CHAPTER II

LITERATURE REVIEW

Relevance of Herbal Medicine to Thailand

The World Health Organization estimates that a large proportion of the world's population relies heavily on traditional practitioners and medicinal plants in order to meet primary health care needs (WHO, 1999). Since safety and efficacy data are often not available for these drugs, the field of herbal medicine and medicinal plants requires additional research and further scholarship in the future.

The art of traditional Thai medicine was passed from father to son and many herbs were used to treat a single disease or symptom. The roots of traditional medical practices in Thailand include a mixture of Indian, Ayurvedic and Thai beliefs. During the Sukhothai period, over one hundred hospitals where traditional medicine was practiced, called Arogaya Sala, were built. This period of history began a long tradition of royal support for traditional medicine methods and techniques that would not be challenged until the nineteenth century. Later, during the Ayutthaya period, the first official textbook of Thai drug recipes was written titled "King Narai's Medicine". This textbook was the precursor for the books used today in programs of traditional medicine instruction. Traditional drug formulations were also recorded during the reigns of King Rama I, II and III when instructions were inscribed on stone tablets at the temples Wat Po and Wat Raj Oros.

King Rama V supported the production of the first medical textbook called "Tumra Paetsart Sonkau" and the national formulary of drugs called "Tumra Chabub Luang". These are two of the official books currently being used by the Thai Food and Drug Administration in their attempts to register traditional medicines.

Principle of Thai traditional medicine

The human body is composed of four elements such as earth, water, wind and fire. When the four elements of the body are in equilibrium, it will be healthy. In contrast, if an imbalance in these elements occurs or disability in any of the four

elements, a person will become ill. Moreover, the imbalance in the four internal elements and illness can also be due to an imbalance in the four external elements as well (Subcharoen, 2001; Sittitanyakit and Termwiset, 2004).

Traditional practitioners believed that herbal healing is based on the healer's belief in the power of nature and earth, and the ability to harness the power of plants and minerals for energy. Herbal treatment emphasizes adjusting the balance of the body elements using the health promotion approach (Vichai, et al., 2005). Moreover, Traditional medicine in Thailand is approached from a holistic perspective, with the idea that many factors contribute to a person's overall health and that a multitude of factors must be targeted to improve health as opposed to focusing exclusively on the narrow perspective of pathological disease.

Trikatu

Trikatu is given along with many other ancient medicines. Trikatu is basically a Sanskrit word that means "three spices". It is containing fruits of black pepper (*Piper nigrum*), long pepper (*Piper longum*) and the rhizomes of ginger (*Zingiber officinalis*) in equal proportions (Johri, et al., 1992). It is a common combination used in stimulating the digestive systems by helping in the production of right amount of gastric juices. It also promotes the secretion of hydrochloric acid from the gastric mucosa and relieves gaseous distention. Trikatu also works well on the respiratory system as it is mucolytic such as anti-mucus and expectorant due to its three pungent ingredients. It removes congestion from the lungs and works as rejuvenator for the respiratory system. It relieves cough, cold, edema, bronchitis, asthma and other breathing problems.

Trikatu is reported to be not only an anti-mucus and digestive power used to tone up gastric and respiratory function, but also to be useful in cases of obesity, high cholesterol, high triglycerides, hypothyroid, and various inflammatory conditions. Trikatu is considered to assist weight loss by maximizing metabolism along with balancing blood-glucose to decrease food cravings. Trikatu acts as an appetite suppressant while simultaneously increasing metabolism. It stimulates the production of many digestive enzymes, thereby decreasing gas, nausea, and constipation, excessive belching, bloating, and indigestion. By helping individuals to achieve and

maintain an ideal weight, this treatment also helps to decrease blood pressure and cholesterol levels. Sivakumar (2004) found that Trikatu reduces low density lipoprotein (LDL) and triglycerides level in the body. It increases high density lipoprotein (HDL) in the body thus improves dyslipidemia and prevents the risk of atherosclerosis and heart attacks. Hence 'Trikatu' can be used as a potent hypolipidemic agent and it can protect cardiac and aortic tissue as well.

The main purpose of Trikatu incorporation into numerous Ayurvedic formulations was most probably to enhance the efficacy of pharmacologically active ingredients. Several groups of investigators now attribute this bioavailability enhancing property of pepper to its main alkaloid, piperine (Kolen and Hussong, 1995). Piperine is an alkaloid with the molecular formula $C_{17}H_{19}O_3N$, which on hydrolysis with alkali gives piperic acid and piperidine (Atal, et al., 1975). The piperine content of pepper is directly proportional to its pungency.

