CHAPTER I

INTRODUCTION

The rhizomes of turmeric, *Curcuma longa*, a plant of the Zingiberaceae family is known as a natural spice food and coloring agent. Turmeric contains tumurin (a water-soluble peptide), essential oil (such as tumerones, atlantones and zingiberene) and the yellow pigment, curcuminoids. The coloring principle of turmeric has been isolated in the 19th century and named curcuminoids. The component of curcuminoids are curcumin, demethoxycurcumin and bisdemethoxycurcumin, their chemical structures are demonstrated in Figure 1. Numerous methods are available for isolating curcuminoids from *C. longa.* Isolation of pure curcumin from plant material is time consuming and pure curcumin sold on the market is therefore, a purified extract containing a mixture of the curcuminoids i.e. curcumin (75-81%), demethoxycurcumin (15-19%) and bisdemethoxycurcumin (2.2-6.6%) (Jayaprakasha, 2005). These percentages of curcuminoids compound depend upon the planting location. However curcumin is the only one component which its pharmacological action as an antioxidant, antimicrobial, anti-inflammatory have been reported (Ammon, 1999: Asai, 2001: Deng, 2006: Desphande, 1997: Fan, 2006: Huang, 1995: Ishita, 2004.). Therefore, it is intention that only the details related to curcumin would be more expressed.

Curcumin is the bis - α, β – unsaturated β – diketone. The bis-keto form is the predominate form in acidic and neutral aqueous solutions and in the cell membrane. Curcumin is unstable at basic pH. Under acidic condition, the degradation of curcumin is much slower. In addition, curcumin is also light sensitive. Therefore, it is recommended that samples containing curcumin should be light protection (Wang et al., 1997).

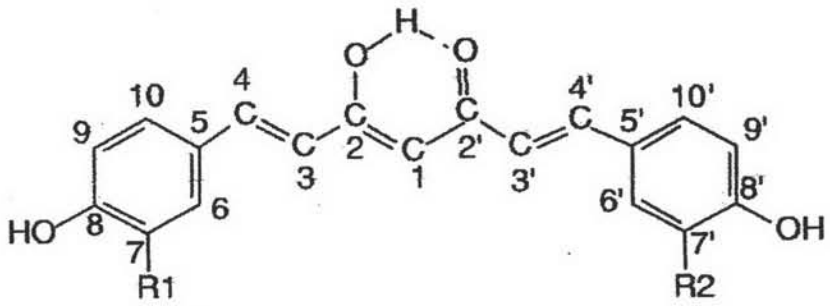

Figure 1. Curcuminoids chemical structure

Compound	R1	R2	Chemical name	M.W.	Molecular formula
Curcumin	OMe	OMe	1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	368	$C_{21}H_{20}O_6$
Demethoxycurcumin	H	OMe	1-(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	338	$C_{20}H_{22}O_5$
Bisdemethoxycurcumin	H	H	1,7-bis(4-hydroxyphenyl) -1,6-heptadiene-3,5-dione	308	$C_{19}H_{24}O_4$

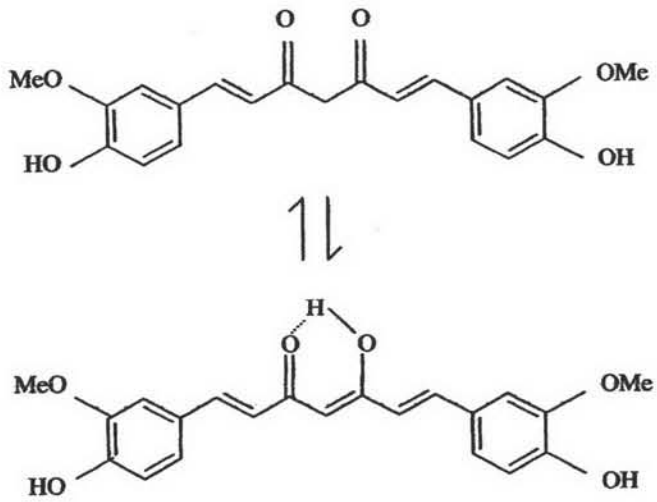

Figure 2. Tautomerism of curcumin under physiological conditions.

At pH 3-7, curcumin acts as an extraordinarily potent H-atom donor. This is due to the keto form of curcumin, the heptadienone linkage between the two methoxyphenol rings contains a highly activated carbon atom. The C-H carbon bonds on this carbon are very weak due to delocalization of the unpaired electron on the adjacent oxygen. In contrast, above pH 8, the enolate form of the heptadienone chain predominates and curcumin acts mainly as an electron donor, (Figure 2) a mechanism more typical for the scavenging activity of phenolic antioxidants. Curcumin is relatively insoluble in water but it is soluble in acetone, dimethylsulphoxide and methanol (Sharma, Gesher and Steward, 2005).

Various *in vitro* and *in vivo* studies curcumin pharmacological activities have been reported mostly emphasizing to its action as an anticancer and antioxidant. Examples of the *in vitro* studies in mouse Dalton's lymphoma cells grown as and mouse macrophages were presented (Kuttan et al., 1985: Chanlei et al., 2006).

In human, lung adinocarcinoma cell lines and the large carcinoma cell lines. The anticancer activity of curcumin was demonstrated, by exposing curcumin (0-160 μM) for 12-72 hours, growth of both cell lines was inhibited in a concentration dependent manner (Pillai et al.,2004).

