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**PREPARATION AND EVALUATION OF BIODEGRADABLE RIFAMPICIN
MICROPARTICLES USING SUPERCRITICAL FLUID TECHNIQUE FOR
PULMONARY DELIVERY**

Miss Vipaluk Patomchaivivat

**A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Pharmaceutics**

Faculty of Pharmaceutical Sciences

Chulalongkorn University

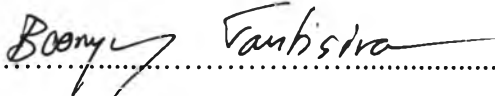
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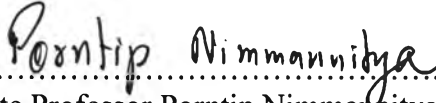
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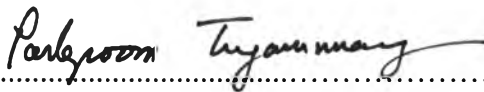
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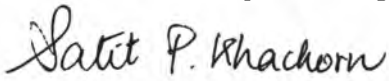
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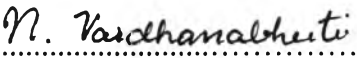
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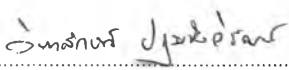
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วิทยานิพนธ์ ปริญญาโท: การเตรียมและประเมินอนุภาคขนาดเล็กที่ย่อยสลายได้ทางชีวภาพของ
 ไรแฟมพิซินโดยใช้เทคนิคสารไหลที่สภาวะเหนือจุดวิกฤตเพื่อนำส่งยาทางปอด (PREPARATION AND
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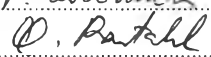
งานวิจัยนี้ใช้เทคนิคสารไหลที่สภาวะเหนือจุดวิกฤต เพื่อเตรียมอนุภาคขนาดเล็กของไรแฟมพิซินและพอลิเมอร์ที่สามารถย่อยสลายได้ทางชีวภาพเพื่อสูดดมเข้าสู่ปอดได้ พอลิเมอร์ที่ใช้ทดลองเป็นกลุ่มของโพลีไฮดรอกซีแอซิด์พอลิเมอร์ ได้แก่ 50:50 พอลิ(ดีแอล-แลคไทด์-โค-ไกลโคลิโด)(PLGA) พอลิ(ดีแอล-แลคไทด์)(DL-PLA) และ พอลิ(แอล-แลคไทด์)(L-PLA) ละลายไรแฟมพิซินและพอลิเมอร์ที่อัตราส่วนต่างๆ ในเมทิลีนคลอไรด์แล้วสเปรย์ลงในคาร์บอนไดออกไซด์ที่อยู่ในสภาวะเหนือจุดวิกฤต ศึกษาถึงผลของชนิดและปริมาณพอลิเมอร์ ความดัน อุณหภูมิ ความเข้มข้นของสารละลาย อัตราการป้อนสาร คอ่ลักษณะของผลิตภัณฑ์ที่ได้ เมื่อใช้พอลิ(ดีแอล-แลคไทด์-โค-ไกลโคลิโด) และพอลิ(ดีแอล-แลคไทด์)ระหว่าง 50 ถึง 100% จะได้ฟิล์มของพอลิเมอร์หรือฟิล์มของพอลิเมอร์กับตัวยา เมื่อใช้พอลิ(ดีแอล-แลคไทด์-โค-ไกลโคลิโด)และพอลิ(ดีแอล-แลคไทด์)ระหว่าง 