The proposed mechanism for the increased bioavailability of drugs coadministered with Trikatu is attributed to the interaction of piperine with enzymes that participate in drug metabolism, such as mixed function oxidases found in the liver and intestinal cells (Bano, et al., 1987; 1991). Interaction with the synthesis of drug chelating molecules in the body such as glucuronic acid has also been proposed. Piperine may also interact with the process of oxidative phosphorylation, or the process of activation/deactivation of certain metabolic pathways, slowing down the metabolism and biodegradation of drugs. This action of piperine results in higher plasma levels of drugs, rendering them more available for pharmacological action (Atal, et al., 1981). These experiments revealed that Trikatu coadministered to rats orally with the drugs isoniazid increased the blood levels of isoniazid as compared to control animals who did not receive Trikatu (Karan, et al., 1998). In subsequent experiments, piperine has been proven to enhance the bioavailability of a number of drugs including rifampicin, diclofenac sodium and pefloxacin (Karan, et al., 1999; Lala, et al., 2004; Madhukar, et al., 2008)

Table 1 Use of three constituents of Trikatu from Ayurvedic literature

General name *Botanical name*	Family	Uses
Ginger *(Zingiber officinale)*	Zingiberaceae	Analgesic, Blood purifier, Carminative, Expectorant, Appetizer, Digestive, Stimulant, Sciatica, Lumbago, Rheumatism, Slip disc, Gout, Chronic arthritis, Muscular trouble, Asthma, Cough, Chronic bronchitis.
Black pepper *(Piper nigrum)*	Piperaceae	Stimulant, Carminative, Antacidic, Anti periodic, Stomachache, Digestive, Throat problem, Liver pain, Muscle pain, Piles, Spleen disorder, Leucoderma, Lumbago, Paralysis, Chronic fever, Vertigo, Arthritis, Urinary disorder, Flatulence, Indigestion.
Long pepper *(Piper longum)*	Piperaceae	Tonic, Alterative, Rejuvenator, Digestive, Carminative Cough, Chronic bronchitis, Sedative, Antidote to snakebite, Throat disorder, Anti-inflammatory, Anti-malarial, Dyspepsia, Lumbago, Splenomegaly.

Sources: Choudhury, et al., 2006

Black pepper

Black pepper is known by the botanical name of *Piper nigrum* and *Piperaceae* family. It might reach South East Asia many centuries earlier. In the seed of black pepper, it mainly contains piperine which is the major alkaloid constituent. It contains 5-9% of the alkaloids piperine and piperidine in volatile oil. Other than this, it also contains terpenes as pinene, limonene, caryophyllene and etc. (Tewtrakul, et al., 2000).

Pharmacology and clinical applications of black pepper

In the Ayurvedic descriptions, black pepper is described as a drug which increases digestive power, improves appetite, cures cold, cough, dyspnoea, diseases of the throat, intermittent fever, colic, dysentery, worms and piles (Atal, et al., 1975) also useful in tooth ache, pain in liver and muscle, inflammation, leucoderma and epileptic fits (Ayier and Kolammal, 1966; Kirtikar and Basu, 1975).

Several studies were reported both under *in vitro* and *in vivo* studies in experimental systems. Neither black pepper nor piperine produces any toxicity. In fact, it exerts liver protective action as evidenced by the studies of several workers. By enzyme modulation, piperine functions as a chemopreventive substance. Dalvi (1991) studied the hepatotoxic effect of piperine on rats by estimating the hepatic mixed function oxidases and serum enzymes as specific markers of hepatotoxicity. An intragastric dose of 100 mg/kg body weight caused an increase in hepatic microsomal enzymes after treatment (cytochrome p-450, cytochrome-b5, NADPH-cytochrome C reductase, benzphetamine N-demethylase, aminopyrine N-demethylase and aniline hydroxylase). On the other hand, an intraperitonial dose of 10 mg/kg did not produce any effect on the activities of the drug metabolizing enzymes. These treatments did not affect those serum enzymes which are specific markers of liver toxic conditions. Thus, piperine exerts significant protection against chemically induced hepatotoxicity.

Koul and Kapil (1993) reported that black pepper reduces *in vitro* and *in vivo* lipid peroxidation and prevents depletion of GSH and total thiols. Lipid peroxidation causes free radical production, which in turn produces tissue damage. GSH conjugates xenobiotics which are excreted out by subsequent glucuronidation. The hepatoprotective action of black pepper was compared with a reference compound, silymarin, a known hepatoprotective drug, and found that piperine has slightly lower activity. A dose dependent increase in the level of the hepatic biotransformation enzymes (glutathione-s-transferase, cytochrome p-450, cytochrome b-5, acid soluble sulfhydryl-SH) was obtained in a feeding experiment study using Swiss albino mice fed with a diet containing 1%, 2 % and 5 % (w/w) black pepper for 10 and 20 days (Singh and Rao, 1993). A lower level of glucuronidation due to the inhibition of the enzyme UDP-glucose dehydrogenase was observed by Reen (1993) in an *in vitro* study. While studying the hypoglycemic action of several plants, Tripathi (1979) reported that pepper fruits are devoid of any significant hypoglycemic action in rabbits. The aqueous extract of pepper leaves in a dose of 10–20 mg/kg led to a moderate increase in the blood pressure of dogs (Sridharan, et al., 1978). Piperine as well as AE (antiepilepsirine) is reported to have detoxifying qualities that may increase the bioavailability of other drugs, hence altering the pharmacokinetic parameter of the epileptic (Bano, et al., 1991).

