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Parallel Synthesis at its Best – Bioanalytics, Screening and Library Generation in SiC Microtiter Plates

The use of silicon carbide materials has revolutionized microwave-assisted synthesis. Since silicon carbide shows extremely fast heating rates under microwave irradiation, distributes heat very quickly and is chemically inert, lots of microwave reactor accessories have been made out of silicon carbide. Besides passive heating elements and even a reaction vessel, microtiter plates are very successfully employed in industrial research.

1 Introduction

Nowadays, the term “time equals money” becomes more and more important, and it is certainly valid for any industrial branch. Consequently, all processes have to be perfectly optimized, while the potential for further optimization is constantly evaluated.

In the field of chemical synthesis, an enormous increase of efficiency can be achieved by using dedicated microwave equipment. Reaction times can be significantly reduced while product purities and yields are improved. Nevertheless, microwave synthesis still bears potential for further improvement of economic efficiency.

This time-saving improvement can be achieved either by using sequential automation, or by performing several chemical reactions in parallel. In fact, sequential processing (even if automated) becomes impractical when a large number of optimization experiments need to be performed. Consequently, applications like library generation, reaction screening or biomedical and bioanalytical (forensic) investigations can be significantly enhanced by performing synthesis of more than 100 reactions in parallel.

This report highlights the use of silicon carbide microtiter plates, assisting the significant enhancement of different applications – ranging from reaction optimization over library generation (both often used in industrial drug discovery processes) to forensic investigations.

2 Equipment

The microwave synthesis reactor platform Multiwave PRO can be equipped with microtiter rotor types employing plates made out of silicon carbide (SiC). This material bears several advantages for microwave chemistry, since it can not only be efficiently heated with microwave irradiation, but also distributes the heat very quickly.

This allows filling completely different mixtures in each well of the plates while still maintaining utmost temperature homogeneity across the whole plate. Furthermore, SiC is chemically inert and resistant to corrosive chemistry.

Silicon carbide plates are available in several formats with different matrices and specifications, serving individual needs of industrial research (see Figure 1 and Table 1). They are heated in the multimode microwave platform Multiwave PRO (Figure 2).

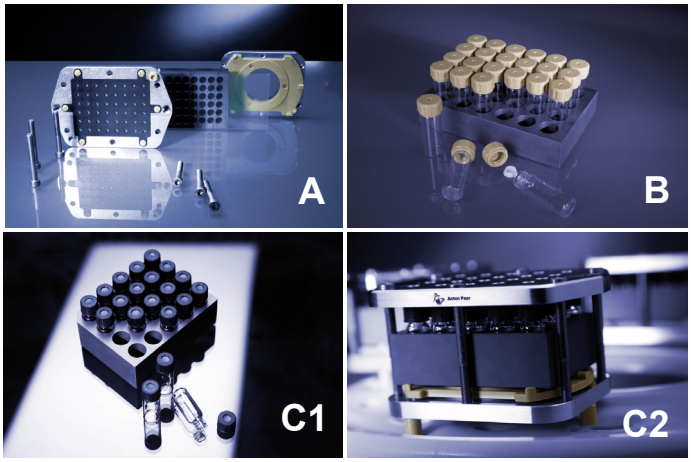


Fig. 1 Different SiC well plate formats (refer to Table 1 for specifications)

Table 1: Specifications of different well plate formats shown in Figure 1 (the maximum temperature is 200 °C for all plates)

Plate Type	Plate Matrix	Filling Volume per Well	max. Pressure
A	6 x 8	0.02 - 0.3 mL	20 bar
B	6 x 4	0.3 - 3 mL	20 bar
C1	5 x 4	0.1 - 1.5 mL	8 bar
C2	5 x 4	0.1 - 1.5 mL	20 bar

Fig. 2 Multiwave PRO with Rotor 4x24 equipped with 4 well plates of type B (Figure 1, type B)

All herein presented applications have been performed in respective silicon carbide microtiter plates (Figures 1 and 2) in Synthos 3000, the predecessor of Multiwave PRO. Using similar accessories, the methods are in general adaptable, however, applying power-controlled Synthos 3000 protocols in Multiwave PRO may require slight adaptations of the microwave power output.

3 Reaction Optimization and Screening

Microwave-assisted optimizations have usually been done in a sequential way, since using differently absorbing reaction mixtures in parallel has a severe impact on the heat distribution. In contrast, with SiC microtiter plates the reaction mixtures can be completely individual, since due to the extremely fast heat distribution within a SiC material, the plates can be heated uniformly and homogeneously regardless of the well contents (Figure 3).

