CHAPTER II

BACKGROUND AND LITERATURE REVIEWS

2.1 Morinda citrifolia

Morinda citrifolia, known commercially as Noni (Figure 2.1), is grown widely throughout the Pacific and is one of the most valuable sources of traditional medicines. All parts of the plant such as leaves, roots, fruit, and bark also have modern medicinal applications. Leaves have been used to treat bacterial infection and inflammation. Roots and bark are used for bacterial infection and termination of pregnancy. Green fruits are used for halitosis infection (bacterial/fungal), menstrual cramps, arthritis, and gastric and oral ulcers. Ripe fruits can be used for infection (bacterial/helminthic) and promotion of lactation and purgatives. One of the most important constituents responsible for many therapeutic properties of this plant is anthraquinones. The compounds can be found in the leaves, barks, but the highest amount of anthraquionones is found in the roots of *M. citrifolia*.

Figure 2.1 *Morinda citrifolia* plant

2.2 Anthraquinones

Anthraquinones is a group of compounds in the roots of *Morinda citrifolia* plants, which is highly soluble in organic solvents such as ethanol and dichlromethane. For a long time, the yellow extract of the roots had been used as dyestuff. Recently, the ability of the anthraquinones to protect human body from various kinds of diseases is becoming more recognized in scientific community, particularly as antioxidant, anti-bacterial, anti-viral, and anti-cancer agents. The basic chemical structure of anthraquinones is shown in Figure 2.2 and some examples of anthraquinones derivatives are shown in Figure 2.3
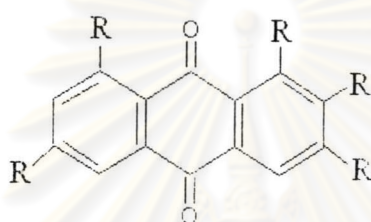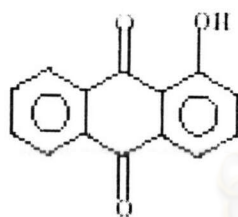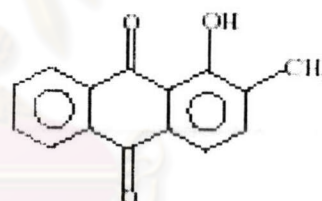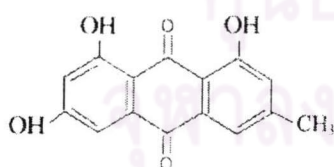

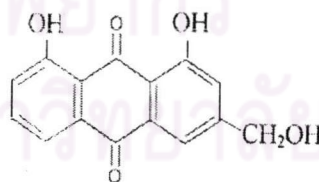Figure 2.2 Basic structures of anthraquinones

1-hydroxy-9,10-anthraquinone
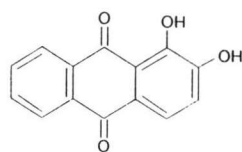(*Karami et al., 2004*)

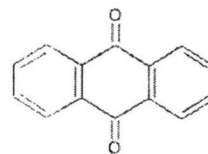1-hydroxy-2-methyl-9,10-anthraquinone
(*Karami et al., 2004*)

Emodin
(*Gow-Chin Yen, 2000*)

Aloe-emodin
(*Gow-Chin Yen, 2000*)

1,2-dihydroxyanthraquinone (Alizarin)	Anthraquinone
(*Gow-Chin Yen, 2000; Epstein, 2003*)	(*Gow-Chin Yen, 2000*)

Figure 2.3 Anthraquinones Derivatives

In this work, alizarin (1,2-dihydroxyanthraquinone) is used as standard compound. The properties of alizarin are shown in Table 2.1

Table 2.1 Properties of alizarin

Name	1,2-Dihydroxyanthraquinone
Formula	$C_{14}H_8O_4$
Molecular weight	240.2148
Melting point (°C)	287 – 289
Boiling point (°C)	430
Solubility at 25 °C (M)	2.5×10^{-6}

2.3 Mechanism of Extraction

Solvent extraction is the most widely used method for extraction of natural products. The process is sometimes called leaching. Generally, when plant materials and solvent come into close contact, the solvent diffuses through the tortuous passages of the solid matrix to solubilize the desired components (solutes). The dissolved solute then diffuses out of the matrix into the bulk liquid solvent. Two main factors that control extraction process are mass transfer (diffusion) and solute solubility. The mass transfer of this process depends on many factors such as size, porosity, and tortuosity of the solid sample matrix. The solubility of solid in the solvent used depends greatly upon the polarities of the solvent, and compounds of interest.

