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CARBON OXIDE NANOPARTICLES FOR CONTROLLED DELIVERY OF CURCUMIN AND  
PEPTIDE NUCLEIC ACID

Miss Sunatda Arayachukeat



A Dissertation Submitted in Partial Fulfillment of the Requirements  
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Faculty of Science

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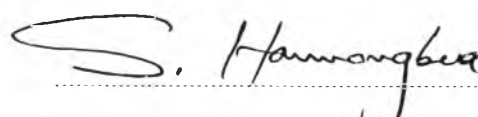
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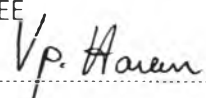
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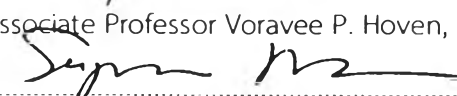
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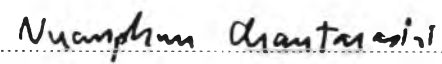
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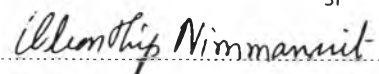
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สุนัดดา อารยชูเกียรติ : อนุภาคระดับนาโนเมตรของคาร์บอนออกไซด์สำหรับการนำส่งแบบควบคุมของเคอร์คิวมินและเปปไทด์นิวคลีอิกแอซิด. (CARBON OXIDE NANOPARTICLES FOR CONTROLLED DELIVERY OF CURCUMIN AND PEPTIDE NUCLEIC ACID) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร. ศุภศร วณิชเวชารุ่งเรือง, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. ดร. ธนาภัทร ปาลกะ, 134 หน้า.

เมื่อทำการออกซิไดซ์กราไฟต์ จากนั้นแยกผลิตภัณฑ์ที่เกิดขึ้นด้วยการปั่นเหวี่ยงที่ความเร็วต่างๆ และตรวจสอบรูปร่างของผลิตภัณฑ์ด้วยภาพถ่ายอิเล็กตรอนแบบส่องกราด (SEM) และ ภาพถ่ายอิเล็กตรอนแบบส่องผ่านชนิดกำลังขยายสูงและวิเคราะห์โครงสร้างด้วยรามานสเปกโตรสโกปี พบว่าสามารถสังเคราะห์และแยกอนุภาคลักษณะกลม ขนาดเส้นผ่าศูนย์กลางเฉลี่ยเท่ากับ  $132.1 \pm 4.74$  นาโนเมตรได้ โดยเป็นอนุภาค  $sp^2$  ไฮบริดคาร์บอน โดยที่ผิวมีหมู่ไฮดรอกซิลอยู่เนื่องจากอนุภาคนี้เป็นการรวมกลุ่มกันของอนุภาคคาร์บอนขนาด 5-10 นาโนเมตร จึงเรียกชื่ออนุภาคนี้ว่า Cluster of carbon oxide nanospheres (CCNs) การทดสอบในเซลล์พบว่า CCNs ไม่มีความเป็นพิษต่อเซลล์ไลน์ไตของเอ็มบริโอมนุษย์ (HEK 293T) เซลล์มะเร็งปากมดลูกมนุษย์ (CaSki) และเซลล์ไลน์มะเร็งเม็ดเลือดขาว(RAW 264.7) ที่ความเข้มข้นสูงถึง 10 ไมโครกรัมต่อไมโครลิตร เมื่อใช้ CCNs ที่สังเคราะห์ได้เป็นตัวนำส่งเคอร์คิวมิน (curcumin) และเปปไทด์นิวคลีอิกแอซิด (peptide nucleic acid, PNA) และตรวจสอบโดย confocal laser scanning fluorescence microscopy (CLSM) พบว่า CCNs สามารถนำส่งสารออกฤทธิ์เข้าสู่นิวเคลียสได้ในขณะที่อนุภาคนำส่งอยู่ในส่วนไซโตพลาสซึม CCNs ช่วยเพิ่มความสามารถในการนำเคอร์คิวมินเข้าสู่เซลล์ไลน์ไตของเอ็มบริโอมนุษย์และนำเปปไทด์นิวคลีอิกแอซิดเข้าสู่เซลล์ไลน์มะเร็งเม็ดเลือดขาว การนำส่งเคอร์คิวมินด้วย CCNs ช่วยเพิ่มฤทธิ์การต้านเซลล์มะเร็งของเคอร์คิวมินให้ดีขึ้นอีกด้วย อย่างไรก็ตามการทดลองประยุกต์ใช้อนุภาคนำส่ง PNA เพื่อใช้รบกวนการแสดงออกของยีนอินเตอร์ลิวคิน 6 (il-6) ยังไม่ประสบความสำเร็จ เนื่องจาก PNA ไม่สามารถจับกับดีเอ็นเอได้อย่างเสถียร