The antioxidant activity of each curcuminoids component (curcumin, demethoxycurcumin and bisdemethoxycurcumin) was expressed *in vitro* utilizing the phosphomolybdenum and linoleic acid peroxidation methods. As ascorbic acid equivalent (μmol/g), antioxidant capability was in the order as curcumin > demethoxycurcumin > bisdemethoxycurcumin (Jayaprakasha et al., 2006). The antioxidant activity of curcumin itself has also been proven in various *in vitro* studies (Deng et al., 2006: Jain et al., 2006).

The chemopreventive activities of curcumin was evidenced by its ability to inhibit cell growth and induce apoptosis in the human ovarian cancer cell line (Shi M. et al., 2006). Furthermore, curcumin was alone used or combined with anticancer drug for cancer treatment. The combination of curcumin with cisplatin resulted in a synergistic antitumor activity, but with doxorubicin, the action could be in additivity or sub-additivity in the hepatic cancer cell line (Notarbartolo M. et al., 2005).

Several in vivo studies either have been performed in animal or human. Oral administration of curcumin to treated mice with carbon tetrachloride, paraquat, and cyclophosphamide showed significantly reduced peroxidation of lipids in tissue liver, lung, kidney and brain (Soundamini et al., 1992).

The preventing of adenomas in mice's intestine, due to effect of curcumin on COX-2 protein level was reported (Perkins et al., 2003: Ying XU et al., 2005: Tunstall et al., 2006).

By feeding curcumin to rat, the lipid metabolism, liver triglyceride and cholesterol concentrations were significantly lower comparing to the control (Asai et al., 2001: Sugiyama et al., 2006). In addition, the co-administration of curcumin with

gentamicin could reduced oxidative damage in rats (Farombi and Ekor, 2006). The antidepressant effect of curcumin in rat was reported by Xu, Y. et al (Xu et al., 2005).

The *in vivo* curcuminoids studies in human were mostly performed in patients. The improvements in morning stiffness, walking time, and joint swelling following 2 weeks of curcumin therapy with phenylbutazone was reported (Deohar et al., 1980). The high dose of oral curcumin 0.5 – 8 g daily for 3 months have been used without any adverse effect in patient with pre-invasive malignant or high risk pre-malignant conditions of the bladder, skin, cervix, stomach or oral mucosa (Cheng et al., 2001).

Even though, pharmacological action of curcumin was explored for many years in both animal and human, its pharmacokinetics has not been well documented. In rodents, curcumin undergoes avid metabolism by conjugation and reduction, and its disposition after oral dosing is characterized by poor systemic bioavailability (Asai, 2000: Christopher et al., 2001: Christopher et al., 2002: Min-Hsiung et al., 1999: Ricky et al., 2001). Pharmacokinetic informations regarding curcumin in humans were mostly studies in cancer patients (Cheng et al., 2001: Ricky et al., 2001: Sharma et al., 2004). Curcumin concentrations could only be detected in plasma 1-2 h after oral administration at the dose of more than 2 grams/day (Cheng et al., 2001).

In Thailand, the used of curcuminoids emphasizing to curcumin was in the capsule and tablet dosage forms. Curmin capsule® containing curcumin 250 mg/cap is manufactured from the Government of Pharmaceutical Organization (GPO). Curcuminoids coated tablet is produced by the Faculty of Pharmaceutical Science, Mahidol University and is in process for commercial, also containing curcumin 250 mg/tab. However, both dosage forms have already been proven for their bioequivalence (Phensri, et al., 2004). Since the dosage form available, many Thai people daily consume curcuminoids whether as part of supplement or part of therapy without pharmacokinetic evidences. Many questions have been raised for its long term used. Therefore, this study is managed for determining pharmacokinetic of curcumin when curcuminoids tablets were daily consumed.

Biomedical analysis of curcumin

The curcuminoids solution exhibit strong light absorption at the wavelength between 420-430 nm. Commercial curcuminoids/turmeric products contain mixture of curcumin, demethoxycurcumin and bisdemethoxycurcumin. It is not possible to quantify the individual curcuminoids with spectrophotometric method. GC methods provide no alternative to HPLC due to the low volatility and thermally labile nature of the curcuminoids (Jayaprakasha, Jaganmohan and Sakariah, 2006). Spectroscopic methods (IR, NMR, MS) are widely used for identification and characterization of the curcuminoids (Anasari et al., 2005: Anchang et al., 2006: Asai and Miyazawa, 2000: Christopher et al., 2001). LC-MS and GC-MS methods were reported for the separation of curcuminoids (Hiserodt et al., 1996). To analyzed curcuminoids and sesquiterpenoids in a fresh turmeric extract, the on-line-HPLC-UV diode array and electrospray mass spectrometer was used but the contamination of organic salt limit the use of mass spectrometer ion source (He et al.,1998).

Very few reports on curcumin analysis in plasma was evidenced. Only HPLC technique was presented with UV or MS detection (Christopher et al., 2001: Cheng, et al., 2001: Pak, et al., 2003: Heath et al., 2003). The only sample preparation used was liquid – liquid extraction either with ethyl acetate or chloroform. In this study the method of Heath et al. was used with modification.

Objectives of the study

1. To determine plasma curcumin concentrations in Thai healthy volunteers following multiple administration of curcuminoids tablets
2. To determine the pharmacokinetic parameters of curcumin in Thai healthy volunteers following multiple administration of curcuminoids tablets

The significance of this study

1. To obtain the pharmacokinetic parameters of curcumin in Thai healthy volunteers following multiple administration of curcuminoids tablets.
2. Pharmacokinetic parameters of curcumin obtained would provide more informationon curcumin for daily used.