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A Direct Comparison of the Mixing Efficiency in Solid-phase Organic Synthesis by Single Bead IR and Fluorescence Spectroscopy

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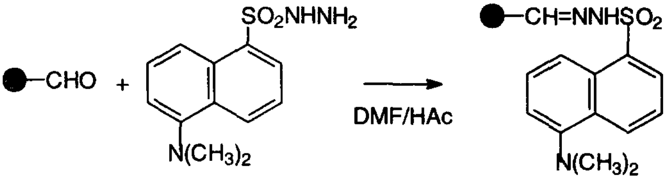
ABSTRACT: The efficiency of six different mixing methods for solid-phase organic synthesis was evaluated for the reaction between formylpolystyrene resin and dansylhydrazine by means of single bead IR and fluorescence spectroscopy. The 360° rotating and nitrogen bubbling methods gave highest yields with mild conditions. © 1997 Elsevier Science Ltd.

Solid-phase organic synthesis (SPOS)[1] techniques are undergoing an exploding growth in the field of combinatorial chemistry.[2] SPOS offers several advantages[2i] over solution phase techniques for achieving high throughput synthesis which significantly impacts on drug and other new chemical entity discoveries. At the same time, SPOS also poses problems that don't exist in solution synthesis. One of the problems is to select the most efficient reaction mixing method for maximizing 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. These methods include gentle rotation mixing at 180° or 360°, nitrogen bubbling, magnetic stirring and vigorous shaking using wrist or orbit shakers. In our experience, solid-phase organic reactions can proceed with a wide range of rates.[3a,c,d] For slow reactions, mixing efficiency is critical even though the soluble reactant can be used in excess. For fast reactions, several synthetic steps can be achieved in a single day if the mixing efficiency is optimal. Therefore, the knowledge of the relative efficiency of available mixing methods is essential for optimizing reactions on solid-phase. This is especially true for designing automated synthesis instruments.

In this paper, we report an evaluation of the efficiency of six different mixing techniques commonly used in the solid-phase synthesis. The reactions were followed by single-bead IR and fluorescence spectroscopy. Infrared signals of functional groups in the product relative to those of the starting material are indicators for the progression of solid-phase reactions.[3a,c] The use of on-resin analytical methods in studying the reaction variability of resin mixtures avoids tedious cleavage, purification and weighing routines a traditional comparative study involves.

Scheme 1



For the purpose of evaluating various mixing techniques, we chose a reaction as shown in Scheme 1.[4] The yield of this reaction was determined directly on-resin by fluorescence spectroscopy as previously described.[5] The yield values obtained by fluorescence measurements report the average reaction progress. During the reaction, the C-H stretch of the aldehyde group at 2728 cm^{-1} decreases while the corresponding $-N(CH_3)_2$ stretch of the hydrazone product at 2790 cm^{-1} increases with time.[5] To further compare the effect of mixing on reactions occurring on individual beads, single bead IR spectra for 80-120 beads in each mixing experiment were obtained.[3a,b] The number of beads with complete product formation (only band at 2790 cm^{-1}) are compared with those having incomplete product formation (mixture of the product band and the starting aldehyde band at 2728 cm^{-1}) in Table I.

We first evaluated the effect of mixing by examining the reaction yield without mixing the reaction mixture. Only 50±6% of reactive sites reacted in 30 min and 21% of all beads have undergone a complete conversion showing the necessity of mixing during solid-phase reactions. The 360° rotation mixing method (16 rmp) is one of the most efficient mixing techniques resulting in a 98±4% product formation. Mixing by 180° rotation works well but needed twice the reaction time to get 99±5% product (Table I).

Mixing the sample by nitrogen bubbling works efficiently with a 99% product formation in 30 min (Table I). Besides the high yield, this mixing method offers additional advantages. It is easy to filter and wash the reaction product on the bubbling equipment directly; the reaction temperature can be controlled from -30°C to 150°C; and it provides an ideal inert atmosphere.

