

*Siti Hendrick Indrawan*

# CDD

## 2016

**The 4<sup>th</sup>**

# Current Drug Development International Conference 2016

**June 1-3, 2016**

**Phuket Graceland Resort & Spa,  
Patong Beach, Phuket, Thailand**



**Proceeding  
The 4th Current Drug Development  
International Conference 2016 (CDD 2016)**

**Editors**

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**CDD2016**

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## Welcome Address

Dear Colleagues,

It is our great pleasure to welcome you to the fourth Current Drug Development (CDD) International Conference 2016. At the first CDD International Conference in 2010, we heard from speakers and participants who shared their valuable experiences and contributions in drug delivery systems and the development of natural medicines. The second CDD International Conference in 2012, which mainly focused on the development of anticancer agents, had distinguished speakers sharing their expertise in advancing anticancer agent from plants and marine life. During the third CDD International Conference in 2014, which highlighted the importance of pharmaceutical biotechnology, we had a great opportunity to meet and discuss the topic with renowned researchers in the field. We had about 200 local and overseas participants share their experiences throughout this conference.

At the fourth CDD International Conference 2016, we are focusing on “Drugs used in aging-associated diseases” and many interesting perspectives on new drug development. I am very glad to see that there is a wide range of participation from the many different countries that are joining this conference. We hope that this will be not only a great opportunity for scientists and researchers to exchange their ideas and expertise, but also that a productive network can be formed here. I also hope that the conference environment will inspire and motivate our young researchers to further their research interests and produce excellent outcomes.

On behalf of the organizing committee, I would like to thank our keynote speakers and invited speakers, the organizing staff from the Drug Delivery System Excellence Center, the Phytomedicine and Pharmaceutical Biotechnology Excellence Center, and the Faculty of Pharmaceutical Sciences at Prince of Songkla University, and the Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang, and the Drug and Cosmetic Research and Development Unit, School of Pharmacy, Walailak University. Your contribution is recognized. Finally, I hope you enjoy the city and have a memorable stay in Phuket.

Best regards,

Pharkphoom Panichayupakaranant, Ph.D.

Chair of the conference

## Organizer Information

### Conference Chair

Pharkphoom Panichayupakaranant  
Director of Phytomedicine and Pharmaceutical Biotechnology Excellence Center

### Organizing Committee

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Juraithip	Wungsintaweekul
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Jiraporn	Chingunpitak
Athip	Sakunphueak

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Juraithip	Wungsintaweekul
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### Organization Support

- Faculty of Pharmaceutical Sciences, Prince of Songkla University
- Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang
- Drug and Cosmetic Research and Development Unit, School of Pharmacy, Walailak University
- Drug Delivery System Excellence Center, Prince of Songkla University
- Phytomedicine and Pharmaceutical Biotechnology Excellence Center, Prince of Songkla University

## Scientific Program

The 4<sup>th</sup> Current Drug Development International Conference (CDD 2016)

