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An Overview of Microwave Assisted Extraction and its Applications in Herbal Drug Research

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ABSTRACT

The application of microwave assisted extraction process for isolation and extraction of phytoconstituents from plant material has gained an increasing importance. The techniques used for extraction since decades have the limitations of requiring longer extraction times, large solvent volumes and cause degradation of thermo labile components. In the present review an attempt is been made to emphasize upon the importance of Microwave Assisted Extraction (MAE) in herbal drug research. The merits of MAE which help to prove it of being better than conventional techniques are discussed. The technique requires less solvent volume, gives high and fast extraction performance and offers protection to thermo-labile constituents. The theory and basic principle of using microwave-energy for extraction, parameters affecting the microwave-extraction efficiency, its statistical optimization strategies and potential applications have also elucidated.

Key words: Microwave-assisted extraction, conventional extraction, optimization strategies, plant research, herbal

INTRODUCTION

Plants have been the source of potential therapeutic agents ever since mankind has evolved. Although several active phytoconstituents and high activity profile drugs have been discovered from plants but the quality and safety related problems of herbal drugs have still been a challenge for researchers. The major reasons for these drawbacks are the lack of high performance, reliable extraction techniques and methodologies for establishing the purity and standard for herbal medicines (Huie, 2002). It is due to these factors that the herbal medicines have still to find their way in order to be accepted in global market. In research related to discovery of new active phytoconstituents, extraction is one of the important steps as it is the starting point for the isolation and purification procedures. An individual plant may consist of several active phytoconstituents existing in abundance along with certain constituents of low activity profile. Thus, there arises a need for the development of extraction and analysis techniques with high performance (Smith, 2003). There has been a need for better and newer extraction techniques, in the herbal drug industry so that the extraction time and the cost of solvent consumption is decreased (Nyiredy, 2004).

Amongst the various traditional and conventional extraction techniques, Soxhlet extraction has been the most widely used. Soxhlet extraction serves not only as a technique for extraction of phytoconstituents but also as a reference to compare newer extraction techniques. Soxhlet extraction has a drawback of having longer extraction period and also the considerable amount of heat energy required for its process. The procedure being time consuming results in more labor-intensive steps and overall the number of samples that can be processed is reduced (Sanghi and Kannamkumarath, 2004; Luque-Garcia and Laque de Castro, 2004). Along with the above mentioned drawbacks, there is also a large consumption of organic solvents in the evaporation and concentration the extract, which poses hazards to the environment (Zuloaga and Madariaga, 1999; Luque and Garcia-Ayuso, 1998). A background about the theory of microwave heating and the principles underlying use of microwave energy for extraction of plant materials is been explained in this review. The crucial parameters which affect the extraction efficiency are discussed with the various optimization methods that can be applied to a process (Punt, 1998). The performance of the microwave extraction technique is also compared to other classical methods, thus explaining the merits of Microwave Assisted Extraction (MAE) technology for plant research (Pare and Belanger, 1993; Poole and Pare, 1994).

MICROWAVE THEORY

Microwaves heat up the molecules by dual mechanism of ionic conduction and dipole rotation (Smith and Carpentier, 1993). Non-ionizing electromagnetic waves positioned between the X-ray and infrared rays in the electromagnetic spectrum with frequency between 300 MHz to 300 GHz are called microwaves. The two types of oscillating perpendicular fields that generate microwaves are the electric field and magnetic field. Both The ionic conduction and dipole rotation are responsible for heating of substances (Bethe, 1947). When the microwaves interact with polar solvents, heating of substance is caused due to any one of the above mentioned phenomena, individually or simultaneously. The electrophoretic migration of ions under the influence of the changing electric field is called Ionic conduction (Anastassiades *et al*., 1992). If the solution offers a resistance to this migration of ions, a friction is generated and the solution is heated. The realignment of the dipoles of the molecule with the rapidly changing electric field is called Dipole rotation (Burfoot, 1967). At a frequency of 2450 MHz the process of heating occurs. The microwaves have a change in the electric component at a speed of 4.9×10^{4} times/ second. There is a generation of heat through frictional force when the solvent molecules try to align themselves with the changing electric field, but the molecules fail to realign themselves (Burkert *et al*., 1993). No heating occurs when the frequency is greater than 2450 MHz and the electrical component changes at a much higher speed (Carroll, 1970; Collier, 1985). No heating occurs when the frequency is less than 2450 MHz and the electrical component changes at a much lower speed. The inference from the above mentioned mechanisms is that only dielectric material or solvents with permanent dipoles get heated up under microwave (Corney, 1988). The value of dissipation factor (tan δ), is a measure of the efficiency with which different solvents heat up under microwave (Encinas, 1993; Fatuzzo and Merz, 1967).

