DOI: 10.1002/ejoc.201000441

Online Monitoring of Microwave-Enhanced Reactions by UV/Vis Spectroscopy

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Keywords: Online monitoring / Microwave chemistry / UV/Vis spectroscopy / Sensors

Microwave-enhanced reactions are very fast in comparison to thermal reactions. They are often finished within a few minutes. Thus, the determination of the end point often fails, because conventional analytical methods are too slow. We developed a fast method using a UV/Vis sensor, which allows a direct online monitoring of the reaction process. The potency of the method is demonstrated by means of five representative reactions.

Introduction

Microwave-enhanced reactions in organic synthesis are well established for a large variety of reactions.[1–3] The reaction time can be shortened in comparison to thermal heating from days or hours to minutes. The problem of such fast reactions is to determine the optimal end point of the conversion. Typical analytical methods controlling the progress of the reaction such as NMR spectroscopy, TLC or HPLC take often longer than the microwave irradiation time. The time range can vary from several minutes to half an hour or even more depending on the treatment of the sample prior to the measurement. Within this time the reaction progresses and by- and decomposition products may develop. This decreases the yield of the major product and increases the efforts to purify the main product.

In principle, UV/Vis[4] and Raman[5] spectroscopy are suitable to monitor the reaction progress in a microwave reactor. However, the UV/Vis method cannot be carried out in the reaction solution itself. A sample needs to be taken from the solution, diluted and measured. Recently, we developed such a real-time observation of reactions by fast FTIR spectroscopy.[6] Unfortunately, this method is not applicable to high-boiling solvents (e.g. DMF, methanesulfonic acid), because the solvent has to be evaporated before the FTIR measurement can take place. Herein, we report a method, which allows direct online observation of the progress of the reaction by using a UV/Vis sensor (in the reaction mixture) and computer software. The experimental setup of the UV/Vis sensor consists of two fiber-optic cables welded together for the incoming and the outgoing beam supported by a quartz glass cover (see Supporting Information).

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201000441.

Results and Discussion

Typically, in order to obtain a valid UV/Vis spectrum, solutions containing 10^{-4} mol/L of an analyte are used. In contrast, chemical reactions are performed in solutions of higher concentrations (0.1–1.0 mol/L). Classical UV/Vis measurements of these solutions are incapable of measurement due to the facts that the absorbance is not linear, and the obtained spectra are complex. However, the spectroscopic sensor used here is able to measure the absorbance in the concentrated solutions of the chemical reaction. Nevertheless, for the monitoring of the ongoing reaction the UV wavelength has to be carefully chosen. The reactions can be carried out in an open flask or in a special quartz-glass vessel, which allows working beyond the boiling point at atmospheric pressure or under pressure. This is exemplified by the following reactions.

Examples 1 and 2

Fluorinated fluorescein-type dyes can be used as reporter molecules in biological systems.[7] They can form stable complexes with tetracysteine motifs engineered into the target proteins of interest.[8] Here, we report a microwave-driven syntheses of the fluorogenic dyes **3** and **6** (Scheme 1), which are carried out in methanesulfonic acid in an open flask.[7] The conversion of the corresponding phenols and anhydrides were monitored between λ = 600 and 700 nm. The conversion to give the dyes **3** and **6** were completed after 15 and 30 min, respectively. Longer reactions times led to decomposition of the products and decrease of the yields.

Whereas conventional approaches yielded 85% (**3**) and 74% (**6**), respectively, with reaction times between 36 and 48 h,[7] the yields of the microwave-supported syntheses are

