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Microwave Assisted Organic Synthesis: An Alternative Synthetic Strategy

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Abstract

Pharmaceutical companies have made major investments in high- throughput technologies for genomic and proteomic research, combinatorial chemistry and biological screening in order to identify more potential drug candidates at a faster pace. However, synthesis and lead compound optimisation remain the bottlenecks in the drug discovery process. Developing chemical compounds with the desired biological properties is time-consuming and expensive. Consequently, increasing interest is being directed towards technologies that allow more rapid synthesis and screening of chemical substances to identify compounds with functional qualities. Microwave heating is a process within a family of electroheat techniques, such as induction, radio frequency, direct resistance or infra-red heating, all of which utilise specific parts of the electromagnetic spectrum. These processes supplement, and in specific cases totally replace, conventional heating or drying systems used in industry. There is hardly any reaction type or name reaction that has not yet been tested in the microwave field. This is because some conventional systems are very bulky, not easy to operate, can pollute the environment due to harmful omissions and above all can be very inefficient. The major advantages of using microwaves are rapid heat transfer, volumetric and selective heating, compactness of equipment, speed of switching on and off and pollution-free environment as there are no products of combustion. Microwave leakage can certainly be kept well below government recommended levels. It has long been established that a dielectric material can be processed with energy in the form of high-frequency electromagnetic waves. The present review article describes the mechanism of microwave heating and comparison of the conventional and microwave assisted organic synthesis to improve the synthesis of the organic molecules.

Key Words: Microwave Synthesis, Multimode reactors, Dielectric loss, Ionic conduction, Dipolar polarization.

Introduction

Microwave technology has been used in chemistry since the late 1970s, but it has only been implemented in organic synthesis since the mid 1980s. The slow uptake of the technology has been attributed to its initial lack of controllability and reproducibility, coupled with a general lack of understanding of the basics of microwave dielectric heating. However, in the past few years, heating chemical reactions by microwave energy has been an increasingly popular theme in the scientific community. Since the first published reports on the use of microwave irradiation to carry out organic chemical transformations by the groups of Gedye and Giguere/Majetich in 1986 [1,2], more than 3500 articles have been published in this fast moving and exciting field, today generally referred to as microwave-assisted organic synthesis (MAOS) .

Microwave dielectric heating drives chemical reactions by taking advantage of the ability of some liquids and solids to transform electromagnetic radiation into heat. A properly designed vessel allows the temperature increase to be uniform throughout the sample, leading to fewer by-products and/or product decomposition. It dramatically reduces reaction time, typically from days or hours to minutes or even seconds, increases product yields and enhances product purity. Arguably, the breakthrough in the field of MAOS on its way from laboratory curiosity to standard practice started in the pharmaceutical industry around the year 2000. Medicinal chemists were among the first to fully realize the true power of this enabling technology. The advantages of this technology have also been exploited in the context of multistep total synthesis and drug discovery, and have additionally penetrated related fields such as polymer synthesis, material sciences, nanotechnology and biochemical processes. Many of the top pharmaceutical, agro- chemical and biotechnology companies were already using MAOS as a forefront methodology for library synthesis and lead optimization as they realize the ability of this enabling technology to speed chemical reactions. The use of microwave irradiation in chemistry has thus become such a popular technique that it might be assumed that, in a few years, most chemists will probably use microwave energy to heat chemical reactions on a laboratory scale. The statement that, in principle, any chemical reaction that requires heat can be performed under microwave conditions has today been generally accepted as a fact by the scientific community [3].

Conventional heating *Vs* microwave assisted heating

Heating reactions with traditional equipment, such as oil baths, sand baths and heating mantles are not only slow but also creates a hot surface on the reaction vessel where products, substrates and reagents often decompose over time. It depends on the thermal conductivity of the various materials that must be penetrated, and results in the temperature of the reaction vessel being higher than that of the reaction mixture. Microwave energy, in contrast, is introduced into the chemical reactor remotely and passes through the walls of the reaction vessel heating the reactants and solvents directly. Consequently, the tendency for the initiation of boiling is reduced, and superheating above the boiling point of the solvent is possible even at atmospheric pressure [4]. Superheating can be generated rapidly in closed vessels typically made out of (nearly) microwave-transparent materials, such as borosilicate glass, quartz, or Teflon, to temperatures as high as 100°C above the normal boiling point of a particular solvent. For every 10°C increase in temperature, the rate of the reaction is approximately doubled. The very

efficient internal heat transfer results in minimized wall effects (no hot vessel surface) which may lead to the observation of so-called specific microwave effects. It is possible, however, that macroscopic or microscopic hotspots resulting from selective heating of specific reagents or catalysts can develop, leading to even faster conversions and the realization of chemistries that cannot be conducted by conventional heating.