Phuket Graceland Resort & Spa, Patong Beach, Phuket, Thailand

Wednesday, June 1, 2016

- 8.00 - 8.45 Registration
- 8.45 - 9.00 Opening remark  
Dean of Faculty of Pharmaceutical Sciences/Prince of Songkla University
- Chairperson: Pharkphoom Panichayupakaranant & Jianbo Xiao**
- 9.00 - 9.45 **Keynote Speaker**  
Aerosol Delivery to The Brain: A New Approach for Treatment of Alzheimer's Disease  
**Prof.Dr.Wellington Pham**  
Vanderbilt University, Institute of Imaging Science, USA
- 9.45-10.15 Alternative Treatment for Elderly from Biomaterials  
**Prof.Dr.Pornanong Aramwit**  
Chulalongkorn University, Thailand
- 10.15-10.45 **Leisure & Break**
- Chairperson: Teerapol Srichana & Ibrahim Jantan**
- 10.45-11.15 Edible Bird's Nest Glycoproteins as the Natural Compounds for Anti-aging and Degenerative Diseases  
**Assoc.Prof.Dr.Chua KienHui**  
Universiti Kebangsaan Malaysia, Malaysia
- 11.15 - 12.00 **Keynote Speaker**  
Formulation and Compaction of Orally Dispersible Tablets  
**Assoc.Prof.Dr.Paul W.S. Heng**  
National University of Singapore, Singapore
- 12.00 - 13.00 **Lunch**
- Chairperson: Kamonthip Wiwattanawongsa & Thipapan Plyduang**
- 13.00 - 13.30 Bacterial Ribosome Selective Antibiotics  
**Prof.Dr.Dev P. Arya**  
Clemson University, USA
- 13.30 - 14.00 Research on A Thai Traditional Remedy for Chronic Diseases  
**Assoc.Prof.Dr. Arunporn Itharat**  
Faculty of Medicine Thammasat University, Thailand
- 14.00 - 14.30 Hair Regeneration by Adipose-derived Stem Cells  
**Assoc.Prof.Dr.Jong Hyuk Sung**  
Yonsei University, Korea
- 14.30 - 15.00 Rational Design and Synthesis of Polysaccharides Based Nano-Particles for Drug Delivery System  
**Dr.Warayuth Sajomsang**  
National Science and Technology Development Agency, Thailand
- 15.00 - 15.30 **Leisure & Break**



Chairperson	Athip Sakunphueak & Hazrulrizawati Abd Hamid Session A Dalah 1 Room	Somchai Sawatdee & Attawadee Sae-Yoon Session B Dalah 2 Room
15.30-15.45	Structure-Stability Relationship of Polyphenols in Cell Culture Medium <b>Jianbo Xiao</b> University of Macau, China	Zwitterionic Carbon Nanodots for Simultaneous Nuclear Imaging and Efficient Drug Delivery <b>Yun Kyung Jung</b> Institution Ulsan National Institute of Science and Technology, Korea
15.45 – 16.00	Inhibition of Nitric Oxide Production by <i>Derris scandens</i> (Roxb.) Benth Extract in UVB-Exposed Human Keratinocytes <b>Sumrit Sukhonthasilakun</b> KhonKaen University, Thailand	Characterization of Plaunotol and Plaunotol-Enriched Extract Complexed with Hydroxypropyl- $\beta$ -Cyclodextrin <b>Wai Mi Aung</b> Prince of Songkla University, Thailand
16.00 – 16.15	Antimicrobial Activity of Commercially Available Curcumin Powder and Ethanol Extract of Turmeric Against Oral Pathogenic Microorganism <b>Mian Waqar Mustafa</b> Prince of Songkla University, Thailand	Molecular interaction studies of amphotericin B formulated with sodium deoxycholate sulfate for inhalation by solid state NMR <b>Faisal Usman</b> Prince of Songkla University, Thailand
16.15 – 16.30	$\alpha$ -Glucosidase Inhibition Activity of Rhinacanthins-rich Extract and Rhinacanthin-C <b>Muhammad Ajmal Shah</b> Prince of Songkla University, Thailand	Microneedle-mediated skin permeation with plasmid DNA complexed transfection reagents <b>Boonnada Pamornpathomkul</b> Silpakom University, Thailand
16.30 – 17.30	<b>Poster presentation (Odd number)</b>	
18.30	<b>Welcome Party</b>	

Thursday, June 2, 2016

Chairperson: Sukanya Dej-adisai & Apichart Atipairin

8.45 – 9.30

**Keynote Speaker**

Effects of standardized extracts of selected medicinal plants and their isolates on modulation of immune functions and inflammation

**Prof.Dr.Ibrahim Jantan**

Universiti Kebangsaan Malaysia, Malaysia

9.30 – 10.15	<b>Keynote Speaker</b> Targeting the gut: Methods and effects in the gastrointestinal tract <b>Prof.Dr.med.Karl-Herbert Schäfer</b> University of Applied Sciences Kaiserslautern, Germany
10.15 – 10.45	A search for natural products for erectile dysfunction <b>Assoc.Prof.Dr. Kornkanok Ingkaninan</b> Naresuan University, Thailand
10.45 – 11.00	<b>Leisure &amp; Break</b>
11.00 – 12.00	<b>Poster presentation (Even number)</b>
12.00 – 13.00	<b>Lunch</b>

