Review Article **CODEN: AJPCFF** **ISSN: 2321 – 0915**

Asian Journal of Phytomedicine
and
Clinical Research

Journal home page: www.ajpcrjournal.com

MICROWAVE ASSISTED EXTRACTION OF PHYTOCONSTITUENTS

U. B. Hadkar[1], Nikita Dhruv[1], Yashwant Malode*[1], Bhagyashri Chavan[2]

*[1]Department of Physical Pharmacy, MET Institute of Pharmacy, Mumbai, Maharashtra, India.
[2]Department of Pharmaceutics, MET Institute of Pharmacy, Mumbai, Maharashtra, India.

ABSTRACT

Now days, microwaves are used for extraction of phytoconstitutents from plant material because of tremendous research interest and potential. Conventional extraction techniques are time consuming and require more solvent and most of them are not suitable for thermolabile constituents. This article throws light on importance of extraction step in herbal research and medicine. The extraction step must be high yielding; fast, selective, less solvent consuming, protecting thermolabile substances and these features are present in the microwave assisted extraction (MAE) technique. In this technique (MAE) heat is generated using microwave energy. The parameters that influence extraction are solvent nature and volume, extraction time, microwave power, matrix characteristics and temperature and some of the potential applications of this extraction technique are given here. The MAE technique is compared with other extraction techniques.

KEY WORDS

Microwave assisted extraction (MAE), Thermolabile, Microwave heating, Dielectric properties and Dissipation factor.

Author for Correspondence:

Yashwant Malode,

Department of Physical Pharmacy,

MET Institute of Pharmacy, Mumbai, Maharashtra,

India.

Email: yashwant060189@gmail.com

INTRODUCTION

The history of plants used for medicinal purpose is probably as old as the history of mankind. Extraction and characterization of several active phyto-compounds from these green factories have given birth to some high activity profile drugs. The potential natural anticancer drugs like vincristine, vinblastine and taxol are the best examples. There has been a growing popularity and faith in the use of herbal medicine worldwide. This may be because of the realization that modern synthetic drugs have undesirable side effects, which may be more problematic than the actual disease itself. The herbal medicine provides a ray of hope through its cocktail of phyto-compounds, which are believed to act in a synergistic manner, providing excellent healing touch with practically no undesirable side effects [1, 2]. Extraction is defined as a process of isolation of soluble material from insoluble residue, which may be liquid or solid, by using different solvents [3]. The fact that one single plant can contain up to several thousand secondary metabolites, makes the need for the development of high performance and rapid extraction methods an absolute necessity [1, 2].

Traditional methods include soxhlet extraction, maceration, percolation, turbo-extraction (high speed mixing) and sonication. These techniques have been used for many decades; however, they are very often time-consuming and require relatively large quantities of environment polluting solvents [4]. The new extraction techniques with shortened extraction time, reduced solvent consumption, decreased pollution and with special care for thermolabile constituents. Novel extraction methods including microwave assisted extraction (MAE), supercritical fluid extraction (SCFE), pressurized solvent extraction (PSE) have drawn significant research attention in the last decade [1, 2]. The present review deals with the application of MAE to the extraction of secondary metabolites from plant material.

History

Microwaves have been used since World War II following the development of radar technology, and later the first commercial application of microwaves was the domestic ovens [4]. The use of microwave energy as a heating source in analytical laboratories started in the late 1970s [5]. The development of microwave assisted extractions was first reported by Ganzler and co-workers [4]. In the beginning of the 1990s various research groups in Europe started using microwave ovens for the extraction of additives from polyalkenes using solvents such as trichloroethane and mixtures of acetone and heptane. In 1991 first application of MAP was performed and dealt with the extraction of essential oils from plant products. In 1993, Onuska and Terry evaluated the extractability of various pesticides in the soil. Extraction parameters such as solvent choice, moisture level and extraction time were investigated. In 1993, Steinheimer extracted atrazine from soils and water samples [6, 7].