Table 1: Comparison of microwave assisted heating and conventional heating.

Sr. No.	Conventional heating	Microwave assisted heating
1	Reaction mixture heating proceeds from an inside surface of reaction vessels.	Reaction mixture heating proceeds directly inside mixture.
2	The vessel should be in physical contact with surface of a higher temperature source (e.g. electric plate heater, oil bath, heating mentle, steam bath, sand bath etc.)	Vessel is kept in microwave cavities.
3	Heating is achieved using both thermal and electric source.	Heating takes place by electromagnetic waves.
4	Heating mechanism involves conduction.	Heating mechanism involves dielectric polarization (dipolar polarization) and conduction.
5	Transfer of energy occurs from the wall, surface of vessel, to the mixture and eventually to the reacting species.	The core mixture is heated directly while surface (vessel wall) is a source of loss of heat.
6	The highest temperature (for an open vessel) that can be achieved is limited by boiling point of particular mixture or solvent.	The temperature of mixture can be raised above its boiling point.
7	Heating is done from out side; therefore the core of the solvent may be as much as 5°C cooler than at the edges.	The core is 5°C hotter than the outside, because of surface cooling. Therefore in microwave heating, we can raise the boiling point of solvent by as much as 5°C, an effect known as super heating.
8	There may be more by-products & more chances of decomposition of products, substrates and reagents	A properly designed vessel allows the temperature increase to be uniform throughout the sample, leading to fewer by-products and/or product decomposition.
9	The heating procedure is controlled to a lesser extent.	The heating procedure is highly controlled since the energy input starts and stops immediately when the power is turned on or off, respectively.
10	There may be unwanted side reactions, so final product is less	Reduction in unwanted side reactions. (Reaction Quenching), so purity of the final

	pure.	product is high.
11	Environmental heat loss is more.	Environmental heat loss is saved.
12	Heating rate is less.	Heating rate is several folds high. Reactions which require many hours or even days to complete, have been accomplished in a minutes.
13	Average reaction time using conventional heating is 6 hours.	Average reaction time using microwave heating is 15 minutes.
14	Less efficiency of heating since all the compounds in a mixture is heated equally. (Lesser selective heating)	High efficiency of heating since specific component can be heated specifically. (More selective heating)
15	Large amount of solvents are used which are hazardous and carcinogenic.	Microwave heating is also known as "GREEN SYNTHESIS" because: (i) Minimal amount of solvent is utilized. (ii) Use of water (supercritical water) in organic reaction, instead of organic solvent, as water in microwave acts as an excellent solvent.
16	Low investment costs.	High investment costs.

Microwave theory

Microwave irradiation is electromagnetic irradiation in the frequency range of 0.3 to 300 GHz as shown in Fig. 1. All domestic "kitchen" microwave ovens and all dedicated microwave reactors for chemical synthesis operate at a frequency of 2.45 GHz (which corresponds to a wavelength of 12.24 cm) to avoid interference with telecommunication and cellular phone frequencies. The energy of the microwave photon in this frequency region (0.0016 eV) is too low to break chemical bonds and is also lower than the energy of Brownian motion. It is therefore clear that microwaves cannot induce chemical reactions.