Long pepper

Piper longum L. (Piperaceae), commonly known as "long pepper", is widely distributed in the tropical and subtropical regions of the world, throughout the Indian subcontinent and South East Asia countries (Kirtikar and Basu, 1980). Long pepper has been used in traditional remedies as well as in the Ayurvedic system of medicine against various disorders (Tripathi, et al., 1999). Dried unripe fruits are used as an alternative to tonic and a root is used in chronic bronchitis, cough and cold (Maitreyi, et al., 2010).

The fruit of long pepper contains a large number of alkaloids and related compounds. Piperine is the major and active constituent of long pepper. It contains 3-5% in long pepper. the most abundant of which is piperine, together with methyl piperine, piperonaline, piperettine, asarinine, pellitorine, piperundecalidine, piperlongumine, piperlonguminine, refractomide A, pregumidiene, brachystamide, brachystamide-A, brachystine, pipercide, piperderidine, longamide and tetrahydropiperine, terahydropiperlongumine, dehydropipernonaline piperidine. Piperine, terahydropiperlongumine, trimethoxy cinnamoyl-piperidine and piperlongumine have been found in the roots of long pepper (Kirtikar and Basu, 1980; Rastogi and Malhotra, 1993).

Pharmacology and clinical applications of long pepper

In view of commercial and medical importance of long pepper, several works have investigated the species chemically and also pharmacologically. An amide namely dehydropipernonaline having coronary vasorelaxant activity was isolated from the fruit of long pepper (Umeyama, et al., 2006). Methanolic extract from dried fruits, roots and nutgalls of *Piper longum*, *Piper sarmentosum*, *and Quercus infectoria* respectively, were examined for their spasmolytic activities using isolated rat or guinea pig ileum and compared with a reference anti-diarrheal drug such as loperamide and an L-type calcium channel blocker such as verapamil. The effect of methanol extract of long pepper fruits was evaluated on adriamycin-induced cardiotoxicity (i.e., biochemical changes, tissue peroxidation damage, and abnormal antioxidant levels) in Wistar rats. Histopathological studies of the heart revealed degenerative changes and cellular infiltration in rats treated with Adriamycin; however, pretreatment with long pepper reduced the intensity of these lesions. The

results indicated that long pepper offered significant protection against Adriamycin-induced oxidative stress and reduced cardiotoxicity by virtue of its antioxidant activity (Wakade, et al., 2008).

The antihyperglycemic and antilipidperoxidative effects of ethanolic extract of long pepper dried fruits in alloxan-induced diabetic rats were studied (Dhar, et al., 1968). The blood glucose level, carbohydrate metabolizing enzymes and the status of lipid peroxidation and antioxidants were assayed using specific colorimetric methods. Oral administration of dried fruits has shown significant anti-hyperglycemic, antilipidperoxidative and antioxidant effects in diabetic rats comparable to that of the standard reference drug glibenclamide (Manoharan, et al., 2007). Methyl piperine significantly inhibited the elevation of total serum cholesterol, and the total cholesterol to HDL-c ratio, in rats fed with a high cholesterol diet (Wang, et al., 1993). The unsaponificable fraction of the oil of long pepper also significantly decreased total serum cholesterol and hepatic cholesterol in hypercholesterolemia mice (Wu and Bao, 1992). The ethanol extract of the long pepper fruit yields piperlonguminine, piperine, and pipernonaline as the main antihyperlipidemic constituents. They exhibited appreciable antihyperlipidemic activity *in vivo*, which was comparable to that of the commercial antihyperlipidemic drug simvastatin (Jin, et al., 2009). Bioassay-guided isolation of chloroform extract of the fruits of long pepper showed an *in vitro* diacylglycerol acyltransferase (DGAT) inhibitory activity, led to isolation of a new alkamide together with four known alkamides. Pharmacological inhibition of acyl CoA: diacylglycerol acyltransferase by alkamides emerged as a potential therapy for the treatment of obesity and type 2 diabetes (Lee, et al., 2006). Guineensine, isolated from chloroform extract inhibited acyl-coenzyme A: cholesterol acyltransferase (ACAT) activity in a dose dependent manner (Lee, et al., 2004). Piperlonguminine has the effect of regulating lipid metabolism by reduce the serum total cholesterol, triglyceride and LDL-c levels. The mechanism is likely related to increasing LDL receptor mRNA expression and decreasing apolipoprotein B mRNA expression (Ma, et al., 2008).

Long pepper is generally assumed to be safe in moderate doses. A single oral dose in experimental animals (3 g/kg body weight) and chronic toxicity studies for 90 days revealed no adverse effects. Studies of isolated constituents in mice reported

