

Microwave-Assisted Pharmaceutical Synthesis: An Overview

Bhupinder Singh Sekhon

PCTE Institute of Pharmacy, Jhande, Baddowal Cantt (Ludhiana) -142 021, India.

Corres.author: sekhon224@yahoo.com
Phone No. – 09876242299

Abstract: Microwave-assisted organic synthesis is an enabling technology for accelerating drug discovery and development processes. Microwave instruments are used principally in three areas of drug research: the screening of organic drug formulae, peptide synthesis, and DNA amplification. The features of microwave-assisted organic synthesis technology include reduction of time for a chemical reaction, instantaneous and uniform heating, carrying out solvent free reactions and possibility of parallel chemical reactions has proved as a bonanza for the researchers involved in drug discovery and development processes like high-speed combinatorial and medicinal chemistry. Microwave-assisted organic synthesis in aqueous medium has resulted in the development of relatively sustainable and environmentally benign protocols for the synthesis of drugs. Microwave-assisted synthesis under controlled conditions has many applications in the field of medical chemistry and pharmaceutical research. This technology has made an impact in several areas of drug discovery related to organic synthesis. It has been used by pharmaceutical companies in target discovery, screening, pharmacokinetics, production of compound libraries and has found application in peptide synthesis.

Keywords: Microwave irradiation; Organic synthesis; Nanoparticles; Pharmaceuticals.

1. Introduction

Microwaves are defined as electromagnetic waves with vacuum wavelengths ranging between 0.1 to 100 cm or, equivalently, with frequencies between 0.3- 300 GHz. Microwave dielectric heating uses the ability of some liquids and solids to transform electromagnetic radiation into heat to drive chemical reactions. This technology opens up new opportunities to the synthetic chemist in the form of new reactions that are not possible using conventional heating.

The interest in the microwave assisted organic synthesis has been growing during the recent years. Drug companies are exploiting microwaves in the area of organic/pharmaceutical synthesis for drug screening and discovery¹⁻⁶. Scientists have demonstrated the potential of microwave -assisted organic synthesis using ionic liquids as solvent, cosolvent, additives and/or catalyst⁷. Despite the wide application of microwave technology in organic chemistry many of specific microwave effects still are not fully explored. The medicinal chemical community has been under increased pressure to produce, in an environmentally

benign fashion, the myriad of drugs required by society in short periods of time. Microwave synthesis is particularly interesting due to its high efficiency, leading to drastically reduced reaction times, higher yield and purer products.

2. Microwave Technology

The development of cleaner technologies is a major emphasis in green chemistry. Among the several aspects of green chemistry, using efficient and less hazardous energy sources such as microwave energy is recommended. Drug companies and research institutions are exploiting microwaves in the area of organic synthesis for drug screening and discovery. Presently, MAOS is an enabling technology for accelerating drug discovery and development processes⁸. Microwave technology uses electromagnetic waves that pass through material and cause its molecules to oscillate, generating heat. In conventional heating, the material's surface heats first and then the heat moves inward. Microwave heating generates heat within the material and heats the entire

volume at about the same rate⁹. Microwave technology has got obvious edge and advantages over conventional methods of heating by virtue of which microwave assisted methods are being employed to carry out various synthetic procedures successfully and effectively, which were otherwise encountering problems earlier. Microwave instruments are used principally in three areas of drug research. i) the screening of drug formulae which are made of organic compounds and the candidate compounds that seem to be numerous, ii) microwave-assisted peptide synthesis, in which peptides are used as drug generics, moreover, synthesis of long chains of peptides is very difficult and the microwave approach has been especially effective in the area of peptides synthesis, iii) the microwave-assisted DNA amplification which is used in disease analysis where there are a number of DNAs that are very difficult to process¹⁰.