2.3.1 Solubility

The solubility of the desired constituents greatly influences the efficiency of extraction the process. It is a measure of solute concentration that is in equilibrium with

the solvent at a given temperature. Generally, it can be said that similar substances are soluble in each other and that the most appropriate extraction solvents or mixtures of solvents should have nearly the same polarities as those of the solutes. A property of a solvent that determines its polarity is the dielectric constant. Highly polar solvent like water has high dielectric constant of 80 at 25 °C. Water-like solvents generally have high dielectric such as methanol and ethanol have dielectric constants of 33 and 24, respectively. Non-polar hydrocarbons such as gasoline have low dielectric constants (ε =2 at 25 °C).

In general, the solute solubility depends on the interaction between the molecules of the solute and the solvent, which is dictated by the molecular structures and the activity coefficient of the solution. However, this is not always the case. Similar molecules such as Phenanthrene and anthracene isomers exhibit very different solubility in benzene (20.7 and 0.81 mol%, respectively). This example demonstrates that the solubility does not only depend on the activity coefficient but also the ratio of fugacity of pure solid and the standard state fugacity according to the following equation.

$$X = \frac{f_{pure-solid}}{\gamma \cdot f^{o}_{subcooled-liquid}} \tag{2.1}$$

where $f_{pure\ solid}$ is fugacity of solid at equilibrium and $f_{subcooled\ liquid}$ is standard state fugacity taken to be that of subcooled liquid.
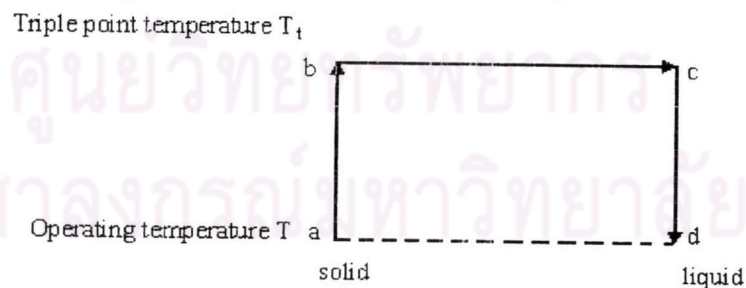

Figure 2.4 Path independence thermodynamic properties

$\Delta M_{a \to d} = \Delta M_{a \to b} + \Delta M_{b \to c} + \Delta M_{c \to d}$ (where M means any state property)!!!

The ratio of the two fugacities are relate to the change of Gibbs energy in going from the state of solid (denoted as state a) to subcooled liquid (denoted as state d) following form equation:

$$\Delta G = RT \, ln\left(\frac{f_{subcooled-liquid}}{f_{pure-solid}}\right) \tag{2.2}$$

The change of Gibbs energy is related to the change of enthalpy and the change of entropy by the following equation:

$$\Delta G_{a \to d} = \Delta H_{a \to d} - T\Delta S_{a \to d} \tag{2.3}$$

To calculate $\Delta H_{a \to d}$ and $\Delta S_{a \to d}$, it is more convenient to employ thermodynamic cycle as shown in Figure 2.4. Because the enthalpy and entropy are not dependent of the path, the $\Delta H_{a \to d}$ and $\Delta S_{a \to d}$ can be calculated from a $\to$ b, b $\to$ c and c $\to$ d.

$$\Delta H_{a \to d} = \Delta H_{a \to b} + \Delta H_{b \to c} + \Delta H_{c \to d} \tag{2.4}$$

The above equation becomes

$$\Delta H_{a \to d} = \Delta_{fus} H_{at\,T_t} + \int_{T_t}^{T} \Delta C_p dT \tag{2.5}$$

where $\Delta_{fus}H$ is the enthalpy of fusion, $\Delta C_p = C_{P\,liquid} - C_{P\,solid}$, the difference between the heat capacity of liquid and the heat capacity of solid, and T_t is the triple point temperature of the solute.