LD_{50} values of piperine, piperlongumine and piperlonguminine as 56.2 ± 3.0, 110.1 ± 7.8, and 115.3 ± 9.5 mg/kg body weight, respectively. Thus, acute toxicity studies did not show any mortality or morbidity when administered to animals during pharmacological study (Chanda, et al., 2009). The ethanolic extract of long pepper fruits reduced the elevated levels of glutathione pyruvate transaminase (GPT), alkaline phosphatase (ALP), and lipid peroxidation (LPO) in liver and serum of radiation treated mice. But, long pepper fruits extract also increased the reduced glutathione (GSH) production to offer the radioprotection (Sunila and Kuttan, 2004). The fruit extract improved the regeneration process by restricting fibrosis, but offered no protection against acute damage or against cirrhotic changes in rodents (Rage, et al., 1984). Treatment with the ethanol extract of long pepper inhibits liver fibrosis induced by carbon tetrachloride (CCl_4) (Christina, et al., 2006). Piperine exerted a significant protection against tertbutyl hydroperoxide and carbon tetrachloride hepatotoxicity by reducing both *in vitro* and *in vivo* lipid peroxidation, enzymatic leakage of GPT and ALP, and by preventing the depletion of GSH and total thiols in the intoxicated mice. Piperine showed lower hepatoprotective potency than silymarin (Indu, et al., 1993).

Ginger

Ginger (*Zingiber officinale Roscoe*, Family *Zingiberaceae*) has a long history for its health benefits and is used as a traditional medicine in the Asian countries. The dried of rhizome extract is one of the main ingredients in Trikatu. The main active compounds are the 6-gingerols and 6-shogaols as well as some phenolic derivatives (Govindarajan, 1982).

Pharmacology and clinical applications of Ginger

Ginger is one of the herbal spices, it is commonly safe to use and proved to be effective against various human ailments. Ginger extract showed antioxidant (Stoilova, et al., 2007; Ahmed, et al., 2008), anti-cancer (Shukla and Singh, 2007), anti-inflammatory (Young, et al. 2005; Habib, et al., 2008) and antithrombotic properties (Thomson, et al., 2002).

Many studies have focused on the effect of ginger on lipids lowering in animals and humans. The results of those studies (Table 2) showed that ginger significantly reduces serum triglyceride and cholesterol level in rats (ElRokh, et al.,

2010; Giri, et al. 1984; Sanjay, et al., 2004; Fuhrman, et al., 2000; Thomson, et al., 2002). Similar effect was also found in cholesterol fed rabbits (Sharma, et al., 1996; Ahmed, et al., 2000; Bhandari, et al., 1998). The lipid lowering of ginger may be a result of elevated the activity of hepatic cholesterol-7a-hydroxylase in the liver, the rate-limiting enzyme in bile acids biosynthesis, or down-regulated HMG-CoA reductase expression. Thereby, ginger could stimulate cholesterol conversion to bile acids, resulting in elimination of cholesterol from the body (Srinivasan and Sambaiah, 1991; Tanabe, et al., 1993; Srinivas, et al., 2009).

In some cases, the hypoglycemic and antidiabetic potential of ginger were investigated and reported to be variable (Table 2). The ethanolic extract has also been shown to lower blood glucose in normal rabbits (Mascolo, et al., 1989) and diabetic rats (Ahmed and Sharma, 1997; Ojewole, 2006; Al-Amin, et al., 2006). However, the ethanolic extract of ginger was reported to elicit no effect on blood glucose in normal rats (Weidner, et al., 2000). Combination therapy often takes advantage of complementary effects of different agents. The combination of garlic and ginger is more effective in reducing serum lipids and blood glucose (Ahmed and Sharma, 1997). Similarly, the combined effect of ginger extract and atorvastatin can reduce cholesterol in hypercholesterolemic rats which are susceptible to liver function abnormalities (Gehan, et al., 2010).

Ginger is usually regarded as safe in small amounts, or approximately 2-4 grams per day (Ernst and Pittler, 2000; Chandra et al., 2002; Bryer, 2005), although certain precautions should be borne in mind. In animal experiments, ginger has not shown any teratogenic effect when applied during pregnancy (Weidner and Sigwart, 1998). Interestingly, Wilkinson (2001) found that ginger tea applied orally to rats was not materno-toxic, but increased fetal loss, although augmenting growth in the surviving fetuses. In a human study, ginger ingested in various forms during pregnancy did not appear to increase the rates of major fetal malformations (Portnoi et al., 2003), and showed neither teratogenic effects (Fischer-Rasmussen, et al., 1990) nor mutagenic activity (Sivaswamy, et al., 1991).

Table 2 Previous studies of ginger extract for lipid lowering and reducing blood glucose

Ginger	Species	Serum					Reference
		TG	TC	LDL	HDL	Glucose	
200 mg/kg	Rabbit (HCD)	down	down	down	up	-	Bhandari, et al., 1998
200 mg/kg	Rat (diabetes)	down	down	-	up	down	Uma, et al., 2005
500 mg/kg	Rat (diabetes)	down	down	-	-	down	Zainab, et al.,2006
100, 200, 400 mg/kg	Rat (HFD)	down	down	down	normal	down	Srinivas, et al.,2009
100, 200, 400 mg/kg	Rat (HCD)	down	down	down	up	-	ElRokh, et al.,2010
250,500,1,000 mg/kg	Rat (HFD)	down	down	down	normal	down	Malik, et al., 2011

HCD, High cholesterol diet; HFD, High fat diet; TG, Total Triglyceride; TC, Total cholesterol; LDL, Low-density lipoprotein and HDL, High-density lipoprotein

Biochemical profiles for assessing Liver Function

The biochemical profile is the most common forms of the database for most diagnostic investigations. Many biochemical parameters tend to have specificity for an organ and/or a limited range of pathological processes. Interpretation of diagnostic biochemical patterns requires an understanding of the pathological implications of each abnormal result. Together with the normal results, these form a pattern which reflects one or more underlying disease process. Investigative biochemical profiles are designed to provide all the data necessary for a broad investigation of internal disease. Profiles with limited data are best used for monitoring an established diagnosis for which the results of a more wide ranging profile have already been obtained.

