

**BINDING INTERACTION BETWEEN DAPTOMYCIN
AND POLYAMIDOAMINE DENDRIMER**

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**A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Pharmaceutical Technology
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อันตรกิริยาการยึดเหนี่ยวระหว่างแคปโทมัยซินกับพอลิอะมิโดเอมีนเดนดริเมอร์

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แคปโทมัยซินคือสารในกลุ่มไซคลิกไลโปเปปไทด์ที่ออกฤทธิ์ในการต่อต้านแบคทีเรียแกรมบวกที่มีอันตรายต่อชีวิตหลากหลายประเภท แต่อย่างไรก็ตามการทดสอบทางคลินิกเบื้องต้นไม่ประสบความสำเร็จเนื่องมาจากการที่สารชนิดนี้มีอันตรกิริยาที่แน่นกับโปรตีนในกระแสเลือด, มีการกำจัดออกอย่างรวดเร็วผ่านทางไต หรือ การกระจายของระดับยาในบริเวณเป้าหมายไม่เพียงพอต่อการรักษา เพื่อให้ผลในการรักษาของแคปโทมัยซินดีขึ้นโดยพัฒนาพอลิอะมีโดเอมีนเดนดริเมอร์หรือแพมแอม เป็นระบบนำส่งยาที่มีอาศัยพันธะโคเวเลนต์ และงานในส่วนนี้อธิบายถึงวิธีศึกษาอันตรกิริยาระหว่างแคปโทมัยซินและแพมแอมเดนดริเมอร์โดยใช้เทคนิค อัลตราฟิวเรชัน, ยูวีดิฟเฟอร์เรนซ์สเปกโตรสโคปี และ ฟลูออเรสเซนซ์สเปกโตรสโคปี อันตรกิริยาระหว่างแคปโทมัยซินกับเดนดริเมอร์ ส่งผลให้มีการเพิ่มขึ้นของความเข้มของแสงฟลูออเรสเซนซ์ที่เปล่งออกมาจากโคโมเร็นในแคปโทมัยซิน การเปลี่ยนแปลงที่เกิดขึ้นอธิบายเชิงปริมาณได้โดยอาศัยแบบจำลองของอันตรกิริยาชนิดเดียวและสองชนิด รวมถึงหลักการถดถอยแบบไม่เป็นเส้นตรงเพื่อหาค่าคงที่ของการแตกตัวและความจุของอันตรกิริยา ซึ่งอันตรกิริยาที่เกิดขึ้นระหว่างแคปโทมัยซินกับแพมแอมเจนเนอร์เรชันห้า (ขนาดโมเลกุล 30 กิโลดาลตัน) เป็นรูปแบบของอันตรกิริยาชนิดเดียว ค่าคงที่ในการแตกตัวในช่วงพีเอช 4-8 มีค่าคงที่ ในขณะที่ค่าความจุของแคปโทมัยซินบนเดนดริเมอร์นั้นสัมพันธ์อยู่กับค่าพีเอช การเรืองแสงที่เปลี่ยนแปลงเนื่องจากอันตรกิริยาระหว่างแคปโทมัยซินกับแพมแอมเจนเนอร์เรชันหก (ขนาดโมเลกุล 60 กิโลดาลตัน) ในช่วงพีเอช 4-7 นั้นสอดคล้องกับแบบจำลองอันตรกิริยาสองชนิด ค่าคงที่ของการแตกตัวที่ตำแหน่งที่มีค่าสัมพรรคภาพสูงมีค่าใกล้เคียงกับค่าคงที่ของการยึดเหนี่ยวของแคปโทมัยซินกับแพมแอมเจนเนอร์เรชันห้า ในขณะที่ค่าคงที่ในการแตกตัวและค่าความจุตำแหน่งที่มีค่าสัมพรรคภาพต่ำผันตามค่าพีเอช มีการทำนายค่าความเข้มของแพมแอมที่เหมาะสมภายใต้สภาวะต่างๆ โดยใช้แบบจำลองทางคณิตศาสตร์เพื่อที่จะได้สภาวะที่ก่อให้เกิดอันตรกิริยาระหว่างแคปโทมัยซินกับและแพมแอมที่เหมาะสมที่สุด นอกจากนี้แบบจำลองระดับโมเลกุลที่แสดงการเกิดอันตรกิริยาระหว่างแคปโทมัยซินกับเดนดริเมอร์ได้ถูกนำเสนอจากค่าคงที่ของอันตรกิริยาและข้อมูลเพิ่มเติม แคปโทมัยซินหันด้านกว้างและสอดคล้องส่วนหางที่เป็นไขมันเข้าไปในแพมแอมเดนดริเมอร์ ผลที่ได้จากการศึกษานี้ทำให้เข้าใจธรรมชาติของอันตรกิริยาระหว่างแคปโทมัยซินและไบโอโมเลกุลที่มีประจุได้ดีขึ้น

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ปีการศึกษา 2550

ลายมือชื่อนิสิต.....