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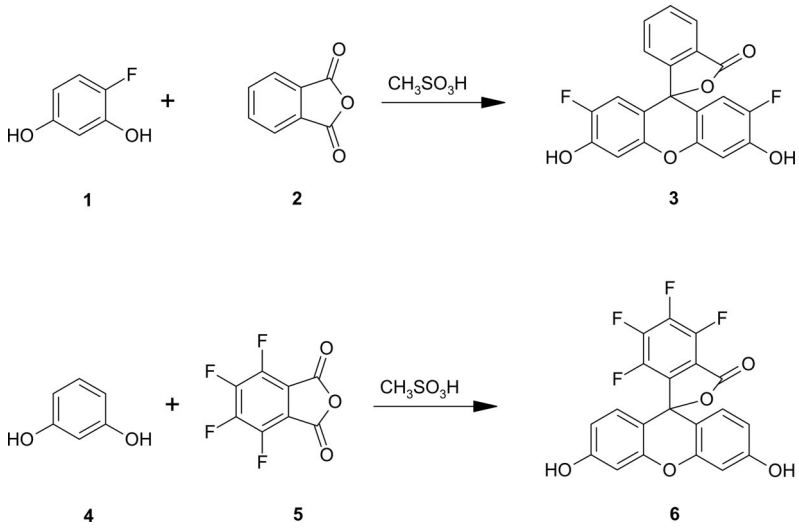


Scheme 1. Syntheses of the fluorinated dyes **3** and **6**.

higher than 90%. Figure 1 shows the overlaid transmission spectra of the reaction solution for the synthesis of compound **3**. The signal of the starting compound (red) at 656 nm disappeared after 3 min. During the reaction, the transmission increased (blue – violet – green). The reaction was finished after 15 min.

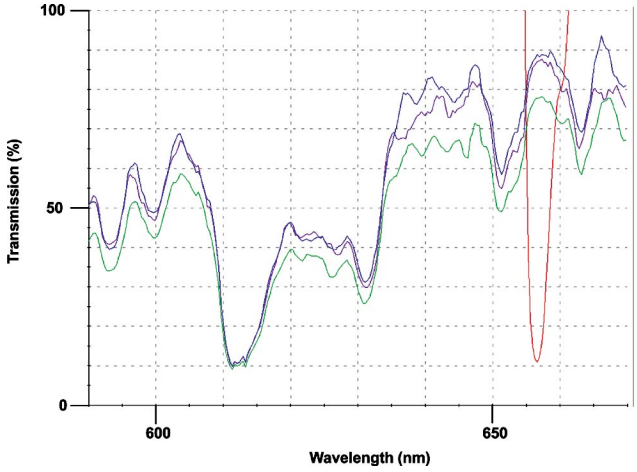


Figure 1. Transmission spectra of the reaction solution for the synthesis of **3**: 0 min (red), 3 min (blue), 10 min (violet) and 15 min (green).

Figure 2 shows the monitoring of the synthesis of compound **6**. After 30 min, no change of the spectrum was observed.

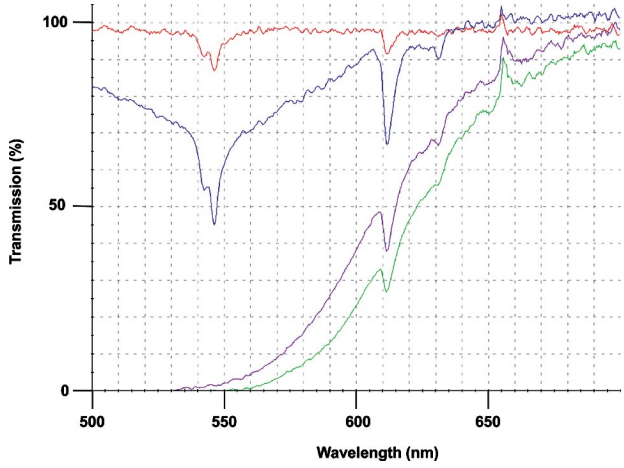
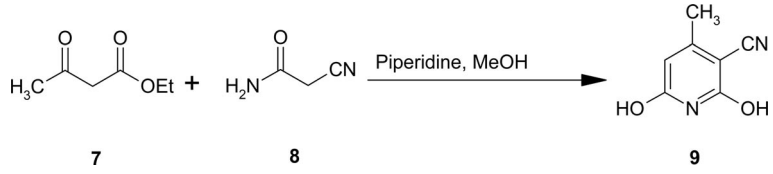


Figure 2. Transmission spectra of the reaction solution for the synthesis of **6**: 0 min (red), 10 min (blue), 20 min (violet) and 30 min (green).

