

# 1. INTRODUCTION

## 1.1. Curcumin - The origin

Natural products are a major source for new drug discovery. Many new drugs developed recently are either natural products or natural product-derived<sup>(113)</sup>. One such plant derived product is curcumin (CUR). Curcumin has been well studied compound for its pharmacological properties. However, it suffers shortfalls such as poor absorption, rapid metabolism and excretion.

### 1.1.1. Turmeric rhizome

Turmeric, rhizome of the perennial plant *Curcuma Longa* belonging to the *Zingiberaceae* (ginger) family, is not only a popular spice in South East Asian cuisine, but has been used for medicinal purposes since ancient times. Turmeric, often available in powdered form, consists of a wide variety of phytochemicals; including curcuminoids, zingiberene, curcumenol, curcumol, eugenol, tetrahydrocurcumin (THC), triethylcurcumin, turmerin, turmerones and turmeronols as well as volatile oils, sugars, proteins and resins<sup>(114-116)</sup>.

### 1.1.2. Curcuminoids

Curcuminoids which represent not less than 3% in the turmeric *Curcuma longa*; are yellow-pigmented fraction of turmeric. Turmeric are responsible for turmeric medicinal properties especially the main component present in the rhizome includes curcumin. It is composed of curcumin(I), desmethoxycurcumin(II), bisdesmethoxycurcumin(III) and the recently identified cyclocurcumin (Figure 36)<sup>(117)</sup>. It is comprised of 70-80% curcumin, 15-25% desmethoxycurcumin and 2.5-6.5% bisdesmethoxycurcumin<sup>(92)</sup>.

Collectively these compounds are referred to as curcuminoids. However the term "curcumin" is often used interchangeably<sup>(118)</sup>.

### 1.1.3. Curcumin

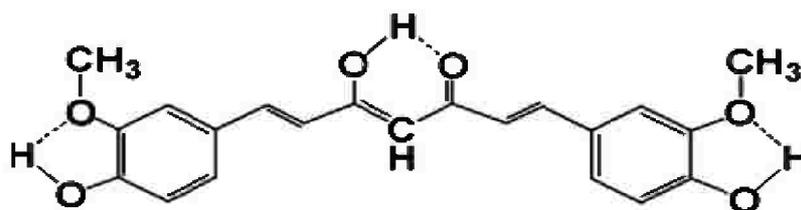
It is a polyphenolic compound. It was first isolated in 1815 and obtained in a crystalline form in 1870. Structurally, curcumin (diferuloylmethane; 1,7-bis(4-hydroxy-3-methoxy phenyl)-1,6-heptadiene-3,5,-dione or diferuloylmethane as shown in figure 1. It is the main ingredient (70-80%) in commercially available curcumin preparations<sup>(118)</sup>. It is particularly abundant in *Curcuma longa* (3.9-12.3%), but curcumin and curcuminoids have also been isolated from a variety of other plant species including *Curcuma Aromatic* (0.11%) and *Curcuma Phaeocalis* (0.89%)<sup>(119)</sup>.

## 1.2. Physicochemical properties of curcumin

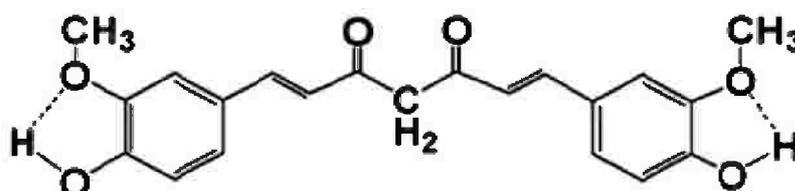
Curcumin is a yellow-orange powder in neutral conditions, brilliant yellow at acidic pH 2.5-7 and red at alkaline pH  $\geq 7$ <sup>(117)</sup>. It is practically insoluble in water but it is readily dissolved in several organic solvents such as alcohols, ketones, esters and organic acids.

It has a melting point of 183<sup>0</sup>C, a molecular formula C<sub>21</sub>H<sub>20</sub>O<sub>6</sub> and a molecular weight 368 g/mol. Spectrophotometrically, the maximum absorption ( $\lambda_{\text{max}}$ ) of curcumin in methanol occurs at 430 nm and in acetone at 415-420 nm.

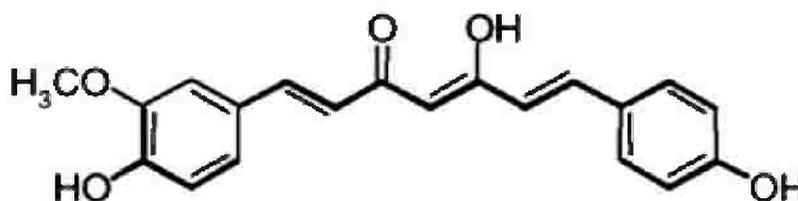
Curcumin is unique in structure for possessing two isomers, enol form and  $\beta$ -diketone form as shown in Figure 36.