Magnetic stirring is the most popular mixing method for solution synthesis. As seen from Table I, this method afforded a 96±4% product formation in 30 min. However, the stirring bar broke resin beads that are usually swollen and softer in organic solvents. This suggests that this method is definitely not suitable for split-mix library synthesis.

Vigorous shaking with a wrist shaker is commonly used in solid-phase synthesis. At the maximum speed of 200 rpm, this method gave a 93±4% product formation. However, only 56±3% conversion was observed with the orbit shaking technique at 200 rpm speed in 30 min, and the reaction yield was not improved with increased reaction time (Table I). This clearly shows that, at a two-fold excess of reagent, the mixing efficiency of this method is not sufficient to drive the reaction to completion in an hour. To approach 100% product formation,

Table I. Mixing efficiency in solid-phase organic synthesis

Method	Time (min)	Total Beads	Incomplete[#] (bead)	Complete[#] (bead)	Yield[*] (%)
No mixing	30	80	63	17	50±6
rotation[a]	30	100	3	97	98±4
180° rotation[b]	30	80	15	65	88±3
	60	80	1	79	99±5
N_2 Bubbling[c]	30	120	4	116	99±4
Mag. Stirring[d]	30	100	3	97	96±4
Wrist Shaking[e]	30	100	5	95	93±4
Orbit Shaking[f]	30	120	66	54	56±3
	60	80	39	41	66±7
	60 (10:1)[g]	80	2	78	n.d.

[#] Single bead IR was recorded as described in reference 3. Recording spectra from 100 beads took 2-3 hours.

[*] All yield values were the average of 2-5 independent fluorescence measurements and procedures for measurement and calculation were as in reference 5.

a: 360° rotation on a Glas-Col Laboratory Rotator at 16 rpm using 10 mg resin in 0.2 ml DMF.

b: 180° rotation on St. John Associates, Inc 180° Shaker at 16 rpm using 10 mg resin in 0.2 ml DMF.

c: Bubbling with Nitrogen at 12 L/min in a reaction vessel/gas/vacuum assembly made by Lab Glass (Vineland, New Jersey) using 100 mg resin in 2 ml DMF.

d: Stirring on Thomas Model 15 Magne-Matic Stirrer at minimum speed using 50 mg resin in 1 ml DMF.

e: Shaking on Burrell Model 75 Wrist Action Shaker at maximum speed (200 rpm) with 50 mg resin in 1 ml DMF.

f: Shaking on LAB-LINE Orbit Shaker placed at a 15° angle at 200 rpm using 10 mg resin in 0.2 ml DMF (the yield is the average of 4 measurements).

g: Hydrazine:formylpolystylene=10:1

the ratio of hydrazine to aldehyde resin has to be raised to 10:1 and the reaction time was doubled.

In summary, mixing is highly important for SPOS. For the reaction studied, the 360° rotation and nitrogen bubbling methods provided high mixing efficiency in solid-phase synthesis under mild conditions. Wrist shaking also showed high mixing efficiency with the maximum setting. Magnetic stirring showed the same high mixing efficiency, but they sometimes broke beads. The method with 180° rotation mixing required longer reaction time, and it thus may not be preferable for slow reactions. Mixing with the orbit shaker did not give satisfactory reaction yields as compared to those obtained by the other techniques. Using the extended reaction time and a ten-fold excess of reactant finally drove this reaction to completion. In practice, using a large excess of reagent and a long reaction time may overcome the shortcoming of this mixing method unless the reaction is very slow.

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4. In all experiments, formylpolystyrene resin (NovaBiochem., 1% DVB, 100-200 mesh) was used. Dansylhydrazine was purchased from Aldrich. The formylpolystyrene resin was shaked with DMF for 30 min before reactions. All reactions were perfomed in DMF at room temperature. The dansylhydrazine concentration was 60 mM in all cases and the excess was always two fold except where noted.

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