**Chairperson: Chua Kien Hui & Panupong Puttarak**

13.00 – 13.30	Development of floating drug delivery systems <b>Assoc.Prof.Dr.Srisagul Sungthongjeen</b> Naresuan University, Thailand
13.30 – 14.00	Chitosan for skeletal drug delivery and tissue engineering <b>Asst.Prof.Dr. Kwunchit Oungbho</b> Prince of Songkla University
14.00 – 14.30	Clinical Drug Development <b>Prof.Dr.Basavaraj K. Nanjwade</b> The Oxford College of Pharmacy, India

**Chairperson**

**Kornkanok Ingkaninan &  
Juraithip Wungsintaweekul**  
**Session A**  
**Dalah 1 Room**

**Chitchamai Ovartlarnporn &  
Namfa Sermkaew**  
**Session B**  
**Dalah 2 Room**

14.40 - 14.55	Antioxidant Capacities of Malayan <i>Murrayakoenigii</i> (Curry Leaves Tree) <b>Rou Chian Ng</b> Universiti Putra Malaysia, Malaysia	Multidimensional Screening Platform for the Simultaneous Targeting of Cancer Pathways <b>Hendrik Luesch</b> University of Florida, USA
14.55 – 15.10	Antioxidant Activity of <i>Etilingeraelator</i> Flower Tea Extracts. <b>Sasiwisa Hosuwan</b> Prince of Songkla University, Thailand	Tamarind seeds polysaccharides (TSP) extracted from <i>Tamarind indica</i> L. as drug delivery system <b>Khanittha Chawanoraset</b> Thailand Institute of scientific and Technological Research (TISTR)
15.10 – 15.25	Antioxidant Activity of Synthetic Eugenol-Based Analogues <b>Sudarat Saenset</b> Naresuan University, Thailand	
15.25 – 15.40	<b>Leisure &amp; Break</b>	

15.40 – 15.55	<p>Production and Characterization of Monoclonal Antibodies against the Isoflavone Glycoside Daidzin</p> <p><b>Gorawit Yusakul</b> Kyushu University, Japan</p>	<p>Isolation and Chemical Profile Confirmation of <i>Tinosporacrispa</i> Extract by UPLC/QToF MS for their antidiabetic potential</p> <p><b>Hazrulrizawati Abd Hamid</b> Universiti Malaysia Pahang, Malaysia</p>
15.55 – 16.10	<p>An In Vitro Study of Wound Healing Effect of Okra Extract as Evidenced by Enhanced Cell Proliferation and Cell Migration</p> <p><b>Sirikhwan Manee</b> Prince of Songkla University, Thailand</p>	<p>Comparison of Electroporation and Iontophoresis effects on Magnesium Ascorbyl Phosphate Permeation through Skin Model</p> <p><b>Soawaluk Poldech</b> Srinakharinwirot University, Thailand</p>
16.10 – 16.25	<p>Heparan Sulfate and Heparin Oligosaccharides as Versatile Molecules for Biological Approaches: Their Structural Heterogeneity and Diversities Challenges</p> <p><b>Nina SuhaityAzmi</b> Universiti Malaysia Pahang, Malaysia</p>	<p>Amino Acids and Peptide-Based Molecular Imprinted Polymers</p> <p><b>Sreenu Madhumanchi</b> Prince of Songkla University, Thailand</p>
16.25 – 16.40	<p>Antibacterial and Effect on Bacterial Growth of Crude Ethyl Acetate Extract of <i>Actinomyces</i> strain PN51B-4-1</p> <p><b>Jirarat Surinta</b> Mahidol University, Thailand</p>	
17.00	<p><b>Closing Ceremony</b></p>	