The goal of the present review is to present microwave-assisted pharmaceutical synthesis with special emphasis on aspects that have relevance to drug discovery. Small molecules can be built in a fraction of the time required by classical thermal methods. In view of this, microwave-assisted technology has rapidly gained acceptance as a valuable tool for accelerating drug discovery and development processes¹¹⁻¹⁵. This technology opens up new opportunities to the synthetic chemist/medicinal chemist, in the form of new reactions that are not possible using conventional heating, improved reaction yields, decreased reaction times and even solvent free reaction conditions. With few exceptions pharmaceutical (organic) materials are diamagnetic and dielectric, i.e. are suitable for microwave heating. This environmentally friendly microwave-induced diffusion for generating activated drug / 3D-matrix nanocomposites has solved the bioavailability-linked problems associated with insoluble drugs. Radiopharmaceuticals that contain isotopes with short half-lives have been synthesized by activation with the use of microwave energy^{16a}. Microwave has been successful in reducing reaction times by up to 50 per cent with improved radiochemical yield of the final product. The most recent applications in the field of MAOS of biologically active compounds both in heterocyclic and in peptide and peptidomimetic optimization have been reported^{17,18}.

The fundamentals and significant outcomes of microwave-assisted organic/pharmaceutical synthesis in aqueous medium were reported which have resulted in the development of relatively sustainable and environmentally benign protocols for the synthesis of drugs and fine chemicals¹⁹. A rapid and efficient microwave-assisted protocol based on the use of cycles of microwave irradiation was reported that greatly

improves a recent synthetic method developed for quinazoline synthesis²⁰. A speedy, high yield and convenient synthesis of Imatinib (a drug used to treat certain types of cancer) was carried out on an aldehydic, super acid-sensitive resin, through an efficient, microwave-assisted synthetic protocol, that may enable the easy preparation of libraries of potential protein kinase inhibitors endowed with large molecular diversity²¹. Microwave-assisted organic synthesis played a key role in the identification of isozyme-selective Akt inhibitors²².

3. Microwave versus Conventional Synthesis

Microwave-assisted organic synthesis has several advantages over conventional reactions in that the microwave allows for an increase in reaction rate, rapid reaction optimization, and rapid analogue synthesis. It also uses both less energy and solvent, and it enables difficult compound synthesis²³. In general, drug discovery can be broken down into five steps: i) target and synthesis design, ii) reaction, iii) work-up (usually extraction and evaporation), iv) purification (usually chromatography), and v) spectral analysis registration. Specifically, microwave synthesis has the potential to impact upon medicinal chemistry efforts in at least three major phases of the drug discovery process: lead generation, hit-to-lead efforts, and lead optimization. Available microwave instrumentation includes i) Single-mode microwave reactors and ii) Multimode microwave reactors²⁴. Microwave chemistry can be carried out very efficiently in a parallel format using dedicated rotors or microtiter plate systems. Several hundred reactions can be performed in a single microwave experiment using multimode microwave devices². Researchers have shown the benefits gained by employing microwave heating in tandem with combinatorial chemistry²⁵. Six fluoroquinolone ribonucleosides synthesized by using microwave irradiation proved superior in time and yield, especially the one step decarboxylation of the carboxyquinolone esters²⁶. Several microwave-assisted protocols that have become integrated into the drug discovery research process in the biotechnology industry were reported²⁷. Eleven flavonoid derivatives synthesized using a modified Baker-Venkataraman rearrangement, and subsequent microwave-assisted closure of the heterocyclic ring displayed antifungal activity against *Aspergillus niger* and *Fusarium oxysporium*, and two of the synthetic flavonoid analogues exhibited significant activity against methicillin-resistant *Staphylococcus aureus*²⁸. Microwave irradiation can be used as a simplistic and general method for the construction of a wide variety of triazoloquinazolinones and benzimidazoquinazolinones²⁹. The basic principles behind microwave technology and recent trends and areas in drug discovery have been reported³⁰.