Example 3

In Scheme 2, the preparation of compound **9** is shown. This substance is an intermediate in the synthesis of thienopyridine being allosteric modulators of the muscarinic re-



Scheme 2. Synthesis of **9**.

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ceptors.[9] Even though the same starting materials were used for the thermal-driven reaction (Figure 3), the yields and the reaction times ranged widely between 0.5 h/18%,[10a] 6 h/65%[10b] and 8 h/65%[10c] in the literature.

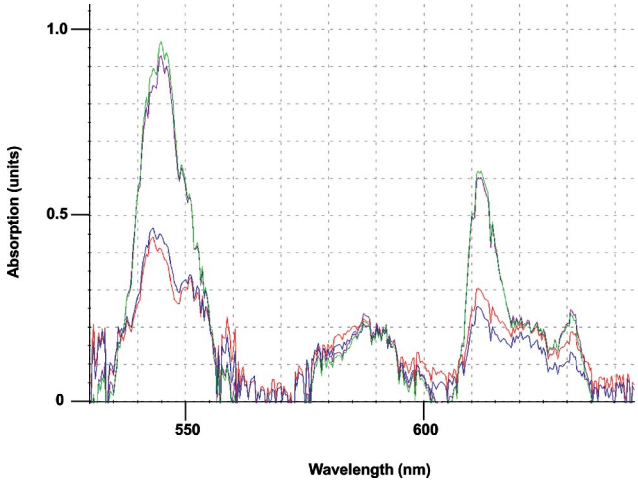


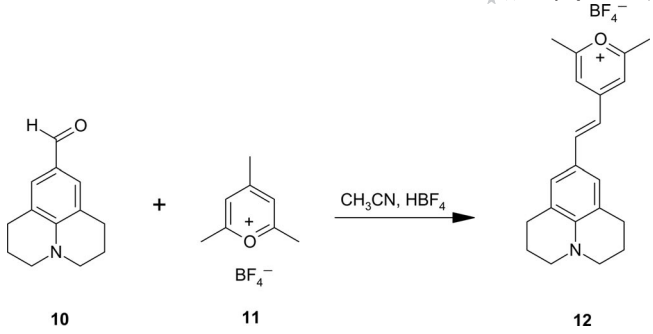
Figure 3. Absorption spectra of the reaction solution for the synthesis of **9**: 0 min (red), 3 min (blue), 5 min (violet) and 10 min (green).

For comparison, we carried out the reaction thermally in 3 d. In the microwave reactor, equipped with a pressure and a UV/Vis sensor, the reaction time was found to be 10 min only. Thereafter, the spectrum did not show any change at all.

Example 4

The pyrylium dye **12** obtained by conversion of the aldehyde **10** with the pyrylium salt **11** is used as a fluorescence marker for proteins.[11] In the classical way, **12** was synthesized according to ref.[12] in 10 min and 65% yield (see Scheme 3).

In the microwave reactor, the reaction was finished in 5 min (Figure 4). The red spectrum shows the absorbance



Scheme 3. Synthesis of **12**.

before the irradiation. The blue curve gives the situation after 3 min and the green one after 5 min of irradiation. There was no significant change of the spectrum after that time. We isolated the product with an 85% yield. This compound is very sensitive. Longer reaction times decreased the yield and made the workup procedure more complicated.

