Using Magnetic Levitation for Three Dimensional Self-Assembly

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Using Magnetic Levitation to Distinguish Atomic-Level Differences in Chemical Composition of Polymers, and to Monitor Chemical Reactions on Solid Supports

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This communication describes a density-based method that uses magnetic levitation to monitor chemical reactions on solid supports, and to distinguish differences in chemical composition of polymers. Solid-supported chemistry is widely used for preparing peptides, nucleic acids, libraries of small molecules, and for capturing reagents for affinity purification and protein target identification. Solid-phase chemistry has the inconvenient feature that there is no inexpensive and rapid method for monitoring the progress of reactions quantitatively on insoluble polymeric supports. The methods currently available can be segregated into two categories: i) colorimetric tests for the presence or absence of certain functional groups and ii) instrumental techniques (e.g., infrared and ultraviolet spectroscopy) that identify functional groups or provide characteristic spectroscopic information (e.g., mass spectroscopy, $^1$H NMR using a magic angle spinning probe, and $^{13}$C NMR). Colorimetric tests are rapid and provide qualitative information about conversion, but are subject to artifacts arising from competing side reactions (false positives) and incomplete reactions (false negatives). Spectroscopic instruments are substantially more informative than colorimetric tests, but they are expensive (> $10,000), usually time-consuming, and inconvenient to use, since they often must be shared by multiple users. An enabling addition to solid-supported chemistry—particularly for the development stages of a solid-supported synthesis—would be a rapid and quantitative method for following the progress of a reaction that does not require specialized or expensive equipment. In essence, what is needed is the procedural equivalent of thin-layer chromatography (TLC) for solid-phase chemistry.

This communication describes an inexpensive, rapid, and straightforward bench-top method that can be used to quantify the progress and kinetics of a reaction on a solid support. The method is based on the concept of magnetic levitation, and involves levitating a sample of beads (taken as an aliquot from a reaction mixture) in a paramagnetic solution (e.g., GdCl$_3$ dissolved in H$_2$O or N,N-dimethylformamide (DMF)) between two 5 cm × 5 cm × 2.5 cm NdFeB magnets oriented in the anti-Helmholtz configuration (Fig. 1).

Polymer beads levitate in the environment depicted in Fig. 1 when the gravitational ($F_g$) and magnetic forces ($F_{mag}$) acting on the beads balance one another (Eqn. 1). In Eqn. 1, $\rho_o$ is the density of the paramagnetic medium (kg·m$^{-3}$), $\rho_i$ is the density of the suspended particle (kg·m$^{-3}$), $V$ is the volume of the particle (m$^3$), $g$ is the acceleration due to gravity (m·s$^{-2}$), $\chi_m$ and $\chi_s$ are the magnetic susceptibilities (unitless) of the paramagnetic medium and the suspended particle respectively, $\mu_0$ is the magnetic permeability of free space (T·m·A$^{-1}$), and $B$ is the applied magnetic field (T).

$$ F_g + F_{mag} = (\rho_i - \rho_o)gV + \frac{(\chi_m - \chi_s)V}{\mu_0}B \cdot \nabla B = 0 $$

(1)

In this configuration of magnets, the “levitation height” $z_0$ (m)—the position along the $z$-axis at which the magnetic and gravitational forces balance each other on the centerline of the $z$-axis between the magnets for a bead with density $\rho_i$—is given by Eqn. 2, where $B_i(T)$ is the magnitude of the magnetic field at the surface of the magnet in the center of the $xy$ plane, and $h$ (m) is the distance between the magnets (see Supporting Information for details).

$$ z_0 = \left[ \frac{g \mu_0 B_i^2}{(\chi_m - \chi_s)4B_i^2} \right] \rho_i + \frac{h}{2} \left[ \frac{1}{\rho_o \mu_0 B_i^2} \right] (\chi_m - \chi_s)4B_i^2 $$

(2)

We have previously applied magnetic levitation for density-based separation of Merrifield resin beads that differed in the level of chlorine-containing functionality per bead, and for detection of the binding of streptavidin to resin-bound biotin. Here we show that: i) covalent modification of polymeric beads sufficiently alters the density of those beads to produce easily measurable changes in $z_0$, and ii) that changes in density and $z_0$ correlate with the progress and kinetics of a chemical reaction on a solid support.