Regiospecific microwave-assisted synthesis of novel purine derivatives as antitumor agents was reported³¹. The pharmacophoric requirements for compounds to exhibit anticonvulsant activity that includes one aryl unit in proximity to a hydrogen donor-acceptor domain and an electron donor have been justified with the molecular orbital surface analysis of the synthesized compounds using microwave-assisted synthesis³². The current state of the art has been reported in the field of microwave assisted synthesis of biologically active compounds both in heterocyclic and in peptide and peptidomimetic optimization³³. A microwave-assisted extraction method followed by LC analysis was developed for the determination of ketoprofen lysine salt in the presence of methyl p-hydroxybenzoate and propyl p-hydroxybenzoate preservatives in topical cream³⁴.

4. Microwave Synthesis for Nanomaterials

Amongst the several methods that exist for synthesizing of nanoparticles, the use of microwave assisted synthesis has shown promise. Synthesis of silver nanoparticles from silver nitrate employing starch as the reductant cum stabilizing agent has been carried out under direct heating, controlled heating and microwave irradiation. The microwave irradiation was considered as better for reduction of silver ions to silver nanoparticles. It also afforded smaller particle sizes and particle size distribution. Compared to conventional methods, microwave assisted synthesis was faster and provided particles with an average particle size of 12 nm. Further, the starch functions as template, preventing the aggregation of silver nanoparticles³⁵. A rapid and reliable synthesis of highly luminescent CdSe/ZnS nanoparticles was developed using a domestic microwave oven³⁶. An elegant scientific breakthrough in terms of the application of microwaves to the synthesis of quantum dots has recently been reported³⁷. A novel synthesis of polymer microcapsules with core-shell morphology comprising of Au@Polymer that is microwave-, photo- and thermo-responsive prepared by a novel microarray technique using a double emulsion process was reported. Scientists further demonstrated a reversible swelling and deswelling behavior of the microcapsule actuated by electromagnetic radiation (visible light and microwave radiation) that can have potential applications to various nanomedicine applications in controlled delivery and release³⁸. Researchers reported recent developments in microwave-assisted synthesis, name reactions and organic transformations, and rapid generation of nanoparticles with uniform size distribution³⁹.

Scientists at the National Institute of Standards and Technology have developed a simplified, low-cost process to synthesize highly uniform and efficient

quantum dots using a laboratory microwave reactor for a range of frequencies which were stable in aqueous solutions for longer than four months³⁷. Nanostructures with smaller sizes, narrower size distributions, and a higher degree of crystallization were obtained under microwave heating than those in conventional oil-bath heating⁴⁰. The Au nanoparticles have been prepared by microwave high-pressure procedure with alcohol as the reducing agent⁴¹. Microwave heating of group-12 and -13 organometallic precursors and group-15 and -16 reactants has demonstrated dielectric heating, a viable method for the preparation of high-quality photoluminescent semiconducting nanoparticles. Additives, for example, tri-octyl phosphine oxide or ionic liquids can have a dramatic effect on the observed growth behavior in the microwave. Increasing microwave power increased the reaction rate and material quality owing to overcoming kinetic barriers. A method has been reported for producing crystalline nanoparticle semiconductor material which includes the steps of mixing a precursor in a solvent to form a reaction mixture and subjecting the reaction mixture to microwave dielectric heating at sufficient power to achieve a superheating temperature of the reaction mixture. A growth-phase reaction was permitted to proceed, wherein nanoparticles were formed in the heated reaction mixture, followed by quenching of reaction to considerably terminate nanoparticle formation⁴².

