

ไมโครอิมัลชันอิเล็กโทรโคเนติกโครมาโทกราฟีสำหรับการแยกและการวิเคราะห์

อะเวอร์เมกทินแมกโครไซคลิกแลกโตน



นายปริญญา สีลานันท์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชาเคมี ภาควิชาเคมี

คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2548

ISBN 974-53-2663-1

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

MICROEMULSION ELECTROKINETIC CHROMATOGRAPHY  
FOR SEPARATION AND ANALYSIS  
OF AVERMECTIN MACROCYCLIC LACTONES

Mr. Parinya Seelanan

A Thesis Submitted in Partial Fulfillment of the Requirements  
for the Degree of Master of Science Program in Chemistry

Department of Chemistry

Faculty of Science

Chulalongkorn University

Academic Year 2005


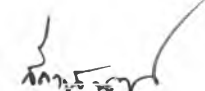


ISBN 974-53-2663-1

**481984**



ปริญญา ศึกษานันท์: ไมโครอิมัลชันอิเล็กโทรโครมาโทกราฟีสำหรับการแยกและการวิเคราะห์อะเวอร์เมกทินแมกโครไซคลิกแลกโตน (MICROEMULSION ELECTROKINETIC CHROMATOGRAPHY FOR SEPARATION AND ANALYSIS OF AVERMECTIN MACROCYCLIC LACTONES) อาจารย์ที่ปรึกษา: ผู้ช่วยศาสตราจารย์ ดร. ธรรมบุญ หนูจักร 70 หน้า. ISBN 974-53-2663-1

ได้พัฒนาเทคนิคไมโครอิมัลชันอิเล็กโทรโครมาโทกราฟี (MEEKC) สำหรับการแยกและการวิเคราะห์อะเวอร์เมกทินแมกโครไซคลิกแลกโตน ได้แก่ อะบาเมกทิน โดราเมกทิน และไอเวอร์เมกทิน โดยใช้ไมโครอิมัลชันบัฟเฟอร์ที่ประกอบด้วย 50 mM ฟอสเฟตบัฟเฟอร์ที่ pH 2.5, 1.1 % v/v *n*-ออกเทนเป็นหยคน้ำมัน, 180 mM โซเดียมโคเดกซิลซัลเฟตเป็นสารลดแรงตึงผิว, 890 mM 1-บิวทานอลเป็นสารลดแรงตึงผิวร่วม และ 30 % v/v เอทานอลเป็นตัวทำละลายอินทรีย์ร่วม ศักย์ไฟฟ้าที่ใช้ -15 kV และอุณหภูมิที่ใช้แยก 25°C พบว่าได้ค่าการแยกของพิกที่ฐานของสารมากถึง 4.9 ด้วยเวลาวิเคราะห์ภายใน 25 นาที มีความแม่นยำและความเที่ยงของวิธีการสูง ได้ใช้วิธี MEEKC ที่พัฒนาขึ้นสำหรับปริมาณวิเคราะห์ของอะเวอร์เมกทินในสูตรผสมทางการค้า พบว่าปริมาณของอะเวอร์เมกทินที่วิเคราะห์ได้จาก MEEKC ในแต่ละสูตรผสมไม่แตกต่างกันมีนัยสำคัญกับที่วิเคราะห์ได้จากไฮเพอร์ฟอร์แมนซ์ลิควิดโครมาโทกราฟี ดังนั้น MEEKC สามารถใช้เป็นอีกวิธีทางเลือกหนึ่งสำหรับปริมาณวิเคราะห์ของอะเวอร์เมกทิน ด้วยเวลาวิเคราะห์ที่เร็วกว่าและปริมาณตัวทำละลายอินทรีย์ที่ใช้น้อยกว่า

ภาควิชา.....เคมี.....ลายมือชื่อนิสิต...............  
 สาขาวิชา.....เคมี.....ลายมือชื่ออาจารย์ที่ปรึกษา...............  
 ปีการศึกษา.....2548.....ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

# # 457 23690 23: MAJOR CHEMISTRY

KEYWORDS: MICROEMULSION ELECTROKINETIC CHROMATOGRAPHY/  
CAPILLARY ELECTROPHORESIS/ AVERMECTINS/ ABAMECTIN/  
IVERMECTIN/ DORAMECTIN

PARINYA SEELANAN: MICROEMULSION ELECTROKINETIC  
CHROMATOGRAPHY FOR SEPARATION AND ANALYSIS OF  
AVERMECTIN MACROCYCLIC LACTONES. THESIS ADVISOR:  
ASST. PROF. THUMNOON NHUJAK, Ph.D., 70 pp. ISBN 974-53-2663-1.