Transaminase

Aspartate aminotransferase (AST) is presented in many tissues and is useful in evaluating muscle and liver damage in body. It is an absolute prerequisite to eliminate extra-hepatic tissue damage as a possible source of serum AST when evaluating the enzyme related to the liver function. In combinations with the physical examination and history, the evaluation of other serum enzymes should aid in differentiating the source of increased AST levels. AST is presented in both the cytoplasm and mitochondria of hepatocytes and will elevate in states of altered membrane permeability. In such cases, levels are expected to be less than in states of frank necrosis, when both cytoplasmic and mitochondrial enzymes are released. Alanine aminotransferase (ALT) is considered to be liver specific. This enzyme is presented in high concentrations in the cytoplasm of hepatocytes. Plasma concentrations increase with hepatocellular, damage/necrosis, hepatocyte proliferation, or hepatocellular degeneration. ALT is a cytoplasmic enzyme, and is considered to be liver specific in body.

Elevation of serum levels of both AST and ALT can occur with states of altered hepatocellular membrane permeability. Because ALT is located only in the cytoplasm, serum levels tend to be relatively higher than AST, as a result of membrane leakage from the hepatocyte. Mitochondrial enzymes are less likely to be released with most of the conditions which result in increased membrane permeability. The magnitude of both AST and ALT elevations in serum is generally related to the

number of hepatocytes affected. However, the level cannot be used to predict either the type of lesion, or whether cell damage is reversible (leakage) or irreversible (frank necrosis). In fact, focal necrosis may yield a lower concentration of both AST and ALT than severe, transient hypoxia in which all cells may be affected resulting in a potentially reversible alteration in membrane permeability and diffuse enzyme leakage. Equally increases in ALT and AST may be relatively mild in cases of severe cirrhosis/fibrosis of the liver since there is no ongoing hepatocellular damage.

Another factor to be considered when interpreting AST and ALT levels is the rate of clearance from plasma. Both enzymes are molecularly too large to permit glomerular filtration and are primarily stereo chemically denatured. The half-life of these enzymes is approximately 2-4 days and some prognostic information may be gleaned with this knowledge. Thus, if an elevated serum level falls by 50% after 2-4 days, the prognosis is generally more favorable than if the enzymes remain persistently elevated or are only slightly decreased after this time period. Finally, it must be remembered that ALT is liver specific and AST is presented in many tissues, but because of organ size and relative enzyme content, it may be used with care to evaluate liver disease.

Lipid Profile

The lipid profile is a group of tests that are often ordered together to determine risk of coronary heart disease. A lipid profile measures total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides. They have been shown to be good indicators of whether someone is likely to have a heart attack or stroke caused by blockage of blood vessels or hardening of the arteries (atherosclerosis).

Normally, lipids are insoluble in water but are soluble in alcohol and other solvents. When dietary fats are digested and absorbed into the small intestine, they eventually reform into triglycerides, which are then packaged into lipoproteins. Dietary fats, including cholesterol, are absorbed from the small intestines and transported into the liver by lipoproteins called chylomicrons. Chylomicrons are large droplets of lipids with a thin shell of phospholipids, cholesterol, and protein. Once chylomicrons enter the bloodstream, an enzyme called lipoprotein lipase breaks down the triglycerides into fatty acid and glycerol. After a 12 to 14 hour-fast, chylomicrons

are absent from the bloodstream. Thus, individuals who are having a lipid profile done should fast overnight to ensure that chylomicrons have been cleared.

The liver removes the chylomicron fragments, and the cholesterol is repackaged for transport in the blood in VLDL, which eventually turns into LDL. LDL consists mainly of cholesterol. Most LDL particles are absorbed from the bloodstream by receptor cells in the liver. Cholesterol is then transported throughout the cells. Diets high in saturated fats and cholesterol decrease the uptake of LDL particles by the liver. LDL particles are also removed from the bloodstream by scavenger cells, or macrophages, which are white blood cells that bury themselves in blood vessels such as arteries. Scavenger cells prevent cholesterol from reentering the bloodstream, but they deposit the cholesterol in the inner walls of blood vessels, eventually leading to the development of plaque. HDL is a separate group of lipoproteins that contain more protein and less cholesterol than LDL. HDL is produced primarily in the liver and intestine, and it travels in the bloodstream, picks up cholesterol, and gives the cholesterol to other lipoproteins for transport back to the liver (Figure 1).