5. Microwave-Assisted Peptide Synthesis

A microwave-assisted, rapid solid phase peptide synthesis procedure has been reported⁴³. The application of microwave heating to solid-phase peptide synthesis is particularly advantageous as the acceleration of coupling and deprotection reactions should lead to shorter cycle times, higher repetitive yields, and ultimately purer peptides⁴⁴. The protocols for the synthesis of cystine-rich peptides in the presence of microwave radiation with Boc-solid phase peptide synthesis have been reported^{45,46}. An instrument and process for accelerating the solid phase synthesis of peptides has been reported. The method includes the steps of deprotecting a protected first amino acid linked to a solid phase resin by admixing the protected linked acid with a deprotecting solution in a microwave transparent vessel while irradiating the admixed acid and solution with microwaves. Then a second amino acid is activated by adding the second acid and an activating solution to the same vessel while irradiating the vessel with microwaves, then followed by coupling of the second amino acid to the first acid while irradiating the composition in the same vessel with microwaves. Finally, the linked peptide from the solid phase resin cleaved by admixing the linked peptide with a cleaving composition in the same vessel while irradiating the composition with microwaves⁴⁷. The applications of microwaves in the

field of peptides and glopeptides have been reported^{48,49}.

6. Polymerase Chain Reactions

Polymerase chain reactions with focused microwave irradiation as the source of heat were demonstrated and the results indicated the possibility to shorten the total reaction time as well as the possibility to perform PCR reactions in millilitre scale⁵⁰. Scientists focused on the microwave technology for advance to the various chemical and biological reactions and the microwave irradiation to rolling circle amplification reaction on controlling the temperature⁵¹. The extract and detection of anthrax DNA from spores and vegetative cells in two steps within 1 min has been reported⁵². Microwave energy is highly focused using thin-film aluminum "bow-tie" structures in a cavity, to extract DNA from whole spores within 20 s, followed by the detection of the released DNA, by employing the microwave-accelerated metal-enhanced fluorescence technique.

7. Some Miscellaneous Aspects

Microwave applications in radiolabelling tracers for Positron Emission Tomography, paralleling and sometimes preceding developments in other areas of microwave-enhanced chemistry were reported⁵³. Dihydropyridones were prepared by microwave-assisted reaction between curcumin and primary amines or their acetates in the presence of Montmorillonite (K-10) as a catalyst⁵⁴. In most pharmaceutical and biotechnologies companies, microwave synthesis is considered the cutting-edge methodology today. The synthetic methodology for the preparation of trimethoprim via microwave-assisted organic synthesis has been reported⁵⁵. The use of microwaves facilitated the preparation of new copolymers (useful for gene delivery), based on α , β -poly-(N-2-hydroxyethyl)-D, L-aspartamide as a polymeric backbone and bearing an oligoamine (such as diethylenetriamine in the side chain) synthetic method beneficial as time and solvents are saved⁵⁶. The development of relatively sustainable and environmentally benign protocols for the synthesis of drugs was the outcomes of microwave-assisted organic synthesis in aqueous medium¹⁹. Greener and expeditious synthesis of bio-active heterocycles using microwave irradiation was reported⁵⁷. The advantages of microwave-assisted technology for the rapid synthesis of novel aspartyl protease inhibitors using dedicated microwave equipment have been reported⁵⁸. A rapid open-vessel focused microwave-assisted extraction method followed by LC analysis was developed for the determination of naproxen in suppositories⁵⁹.

New sildenafil analogues characterized by the presence on the sulfonyl group in the 5' position of novel N-4-substituted piperazines or ethylenediamine moiety, were prepared by microwave-assisted synthesis and several analogues were significantly more lipophilic than sildenafil⁶⁰. An convenient microwave-assisted synthesis (incorporating parallel and nonparallel combinatorial methods) of N-alkylated glycine methyl esters has been reported⁶¹. An efficient two-step synthesis of bezafibrate from tyramine under microwave conditions has been developed using SmithReactionKit and Coherent Synthesis Technology⁶².