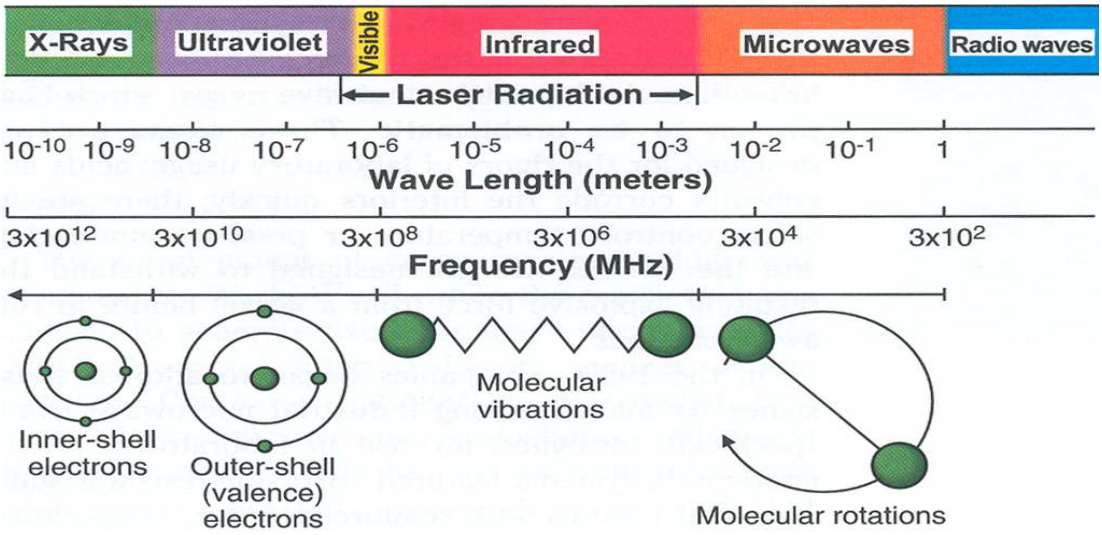
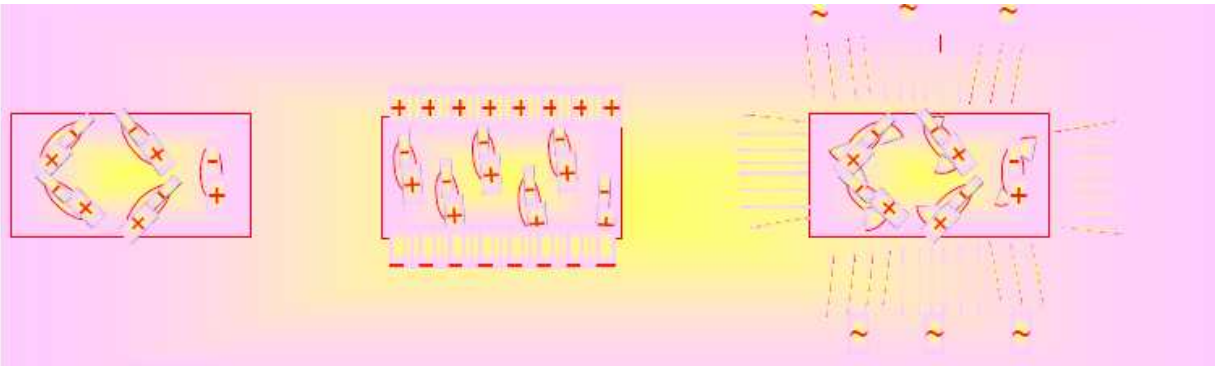


Fig. 1 Electromagnetic Spectrum

Microwave-enhanced chemistry is based on the efficient heating of materials by "microwave dielectric heating" effects. This phenomenon is dependent on the ability of a specific material (solvent or reagent) to absorb microwave energy and convert it into heat. The electric component of an electromagnetic field causes heating by two main mechanisms: dipolar polarization and ionic conduction as shown in Fig. 2. Irradiation of the sample at microwave frequencies results in the dipoles or ions aligning in the applied electric field.



No constraint *Continuous electric field* *Alternating electric field With high frequency*

Fig. 2 Microwave Heating Mechanism

As the applied field oscillates, the dipole or ion field attempts to realign itself with the alternating electric field and, in the process, energy is lost in the form of heat through molecular friction and dielectric loss. The amount of heat generated by this process is directly related to the ability of the matrix to align itself with the frequency of the applied field. If the dipole does not have enough time to realign, or reorients too quickly with the applied field, no heating occurs. The allocated frequency of 2.45 GHz used in all commercial systems lies between these two extremes and gives the molecular dipole sufficient time to align in the field, but not to follow the alternating field precisely [5].

The second major heating mechanism is the ionic conduction mechanism. During ionic conduction, as the dissolved charged particles in a sample (usually ions) oscillate back and forth under the influence of the microwave field, they collide with their neighboring molecules or atoms. These collisions cause agitation or motion, creating heat. Thus, if two samples containing equal amounts of distilled water and tap water, respectively, are heated by microwave irradiation at fixed radiation power, more rapid heating will occur for the tap water sample due to its ionic content. The conductivity principle is a much stronger effect than the dipolar rotation mechanism with regard to the heat-generating capacity. This capacity of a particular material (for example, a solvent) under microwave irradiation conditions is dependent on its dielectric properties. The ability of a specific substance to convert electromagnetic energy into heat at a given frequency and temperature is determined by the so-called loss factor tanδ. This loss factor is expressed as the quotient $\tan\delta=\varepsilon''/\varepsilon'$, where ε'' is the dielectric loss, which is indicative of the efficiency with which electromagnetic radiation is converted into heat, and ε' is the dielectric constant describing the ability of molecules to be polarized by the electric field.