Similarly, the entropy change from a to d can be determined from the following equation

$$\Delta S_{a \to d} = \Delta S_{a \to b} + \Delta S_{b \to c} + \Delta S_{c \to d} \tag{2.6}$$

which can be written as follows:

$$\Delta S_{a \to d} = \Delta_{fus} S_{at\,T_t} + \int_{T_t}^{T} \Delta C_p dT \tag{2.7}$$

where $\Delta_{fus}S$ is entropy of fusion which is related to $\Delta_{fus}H$ by the following equation:

$$\Delta_{fus} S = \frac{\Delta_{fus} H}{T_t} \tag{2.8}$$

substituting Equations (2.7), (2.5) and (2.3) into Equation (2.2), and assuming that ΔC_P is constant over the temperature range $T_t \to T$, we obtain the following equation.

$$ln\left(\frac{f_{subcooled-liquid}}{f_{pure-solid}}\right) = \frac{\Delta_{fus} H}{RT_t}\left(\frac{T_t}{T} - 1\right) - \Delta C_p\left(\frac{T_t}{T} - 1\right) + \Delta C_p \, ln\left(\frac{T_t}{T}\right) \tag{2.9}$$

This equation gives an expression for the ratio of the fugacities, which can be substituted (2.8) in equation (2.9) to give the expression for the solubility as follows.

$$ln\ X = -\frac{\Delta_{fus}S}{R}\left(\frac{T_t}{T}-1\right) + \Delta C_p\left(\frac{T_t}{T}-1\right) - \Delta C_p\ ln\left(\frac{T_t}{T}\right) - ln\ \gamma \qquad (2.10)$$

As an approximation, the term of ΔC_P can be neglected and it is permissible to substitute melting temperature for triple point temperature Then, Equation (2.10) becomes:

$$ln\ X = \frac{-\Delta_{fus}S}{R}\left(\frac{T_m}{T}-1\right) - ln\ \gamma \qquad (2.11)$$

To represent the values at equilibrium or saturation, the superscript, SAT, is used and the equation becomes:

$$ln\ X^{SAT} = \frac{\Delta_{fus}S}{R}\left(1-\frac{T_m}{T}\right) - ln\ \gamma^{SAT} \qquad (2.12)$$

This equation shows that the solute solubility depends on temperature and intermolecular forces between solute and solvent as represented by the activity coefficient. For an ideal solution, the activity coefficient is equal to 1. For nonideal solution, activities coefficient is not equal to 1. Many solubility estimation methods such as Robbins chart, UNIFAC model, Hansen solubility parameter, and Margules equation can be used to estimate of the value of activity coefficient, and thus solubility. The knowledge of solute solubility in extraction solvents at various conditions is useful for the design of the process.

2.4 Methods of Extraction

2.4.1 Conventional Solvent Extraction

2.4.1.1 Maceration in organic solvent

Maceration involves soaking plant samples in organic solvent for an extended period of time up to 2 to 3 days. This method is simple and cheap but it requires long extraction time.

2.4.1.2 Soxhlet extraction

Soxhlet extraction involves removal of extracting compounds from plant materials by dissolution into a refluxing liquid solvent that is brought into contact with the plant materials. The apparatus used is shown in Figure 2.5. Typically, plant material is placed

in a thimble case which, in turn, is placed in a cavity that is gradually filled with the extracting liquid, resulted by condensation of vapors of the solvent from a distillation flask. A portion of liquid with dissolved solutes is pulled out of the cavity back into the distillation by means of a siphon. Thus, as the process continues, the concentration of the solute in the bulk liquid in the distillation flask continues to increase. This extraction is carried out until virtually all the extractable compounds are exhausted. This method has several advantages, the most important one is that the sample is continuous and repeatedly brought into contact with fresh portions of the solvent. Furthermore, the temperature of the system is close to the boiling point of the solvent, which increases the extraction kinetics of the system compared to solvent extraction at ambient temperature. On the other hand, the method also has several disadvantages including long extraction time, which reduces sample throughput. Furthermore, because heat is constantly supplied during this long extraction period, degradation of thermally labile compounds may occur.

2.4.2 Accelerated solvent extraction

Recently, more studies have been directed toward accelerating the process of conventional extraction. This can be done by several ways including using pressurized solvent extraction, ultrasound assisted extraction, and microwave assisted extraction.

2.4.2.1 Pressurized solvent extraction

Compared with normal fluid, fluids under high pressure and high temperature have special characteristics such as low viscosity and high diffusion coefficient. These characteristics enhance mass transfer in extraction process. Pressurized fluid, especially supercritical carbon dioxide and subcritical water have been focused as an environmentally benign extraction process. Because of low critical temperature (304.1 K) supercritical carbon dioxide has been mostly used for extraction process of natural products. Water has a high critical point temperature (647 K), thus water beyond the critical point is highly corrosive and is not suitable for the purpose of extraction. However, subcritical water, which refers to water at the temperature between the boiling point temperature and critical temperature, have been shown to have high potential for extraction of slightly polar compounds from plant materials. The review of this method and the study on its application for extraction of natural compounds inclduing

anthraquionones can be found elsewhere (*Pongnaravane, 2003 and Pitipannapong, 2003*).

