectra of PS-PEG resins are easily obtained with single bead FTIR or other FTIR techniques. The

main difference between IR spectra of PS resins and PS-PEG resins is the $CH_2$ vibrational band of the latter is prominent (see Fig. 7). This does not interfere with the analysis in most cases. For PS beads, the flattened beads usually improves the spectral quality. [33] PS beads can be flattened with a compression cell or simply by hand-press the beads between two NaCl plates. However, the elasticity of PS-PEG resin beads is so low that a slight press will shatter the bead. Another PS-PEG resin, NovaGel has an improved elastic property and can be flattened for FTIR studies. The single bead FTIR measurement of PS-PEG resins is usually done with the globular bead when flattening is not possible. Spectra with reasonable quality can be generated and used for the monitoring of organic reactions on PS-PEG resins.

A common problem in SPOS practice is that reaction conditions can not simply be transfered from one kind of support to another. A set of reaction conditions may work well for polystyrene resins, but may fail completely for pin- or PS-PEG resin-based synthesis. To understand the effect of various supports on reactions is critical for a successful synthesis.

PS contains mainly 1% DVB cross-linked polystyrene backbones (~96–98% by weight) which is highly hydrophobic. Due to the short linker length, reactions on PS resin tend to be affected more by the hydrophobic PS backbones. The architecture of PS-PEG resin is based on a very small portion of crosslinked 1% DVB-PS backbones extensively grafted with long PEG linkers (40–60 ethylene oxide units). The PEG content is up to 70% of the resin weight. Therefore, the mechanical, physico-chemical behavior of the resin is determined by the PEG chain. Because the reactive sites in PS-PEG resins are located at the end of long and flexible spacers and totally separated from the PS backbone, they are not affected by PS matrix. Additionally, the domi-

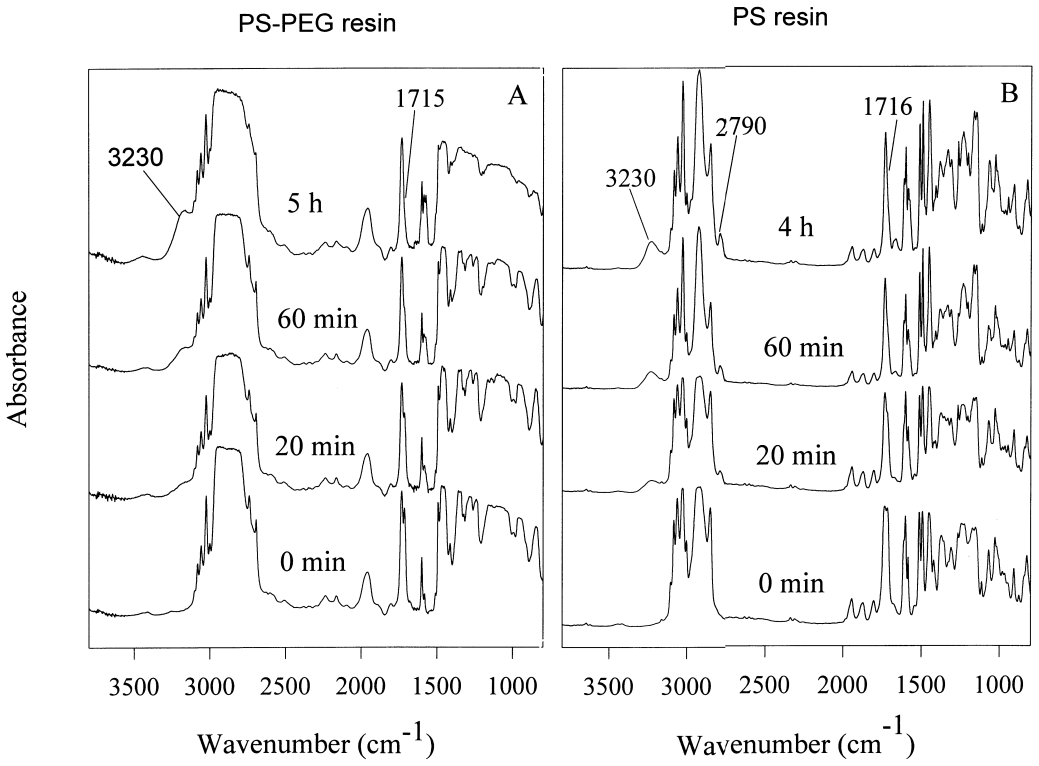


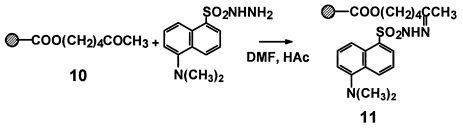

Fig. 7. IR spectra taken from a single bead (A) or a single flattened bead (B) at various times during the reaction in Scheme 5 on PS-PEG-(A) or PS-based (B) resins.

nant PEG linkers exhibit greater miscibility with most solvents including water. PS-PEG is generally regarded as providing a quasi-homogeneous and 'solution-like' reaction support. It is commonly assumed that reactions carried out on PS-PEG resins proceed faster than those carried out on PS resins. Peptide synthesis reactions would be more favored on PS-PEG resins since it is more polar than PS resins. However, peptide bond formation is only a special reaction. In SPOS, a different set of rules may apply.

