

การเตรียมอนุภาคโคออร์ดิเนชันระดับนาโนเมตรชนิดใหม่จากการรวมตัวกันเองของ
สารลดแรงตึงผิวและไอออนกาโดลิเนียมเพื่อให้อนุพันธ์เคอร์คูมินเสถียรในสารละลายบัฟเฟอร์



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PREPARATION OF NOVEL SELF-ASSEMBLED COORDINATION NANOPARTICLES
FROM SURFACTANTS AND GADOLINIUM ION TO STABILIZE CURCUMIN
DERIVATIVES IN BUFFERED SOLUTION

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

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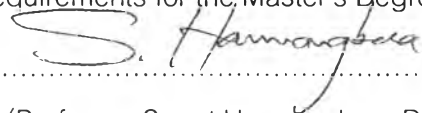
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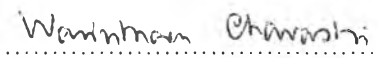
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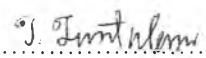
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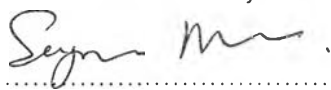
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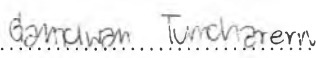
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ชลธิชา สาทับ : การเตรียมอนุภาคโคออร์ดิเนชันระดับนาโนเมตรชนิดใหม่จากการรวมตัวกันเองของสารลดแรงตึงผิวและ ไอออนกาโดลิเนียมเพื่อทำให้อนุพันธ์เคอร์คูมินเสถียรในสารละลายบัฟเฟอร์. (PREPARATION OF NOVEL SELF-ASSEMBLED COORDINATION NANOPARTICLES FROM SURFACTANTS AND GADOLINIUM ION TO STABILIZE CURCUMIN DERIVATIVES IN BUFFERED SOLUTION) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : ศ. ดร.รัชชัช ต้นทุลานี, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : ผศ. ดร.บุญยรัตน์ ธรรมพัฒนกิจ, 121 หน้า.

เคอร์คูมินเป็นสารธรรมชาติจำพวกโพลีฟีนอล พบในรงควัตถุหลักที่สกัดได้จากเหง้าของขมิ้นชัน (ชื่อวิทยาศาสตร์คือ *Curcuma longa* Linn.) มีฤทธิ์ในการยับยั้งการเจริญเติบโตของเซลล์มะเร็งได้ค่อนข้างสูง แต่ข้อเสียของเคอร์คูมินได้แก่ การไม่ละลายและไม่เสถียรในน้ำ ส่งผลให้มีชีวปริมาณการออกฤทธิ์ที่ต่ำ ในงานวิจัยนี้คณะผู้วิจัยได้รายงานการเตรียมอนุภาคโคออร์ดิเนชันระดับนาโนเมตรชนิดใหม่จากการรวมตัวกันเองของสารลดแรงตึงผิวและ ไอออนกาโดลิเนียมและบัฟเฟอร์เพื่อทำให้อนุพันธ์เคอร์คูมินเสถียรในสารละลายบัฟเฟอร์ การศึกษาผลของชนิดของบัฟเฟอร์ที่ประกอบด้วย HEPES MOPS Tris และ phosphate และผลของชนิดของสารลดแรงตึงผิวชนิด SDS CTAB และ Triton X-100 ที่มีต่อลักษณะโครงสร้างภายนอกและสมบัติของโครงสร้างโมเลกุล โดยใช้เทคนิคอินฟราเรดสเปกโทรสโกปี กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด (SEM) กล้องจุลทรรศน์อิเล็กตรอนแบบส่องผ่าน (TEM) การดูดกลืนรังสีเอ็กซ์ (XAS) อะตอมมิกสเปกโทรสโกปีจากการใช้พลาสมา (ICP-AES) และเอ็กซ์เรย์ดิฟแฟรกชัน (XRD) พบว่า การเกิดอนุภาคโคออร์ดิเนชันระดับนาโนเมตรจาก Gd^{3+} , SDS และ HEPES ที่มีชื่อว่า $GdSH$ ให้โครงสร้างที่มีลักษณะทรงกลมและเหมาะสมในการทดลองงานด้านชีวภาพ นอกจากนี้อนุพันธ์เคอร์คูมินที่ถูกห่อหุ้มด้วยอนุภาคระดับนาโนถูกตรวจสอบด้วยเทคนิคยูวี-วิจิเบิล ฟลูออเรสเซนซ์และอินฟราเรดสเปกโทรสโกปี จากนั้นศึกษาความเสถียรของอนุพันธ์เคอร์คูมินด้วยเทคนิคยูวี-วิจิเบิลและฟลูออเรสเซนซ์สเปกโทรสโกปี พบการลดลงของความเข้มของการคายพลังงาน (normalized fluorescence intensity) ของเคอร์คูมินในอนุภาคนาโนน้อยกว่าเคอร์คูมินที่ไม่ถูกห่อหุ้มด้วยอนุภาคนาโนถึง 2 เท่า เมื่อเวลาผ่านไป 2 ชั่วโมง ซึ่งสอดคล้องกับผลของการทดสอบฤทธิ์ทางชีวภาพ ที่บ่งชี้ว่าอนุภาค $GdSH$ สามารถเพิ่มสมบัติในการยับยั้งเซลล์มะเร็งถ้าใส่ใหญ่ SW620 เมื่อเปรียบเทียบกับเคอร์คูมินธรรมดาทั้งในสารละลาย DMSO และ HEPES buffer ด้วยค่า IC_{50} ในรูปแบบของเคอร์คูมินที่มีปริมาณเท่ากันเท่ากับ 8.0, 10.3 $\mu g/mL$ และไม่เป็นพิษ ตามลำดับ นอกจากนี้ภาพถ่ายแบบคอนโฟคอลฟลูออเรสเซนซ์แสดงให้เห็นว่าเคอร์คูมินที่อยู่ภายในอนุภาค $GdSH$ สามารถเข้าไปภายในเซลล์ได้อย่างรวดเร็วและเพิ่มความสว่างแสงฟลูออเรสเซนซ์ได้อีกด้วย จากผลการทดลองคาดว่างานวิจัยนี้จะมีประโยชน์ในการพัฒนาความเสถียรของเคอร์คูมินเพื่อใช้ประโยชน์ในงานด้านชีวภาพต่อไป