Microemulsion electrokinetic chromatography (MEEKC) was developed for quantitative analysis of avermectins, such as abamectin, doramectin and ivermectin, in commercial formulations, using the microemulsion buffer containing a 50 mM phosphate buffer at pH 2.5, 1.1 % v/v *n*-octane as oil droplets, 180 mM sodium dodecyl sulfate as surfactant, 890 mM 1-butanol as co-surfactant and 30 % v/v ethanol as organic co-solvent; applied voltage of -15 kV; and separation temperature at 25 °C. Achieved baseline resolution up to 4.9 was obtained with analysis time within 25 min. High accuracy and precision of the method were obtained. The developed MEEKC method was used for quantitative analysis of avermectins in commercial formulations. The contents of avermectins in commercial formulations determined by MEEKC were found to be insignificantly different with those determined by high performance liquid chromatography (HPLC). Therefore, MEEKC can be used as an alternative method to HPLC for quantitative determination of avermectins with shorter analysis time and lower amount of organic solvent consumption.

Department.....Chemistry..... Student's signature.....  
Field of study.....Chemistry ..... Advisor's signature.....  
Academic year.....2005..... Co-advisor's signature.....

## ACKNOWLEDGEMENTS

First of all, I am really appreciated and would like to express my deepest gratitude to Assistant Professor Dr. Thumnoon Nhujak, my thesis advisor, for his professionalism, discerning guidance, attentive supervision, forbearance, motivation and devotion toward my study. My special thanks also extend to the thesis committee for their valuable comments. In addition, I wish to thank the Department of Chemistry, the Faculty of Science, Chulalongkorn University, for all facilities and grant. Moreover, this work is granted by the Graduate School, Chulalongkorn University, and I am much indebted. I would like to thank the Institute of Biotechnology and Genetic Engineering for the information and instrumentation used for my initial study on HPLC.

I gratefully acknowledge Mr. Pichai Manichote and all laboratory staffs of Ladda Co., Ltd., Thailand, for their kindness, useful suggestions and information of insecticide commercial formulation. Thank to Dr. Teerachai Wongcharu, a veterinary surgeon of Pfizer (Thailand) Co., Ltd., for the beneficial knowledge of antiparasitic agents. I would like to thank Mr. Pichachan Pangchan, an animal husbandry scientist and also my nice friend, for providing a sample of avermectins.

I am grateful to all members of Separation and Chromatography Research Group, especially all members of CE group, for their helpfulness and suggestions. In particular, special thanks go to Monphichar and Warangkana for moral supports and cheerful willingness. Also, heartfelt friendship and encouragement from Oraphan, Urairat and Duangkamol, my lovely friends, are truly appreciated. Thanks also extend to everyone who has contributed suggestions and supports throughout this research.

I wish to thank Mrs. Krongrat Trisatayapan, my beloved aunt, for her unconditional love, cheer and supports through to the end. Finally, I am eternally grateful to my parents and the rest of my family members for their endless love and warmth, the greatest things in my life I have ever had.

Parinya Seelanan

# CONTENTS

	PAGE
ABSTRACT (IN THAI) .....	iv
ABSTRACT (IN ENGLISH).....	v
ACKNOWLEDGEMENTS .....	vi
CONTENTS.....	vii
LIST OF TABLES .....	x
LIST OF FIGURES .....	xi
LIST OF ABBREVIATIONS AND SYMBOLS .....	xiv
CHAPTER I INTRODUCTION.....	1
1.1 Avermectin Macrocyclic Lactones .....	1
1.2 Analysis of Avermectins.....	3
1.3 Motivation of This Work .....	3
1.4 Aims and Scope .....	5
CHAPTER II THEORY .....	5
2.1 Introduction and Modes of Capillary Electrophoresis .....	6
2.2 Electrophoretic Mobility.....	8
2.3 Electroosmosis .....	8
2.4 Migration Behaviour of Analytes in CE .....	10
2.5 Microemulsion Electrokinetic Chromatography (MEEKC).....	11
2.6 Retention in MEEKC.....	14
2.7 Peak Efficiency, Peak Broadening and Resolution.....	16
2.7.1 Peak efficiency.....	16
2.7.2 Peak broadening.....	17
2.7.2.1 Longitudinal diffusion .....	18
2.7.2.2 Intermicelle mass transfer in the aqueous phase.....	19
2.7.2.3 Sorption-desorption kinetics in microemulsion solubilisation.....	20
2.7.2.4 Thermal dispersion.....	21
2.7.2.5 Micellar polydispersity .....	22
2.7.3 Resolution .....	23

