

An Efficient Monitoring Technique for Solid-Phase Reactions by KBr Pellets/FT-IR Using Methyl *p*-Aminobenzoate Synthesis Assisted by Microwave Radiation on Merrifield Resin

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O uso de pastilhas de KBr em espectroscopia de infravermelho foi investigado para a monitorização de reações em fase sólida. A síntese do *p*-aminobenzoato de metila na Resina Merrifield (RM) é descrita como um modelo para esta técnica, a qual permite que o progresso das três etapas da síntese sejam monitoradas.

The use of KBr pellets in infrared spectroscopy has been investigated for monitoring solid-phase reactions. Synthesis of the methyl *p*-aminobenzoate on Merrifield resin (MR) is described as a model for this technique, which allows the progress of its three on-bead reaction steps to be monitored throughout their duration.

Keywords: Merrifield resin, infrared spectroscopy, solid phase organic synthesis, microwave-assisted reactions

Introduction

Solid Phase Organic Synthesis (SPOS)¹ involves the synthesis of molecules on chemically-inert insoluble polymers. Syntheses on solid support are generally quicker because unwanted side products are removed by simple filtration and washing at each stage. High yields are often obtained because each reaction step can be driven virtually to completion by use of excess reagents. Furthermore, purification need to be performed only once, following cleavage of the final product from the resin.

The ability to monitor solid-supported reactions is highly desirable when an ever-increasing diversity of chemical processes are becoming amenable to SPOS and, hence, the production of combinatorial libraries for the rapid discovery of novel drug candidates.

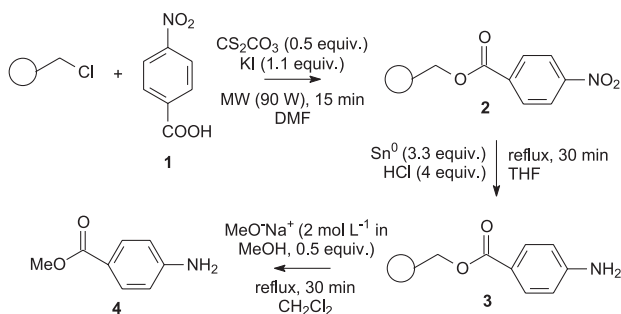
Even though the two classical approaches to control reactions, consisting of chromatography and NMR methodologies, work well in solution phase, some difficulties have been found to extending these methods to monitoring on-bead reactions. The main consequence is that solid-phase reactions are often left to run for longer than necessary (to ensure a complete reaction). Some methods have been employed in SPOS such as NMR gel-phase spectroscopy or magic angle NMR spectroscopy,²⁻⁶ fluorescence spectroscopy,⁷ and Fourier Transform Infrared

Spectroscopy (FT-IR) including Raman FT-IR,^{8,9} Attenuated Total Reflection¹⁰ and FT-IR microspectroscopy¹¹ and KBr pellets by Infrared difference spectra.¹² Though invaluable, these techniques require instrumentation that is often prohibitively expensive. The necessity for developing a new and accessible technique to monitor SPOS reactions allowed development of techniques to analyse reactions on the solid-phase. FT-IR is an useful technique applied by practitioners of SPOS, and infrared difference methodology is an useful alternative, but in this work, it is reported the use of conventional FT-IR KBr pellet methodology^{13,14} to monitor the reaction directly on the polymer-support during the course of a solid-phase synthesis. This is without the need to accurately weigh the sample, or by using a control sample (reference pellet of polystyrene bead) to obtain infrared difference spectra. The goal of this work is to report an adaptation of the use of FT-IR/KBr pellets spectroscopy to monitor SPOS reactions. We chose a simple, inexpensive, non-hazardous and classical synthesis: the SPOS of methyl *p*-aminobenzoate from *p*-nitrobenzoic acid (Scheme 1).¹⁵

Results and Discussion

Samples were removed at regular time intervals, washed, and their IR spectra recorded (see Experimental part). The scattering phenomena often observed using KBr pellets, were eliminated by a simple baseline correction.