Other examples of the overall yield improvement in MW-assisted synthesis over that obtained with conventional methods include: fast and convenient synthesis of fenclofenac (a non-steroidal anti-inflammatory drug) using Ullmann ether coupling and the Willgerodt-Kindler reaction; development of bezafibrate (a well-known lipid- and cholesterol-lowering drug) synthesis; the efficient two-step synthesis of atenolol from p-hydroxyphenylacetamide; integrating microwave synthesis and flash purification as enabling tools in drug discovery⁶³. Total synthesis of viscolin, an anti-inflammatory 1, 3-diphenylpropane isolated from *Viscum coloratum*, employing microwave-assisted Wittig olefination reaction was reported⁶⁴. The current developments and future potential offered by continuous flow microwave mediated synthesis has been reported⁶⁵. Microwave-assisted extraction was applied for the extraction of astaxanthin from *Haematococcus pluvialis* and the results showed that the extracts presented strong ability of inhibiting the peroxidation of linoleic acid, exhibited strong radical-scavenging properties against the DPPH, as well as strong reducing power⁶⁶.

8. Conclusions and Perspectives

The obvious features of microwave technology like reduction of time for a chemical/pharmaceutical reaction, instantaneous and uniform heating, carrying out solvent free reactions and possibility of parallel chemical reactions has proved as a bonanza for the researchers involved in drug discovery and development processes like high-speed combinatorial and medicinal chemistry. Microwave synthesis in macro-scale (synthesis of active pharmaceutical ingredient synthesis), microscale (integrated 'lab-on-a-chip' type approaches where synthesis and biological screening are integrated) and meso-scale flow units should be actively pursued. The combination of ionic liquids and microwave heating encourage scientists to initiate new unexplored areas of complex pharmaceutical systems. Due to high polarity of ionic liquids and stability at elevated temperatures, these are attractive solvents or co-solvents in microwave synthesis.

References

1. Kappe C. O., Dallinger D., The impact of microwave synthesis on drug discovery. *Nat. Rev. Drug Discovery*, 5: 51-63, 2006.
2. Kappe C. O., Dallinger D., Murphree S. S., Practical microwave synthesis for organic chemists - Strategies, instruments, and protocols. 1st Ed. Wiley-VCH, Verlag GmbH & Co. KGaA, Weinheim, 2009.
3. Tierney J.P., Lidström P., Eds., Microwave assisted organic synthesis. Blackwell Publishing, Oxford, 2005.
4. Larhed M., Hallberg A., Microwave-assisted high speed chemistry: A new technique in drug discovery. *Drug Discovery Today*, 6: 406-416, 2001.
5. Microwave-assisted synthesis in the pharmaceutical industry -a current perspective and future prospects. http://www.analis.be/tempFiles/1033941353_0.8554651/Microwave-assisted_synthesis_in_the_pharmaceutical%20industry_DDW_summer%202006.pdf
6. Lidstrom P., Tierney J., Wathey B., Westman J., Microwave assisted organic synthesis-a review. *Tetrahedron.*, 57: 9225-9283, 2001.