	<b>PAGE</b>
2.8 Instrumentation .....	24
2.8.1 Basic apparatus of CE .....	24
2.8.2 Injection system .....	25
2.8.3 Detection system .....	25
2.9 Quantitative Analysis in CE.....	26
2.9.1 Peak area and corrected peak area .....	26
2.9.2 Internal standard.....	28
CHAPTER III EXPERIMENTAL.....	29
3.1 Chemicals and Materials.....	29
3.2 CE Analysis .....	29
3.2.1 CE conditions.....	29
3.2.2 Preparation of microemulsion buffer .....	30
3.2.3 Preparation of standard solutions.....	30
3.3 MEEKC Optimisation .....	31
3.3.1 Effect of types and concentration of organic co-solvents.....	31
3.3.2 Effect of SDS concentration .....	31
3.3.3 Effect of co-surfactant types .....	31
3.3.4 Effect of temperature .....	32
3.3.5 Effect of applied voltage .....	32
3.3.6 Effect of pressure .....	32
3.4 Validation of MEEKC Method.....	32
3.4.1 Limit of detection (LOD) and limit of quantitation (LOQ) .....	33
3.4.2 Calibration plots.....	33
3.4.3 Accuracy and precision.....	33
3.5 HPLC Analysis .....	34
3.3.1 HPLC conditions.....	34
3.3.2 Calibration plots.....	34
3.6 Applications to Real Samples .....	35



	<b>PAGE</b>
CHAPTER IV RESULTS AND DISCUSSION.....	35
4.1    Choices of CE Conditions.....	35
4.1.1 pH and concentration of buffer .....	35
4.1.2 Diameter and length of capillary.....	38
4.1.3 Detection wavelength.....	38
4.2    MEEKC Optimisation.....	38
4.2.1 Effect of types and concentration of organic co-solvents.....	39
4.2.2 Effect of SDS concentrations.....	43
4.2.3 Effect of co-surfactant types .....	46
4.2.4 Effect of temperatures.....	49
4.2.5 Effect of applied voltages .....	49
4.2.6 Effect of pressure .....	54
4.3    Validation of MEEKC Method.....	55
4.3.1 Limit of detection (LOD) and limit of quantitation (LOQ) .....	55
4.3.2 Calibration plots.....	55
4.3.3 Accuracy and precision.....	57
4.4    Application to Real Samples.....	59
CHAPTER V CONCLUSION AND FUTURE WORK .....	64
REFERENCES .....	66
VITA.....	70

## LIST OF TABLES

<b>TABLES</b>	<b>PAGE</b>
3.1 Sampling weight of commercial avermectins and dilution factor for analysis by MEEKC and HPLC.....	35
4.1 LOD and LOQ of individual avermectin.....	55
4.2 Concentration ranges and linear equations for calibration plots.....	56
4.3 MEEKC analysis of the amounts of standard avermectins spiked in the microemulsion and the diluted solution of samples, $n = 5$ runs.....	58
4.4 Intraday and interday precision in migration time and $A_{\text{corr, ratio}}$ of 100 ppm avermectin each .....	59
4.5 The amounts of avermectins in samples determined by MEEKC and HPLC .....	63

## LIST OF FIGURES

FIGURES	PAGE
1.1 Chemical structures of avermectin macrocyclic lactones.....	1
2.1 Separation mechanism of (a) CZE, (b) MEKC and (c) MEEKC.....	7
2.2 Electroosmotic flow (EOF).....	9
2.3 Flow profile and peak shape in CE .....	10
2.4 Migration behaviour of the analytes .....	11
2.5 Schematic of microemulsion.....	12
2.6 Schematic of the separation principles of MEEKC: (a) high EOF and (b) low EOF .....	13
2.7 Typical elution order for analytes in MEEKC: (a) high EOF and (b) low EOF, where $k_A > k_B > k_C$ . .....	13
2.8 Gaussian peak.....	16
2.9 Schematic diagram of a basic CE instrument. ....	24
4.1 UV absorption of abamectin in the microemulsion buffer containing 50 mM phosphate buffer at pH 2.5, 1.1 %v/v <i>n</i> -octane, 890 mM 1-butanol and 180 mM SDS, at 245 nm within (a) 2 hours and (b) 7 days.....	37
4.2 MEEKC separation of avermectins using (a) no organic co-solvent and 30% v/v organic co-solvent as (b) acetonitrile (ACN), (c) methanol (MeOH), (d) ethanol (EtOH), and (e) 2-propanol (2-PrOH) in a pH 2.5 50 mM phosphate buffer containing 1.1 % v/v <i>n</i> -octane, 180 mM SDS and 890 mM 1-butanol. CE conditions: uncoated fused silica capillary 50 $\mu$ m I.D. $\times$ 40.2 cm (30 cm to detector), temperature 25 $^{\circ}$ C, voltage -15 kV, 0.5 psi pressure injection for 3 s and UV detection at 214 nm, DB = dodecyl benzene used as microemulsion marker, I = ivermectin, D = doramectin, C = abamectin B <sub>1a</sub> , and ISTD = internal standard.....	41
4.3 Effect of types and concentration of organic co-solvents on (a) retention factor of A, (b) selectivity of D and A, (e) average efficiency of D and A, and (f) resolution of D and A. Acetonitrile( $\diamond$ ), methanol ( $\square$ ), ethanol ( $\Delta$ ), and 2-propanol ( $\times$ ). Other CE conditions shown in Figure 4.2. Each symbol is the average value obtained from duplicate runs .....	42