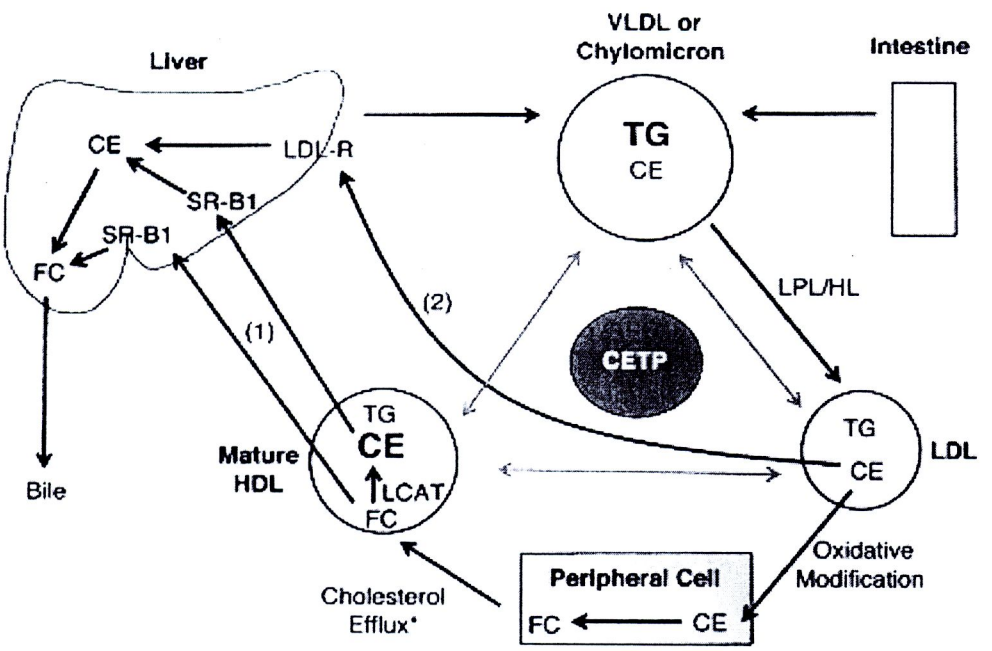

Figure 1 Lipid transport.

Sources: Philip and John, 2006

A lipid profile should be done after a nine to twelve hour-fast without food, liquids or medication. If fasting is not possible, the values for total cholesterol and HDL may still be useful. If total cholesterol is 200 mg/dl or higher or HDL is less than 40 mg/dl, the individual will need to have a follow-up lipoprotein profile done to determine LDL and triglyceride levels. Depending on the physician's request, the lipid profile may include the ratio of cholesterol to HDL. This ratio is sometimes used in place of total blood cholesterol. The ratio is obtained by dividing the HDL level by the total cholesterol.

The National Cholesterol Education Program, the American College of Cardiology, and the American Heart Association recommend diet and lifestyle modification as the first line of defense against abnormal blood lipids. These recommendations include a diet low in total fat, saturated fat, and cholesterol; a diet high in fiber; weight loss or weight management; increased physical activity; smoking cessation; increased intake of plant sterols (e.g., margarines and salad dressings made with soybean sterols) and daily use of a low-dose aspirin. Cholesterol-lowering drugs works to lower LDL by reducing cholesterol synthesis and by binding bile acids in the small intestines. However, there are possible side effects to these drugs that patients should be aware.

Table 3 Optimal, borderline, and high levels for each lipid component

Element	Optimal	Borderline	High risk
LDL Cholesterol	<100 mg/dl	130–159 mg/dl	160+ mg/dl
HDL Cholesterol	>60 mg/dl	35–45 mg/dl	<35 mg/dl
Triglycerides	<150 mg/dl	150–199 mg/dl	>200 mg/dl
Total Cholesterol	<200 mg/dl	200–239 mg/dl	>240 mg/dl
Cholesterol to HDL Ratio	<4	5	>6

Source: The National Cholesterol Education Program, the American College of Cardiology, and the American Heart Association

Proteomics

Proteomics is the study of all proteins synthesized in a cell or an organism. It is the newly developed science for the study of proteins. Genomics was used as a means to improve our understanding of disease with the hope that a comprehensive knowledge of an organism genetic makeup would lead to more efficient drug discovery. Although useful, DNA sequence analysis alone does not lead efficiently to new target identification, since one cannot easily infer the functions of gene products and protein pathways from DNA sequence. Most large pharmaceutical companies now have a proteomics oriented biotech or academic partner or have started their own proteomics division. Common applications of proteomics in the drug industry include target identification and validation, identification of efficacy and toxicity biomarkers from readily accessible biological fluids, and investigations into mechanisms of drug action or toxicity. These proteins may serve as potential therapeutic targets and or may be used to treat patients for clinical (Seyed, 2009).

Protein Expression Profiling by gel electrophoresis is a primary analysis tool used to characterize the expression of proteins in the pharmaceutical industry. Large numbers of proteins, mostly protein variants, are identified with these methods, and highly expressed proteins are easily located. The resulting differences in protein expression due to treatment with various stimulating factors are the basis for comparative gel electrophoresis maps. Proteins or peptides after separation by electrophoresis are identified by determining the sequence of amino acids comprising them. Traditionally, this was done by Edman degradation, which determined one amino acid at a time from the N-terminus of the proteins or peptides. However, the process of the identification of proteins was revolutionized by the development and application of the mass spectrometer in conjunction with the advances in genomics and bioinformatics, which made the gene and protein data available for the assignment of a particular peptide sequence to a protein and to the encoding gene.