20 ถึง 40% จะได้อนุภาคที่มีขนาดใหญ่กว่า 18 ไมครอนและเมื่อใช้พอลิ(แอล-แลคไทด์)ระหว่าง 70 ถึง 100% จะได้อนุภาคทรงกลมขนาดเล็ก เมื่อใช้พอลิ(แอล-แลคไทด์)ที่ 60% จะได้ทั้งอนุภาคทรงกลมขนาดเล็กและอนุภาคที่มีรูปร่างไม่แน่นอน เมื่อลดปริมาณของพอลิ(แอล-แลคไทด์)ลงอีกจะพบเพียงอนุภาคที่มีรูปร่างไม่แน่นอน อนุภาคขนาดเล็กที่ผลิตโดยใช้ปริมาณของพอลิ(แอล-แลคไทด์) 60% และไรแฟมพิซิน 40% กักเก็บยาได้ดี (23.30%) และมีขนาดอนุภาคเฉลี่ยโดยปริมาตรเท่ากับ 4.07 ไมครอน แต่ปลดปล่อยยาค่อนข้างรวดเร็ว อนุภาคขนาดเล็กที่ผลิตโดยใช้ปริมาณของพอลิ(แอล-แลคไทด์) 70% และไรแฟมพิซิน 30% เป็นสูตรตำรับที่เหมาะสมเนื่องจากสามารถกักเก็บยาได้ดี (16.33%) มีขนาดอนุภาคเฉลี่ยโดยปริมาตรเท่ากับ 3.40 ไมครอน และสามารถปลดปล่อยอย่างต่อเนื่องได้ตลอด 24 ชั่วโมง อนุภาคขนาดเล็กที่ผลิตโดยใช้ปริมาณของพอลิ(แอล-แลคไทด์) 80% และไรแฟมพิซิน 20% กักเก็บยาต่ำ (8.13%) มีขนาดอนุภาคเฉลี่ยโดยปริมาตรเท่ากับ 3.37 ไมครอน และปลดปล่อยยาค่อนข้างช้า ขนาดอนุภาคเฉลี่ยแบบแอโรไดนามิก (mass median aerodynamic diameter) ของสูตรตำรับที่เตรียมได้จากพอลิ(แอล-แลคไทด์) 70% และไรแฟมพิซิน 30% กับแลคโตสขนาดเล็กลงกว่า 45 ไมครอน ในอัตราส่วน 1:2 เท่ากับ 4.86 ไมครอน และเมื่อผสมกับแลคโตสขนาดระหว่าง 45 ถึง 90 ไมครอนในอัตราส่วน 1:2 เท่ากับ 4.29 ไมครอน อนุภาคของพอลิ(แอล-แลคไทด์)กับเก็บไรแฟมพิซินที่เตรียมจากกระบวนการคั่งตัวทำละลายที่ใช้สภาวะสารไหลเหนือจุดวิกฤต (supercritical antisolvent process) เหมาะสมสำหรับใช้เตรียมผลิตภัณฑ์ยาสูดดมแบบผงแห้งเพื่อนำส่งยาทางปอดเนื่องจากมีขนาดของอนุภาคที่เหมาะสม ไม่พบว่ามีสารละลายของไรแฟมพิซินเมื่อเตรียมตัวยาคด้วยกระบวนการที่ใช้สภาวะสารไหลเหนือจุดวิกฤต การวิเคราะห์โดยใช้ XRD, FTIR และ DSC แสดงว่าไรแฟมพิซินที่ผ่านกระบวนการดังกล่าวได้สารที่มีรูปผลึกไม่เหมือนกับรูปผลึกที่มีการรายงานก่อนหน้านี้ น้ำหนักโมเลกุลและแหล่งผลิตพอลิเมอร์มีอิทธิพลต่อคุณสมบัติของอนุภาคขนาดเล็กที่ได้ ประสิทธิภาพในการต่อต้านการเจริญเติบโตของเชื้อไมโครแบคทีเรียทูปอร์คูโลซิสของอนุภาคพอลิ(แอล-แลคไทด์)กักเก็บไรแฟมพิซินที่เตรียมจากกระบวนการที่ใช้สภาวะสารไหลเหนือจุดวิกฤตยังเท่าเทียมกับตัวยาไรแฟมพิซินก่อนผ่านกระบวนการ

