

EXTRACTION OF α -CHYMOTRYPSIN USING SODIUM BIS (2-ETHYLHEXYL) PHOSPHATE (NaDEHP) REVERSE MICELLAR SYSTEM



Ms. Wassana Sooksomsin


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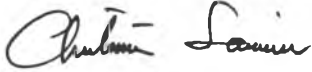
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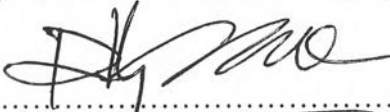
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By : Wassana Sooksomsin
Program : Petrochemical Technology
Thesis Advisors : Assoc. Prof. Chintana Saiwan
Dr. Pomthong Malakul
Prof. Erdogan Gulari

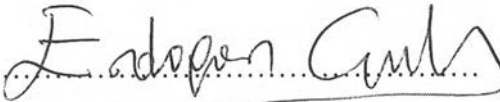
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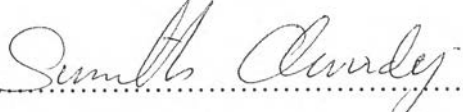

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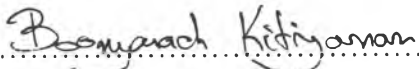
Thesis Committee:


.....
(Assoc. Prof. Chintana Saiwan)


.....
(Dr. Pomthong Malakul)


.....
(Prof. Erdogan Gulari)


.....
(Assoc. Prof. Sumaeth Chavadej)


.....
(Dr. Boonyarach Kitiyanan)

ABSTRACT

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Recently, liquid-liquid extraction using reverse micelles has been proposed as an effective method to separate, concentrate, and purify proteins from a complex biomedium such as fermentation broth with scaled-up ability. In this study, the reverse micellar system of sodium bis (2-ethylhexyl) phosphate (NaDEHP)/isooctane/brine was used to extract α -chymotrypsin from an aqueous phase. The influence of factors affecting the extraction efficiency such as pH, salt concentration, protein loading, and type of cosurfactants were examined. The results showed that extraction efficiency is strongly affected by pH and salt concentration in the aqueous phase. At near neutral pH and low salt concentration, high extraction efficiency (>90%) was obtained. At pH above pI of the protein (pH 8.5), extraction of the protein into the reverse micelles decreased dramatically. Increasing salt concentration resulted in a decline in the proteins transferred into the micellar phase due to lessening attractive interaction. Back extraction of proteins was performed by contacting the micellar phase with a divalent cation aqueous solution, which caused the reverse micelles to destabilize, thus releasing a quantitative amount of protein back into the aqueous solution. After back extraction, the enzymatic activity of the recovered proteins was also examined using a simple hydrolysis reaction.

บทคัดย่อ

วาสนา สุขสมสิน : การสกัดอัลฟาดีโมทริปซิน โดยใช้ระบบรีเวอร์สไมเซลล์ของโซเดียมบิส(ทูเอททิลเฮกซิล) ฟอสเฟส (โซเดียมดีอีเอชพี) (Extraction of α -Chymotrypsin using Sodium Bis(2-Ethylhexyl) Phosphate (NaDEHP) Reverse Micelle System) อ.ที่ปรึกษา : รศ.ดร.จินตนา สายวรรณ, ดร. ปมทอง มาลากุล ณ อยุธยา, และ ศ. เออร์โดแกน กุลารี่ 55 หน้า ISBN 974-03-1577-1

ในปัจจุบันการใช้การสกัดระหว่างของเหลว-ของเหลว แบบรีเวอร์สไมเซลล์ เป็นวิธีการที่มีประสิทธิภาพในการแยก เพิ่มความเข้มข้นและทำโปรตีนให้บริสุทธิ์จากตัวกลางทางชีวภาพที่มีความซับซ้อน เช่น น้ำจากการหมักที่อาจมีปริมาณมาก การศึกษานี้ใช้ระบบรีเวอร์สไมเซลล์ของโซเดียมบิส (ทูเอททิลเฮกซิล) ฟอสเฟส (โซเดียมดีอีเอชพี)/ไอโซออกเทน/น้ำเกลือ เพื่อสกัดอัลฟาดีโมทริปซินจากน้ำ และศึกษาปัจจัยที่มีอิทธิพลต่อประสิทธิภาพในการสกัดต่างๆ เช่น ค่าความเป็นกรด-ด่าง (พีเอช) ความเข้มข้นของเกลือ ความเข้มข้นของโปรตีนและชนิดของโคเซอร์แฟกแตนท์ ผลการทดลองแสดงให้เห็นว่าพีเอชและความเข้มข้นของเกลือในน้ำนั้นมีผลกระทบต่อประสิทธิภาพในการสกัดเป็นอย่างมาก ที่พีเอชเป็นกลางและความเข้มข้นของเกลือต่ำ พบว่าประสิทธิภาพของการสกัดมีมากกว่า 90 % ที่พีเอชสูงกว่าค่าพีไอของโปรตีน (พีเอช 8.5) การสกัดโปรตีนเข้าสู่รีเวอร์สไมเซลล์ลดลงอย่างมาก ความเข้มข้นของเกลือที่เพิ่มขึ้นมีผลทำให้การถ่ายโอนของโปรตีนสู่รีเวอร์สไมเซลล์เฟสลดลง เนื่องจากแรงดึงดูดระหว่างรีเวอร์สไมเซลล์และผิวของโปรตีนลดลง การสกัดย้อนกลับของโปรตีนทำได้โดยการผสมไมเซลล์เฟสกับสารละลายที่มีประจุสองบวกเพื่อสลายความเสถียรของรีเวอร์สไมเซลล์ ซึ่งทำให้ได้โปรตีนในปริมาณมากกลับมาอยู่ในน้ำ หลังจากทำการสกัดย้อนกลับแล้ว ยังได้ทำการทดสอบความว่องไวในการทำปฏิกิริยาของเอนไซม์ที่สกัดกลับมาได้โดยปฏิกิริยาไฮโดรไลซิสด้วย

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