Microwave-Assisted Extraction (MAE)

Microwaves serve two major purpose - communication and as energy vectors [2, 8, 9]. Microwaves are electromagnetic radiations with a frequency from 0.3 to 300 GHz. In order to avoid interferences with radio communications, domestic and industrial microwaves generally operate at 2.45 GHz [4]. The latter application is the direct action of waves on materials that have the ability to convert a part of the absorbed electromagnetic energy to heat energy. Microwaves are made up of two oscillating perpendicular field's i.e. electric field and magnetic field and the former is responsible for heating. Unlike conventional heating which depends on conduction – convection phenomenon with eventually much of the heat energy being lost to the environment, in case of MAE, heating occurs in a targeted and selective manner with practically no heat being lost to the environment as the heating occurs in a closed system. This unique heating mechanism can significantly reduce the extraction time (usually less than 30 min) as compared to Soxhlet.

The principle of heating using microwave is based upon its direct impact with polar materials or solvents and is governed by two phenomena: ionic conduction and dipole rotation, which in most cases occurs simultaneously. Ionic conduction refers to the electrophoretic migration of ions under the influence of the changing electric field. The resistance offered by the solution to the migration of ions generates friction, which eventually heats up the solution. Dipole rotation means realignment of the dipoles of the molecule with the rapidly changing electric field [2, 8, 9]. With a frequency of 2.45 GHz, this phenomenon occurs 4.9×10^4 times per second and the resulting heating is very fast. The larger the dielectric constant of the solvent, the more optimal the heating. Consequently, unlike classical conductive heating methods, microwaves heat the whole sample simultaneously [4].

With frequency greater than 2450 MHz the electrical component even changes at a much higher speed as a result the molecules not get sufficient time to even start to align themselves with the external field as a result no heating occurs. If the frequency is less than 2450 MHz the electrical component changes at a much lower speed and the molecules get sufficient time to align themselves with the electric field, thus there occurs no heating. The above mechanisms clearly indicate that only dielectric (Table No.1) material or solvents with permanent dipoles do get heated up under microwave. The efficiency with which different solvents heat up under microwave depends on the dissipation factor (tanε), which is indeed the measure of the ability of the solvent to absorb microwave energy and pass it on as heat to the surrounding molecules. The dissipation factor is given by the equation:

$$\tan \varepsilon = \varepsilon'' / \varepsilon',$$

Where,

ε’’ = dielectric loss (the efficiency of converting microwave energy into heat).

ε’ = dielectric constant (measure of the ability to absorb microwave energy).

Extraction principle

The dried plant material used for extraction contains cells having traces of moisture that serves as the target for microwave heating. The moisture when heated up inside the plant cell due to microwave effect, evaporates and generates tremendous pressure on the cell wall due to swelling of the plant cell. The pressure pushes the cell wall from inside, stretching and ultimately rupturing it, which facilitates leaching out of the active constituents from the ruptured cells to the surrounding solvent thus improving the yield of phytoconstitutents. The extraction efficiency can be increased if the plant matrix is impregnated with solvents with higher heating efficiency under microwave (higher tan ε value). The effect of microwave energy is strongly dependent on the dielectric susceptibility of both the solvent and solid plant matrix. Most of the time, the sample is immersed in a single solvent or mixture of solvents that absorb microwave energy strongly. Temperature increases penetration of the solvent into the matrix and constituents are released into the surrounding hot solvent. However in some cases only selective heating of sample matrix is brought about by immersing the sample in a microwave transparent solvent (hexane, chloroform). This approach is particularly useful for thermolabile components to prevent their degradation [2].

Instrumentation

There are two types of commercially available MAE systems

Closed extraction vessels and focused microwave ovens (Figure No.1)[10]. The former performs extraction under controlled pressure and temperature. The latter is also named as focused microwave assisted.

Soxhlet or solvent extraction (FMASE), in which only a part of the extraction vessel containing the sample is irradiated with microwaves. However, both the above-mentioned systems are available as multimode and single- mode or focused systems. A multimode system allows random dispersion of microwave radiation within the microwave cavity, so every zone in the cavity and sample it contains is evenly irradiated. Single mode or focused systems allows focused microwave radiation on a restricted zone where the sample is subjected to a much stronger electric field than in the previous case. Even a modified multimode domestic microwave oven operates as an open vessel extraction system.

Principle elements of a microwave device both multi- mode and focused microwave devices comprise of four major components:

(a) Microwave generator: magnetron, which generates microwave energy.