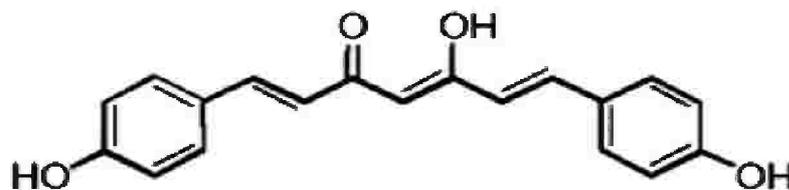
Enol form of curcumin



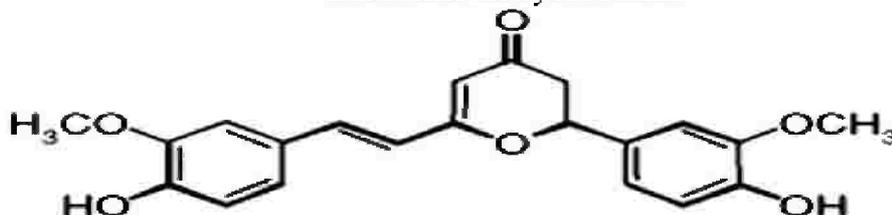
keto form of curcumin



Desmethoxycurcumin



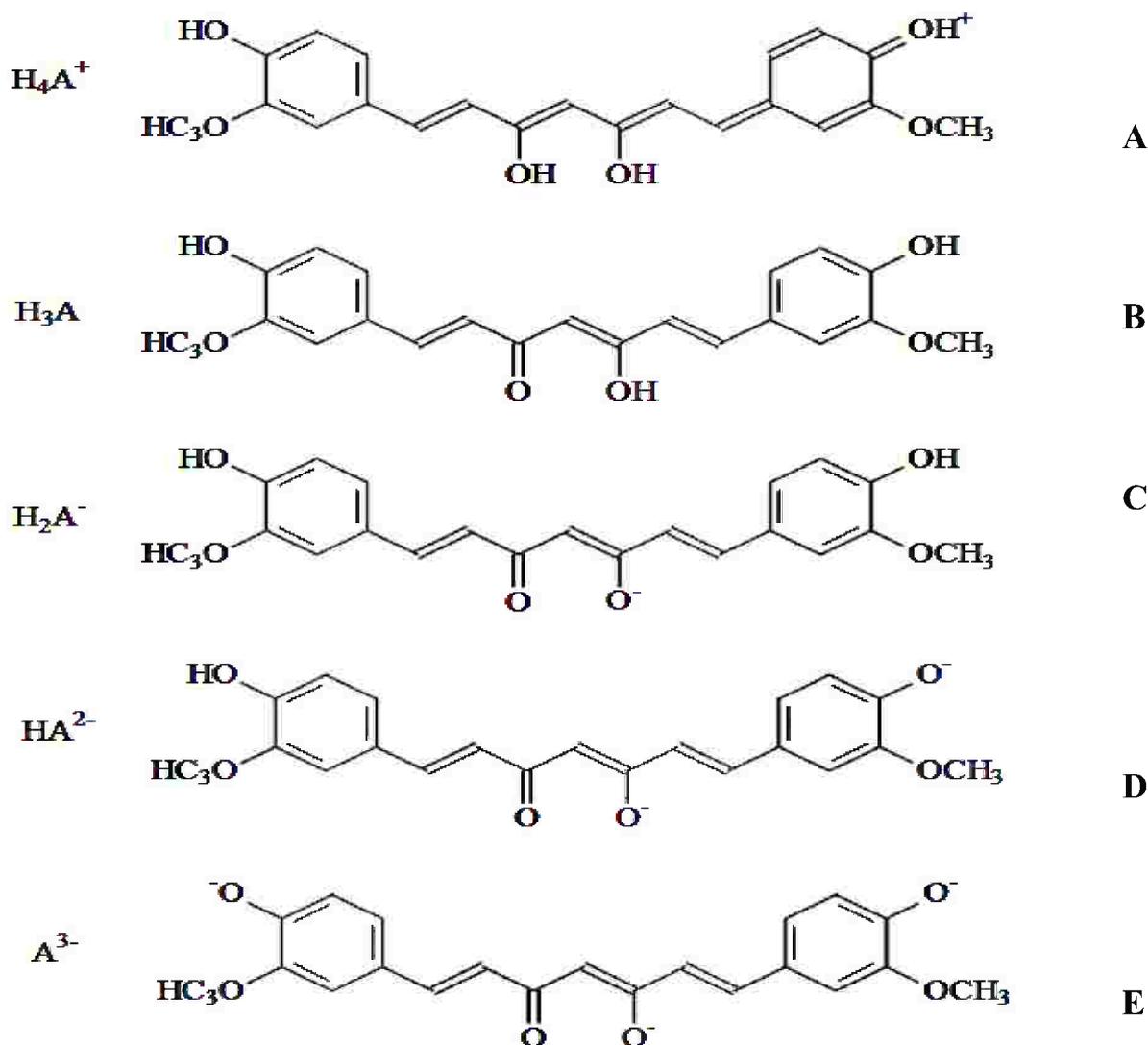
Bisdesmethoxycurcumin



Cyclocurcumin

Figure 36: Molecular structure of curcuminoids<sup>(120)</sup>.

In addition, the enol form of curcumin has three ionizable protons corresponding to the enolic group and two phenolic groups. At  $\text{pH} < 1$ , aqueous solutions of diferuloylmethane have a red color which indicates the protonated form ( $\text{H}_4\text{A}^+$ ). In the  $\text{pH}$  range 1-7, the majority of diferuloylmethane species are in the neutral form ( $\text{H}_3\text{A}$ ). At  $\text{pH} > 7.5$ , the color changes to red. The  $\text{pK}_a$  values for the dissociation of the three acidic protons in compound 1 (forms  $\text{H}_2\text{A}^-$ ,  $\text{HA}^{2-}$  and  $\text{A}^{3-}$ ) have been determined to be 7.8, 8.5 and 9.0 respectively as shown in Figure 37.