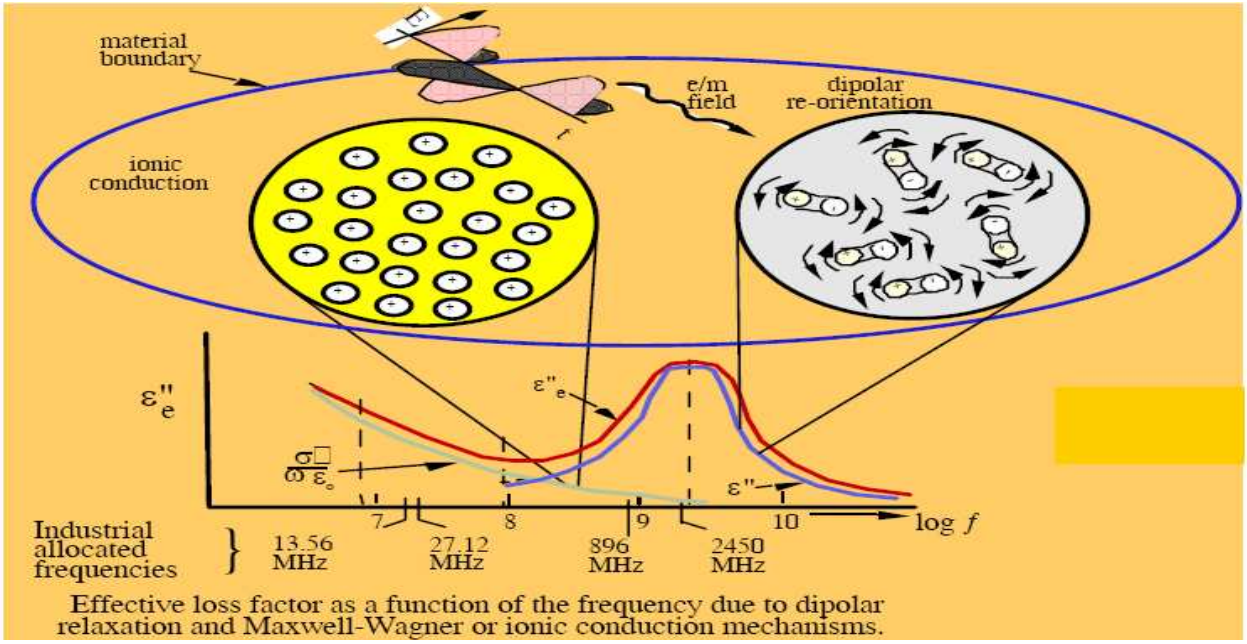


Fig. 3

The two components of the complex relative permittivity shown plotted as a function of the frequency in Fig. 3 **[6]**, for a dipolar liquid or for a wet dielectric, where the losses at microwave frequencies are due to re-orientation polarization. The conductivity effects of ionic species, shown by the light blue response, dominate at radio frequencies, while the combined loss is shown by the red response.

A reaction medium with a high tanδ value is required for efficient absorption and, consequently, for rapid heating. The loss tangents for some common organic solvents are summarized in Table-2.

Table:2 Loss tangents (tan δ) of various solvent (2.45 GHz 20^0 C)

Solvent	tan δ	Solvent	tan δ
Ethylene glycol	1.350	N,N-dimethylformamide	0.161
Ethanol	0.941	1,2-dichloroethane	0.127
Dimethyl sulfoxide	0.825	Water	0.123
2-propanol	0.799	Chlorobenzene	0.101
Formic acid	0.722	Chloroform	0.091
Methanol	0.659	Acetonitrile	0.062
Nitrobenzene	0.589	Ethyl acetate	0.059
1-butanol	0.571	Acetone	0.054
2-butanol	0.447	Tetradydrofuran	0.047
1,2-dichlorobenzene	0.280	Dichloromethane	0.042
1-methyl-2-pyrolidone	0.275	Toluene	0.040
Acetic acid	0.174	Hexane	0.020

In general, solvents can be classified as high (tanδ>0.5), medium (tanδ 0.1–0.5), and low microwave absorbing (tanδ<0.1). Ethylene glycol, ethanol, DMSO, methanol are the solvents having high value of loss factor.