2.4.2.2 Ultrasonic extraction

Ultrasound is a high frequency wave at more than 20.000 Hz, the frequency higher than what human can hear. It is produced by the vibration caused by a rapidly alternating electrical poteintial of a synthetic piezolelectric crystal. The crystal expands or contracts when an electrical potential is applied. As the ultrasound crystal vibrates, it sends an ultrasound wave that consists of alternating compression (positive pressure) and rarefraction (expansion or negative pressure) zones into the media (Figure 2.5).
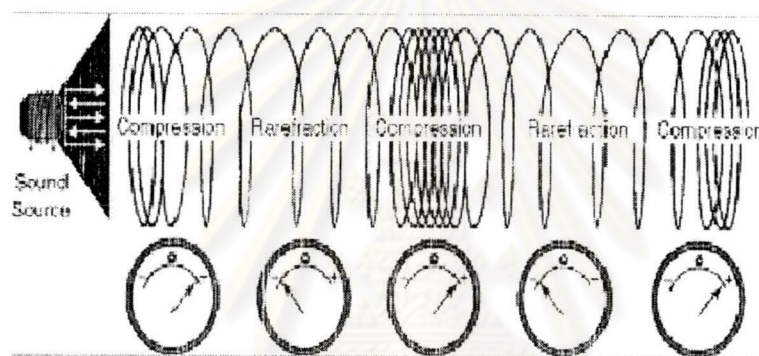

Figure 2.5 Characteristic of ultrasound
(reference www.variclean.nl)

Ultrasound has been applied for extraction of secondary metabolites from various plant tissues such as leaves of tea, mint, sage, chamomile, ginseng, arnica, and gentian (*Vinatoru et al., 1997; Mason et al., 1994; Salisova et al., 1997; Hromadkova et al., 1999; Li et al., 1994*). These investigators found that the use of ultrasound assisted methods can enhance the efficiency of product release generally by shortening the time of extraction processes. Most of these studies described that the enhanced efficiency of this technique is attributed to the phenomenon called acoustic cavitation which causes mass transfer and tissue disruption within the system. Cavitation phenomenon occurs in liquid media subjected to acoustic disturbances when the acoustic pressure during the rarefaction phase of the cycle reduces the total pressure to a specific threshold value. This causes the growing, collapsing, microstreaming of bubbles of existing gases in the medium. This threshold value of the acoustic pressure amplitude is a function of a

number of physical parameters that describe the state of the medium. These include temperature, pressure, frequency, kind and amount of dissolved gases, and previous history of the medium. In general, the threshold for cavitaiton increases with increasing frequency, sample, pressure, and sample viscosity, and decreases with increasing gas content and temperature.

Acoustic cavitation

Acoustic cavitation is an important phenomena occurring under the influence of ultrasound. As ultrasound passes through a liquid, the expansion cycles exert negative pressure on the liquid, pulling the molecules away from one another. If the ultrasound is sufficiently intense, the expansion cycle can create cavities or microbubbles in the liquid. This will occur when the negative pressure exceeds the local tensile strength of the liquid, which varies according to the type and purity of liquid. Once formed, small gas bubbles irradiated with ultrasound will absorb energy from the sound waves and grow during the expansion cycles and recompressed during the compression cycles. Cavity growth and recompression depend on the intensity and the frequency of the sound and the properties of the liquid.

At equilibrium, the pressure just outside the bubble surface and the pressure inside the bubble are related by the following equation:

$$P_V + P_G = P_H \pm P_A + \frac{2\sigma}{R_0}$$

Here, P_V is partial pressure due to vapor content and P_G is partial pressure due to gas content in the bubble. P_H is hydrostatic pressure or base pressure and P_A is acoustic pressure. The + and − signs prepresent the compression and rarefaction cycles of ultrasound respectively. σ is the surface tension and R_0 is the bubble radius. During bubble growth, the value on the right hand side is higher than that on the left hand side, while the opposite is true for compression phase.

