

การพัฒนาโปรตีนในรูปผงแห้งสำหรับนำส่งทางจมูกสู่สมองผ่านส่วนรับกลิ่นและส่วนหายใจ

นายวิทยา นาคาชน



ห้องสมุดคณะวิทยาศาสตร์

จุฬาลงกรณ์มหาวิทยาลัย

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ปีการศึกษา 2556
ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย



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DEVELOPMENT OF DRY PROTEIN POWDER FOR INTRANASAL DELIVERY TO BRAIN
VIA OLFACTORY AND RESPIRATORY REGIONS

Mr. Wittaya Nakachon



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วิทยา นาคาชน : การพัฒนาโปรตีนในรูปผงแห้งสำหรับนำส่งทางจมูกสู่สมองผ่านส่วนรับกลิ่นและส่วนหายใจ. (DEVELOPMENT OF DRY PROTEIN POWDER FOR INTRANASAL DELIVERY TO BRAIN VIA OLAFACTORY AND RESPIRATORY REGIONS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ศ. ภญ. ดร.กาญจน์พิมล ฤทธิเดช , 161 หน้า.

วัตถุประสงค์ของการศึกษาครั้งนี้ คือพัฒนาและประเมินลักษณะโปรตีนในรูปผงแห้งสำหรับการนำส่งยาจากจมูกไปยังสมอง แอลบูมินซีรัมจากวัวถูกเลือกเป็นโปรตีนต้นแบบ เทคนิคใหม่ที่ใช้กระบวนการพลังงานต่ำและการลดขนาดนำมาเตรียมโปรตีนในรูปผงแห้ง สูตรตำรับที่เลือกแล้ว 6 สูตร ถูกบดให้เป็นผงละเอียดจากนั้นประเมินสัณฐานวิทยาของผง อันตรกิริยาทางเคมีฟิสิกส์ คุณสมบัติการยึดติดเยื่อเมือก การปลดปล่อยยานอกกาย และการซึมผ่านเยื่อเมือกที่รับกลิ่นและเยื่อเมือกหายใจของสุกร จากผลการทดลองแสดงให้เห็นว่า ผงที่เตรียมได้จากการบดด้วยลมพ่น (jet milling) มีลักษณะขอบมน ขนาดอนุภาคเฉลี่ยมัธยฐานประมาณ 6.4-9.4 ไมครอนและการกระจายขนาดอนุภาคแคบ นอกจากนี้ ไม่พบฟิโคใหม่ หรือการเปลี่ยนแปลงฟิโคอย่างมีนัยสำคัญจากเทอร์โมแกรม การเลี้ยวเบนรังสีเอกซ์ และสเปกตรัมเอฟทีไออาร์ สูตรตำรับผงมีแนวโน้มของคุณสมบัติการยึดติดเยื่อเมือกดีกว่าตำรับควบคุม การประเมินคุณภาพของโปรตีนแสดงให้เห็นว่ากระบวนการพลังงานต่ำไม่มีผลต่อโครงสร้างทุติยภูมิ ในทางตรงกันข้ามการลดขนาดมีผลเสียอย่างมาก มีเพียงสูตร เอส-6 (S-6) ซึ่งประกอบด้วย พอลิไวนิล คาโพรแลคแตม-พอลิไวนิล แอซีเทต-พอลิเอธิลีน ไกลคอล กราฟท์ โคพอลิเมอร์ และ พอลิเอธิลีน ไกลคอล (ร้อยละ 60 โดยน้ำหนักต่อน้ำหนัก) ที่คงสภาพโครงสร้างทุติยภูมิได้ นอกจากนี้ การศึกษาการซึมผ่านพบว่าสารละลายแอลบูมินซีรัมของวัวที่ติดสารเรืองแสงซึมผ่านสูงกว่าตำรับผงเล็กน้อยอย่างไม่มีนัยสำคัญทางสถิติ ในเยื่อเมือกหายใจปริมาณโปรตีนที่ได้กลับคืนจากตำรับผงต่ำกว่าจากตำรับสารละลายซึ่งสามารถอธิบายได้ว่า ตำรับผงเพิ่มการซึมผ่านเยื่อเมือกได้ดีกว่าตำรับสารละลายซึ่งพิสูจน์ได้จากการศึกษาวิจัยขึ้นเนื้อเยื่อไตกล้องจุลทรรศน์เรืองแสง จากผลการทดลองทั้งหมด สูตรตำรับโปรตีนในรูปผงแห้งที่เตรียมด้วยกระบวนการพลังงานต่ำและการลดขนาดที่เหมาะสมอาจใช้เป็นระบบนำส่งยาทางจมูกที่มีแนวโน้มและศักยภาพในการนำส่งยาไปยังสมอง