Other common solvents without a permanent dipole moment such as carbon tetrachloride, benzene, and dioxane are more or less microwave transparent. A low tanδ value does not preclude a particular solvent from being used in a microwave-heated reaction. Since either the substrates or some of the reagents/catalysts are likely to be polar, the overall dielectric properties of the reaction medium will in most cases allow sufficient heating by microwaves. Furthermore, polar additives such as ionic liquids, for example, can be added to low-absorbing reaction mixtures to increase the absorbance level of the medium.

Equipment

The early pioneering experiments for MAOS were performed in domestic, sometimes modified, kitchen microwave ovens. In this type of microwave oven, the irradiation power is generally controlled by on/off cycles of the magnetron (pulsed irradiation), and it is typically not possible to monitor the reaction temperature in a reliable way. It has received criticism owing to reports of low reproducibility, uncontrolled heating and an inability to stir reactions during irradiation resulting in splashing of the chemicals. Also, the use of open beakers covered with a watch glass in domestic ovens has been considered hazardous. In contrast, the current trend is to use dedicated instruments which have only become available in the last few years for chemical synthesis. Most of today's commercially available dedicated microwave reactors for synthesis feature built-in magnetic stirrers, direct temperature control of the reaction mixture with the aid of fiber-optic probes or IR sensors, and software that enables on-line temperature/pressure control by regulation of microwave power output.

Since 2003, suppliers of microwave instrumentation for organic synthesis have also moved towards combinatorial/ high-throughput platforms, addressing the needs of the drug discovery industry.

Currently, two different philosophies with respect to microwave reactor design are emerging: multimode and monomode reactors [7-8]. In the so-called multimode instruments, the microwaves that enter the cavity are being reflected by the walls and load typically over the large cavity. A mode stirrer ensures that the field distribution is as homogeneous as possible. Industrially designed microwave systems typically resemble domestic microwave ovens with multimode cavities. These have seen successful, but limited, use in medicinal chemistry applications. Multimode cavities propagate a microwave field with multiple modes of energy, each with varying intensity; this causes a problem with positional sensitivity – more commonly known 'hot spots'.

In the much smaller monomode or single-mode cavities, only one mode is present and the electromagnetic irradiation is focused directly through an accurately designed wave guide onto the reaction vessel mounted in a fixed distance from the radiation source. Recently, chemists began using single-mode cavities to great benefit in synthetic chemistry, due to the higher degree of field uniformity and repeatability that they offer.

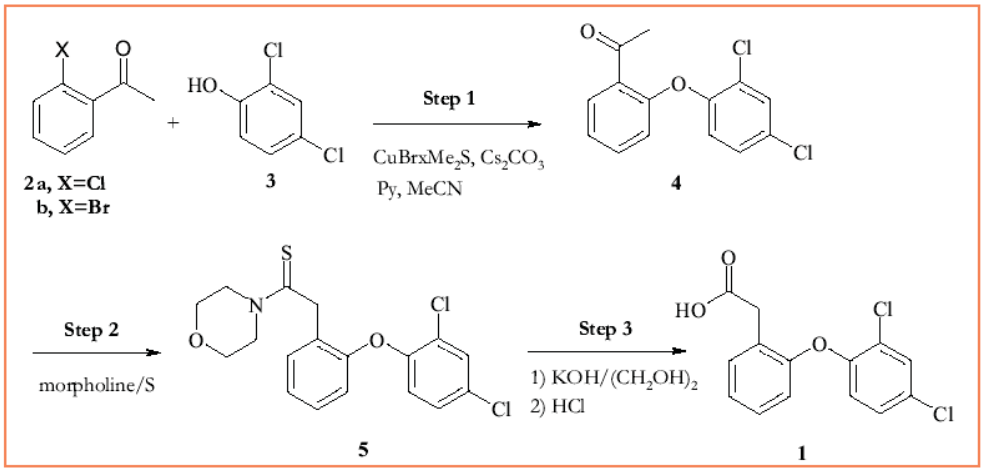
For high-throughput applications, the key difference between the two types of reactor systems is that, in multimode cavities several reaction vessels can be irradiated simultaneously in multi- vessel rotors (parallel synthesis), where as in monomode systems only one vessel can be irradiated at a time. In the latter case, high throughput can be achieved by integrated robotics that move individual reaction vessels in and out of the microwave cavity (Automated sequential synthesis).