The dissipation factor is given by the equation:

$$\tan \delta = \varepsilon'' / \varepsilon \quad (1)$$

where, ε'' indicates the efficiency of converting microwave energy to heat i.e., the dielectric loss. ε is the measure of the ability to absorb microwave energy, i.e., the dielectric constant (Feynman *et al*., 1966).

Due to their lower ε values polar solvents like ethanol and methanol undergo lesser microwave absorption compared to water but as the tan δ values for them remain increased, overall heating efficiency for both the solvents remain higher (Gledhill, 1985; Hartfield and Miller, 1988). Solvents like hexane and other less polar solvents like chloroform produce no heat as they remain transparent to microwave (Lamb and Retherford, 1947).

PRINCIPLE OF MAE

The target for heating in dried plant material is the minute microscopic traces of moisture that occurs in plant cells. The heating up of this moisture inside the plant cell due to microwave effect, results in evaporation and generates tremendous pressure on the cell wall. The cell wall is pushed from inside due to the pressure and the cell wall ruptures. Thus the exudation of active constituents from the ruptured cells occurs, hence increasing the yield of phytoconstituents (Gordy *et al.*, 1953; Goldman, 1962). The yields from plant matrices can also be enhanced if the plant matrix is soaked with solvents with higher tan δ value. The ether linkages of cellulose are hydrolyzed and are converted into soluble fractions within a few minutes. Higher yields can be obtained also by increasing the temperature, which facilitates faster penetration of solvent into the cell walls. It is observed that when one compares the Scanning Electron Micrographs (SEM) of untreated samples, heat-reflux extraction sample and MAE samples, there may be no structural difference between heat-reflux extraction and those of untreated samples, whereas there is a complete rupture of cell wall in the MAE treated samples (Jassie *et al.*, 1997). In extraction by heat-reflux, extraction takes place by a series of permeation and solubilization processes to bring the analytes out of the matrix and in the case of MAE the process is based on exposing the analytes to the solvent through cell rupture. Desorption of components from plant matrix occurs in MAE. The free water molecules present in the gland and vascular systems of plant matrices are heated and this causes a localized heating and expansion of their walls, thus resulting into the flow of constituents outside the cells. The dielectric susceptibility of the solvent and matrix is the factor that affects utilization of microwave energy in the process (Delhaes and Drillon, 1987). In order to prevent the degradation of thermo-labile components the sample matrix is immersed in a microwave transparent solvent like hexane (Devreese *et al.*, 1979).

INSTRUMENTATION

Microwave systems for extraction and laboratory use are available in two forms:

- Closed extraction vessels/Multi-mode microwave ovens and
- Focused microwave ovens

The extraction in a closed extraction vessel/ Multi-mode microwave oven is brought about by controlled pressure and temperature. Whereas in focused microwave assisted Soxhlet or solvent extraction (FMASE), as the name indicates, only the part of the extraction vessel containing the sample is focused for irradiation with microwave. Both the closed vessel type and the focused type are available commercially 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 is irradiated evenly (Moen *et al.*, 1991).

Principle components of a microwave device: The microwave extraction assembly comprises of four major components. A Microwave generator also called as the magnetron is responsible for generation of microwaves, a Wave guide is used to direct the propagation of microwave from the source to the microwave cavity. The third component is the applicator, where the sample holder along with the sample is placed. The next component is the Circulator which regulates the movement of microwaves only in the forward direction. In case of multi- mode systems the applicator is a closed cavity inside which a random dispersion of microwaves is brought about (Kristenson, 2006). Beam reflectors or turntables help in bringing about a uniform distribution of microwave energy inside the cavity, irrespective of the position of placement of sample. In case of focused microwave systems, the microwave waveguide acts as the applicator and the extraction vessel is placed directly in the cavity. Only a few inches of the bottom of the vessel is exposed to the microwaves and as glass is transparent to microwaves the upper region of the vessel remains cool. Thus an effective condensing mechanism is a result of such an inbuilt design of the microwave.