Friday, June 3, 2016

8.00 – 17.00

Excursion 1 & 2

### Poster Presentation

#### Session A: Pharmaceutical Biotechnology and Herbal Medicines & Dietary Supplements

Paper Code	Paper name	Name Surname	
<b>Pharmaceutical Biotechnology</b>			
PA001	Biotransformation of dehydroepiandrosterone by <i>Aspergillus candidus</i>	Ali	Kuru
PA002	Functional consequences of BARD1 missense mutations on ubiquitin ligase activity	Apichart	Atipairin
PA003	Antioxidant Effect of Imperatorin against Ultraviolet B Irradiation -induced ROS in Human Keratinocytes	Kanuengnit	Choochuay
PA004	Differential mRNA expression of homologous recombination-related genes in breast cancer cells treated with cisplatin	Khwanjira	Hongthong
PA005	Anthraquinone variation in <i>Cassia tora</i> plantlet	Masetoh	Haling
PA006	Construction of Genomic Library of <i>Streptomyces</i> sp. KBI	Nuttapon	Songnaka
PA007	Synergistic effects of combined olaparib and RAPTA-EA1 in <i>BRCA1</i> -associated breast cancer cell lines	Pornvichai	Temboot
PA008	Effect of Dilution Susceptibility Testing Methods on Antibacterial Activity of Chitosan	Supreede	Sungkarak
<b>Herbal Medicines &amp; Dietary Supplements</b>			
PA009	Screening of anti-breast cancer activity from Thai and Indian medicinal plants extract	Aisah	Yusoh
PA010	Formulation Development and Evaluation of Multifunctional Cosmetic Cream	Attawadee	Sae-Yoon
PA011	Simultaneous HPLC Quantitative Analysis of Phenolic Compounds in <i>Pluchea indica</i> Leaf Extract	Boonyadist	Vongsak
PA012	Cytotoxic Effect of Thai Medicinal Herbal Extracts on Human Cancer Cell Lines	Juthathip	Poofery
PA013	Antimicrobial activity of endophytic fungi isolated from <i>Eurycoma longifolia</i> Jack.	Kusuma	Jitsaeng

Paper Code	Paper name	Name Surname	
PA014	Antibacterial Potential of Singawalang ( <i>Petiveria Alliacea</i> ) Against <i>Mycobacterium Tuberculosis</i> H37Rv	Mulyani	Yani
PA015	Effects of carbazole alkaloid from <i>Murraya Koenigii</i> on proliferation and MMP-13 mRNA expression in PC3 cell line and HUVECs	Nurdina	Charong
PA016	Green extraction method for <i>Garcinia cowa</i> leaf extracts containing chamuangone	Pirunrat	Sae-Lim
PA017	Efficacy Evaluation of a Multifunctional Cosmetic Cream	Pisit	Rakkong
PA018	Anti-oxidative Activity of <i>Clerodendrum inerme</i> (L.) Gaertn. Extracts	Poonsit	Hiransai
PA019	In Vitro Bioactivities Study of <i>Portulaca oleracea</i> Linn. Extract in HaCaT cell	Pornsawan	Juntong
PA020	Inhibition of Lipopolysaccharide-Induced Nitric Oxide production By Isolated Compounds from <i>Chromolaena odorata</i> (L.) in RAW 264.7 Macrophages	Rana	Dhar
PA021	Cytotoxic effect of <i>Piper cubeba</i> Linn extract for breast cancer treatment.	Saowanee	Muangchanburi
PA022	Preliminary study on the effect of a topical alcohol free curcumin preparation on a modified acetic acid-induced buccal mucosal ulcer in hamster model	Sineenat	Kuadkaew
PA023	Stability-indicating HPLC Method for Determination of Berberine Hydrochloride in <i>Arcangelisia flava</i> Extract	Sirinan	Khongphan
PA024	Nitric oxide inhibitory activity of topical gel containing an aqueous noni fruit extract	Sonsawan	Kongpuckdee
PA025	Inhibition of Nitric Oxide Production by <i>Derris scandens</i> (Roxb.) Benth Extract in UVB-Exposed Human Keratinocytes	Sumrit	Sukhonthasilakun
PA026	Enzymes inhibitory activity of isolated phytoconstituents from <i>Euphorbia hirta</i> for treatment and management of metabolic syndrome	Sunil	Kumar
PA027	Antioxidant activity of <i>Bauhinia aureifolia</i> K. & S.S. Larsen (stems) extract	Supat	Langyanai