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Scheme 1. *p*-Aminobenzoic acid methyl ester synthesis on Merrifield Resin.

Pleasingly, all spectra were obtained with high quality, and the presence of a straight low-noise baseline allowed real-time detection of reaction intermediates. The methodology used for monitoring the reactions is based on the ratio of peak heights B/A (Figures 1 and 2) which minimizes the error associated with overlapping bands. Since the polymeric structure of the solid support remains unaltered during the entire reaction, it can be used as an internal reference, allowing the comparison of the additional and subtracted peaks. The equilibrium state is reached when this ratio becomes constant. At the beginning, any peak can be selected for this purpose, as long as it remains constant during the reaction. For our internal

standard we chose the stretching vibration at 1450 cm^{-1} for the C=C-C aromatic (defined as B on the Figures) from Merrifield resin (MR). For all spectra, the ratios were recorded comparing the different height values of the non-constant band (A). All the FT-IR spectra presented here are fully consistent with the synthetic pathway proposed.

The reported FT-IR results further confirmed that by employing both SPOS and microwave-assisted strategies, we can obtain the product in a very short time: 66 min as a total time, as observed in Scheme 1.

First step - anchoring of template 1 on a MR

The coupling reaction of *p*-nitrobenzoic acid **1** onto MR was studied by the additional band appearance at 1740 cm^{-1} (Figure 1), corresponding to stretching vibration of the C=O bond. The spectra displayed in Figure 1 clearly show the progress of the anchoring step. The classical coupling reaction between MR and a carboxylic group generally requires several hours.¹⁶ However, the use of microwave radiation drastically shortened the reaction time to 6 min, indicating the success of this technology for polymer supported/solid phase reactions.^{17,18} Figure 3 displays the way in which the ratio B/A decreased with time and reached a plateau after approximately 6 min.

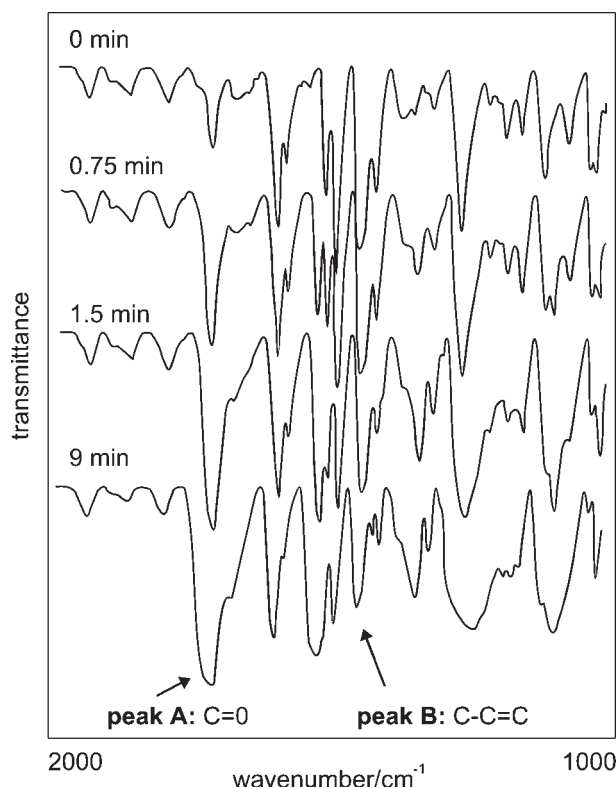


Figure 1. FT-IR spectra of **2** at various times during the coupling reaction.