Advantages of closed-vessel systems: In a closed vessel system higher temperatures can be reached due to the increased pressure inside the vessel that raises the boiling point of the solvents used. There is considerably no loss of volatile substances in a closed system vessel and very less volume of solvent is required. There is no need of addition of solvent/s repeatedly and hence risk of air-borne contamination is lowered.

Drawbacks or limitations of closed-vessel systems: The disadvantage of a closed vessel system include the risk involved in use of high pressures and the limited amount of sample that can be processed. The material like PTFE (polytetraflouroethylene) that is used for vessel construction does not allow use of high temperatures and while using volatile compounds the vessel must be opened only after a cooling step to avoid loss of extracted volatile constituents.

Advantages of open-vessel and over pressurized/closed-vessel: Open vessels have an increased safety as they can be operated at atmospheric pressure and the reagents can be added at any time during the treatment. The material to be used for vessels of the oven may be PTFE, glass or quartz and excess of solvents can be removed easily. The major advantage of the instrument is the ability to process large samples without the requirement of a cooling process. The equipment can be obtained at a low cost and a complete automation with Open-vessel operation can be done.

Short comings of open-vessel systems: In open vessel systems the methods used are usually less precise than the ones used in closed-vessel systems. The open vessel system cannot process many samples simultaneously, while closed-vessel systems can handle about 10-14 samples at a time. To obtain extraction efficiencies similar to those of closed-vessel systems, the open vessel systems require longer extraction times.

FACTORS AFFECTING MAE

Properties of solvent (nature and volume): The basic factor which affects an extraction process is the choice of appropriate solvent. In an experimental setup the choice of the solvent will be based upon the solubility of the desired analyte, the solvent-matrix interaction and the property of the solvent to absorb microwaves (Chen *et al.*, 2008). The selected solvent should have a high selectivity towards the analyte of interest than the other matrix components and also a good compatibility with further chromatographic analytical steps. Solvents which are transparent to microwaves, do not heat up under microwave and those with good microwave absorbing capacity get heated up faster and enhance the extraction process. Hexane is an example of microwave transparent solvent whereas ethanol is an excellent microwave absorbing solvent. In order to get optimum extraction yields, researchers even use mixtures of high and low microwave absorbing solvents. As discussed earlier, we have known that inner glandular and vascular systems of the plant material have some moisture content. The rapid heating of these water molecules ruptures the cell walls and the phytoconstituents are released into the solvent environment. For extraction of volatile oils from several aromatic herbs Solvent free MAE (SFMAE) has been designed, where the moisture content within the plant matrix itself serves extraction and no solvent is used. The matrix: solvent ratio also plays an important role in extraction (Luque-Garcia and Luque de Castro, 2003). The solvent volume should be sufficient enough to immerse the plant matrix completely in the solvent throughout the entire irradiation process. In Conventional extraction methods, a higher ratio of solvent volume to solid matrix gives better extraction yields, whereas in case of MAE a higher solvent: matrix ratio may not give better yield due to non-uniform distribution and exposure to microwaves.

Time of extraction: For MAE, time period of heating is another important factor that influences the extraction process. The quantity of analyte extracted can be increased with an increase in the extraction time, but also there is an associated risk of degradation of thermo labile components (Al-Harahsheh and Kingman, 2004). Varying time periods are required for extraction of different matrices, but exposure of even few seconds have demonstrated to give excellent yields. The dielectric properties of the solvent influence irradiation time optimisation. Longer exposure with microwave absorbing solvents like water, may risk the future of thermo labile constituents.

Microwave-power: The factors microwave power and irradiation times influence each other to a great extent. In order to optimize a MAE procedure a combination of low or moderate power with longer exposure is a generally selected. Although due to the use of high power there is an associated risk of thermal degradation/deterioration, reports also exist which shows that varying of power from 500 W to 1000 W had no significant effects on the yield of flavonoids (Raner *et al.*, 1993).