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CHONTICHA SAHUB: PREPARATION OF NOVEL SELF-
ASSEMBLED COORDINATION NANOPARTICLES FROM
SURFACTANTS AND GADOLINIUM ION TO STABILIZE
CURCUMIN DERIVATIVES IN BUFFERED SOLUTION. ADVISOR:
PROF. THAWATCHAI TUNTULANI, Ph. D., CO-ADVISOR: ASST.
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Curcumin is a natural polyphenol found as a major pigment extracted from turmeric rhizomes (*Curcuma longa* Linn.). Effective inhibition of the growth of cancer cells by curcumin is relatively high. However, the disadvantages of curcumin derivatives are water-insolubility and instability resulting in exceedingly poor bioavailability. In this study, we have reported novel coordination nanoparticles prepared by self-assembly of surfactants and gadolinium ion (Gd^{3+}) to stabilize curcumin derivatives in buffer solution. Effects of various buffers including HEPES, MOPS, Tris and phosphate and various surfactants including SDS, CTAB and Triton X-100 towards the morphology and structural properties of the nanoparticles were investigated by FT-IR, SEM, TEM, XAS, ICP-AES and XRD techniques. It was found that the formation of coordination nanoparticles from Gd^{3+} , SDS and HEPES, namely, **GdSH CNPs** showed excellently uniform spherical nanoparticles and a possibly suitable candidate for biological tasks. Moreover, the curcumin derivatives encapsulated in self-assembled coordination nanoparticles were prepared and then characterized by UV-visible, fluorescence and FT-IR spectroscopies. The stability of curcumin derivatives was also examined by both of UV-visible and fluorescence spectroscopies and the results showed that the decrease of the normalized fluorescence intensity of curcumin incorporated in coordination nanoparticles was less than 2-fold that of free curcumin during the 2 h period. As compared to the corresponding cytotoxicity assay studies of free curcumin in DMSO and HEPES buffer solution with IC_{50} values of 10 $\mu g/mL$ and non-toxicity, respectively, **GdSH CNPs** can enhance *in vitro* anti-cancer activity of curcumin to SW620 colon cancer cells with IC_{50} values of 8.0 $\mu g/mL$ in term of curcumin-equivalent dose. Additionally, as the results of the confocal fluorescence images, the curcumin immobilized **GdSH CNPs** could be immediately taken to cancer cells and enhanced the fluorescence brightness. In these approaches, our novel nanoparticles would be beneficial to further development of stability of curcumin for biological tasks.

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LIST OF ABBREVIATIONS AND SYMBOLS

$^1\text{H-NMR}$	Proton nuclear magnetic resonance
equi.	Equivalent
g	Gram
μg	Microgram
h	Hour
min	minute
Hz	Hertz
J	Coupling constant
mmol	Millimole
mL	Milliliter
nm	Nanometer
M	Molar
mM	Milimolar
δ	Chemical Shift
ppm	Part per million
ppb	Part per billion
s, d, t, m	Splitting patterns of $^1\text{H-NMR}$ (singlet, doublet, triplet, multiplet)
CNPs	Coordination Nanoparticles
DLS	Dynamic light scattering
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
ICP-AES	Inductively Coupled Plasma-Atomic Emission Spectroscopy
FT-IR	Fourier transform infrared spectroscopy
XAS	X-ray absorption
XANES	X-ray absorption near-edge structure
XRD	X-ray diffraction patterns
CLSM	Confocal laser scanning microscopy