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Articles

Direct Infrared Spectroscopic Analysis of Reagent Partitioning in Polystyrene Bead Supported Solid Phase Reaction Chemistry

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Throughout the evolution of polymer support based combinatorial synthesis, many issues have been raised concerning chemistry at the interior of the support bead. Long standing questions regarding size exclusion, reagent diffusion, and reaction kinetics remain unresolved, largely due to limited analytical capabilities which address chemical activity at the core of the solid phase support bead. A variety of novel infrared spectroscopic techniques for the direct evaluation of intrabead reagent dynamics are presented in this work. Specifically, these spectroscopic tools facilitate analysis and understanding of reagent penetration and intrabead reagent mobility parameters. Data from these studies downplay size exclusion as a consequential factor in the delivery of small molecule reagents throughout synthesis on polystyrene-based resins. Experiments presented here, however, illustrate the first direct spectroscopic evidence of thermodynamic reagent partitioning between the support bead and surrounding solvent as an essential factor in efficient delivery of reagent to the solid phase support interior.

Solid phase synthesis has undergone a dramatic resurgence with the introduction of resin-based combinatorial synthesis throughout the pharmaceutical and agrochemical industries. While tremendous advances have been made with respect to resins, linkers, and documented reaction methodologies, far less is understood about the interior bead environment and its effect on reaction kinetics. In general, solid phase reactions are generally thought to have attenuated kinetics with respect to their solution phase analogues. A number of works[1,2] evaluating the reactive site microenvironment influence on reaction kinetics have been published, yet none provide clear definition or direct analysis of reagent partition.

The infrared methodology of this report provides the first opportunity for direct analysis of the interior microenvironment of a solvent swollen resin bead as distinguished from the surrounding solvent. Single bead analysis performed in conjunction with the previously reported[3] infrared microscope and flow cell permits identification and characterization of "reagent partitioning" as a powerful factor in the variant kinetics of solution- vs polymer-based solid phase reactions. Reagent partition refers to either extraction or rejection of reagent by the support bead from the surrounding solvent. The direction and degree of reagent partition are a function of the solvation characteristics of the solvent swollen bead vs the solvent itself for the particular reagent of interest. Hence, by altering equilibrium reagent concentration in a gel phase bead relative to the surrounding solvent, reagent partition can be a primary deterrent to efficient delivery of reagent to the bead interior.

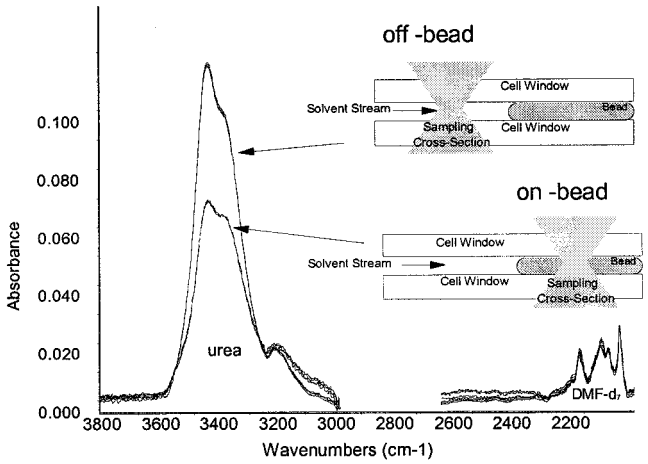

Figure 1. Reagent partitioning of urea in DMF.

In this analysis of reagent partition, sample-modulated infrared experiments were conducted by mounting a bead[4] in the previously described flow cell used in conjunction with the infrared microscope. A reagent stream containing only nondeuterated solvent was introduced into the flow cell to swell the beads. As beads swell, the cell windows contacting the bead permit expansion only in the plane parallel to the cell windows which results in a flattened bead with parallel surfaces as illustrated in the Figure 1 inset. An infrared spectral background was then collected from an open area of the cell in which only solvent was present (Figure 1, off-

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bead). The infrared microscope stage was then moved to position a bead directly in the infrared beam (Figure 1, on-bead), and a second background spectrum which included the solvent swollen bead was collected.

The reagent stream was then switched to a 5 mL solvent solution containing 50 μL deuterated solvent and 25 μL of the particular analyte in question. Using the real time spectral display mode of the infrared spectrometer, analyte diffusion equilibrium was observed to be established in less than 1 min at the bead core. In subsequent experiments, a time delay (3 min) was introduced between changes in the reagent stream and spectral acquisition to ensure diffusion equilibrium for the "on-bead" spectra.

After diffusion equilibrium, an "on-bead" spectrum was collected using corresponding "on-bead" background. Since bead and nondeuterated solvent were present in both the background and the spectrum, their absorbance features ratio out in the single beam to absorbance conversion leaving only deuterated solvent and analyte spectral features in the final spectrum. The microscope stage was then moved to the "off-bead" position, and a spectrum was collected from the reagent stream using the off-bead background. Again, cell and nondeuterated solvent spectral features ratio out of the single beam to absorbance conversion leaving only deuterated solvent and analyte absorbances.