### *3.2. Examples*

In order to compare the reaction rate on PS- and PS-PEG -resins, several reactions were carried out on these two resins under identical conditions. An example shown in Fig. 7 is the synthesis of a dansyl hydrazone. Resin-bound **10** reacted with dansylhydrazine under identical conditions on PS-PEG and PS resins (Scheme 5). Single bead IR spectra for the transformation from **10** to **11** is monitored. The ester carbonyl bands at 1734 $cm^{-1}$ remains while the ketone carbonyl band at 1716 $cm^{-1}$ disappears accompanying the formation of dansylhydrazone. The formation of the band for hydrazone N–H stretch at 3230 $cm^{-1}$ and the band for $N(CH_3)_2$ stretch on the dansyl group at ~2790 $cm^{-1}$ on PS resin product proved the product formation. Although the product band at 2790 is not observed due to the overlaps between the strong $CH_2$ band and the hydrazone signal in PS-PEG resin, the appearance of the band at 3230 and the disappearance of the band at 1716 $cm^{-1}$ are evident.

Due to the overlap between the ester and the ketone carbonyl bands, a peak deconvolution analysis using PeakFit (Jandel Scientific, San Rafael, CA, USA) was carried out for a clear-cut quantitation. As an example for the fitting method, the fitting of the data for PS reaction at 0 and 60 min (taken from Fig. 7) is shown in Fig. 8. It demonstrates that overlapping bands can be resolved to aid a quantitative analysis. The areas of the resolved ketone carbonyl band after PeakFit analysis was used to obtain the time course for these reactions. Results of area integrations are plotted in Fig. 9. In this case, the reaction on PS resin is 2.2 times faster than that on PS-PEG resin.




Scheme 5.

These data and results from studies on other reactions (Schemes 3, 4 and 6) are listed in Table 1. Based on these results and other studies [44], we speculate that the effect of polymer matrix on the reaction rate is similar to the effect of solvent on a solution-phase reaction rate. Our data are consistent with the conclusion reached by Czainik [45] that solid supports are like solvents. The reaction rate on resin supports should be proportional to the compatibility of the reagent with the solvent and the adsorption coefficient of the reagent into the resin. The selective adsorption of a compound into the resin depends on its similarity to the resin in terms of molecular interactions such as hydrogen bonding,

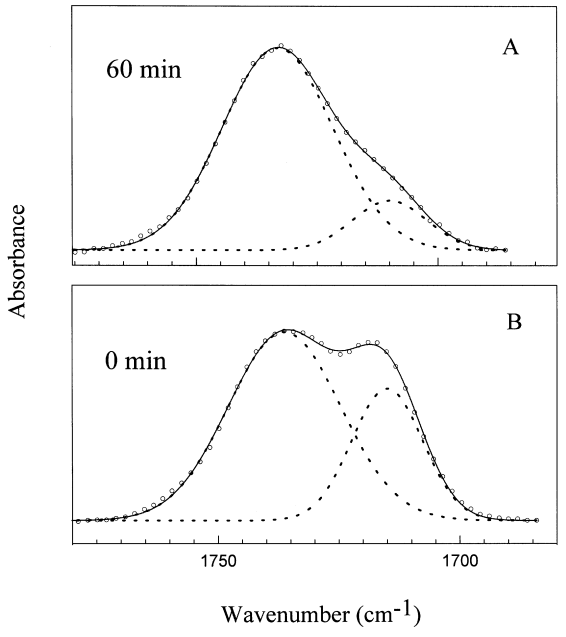



Fig. 8. PeakFit analysis of overlapping IR bands. Overlapping IR bands at ~1716 $cm^{-1}$ from Fig. 6B were analyzed with the PeakFit program (Jandel Scientific, San Rafael, CA, USA).