Characteristics of matrix: The matrix characteristics like particle size and the nature of the material will affect the recoveries of the compounds to a considerable extent. Finer the particle size of the sample larger is the surface area and better is the penetration of microwaves (Huie, 2002). Finer particle size of matrix, may sometimes pose a problem during separation of matrix from solvent after the extraction process. Additional step of centrifugation or filtration can serve as a remedy to this issue. If the samples are pretreated with microwave absorbing solvents, the solvent gets impregnated in to the cells and on microwave irradiation dual heating of the solvent in the surrounding environment and in the cells takes place (Talebi, 2004). Some researchers apply the technique of pre-leaching extraction, (soaking of matrix with solvent prior to irradiation) to get better yields. An increase in the yield of tashinones was found after pre-leaching for 2 min at room temperature (Pan *et al.*, 2001). While working with closed vessel system care must be taken for the microwave power and temperature used, as both are closely related to each other. The increase in temperature results in an improved extraction efficiency as desorption of analyte from active sites in the matrix increases. At higher temperatures surface tension and solvent viscosity decreases and solvents have higher capacity to solubilize the analytes by improving sample wetting and matrix penetration respectively.

STRATEGIES OF OPTIMIZATION IN MAE PROCESS

In order to design an experimental technique with efficient working, it is necessary to optimize the conditions and strategy of designing. Similarly optimization strategies need to be applied to a MAE as it is influenced by many factors which interact with one another. An optimization strategy known as Orthogonal array, was given by Taguchi, where interaction of various factors on the experiment output can be studied. One can study the effects of multiple factors on the extraction process and solve problems in experiments by applying Taguchi approach to investigative trial experiments. Application of Taguchi method can improve the product and process quality (Roy, 2001). The best performance can be obtained by determining the appropriate combination of design factors in an experiment. The product/process can be made uninfluenced by uncontrollable factors and a consistent performance can be achieved. Several multivariate approaches like Response surface methodology-RSM are also used for optimization. RSM is application of statistical and mathematical techniques to develop, improve and optimize the design process. In application of RSM one has to study the unknown relation between a set of variables and the system output. In traditional and conventional approaches, a design is evaluated based upon changes in the response obtained from previous experimentation. A procedure can be improved by incorporating design criteria into a set of mathematical framework (Osborne and Armacost, 1996). Various statistical softwares available commercially that can be used for the optimization are MINITAB, STATGRAHICS, STATGRAPHICS PLUS and DOE software.

MICROWAVE VS. CONVENTIONAL EXTRACTION TECHNIQUES-APPLICATIONS

Several reports have been published on the comparison of MAE with other available conventional techniques (Thuery and Grant, 1992). As mentioned above, in most of the cases Soxhlet is used as control for comparison of any new extraction methodology. Efficiency, yields and recoveries can be enhanced by study of three crucial variables, namely irradiation time, irradiation power and the extracting solvent. MAE can also be used to study the changes in cell structure by damage due to microwaves using scanning electron microscopy and light microscopy. The use of microwaves finds its application not only in plant matrix extraction but also in Pesticides, organic pollutants, phenols, metals and polymers.

The review emphasizes the importance of MAE process as a novel tool for extraction of plant constituents in a cost effective and efficient manner. The authors also summarize the application of MAE to their research work carried out in the field of extraction and Isolation of phytoconstituents from plant products, wherein MAE has proved to be a boon. Extraction and isolation of a potent biomarker-Catechin was carried out from the leaves of *Anacardium occidentale* with the help of MAE. Various parameters affecting the extraction procedures were studied and optimized to design the method (Tatke *et al*., 2010a). A novel extraction procedure for isolation of achyranthine from *Achyranthes aspera* L. was developed (Tatke *et al*., 2010b). Several phytoconstituents have found to give an increase in their extractive yields when compared to their yields when subjected to conventional extraction techniques (Tatke *et al*., 2010c; Tatke, 2010d, e).

