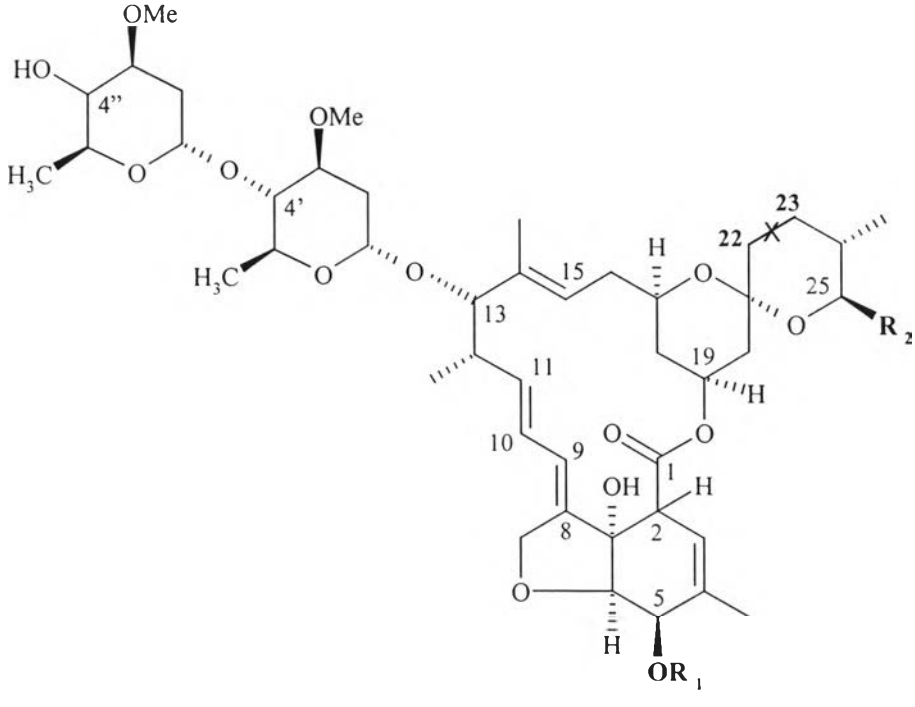
CHAPTER I

INTRODUCTION

1.1 Avermactin Macrocyclic Lactones

Avermactins are antibiotic macrocyclic lactone compounds produced by fermentation of soil bacterium *Streptomyces avermitilis*. These antibiotics are first discovered in Japan, 1979 and are very potent chemical drugs used in agriculture and farm animals for treatment of a broad spectrum of parasitic diseases [Burg *et al.* 1979, Campbell *et al.* 1983]. The structures of avermectins are closely related to complex 16-membered macrocyclic lactones as shown in Figure 1.1.



Avermectins	R_1	R_2	C_{22}-C_{23}
A_{1a}	CH_3	sec-butyl	-CH=CH-
A_{1b}	CH_3	isopropyl	-CH=CH-
B_{1a}	H	sec-butyl	-CH=CH-
B_{1b}	H	isopropyl	-CH=CH-
Ivermectin	H	> 80% sec-butyl < 20% isopropyl	$-CH_2-CH_2-$
Doramectin	H	cyclohexyl	-CH=CH-

Figure 1.1 Chemical structures of avermectin macrocyclic lactones [Campbell *et al.* 1983].

From structures of avermectins, macrocycle is the backbone, and the related compounds are classified by the substituted alkyl groups of R_1 and R_2 and the type of chemical bond between C_{22} and C_{23}. Fermentation of actinomycete *Streptomyces avermitilis* produces homologous pair of related compounds which are avermectin A_1, A_2, B_1 and B_2. These related compounds are further divided into the major components A_{1a}, A_{2a}, B_{1a} and B_{2a} and minor components A_{1b}, A_{2b}, B_{1b} and B_{2b} [Campbell 1989]. Avermectin B_1 is the major component and the most important due to high potency against a broad spectrum of endo- and ectoparasites of farm animals and many agricultural mite and insect pests. Avermectin B_1 also serves as starting material for the semisynthetic 22,23-dihydroanalog, which yields ivermectin. Avermectins are highly hydrophobic substances and dissolve in most organic solvent. The solubility in water of avermectins is correspondingly low, which is approximately 0.006-0.009 ppm. Avermectins has maximum UV absorption at 245 nm as a result of 2 of 5 double bonds conjugated as an 8,9,10,11-diene function [Campbell 1989]. This result is an advantageous property for analytical detection.

Normally, avermectins in commercial uses are abamectin, ivermectin and doramectin. Abamectin or avermectin B_1 consists of avermectin B_{1a} as a major component and avermectin B_{1b} as a minor component. Generally, abamectin product is defined as a mixture containing more than 80% avermectin B_{1a} and less than 20% avermectin B_{1b}. Abamectin is used as insecticide, acaricide or parasiticide for plants or farm animals. Among avermectins, abamectin plays an important role in many agricultural applications at present. Abamectin is applied in controlling of mites and other insect pests on various crops. Moreover, there are some applications of using abamectin in cattle and other farm animals [Vercruysse *et al.* 2002].

Ivermectin or 22,23-dihydroavermectin B_1 is obtained by either fermentation of *Streptomyces avermitilis* or reduction of abamectin. Ivermectin consists of a mixture containing 22,23 dihydroavermectin B_{1a} at least 80% and 22,23 dihydroavermectin B_{1b} less than 20%. Ivermectin is commonly used as antiparasitic or anthelmintic agent for many farm animals such as cattle, horse, sheep, swine and dog.