Illustrated Example

The bottleneck of parallel synthesis is typically optimization of reaction conditions to afford the desired products in suitable yields and purities. Since many reaction sequences require a heating step for extended time periods, these optimizations are often difficult and time-consuming.

Microwave-assisted heating has been shown to be an invaluable optimization method since it reduces reaction times dramatically, typically from days or hours to minutes or seconds. Many reaction parameters can be evaluated in a few days to optimize the desired chemistry. Compound libraries can then be synthesized rapidly using the new technology, either in a parallel or sequential mode.

The rapid synthesis of the non-steroidal anti-inflammatory agent Fenclofenac (2-(2,4-dichlorophenoxy)phenyl acetic acid) under microwave irradiation is reported (Scheme 1). Fenclofenac is a non-steroidal anti-inflammatory drug with analgesic and anti-inflammatory activity. Conventionally, the synthesis of Fenclofenac has been undertaken in 3 steps from 2'-chloroacetophenone. However, this conventional approach requires a total reaction time of 5 days and the final yield is relatively low (31%).



Scheme 1 Synthesis of Fenclofenac

The key step in the synthesis of Fenclofenac (1) is the step-1, Ullmann ether coupling of halide (2) with 2,4-dichlorophenol (3) in the presence of $CuBrxSMe_2$ complex, a base and a small amount of pyridine. In literature it was reported that Cs_2CO_3 is superior to K_2CO_3 or NaOH as a base resulting in higher yields. The reaction temperature is also important. When the reaction temperature was increased to 180–190°C, debromination of 2'-bromoacetophenone occurred (2b)

resulting in decreased yields. Lower temperatures (100–120°C) were not sufficient for quantitative conversion. Optimization of the reaction parameters led to synthesis of diaryl ether (4) in a very good yield.

The second step in the synthesis of Fenclofenac (1) involved carbonyl transformation (the Willgerodt-Kindler reaction) in the presence of sulphur and a secondary amine (morpholine) leading to the terminal thioamide (5). It was also reported that initial experiments under microwave conditions resulted in low yields of product and some unidentified by-products, probably caused by the large excess of morpholine. When DMF was used as a solvent instead of morpholine, there was no significant increase in the yields. It became apparent that the concentrations of the reaction components are crucial for the Willgerodt reaction. When the concentration of the starting material (4) was increased to 0.20 mmol/mL a 74% HPLC purity of thioamide (5) was achieved.

The final step in the synthesis of Fenclofenac (1) was hydrolysis of thiomorpholide (5), a reaction that conventionally requires prolonged reaction times of up to 72 hours. Although microwave irradiation with 20 eq of KOH in methanol at 160°C allowed a significant reduction in the reaction time (from 72 h to 1.5 h), conditions for quantitative hydrolysis were inconvenient from a practical point of view (long reaction time andvery high pressure, ~17 bars). Changing the solvent to ethylene glycol, however, allowed satisfactory conversion of thioamide (5) to Fenclofenac (1) with an overall yield of 52% from 2'-bromoacetophenone (2b) (31% with conventional heating).

Table:3 Results of Fenclofenac synthesis by Microwave irradiation and Conventional heating.

		Microwave Synthesis	Conventional
Step 1	Temp (°C)	150	125
	Time	30 min.	24 h
	Yield (%)	88	45
Step 2	Temp (°C)	150	Reflux
	Time	30 min.	24 h
	Yield (%)	66	75
Step 3	Temp (°C)	200	Reflux
	Time	30 min.	72 h
	Yield (%)	89	93
Overall Yield (%)		52	31

This report summarizes a convenient approach to the synthesis of Fenclofenac. The reaction time has been decreased from 5 days to 1.5 h with an improvement in total yield from 31% to 52%. The user-friendly software of Microwave Synthesis in combination with highly automated dispensing and heating devices allowed the speedy optimization of the reactions. Parameters such as solvent, catalyst, concentration and reagent stochiometry turned out to have a great influence on the outcome of the synthesis of Fenclofenac. [9]

There are several examples of the reactions taking place efficiently by microwave irradiation including *N*-acylation reactions like Alkyl/aryl coupling, Condensation, (De)protection, Heterocycles synthesis, Organocatalysis, Oxidation, Reduction, Radical reactions as well as Alkylation reactions like Nucleophilic substitution, Cycloaddition, (Trans)esterification, Organometallic reactions, Metathesis, Rearrangement, Olefination reactions.