The ability to resolve differences in the chemical composition of polymeric beads by levitation is evident in Fig. 2. This figure shows the correlation of $z_0$ with $\rho_i$ for 10 derivatives of 4-benzyloxybenzaldehyde polystyrene (diameter = 35–75 $\mu$m, loading level = 3.5 mmol –CHO/g resin, ~350 pmol –CHO/ bead). We prepared these derivatives using reductiveamination reactions (Eqn. 3):

$$ \text{H}_{-\text{N},\text{R}} \xrightarrow{\text{H}_\text{N,N}, \text{AcO}^- \text{H}, \text{NaNCB}_{3}, \text{DMF}} \text{H}_{-\text{N},\text{R}} \text{polystyrene} \text{ (35–75 $\mu$m) } $$

(3)
We used 10 equivalents of amine and NaBH₄(CN) in each reaction (dissolved in 5% CH₃COOH–DMF), and agitated the beads for 24 h to ensure complete conversion. We washed the beads (3 × 3 min each of DMF, CH₃Cl, CH₃OH, and H₂O) to remove excess reagent from the polymer, and stained aliquots of beads from each reaction with 2,4-dinitrophenylhydrazine to confirm complete conversion of the aldehyde.⁵

We measured z₀ by suspending ~100 beads from each reaction in a cuvette containing 650 mM GdCl₃ in water, and positioning the cuvette along the central axis between the magnets. The concentration of GdCl₃ was chosen empirically to adjust the density of the medium approximately to the density of the beads. Initially the beads were dispersed in solution, but after ~5 minutes they began to coalesce to form a cloud in one position between the magnets. After ~15 min the cloud of beads had coalesced to form a cloud in one position between the magnets. After ~15 min the cloud of beads had coalesced to form a cloud in one position between the magnets. After ~15 min the cloud of beads had coalesced to form a cloud in one position between the magnets.

Each bead in this experiment contained ~350 pmol of small molecule. A difference in chemical composition of a single atom between the small molecules led to measurable differences in z₀ (and ρ) between the corresponding beads (e.g., the differences in z₀ between derivatives b, g, and j shown in Fig. 2B reflect differences of one fluorine atom). A plot of z₀ versus ρ for each set of beads reveals a linear relationship between density of a polymer and its equilibrium levitation height (Fig. 2A), as expected from Eqn. 2. We measured ρ, for each polymer using sink–float techniques at various concentrations of CaCl₂ in water (see Supporting Information); these measurements are tedious, and impractical as a method of following chemical reactions.

The method also is sensitive to changes in chemical composition (and, hence, density) of a polymer during the course of a chemical reaction (Fig. 3). We demonstrate this sensitivity by monitoring the condensation reaction of 2,5-diodobenzoic acid to leucine-derivatized Wang polystyrene (diameter = 75–150 µm, 1.8 mmol –NH₂/g resin, ~1 mmol –NH₂/bead) at 0 °C using five equivalents of O-benzotriazol-1-yl-N,N,N′,N′-tetramethyluronium-hexafluoro-phosphate (HBTU) and five equivalents of diisopropylethyl amine (DIEA) in DMF (Fig. 3). We withdrew aliquots of beads from the reaction mixture at different times throughout the course of the reaction, and washed the beads immediately to remove excess reagents. We suspended ~100 beads from each aliquot in a cuvette containing 650 mM GdCl₃ in DMF, placed the cuvette between the NdFeB magnets, and waited ~15 min for the beads to reach an equilibrium levitation height.

Fig. 4A demonstrates the ease with which levitation can be used to monitor solid-phase reactions. The levitation height of the beads decreases as their density increases until the reaction reaches completion. The beads formed tight clusters at the beginning and end of the reaction, but displayed increased dispersion when the reaction approached 50% completion. The polymer beads are polydisperse in size (they vary in diameter between 75–150 µm), but are equal in density. During the course of the reaction, however, this polydispersity leads to variations in accessibility of reagents to the interior of the beads (~99% of the amines are on the interior of the beads)¹⁰, and results in slight differences in chemical composition (and density) between beads.¹¹ Once the reaction reaches completion and all of the available amines react, the chemical composition of the beads becomes uniform. Fig. 4B correlates z₀ and ρ, (measured independently using the sink–float technique) as the reaction progresses for each of the data points summarized in Fig. 4A.