Pesticides: Several reports on MAE of agricultural and associated surface and ground water soils for triazines have been published (Steinheimer, 1993). Extraction of weathered soils was carried out to extract the fungicide hexaconazole. Microwave extractions of dacthal chlorpyriphos, chlorothalonil, diazinon, permethrin methoxychlor and azinphos-methyl have also been reported (Pylypiw and Incorvia, 1997). Extractions of imidazolinone herbicides and sulfonylurea herbicides have been reported (Stout *et al.*, 1996, 2000).

Persistent organic pollutants: Persistent Organic Pollutants (POPs) are hazardous contaminants that have detrimental effect on ecosystem and human health. Many contaminants like PAHs, base/neutral compounds, phenols and OCPs were extracted from various soils and sediments (Erickson, 1997; Zakharov, 1990). Solvents alone as well as in combination like that of acetone-hexane, have being used for MAE (Wania and Mackay, 1993). Extraction volume is one of the important parameters of extraction and more recent papers report 30 mL to be appropriate (Report, 1990).

Phenols: An excellent report for extraction of 14 phenol compounds from reference soils and sediments using acetone-hexane (1:1) as solvent has been reported (Lopez-Avila, 1994). Measurements of spiked and real soil samples for phenol and methyl phenol isomers have also been reported. The recoveries obtained in soils containing more than 5% charcoal for MAE was not found to increase significantly as compared to supercritical fluid extraction and derivatisation process (Llompart *et al.*, 1997).

Metals: In an extraction method for Methylmercury (MeHg) both spiked sediments and reference materials were used (Lorenzo *et al.*, 1999). In a MAE method using 10 mL toluene with 400 mL 6 M HCl at 120°C for 10 min, recoveries for certain metals produced equivalent results as compared to a conventional acid leaching extraction (Vazquez *et al.*, 1997). Determination of various metals like Pb, Zn and Cu from soils using sequential microwave extraction procedure has been reported (Campos *et al.*, 2000). Investigations for determination of heavy metals in sewage sludges by MAE procedures have been reported and they emphasize the replacement of traditional shaking methods by MAE (Perez-Cid *et al.*, 1999).

Polymers: Extractions of additives like polypropylene (PP) and polyethylene (PE) in the field of polymer research was carried out by Freitag and John using domestic ovens. From the powdered (1:1) drug and solvent ratio, about 90% of the substances were recovered (Freitag and John, 1990). The combinations of solvent mixtures like cyclohexane-IPA and acetone-heptane have also been reported to give good yields (Nielson, 1991). MAE is also been applied for extraction of pellets, freeze ground particles as well as freeze-ground polymers (Vandenburg *et al.*, 1999).

Pharmaceuticals and natural products: Pharmaceutical industry deals with many analytical procedures for preparation of samples, but there are very few reports involving the use of MAE. Food processing industry uses MAE for processing of vitamins in foodstuffs (Greenway and Kometa, 1994). Ergosterol was extracted from hyphae and spores using SFE and the process resulted in yields lower than MAE (Young, 1995). Aromatic compounds like terpenes were found to give good recoveries using MAE.

Miscellaneous: Reports of extraction of Phthalate esters from marine sediments and soils by MAE have been found (Chee *et al.*, 1996). Experiments have been carried out to trap airborne substances like lindane and metazachlor on solid adsorbents and then getting them desorbed by microwave heating (Scharf and Sarafin, 1995). Aromatic contaminants like toluene and chlorobenzene have been reported to be extracted from soil and water (Pare *et al.*, 1997). Determination of polyaromatic hydrocarbons by MAE has been reported in sediments (Haque, 1999; Cresswell and Haswell, 1999).

CONCLUSIONS

The analysis and extraction of plant matrices play an important role in development, modernization and quality control of herbal formulations. With the increase in the demand for herbal drugs in the global market, strict quality control guidelines are been laid down, which the researchers have to follow for herbal formulations. Stringent quality control requirements demand faster and better sample preparation techniques; so that within an estimated time frame conclusions are obtained with investigative experiments and scale up can be attempted. To serve this purpose need for techniques with attributes like efficiency and economy is felt. MAE can prove as a boon to natural product research, if applied to research in discovery of new and effective compounds from plants.

There are only few reports for application of MAE to herbal drug research. Hence, there is a need to boost the acceptance and utility of this novel extraction technique for its applications in herbal drug industry.

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