Mass spectrometry

Mass spectrometry-based formats and industry preferences are still evolving. Proteomics applications that involve LC/MS are at similar stages of growth as drug metabolism applications during the late 1980s and early 1990s. To date, the

predominant application involves the qualitative analysis of proteins via automated database searching (i.e., protein expression profiling). Sensitive and accurate mass spectrometry approaches for quantitation of proteins appear to be destined for major advances. Mass spectrometry is unsurpassed capacity for accurate protein identification and quantitation. The principles of the mass spectrometer originated that molecules can be ionized, and the ionized molecules can be separated based on their mass-to-charge ratio by applying a magnetic force. The positively charged particles are the ionized molecules, whereas the negatively charged particles are the electrons. The results yield information about their molecular weights and chemical structure.

Components of the Instrument

A spectrometer consists of the following five major components: a port or device for the introduction of sample into the machine, a device for ionization of molecules, an analyzer for the separation of ionized molecules on the basis of their mass to charge (m/z) ratio, a detector that monitors the presence of the separated ions and records them, and a high vacuum system to allow free movement of ions within the spectrometer

Ion sources

In a mass spectrometer the role of the ion source is to create gas phase ions. Analyte atoms, molecules, or clusters are transferred into gas phase and ionized either concurrently (as in electrospray ionization) or through separate processes (as in the glow discharge). The choice of ion source depends heavily on the application. So called soft ion sources can produce intact ions of large fragile molecules.

Electrospray ionization (ESI) was first introduced by Dole and coworkers (1968) and coupled to MS by Yamashita and Fenn (1984). In ESI, a sample is vaporized by high voltage and then ions are generated as the solution of proteins or peptides is forced through a fine syringe. The sample is dissolved in a polar and transported through a needle placed at high positive or negative potential (Yamashita, et al., 1984; Aleksandrov, et al., 1984; Fenn, et al., 1989). The high electric potential (1 to 4 kV) between the needle and nozzle causes the fluid to form a Taylor cone, which is enriched with positive or negative ions at the tip. A spray of charged droplets is ejected from the Taylor cone by the electric field. The droplets shrink through evaporation, assisted by a warm flow of nitrogen gas passing across the front of the

ionization source (Figure 2). Ions are formed at atmospheric pressure and pass through a cone shaped orifice, into an intermediate vacuum region, and from there through a small aperture into the high vacuum of the mass analyzer. ESI has been used in conjunction with all common mass analyzers. The exact mechanism of ion formation from charged droplets has still not been fully elucidated and there are different theories proposed (Mora, et al., 2000; Iribarne, et al., 1976).

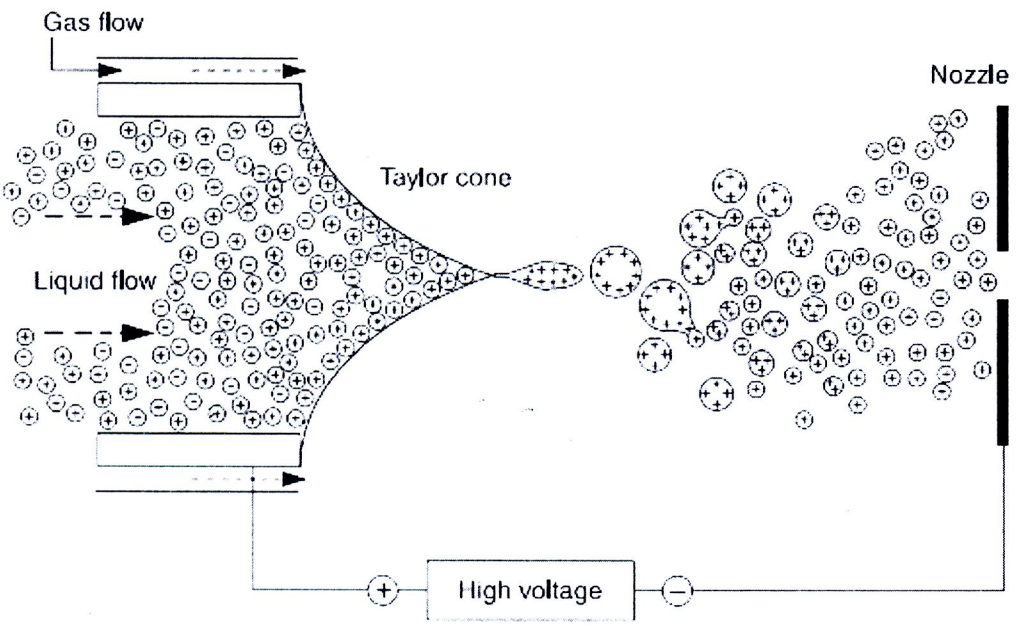

Figure 2 Schematic of electrospray ionization (ESI)