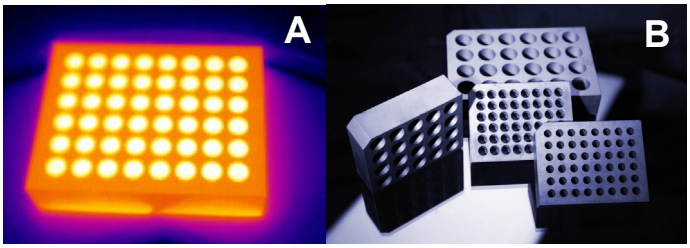
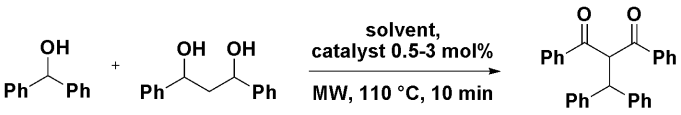


Fig. 3 (A) IR picture of microwave-heated SiC plate type A (Table 1), showing the uniform heat distribution. (B) Available SiC microtiter plates. All show similar, homogeneous heating under microwave irradiation.

In Figure 4 a catalyst and solvent screening for a C-C coupling reaction of diphenylmethanol and dibenyozlmethane is shown. Employing the plates of type C2 (Figure 1), 5 different solvents and four metal catalysts were evaluated in parallel to determine the most efficient combination.



solvent

catalyst	DCM	$MeNO_2$	MeCN	THF	toluene
$Sc(OTf)_3$	95	98	82	84	94
$Cu(OTf)_2$	94	98	71	94	93
$Zn(OTf)_2$	53	97	89	37	25
$FeCl_3$	97	95	82	30	69

solvent/catalyst

catalyst-concentration	$MeNO_2$		DCM		
	$Sc(OTf)_3$	$FeCl_3$	$Sc(OTf)_3$	$FeCl_3$	$Cu(OTf)_2$
3 mol%	96	92	99	99	97
2 mol%	95	82	96	95	96
1 mol%	89	29	90	83	91
0.5 mol%	82	26	92	51	92

Fig. 4 Microwave-assisted C-C coupling with respective yields, depending on the conditions.

The tables in Scheme 1 show the crude product purities, and it can be seen at the first glance that this C-C coupling is best performed in DCM with 3 mol% of $FeCl_3$ as a catalyst.

Using the parallel SiC plate equipment, all 40 reactions for the optimization can be performed simultaneously, which takes approximately 20 min reaction time in total in order to find out the optimum conditions for the respective C-C coupling reaction. Performing the reactions sequentially, it would take 13.5 hours to finish the same optimization studies!

4 Synthesis of Compound Libraries

Besides reaction screening, one of the most obvious applications of SiC microtiter platforms is the generation of compound libraries. Once a reaction has been optimized, the scope of the reaction can be evaluated by using different derivatives of starting materials and reagents in order to generate diverse compound libraries.

As an example for library generation, Figure 5 shows the Hantzsch synthesis of different dihydropyridines, where six aldehyde substrates and four β-dicarbonyl compounds were used. Aqueous ammonium hydroxide was employed both as the ammonia source and as the solvent for the reaction, and the reaction mixtures were heated for 10 min at 140 °C. The results varied considerably for this library, the best conversions were obtained with ethyl acetoacetate as the dicarbonyl component. Of the aldehydes screened, pyridine-2-carboxaldehyde and 3,4-methylenedioxybenz-aldehyde gave the best results.