7. Martínez-Palou R., Ionic liquid and microwave-assisted organic synthesis: A "green" and synergic couple. *J. Mex. Chem. Soc.* 51: 252-264, 2007.
8. Vasudevan A., Microwave-assisted organic synthesis an enabling technology with disruptive potential. *Drug Discovery World*, Fall 2008.
9. Dubey R., Dwivedi S., Mehta K., Joshi H., New era in the field of synthetic chemistry: Microwave assisted synthesis. *Pharmainfo.net*, Vol. 6, Issue 3, 2008.
10. <http://www.pharmaceutical-technology.com/features/feature43413/>
11. Mavandadi F., Pilotti Å., The impact of microwave-assisted organic synthesis in drug discovery. *Drug Discovery Today*, 11:165-174, 2006.
12. Mavandadi F. M., Lidström P., Microwave-assisted chemistry in drug discovery. *Curr. Top. Med. Chem.*, 4: 773-792, 2004.
13. Kappe C. O., Stadler A., Microwaves in organic and medicinal chemistry. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, 2005.
14. Dzierba C. D., Combs A. P.. Microwave-assisted chemistry as a tool for drug discovery. *Ann. Rep. Med. Chem.*, 37:247-256,2002.
15. Wathey B., Tierney J., Lidström P., Westman J., The impact of microwave-assisted organic chemistry on drug discovery. *Drug Discovery Today*, 7: 373-380, 2002.
16. John R. Jones J. R., Lu S., Microwave-enhanced radiochemistry. *Microwaves in organic synthesis*. Wiley-VCH Verlag GmbH & Co. KGaA, 435-462, 2002.
17. Colombo M., Peretto I., Chemistry strategies in early drug discovery: an overview of recent trends. *Drug Discovery Today*, 13: 677-684, 2008.
18. Santagada V., Frecentese F., Perissutti E., Fiorino F., Severino B., Caliendo G., Microwave assisted synthesis: a new technology in drug discovery. *Mini Rev. Med. Chem.*, 9: 340-358, 2009.
19. Polshettiwar V., Varma R. S., Aqueous microwave chemistry: a clean and green synthetic tool for rapid drug discovery. *Chem. Soc. Rev.*, 37:1546 – 1557, 2008.
20. Chilin A., Marzaro G., Zanatta S., Guiotto A., A microwave improvement in the synthesis of the quinazoline scaffold. *Tetrahedron Lett.*, 48: 3229-3231, 2007.
21. Leonetti F., Capaldi C., Carotti A., Microwave-assisted solid phase synthesis of Imatinib, a blockbuster anticancer drug. *Tetrahedron Lett.*, 48: 3455-3458, 2007.
22. Shipe W. D., Wolkenberg S. E., Lindsley C. W., Accelerating lead development by microwave-enhanced medicinal chemistry. *Drug Discovery Today: Technologies*, 2: 155-161, 2005.
23. Algul O., Kaessler A., Apcin Y., Yilmaz A., Jose J., Comparative studies on conventional and microwave synthesis of some benzimidazole, benzothiazole and indole derivatives and testing on inhibition of hyaluronidase. *Molecules*, 13: 736-748, 2008.
24. Stadler A, Yousefi B H., Dallinger D., Walla P., Van der Eycken E., Kaval N., Kappe C. O., Scalability of microwave-assisted organic synthesis. From single-mode to multimode parallel batch reactors. *Org. Process Res. Dev.*, 707-716, 2003.
25. Lidström P., Westman J., Lewis A., Enhancement of combinatorial chemistry by microwave-assisted organic synthesis. *Comb. Chem. High Throughput Screening*, 5: 441-458, 2002.