Source: Westman and Brinkmalm, 2002

Sample preparation requires only dissolution of the sample to a suitable concentration in a mixture of water and organic solvent, commonly methanol, isopropanol, or acetonitrile. A trace of formic acid or acetic acid is often added to aid protonation of the analyte molecules in the positive ionization mode. In negative ionization mode, ammonia solution or a volatile amine is added to aid deprotonation of the analyte molecules. The sensitivity of ESI-MS is good, with low femtomole or attomole detection levels for many peptides. However, the sensitivity of ESI is a function of the concentration of the injected sample. High flow rates, that is, 1 to 1,000

mL/min in conventional ESI-MS, result in high sample consumption. It is therefore advantageous to use the lowest possible flow rate. A recent version of electrospray ionization called "nanospray ionization" has become more popular. In nanospray ionization, a much smaller volume of liquid as little as 1 nL/min is passed through the charged capillary needle. This results in generation of a finer spray with much reduced size of the ionized droplets and considerably higher sensitivity (Wilm, et al., 1996).

Mass analyzers

After the process of ionization, the ionized molecules of proteins or peptides enter the section of the mass spectrometer called the mass analyzer, where they are separated based on their mass-to-charge ratio by electric and/or magnetic fields or by measuring the time taken by an ion to reach a fixed distance from the point of ionization to the detector. Different kinds of analyzers are available for the separation of ionized molecules. Among the different kinds of analyzers, two particular kinds, called the quadrupole and the time-of-flight (TOF) analyzers, are the most important from the point of proteomics for their use in mass spectrometers. A particular spectrometer may use one or the other or at times a combination of both quadrupole and TOF analyzers. The separation should also be independent of the chemical conformation of the species. All mass analyzers presently in use are based on electromagnetism so ions are required to obtain separation. Therefore, an ion source has to be coupled to the analyzer. The analyzer will then separate ions coming from the source according to their m/z. There are several types of mass analyzers used in mass spectrometric research and they can be divided into different categories, such as magnetic or pure electric, scanning or non-scanning (pulse based), and trapping or non-trapping analyzers (Blaum, et al., 2006).

Ion trap analysers use a similar principle to quadrupole mass analysers but employ a system of entrance, exit and end-cap electrodes together with a ring electrode that surrounds the trap cavity (Figure 3). As with quadrupole so with ion trap, for each ion type with a given value of m/z there is a corresponding value of $\varphi 0$ when interactions between ion type and external quadrupole field are such as to enable the trapping of ion within the analyzer prior to release for detection. Ion traps are relatively inexpensive, quite sensitive and robust, so are fairly widespread, despite being less accurate than TOF and quadrupole mass analysers.

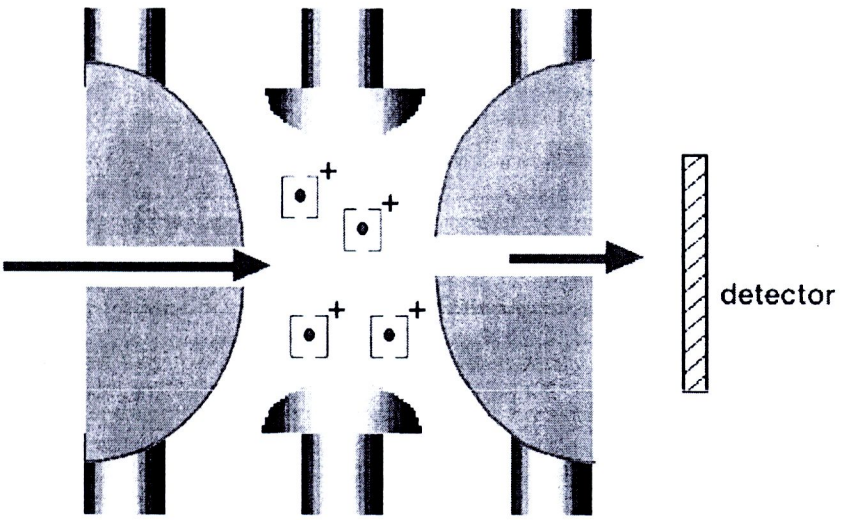

Figure 3 Schematic of an Ion Trap Mass Analyser.

Detectors

This is the final component of a mass spectrometer. Its purpose is to detect and record the presence of ions coming out of the mass analyzer hitting the detector. An electron is emitted when an ion hits the recorder and creates a small current. The low level of signal from a small number of ions coming out of the mass analyzer is amplified from 1,000 to 1 million times to become delectable and then recorded. A detector may use an electron multiplier or a photomultiplier. Photomultipliers first convert an electron produced by the ion hitting the detector plate into photon, which is detected by a phosphorescent plate in a sealed tube. Photomultipliers are preferred in a detector because they are located in a sealed tube, which reduces the noise-to-signal ratio by not allowing any outside interference to come out from the mass analyzer. All mass spectrometer are equipped with photomultipliers. These signals are then recorded on a graph by plotting the amount of signal versus m/z ratio. Mass spectrometer graphs usually show the presence of proteins/peptides of different molecular size and their abundance.