(b) Wave guide: which is used to propagate the microwave from the source to the microwave cavity?

(c) The applicator: where the sample is placed.

(d) Circulator: this allows the microwave to move only in the forward direction.

The advantages of closed-vessel systems can be summarized as follows.

They can reach higher temperatures because the increased pressure inside the vessel raises the boiling point of the solvents used. The higher temperatures in turn decrease the time needed for the microwave treatment. Loss of volatile substances during microwave irradiation is virtually completely avoided. Less solvent is required. Because no evaporation occurs, there is no need continually to add solvent to maintain the volume. The risk of contamination is avoided as a result there is little or no risk of airborne contamination. The fumes produced during an acid microwave extraction are contained within the vessel, so no provision for handling potentially hazardous fumes need to be made.

By contrast, closed-vessel systems are subject to several shortcomings, as follows.

The high pressures used pose safety (explosion) risks. The amount of sample that can be processed is limited. The usual constituent material of the vessel, PTFE (polytetraflouro ethylene), does not allow high solution temperatures. The single-step procedure excludes the addition of reagents or solvents during operation. The vessel must be cooled down before it can be opened after the treatment to avoid loss of volatile constituents. Atmospheric pressure (open-vessel) microwave sample preparation can be even more effective than closed-vessel methods. The use of atmospheric pressure provides substantial advantages over pressurized vessels. Increased safety since operating at atmospheric pressure with open vessels. The ability to add reagents at any time during the treatment, to use vessels made of various materials, including PTFE, glass and quartz. The excess solvent can be removed easily. Large samples can be easily processed. Cooling down or depressurization is not required. The cost of the equipment is low. It has the ability to quantitatively remove desired component. Fully automatic operation. Open-vessel operation is more suitable with thermolabile species as it uses low temperatures relative to closed vessel systems.

Open-vessel systems have several shortcomings.

The ensuing methods are usually less precise than those developed using closed-vessel systems. The open systems cannot process many samples simultaneously, while closed-vessel systems can handle 8-14 samples at a time. The operation times required to obtain results similar to those of closed-vessel systems are usually longer.

FACTORS AFFECTING MAE [2, 5]

Solvent nature

A correct choice of solvent is important for obtaining an optimal extraction process. Solvent choice for MAE is dictated by the solubility of the target analyte, by the interaction between solvent and plant matrix, and finally by the microwave absorbing properties of the solvent. Preferably the solvent should have a high selectivity towards the analyte of interest excluding unwanted matrix components. Another important aspect is the compatibility of the extracting solvent with further chromatographic analytical steps. Hexane is transparent to microwave and so does not heats up under microwave, whereas ethanol has good microwave absorbing capacity and hence heats up faster and can enhance the extraction process.

Solvent volume

Volume of the extracting solvent is also a critical factor. The solvent volume must be sufficient to ensure that the plant matrix is always entirely immersed in the solvent throughout the entire irradiation time. Generally, a higher ratio of solvent volume to solid matrix may be effective in conventional extraction methods. However, in MAE a higher ratio may yield lower recoveries, which may be due to inadequate stirring of the solvent by microwaves.

Extraction time

As in other extraction technique, time is another parameter whose influence needs to be taken into account. Generally, by increasing the extraction time, the quantity of analytes extracted is increased, although there is the risk that degradation may occur. Often 15-20 min is sufficient, but even 40 sec have been demonstrated to have given excellent recovery. In the extraction of artemisnin an overall high of 92% extraction was achieved within 12 min after which extraction yield dropped down, as over exposure may lead to thermal degradation of effective constituents. A proper study on optimization of extraction time is vital because extraction time may vary with the plant part used. Irradiation time is also influenced by the dielectric properties of the solvent. Solvents like water, ethanol, and methanol may heat up tremendously on longer exposure thus risking the future of thermolabile constituents[11].

Microwave Power

Microwave power and irradiation time should be critically selected. A combination of low or moderate power with longer exposure may be a wise approach. High power with prolonged exposure always involves the risk of thermal degradation. At higher temperature when kept at higher power, rapid rupture of cell wall takes place as a result together with the desired analytes impurities are also leached out into the solvent. Whereas at low power levels the cell wall rupture might take place gradually which enables selective MAE. In closed vessel systems, the chosen power settings depends on the number of samples to be extracted during a single extraction run, as up to 12 vessels can be treated in a single run. The power must be chosen correctly to avoid high temperature, which could lead to solute degradation and high pressure inside the vessel.