Paper Code	Paper name	Name Surname	
PA028	Study on antioxidant activity of Thai medicinal plants in Songkhla Rajabhat University	Supat	Langyanai
PA029	Study on Culture Condition of the Mycelial Growth of <i>Schizophyllum commune</i>	Surachai	Techaoei
PA030	ROS Scavenging Effect of Isolated Interruptins from <i>Cyclosorus terminans</i>	Suriya	Chaiwong
PA031	Increased multidrug transporter activity in human oral squamous cell carcinoma cells is associated with cisplatin resistance	Warangkana	Chunglok

**Session B: Pharmaceutical Chemistry, Pharmaceutical Technology & Drug Delivery System and Biopharmaceutics**

Paper Code	Paper name	Name Surname
<b>Pharmaceutical Chemistry</b>		
PB001	Carbon Dioxide Supercritical Fluid Extraction of <i>t</i> -Resveratrol from <i>Arachis hypogaea</i> Using Central Composite Design	Amornrut Chaidedgumjorn
PB002	Phytochemicals and cytotoxicity studies of <i>Smithatris supraneanae</i> W.J. Kress & K.Larsen	Khanittha Chawanoraset
PB003	Simultaneous Quantitative HPLC Method for Determination of <i>trans</i> -Resveratrol and Its Glucoside in Thai Beverages	Malai Satiraphan
PB004	Development of <i>in vitro</i> assay for the detection of fetal hemoglobin inducers	Patamaporn Pruksakorn
PB005	Synthesis of Benzhydrol and Its Acetamido Analogues as Antituberculosis Agents	Pitikan Kanjanapruk
PB006	Synthesis of Some Biologically Important Thiazolidinone Compounds	Sedat Sevmeszler
PB007	Antiproliferative compound of <i>Dipterocarpus obtusifolius</i> root	Woranan Chancherdtha
<b>Pharmaceutical Technology &amp; Drug Delivery System</b>		
PB008	Analytical Method Validation for Determination of Quinine Sulfate in Extemporaneous Oral Suspension	Aitsara Mitchuayrod
PB009	Development of Lidocaine Mucoadhesive Films	Araya Raiwa
PB010	Influence of hydrophobicity or hydrophilicity of nano silica in flow property of cohesive API in a powder mixing: a comparative evaluation	Bappaditya Chatterjee
PB011	Water Absorption and Mucoadhesive Properties of <i>Scaphium macropodum</i> Beaum. (Malva Nut)	Benjabhorn Sethabouppha

Paper Code	Paper name	Name Surname
PB012	Effect Of Incubation Time On Protein Loading And Encapsulation Efficiency Of Ovalbumin-Alginate Microspheres	Dewi Melani Hariyadi
PB013	Activity of Cyprofloxacin from implant with cross link agent genipin in composites to <i>Staphylococcus aureus</i> ATCC25923 dan <i>Escherichia coli</i> ATCC 25922	Esti Hendradi
PB014	Preparation and Characterization of Chlortetracycline Hydrochloride Solid Dispersions	Jenjira Apiwongngam
PB015	Screening of Cytotoxic and Antioxidant Activities of <i>Jatropha curcas</i> Linn Latex Extracts	kittiya Tinpun
PB016	Formulation Development of Mucoadhesive Microspheres from Extract of <i>Piper betle</i>	Namfa Sermkaew
PB017	Antimicrobial Effect of <i>Piper betle</i> Leaf Extract for Foot Anti-malodor	Natamon Dorkbuakaew
PB018	In vitro Antioxidant Activity of Mouthwash Containing Stingless Bee's Propolis Extract	Natthan Charemsriwilaiwat
PB019	Increasing of Lycopene's Antioxidant Stability In Solid Lipid Nanoparticle (SLN) and Nanostreture Lipid And Carrier (NLC) in Use As Antiaging	Noorma Rosita
PB020	Development of Pluronic Lecithin Organogel loaded <i>Kaempferia parviflora</i> Extract for Transdermal Delivery	Paisit Wattanasri
PB021	Effect of skin penetration enhancers containing liposome on ATRA loading capacity	Ponwanit Charoenputtakun
PB022	Effect of Tween on transport function of organic cation transporter 2	Sirima Soodvilai
PB023	<i>In vitro</i> release kinetic of sildenafil dry foam tablets	Somchai Sawatdee
PB024	Development of Dong quai extracts-loaded solid lipid nanoparticles cream and it preliminary charaterization	Somkamon Manchun