There are two types of cavitation: transient cavitation and stable cavitation. In transient cavitation, filled gas and vapor bubbles undergo irregular oscillation, growing

several times their initial size, and implodes violently while producing intense local heating or "hot spots" having temperature about 5000 °C and pressure about 1000 atm (*Suslick, 1999; Thompson, 2000*) (Figure 2.6).

When cavitation collapse occurs in a liquid close to a solid surface, the dynamics of cavity collapse change dramatically. In pure liquid, the bubble retains its spherical shape during collapse, but close to the solid boundary, bubble collapse becomes nonspherical and produces high-speed jets of liquid (Figure 2.7). These liquid jets driving into the surface have been observed at the speed close to 400 km/h and the impact of the jets on the solid surface causes disruption of the surface. In ultrasonic extraction of plant tissue, this causes tissue disruption, and thus better contact between solvent and plant material. Furthermore, the local heating due to transient cavitaiton causes supersaturation of slightly soluble compounds, hence increases its solubility in the liquid medium (*Thompson et al., 2000*).

Stable cavitation, on the other hand, occurs when the bubble does not collapse completely during the compression, then at certain sizes it will begin to pulsate in time with the frequency of the acoustic vibrations. These stable cavitation bubbles induce around them a microstreaming which also enhance the mass transfer in the fluid.
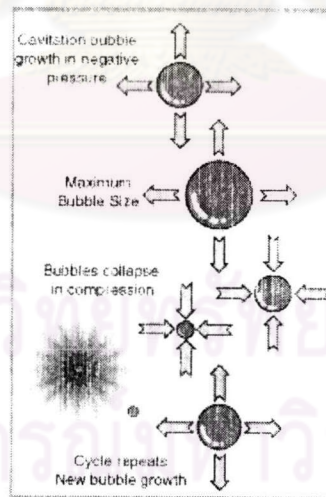

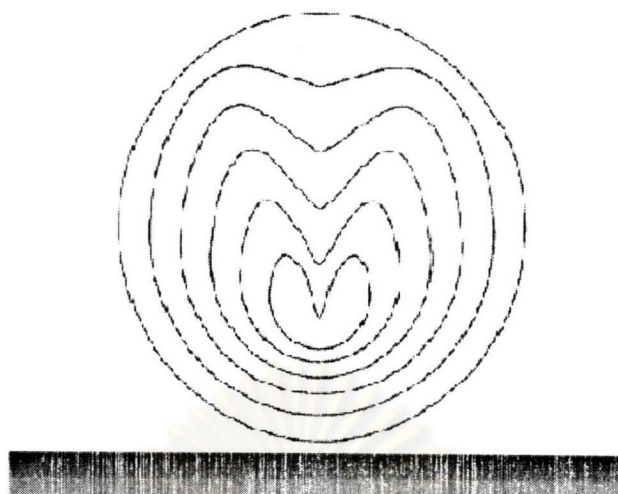Figure 2.6 Cavitation and implosion
(reference www.variclean.nl)

Figure 2.7 Formation of a liquid microjet during bubble collapse near an extended surface

Factors effecting acoustic cavitation

As mentioned, cavity growth and recompression depend on the characteristics of ulstrasound and the properties of the liquid through which the sound is irradiated. Specific factor effects are summarized here.

Acoustic pressure (or intensity)

At high intensities, a small cavity can grow rapidly during rarefaction cycles and collapsed violently during compression cycles.

Acoustic frequency

At high frequency bubbles grow and recompress rapidly, the bubble does not easily reach the critical size, thus at high frequency, the transient cavitation pressure threshold increase. In other words, transient cavitation does not occur easily. High frequency ultrasound usually results in stable cavitation.

Surface tension

Surface tension is related to the cohesive force of the liquid molecules or a tendency in which the liquid reduces its exposed space. Liquid with high surface tension has higher intermolecular interactions and thus in this liquid, ultrasonic cavitation is

more difficult to occurs. On the other hand, the degree of cavitation is more violent in this liquid.

Hydrostatic pressure

At high hydrostatic pressure, the bubble growth occurs less readily but once the cavity occurs, the collapse can be violent.

Liquid viscosity

When the liquid viscosity is high, the molecular interaction between the liquid molecules is also high. Caviation does not occur easily and degree of cavitation is also low.

Liquid temperature

When the temperature of the liquid increases, the molecular interaction between the liquid molecules decreases, thus increasing the chance for ultrasonic cavitation. Furthermore, the effect is similar to decreasing the viscosity of the liquid, thus the occurrence and the degree of cavitaion are increased at high liquid temperature.