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KEYWORD : RIFAMPICIN / SUPERCRITICAL FLUID TECHNIQUE / PULMONARY DELIVERY / MICROPARTICLES/ BIODEGRADABLE POLYMER

VIPALUK PATOMCHAIWIWAT: PREPARATION AND EVALUATION OF BIODEGRADABLE RIFAMPICIN MICROPARTICLES USING SUPERCRITICAL FLUID TECHNIQUE FOR PULMONARY DELIVERY. THESIS ADVISOR: ASSOC. PROF. POJ KULVANICH, Ph.D., THESIS CO-ADVISOR: ASSIST. PROF. ORNLAKSANA PAERATAKUL, Ph. D., 187 pp. ISBN 974-17-4558-3

It is of interest to apply a supercritical fluid technology for production of inhalable biodegradable microparticles of rifampicin. The polyhydroxy acids[poly(DL-lactide-co-glycolide) copolymer composition 50:50 (PLGA), poly(DL-lactide)(DL-PLA), and poly(L-lactide)(L-PLA)] were used for preparation of drug-loaded microparticles. The solutions of rifampicin and polymer in methylene chloride at various ratios were sprayed into supercritical carbon dioxide. The effect of the type and content of polymer, operating pressure, temperature, solution concentration and feed rate of solution on the characteristics of products were investigated. With 50-100% polymer content of DL-PLA and PLGA, polymer or polymer-drug film occurred. The DL-PLA and PLGA microparticles of 20-40% polymer content had volumetric mean diameter larger than 18 μm and exhibited irregular shape particles forming large and porous agglomerates. The spherical drug loaded microparticles of L-PLA polymer was formed at high polymer content (70-100%). The microparticles prepared using 60% L-PLA polymer content was in spherical and irregular shapes. The shape of L-PLA microparticles became more irregular with decreasing polymer content. The microparticles prepared from 60 % L-PLA and 40 % rifampicin had good drug loading (23.30%) and a mean size of 4.07 μm but their release of drug was rather rapid. The microparticles prepared from 70 % L-PLA and 30 % rifampicin was the preferred formula because it had good drug loading (16.33%) with mean of 3.40 μm and showed sustained release property throughout 24 hours. The microparticles prepared from 80% L-PLA and 20% rifampicin had low drug loading (8.13%) with mean size of 3.37 μm and their releases of drug was rather slow. The mass medium aerodynamic diameter of the microparticles prepared from 70 % L-PLA and 30 % rifampicin with lactose (< 45 μm) in 1:2 ratio and with lactose (45-90 μm) in 1:2 ratio were 4.86 μm and 4.29 μm , respectively. L-PLA rifampicin loaded microparticles prepared by supercritical anti-solvent (SAS) process was of a suitable size to be used in dry powder inhaler formulation for pulmonary delivery. SAS process showed that no decomposition of rifampicin occurred during the processing. The analysis from XRD, FTIR and DSC indicated that processed rifampicin produced by SAS technique was not corresponding to that of rifampicin in the previous reports. Not only the molecular weight of polymer but also the source of polymer influenced on the characteristics of microparticles. The bactericidal efficacy of L-PLA rifampicin loaded microparticles produced by SAS technique against *Mycobacterium Tuberculosis* was similar to that of unprocessed rifampicin.

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ABBREVIATIONS

| | |
|--------------------|--|
| bar | 10^5 Pa (one newton per square meter) |
| °C | degree Celsius |
| cm | centimeter |
| cm ² | square centimeter |
| CV | coefficient of variation |
| D _{10%} | particle diameters at a cumulative fraction of the volume distribution at 10 percent (μm) |
| D _{50%} | particle diameters at a cumulative fraction of the volume distribution at 50 percent (μm) |
| D _{90%} | particle diameters at a cumulative fraction of the volume distribution at 90 percent (μm) |
| DL-PLA | poly(DL-lactide) |
| DL-PLGA | poly(DL-lactide-co-glycolide) copolymer |
| e.g. | <i>exempli gratia</i> , 'for example' |
| et al. | <i>et alii</i> , 'and others' |
| g | gram |
| HPLC | high performance liquid chromatography |
| hr | hour |
| Kg | kilogram |
| L-PLA | L-poly(lactide) |
| mg | milligram |
| min | minute |
| ml | milliliter |
| mm | millimeter |
| MPa | Megapascals |
| nm | nanometer |
| MW | molecular weight |
| N | normal (concentration) |
| N/m ² | Newton per square meter |
| N-s/m ² | Newton-second per square meter |

| | |
|---------------|---|
| Pa | pascal |
| PBS | phosphate buffer in saline |
| pH | the negative logarithm of the hydrogen ion concentration |
| ppm | part per million |
| psi | pound per square inch |
| r^2 | coefficient of determination |
| rpm | revolution per minute |
| SAS | supercritical anti-solvent |
| SD | standard deviation |
| sec | second |
| SEM | Scanning Electron Microscope |
| SF | Supercritical Fluid |
| Span | The polydispersity of the microparticles was $[D_{90\%}-D_{10\%}]/D_{50\%}$ |
| T_g | glass transition temperature |
| T_m | melting temperature |
| μl | microliter |
| μg | microgram |
| μm | micrometer |
| USP | The United States Pharmacopoeia |
| Vs | versus |
| %w/w | % weight by weight |
| %w/v | % weight by volume |