**Figure 37:** Protonated curcumin at different  $\text{pH}$ s; (A)  $\text{pH} < 1$ , (B)  $\text{pH}$  1-7, (C, D, E)  $\text{pH} > 7$

It was reported that curcumin is stable at acidic  $\text{pH}$  but unstable at neutral and basic  $\text{pH}$  where 90% of curcumin is decomposed within 30 min in 0.1M phosphate buffer and serum-free medium ( $\text{pH}$  7.2 at  $37^\circ\text{C}$ ) to trans-6-(40-hydroxy-30-methoxyphenyl)-2,4-dioxo-5-hexenal which encountered to be major metabolite while vanillin, ferulic acid, feruloyl methane which are minor metabolites<sup>(121)</sup> as illustrated in Figure 38.

Decomposition of curcumin is  $\text{pH}$  dependant where the rate of decomposition is higher under neutral-basic conditions<sup>(122)</sup>. It was suggested that the seven carbon  $\beta$ -diketone

linker in curcumin is responsible for its instability<sup>(123)</sup> leading to its hydrolytic degradation starting from the nucleophilic  $\text{OH}^-$  ion to the carbonyl carbon in the keto-enol moiety<sup>(124)</sup>.

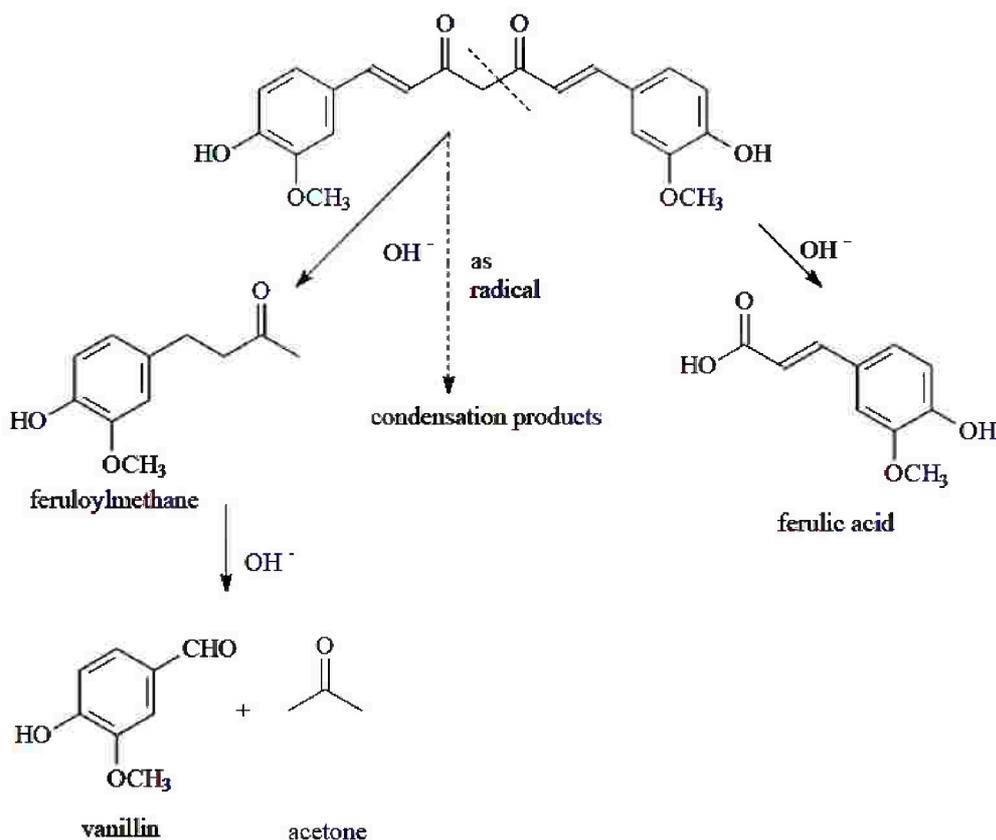


Figure 38: Degradation products of curcumin in alkaline pH.

### 1.3. Bioavailability and metabolism of curcumin

#### 1.3.1. Oral bioavailability

Studies have suggested a strong intrinsic activity and efficacy of curcumin as a therapeutic agent for various ailments. However, studies over the past three decades related to absorption, distribution, metabolism and excretion of curcumin have revealed poor absorption and rapid metabolism of curcumin that severely curtails its bioavailability<sup>(125)</sup>.