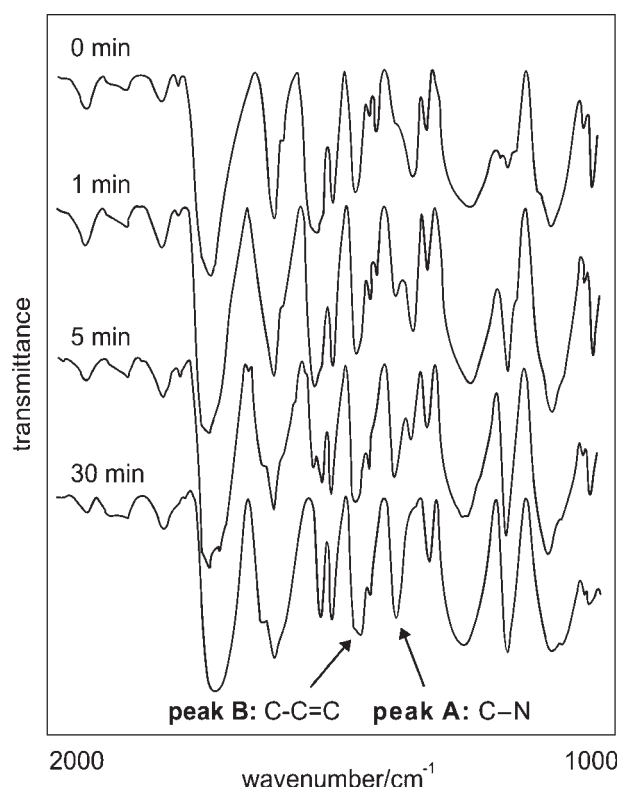


Figure 2. FT-IR spectra of **3** at various times of the reduction reaction.

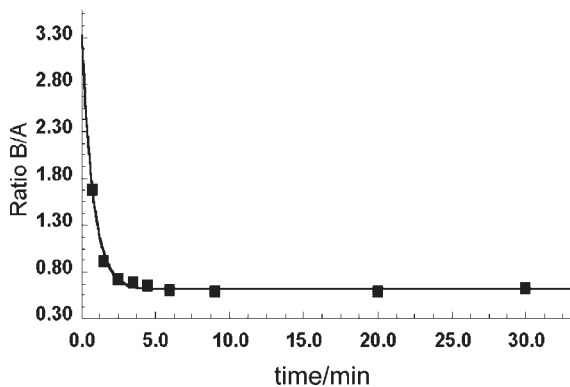


Figure 3. Time course of the coupling reaction.

Second step – reduction of nitro group

The formation of the aromatic amino group by a classical solution-phase protocol has shown that the reduction of the nitro group with the Sn⁰/HCl mixture could be a convenient method for application to solid-phase reactions in the presence of MR. In fact, this reductive condition allows formation of the amine in the same manner as solution chemistry, as can be observed in Figure 2 and in Figure 4. The spectrum revealed a new band at 1373 cm⁻¹ attributed to C-N vibrations, and the disappearance of the band at 1310 cm⁻¹, corresponding to NO₂ group stretching frequencies.¹⁹ In order to monitor this reaction we chose the following absorption bands (Figure 2): A = 1373 cm⁻¹, stretching of the phenyl C-N bond, and B = 1450 cm⁻¹, attributed to the polystyrene matrix.

The Figure 4 corresponds to a ratio of peak heights B/A, and from that we can observe the reduction was complete after 30 min.

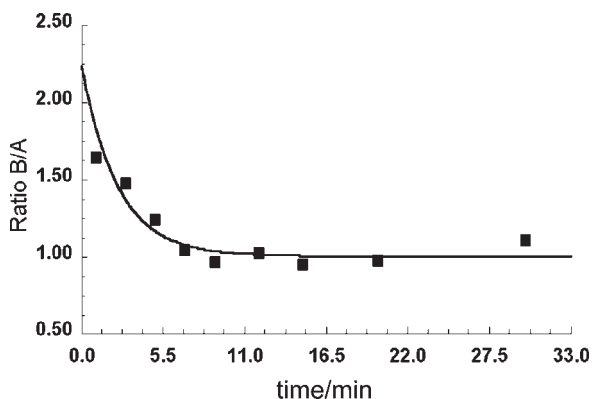


Figure 4. Time course of the reduction reaction.