Matrix characteristics

The plant particle size and the status in which it is presented for MAE can have a profound effect on the recoveries of the compounds. The particle size of the materials used for extraction is generally in the range of 100μm to 2 mm. Fine powder can enhance the extraction by providing larger surface area, which provides better contact between the plant matrix, and the solvent, also finer particles will allow much deeper penetration of the microwave. One of the disadvantages associated with the use of finer particles is difficulty of separation of the matrix from the solvent after microwave irradiation. Generally, centrifugation or filtration is used for separation. The plant matrix may be selectively heated using microwave with the extracting solvent surrounding the sample transparent to microwave. In many cases the natural moisture content of the matrix improves the extraction recoveries, as in the case of extraction of essential oil from various plant.

Pre-Leaching

Soaking of the dried plant material in the extracting solvent prior to MAE has resulted in improved yield. This phenomenon is called pre-leaching extraction. Pre-leaching time at room temperature before MAE increased the percentage extraction of tanshinones and maximum yield was obtained when pre-leaching time was 45 minutes.

Temperature

Microwave power and temperature are interrelated to each other and need to be given special attention, particularly when working with closed vessel system. In closed vessel systems, temperature may reach well above the boiling point of the solvent. Temperatures attained by some commonly used microwave solvents under pressurized conditions are given in Table No.2. This elevated temperature does indeed result in improved extraction efficiencies since desorption of analyte from active sites in the matrix will increase. Additionally solvents have higher capacity to solubilize analytes at higher temperature while surface tension and solvent viscosity decreases with temperature, which will improve sample wetting and matrix penetration respectively. Increase in temperature is also associated with increase in pressure in closed systems, which can raise safety concerns. Temperature was found to be a significant factor in the extraction of paclitaxel. Temperature can be effectively controlled in open vessel system by proper combinations of extracting solvents which heat up differently.

Advantages [2, 5, 7, 8, 12]

- Extremely short extraction times, 15 to 30 minutes.
- High sample throughput, 8 to 16 samples in one extraction run.
- Exact reaction control by temperature and pressure sensors.
- Automated.
- Automation provides better accuracy and precision.
- The heating of the bulk as opposed to transferring heat from the surface, inwards, is

more efficient, uniform and less prone to overkill.

- Suitable for thermolabile constituents.
- Can even extract minute traces of constituents including heavy metals and pesticide residue from a few milligram of plant sample.
- Safe- A built-in solvent detector turns off microwave energy in the presence of flammable organic solvent and the safety back-up system turns off microwave energy if the exhaust system is not functioning properly.
- Cost-Effective- In processing applications, the ability to instantaneously shut the heat source makes enormous difference to the product quality and hence the production economics. Perform over 500 microwave assisted extractions with the same amount of solvent used for 32 soxhlet extractions. A dramatic cost savings is realized from the reduced quantity of solvent.
- Provides agitation during extraction, which improves the mass transfer and extraction time is reduced.
- Instrumental set up like soxwave combines both the features of soxhlet and advantages of microwave, thus making extraction even more effective.
- Quality consistency as well as positive environmental impact.
- MAE is simple, rapid and low solvent consuming process.

Limitations [13-17]

- The use of various instruments from different companies and with different characteristics (power density, volume, waveguides etc.).
- The indistinct use of monomode and multimode instruments with and without control of the incident power, respectively.
- The general use, until 2000, of domestic kitchen-type microwave instruments, the results of which are considered to be non-reproducible.
- The low penetration depth of microwave irradiation, which hinders the scale up of reactions assisted by microwaves.
- An additional centrifugation or filtration step is required.
- Efficiency of MAE can be poor when target compounds or solvents are non polar.
- It has initial high capital cost.