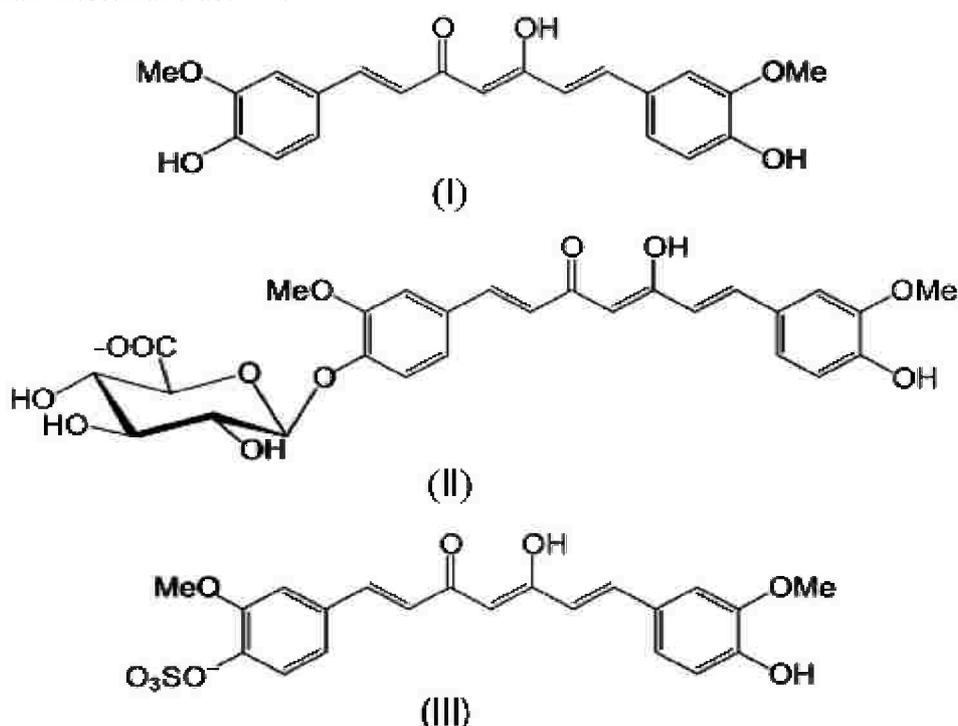
All *in vivo* trials of CUR showed controversy data with differences in given doses and detected quantities in plasma. In animal studies, it was reported that oral dose 100 mg/kg administrated to mice yielded a peak plasma concentration of free curcumin that was only 2.25  $\mu\text{g/ml}$ <sup>(126)</sup> while in case of oral administration of 500 mg/kg, peak concentrations was 1.8 ng/ml of free curcumin were detected in the plasma<sup>(127)</sup>. In other study, it showed 0.06  $\mu\text{g/ml}$  after the oral administration with the same dose<sup>(128)</sup>. Another study reported that maximum serum curcumin after oral administration of 1g/kg in rats is 0.5  $\mu\text{g/ml}$  after 45 min<sup>(129)</sup>. Similarly Marczyklo *et al*<sup>(130)</sup> showed a maximum serum curcumin concentration of  $6.5 \pm 4.5$  nM reached after 0.5h after oral dose of curcumin. These results assure the poor oral

bioavailability of curcumin. In a recent study, it was reported that there is no free curcumin detected in the human plasma after oral administration of 10 gm or 12 gm curcumin as a single dose and subsequently results refer exclusively to curcumin conjugates<sup>(131)</sup>.

Following oral administration (up to 8g/day), only trace amount of curcumin was detected in the blood<sup>(132)</sup>. In addition, oral bioavailability of curcumin in such case was only 1% in rats<sup>(128)</sup>. In clinical trial quantifiable serum levels were not achieved until doses of up to 3.6 gm<sup>(133)</sup>, therefore the dose of oral curcumin at 3.6 daily was recommended for phase II clinical trial of treating advanced colorectal cancer<sup>(134)</sup>.

### 1.3.2. Systemic metabolism of curcumin

In animal studies, curcumin undergoes rapid metabolic reduction and conjugation resulting in poor systematic bioavailability after oral administration<sup>(126, 127)</sup>. The major metabolites of curcumin shown in Figure 39 identified in rat plasma. They included curcumin glucouronide and curcumin sulfate based on enzymatic hydrolysis studies. Hexahydrocurcumin, hexahydrocurcuminol and hexahydrocurcumin glucuronide were also present in minor amounts<sup>(127)</sup>. It is reported that glucuronide conjugate of curcumin predominates over sulphate conjugate in plasma with no evidence of a mixed conjugate being formed and these results indicate that curcumin is absorbed after oral administration and could be transported to sites other than intestinal tract<sup>(131)</sup>.



**Figure 39: Structure of curcumin (I), curcumin glucuronide (II) and curcumin sulphate (III).**

It is reported in *In-vitro* models that curcumin metabolism (i.e. conjugation and reduction) is taken place in the intestinal tissues and liver in both human and rats. Where it was reported that conjugation of curcumin in humans to sulphate conjugate in the intestine exceeds that in liver by 2.6 times and to glucuronide conjugate in the human intestine exceeds that in liver by 2.7 times. Reduction to hexahydrocurcumin took place in human intestine

more than in liver by 0.8 times. In contrast, curcumin metabolism takes place in the liver of the rat more than in the intestinal tissue<sup>(135)</sup>.

It is believed that enzymes sulphotransferase "SULT 1A1" and "1A3" which are among five isoenzymes of the human phenol xenobiotic-metabolizing SULT subfamily which are expressed in the gastrointestinal tract<sup>(136)</sup> play a role in conjugation of curcumin to its sulphate conjugate to be the first curcumin metabolite identified in human feces, on other hand it is concluded that there is a variety of other ubiquitous and nonspecific oxido-reductase reduce curcumin and thus contribute to the formation of curcumin reduction products *in vivo*<sup>(127)</sup>. The effect of CYP 3A4 enzymes in the intestinal metabolism for curcumin<sup>(137)</sup> is also reported.

## **1.4. Biological activity**

### **1.4.1. Biological activity for parent compound**

Clinical activity of curcumin has yet to be confirmed however preclinical animal model showed that curcumin inhibits the tumor formation in the skin, stomach, duodenum and colon of mice and in the tongue, colon, mammary glands, sebaceous glands of rats. It has antineoplastic and anti-inflammatory properties<sup>(138)</sup>.

Curcumin has been shown to attenuate thermal hyperplesia in mouse model of diabetic neuropathic pain mediated via inhibition of tissue necrosis factor  $\alpha$  (TNF- $\alpha$ ) and nitric oxide (NO) release<sup>(139, 140)</sup>. It inhibits breast tumor growth and spontaneous pulmonary metastasis in mice<sup>(141)</sup>. Also, it is encountered to be anti-inflammatory, anti-oxidant, anti-bacterial, anti-virus, anti-tumor and hyperlipidemic activities<sup>(142-144)</sup>.