In these experiments, inclusion of 1% deuterated solvent along with the analyte in question served several purposes. First, since both on- and off-bead backgounds were collected in the presence of solvent, the protonated solvent spectra ratios out of all subsequent spectra leaving no spectral absorbance with which to determine the amount of solvent present between the on-bead and off-bead experiments. The presence of the deuterated solvent provides a solvent spectral absorbance required for calculation of analyte-to-solvent ratios in the on- and off-bead experiments. Inclusion of deuterated solvent in the analyte-containing fraction also provides a spectral probe by which the rate of solvent exchange in the bead can be measured. Without this test, it could be argued that the channeling of solvent in the cell around the bead is responsible for low reagent delivery. Triplicate samplings of on-bead and off-bead spectra in which urea was used as the analyte and DMF as the solvent are presented in Figure 1. In this figure, each set of triplicates appears as a single spectrum due to their very close overlay. In Figure 1, spectra have been graphically normalized to the DMF-d_7 band intensity to illustrate differences in the urea/DMF-d_7 band intensity ratios.

A partition coefficient was calculated according to eq 1. From eq 1, a reagent−solvent combination which undergoes no partition will yield a coefficient of 1.0. A coefficient below 1.0 indicates a partitioning rejection of the reagent by the swollen bead matrix relative to the reagent solvent. A coefficient above 1.0 indicates a partitioning of the reagent into the solvent swollen bead matrix.

$$\text{partition coefficient} = \frac{\left(\dfrac{\text{analyte absorbance}}{\text{deuterated solvent absorbance}}\right)_{\text{on-bead}}}{\left(\dfrac{\text{analyte absorbance}}{\text{deuterated solvent absorbance}}\right)_{\text{off-bead}}} \quad (1)$$

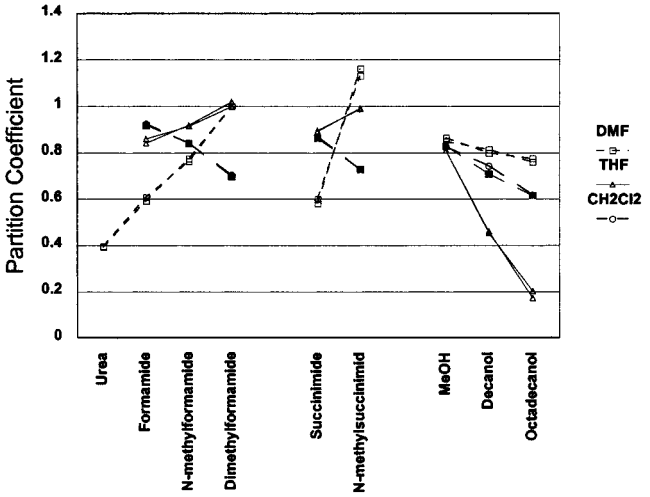

Figure 2. Solvent-dependent partition trends.

Figure 2 illustrates the solvent/bead partition coefficients obtained for aminomethylstyrene beads over a range of amides, imides, and alcohols using DMF, THF, and methylene chloride as solvents, respectively. Dual bars indicate repeat experiments obtained from sequential off-bead/on-bead cycles.

In Figure 2, increased bead uptake of amides and imides occurs with increased nitrogen alkylation for DMF and THF solvents, while the opposite trend was observed in CH_2Cl_2. In the case of homologous alcohols, a decrease in reagent uptake by the bead was noted with increased chain length for all solvents. Although amide and imide series of Figure 2 disprove size exclusion as an overriding factor in the delivery of reagent to the bead core, an additional experiment was devised to discount size exclusion as a factor in the broader size range of the hydroxyl experiments which demonstrated reduced concentration at the bead core as a function of chain length in all solvents.

Figure 3 presents an on-line experiment in which an on-bead background spectrum was collected from a bead swollen in a DMF solvent stream. At 25 min, the input stream was switched to DMF-d_7/octadecanol and the rate of elution of octadecanol into the bead core was monitored relative to that of the DMF-d_7 tagged solvent. At 34 min, the solvent stream was switched back to 100% DMF to permit wash out of the DMF-d_7 and octadecanol to be observed. The real-time intensity profiles for that experiment are presented in Figure 3 together with the corresponding scaled subtraction.

In Figure 3, the DMF-d_7 and octadecanol absorbance vs time traces were scaled in intensity to permit superposition. Only a very slight phase delay is noted at both the introduction and wash cycles for octadecanol relative to solvent. This delay is most easily observed as the positive and negative spikes in the difference spectrum between the two traces obtained by spectral subtraction. Therefore on the time scale of the Figure 2 experiments, diffusion equilibrium was reached very quickly, and latent hydroxyl concentrations are not due to retardation of diffusion parameters as a result of size exclusion. Hence, observed partition was based in the thermodynamic properties of the system.