Liquid vapor pressure

In liquid with high vapor pressure, bubbles can more easily grow and thus the cavitation occurs easily. However, the degree of violence during the collapse is low. On the other hand, liquid with low vapor pressure produces cavitation bubbles that implode with relatively greater intensity, but results in fewer bubbles and a higher cavitation threshold.

Existing gases

When liquid contains other existing gases, the partial pressures due to the gases within the bubbles enhance the bubble growth. On the other hand, the existence of these gases will cushion the collapse making it less violent.

Types of ultrasonic equipment

There are two types of ultrasound source generally used in biological and biochemical experiments: ultrasonic bath and ultrasonic probe (Figure 2.8). Ultrasonic

bath is designed for cleaning purposes commonly operated at frequencies between 20 kHz and 50 kHz. Frequencies above 50 kHz are more commonly used for high precision cleaning. Ultrasonic probe was first commercialized for biological cell disruption and lab-scale emulsification. The latter system consists of an acoustic horn conducting the sound energy from transducer to reaction medium. In our study, ultrasonic bath will be investigated for its effects on extraction of anthraquinones from root tissues of *Morinda citrifolia*.
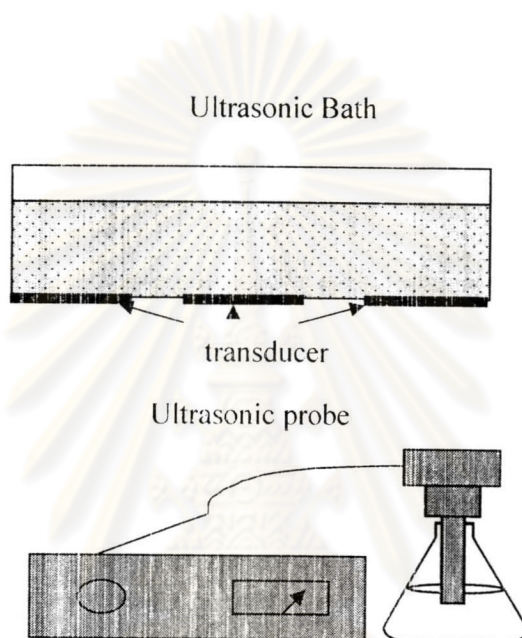
Figure 2.8 Diagram of ultrasound instruments.

2.4.2.3 Microwave extraction

Microwave is a form of electromagnetic radiation, which has a frequency range from 300 to 300,000 MHz. The most commonly used frequency for commercial microwave ovens is 2450 MHz, and has the energy output of 600-900 W. Other than its commonly known application for domestic food heating, microwave is also used for radar and telecommunications transmission.

Like all the other kinds of waves, microwave alternates between positive and negative cycles above and below a horizontal baseline. As the positive half cycle of the microwave travel through a polar liquid medium, the negative portion of the liquid molecules are attracted and attempt to align themselves with this positive field of energy.

Then, when the microwave energy alternates to the negative half cycle, the opposite occurs. The negative part of the molecules is repelled and the positive part would be attracted. In polar liquids, the liquid molecules are said to be able to absorb microwave. Other non-polar liquids or solids such as glass, paper, and plastics are transparent to microwave. Heating effect of microwave occurs when the molecules of the polar liquids could not align themselves quite quickly enough to the electric field of microwave. This is due to the high frequency of electrical field (2,450 Hz), the molecules do not have enough time to line up with the field during the positive wave cycle before the electric field is switched to the negative cycle. This discrepancy causes the microwave energy to be dissipated as heat. The lag between the resultant polarization and electric field is depicted in Figure 2.9.

The heating action of microwave has been employed in extraction of organic compounds to promote dissolution of the compounds of interest into the extraction solvent. The process has been shown to increase the yield and reduce the process time compared with other conventional solvent extraction methods.
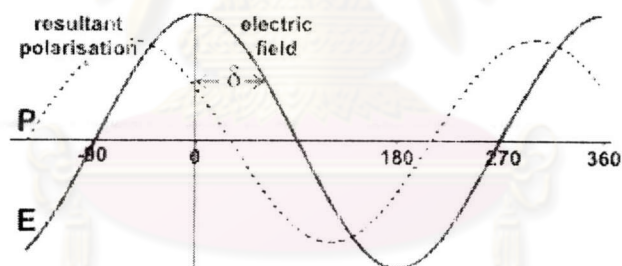
Figure 2.9 Resultant polarization and electric field