The mechanisms of therapeutic action of curcumin are due its structure that allows interacting with several molecular targets include inhibition of several cell signaling pathways at multiple levels, immune-modulation, effects on cellular enzymes such as cyclooxygenase and glutathione S-transferase and effects on angiogenesis and metastasis. It has the ability to affect gene transcription and to induce cell cycle arrest and apoptosis<sup>(145, 146)</sup>.

### **1.4.2. Bioactivity of Curcumin metabolites**

Whether curcumin metabolites are as active as curcumin itself is not clear. While most studies indicate that curcumin glucuronides and tetrahydrocurcumin are less active than curcumin itself<sup>(127, 147, 148)</sup> while other studies suggest that they may actually be more active than curcumin<sup>(149-152)</sup>. Where it was reported that tetrahydrocurcumin (THC) was found to show better antidiabetic and antioxidant activity than curcumin in type 2 diabetic rats<sup>(151)</sup>. On other hand, THC was found to possess lower anti-inflammatory and antiproliferative<sup>(153)</sup> and reduced ability to inhibit COX-2 expression compared to curcumin<sup>(127)</sup>.

## 1.5. Lipid based delivery systems for curcumin

Although many lipid based delivery systems were introduced to enhance the oral bioavailability of curcumin like application of liposomes<sup>(154)</sup>, self nano-emulsifying delivery system<sup>(155)</sup>, self micro-emulsifying delivery systems<sup>(156)</sup>, application of lipid formulation classification system (LFCS) class IV<sup>(157)</sup>, none of these approaches were developed to be administrated in oral dosage form except phospholipid complex formation<sup>(129, 158)</sup> known as Phytosome technology. This technology was the only commercialized delivery system for curcumin which is patently developed by (Indena, SpA, Italy) under trade name "Meriva™".

Due to gaining interest in utilizing curcumin-phospholipid complex (phytosome), our current work cared about synthesis of lower cost locally prepared complex similar to Meriva™ and to develop a higher strength of curcumin in its complexed form in a soft gelatin capsule which is discussed in details in chapter one.

In chapter two, our work cared about using the cheapest type of phospholipid expressed as "Phosal®" to develop a less complicated delivery system to enhance the oral bioavailability for curcumin named as phospholipid dispersion technique.

This part of current thesis is divided into two chapters:

**Chapter I:** Preparation and evaluation of soft gelatin capsule containing curcumin-phospholipid complex.

**Chapter II:** Preparation and evaluation of curcumin self phospholipid nano dispersion: As a novel delivery system to enhance curcumin systemic bioavailability.

## **CHAPTER I**

# **PREPARATION AND EVALUATION OF SOFT GELATIN CAPSULES CONTAINING CURCUMIN- PHOSPHOLIPID COMPLEX**

# 1. INTRODUCTION

Although various pharmacological use for herbal derived drugs, many reasons stand against its medicinal use because of its poor oral bioavailability, specifically those containing polyphenolic rings in their chemical structure such as flavonoids and other water soluble constituents like terpenoids and tannins<sup>(159)</sup>. Some of these reasons are low aqueous solubility of these substances, high molecular weight/size, poor membrane permeability<sup>(160, 161)</sup> and low stability in the presence of gastric fluids<sup>(162)</sup>. One of the methods to counter latter reasons is application of a novel delivery system known as Phytosome or herbesomes<sup>(159)</sup>.

Phytosomes were developed at Indena SpA (Milan, Italy) in the late Eighties in herbal formulations. This provides better pharmacokinetic and pharmacodynamic behavior than conventional botanical extracts. This technique incorporates the phospholipid molecules containing phosphatidylcholine in their structure to form complexes with herbal extracts or its constituents producing a lipid compatible molecular complex and a compound soluble in both water and lipid environment.

Water soluble drugs as Gentiopiricin can be incorporated in phospholipid complex to enhance their bioavailability by increasing penetration through the lipoidal plasma membrane<sup>(163)</sup> while phospholipid complex for poorly water soluble drugs increase their bioavailability by improving their solubility in gastric fluids<sup>(164, 165)</sup>. Some of the advantages of phyto-phospholipid complexes over pure plant drugs are presented in Figure 40.

## 1.1. Phytosome as a delivery system

Phytosome technology is encountered to be one of the most safe and convenient system among other drug delivery systems in enhancement of oral bioavailability of poorly water soluble drugs.