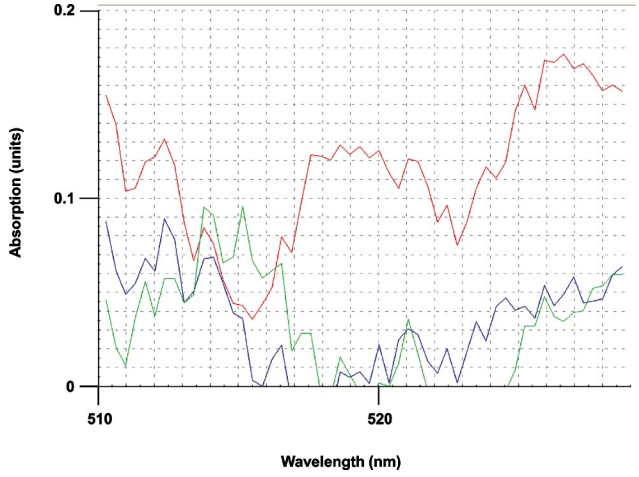
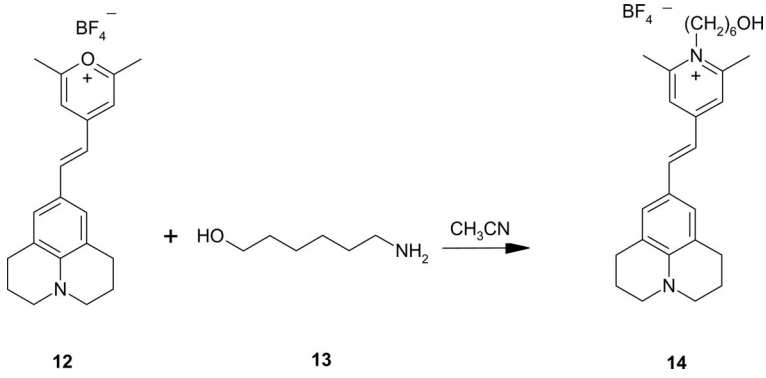


Figure 4. Absorption spectra of the reaction solution for the synthesis of **12**: 0 min (red), 3 min (blue) and 5 min (green).

Example 5

Compound **14** (Scheme 4) is used as a fluorescence marker for allosteric modulators of the muscarinic recep-



Scheme 4. Synthesis of **14**.

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tors as an alternative to radioactive labeling.[13] The reaction was carried out in the classical way, but the yields fluctuated strongly, and the workup was complicated (Figure 5). In the microwave reactor, the reaction was finished within 8 min with an 82% yield. The by-products could be easily removed by column chromatography.

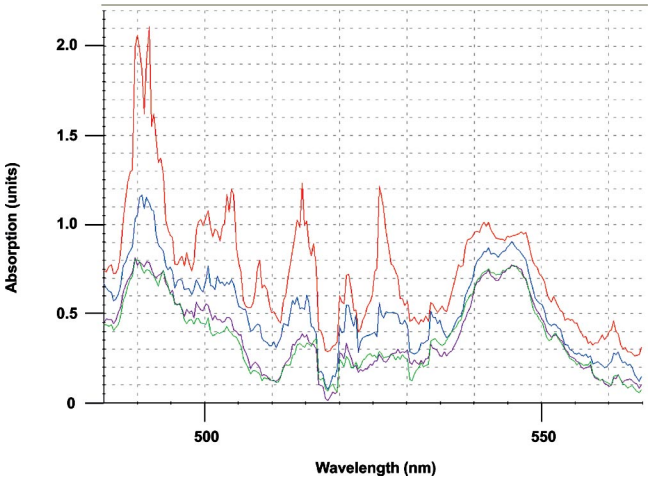

Figure 5. Absorption spectra for the reaction solution for the synthesis of **14**: 0 min (red), 3 min (blue), 5 min (violet) and 8 min (green).

