

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 to prepare peptides, nucleic acids, libraries of small molecules, and capturing reagents for affinity purification and protein target identification.^{1–3} 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.⁴ The methods currently available can be segregated into two categories: (i) colorimetric tests for the presence or absence of certain functional groups^{5,6} and (ii) instrumental techniques (e.g., infrared and ultraviolet spectroscopy) that identify functional groups or provide characteristic spectroscopic information (e.g., mass spectroscopy, ¹H NMR using a magic angle spinning probe, and ¹³C NMR).^{1,3}

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).⁶ 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 benchtop 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 levitation⁷ and involves levitating a sample of beads (taken as an aliquot from a reaction mixture) in a paramagnetic solution (e.g., GdCl₃ dissolved in H₂O or *N,N*-dimethylformamide (DMF)) between two 5 cm × 5 cm × 2.5 cm NdFeB magnets oriented in the anti-Helmholtz configuration (Figure 1).⁸

Polymer beads levitate in the environment depicted in Figure 1 when the gravitational (F_g) and magnetic forces (F_{mag}) acting on the beads balance one another (eq 1). In eq 1, ρ_m is the density of the paramagnetic medium ($\text{kg}\cdot\text{m}^{-3}$), ρ_s is the density of the suspended particle ($\text{kg}\cdot\text{m}^{-3}$), V is the volume of the particle (m^3), g is the acceleration due to gravity ($\text{m}\cdot\text{s}^{-2}$), χ_m and χ_s are the magnetic susceptibilities (unitless) of the paramagnetic medium and the suspended particle respectively, μ_0 is the magnetic permeability of free space ($\text{T}\cdot\text{m}\cdot\text{A}^{-1}$), and B is the applied magnetic field (T).

$$\vec{F}_g + \vec{F}_{\text{mag}} = (\rho_s - \rho_m)V\vec{g} + \frac{(\chi_m - \chi_s)}{\mu_0}V(\vec{B}\cdot\nabla)\vec{B} = 0 \quad (1)$$

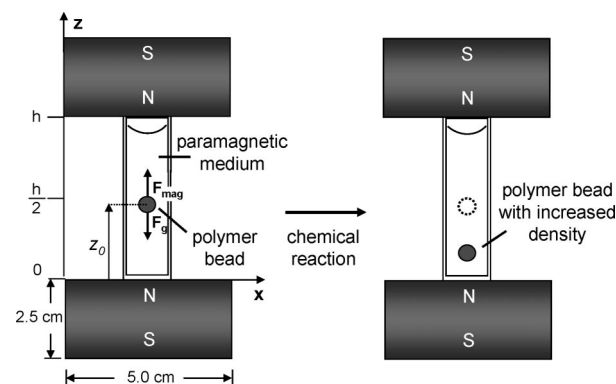


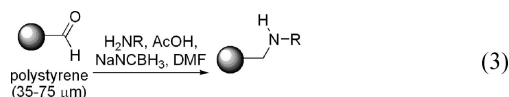
Figure 1. Schematic representation of the apparatus used for monitoring solid-supported reactions. A polymer bead levitates in this device at an equilibrium position z_0 when the magnetic (F_{mag}) and gravitational (F_g) forces acting on the bead balance each other.

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 ρ_s —is given by eq 2, where B_0 (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).

$$z_0 = \left[\frac{g\mu_0 h^2}{(\chi_s - \chi_m)4B_0^2} \right] \rho_s + \left[\frac{h}{2} - \frac{\rho_m g \mu_0 h^2}{(\chi_s - \chi_m)4B_0^2} \right] \quad (2)$$

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.⁸ 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) 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 Figure 2. This figure shows the correlation of z_0 with ρ_s for 10 derivatives of 4-benzyloxybenzaldehyde polystyrene (diameter = 35–75 μm , loading level = 3.5 mmol –CHO/g resin, ~350 pmol –CHO/bead). We prepared these derivatives using reductive amination reactions (eq 3):



We used 10 equiv of amine and NaBH₃(CN) in each reaction (dissolved in 5% CH₃COOH–DMF) and agitated the beads for 24 h