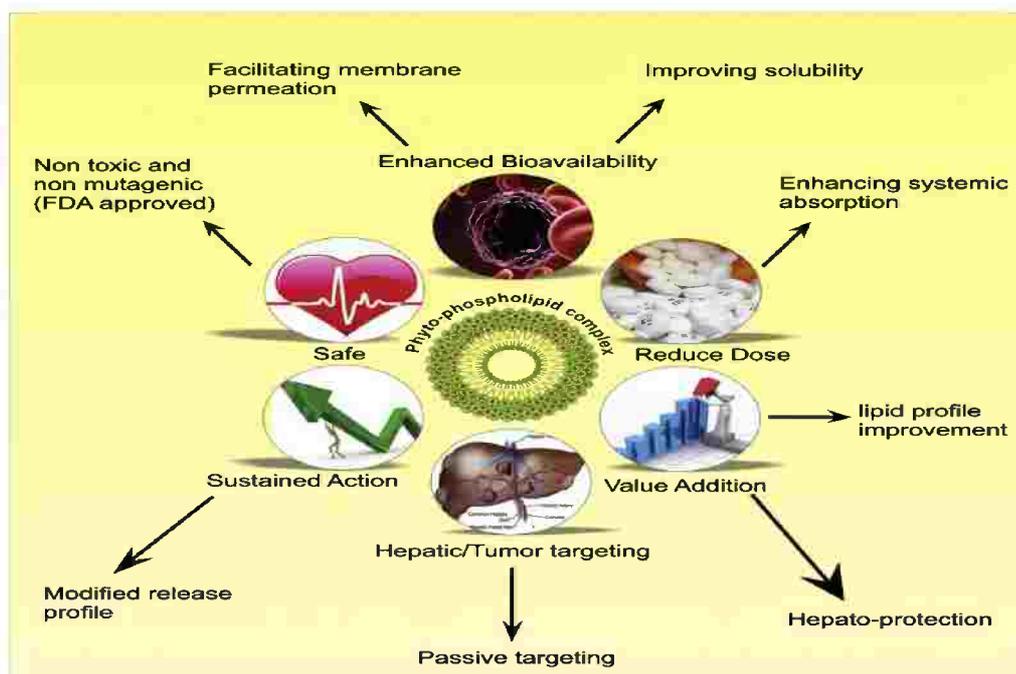


Figure 40: Benefits of phyto-phospholipid complexation<sup>(166)</sup>.

This is because of a similarity between its structural components and lipid content of the mammalian cell membrane which makes them compatible with human physiological system, so phospholipids are encountered to be unique.

Phytosome is the result of a uniquely interaction between phospholipid molecule and polyphenolic compounds via chemical bonds between them. Many possible interaction mechanisms were suggested in many researches such as:

- Hydrogen bond formation between polar head of the phospholipid molecule and – OH group of phenolic rings existing in the structure of the drug molecule<sup>(166)</sup> leading to encapsulation of drug molecule in the polar head of phospholipids<sup>(167, 168)</sup> as reported for naringenin, puerarin, gallic acid and gymnemic acid<sup>(169)</sup>, such bonding is encountered to be the main reported cause for better stability profile of phytosomes compared to liposomes<sup>(170)</sup>.
- Formation of Vander Waals forces between the two moieties had also studied by some researchers<sup>(171)</sup>. When treated with water, phytosomes unify themselves into several assemblies and the drug being entangled as shown in Figure 41.

In Figure 42, a typical interaction between phyto-phospholipid complex and the plasma membrane of the mammalian cell leads to increase the permeability of poorly water soluble drugs. Where the small sized micelle with phospholipids has the ability to penetrate the lipoidal membrane of luminal absorptive cells in an energy-independent manner without damaging the cellular lipid bilayer leading to non cytotoxic migration<sup>(172)</sup>.

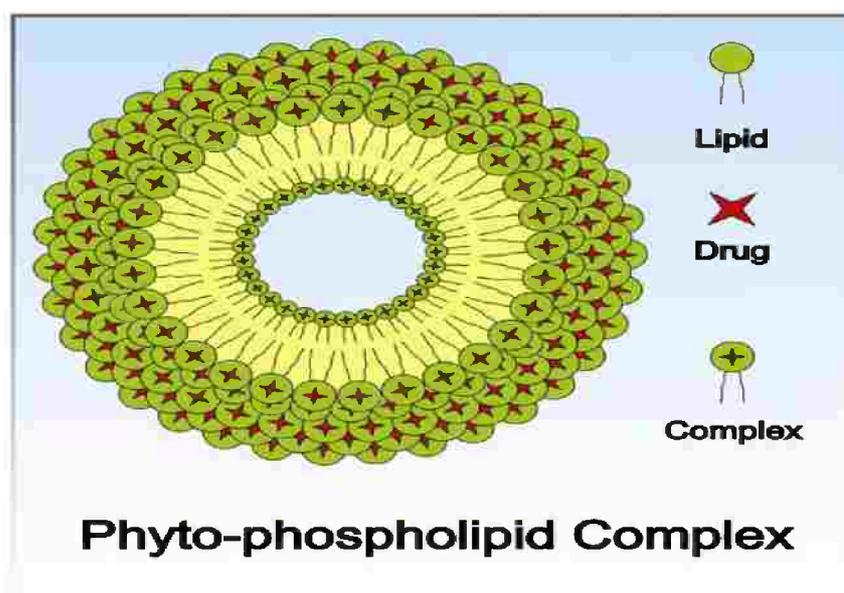
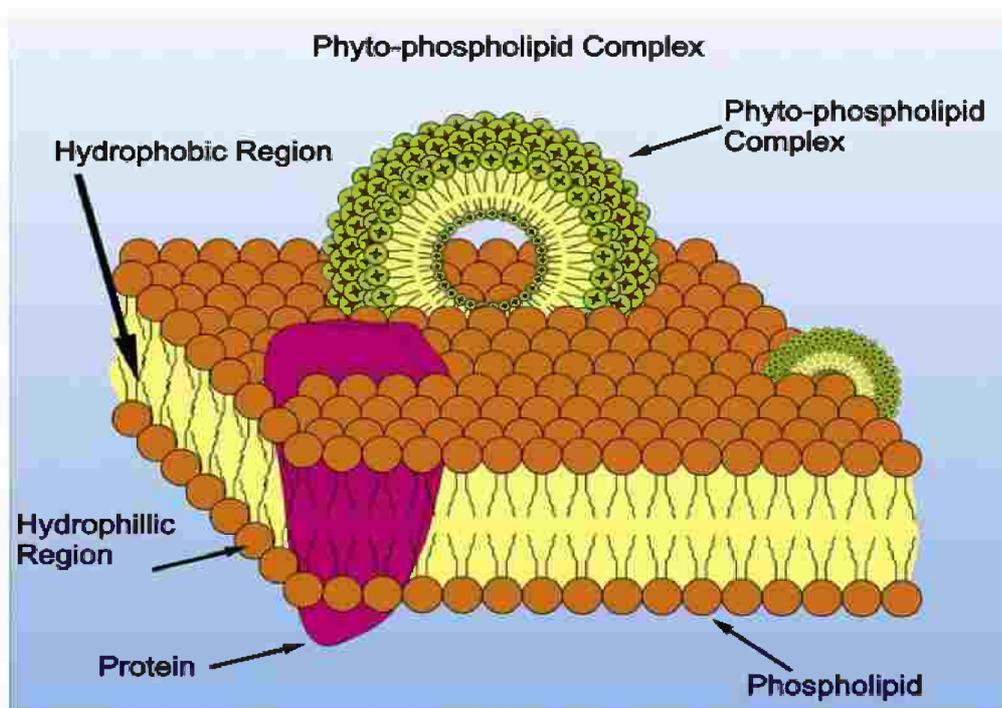