Paper Code	Paper name	Name Surname
PB025	Formulation and Characterization of Capsaicin-loaded Microemulsions for Transdermal Drug Delivery: Trial and Error Method vs Response Surface Method	Sureewan Duangjit
PB026	Formulation and Evaluation of Topical Antimicrobial Tea Tree Oil Gels	Suwannee Panomsuk
PB027	Fabrication of clotrimazole-microemulsion nanofibers for oral candidiasis application	Teeratas Kansom
PB028	Selection of Stabilizers for Preparation of Alpha-mangostin Nanosuspension	Tharahpi Aung
PB029	Development and Evaluation of Hydrogel Formulation of Gac Fruit Extract	Thipapun Plyduang
PB030	Bioactivity and safety studies of nanofilm from povidone-iodine spray <i>in vitro</i>	Titpawan Nakpheng
PB031	Effect of different types and amount of fatty acid content in corn oil and virgin coconut oil (VCO) on the characteristic, release rate, penetration and effectiveness of para methoxycinnamic acid (PMCA) in nanoemulsion	Tristiana Erawati M.
PB032	Advanced Characterization of Ethanolic Nano Lipid Vesicles Loaded with Raloxifene HCl for Transdermal Drug Delivery	Uttam Kumar Mandal
PB033	Preparation and characterization of silymarin-loaded amphiphilic chitosans micelles	Wajee Tipparos
PB034	The physicochemical properties of indomethacin topical spray	Wilaiporn Buatong
PB035	Impact of Foam Powder Level and PEO Grade on Properties of Floating Matrix Tablet Prepared by Hot Melt Extrusion	Worawut Kriangkrai
<b>Biopharmaceutics</b>		
PB036	The Study of Suitable Conditions for Effective Use of Insulin Human Reference Standard	Chanida Karnpracha
PB037	Liquid chromatography-tandem mass spectrometry with electrospray ionization method for quantitation of donepezil in human plasma and its application to a bioequivalence study	Ekawan Yoosakul

Paper Code	Paper name	Name Surname
PB038	Valproic acid attenuates MPP <sup>+</sup> -mediated toxicity in human neuroblastoma SH-SY5Y cells involve Erk pathway	Jakkapong Muangsab
PB039	Antibacterial and Effect on Bacterial Growth of Crude Ethyl Acetate Extract of Actinomycetes strain PN51B-4-1	Jirarat Surinta
PB040	Rapid and simple LC-MS/MS method for the determination of atorvastatin in human plasma	Nattawut Sukkhum
PB041	Biopharmaceutics Classification System (BCS)-based Biowaiver for Immediate Release Solid Oral Dosage Forms of Pregabalin 75 and 150 mg Capsules (Pregabalin GPO)	Nawaphon Saelim
PB042	Pharmacokinetic Modeling to Predict the Phenytoin Level in Blood by Using STELLA Program	Pattarawit Rukthong
PB043	Evaluation of Survival Rate of Encapsulated <i>Lactobacillus fermentum</i> LF026 in Yoghurt	Sirima Suvarnakuta Jantama
PB044	Synergistic Effect of Diterpenoid from <i>Trigonostemon reidioides</i> and Cisplatin on Antiproliferation of Human Oral Squamous Carcinoma Cells	Sirinya Thungtong
PB045	Protective effect of fenofibrate on cisplatin toxicity in human renal proximal tubular cells	Sunhapas Soodvilai