Conclusions

The examples clearly demonstrate that monitoring of fast microwave-supported reactions is possible by online measurement of the UV/Vis spectra in solution. However, pretreatment of the reaction samples for NMR or HPLC measurements can take up to half an hour in addition of the measurement itself. TLC does not need a sample preparation in principle, but the development time is mostly at least 10 min. Thus, the UV/Vis method presented here is optimal for the identification of the endpoint of fast microwave-enhanced reactions. Overreactions occurring during NMR, HPLC or TLC monitoring can be avoided. It is also suitable for high-boiling solvents (e.g. DMF, DMSO), where the FTIR method fails, because it needs the complete removal of the solvent.[6] However, the starting materials and the products should not absorb near the maximum absorption of the solvent, and the products and the reactants must have different UV/Vis spectra in some areas. These requirements can be easily fulfilled.

Experimental Section

General Procedure for the Online Monitoring: The spectrum analyses were done with a modified UV/Vis spectrometer (Ocean Optics, Ostfildern, Germany) with a special cell for direct control (made by MLS GmbH) for quantitative control of the reactions. The spectrometer was connected to a PC with the Spectral Suite software. The UV/Vis spectra were measured continuously with 2 s integration time and saved either every 2 min or in shorter intervals depending on the length of the reaction time. As soon as the spectra do not change, the microwave irradiation was stopped and the workup of the reaction mixture started. An MLS Ethos 1600 microwave system (MLS GmbH, Leutkirch, Germany) was used, and the reactions were performed in an open flask/reaction vessel (Examples 1 and 2) and with a special quartz batch reactor (Examples 3–5).

General Procedure for the Syntheses of Fluorinated Fluoresceins: 20 mmol of the resorcin and 10 mmol of the phthalic anhydride were dissolved in 50 mL of methanesulfonic acid in a flask equipped with a temperature and a UV/Vis sensor and heated in the microwave oven (3 min to 120 °C). The reaction temperature was kept constant until no significant change of the UV/Vis spectrum was observed. The mixture was poured into 400 mL of ice/water and stirred for 1 h. The precipitate was filtered off, washed neutral and dried in vacuo. The residue was dissolved in acetic anhydride (50 mL) and pyridine (4 mL) and heated up to 65 °C within 2 min. The temperature was kept constant for 5 min, poured into 400 mL of ice/water and stirred for 30 min. The aqueous layer was extracted with chloroform (3 × 150 mL), and the combined organic layers were dried with Na_2SO_4. After evaporation, the residue was dissolved in 100 mL of methanol and 100 mL of THF; 25% ammonia (10 mL) was added, the solution was stirred for 2 h, and the mixture was poured into water (500 mL). The solution was acidified with 10% HCl until pH = 2 was reached. The residue was filtered, washed and dried in vacuo.

2,7-Difluorofluoresceine (3): Yield 91%.

3,4,5,6-Tetrafluorofluoresceine (6): Yield 93%.

Analytical data were in agreement with those of ref.[7]

3-Cyano-2,6-dihydroxy-4-methylpyridine (9): Cyanoacetamide **8** (59 mmol, 5.0 g) was dissolved in 50 mL of methanol. Ethyl acetacetate **7** (59 mmol, 7.5 mL) and piperidine (60 mmol, 6.0 mL) were added, and the mixture was heated (Methods A and B). The mixture was allowed to cool down to 25 °C, the solid was filtered off and then dissolved in hot water. Afterwards, the solution was acidified slowly until pH = 5. The white solid was filtered off again, washed with ice-cold water and dried in vacuo. Method A: 3 d of reflux, yield 4.57 g (52%). Method B: Microwave, 3 min to 100 °C, 10 min at 100 °C, yield 3.25 g (37%). Analytical data were in agreement with those of ref.[10]

2,6-Dimethyl-4-[(*E*)-2-(2,3,6,7-tetrahydro-1*H*,5*H*-pyrido[3,2,1-*ij*]-quinolin-9-yl)vinyl]pyranylium Tetrafluoroborate (12): Julolidin-9-carbaldehyde (**10**) (30 mmol, 6.04 g), 2,4,6-trimethylpyrylium tetrafluoroborate (33 mmol, 6.93 g) and 1 drop of HBF_4 were dissolved in 60 mL of acetonitrile and heated up in a microwave oven to 100 °C within 3 min. The temperature was kept constant at 100 °C. After 5 min, the reaction was finished, and the solvent was removed. The crude product was purified by column chromatography on silica gel. The by-products were eluted with $CHCl_3$/MeOH (25:1) and the main product with $CHCl_3$/MeOH (9:1). Yield 10.04 g (85%). Analytical data were in agreement with those of ref.[12]