Doramectin or 25-cyclohexylavermectin B_1, like abamectin and ivermectin, can also be produced by fermentation of *Streptomyces avermitilis*. In contrast, the substituted

alkyl group at C_{25} position of doramectin is a cyclohexyl group resulting from a mutant *Streptomyces avermitilis* strain [Shoop *et al.* 1995]. However, its spectrum of activity is very nearly identical to avermectin B_1 and this lipophilic cyclohexyl group of doramectin also produces greater half-life in tissue [Shoop *et al.* 1995]. At present, doramectin is usually used for cattle and some farm animals.

1.2 Analysis of Avermectins

Most of previous works have been reported on the analysis of avermectins residue in various samples. For example, residues of total avermectin B_1 and its major photodegradation isomer, 8,9-Z-avermectin B_1 were determined by high performance liquid chromatography (HPLC) with fluorescence detection of derivative avermectins in tomatoes [Prabhu *et al.* 1992], apples [Cobin *et al.* 1995], hops [Cobin *et al.* 1996a], wine [Cobin *et al.* 1996b], fruits and vegetables [Diserens *et al.* 1999], and milk [Chou *et al.* 2004]. Some work has been reported as a comparison of UV, fluorescence and mass spectrometry detection in HPLC for analysis of avermectin residues in citrus fruits [Valenzuela *et al.* 2001] and soil [Brewer *et al.* 2004]. Moreover, there is also a number of works involving analysis of avermectins residue in animal tissue and plasma using detection methods as fluorescence [He *et al.* 2005] and mass spectrometry [Yoshii *et al.* 2000, Valenzuela *et al.* 2001, Wu *et al.* 2001, Pozo *et al.* 2003, Pereira *et al.* 2004, Grimalt *et al.* 2005, Turnipseed *et al.* 2005].

1.3 Motivation of This Work

As previously mentioned, fermentation of the actinomycete *Streptomyces avermitilis* produces homologous pair of related compounds that have abamectin as the dominant product. Commercially, it is impractical and not economic to isolate pure abamectin from another by products. Therefore, commercial products used in agriculture, especially abamectin insecticide product, are a mixture of avermectins.

It should be noted that specific strains of actinomycete *Streptomyces avermitilis* are important for fermentation to obtain each of these three avermetins (abamectin, ivermectin and doramectin). From literature review, the specific strains of abamectin, ivermectin and doramectin are still not disclosed and the trading information of

specific strains in commercial is rarely found. Nowadays, commercial avermectins used in Thailand are imported. Researchers at the Institute of Biotechnology and Genetic Engineering, Chulalongkorn University has investigated on the isolation of *Streptomyces avermitilis* from soil samples in order to produce abamectin by fermentation. Furthermore, according to the regulation, the content of abamectin in formulation must be in the range of 1.57 to 2.03 % weight by volume, issued by the Department of Agriculture, the Ministry of Agricultural and Cooperatives, Thailand. Therefore, a quantitative method is important for screening and monitoring abamectin in fermented products and for quality control of abamectin content in formulation.

Due to extremely lipophilic property of avermectins [Campbell 1989], the commercial products of avermectins usually consist of emulsifier, modifier, mineral oil and/or other oils used as solvent to improve their solubility. Thus, a direct method for HPLC analysis of samples requires long analysis time to remove highly hydrophobic compounds that strongly retain in an HPLC column, approximately 1 hour per sample, and uses a large amount of organic solvents. Capillary electrophoresis (CE) has been shown to be a powerful and alternative method to HPLC for direct analysis of samples without the need of matrix removal, and with fast flushing matrix after each run [Nhujak *et al.* 2005, Nhujak *et al.* 2006]. Additional advantages of CE include high efficiency, short analysis time and many applications for both hydrophobic and hydrophilic compounds [Suzuki *et al.* 1998, and Marsh *et al.* 2005]. It is well-known that CE is mostly used for analysis of charged analytes, therefore, CE analysis of highly hydrophobic neutral compounds of avermectin macrocyclic lactones is a challenging work.

Recently, microemulsion electrokinetic chromatography (MEEKC), a new mode of CE, has been reported to be an alternative way for analysis of varieties of hydrophobic compounds such as steroid [Vomastova *et al.* 1996], fat-soluble vitamins [Pedersen-Bjergaard *et al.* 2000], polymer additives [Hilder *et al.* 2001], UV filter in sunscreen lotion [Klampfl *et al.* 2003], capsaicins [Jungmanotham *et al.* 2004] and curcuminoids [Nhujak *et al.* 2006]. Up to date, CE has not been previously reported for analysis of avermectins.

1.4 Aims and Scope

The aims of this work are to develop MEEKC as an alternative method for analysis of avermectins and to compare MEEKC and HPLC for determination of avermectins in commercial products. MEEKC separation was carried out by using microemulsion containing a phosphate buffer at pH 2.5, *n*-octane as oil droplet, sodiumdodecyl sulfate (SDS) as surfactant, 1-butanol as co-surfactant and appropriate types and amounts of organic co-solvent. Furthermore, factors, such as SDS concentration, types of co-surfactants, temperatures and voltages for separation, will be investigated in order to obtain suitable resolution of analytes. Validation of an MEEKC method will be studied on accuracy, precision, and the limits of detection and quantitation. Moreover, the developed MEEKC method will be applied for quantitative analysis of avermectins in commercial products, and the determined amount of avermectins obtained from MEEKC and HPLC will be compared. It is expected that this developed MEEKC method can be used as an alternative method for quantitative analysis of avermectins in commercial formulations and other forms of samples.