## Effect Of Incubation Time On Protein Loading And Encapsulation Efficiency Of Ovalbumin-Alginate Microspheres

**Dewi Melani Hariyadi<sup>1\*</sup>, Esti Hendradi<sup>1</sup>, Ani Putri Ayu Setyawati<sup>1</sup>**

<sup>1</sup>Pharmaceutics Department, Faculty of Pharmacy, Universitas Airlangga (UNAIR), Surabaya, Indonesia

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**Abstract-** The aim of this research was to investigate effect of incubation time that was important part on determination protein content method toward encapsulation efficiency and protein loading of microspheres. Determination method of encapsulation efficiency and protein loading used on this research by addition of sodium citrate solution 0.5 M pH 8.5 for 3, 6, 9, and 12 hours. Results showed that encapsulation efficiency and protein loading of ovalbumin-alginate microspheres after 3, 6, 9 and 12 hours incubation were above 99% and above 38% respectively. Based on statistical analysis, it was found that no significant differences between encapsulation efficiency and protein loading at different incubation times. Results of size before incubation was 5.87 $\mu$ m and after 3, 6, 9 and 12 hours incubation was 2.45 $\mu$ m; 2.38 $\mu$ m, 2.39 $\mu$ m, and 2.42 $\mu$ m respectively. Different incubation times produced similar size of particle; and same high encapsulation efficiency and protein loading as size. In addition, Biuret test demonstrated that protein was still existed after several hours' incubation.

**Keywords:** ovalbumin, alginate microspheres, incubation time, efficiency encapsulation, protein loading

### Introduction

The development of proteins and peptides for therapeutic purposes has been widely increased. However, some drawbacks such as low stability, easily and quickly deactivated, short half-life and difficulties in oral absorption [1]. Microspheres for oral delivery system are an alternative to deliver the protein effectively. There was another problem in the development of microspheres related with accuracy of active content and precision in the determination of the amount of protein which is encapsulated in the microspheres. Some factors that affected microencapsulation techniques including manufacture process and microsphere formulations on the encapsulation efficiency of protein, protein loading as well as the amount of protein release.

Addition of sodium citrate pH 8.5 is one way method to break the microspheres and determine the protein content [2]. This method had advantages compare to other protein determination methods, especially in avoiding use of organic solvent, exposure from heat denaturation, overheating or over stirring and addition of chemical agents such as ammonium salt, heavy metals and alcohol. The disadvantages method was mostly resulted in irreversible denaturation [3]. According to Bilati *et al.*, 2005, several factors influenced the amount of protein released for example affinity of active agent to polymer, protein adsorption, instability during incubation period [4]. Ovalbumin-loaded alginate microspheres used Calcium Chloride as crosslinker was produced by ionotropic gelation method. Sodium alginate is used as polymer in the microspheres because of safe, cheap and biodegradable. Ca<sup>2+</sup> was selected to crosslink with alginate forming hydrogel microspheres and has ability to produce strong gel and high potential[5].

The determination of protein using the current study was aiming to dissolve and break the microspheres. CBB (Coomassie Brilliant Blue) reagent to determine ovalbumin content has been used commonly resulting accurate, high sensitivity, fast and reproducible results. In previous study, same method has been conducted to determine ovalbumin content and encapsulation efficiency calculation; however 12 hours incubation time was the only time used [2]. Some researchs suggested that short incubation time was also efficient in producing as same high efficiency and loading as longer incubation time [6]. Therefore, this current study investigated effect of incubation time on the determination of protein loading and encapsulation efficiency.