(*E*)-1-(6-Hydroxyhexyl)-2,6-dimethyl-4-[2-(2,3,6,7-tetrahydro-1*H*,5*H*-benzo[*ij*]quinolizidin-9-yl)ethenyl]pyridinium Tetrafluoroborate (14): Compound **12** (10 mmol, 3.93 g) and the amino alcohol **13** (10 mmol, 1.17 g) were dissolved in 60 mL of acetonitrile and heated to 100 °C within 3 min. The temperature was kept constant at 100 °C, and the reaction was finished in 8 min. The solvent was evaporated, and the residue was purified by column chromatography on silica gel. The by-products were eluted with $CHCl_3$ and the main product with $CHCl_3$/MeOH (25:1). Yield: 4.05 g (82%) of a dark-red solid, m.p. 147 °C (dec.). R_f = 0.41 ($CHCl_3$/MeOH,

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25:1). ^{1}H NMR (400 MHz, CD_3CN): δ = 1.41–1.52 (m, 7 H, 3′-H, 4′-H, 5′-H, OH), 1.72–1.79 (m, 2 H, 2′-H), 1.92 (t, J = 6.0 Hz, 4 H, 2′′′-H, 6′′′-H), 2.68 (s, 6 H, 2 × CH_3), 2.72 (t, J = 6.0 Hz, 4 H, 1′′′-H, 7′′′-H), 3.52 (q, J = 5.0 Hz, 2 H, 6′-H), 4.21 (t, J = 8.5 Hz, 2 H, 1′-H), 6.77 (d, J = 16.0 Hz, 1 H, 2′′-H), 7.09 (s, 2 H, 8′′′-H, 10′′′-H), 7.53 (s, 2 H, 3-H, 5-H), 7.54 (d, J = 6.0 Hz, 1 H, 1′′-H) ppm. ^{13}C NMR (100 MHz, CD_3CN): δ = 21.3 (2 × CH_3), 22.2 (C-2′′′, C-6′′′), 26.0 (C-3′), 27.1 (C-4′), 28.1 (C-1′′′, C-7′′′), 29.3 (C-2′), 33.3 (C-5′), 50.6 (C-3′′′, C-5′′′), 62.4 (C-6′), 116.1 (C-2′′), 122.3 (C-7a′′′, 10a′′′), 122.6 (C-9′′′), 128.8 (C-8′′′, C-10′′′), 143.3 (C-1′′), 146.6 (10b′′′), 154.4 (C-2, C-6) ppm. FTIR (ATR): 3547 (OH), 2937 and 2858 (CH), 1557 and 1517 (C=C), 1407, 1356, 1287 1056. $C_{27}H_{37}BF_4N_2O$ (492): calcd. C 65.60, H 7.57, N 5.70; found C 65.58, H 7.65, N 5.59.

Supporting Information (see footnote on the first page of this article): General information and general procedure for the syntheses of the compounds by using a UV/Vis sensor, atom numbering of the ring system of (*E*)-1-(6-hydroxyhexyl)-2,6-dimethyl-4-[2-(2,3,6,7-tetrahydro-1*H*,5*H*-benzo[*ij*]quinolizidin-9-yl)ethenyl]-pyridinium tetrafluoroborate (**14**), images of the UV/Vis sensor for open-flask and QS-Batch.

Acknowledgments

This work was partially supported by the Deutsche Forschungsgemeinschaft (No. HO1368/12-1) and by Prof. M. J. Lohse of the Institute of Pharmacology and Toxicology, University of Würzburg.

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Received: April 1, 2010
Published Online: May 27, 2010

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