The conversion of starting material to product measured by magnetic levitation matches the conversion measured by ¹H NMR within the 95% confidence interval. For measurements of conversion by levitation, we assumed that the value of z₀ for the beads reflected the mole fraction of starting material, and we used z₀ for each set of beads to calculate the concentration of unreacted amine at different time points during the course of the reaction using Eqn. (4):

\[
[\text{NH}_2]_{\text{experimental}} = \frac{z_{\text{experimental}} - z_{\text{NH}_2,0\%\text{conversion}}}{z_{\text{NH}_2,100\%\text{conversion}} - z_{\text{NH}_2,0\%\text{conversion}}}[\text{NH}_2]_{0\%\text{conversion}}
\]
Since the reagents for the reaction were used in five-fold excess relative to the quantity of polymer-bound –NH₂, the reaction followed pseudo first-order kinetics. Fig. 4C gives the data from three independent reactions. Both magnetic levitation and NMR yield similar rates for the pseudo-first order reaction (T₁/₂ = 23 ± 4 min (NMR) and T₁/₂ = 18 ± 2 min (levitation)) (Fig. 4C).

We conclude that magnetic levitation provides a sensitive, density-based approach for distinguishing relative differences in chemical functionality on polymeric beads, and for monitoring the progress and kinetics of solid-phase chemical reactions. This technique has the following useful characteristics: i) it is exceedingly simple (the levitation height can be measured easily by eye and quantified using a ruler), ii) it is inexpensive (5 cm × 5 cm × 2.5 cm NdFeB magnets cost $5 each, and GdCl₃ costs $0.39 per gram), iii) it is rapid (measurements require 15 minutes), iv) it requires only a small amount of sample (a single bead in a capillary tube works as well as groups of beads in a cuvette), v) and it does not destroy the sample. The speed and ease of this method is reminiscent of thin-layer chromatography (TLC) for solution-phase chemistry. The method, however, does not provide information about the chemical composition of the sample, nor does it reveal potential byproducts formed during the reaction.

We believe this technique will find broad applications as a rapid bench-top tool for monitoring and analyzing chemical and biochemical transformations on solid supports; in-depth information about the exact chemical composition of a polymer bead is best obtained with more accurate (but more expensive) techniques such as NMR spectroscopy. Studies focusing on the limitations and sensitivity of this technique are underway.

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Supporting Information: is available free of charge via the Internet at http://pubs.acs.org.

References:
(12) These values are for bulk pricing; individual 5 cm × 5 cm × 2.5 cm NdFeB magnets are available for $15–20 at www.magnet4less.com and GdCl₃ 6H₂O costs $2 per gram from Sigma-Aldrich.

For ¹H NMR experiments, we cleaved the product and starting material from the polymer (using trifluoroacetic acid) for each sample of beads collected from the reaction. We obtained the ratio of product to starting material by integration of ¹H NMR spectra (we integrated leucine ß-hydrogens, which are resolved between product and starting material by 0.71 ppm in the ¹H NMR spectrum).

Figure 4. (A) Photographs of clusters of levitating polymer beads (leucine-derivated Wang polystyrene (diameter = 75–150 µm, 1.8 mmol –NH₂/g resin, –1 mmol –NH₂/head) (~100 beads/cluster) taken at different times throughout the course of the reaction shown in Figure 3. We levitated the beads in 650 mM GdCl₃, in DMF at 23 °C. (B) Plot showing the correlation of density of the polymer beads with their levitation height; each data point corresponds to a sample of beads shown in (A). The error bars represent the standard deviation from three independent measurements. (C) Pseudo-first order kinetics plots showing the rate of consumption of polymer-bound amine determined by ¹H NMR (•) and by levitation (○) from three independent measurements. The data were fit with linear least-squares lines: (i) levitation (○): y = -0.038x + 0.730, R² = 0.992; and (ii) ¹H NMR (•): y = -0.031x + 0.583, R² = 0.993.
This communication describes a density-based method that uses magnetic levitation for monitoring solid-supported reactions and for distinguishing differences in chemical composition of polymers. The method is simple, rapid, and inexpensive, and is similar to thin-layer chromatography (TLC; for solution-phase chemistry) in its potential for monitoring reactions in solid-phase chemistry. The technique involves levitating a sample of beads (taken from a reaction mixture) in a cuvette containing a paramagnetic solution (e.g., GdCl₃ dissolved in H₂O) positioned between two NdFeB magnets. The vertical position at which the beads levitate corresponds to the density of the beads and correlates with the progress of a chemical reaction on a solid support. The method is particularly useful for monitoring the kinetics of reactions occurring on polymer beads.