Application

Chemistry applications have ranged from conventional solution phase synthesis to protocols involving polymer-supported reagents or scavengers, in addition to solid or flavorous phase techniques [10-11].

With the most recent advances in reactor technologies such as continuous-flow microwave systems, even process chemists are now taking MAOS. Most recently, microwaves have also been used to speed up biochemical processes such as polymerase chain reaction or enzyme-mediated protein mapping. The full scope and potential of this technology may not yet have been realized.

Future Trends

A common requirement associated with the introduction of a new technology is the possibility to scale-up the respective processes, first to a pilot plant-scale and eventually to the production scale. The aim of using microwave processing is to accelerate reactions in order to avoid disadvantageous reaction parameters (*i.e.* long reaction times, secondary reaction time, solvent use, excess components *etc.*). A further goal for process improvement is to transfer a batch operation to a continuous operation after they have undergone a process analysis. For this purpose, usually the first step is to repeat the known reaction conditions used in the conventional reactions in the microwave field.

Table: 4 Comparison of the currently available microwave systems for synthetic applications

Manufacturer MLS/Milestoned	Sharp	Personal Chemistry	CEM	MLS/Milestoned
Type	domestic MW oven R-220A	Emrys™ Creator	Discovery™	ETHOS™ MR
Irradiation modus	Multimode	monomode	monomode	Multimode
Max. power	800 W, pulsed	300 W, unpulsed	300 W, unpulsed	1000 W, pulsed or unpulsed
Cavity volume	15.7 L	< 1 L	< 1 L	42.8 L
Maximum power density in empty cavity	around 50 W L21	> 300 W L21	> 300 W L21	around 23 W L21
Reaction scale	max. 100 g in dry reactions	< 20 g	< 50 g	up to 3000 g depending on reactor

Some other modified or unmodified domestic microwave ovens are used for chemical reactions, e.g. Panasonic NN-S740WA-1200 W, Anton Paar Synthos 3000, Biotage.

Often a similar experimental setup is used (reflux apparatus). Starting from this point, all conventional reaction conditions must be re-evaluated. The introduction of a new technology in organic technical synthesis allows for the questioning of old preparation protocols. It would be advantageous to produce a check list for each reaction that critically questions the known synthetic protocols, analyses them, and provides potential new solutions [12].

The development of microwave systems for further applications in organic chemistry is going on in several directions: one trend is the development of small devices or devices that are tailored to a special application. The small devices (Table 4) allow for the reaction of millimolar (mmol) amounts in a short time (several minutes) with comparatively high power input [13,14]. These devices possess a small microwave cavity (51 L) and have a reactor installed directly in the waveguide; often only small and closed vessels similar to GC-vials can be used.

CEM also produces a wide range of multimode devices, which are mainly used for sample preparation (digestion, drying, ashing) and sometimes also for carrying out organic syntheses. However, according to the previous explanations, the EMRYS and the Discovery systems do not represent real monomode systems, but rather are multimode systems with a high power density.
The second development direction is the design of different reactor kits that can be integrated into the same basic system (ETHOS system, MLS/Milestone). This system allows for the realisation of a concept for the comparative transition of classical thermal reactions into the microwave field as described in Fig. 4.