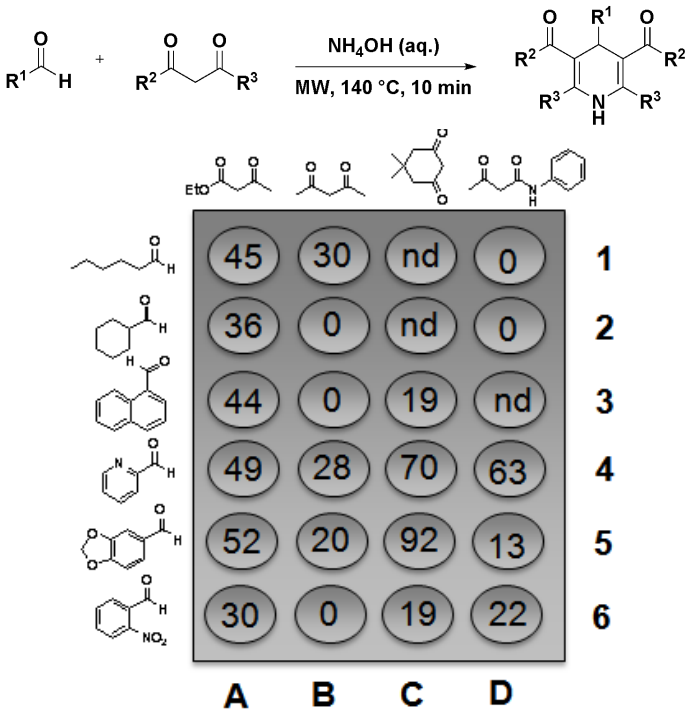


Fig. 5 Synthesis of dihydropyridines using SiC plate system B (Figure 1). The number in the wells corresponds to the product conversions in the Hantzsch syntheses.

The application of sealed SiC microtiter plates is highly advantageous for library generation, since up to 192 different products (depending on the plate type, Figure 1) can be synthesized in parallel.

5 Forensic Applications

For bioanalytic applications the plates of type C (C1 and C2, Figure 1) are the most widely used plate formats because of the unique possibility to use standard HPLC/GC autosampler vials as microwave reaction containers. Consequently, the big issue of losing important material when transferring the reaction mixture from the reaction vial to the analysis vial is solved. Typical bioanalytic applications are derivatization reactions, which make it possible to quantify e.g. drugs in human blood.

As an example, the derivatization for the analysis of heroine is shown in Figure 6. Heroine is metabolized to 6-monoacetylmorphine (6-MAM), which cannot be directly analyzed. Therefore, a derivatization (pentafluoro-propionylation, PFP) to 3-PFP-6-MAM is usually performed.

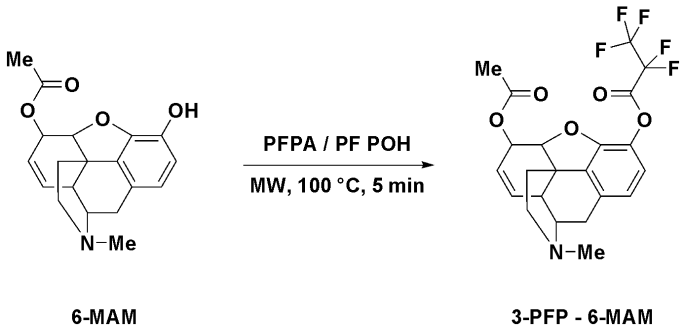


Fig. 6 Derivatization reaction for the analysis of heroine in human blood.

With this example (among others) it has been shown, that with Multiwave PRO and its special SiC plates format (plate C1 and C2 , Figure 1), rapid, microwave-assisted derivatizations be easily and conveniently performed in HPLC/GC vials. This allows for analysis without transferring the reaction mixture to another vial. Furthermore, additional increase of efficiency is provided due to the possibility of analyzing up to 80 blood samples in parallel.

6 Conclusion

Silicon Carbide microtiter plates have proven to be very valuable tools for high-throughput experimentation, combining the benefits of both high-speed sealed vessel microwave chemistry and parallel processing. Different modifications allow their use not only for common library synthesis, but also for efficient reaction optimization/ screening and in a variety of (bio)analytical applications.

Reaction volumes can be as small as 50 µL and can reach up to 3 mL at a maximum temperature/pressure limit of 200 °C/20 bar. Besides the possibility to reach such high pressure/temperature conditions, SiC plates can hold standard analysis autosampler vials which allow performing reactions as well as analytics in the same vial. Last but not least, a big advantage of SiC plates is that completely diverse reaction mixtures utilizing different solvents can be used in one and the same microwave experiment, since the heating efficiency of SiC under microwave irradiation is very high per se and therefore independent of the used solvents.

7 Outlook

It can be expected that these high-throughput plates will be used for many different applications in the future, whenever operating at high-temperature / high-pressure conditions and under accurate temperature control is necessary, and miniaturization and parallelization is important. Compared to the use of common automated sequential processing techniques in monomode reactors, like Monowave 300, the use of the SiC microtiter plates allows a significant saving of time and efficiency.

8 References

This document is based on the following review:

„Parallel Microwave Chemistry in Silicon Carbide Microtiter Platforms: A Review“

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