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ลายมือชื่อนิสิต สุนัดดา อารยชูเกียรติ

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NUCLEIC ACID (PNA) / DRUG DELIVERY SYSTEM

SUNATDA ARAYACHUKAT: CARBON OXIDE NANOPARTICLES FOR  
CONTROLLED DELIVERY OF CURCUMIN AND PEPTIDE NUCLEIC ACID.  
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Graphite was oxidized, and the resulting products were separated by centrifugation at various speeds. The obtained products were morphologically characterized by scanning electron microscopy (SEM) and high resolution transmission electron microscopy (HR-TEM), and structural characterized by Raman spectroscopy. It was found that the spherical particles with diameter of  $132.1 \pm 4.74$  nm could be prepared. The particles were  $sp^2$ -hybridized carbon with hydroxyl functionality at the surface. Since the obtained particles consisted of aggregated 5-10 nm carbon spheres, they were named cluster of carbon oxide nanoparticles (CCNs). CCNs showed no cytotoxicity against human embryonic kidney cell line, HEK 293T, human cervical carcinoma cell line, CaSki and murine macrophage cell line, RAW 264.7, at the concentration up to 10  $\mu\text{g}/\text{mL}$ . CCNs were used as carriers for curcumin and peptide nucleic acid (PNA). Confocal laser scanning fluorescence microscopy (CLSM) showed that CCNs could deliver both actives into nucleus while CCNs remained in the cytoplasm. CCNs could increase uptake of curcumin in HEK 293T cells and of PNA in RAW 264.7 cells. The delivery of curcumin by CCNs also enhanced anticancer activity of curcumin. However, the application of CCNs for PNA delivery for the anti-IL6 gene was unsuccessful due to unstable PNA-DNA complex.

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## LIST OF ABBREVIATIONS

acpcPNA	(1S, 2S)-2-aminocyclopentanecarboxylic acid
ATCC	American type culture collection
ATR-FTIR	Attenuated total reflectance-fourier transform
CaSki	Human epidermoid cervical carcinoma cell lines
CCNs	Cluster of carbon oxide nanospheres
CM	Complete medium
CLSM	Confocal laser scanning fluorescence microscopy
Da	Dalton
DLS	Dynamic light scattering
DMEM	Dulbecco's Modified Eagle medium
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EA	Elemental analysis
EDCI	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide
ELISA	Enzyme-linked immunosorbent assay
eV	Electron volt
FBS	Fetal bovine serum
g	Gravity
GOShs	Graphene oxide sheets
GNP	Graphene nanopellet
h	Hours





HR-TEM	High-resolution transmission electron microscopy
HEK293T	Human embryonic kidney cell line
HEPES	N-2-hydroxyethylpiperazine-N'-2- ethanesulfonic
IL6	Interleukin 6
MTT	3-(4,5-dimethylthiazol-2-yl)-2-5- diphenyltetrazolium bromide
µg	Microgram
µL	Microliter
µM	Micromolar
mW	Milliwatt
mL	Milliliter
min	Minute
MWCO	Molecular weight cut-off
NHS	N-hydroxy succunamide
NF-κB	NF-κB specific sequences; Bz-GGGATTTTCCCA-LysNH <sub>2</sub>
ng	Nanogram
nm	Nanometer
nM	Nanomolar
PBS	Phosphate buffer solution
PCR	polymerase chain reaction
PNA	Random sequences; Bz-TGTCAACTGACT-LysNH <sub>2</sub>



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ppm	Parts per million
qRT- PCR	Quantitative real time polymerase chain reaction
RAW 264.7	Mouse leukaemic monocyte macrophage cell line
RNA	Ribonucleic acid
rpm	Revolution per minute
RPMI 1640	Roswell park memorial institute 1640 medium acid
RT-PCR	Reverse transcription polymerase chain reaction
SEAD	Selected area electron diffraction analysis infrared spectroscopy
SEM	Scanning electron microscope
TAMRA	Tetramethylrhodamine-5-carbonyl azide
TEM	Transmission electron microscope
TGA	Thermo-gravitational analysis
XRD	X-ray diffraction analysis
XPS	X-ray photoelectron spectroscopy
w/ w	Weight by weight
UV/Vis	Ultraviolet/Visible
U	Unit
°C	Degree celsius
%	Percent
LE	Loading efficiency
LC	Loading capacity



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