ลายมือชื่ออาจารย์ที่ปรึกษา.....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

ร.ศ.ดร. นพ. ธีระเกียรติ ธีระขันธ์


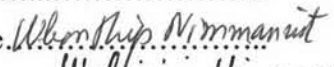
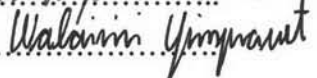
**#4576961233: MAJOR PHARMACEUTICAL TECHNOLOGY (INTERNATIONAL)
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**BOONTARIKA CHANVORACHOTE : BINDING INTERACTION
BETWEEN DAPTOMYCIN AND POLYAMINOAMINE DENDRIMER.
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PROF. WALAISIRI YIMPRASERT, Ph. D. 188 pp.**

Daptomycin is a cyclic lipopeptide agent that highly active against a broad spectrum of fatal gram-positive pathogens. However, initial clinical trials resulted in treatment failure due to the high degree of daptomycin protein binding, rapid renal clearance or inadequate distribution to the target sites. In order to improve therapeutic outcomes of daptomycin, a non-covalent carrier system was developed using polyamidoamine (PAMAM) dendrimers. The study reported herein describes methods for characterization of an interaction between daptomycin and positively charged PAMAM dendrimers using ultrafiltration, UV difference spectroscopy and fluorescence spectroscopy. Binding of daptomycin to dendrimers led to fluorescence enhancement at the kynurenine residue on the daptomycin. These changes were quantitatively described by one- and two-site binding models by using nonlinear regression to estimate binding capacity and affinity constants. The binding of PAMAM dendrimer generation 5 (molecular size of 30 kDa) was described by a single site binding model. The estimated binding dissociation constants in pH range of 4.0-8.0 remained constant whereas the capacity constant was pH dependent. The fluorescence changes due to the binding of daptomycin to the PAMAM dendrimer generation 6 (molecular size of 60 kDa) in a pH range of 4.0 – 7.0 resulted in biphasic binding profiles, explained by a two site binding model. The estimated binding constants at high affinity site were similar to the results observed from the binding of daptomycin to PAMAM generation 5, whereas the binding dissociation and capacity constants of the low affinity sites were both sensitive to pH. The optimum total concentrations of PAMAM dendrimer in different conditions were predicted using the mathematical model in order to gain the optimum condition of complex formation. In addition, the molecular model of interaction between dendrimer and daptomycin was proposed based on the binding parameters and supporting information. The daptomycin appeared to orientate in latitudinal dimension and insert its lipid tail into the PAMAM dendrimer. The results of these investigations can provide an insight into the nature of daptomycin interactions with charged biomolecules.

Field of study Pharmaceutical Technology

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Student's signature.....
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LIST OF ABBREVIATIONS

%	percentage
ϵ	molar absorptivity
λ	wavelength
μg	microgram (s)
μl	microlitre (s)
μM	micromolar (s)
$^{\circ}\text{C}$	degree Celsius
\AA	Angstrom
ANOVA	analysis of variance
cm	centrimeters
Da	Dalton
Dap	daptomycin
Den	dendrimer
Den-Dap	complex between daptomycin and dendrimer
et al.	et alii, and others
g	gram (s)
HCl	hydrochloric acid
K	association constant
K_d	dissociation constant
L	ligand
mg	milligram (s)
min	minute (s)
mM	milimolar (s)
n	capacity constant
NaOH	sodium hydroxide
nm	nanometers
PAMAM	Polyamidoamine
pH	the negative logarithm of hydrogen ion concentration
Q	quencher
R	total number of independent binding sites

R^2	square of correlation coefficient
R_g	average radius of gyration
S	substrate
SD	standard deviation
UV	Ultraviolet
w/v	weight per volume
ΔE	molar signal coefficient
ΔF	difference in fluorescence intensity