Third step – release of final product from MR

The release of the final product from MR with sodium methoxide was accomplished in 30 min, as monitored by the disappearance of the ester absorption (1740 cm⁻¹). At

the same time, the reaction was followed by TLC (EtOH/CH₂Cl₂ 1:4), and the methyl *p*-aminobenzoate was isolated and characterized by ¹H-NMR (overall yield: 35%).

Conclusions

In this paper we have demonstrated that the use of an FT-IR KBr pellet methodology could assist the monitoring of different reactions in SPOS, using MR. The qualitative analytical technique presented here is an uninterrupted and non-destructive method. The advantages also include low cost, availability, high resolution, accuracy, use of small amounts of samples, with easy and fast sample handling. Indeed, this technique proves that the synthetic pathway described in this paper is very fast and demonstrates that solid phase reactions can be carried out with short reaction times.

Experimental

Instrumental

All IR spectra were recorded on a Shimadzu DR 8001 FT-IR Spectrophotometer in transmittance mode, with 4 cm⁻¹ resolution and with 40 accumulations. The frequency range 4000 to 600 cm⁻¹ was covered. The samples were collected in a syringe and washed, including a final wash with diethyl ether giving samples ready to be analyzed. The KBr (150 mg) pellets were prepared on a quartz mortar with about 4.5 mg (*ca.* 3%) of sample (the amount of sample ensured that absorbance values conformed to the Lambert-Beer law). The sample was then transferred to a metal container and pressed at 8 ton for 5 min to afford pellets of width 0.55 mm. The baseline correction is only required on troughs which will be compared. The ¹H NMR spectrum was recorded using a Varian XL-200 at 200 MHz, and CDCl₃ as solvent. Chemical shifts are reported as *d* values (ppm) relative to TMS (0.0 ppm).

Synthetic procedures (Scheme 1)

p-Nitrobenzoic acid and other reagents were obtained from commercial sources. All solvents were distilled prior to use. Abbreviations: THF = tetrahydrofuran, DMF = dimethylformamide, Et₂O = diethyl ether, MeOH = methanol. Merrifield resin (1% cross-linked, 2 – 2.5 mmol/g, particle size: 200–400 mesh) was purchased from ACROS Organics, New Jersey, US.

Coupling reaction

A suspension of Merrifield resin (600 mg) in anhydrous DMF (5 mL) was treated with *p*-nitrobenzoic acid (**1**, 1

equiv., 300.9 mg), Cs_2CO_3 (0.5 equiv., 293.4 mg) and KI (1.1 equiv., 273.93 mg). The reaction was performed in a domestic microwave oven (Sharp® Carousel II, 900 W), using 10% power for 15 min, to ensure the completion of the reaction. The samples were carefully removed with a syringe, filtered, washed with MeOH, then Et_2O , and the spectra recorded.

Reduction of nitro group

A suspension of the resin-bound **2** (200 mg) in THF was treated with Sn^0 (3.3 equiv., 195.8 mg) and HCl (36%, 9.8 equiv.) and stirred for 30 min at reflux. The samples were carefully removed with a syringe, filtered, washed with THF/water (1:1 v/v), then Et_2O , and the spectra recorded.

Cleavage step

To a suspension of **3** (200 mg) in THF was added 1.25 mL of sodium methoxide (0.2 mol L^{-1} in MeOH, 0.25 mmol, 0.5 equiv.) and refluxed for 30 minutes. The resin was filtered off and washed with MeOH, then Et_2O . The organic layer was dried with Na_2SO_4 and evaporated under vacuum to give 28.8 mg of **4**, without additional purification (overall yield = 35%). ^1H NMR (CDCl_3 , δ ppm): 3.8 (3H, s, $\text{CH}_3\text{-O}$), 6.4 (2H, d, J 8.5 Hz, Ar-H), 7.8 (2H, d, J 8.5 Hz, Ar-H).

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