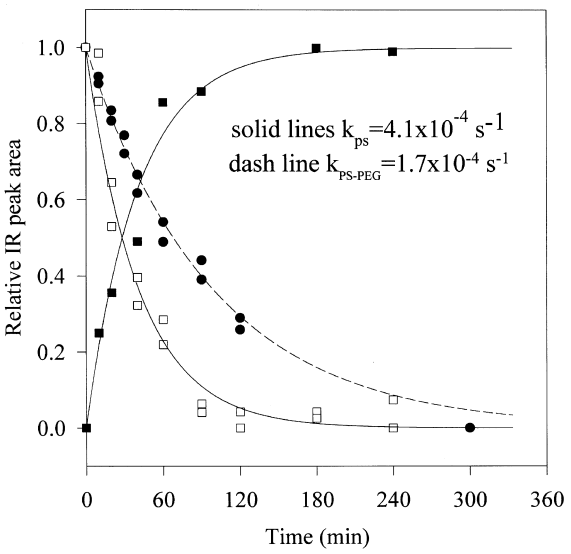
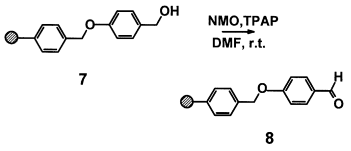
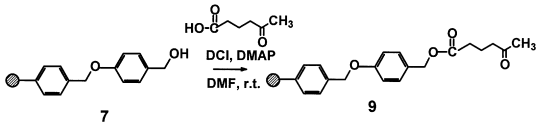


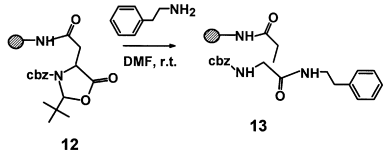

Fig. 9. Integrations of peak areas for band at 3230 $cm^{-1}$ for PS-based resin (solid squares) and PeakFit resolved peak areas at 1715/1716 $cm^{-1}$ (solid circles for PS-PEG- and open squares for PS-based resins). Solid lines are a theoretical time course with a rate constant of $4.1\times10^{-4}$ $s^{-1}$ and the dashed line is a theoretical time course with a rate constant of $1.7\times10^{-4}$.




Scheme 3.




Scheme 4.




Scheme 6.

Table 1
Comparison of reaction rates on PS- and PS-PEG-based resins

| Reaction scheme | $k_{PS}$ ($s^{-1}$) | $k_{PS\text{-}PEG}$ ($s^{-1}$) | $k_{PS\text{-}PEG}/k_{PS}$ |
|---|---|---|---|
| 3 | $4.6\times10^{-4}$ | $1.8\times10^{-3}$ | 3.9 |
| 4 | $2.2\times10^{-4}$ | $2.3\times10^{-4}$ | 1.0 |
| 5 | $3.1\times10^{-3}$ | $1.8\times10^{-3}$ | 0.6 |
| 6 | $1.13\times10^{-4}$ | $6.26\times10^{-6}$ | 0.055 |

hydrophobicity, and polarity. There is no single polymer support that favors all reactions. Depending on whether the SPOS requires polar or non-polar medium, PS-PEG - or PS-based resins respectively should be chosen.

## 4. The monitoring of reactions on multipin crowns

Chiron ''Crowns'' [46] or ''microtubes'' (e.g. IRORI, San Diego, CA, USA) are surface coated polymers with various dimensions. Reactive groups coupled with a linkers are grafted on to the polymer. In this section, the monitoring of reactions directly on crowns using FTIR internal reflection spectroscopy [47] is described.

### *4.1. Techniques*

Internal reflection spectroscopy [48], also known as attenuated total reflectance (ATR), is a versatile, non-destructive technique for obtaining the infrared spectrum of the surface of a material or the spectrum of materials either too thick or too strongly absorbing to be analyzed by standard transmission spectroscopy.

Direct observation of the chemical synthesis on crowns is possible with FTIR accessories like the SplitPea™, [49–52] the Golden Gate [53] and the DuraSamplIR [54] which combine internal reflection with a FTIR spectrophotometer. In internal reflection spectroscopy, no sample preparation is needed, i.e. the crown is simply clamped on to the internal reflection element. This makes it a straightforward, extremely convenient and efficient method for the direct spectral analysis of crowns.

### *4.2. Example*

A combinatorial chemistry synthesis on crowns sometimes starts with the removal of the 9-Fluorenylmethoxycarbonyl (Fmoc) protecting group from the linker. This is illustrated in Fig. 10 where the spectra of a crown with a hexamethylene–diamine–glycine linker, Fmoc-protected (upper curve), and of the same crown after the removal of the protecting group (lower curve) are overlaid. In the lower curve it is clearly seen that the bands due to the Fmoc protecting group, marked with ''F'', have disappeared.

Very limited analytical methods are available for analyzing surface modified polymer samples such as ''crowns'' and ''microtubes''. The above example shows that internal reflection measurements are a convenient, quick and reliable means for direct analysis of the surface of crowns. It is particularly advantageous for the monitoring of combinatorial chemistry reactions which take place in a thin stratum above the ''crown'' or ''microtube'' polymer. The resulting spectra therefore have a high information content regarding the synthesis rather than the underlying crown polymer.