26. Adams M. M., Bats J. W., Nikolaus N. V., Witvrouw M., Debysers Z. E., Engels J. W., Microwave-assisted synthesis of fluoroquinolones and their nucleosides as inhibitors of HIV integrase. *Collect. Czech. Chem. Commun.*, 71: 978 - 990, 2006.
27. Sandoval W. N., Pham V. C., Lill J. R., Recent developments in microwave-assisted protein chemistries – can this be integrated into the drug discovery and validation process? *Drug Discovery Today*, 13: 1075-1081, 2008.
28. Ghani S. B. A., Weaver L., Zidan Z. H., Ali H. M., Keevil C. W., Brown R. C. D., Microwave assisted synthesis and antimicrobial activities of flavonoid derivatives. *Bioorg. Med. Chem. Lett.*, 18: 518-522, 2008.
29. Mourad A. E., Aly A.A., Farag H. H., Beshr E.A., Microwave assisted synthesis of triazoloquinazolinones and benzimidazoquinazolinones. *Beilstein J. Org. Chem.*, 3:11, 2007.
30. Mavandadi F., Pilotti A., The impact of microwave-assisted organic synthesis in drug discovery *Drug discovery Today*, 11: 165-174, 2005.
31. Conejo-García M.A., Gallo A., Espinosa J.M., Campos. Latest advances on regiospecific microwave-assisted synthesis of novel purine derivatives as antitumor agents. *Expert Opin. Ther. Pat.*, 18: 211-222, 2008.
32. Shalini M., Yogeewari P., Sriram D., Induja S., Microwave-assisted synthesis, anticonvulsant activity and quantum mechanical modelling of N-(4-bromo-3-methylphenyl) semicarbazones. *J Zhejiang Univ Sci B*, 8: 45–55, 2007.
33. Santagada V., Frecentese F., Perissutti E., Fiorino F., Severino B., Caliendo G., Microwave assisted synthesis: a new technology in drug discovery. *Mini Rev. Med. Chem.*, 9: 340-358, 2009.
34. Labbozzetta S., Valvo L., Paola Bertocchi P., Alimonti S., Gaudiano MC., Manna L., Focused microwave-assisted extraction and LC determination of ketoprofen in the presence of preservatives in a pharmaceutical cream formulation. *Chromatographia*, 69: 365-368, 2009;
35. Sreeram K. J., Nidhin, B M., Nair U., Microwave assisted template synthesis of silver nanoparticles. *Bull. Mater. Sci.*, 31: 937–942, 2008,.
36. Ziegler. J., Merkulov A., Grabolle M., Resch-Genger U., Nann T., High-quality ZnS shells for CdSe nanoparticles: rapid microwave synthesis. *Langmuir*, 23: 7751-7759. 2007.
37. Roy M. D., Herzing A.A., De Paoli Lacerda S.H., Becker M. L., Emission-tunable microwave synthesis of highly luminescent water soluble CdSe/ZnS quantum dots. *Chem. Commun.*, 18: 2106, 2008.
38. http://www.uml.edu/nano/Research/NanoMedicine/aupolymer_microcap.html
39. Polshettiwar V., Nadagouda M. N., Varma R.S., Microwave-assisted chemistry: A rapid and sustainable route to synthesis of organics and nanomaterials. *Aust. J. Chem.*, 62: 16-26, 2009.
40. Tsuji M., Hashimoto M., Nishizawa Y., Kubokawa M., Tsuji T., Microwave-assisted synthesis of metallic nanostructures in solution. *Chem.--Eur. J.*, 11; 440 – 452, 2004.
41. Jiang Z. L., Feng Z. W., Shen X. C., Microwave Synthesis of Au nanoparticles with the system. *Chin. Chem. Lett.*, 12: 551 – 554, 2001.
42. Strouse G. F., Gerbec J. A., Nanoparticle synthesis and associated methods. Patent IPC8 Class: B01J1912FI, USPC Class: 20415743.
43. Erdélyi M., Gogoll A., Rapid microwave-assisted solid phase peptide synthesis. *Synthesis*, No. 11, 1592–1596, 2002
44. White P., Collins J., Cox Z., Comparative study of conventional and microwave assisted synthesis; <http://www.emdbiosciences.com/SharedImages/novabiochem/P69-APS.pdf>
45. Čemažar M., Craik D. J., Microwave-assisted Boc-solid phase peptide synthesis of cyclic cysteine-rich peptides. *J. Pept. Sci.*, 14: 683 – 689, 2007.
46. Rybka A.C., Frank H.G., Microwave assisted peptide synthesis — A tool to replace classical SPPS? *American Peptide Symposia*, Volume 9, Sylvie E. Blondelle, Ed, In: *Understanding Biology Using Peptides*, Part 3, 148-149, Springer New York, 2006.
47. Collins. J.M., Lambert J. J., Collins M. J., Microwave-assisted peptide synthesis, Patent US7393920, July 01, 2008.
48. Matsushita T., Hinou H., Fumoto M., Kurogouchi M., Fujitani N., Shimizu H., Nishimura S. I., Construction of highly glycosylated mucin-type glycopeptides based on microwave-assisted solid-