FIGURES	PAGE
4.4 Electropherograms of avermectins in MEEKC using different concentration of SDS (a) 130, (b) 150, (c) 180, (d) 200 and (e) 220 mM. Other CE conditions as shown in Figure 4.2d.....	44
4.5 Effect of SDS concentration on (a) migration time, (b) retention factor, (c) selectivity, (d) efficiency, and resolution of avermectins. Other CE conditions as shown in Figure 4.2d. Each symbol is the average value obtained from duplicate runs.....	45
4.6 Electropherograms of avermectins in MEEKC using different types of 890 mM co-surfactants as (a) 1-butanol, (b) 2-butanol, (c) tert-butanol, (d) isoamyl alcohol, (e) 4-methyl-2-pentanol, and (f) 1-hexanol. Other CE conditions as shown in Figure 4.2d.....	47
4.7 Effect of types of co-surfactants on (a) migration time, (b) electrophoretic mobility, (c) retention factor, (d) selectivity, (e) efficiency, and (f) resolution of avermectins. Other CE conditions as shown in Figure 4.2d. Each symbol is the average value obtained from duplicate runs .....	48
4.8 Electropherograms of avermectins in MEEKC using different temperatures (a) 25, (b) 30, (c) 35, and (d) 40°C. Other CE conditions as shown in Figure 4.2d.....	50
4.9 Effect of temperature on (a) migration time, (b) electrophoretic mobility, (c) retention factor, (d) selectivity, (e) efficiency, and (f) resolution of avermectins. Other CE conditions as shown in Figure 4.2d. Each symbol is the average value obtained from duplicate runs .....	51
4.10 Electropherograms of avermectins in MEEKC using different applied voltages (a) -15, (b) -20, and (c) -25 kV. Other CE conditions as shown in Figure 4.2d .....	52
4.11 Effect of applied voltage on (a) migration time, (b) electrophoretic mobility, (c) retention factor, (d) selectivity, (e) efficiency, and (f) resolution of avermectins. Other CE conditions as shown in Figure 4.2d. Each symbol is the average value obtained from duplicate runs .....	53
4.12 Electropherograms of avermectins in MEEKC using -15 kV and different applied pressure (a) 1.0, (b) 0.8, (c) 0.6, (d) 0.1 psi and (e) without pressure. Other CE conditions as shown in Figure 4.2d .....	54
4.13 Calibration plots between $A_{\text{corr, ratio}}$ and the concentration of ivermectin (I), doramectin (D) and abamectin B <sub>1</sub> (A). Each point was run in triplicate and with RSD < 4% .....	56

<b>FIGURES</b>	<b>PAGE</b>
4.14 An example of electropherograms of avermectins formulations of samples A1 (a and b), A2 (c), A3 (d), D1 (e) and I1 (f). UV detection at 214 nm (a) and 245 nm (b-f). Other CE conditions as shown in Fig. 4.2d...	60
4.15 An example of HPLC chromatograms of (a) mixed standards and avermectins formulations of samples A1 (b), A2 (c) and A3 (d) using HPLC conditions as described in Section 3.5.1. ....	61