### Materials and Method

Alginate solution (2.5%) containing ovalbumin (2.5%) was sprayed into crosslinking agent CaCl<sub>2</sub> solution (1.5M) and was stirred continuously for 2 hours at 1000 rpm. The microspheres were collected by centrifugation at 2500 rpm for 6 minutes. 5% Lactose as lyoprotectant was added and finally microspheres were freeze dried 29 hours at -80°C. The particle size and morphology of microspheres was characterized by optical microscope. Freeze-dried ovalbumin-alginate microspheres were then incubated for 3,6,9 and 12 hours into Sodium Citrate pH 8.5 and were named F1, F2, F3 and F4 respectively (Table 1). Protein loading, encapsulation efficiency were then calculated using Protein Quantification Assay

(Coomassie Brilliant Blue/ CBB Assay). To investigate the presence of ovalbumin protein after several hours' incubation times, Biurett test was conducted.

## Results and Discussion

Aerosolization technique produced spherical ovalbumin-Ca alginate microspheres with average particle size of 5.87  $\mu\text{m}$ . Following incubation, particle size of microspheres were measured as well as protein loading and encapsulation efficiency. Summary of the particle size, protein loading and encapsulation efficiency of four formulas is presented in Table 1

**Table 1** Particle size, protein loading and encapsulation efficiency of ovalbumin-Ca alginate microspheres

Formula	Protein Loading (%)	Encapsulation Efficiency (%)	Particle Size ( $\mu\text{m}$ )
F1	38.25 $\pm$ 4,61	99.10 $\pm$ 0,93	2.45
F2	38.25 $\pm$ 4,63	99.09 $\pm$ 0,96	2.38
F3	38.24 $\pm$ 4,62	99.08 $\pm$ 0,97	2.39
F4	38.24 $\pm$ 4,58	99.09 $\pm$ 0,88	2.42

Particle size of the microspheres before incubation (5.87 $\mu\text{m}$ ) was bigger than after 3, 6, 9 and 12 hours incubation at around 2.4 $\mu\text{m}$ . This smaller size may be due to Ca-alginate microspheres especially the crosslinked part ( $\text{Ca}^{2+}$ ) were dissolved in the alkalized solution of sodium citrate pH 8.5 caused microspheres erosion, irregular morphology until formed clear solution. This is as mentioned by Rowe et al [5]. Replacement of  $\text{Ca}^{2+}$  with  $\text{Na}^+$ , reduced density of alginate gel and affected on the gel strength [7]. The complete process of microspheres from diffusion, dissolution, and erosion commonly was taken longer, however this study found that after 3, 6, 9 and 12 hours there was no significant differences of particle size of about 2.4  $\mu\text{m}$  indicated that after 3 hours the microspheres were completely dissolved to release the ovalbumin protein.

For protein loading and encapsulation efficiency, results showed that no significant differences between all formulas after different time incubation. Based on particle size, shortened incubation time (3 hours) produced same size as longer time (12 hours), this caused ovalbumin protein released from alginate microspheres therefore loadings and efficiency were calculated. Some literatures mentioned that addition of NaOH, DMSO and SDS were able to obtain protein recovery and loadings of protein from PLGA microspheres after 1 hour incubation. Higher encapsulation efficiency was also reported using SDS alkalized after 12 hours. They discussed that the optimum method for production of microspheres was able to break the microspheres and released the active agent in any incubation time. This explanation confirmed that the method of this study using sodium citrate at pH 8.5 is the best method to break the microspheres completely; therefore different incubation time did not affect the protein loadings and efficiency.

In order to confirm whether protein was present after several hours incubation, Biurett test showed that protein was identified and existed by violet color in the solution after 3, 6, 9 and 12 hours incubation. All results in this study suggested further recommendation that it was only needed 3 hours incubation to break the Ovalbumin-Ca alginate microspheres and released the protein.

## Conclusion

No significant differences were found in particle size, protein loading and entrapment efficiency following incubation of ovalbumin-Ca alginate microspheres in 3, 6, 9 and 12 hours. This study recommended that determination of optimum protein loading and encapsulation efficiency can be achieved during 3 hours incubation.

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