phase syntheses and enzymatic modifications. *J. Org. Chem.*, 2006, 71, 3051-3063.

49. Collins J. M., Leadbeater N. E., Microwave energy: a versatile tool for the biosciences. *Org. Biomol. Chem.*, 5, 1141- 1150, 2007.

50. Fermér C., Nilsson P., Larhed M., Microwave-assisted high-speed PCR. *Eur. J. Pharm. Sci.*, 18:129-132, 2003.

51. Yoshimura T., Nishida K., Uchibayashi K., Ohuchi S., Microwave assisted rolling circle amplification. *Nucleic Acids Symp. Ser.*, No. 50, 305-306, 2006.

52. Aslan K., Previte M.J.R., Zhang Y., Gallagher T., Baillie L., Geddes C. D., Extraction and detection of DNA from bacillus anthracis spores and the vegetative cells within 1 min. *Anal. Chem.*, 80: 4125-4132, 2008.

53. Stone-Elander S., Elander N., Microwave applications in radiolabelling with short-lived positron-emitting radionuclides. http://www.resonanceinstruments.com/JLCR_MwaveRev.pdf

54. Elias R.S., Saeed B.A., Saour K.Y., Al-Masoudi N. A., Microwave-assisted synthesis of dihydropyridones from curcumin *Tetrahedron Lett.*, 49: 3049-3051, 2008.

55. Mavandadi F., Microwave technology in process optimization. In: Gadamasetti K.G., Braish T., Process chemistry in the pharmaceutical industry, Volume 2, 2nd Edition, CRC Press, Chapter 25, pp 403-426, 2008.

56. Cavallaro G., Licciardi M., Scirè S., Giammona G., Microwave-assisted synthesis of PHEA-oligoamine copolymers as potential gene delivery systems. *Nanomedicine*, 4: 291-303, 2009.

57. Polshettiwar V., Varma R.S., Greener and expeditious synthesis of bio-active heterocycles using microwave irradiation. *Pure App. Chem.*, 80: 777-790, 2008.

58. Ersmark K., Larhed M., Wannberg J., Microwave-enhanced medicinal chemistry: a high-speed

opportunity for convenient preparation of protease inhibitors. *Curr. Opin. Drug Discov. Devel.*, 7: 417-427, 2004.

59. Labbozzetta S., Valvo L., Bertocchi P., Manna L., Focused microwave-assisted extraction and LC determination of the active ingredient in naproxen-based suppositories. *J. Pharm. Biomed. Anal.*, 39: 463-468, 2005.

60. Flores Toque H. A., Priviero F. B., Teixeira C. E., Perissutti E., Fiorino F., Severino B., Frecentese F., Lorenzetti R., Baracat J.S., Santagada V., Caliendo G., Antunes E., De Nucci G., Synthesis and pharmacological evaluations of sildenafil analogues for treatment of erectile dysfunction. *J. Med. Chem.*, 51: 2807-2815, 2008.

61. Santagada V., Frecentese F., Perissutti E., Fiorino F., Severino B., Cirillo D., Terracciano S., Caliendo G., Efficient microwave combinatorial parallel and nonparallel synthesis of N-alkylated glycine methyl esters as peptide building blocks. *J. Comb. Chem.*, 7: 618-621, 2005.

62. Efficient and versatile microwave-assisted synthesis of bezafibrate employing coherent synthesisTM, <http://www.biotage.com/DynPage.aspx?id=21997>

63. <http://www.biotage.com/DynPage.aspx?id=21997>.

64. Su. C.R., Shen Y.C., Kuo P.C., Leu Y.L., Damu A.G, Wang Y.H., Wu T. S., Total synthesis and biological evaluation of viscolin, a 1,3-diphenylpropane as a novel potent anti-inflammatory agent. *Bioorg. Med Chem. Lett.*, 16: 6155-6160, 2006.

65. Baxendale R I., Hayward J.J., Ley S.V., Microwave reactions under continuous flow conditions. *Comb. Chem. High Throughput Screening*, 10: 802-836, 2007.

66. Liyan Z., Guitang C., Guanghua Z., Xiaosong H., Optimization of microwave-assisted extraction of astaxanthin from haematococcus pluvialis by response surface methodology and antioxidant activities of the extracts. *Separation Science and Technology*, 44: 243-262, 2009.