With these advantages, macro-internal reflection spectroscopy is an excellent tool for directly checking successive steps of a combinatorial synthesis on

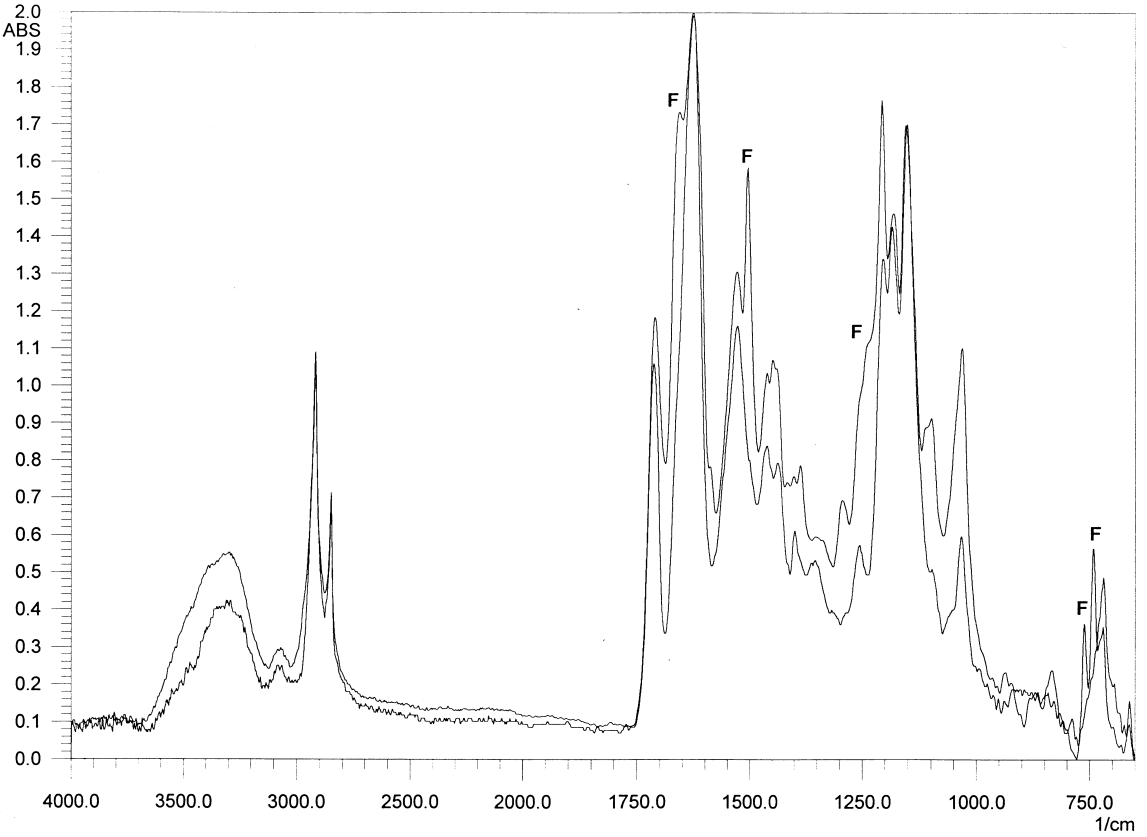



Fig. 10. Internal reflection spectra of crowns with hexamethylene–diamine–glycine linker, Fmoc-protected (upper curve), and Fmoc-deprotected (lower curve). The bands due to the Fmoc protecting group are marked with ''F''. The spectra were obtained, in the 4000–650 $cm^{-1}$ range with 32 scans at 4 $cm^{-1}$ resolution, by means of a Bruker IFS 28 spectrophotometer equipped with a SplitPea™ accessory. The background spectra were scanned with the clean silicon internal reflectance element as the reference. The crowns were obtained from Chiron Mimotopes Pty., Clayton, Victoria, Australia.

polymer surface. It is extremely helpful for the elaboration of a synthesis when reaction conditions are to be optimized.

## 5. Concluding remarks

As a TLC-equivalent method, the single bead FTIR is a simple, sensitive, fast, and convenient analytical method to monitor SPOS without stopping the reaction or cleaving the product. As with TLC, single bead FTIR provides a wide range of information such as quanlitative assessment, quantitative determination, and reaction kinetics. Studies with the single bead FTIR have not only provided a tool for daily monitoring of the solid-phase reactions, but a way to understand the properties of polymer-bound substrate and the nature of polymer-supported organic reactions. It has assisted in the selection of a wide range of reaction conditions rapidly for SPOS in the rehearsal phase of combinatorial chemistry. Due to its convenience and efficiency, FTIR internal reflection spectroscopy has evolved as a useful analytical methodology for monitoring of combinatorial chemistry reactions directly on polymer surface.

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