Figure 41: Structure of a phyto-phospholipid complex micelle<sup>(166)</sup>.



**Figure 42: Interaction of the phyto-phospholipid complex with the biological membrane<sup>(166)</sup>.**

## 1.2. Methods of preparation of phytosome

### 1.2.1. Solvent evaporation method<sup>(166)</sup>

It is the most frequently used method to prepare phytosomes, where the drug and the phospholipid are placed in the same flask containing a suitable solvent for both components such as dichloromethane or ethanol. The reaction is allowed to take place at a fixed duration of time and a suitable temperature. Then the solvent is removed by application of rotary evaporator yielding a semi-solid mass for drug-phospholipid complex.

### 1.2.2. Antisolvent precipitation process (Salting out technique)

This method was introduced by Sikarwar *et al*<sup>(173)</sup> in the preparation of marsupsin-phospholipid complex. They dissolved soy lecithin in diethyl ether while marsupsin was dissolved in distilled water. The drug solution was added drop wise to phospholipid solution leading to formation the complex. Also, this method was utilized by incorporating n-hexane as the antisolvent to salt out the drug-phospholipid complex from the organic solvent<sup>(174)</sup>. Where Mukherjee *et al*<sup>(175)</sup> used this method in the preparation of phyto-phospholipid complex of andrographolide by using dichloromethane as the reaction medium and n-hexane as the anti solvent for final precipitation of the product. Then the solution is evaporated off and residue dried under vacuum.

### 1.2.3. Anhydrous co-solvent lyophilization method

Jain *et al* <sup>(176)</sup> utilized this method to prepare rutin-phospholipid complex where the drug and the phospholipids were dissolved separately in each vessel. The two solutions were mixed together mechanically then the complex was isolated by lyophilization technique.

### 1.3. Merits of phytosome over other conventional dosage forms

- It enhances the absorption of lipid insoluble polar phytoconstituents through oral as well as topical route showing better systematic bioavailability <sup>(166, 170, 177)</sup>.
- High drug entrapment <sup>(170)</sup>.
- Dose reduction of the active constituents due to enhancement of bioavailability <sup>(170)</sup>.
- On using other hepatoprotective substances, phospholipids used in phytosome formation give a synergetic effect with them <sup>(171)</sup>.
- Phytosomes show better stability profile over liposomes due to formation of chemical bonds <sup>(177)</sup>.
- Enhancement of percutaneous absorption for phyto-constituents in the form of phytosomes <sup>(170)</sup>.

### 1.4. Some biological applications of phytosome

#### 1.4.1. Anti-inflammatory properties

Phytosome complexes of glycyrrhetic acid which is encountered to be a powerful anti-inflammatory with anti-allergic triterpenoid and silymarin, a free radical scavenger flavanolignan endowed with anti-phlogistic activity were compared with uncomplexed constituents by using croton oil induced dermatitis as an inflammation model involving both tissue and vascular damage to evaluate the anti-inflammatory activity of phytosome complexes in animals. It was found that phospholipid complex formation with glycyrrhetic acid leading to prolong its activity over 24 hours. Moreover, it also induces 80% reduction of edema. On the other hand, complexed form of silymarin reduce the edema by 76% in 6 hours as compared to that with free form 33% even after 12 hours in the croton oil test in mice <sup>(178)</sup>.

#### 1.4.2. Anti-aging properties

Because of the reported effect of *G.biloba* orally on improvement of the peripheral circulation <sup>(178)</sup>. Its complexes form with phospholipid open new opportunities to be used as active ingredients in the cosmetic field. It was examined topically in the improvement of microcirculation of meliorated skin aging associated to dystrophic alteration of the epidermis and dermis. Also, use of Silymarin phytosome on aging skin was also reported <sup>(178)</sup>.

#### 1.4.3. Anti cancer properties

Plant derived drugs as silymarin and silybin have received a great attention in the last few years due to their anticancer, chemoprotective, hypocholesterolemic, cardioprotective, neuroactive and neuroprotective activities <sup>(179)</sup>. Phase I and pharmacokinetic study has been carried on silybin phytosome. This study concluded that 13 gm daily dose of silybin-phytosome seems to be well tolerated in patients with advanced prostate cancer and this dose was recommended for phase II study <sup>(180)</sup>.