**LIST OF ABBREVIATIONS AND SYMBOLS**

A	abamectin B <sub>1</sub>
BGE	background electrolyte
D	doramectin
I	ivermectin
CE	capillary electrophoresis
CEC	capillary electrochromatography
CGE	capillary gel electrophoresis
CIEF	capillary isoelectric focusing
CITP	capillary isotachopheresis
CMC	critical micelle concentration
CZE	capillary zone electrophoresis
DB	dodecyl benzene
EOF	electroosmotic flow
HPLC	high performance liquid chromatography
I.D.	internal diameter
ISTD	internal standard
LC	liquid chromatography
MEKC	micellar electrokinetic chromatography
MEEKC	microemulsion electrokinetic chromatography
MS	mass spectrometry
RSD	relative standard deviation
SDS	sodium dodecyl sulphate
<i>A</i>	peak area
<i>A</i> <sub>aq</sub>	analyte molecule in the aqueous phase
<i>A</i> <sub>mc</sub>	analyte molecule in the microemulsion phase
<i>A</i> <sub>corr</sub>	corrected peak area
<i>B</i>	constant value (2400 K)
<i>c</i>	concentration of analyte
<i>D</i>	diffusion coefficient
<i>D</i> <sub>aq</sub>	diffusion coefficient of the solute in the aqueous phase
<i>D</i> <sub>mc</sub>	diffusion coefficient of the solute in micelle (microemulsion) phase

$D_{th}$	diffusion coefficient of the solute by thermal dispersion
$E$	electric field strength
$e$	electronic charge
$F$	Faraday's constant
$H$	plate height
$H_{aq}$	plate height due to intermicelle mass transfer in the aqueous phase
$H_l$	plate height due to longitudinal diffusion
$H_{mc}$	plate height due to sorption-desorption kinetics in microemulsion solubilisation
$H_{pd}$	plate height due to micellar polydispersity
$H_t$	plate height due to thermal dispersion
$I$	ionic strength
$I_A$	electric current
$K$	distribution constant
$k$	retention factor
$k_A$	retention factor of analyte A
$k_B$	retention factor of analyte B
$k_C$	retention factor of analyte C
$k_d$	desorption rate constants
$k_s$	sorption rate constants
$k^*$	boltzmann constant
$L$	total capillary length
$l$	the length of capillary to detector
$l_{inj}$	length of analyte injected
$N$	the number of theoretical plate, or peak efficiency
$n_{aq}$	the amount of analyte in aqueous phase
$n_{mc}$	the amount of analyte in microemulsion phase
$\Delta P$	pressure difference across the capillary
$Q$	amount of analyte
$Q_{inj}$	quantity of sample injected
$Q_{determined}$	the determined amount of analyte in the diluted sample after spiking standard
$Q_{sample}$	the determined amount of analyte in the diluted sample solution before spiking standard

$Q_{\text{spiked}}$	the amount of spiked standard
$R$	gas constant
$R_s$	resolution
$r$	internal capillary radius
$r_h$	hydrodynamic radius
$T$	absolute temperature
$t$	time
$t_0$	retention time of unretained compound
$t_{\text{eo}}$	migration of EOF
$t_{\text{inj}}$	injection time
$t_m$	migration time
$t_{\text{mc}}$	retention time of microemulsion
$t_R$	retention time
$t_{R,A}$	retention time of analyte A
$t_{R,B}$	retention time of analyte B
$t_{R,C}$	retention time of analyte C
$t_{\text{mc}}^*$	mean life-time of analyte in microemulsion (micelle)
$V$	applied voltage
$V_F$	volume flow of the analyte passing the detector
$V_{\text{inj}}$	volume of sample injected
$v_{\text{eo}}$	electroosmotic velocity
$v_{\text{ep}}$	electrophoretic velocity
$v_{\text{net}}$	total electrophoretic velocity
$w$	the amount of sampling weight
$w_b$	peak width at base
$w_h$	peak width at half height
$x_{\text{aq}}$	mole fraction of analyte in aqueous phase
$x_{\text{mc}}$	mole fraction of analyte in microemulsion phase
$z$	charge of an ion
$\alpha$	selectivity
$\epsilon$	permittivity
$\phi$	volume of the aqueous phase to microemulsion phase
$\eta$	viscosity



$\kappa$	electrical conductivity
$\lambda_s$	thermal conductivity
$\mu$	electrophoretic mobility
$\mu_{eo}$	electroosmotic mobility
$\mu_{obs}$	observed mobility
$\mu_{mc}$	mobility of microemulsion
$\mu_{net}$	total mobility
$\mu^0$	absolute mobility at zero ionic strength
$\sigma$	standard deviation of peak in distance unit
$\sigma_{\mu,mc}$	standard deviation of electrophoretic mobility of microemulsion (micelle)
$\sigma^2$	peak variance
$\sigma_{diff}^2$	peak variance due to longitudinal diffusion
$\sigma_{th}^2$	peak variance due to thermal diffusion
$\tau$	standard deviation of peak in time unit
$\zeta$	zeta potential