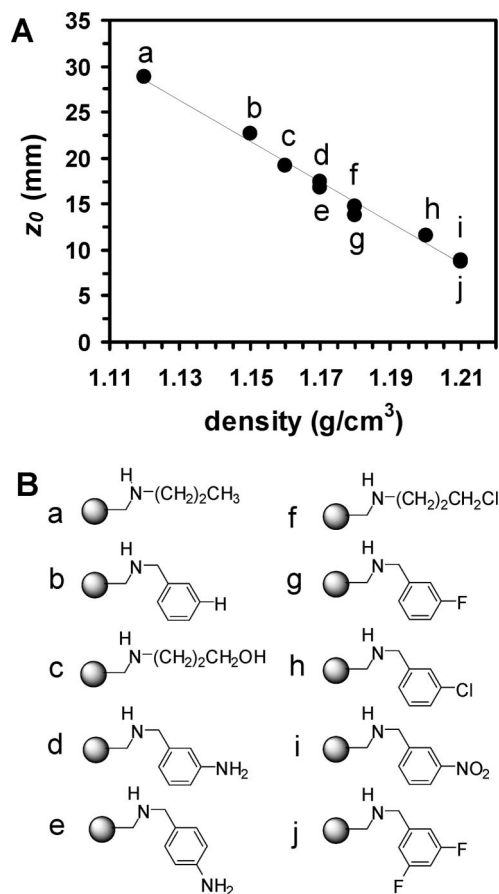


Figure 2. (A) Graph of z_0 versus ρ_s for 10 different derivatives of the 4-benzyloxybenzaldehyde polystyrene beads levitated in a 650 mM aqueous solution of GdCl_3 at 23 °C. The linear least-squares fit is described by the following equation: $y = -221x + 276$; $R^2 = 0.984$. (B) Chemical structures for each of the data in (A).

to ensure complete conversion. We washed the beads (3×3 min each of DMF, CH_2Cl_2 , CH_3OH , and H_2O) to remove excess reagent from the polymer and stained aliquots of beads from each reaction with 2,4-dinitrophenylhydrazine to confirm complete consumption of the aldehyde.⁹

We measured z_0 by suspending ~ 100 beads from each reaction in a cuvette containing 650 mM GdCl_3 in water and positioning the cuvette along the central axis between the magnets. The concentration of GdCl_3 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 min 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 tight cluster; we took the center point of this cluster to be z_0 , which we measured using a ruler with mm-scale markings.

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 z_0 (and ρ_s) between the corresponding beads (e.g., the differences in z_0 between derivatives **b**, **g**, and **j** shown in Figure 2B reflect differences of one fluorine atom). A plot of z_0 versus ρ_s for each set of beads reveals a linear relationship between the density of a polymer and its equilibrium levitation height (Figure 2A), as expected from eq 2. We measured ρ_s for each polymer using sink–float techniques at various concentrations of CaCl_2 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 (Figure 3). We demonstrate this sensitivity by monitoring the condensation reaction of 2,5-diiodobenzoic acid to leucine-derivatized Wang polystyrene (diameter = 75–150 μm , 1.8 mmol $-\text{NH}_2/\text{g}$ resin, ~ 1 nmol $-\text{NH}_2/\text{bead}$) at 0 °C using 5 equiv of *O*-benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate (HBTU) and 5 equiv of diisopropylethyl amine (DIEA) in DMF (Figure 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 GdCl_3 in DMF, placed the cuvette between the NdFeB magnets, and waited ~ 15 min for the beads to reach an equilibrium levitation height.

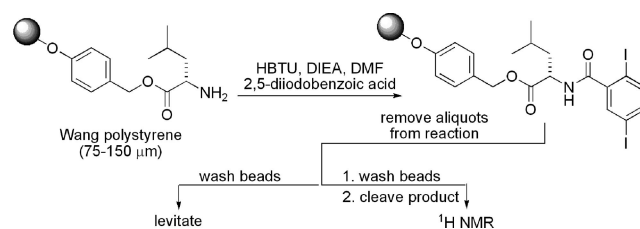


Figure 3. Schematic describing the process for monitoring conversion for a condensation reaction of a solid-supported amine with a carboxylic acid.