Like in any typical solvent extraction, the dominating factors that govern the extraction efficiency of microwave assisted extraction include the solubility of the compound being extracted in the solvent, the mass transfer kinetics of the product and the strength of the solute/matrix interactions. In microwave assisted extraction, the dissolution is enhanced by increasing the solvent temperature when under the influence of the electromagnetic field. Thus, the success of microwave assisted extraction can be said to be determined by two parameters defining the dielectric properties of the solvent. The first is the dielectric constant or relative permittivity, ε', which describes the

polarizability of the molecule to an electric field. It is a measure of the ability of a material to store electromagnetic radiation. The second is the dielectric loss factor, ε'', which measures the efficiency in which the absorbed microwave energy can be converted into heat inside a material when an electric field is applied. Thus, in choosing an appropriate solvent for microwave assisted extraction, one must consider the solubility, dielectric constant, and the dissipation factor, δ, which is the ratio of the dielectric loss constant and the dielectric constant, or expressed mathematically by:

$$\delta = \frac{\varepsilon''}{\varepsilon'} \qquad (2.13)$$

where ε'' is a dielectric loss factor (F/m) and ε' is a dielectric constant or relative permittivity (F/m).

To achieve the maximum heating effect, one must choose a solvent that has a high dielectric constant and dissipation factor. Dielectric constants and dissipation factors of some common solvents are shown in Table 2.2.

Table 2.2 Dielectric constant of some common solvent

Solvent	Dielectric constant (ε')	Dielectric loss factor (ε'')	Dissipation factor/ $\tan \delta \times 10^{-4}$
Acetone	20.7	11.5	5555
Methanol	32.7	20.9	6400
Water	78.3	12	1570
Ethanol	24.3	6.1	2500
Hexane	1.88	0.00019	.10
Ethyl Acetate	6.02	3.2	5316

Literature review

Ultrasonic extraction is employed for the extraction organic and inorganic material such as bioactive principles in organic compounds such as (*Vinatoru et al., 1997; Salisova et al., 1997*) and Ca, Mn, Mg and Zn (*Korn et al., 2001*) from plants material. Review of previous work on the environmental applications of ultrasound-assisted extraction is summarized in Table 2.3. Many investigators compare ultrasound-assisted extraction with classical extraction methods (*Hromadkova et al., 2003; Pappas et al., 2002; Salisova et al., 1997; Schinor et al., 2003; Kovacikova et al., 1999; Vinatoru et al., 1997; Albu et al., 2004*) and describe the effect of ultrasound intensity on extraction efficiency. All of these studies demonstrated that ultrasonic assisted extraction was more efficient than conventional solvent extraction and that as the intensity and temperature increased the efficiency of extraction increased. (*Li et al., 2004; Korn et al., 2001; Palma et al., 2002; Salisova et al., 1997; Rostagno et al., 2003*)

Apart from ultrasound assisted extraction, microwave assisted extraction has also been employed for the extraction of organic compounds from solid matrices such as soil, seed, food and feeds (*Salgo et al., 1986*). It was found to be more efficient than Soxhlet extraction for polar compounds. The review of previous work on environmental applications of microwave-assisted extraction is summarized in Table 2.4. In these works, the microwave assisted extraction was found to be more efficient than conventional solvent extraction (*Shu et al., 2003; Garcia et al., 2003; Liu et al., 2004; Carrillo et al., 2003*) as the method is able to reduce the extraction time compared to that required in the conventional solvent extraction. It is also able to yield a higher amount of the extracted compounds, especially when the power of microwave was increased. (*Li et al., 2004; Shu et al., 2003; Carrillo et al., 2003*).

Table 2.3 Review of previous investigation of ultrasonic extraction of plant materials

References	Material	Extracted	Conditions					Conclusions
			frequency & power	type of solvent	time(min)	temp.(°C)	
Salisova et al. (1997)	*Salvia officinalis*	pharmaceutically active compounds	37-42 kHz (130 W)	65% methanol	1, 3, 5, 12 , 24 hr	20 , 30, 50	stirring, at 30°C for 12 hr and the use of ultrasonic horn required less time than that of ultrasonic bath.
Rostagno et al. (2003)	soybeans	isoflavones	24 kHz (200 W)	methanol ethanol MeCN	10, 20	10, 60	The best condition was 20 min extraction at 60 °C with 50%ethanol.
Palma et al. (2002)	grapes	tartaric and malic acids	24 kHz (200 W)	methanol water	5, 15, 30	20, 30, 50, 70	Ultrasonic extraction gave the best yield. The optimum condition was 30 min extraction at 70 °C in 100 ml water using a 2 mm ultrasonic probe with 30 % power amplitude.