Applications

A. Extraction of plant constituents

Polyhenols from Green Tea [18].	Carotenoids from Paprika powders [19]
Glycyrrhizic acid from liquorice root [20]	Saikosaponins from *Bupleurum falcatum* roots [21]
Cocaine and benzoylecgonine from coca leaves[22]	Carnosic acid from Rosemary
Canola oil from Canola	Oil from evening primrose and borage seeds
Alkamides from *Echinacea purpurea* L. roots [23]	Pierine from black pepper
Oil from olive seeds [24,25]	Lipids from several oleaginous seeds [24,25]
Essential oil from Mint Leaves [26]	Essential oil from *Cuminum cyminum* and *Zanthoxylum bungeanum* [27]

Camptothecin from *Nothapodytes foetida* [28]	Essential oil from *Lippia alba* [29]
Sanguinarine and chelerythrine from *Macleaya cordata* [30]	Diterpenes like tanshinones from *Salvia miltiorrhiza* [31]
Geniposidic acid and chlorogenic acid from *Eucommia ulmodies* [32]	Solanesol from Tobacco leaves [33]
Embelin from *Embelia ribes* [34]	Artemisnin from *Artemisia annua* L. [35]
Furanocoumarins from *Pastinaca sativa* [36]	Coumarin and melilotic acid from *Melilotus officinalis* L. [37]
Phenolic compound and *Hypericum perforatum* and *Thymus vulgaris* [38]	Pigments from *Capsicum annum* [39]
Saponin from *Panax ginseng* [40]	Paclitaxel from *Taxus baccata* [41]
Oleuropein and related biophenols *Olea europeaea* [42]	Anthraquinones from *Morinda citrifolia* [43]
Microalgal pigments from *Cylindrotheca closterium, Dunaliella tertiolecta* [44]	Mucilage from the fruits of *Abelmoschus esculentus plant* [45]
Fat from cocoa powder and cocoa nibs [46]	Alpha-tocopherol: extraction from rice bran [47]
Flavonolignan from silybinin [48]	Saponins from chickpea *(Cicer arietinum L)* [49]
Flavonoids from Chinese herb *Radix puerariae (Ge Gen)* [50]	

B. Extraction of Other Organic Compounds from Soil and Sediments

Organochlorine pesticides, PCBs from soil [51, 52]	Phenolic compounds from soil [53,54]
PAHs, PCBs, neutral and basic compounds, phenols, pesticides from soil and sediment [55]	Triazines from soil [56,57]
Fungicides from soil [58]	Herbicides from soil [59]
PAHs, phenols, organochlorine pesticides, neutral and basic compounds soil and sediment[26,60]	PCDDs/PCDFs from soil [61]

PAHs from soil [62,63]	Organochlorine pesticides from soil and sediment [64]
Organochlorine and organophosphorus pesticides, arochlors from soil [65]	TPH from soil [66]
PAHs from coal [67]	Dioxins and furans from soil [68]

C. Miscellaneous

Packaging [69,70]	Defrosting [71, 72,73]
Browning [74,75,76]	Microbial inactivation [77]
Tempering [78]	Dehydration [79]
Blanching [80]	Aroma extraction [81]

TPH: Total Petroleum Hydrocarbons, PUF: Polyurethane Foam).
PAHs polycyclic aromatic Hydrocarbons, PCBs polychlorobiphenyls.

Commercially Available Microwave Extraction Models [5]

- CEM MES 1000
- CEM MDS 2100
- Milestone 1200 Mega Unit
- MLS 1200
- CEM MPS 1000
- Prolabo
- Prolabo microdigest 301.

Comparison with other methods of extraction [8]

Traditionally, plant materials were subjected to mechanical shear to release the volatiles in virgin state. There are today various closely controlled sophisticated methods of extraction from distillation, through leaching to super- or sub-critical solvent extraction. Among the various available methods, microwave assisted extractions are also well reported and show the highest promise.