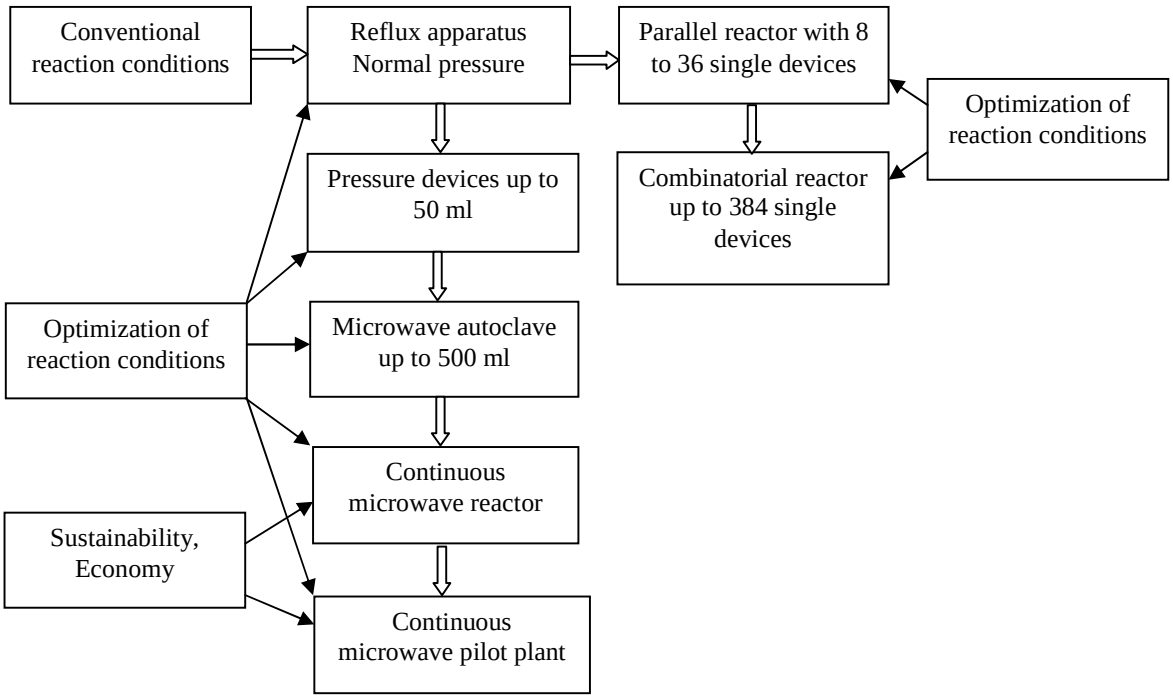


Fig. 4. Development concept for microwave assisted chemical reactions and processes.

The use of different reactors for different requirements and applications allows for a flexible reaction engineering in which reaction parameters can be precisely documented. Through the exact reproduction of conventional conditions, it is possible to simultaneously compare classical and microwave-assisted reactions. With this construction kit, reactions from the mmol-scale to the mol-scale can be performed. Furthermore, the transition from a batch operation to a continuous operation is also imaginable. This has already been described for some reaction types. Derived from the basic model, a robust beginner system (PRAKTIKA, MLS GmbH) is now available with simple measurement technology that allows for easy integration of microwave assisted reactions into laboratory classes. Further, a pilot plant device was derived from the base mode in which first studies on the real scale-up were performed. The ETHOS 4000/4001 devices can already process reaction mixtures of 5–10 kg per hour and are thus already suitable for the production of high-priced fine chemicals like pharmaceuticals [12].
The goal of all these investigations was to obtain a holistic view and to question all reaction parameters employed so far in order to discover new unconventional ways for carrying out long-known reactions.

References

[1] R. Gedye, F. Smith, K. Westaway, H. Ali, L. Baldisera, L. Laberge, J. Rousell, *Tetrahedron Letters*., **1986**, 27, 279–282.
[2] R.J. Giguere, T.L. Bray, S.M. Duncan, G. Majetich, *Tetrahedron Letters*, **1986**, 27, 4945–4948.
[3] C. Oliver Kappe, D. Dallinger, S. Shaun Murphree; Practical Microwave Synthesis for Organic Chemists: Strategies, Instruments, and Protocols, WILEY-VCH, Weinheim, **2009**.
[4] C. Oliver Kappe, A. Stadler; Microwaves in Organic and Medicinal Chemistry, WILEY-VCH, Weinheim, **2005**.
[5] C. Oliver Kappe; Controlled Microwave Heating in Modern Organic Synthesis, Wiley-VCH, Weinheim, **2004**.
[6] A. C. Metaxas, R. J. Meredith; Industrial microwave heating, IEE, **1993.**
[7] P. Lidström, J. Tierney, B. Wathey, J. Westman, *Tetrahedron*, **2001** 57, 9225–9283.
[8] Nicolaou, K.C et al. *J. Am. Chem. Soc*., **1997**, 119, 3421-3422.
[9] B. Hayes; Microwave Synthesis Chemistry at the Speed of Light, CEM Publishing: Matthews, North Carolina, **2002**.
[10] D.Bradley, *Modern Drug Discovery*, **2001**, 4, 32-36.
[11] B. Wathey, J. Tierney, P. Lidström, J. Westman, *Drug Discovery Today*, **2002,** 7, 373–380.
[12] M. Nüchter, B. Ondruschka, W. Bonrath, A. Gum, *Green Chem*., **2004**, 6, 128–141.
[13] R. S. Varma, V. V. Namboodiri, *Chem. Commun*., **2001**, 643.
[14] J.S. Schanche, *Mol. Diversity*, **2003**, 7, 293.