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The purpose of this study was to develop and characterize dry protein powder for nose-to-brain drug delivery. Bovine serum albumin (BSA) was selected as a model protein. A novel technique utilizing low energy process equipped with comminutions was used to prepare dry protein powder. Six selected formulations were pulverized, and then powder morphology, physicochemical interactions, mucoadhesive properties, *in vitro* drug release, and permeation through porcine olfactory and respiratory mucosae were carried out. The results indicated that obtained powders from jet milling had roundish edge and median particle size of about 6.4-9.4 micron with narrow size distribution. Furthermore, there was no new or significant peak shift observing from thermograms, X-ray diffractograms, and Fourier transform infrared spectra. Powder formulations tended to have better mucoadhesive properties than a control. Protein integrity determination revealed that low energy process made a scarcely detrimental effect on protein secondary structure. On the other hand, the comminution had a potential impact. Only S-6 formulation containing polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer and polyethylene glycol (60% w/w) could maintain protein secondary structure. Additionally, *in vitro* permeation study showed that native BSA labeled fluorescence (FITC-BSA) solution had slightly higher permeation than powder formulation with no statistical significance. In respiratory mucosa, recovery amount of FITC-BSA from powder formulation was lower than that from solution formulation. It could be explained that powder formulation enhanced permeation through the mucosa more than solution, proven by histological study under fluorescence microscope. According to the results, dry protein powder formulation prepared by low energy process and appropriate comminution seem to be a promising and potential intranasal delivery system for brain targeting.

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Student's Signature

Wittaya Nakachon

Advisor's Signature

Prof. Dr. Garnpimol C. Ritthidej



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ABBREVIATIONS

θ	=	ellipticity
$^{\circ}\text{C}$	=	degree Celsius
μg	=	microgram
μl	=	microliter
μm	=	micrometer
A	=	colloidal silicon dioxide; Aerosil [®] 200
AIC	=	Akaike Information Criterion
BB	=	Brilliant Blue
BBB	=	blood-brain barrier
BCA	=	bicinchoninic acid
BSA	=	bovine serum albumin
C	=	low molecular weight chitosan
CD	=	circular dichroism
cm	=	centimeter
CM	=	cryomill
CNS	=	central nervous system
CSF	=	cerebrospinal fluid
Da	=	dalton
DSC	=	differential scanning calorimetry
e.g.	=	<i>exempli gratia</i> , 'for example'
EE	=	poly (butyl methacrylate-co-(2-dimethylaminoethyl) methacrylate-co-methyl methacrylate); Eudragit [®] E PO
EL	=	methacrylic acid and ethyl acrylate copolymer; Eudragit [®] L 100-55
et al.	=	<i>et aliti</i> , 'and others'
FT-IR	=	Fourier transform infrared spectroscopy
g	=	gram
HE	=	hydroxypropyl methylcellulose E5; HPMC E5, Methocel E5LV
HK	=	hydroxypropyl methylcellulose K15M; HPMC K15M, Methocel K15M
hr	=	hour



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JM	=	jet mill
MCC	=	mucociliary clearance
MW	=	molecular weight
MS	=	multiple sclerosis
MSC	=	Model Selection Criterion
mg	=	milligram
min	=	minute
ml	=	milliliter
OSN	=	olfactory sensory neuron
P or PEG	=	polyethylene glycol 3350
PBM	=	planetary ball mill
PBS	=	phosphate buffer solution
pH	=	the negative logarithm of the hydrogen ion concentration
R^2	=	coefficient of determination
R^2_{adjusted}	=	adjusted coefficient of determination
RH	=	relative humidity
S	=	polyvinyl caprolactam - polyvinyl acetate - polyethylene glycol graft copolymer; Soluplus [®]
SD	=	standard deviation
SDS-PAGE	=	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
sec	=	second
SEM	=	scanning electron microscope
SNF	=	simulated nasal fluid
T	=	talc
TEER	=	transepithelial electrical resistance
w/v	=	weight by volume
w/w	=	weight by weight
XRPD	=	X-ray powder diffraction
ρ	=	density