Table No.1: The dielectric constants and dissipation factors for solvents commonly used are given in table no.1 [2, 8, 9]

S.No	Solvent	Dielectric loss (ε'')	Dielectric constant (ε')	Tanε
1	Methanol	15	24	6400
2	Acetone	11.5	20.7	5555
3	Ethyl Acetate	6	3.3	5316
4	Ethanol	1.6	7	2286
5	Water	12	80	1500
6	Hexane	0.00019	1.9	0.1

Table No.2: Boiling temperature and temperature under microwave at 175 *psig* of different solvents

S.No	Solvent	Boiling temperature ^{0}C	Temperature at 175 psig
1	Dichloromethane	40	140
2	Acetone	56	164
3	Methanol	65	151
4	Hexane	69	-
5	Ethanol	78	164
6	Acetonitrile	81	194
7	2- propanol	82	145
8	Petroleum Ether	40-80	-
9	Acetone: Hexane(1:1)	52	156

Table No.3: Comparison of microwave assisted extraction with other methods

S.No	Parameters	Soxhlet Extraction	Sonication	Microwave Assisted Extraction	Supercritical Fluid Extraction
1	Sample wt. (gms) *	5.00-10.00	5.00-30.00	0.50-1.00	1.00-10.00
2	Solvent	*	Acetone, Toluene	Hexane/Ethanol	CO_2
3	Solvent Volume(ml)	>300.00	300.00	10.00-20.00	5.00-25.00
4	Vessel Volume(ml)	500.00-1000.00	500.00	<100.00	5.00-25.00
5	Temprature(^{0}C)	Boiling Point	Room Temp.	40 to 100	50,200
6	Time	16hrs.	30min.	30-45sec	30-60min.
7	Pressure(atm.)	Ambient Atm.	Ambient Atm.	1.0-5.0	150.0-650.0

*Dependent on the concentration and type of sample.

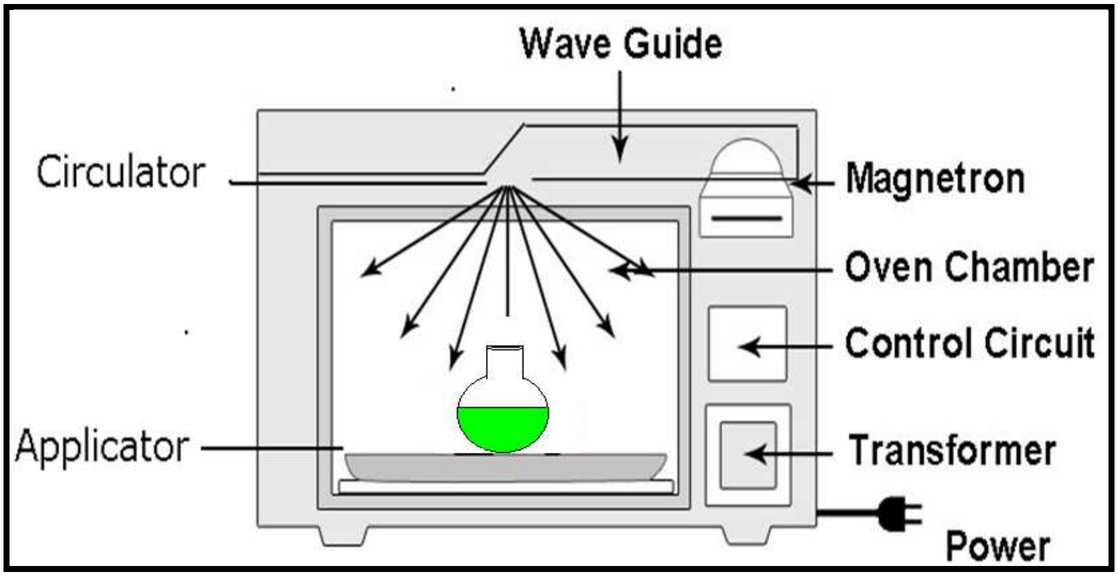

Figure No.1: Schematic diagram of MAE [10]

CONCLUSION

The obvious features of microwave technology like reduction of time for extraction of herbal constituents, chemical/pharmaceutical reaction, instantaneous and uniform heating, carrying out solvent free extraction has proved as a bonanza for the researchers involved in herbal drug extraction, isolation and development processes. 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.

ACKNOWLEDGMENT

I am cordially thankful to Dr. Abha Doshi (Principal, MET Institute of Pharmacy, Mumbai) for providing required facilities during writing paper.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Yashwant Malode. *et al.* Microwave assisted extraction of Phytoconstituents, *Asian Journal of Phytomedicine and Clinical Research*, 2(3), 2013, 73 - 86.