References	Material	Extracted	Conditions					Conclusions
			frequency & power	type of solvent	time(min)	temp.(°C)		
Korn et al. (2001)	vegetable (lettuce and cabbage)	Ca, Mn, Mg,Zn	47 kHz	de-ionised water	0, 10, 20, 30	25, 30, 35		Ultrasound offered a fast extraction means, and the best condition was extraction with 0.14mol/L HNO_3 for 10 min.
Elisandra et al. (2003)	Chresta exsucca Chresta scapigera Chresta sphaerocephala	steroids triterpenoids	60 Hz (125 W)	dichloromethane methanol	30	30		The use of ultrasound was effective for the steroids and triterpenes and was able to decrease the total time required for extraction.
Albu et al. (2004)	rosemary	carnosic acid	40 kHz	butanone ethanol ethyl acetate water:ethanol	15, 30, 45	47-53		Compare with various methods, ultra-sonication was more effective and dry materials gave higher yield than wet materials.

References	Material	Extracted	Conditions				Conclusions
			frequency & power	type of solvent	time(min)	temp.(°C)	
Wu et al. (2001)	ginseng root	ginseng saponins	38.5 kHz (810 W)	pure ethanol water-saturated n-butanol 10%methanol	1-2 hr	25-27	Sonication was more effective extraction method than classical Methods.
Hromadkova et al. (2003)	buckwheat	hemicellulose	20 Hz (100 W)	3% NaOH 5% NaOH	5, 10	40, 60	Ultrasound increased product yield and required short extraction time and the product structural and molecular properties were preserved.
Hromadkova et al. (2003)	roots of valerian	polysaccha-rides	20 kHz (600 W)	ethanol	2 hr for every 3 days	room temp.	The use of ultrasonic increased the yield of polysaccharides extracted from the roots of valerian.

Table 2.4 Review of previous work on the environmental and plant materials applications of microwave-assisted extraction

References	Material	Extracted	Conditions					Conclusions
			frequency & power	type of solvent	time(min)	temp.(°C)	power (W)	
Li et al. (2004)	E.ulmodies	geniposidic chlorogenic acid	2450 MHz (700 W)	methanol water	10, 30, 50 s	room temp	700 (90, 70, 50%)	The optimum extraction condition for geniposidic acid was 50% microwave power for 40 s with 80% aqueous methanol (20ml/g) and that for chlorogenic acid was 50% microwave power for 30 s with 20% aqueous methanol (20ml/g).
Liu et al. (2004)	sediment	endocrine disrupting chemical	2450 MHz (1200 W)	methanol ethyl acetate acetone hexane dichloromethane	5, 15, 25, 40	90, 110, 130	1200, 600	The most efficient ultrasonic extraction of the target compounds was achieved using methanol of 110°C and 15 min of holding time.

References	Material	Extracted	Conditions					Conclusions
			frequency & power	type of solvent	time(min)	temp.(°C)	power W	
Garcia et al. (2003)	soil	polychlorimated biphenyles	2450 MHz	n-hexane-acetone	90	-	150	Microwave extraction was more effective than conventional soxhlet extraction.
Carrillo et al. (2003)	solid wool	wool wax	2450 MHz (750 W)	toluene hexane acetone acetone/hexane	2-15	-	750 (10-100%)	The optimum ultrasonic extraction condition was extraction of 1.25g of sample with 90% power in 10 ml of hexane:acetone (1:1) for 8 min.

References	Material	Extracted	Conditions					Conclusions
			frequency & power	type of solvent	time(min)	temp.(°C)	power (W)	
Shu et al. (2000)	licorice root	glycyrrhizic	2450 MHz (700 W)	water ethanol ethanol-water ammonia s. ethanol-water-ammonia	15 s power on 15 s power off 3 s for heating 15 s for cooling	85-90	700	Microwave was suitable for fast extraction. Under appropriate MAE conditions extraction time of 4-5 min with 50-60% ethanol with 1-2% ammonia.
Shu et al. (2003)	ginseng root	ginsenosides	2450 MHz	water ethanol	1, 2, 5, 10, 15	-	30, 150	Fifteen minute microwave assisted extraction with 70% and 30% ethanol- water solutions, 150 W were more effective than conventional solvent extraction with 70% ethanol-water solutions for 10 hr.