Many researches were held on Turmeric due to its potential value in cancer management<sup>(181)</sup>. Compared bioavailability of pure curcumin,(i.e. Turmeric active constituent) and Meriva<sup>TM</sup> has been evaluated in rats, showing that Meriva<sup>TM</sup> is five times more absorbed than the parent compound<sup>(130)</sup>. Also recent studies have confirmed that the interaction between curcumin and lipid layers plays a fundamental role in the wide bioactivity of this compound<sup>(182)</sup> and Meriva<sup>TM</sup> thus, appears to be a favorite candidate to enhance biological action of curcumin through a higher bioavailability.

#### **1.4.4. Weight management**

Role of Green select PHYTOSOME was studied in the treatment of obesity and its efficacy to potentiate weight loss in dieting obese objects<sup>(183)</sup>

#### **1.5. Phytosomes as commercial products<sup>(178)</sup>**

Due to successful application of phytosomes, many herbal products were produced and commercially distributed as following:

**Table 11: Commercial phytosome products**

Trade name	Phytoconstituent complexed with phospholipid	Indication
Escin $\beta$ - sitosterol phytosome®	Escin $\beta$ - sitosterol from horse chestnut fruit	Anti-oedema
Siliphos®	Silybin from milk thistle seed	Hepatocyte protection
Silymarin Phytosome®	Silymarin from milk thistle seed	Anti-hepatotoxic
Meriva™	Curcuminoids from turmeric rhizome	Osteoarthritis
Virtiva®	Ginkgoflavonglucosides, ginkgolides, bilobalide from ginkgo biloba leaf	Vasokiretic
Ginselect phytosome®	Ginsenosides from Panax ginseng rhizome	Skin elasticity improver, adaptogenic
Leucoselect phytosome®	Polyphenols from grape seed	Anti-oxidant, capillarotropic
Centella phytosome®	Triterpenes from centella asiatico leaf	Cicatrizing, trophodermic
18 $\beta$ -glycyrrhetic acid phytosome®	18 $\beta$ -glycyrrhetic acid from licorice rhizome	Soothing
Crataegus Phytosome®	Vitexin-2°-O-rhamnoside from hawthorn flower	Antioxidant
Ginkgo biloba Dimeric Flavonoids phytosome®	Dimeric flavonoids from ginkgo biloba leaf	Lipolytic, vasokinetic
Ginkgo biloba Terpenes phytosome®	Ginkgolides and bilobalide from ginkgo biloba leaf	Soothing
Sericoside phytosome®	Sericoside from Terminalia Sericea bark root	Anti-wrinkles
Greenselect phytosome®	Polyphenols from green tea leaf	Prevention of free radical-mediated issue damages and weight management
Visnadex®	Visnadin from Amn visnaga umbel	Vasokinetic
PA <sub>2</sub> Phytosome®	Proanthocyanidin A <sub>2</sub> from chestnut bark	Anti-wrinkles, UV- protectant

## **AIM OF THE WORK**

Due to various, valuable therapeutic applications of CUR-SPC complex (PHYTOSOME), Indea SpA (Milano, Italy) has patented a commercial product Meriva™ in the form of a dry powder form able to be encapsulated in a hard gelatin capsule. This approach is considered the only available successful mean to deliver CUR with high systematic bioavailability in a form of an oral dosage form.

Our work at this chapter is aiming to:

- Develop a locally prepared CUR-SPC complex in a dry powder form resemble to patently Indena product (Meriva™) to produce a low cost price CUR-SPC complex with a simple production process if compared to the high cost of Meriva™ reaching to more than 220€ for one kilogram for 20% CUR content
- Formulation of CUR phytosome in softgels using lipophilic/hydrophilic vehicle as an approach to increase CUR dose per unit capsule utilizing bioactive excipients which in turns inhibit systematic metabolism of CUR at intestinal wall.

## 2. EXPERIMENTAL

### 2.1. Materials

- Curcumin powder was purchased from Shenzhen chemrider, China
- The soy phosphatidylcholine (SPC) phospholipid, Lipoid S 100 was a gift from Lipoid Co., Ludwigshafen, Germany.
- Curcumin - phospholipid S 100 complex was a gift from Indena SpA, Italy.
- Colloidal silicon dioxide (Aerosil 200) was purchased from Evonik Degussa, UK
- Sodium lauryl sulphate was purchased from Basf, USA
- Capric/caprylic triglyceride (Miglyol 812) was purchased from Gattefosse' Corp, USA
- Kollisolv P 124 was a gift sample from Basf, USA
- Polyoxyl 35 castor oil was purchased from Basf, USA
- Polyoxyl 40 hydrogenated castor oil was purchased from Basf, USA
- Soya bean oil was purchased from Crouda, USA
- Tween 80 was purchased from Adina chemicals, UK
- Dichloromethane, Chloroform and methanol were of analytical grade.
- Polyethylene glycol 400 (PEG 400) was purchased from Dow chemical company, USA

## 2.2. Equipments

- Eight stations dissolution apparatus,( SR 8 plus, Hanson research, USA)
- FTIR spectrometer (Perkin Elmer Life and Analytical Sciences, Shelton, CT, USA)
- DSC 6 differential scanning calorimeter (Perkin Elmer, USA)
- Malvern Zeta Sizer (Malvern Instruments, UK)
- Transmission electron microscope (model JEM-100S microscope, Jeol, Japan)
- Scanning electron microscope (model JSM-6360LV, Jeol, Japan)
- Thermostatically shaking water bath,( Bunsen, India)
- UV-1800 double beam spectrophotometer (Shimadzu, Japan)
- Electric mixer,(IKA T25, Germany)
- Sensitive electronic balance,(AND, Japan)
- Silica gel 60 F<sub>254</sub> precoated TLC plate, (Merk, Germany)