Close examination of the interior bead microenvironment, however, suggests that the problem may be more severe than

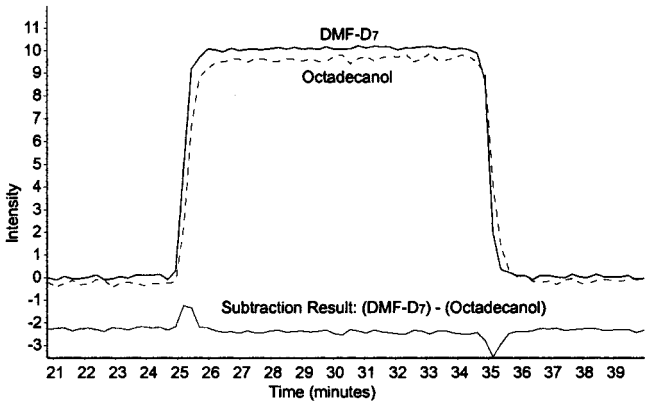

Figure 3. Reagent equilibration.

initially indicated by the Figure 2 data. On a molecular scale, the bead is a highly porous material with many pockets and holes capable of containing analyte in a solvent microenvironment isolated from the surrounding polymer. Figure 4A presents spectra of the amide III (mixed CN and NH vibrations) region for an experiment in which methylene chloride was used as the solvent and succinimide as the partitioned analyte. The on-bead and off-bead spectra indicate a high-frequency shift of the amide III band collected on-bead, relative to the off-bead spectrum collected in neat solvent.

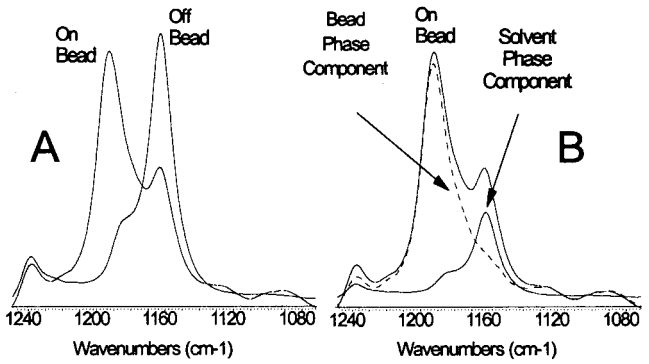

Figure 4. Solvent/reagent/bead molecular interaction.

Spectra presented in Figure 4B indicate the "on-bead" band can be resolved into two components through spectral subtraction of the scaled off-bead spectrum. The band labeled "solvent phase component" is a scaled attenuation of the off-bead spectrum in Figure 4A. This band corresponds to a succinimide fraction which, although collected in the on-bead mode, actually had no interaction with the bead itself and hence shows no frequency perturbation relative to succinimide in the "neat" solvent.

The band labeled "bead phase component" is the direct result of subtracting the solvent phase component spectrum from the primary on-bead spectrum. Hence, the solvent phase component and bead phase component bands sum to equal the on-bead absorbance. The frequency shift of the bead phase component spectrum suggests matrix modification relative to "neat" solvent and is therefore indicative of true interaction between reagent and the polymer bead matrix. Since only that fraction of reagent in this proximity to the polymer itself is in a position to react with the functionalized solid phase support, simple examination of the on-bead/off-bead ratios presented in Figure 2 may suggest a misleadingly high partition coefficient in the case of analyte rejection. In the case of reagent extraction, the result would be low estimations of reagent concentrations available for reaction at the bead interior.

In a case where modest attenuation of a reagent spectrum is observed for on-bead vs off-bead experiments yet no shift is observed in the reagent spectrum between the on/off bead phases, it may be that little or no reagent was delivered to dense polymer regions. In that case, the infrared experiment would simply observe reagent in voided regions where solvation by the bead is not a factor and reagent is not in proximity to react.

In conclusion, by altering equilibrium reagent concentration in a gel phase bead relative to the surrounding solvent, reagent partition can be demonstrated to be a primary deterrent to efficient delivery of reagent to the bead interior. The nature of the infrared experiments presented in this paper mandate a nonreactive analyte be used in the determination of reagent partition as outlined above. In a "true" synthesis, functionality of a resin and its affinity to reagent could change drastically throughout the course of a reaction. Although the potential overwhelming diversity of resin functionalization obtained throughout actual synthesis prevents meaningful characterization of reagent partition on an ad hoc basis for each specific resin, understanding of the reagent partition phenomenon and its potential for influencing reaction parameters throughout a synthetic sequence remain valuable. Identification of the partition phenomenon adds further credence to earlier infrared work[10] which minimized the role of size exclusion in the attenuation of reaction kinetics for small molecule synthesis.

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