Figure 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 and 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 ($\sim 99\%$ of the amines are on the interior of the beads)¹⁰ and results in slight differences in chemical composition (and density) between beads.^{1,11} Once the reaction reaches completion and all of the available amines react, the chemical composition of the beads becomes uniform. Figure 4B correlates z_0 and ρ_s (measured independently using the sink–float technique) as the reaction progresses for each of the data points summarized in Figure 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 z_0 for the beads reflected the mole fraction of starting material, and we used z_0 for each set of beads to calculate the concentration of unreacted amine at different time points during the course of the reaction using eq 4:

$$[-\text{NH}_2]_{\text{experimental}} = \left(\frac{z_{\text{experimental}} - z_{100\% \text{ conversion}}}{z_{0\% \text{ conversion}} - z_{100\% \text{ conversion}}} \right) [-\text{NH}_2]_{0\% \text{ conversion}} \quad (4)$$

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 α -hydrogens, which are resolved between product and starting material by 0.71 ppm in the ^1H NMR spectrum).

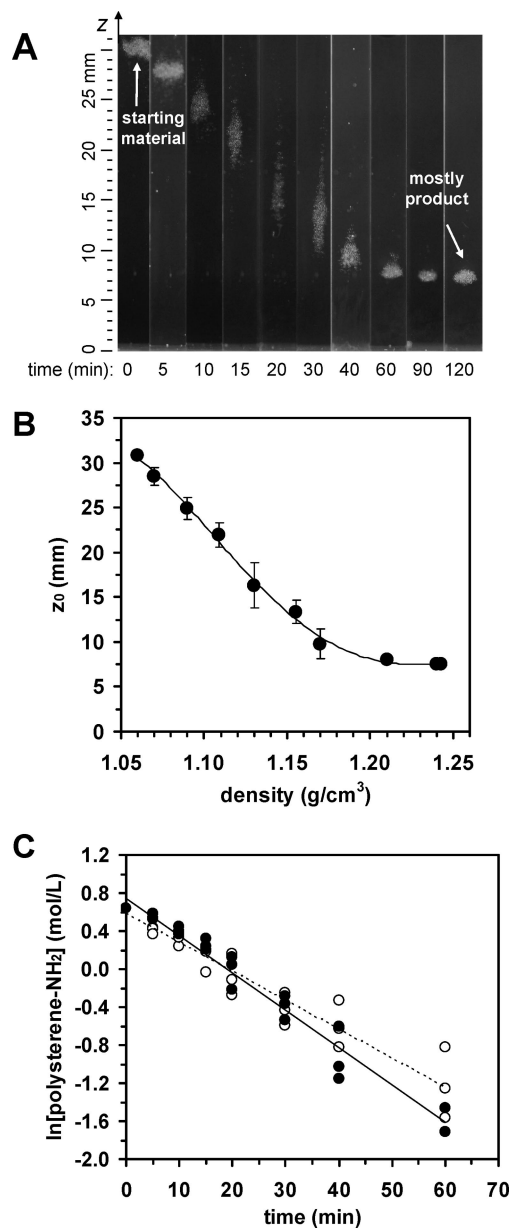


Figure 4. (A) Photographs of clusters of levitating polymer beads (leucine-derivatized Wang polystyrene (diameter = 75–150 μm , 1.8 mmol $-\text{NH}_2/\text{g}$ resin, ~ 1 nmol $-\text{NH}_2/\text{bead}$) (~ 100 beads/cluster) taken at different times throughout the course of the reaction shown in Figure 3. We levitated the beads in 650 mM GdCl_3 in DMF at 23 $^\circ\text{C}$. (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 (\circ) and by levitation (\bullet) from three independent measurements. The data were fit with linear least-squares lines: (i) levitation (—): $y = -0.038x + 0.730$, $R^2 = 0.992$; and (ii) ^1H NMR (---): $y = -0.031x + 0.583$, $R^2 = 0.993$.

Since the reagents for the reaction were used in 5-fold excess relative to the quantity of polymer-bound $-\text{NH}_2$, the reaction followed pseudo-first-order kinetics. Figure 4C gives the data from three independent reactions. Both magnetic levitation and NMR

yield similar rates for the pseudo-first-order reaction ($T_{1/2} = 23 \pm 4$ min (NMR) and $T_{1/2} = 18 \pm 2$ min (levitation)) (Figure 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 \times 5 cm \times 2.5 cm NdFeB magnets cost \$5 each, and GdCl_3 costs \$0.39 per gram);¹² (iii) it is rapid (measurements require 15 min); (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 are reminiscent of thin-layer chromatography (TLC) for solution-phase chemistry. The method, however, neither provides information about the chemical composition of the sample nor reveals potential byproducts formed during the reaction.

We believe this technique will find broad applications as a rapid benchtop 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 Available: Additional experimental information. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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