สัญญาเลขที่ MRG5080406

รายงานวิจัยฉบับสมบูรณ์

โครงการ

ศึกษาแบบจำลองพื้นฐานการควบคุมการปลดปล่อยและการเก็บรักษาอะซาไดแรคตินด้วยพอลิเมอร์ย่อยสลาย

โดย

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มิถุนายน 2552

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(ความเห็นในรายงานนี้เป็นของผู้วิจัย สกอ และ สกว.ไม่จำเป็นต้องเห็นด้วยเสมอไป)

บทคัดย่อ

รหัสโครงการ MRG5080406

ชื่อโครงการ ศึกษาแบบจำลองพื้นฐานการควบคุมการปลดปล่อยและการเก็บรักษาอะซาไดแรคตินด้วยพอลิเมอร์ย่อยสลาย

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ปัจจุบันนี้มีการใช้สารกำจัดศัตรูพืชชนิดสังเคราะห์เป็นจำนวนมากเพื่อใช้ในงานเกษตรกรรม ทำให้เกิดสารตกค้างต่อสิ่งแวดล้อม สารกำจัดศัตรูพืชจากธรรมชาติถูกนำมาใช้เพื่อแก้ไขปัญหานี้ สารสกัดสะเดาหรือตัวย่อ neem Aza-A มีสารอะซาดิแรคตินเป็นองค์ประกอบ ซึ่งมีสมบัติยับยั้งการกินอาหาร และการเจริญเติบโตของแมลงศัตรูพืช อย่างไรก็แล้วแต่การใช้สารสกัดสะเดามีปัญหาในการใช้งาน เนื่องจากสารชนิดนี้สลายตัวอย่างรวดเร็วในสภาพสิ่งแวดล้อม ดังนั้นขบวนการห่อหุ้มสารสกัดสะเดาด้วยเมนเบรน เป็นการช่วยควบคุมการปลดปล่อยของสารชนิดนี้ และช่วยให้สารสกัดสะเดามีความเสถียรภาพต่อสิ่งแวดล้อม วิธีที่ใช้ควบคุมการปลดปล่อยของสารสกัดสะเดาสำหรับการศึกษานี้มี 4 วิธี ลำดับแรกยางไซไคลถูกเตรียมขึ้นใช้เป็นสารเคลือบผิวของผนังของแคปซูลเพื่อลดการติดระหว่างแคปซูล สำหรับวิธีแรกในการเตรียมแคปซูลเพื่อการควบคุมการปลดปล่อยของสารสกัดสะเดาประสบความสำเร็จโดยใช้วิธีโฟม ปัจจัยที่ศึกษาสำหรับการเตรียมแคปซูล คือ จำนวนชั้นเคลือบของยางธรรมชาติ ชนิดของโฟม และขนาดของโฟม ผลจากเทคนิคอิเล็กตรอนชนิดส่องกราด (SEM) ชี้ให้เห็นว่าผนังของแคปซูลที่เตรียมมีผิวเรียบ และไม่มีรูพรุน ปริมาณสารสกัดสะเดาถูกตรวจสอบโดยเทคนิค HPLC หรือ UV สเปกโทรสโกปี ประสิทธิภาพการห่อหุ้มสารสกัดสะเดาด้วยโฟมจากพอลิไวนิลแอลกอฮอล์มีประสิทธิภาพที่ดีกว่าโฟมชนิดอื่นๆ เนื่องจากขนาดรูพรุนขนาดเล็ก และสมบัติความชอบน้ำของพอลิเมอร์เมทริกซ์ การบวมตัวของแคปซูลลดลงเมื่อเพิ่มจำนวนชั้นของยางธรรมชาติ วิธีที่สองเป็นการเตรียมไมโครแคปซูลของสารสกัดสะเดาด้วยวิธีการพ่นฝอย โดยใช้พอลิไวนิลอะซีเตทที่มีระดับเปอร์เซ็นต์ไฮโดรไลซิสที่ 0 40 และ 87% เป็นเมทริกซ์ สภาวะที่เหมาะสมในการเตรียมแคปซูลวิธีนี้ถูกตรวจสอบ ตัวแปรที่มีผลต่อการเตรียมแคปซูลได้แก่ ปริมาณสารเชื่อมโยง และปริมาณสารสกัดสะเดา ความเป็นผลึกของพอลิเมอร์เมทริกซ์ถูกศึกษาจากผลจากเทคนิคSEM ชี้ให้เห็นว่าผนังของแคปซูลที่เตรียมมีผิวเรียบ และไม่มีรูพรุน การบวมตัวของแคปซูลลดลงเมื่อเติมสารเชื่อมโยง เวลาการเก็บตัวอย่างที่เวลาต่างๆ แคปซูลที่เตรียมมาจากพอลิไวนิลอะซิเตตที่มี 87% ไฮโดรไลซิสต่อน้ำกลั่นที่อัตราส่วน 1:40 ให้ความเข้มข้นของสกัดสะเดาที่ถูกห่อหุ้มได้มากที่สุด ระดับการปลดปล่อยของสารสกัดสะเดาขึ้นอยู่กับปัจจัยที่ใช้ในการเตรียม นอกจากนี้ผลของเสถียรภาพต่อแสงยูวีของสารสกัดสะเดาทั้งชนิดห่อหุ้มและไม่ห่อหุ้มถูกศึกษา วิธีที่สามนี้การเตรียมแคปซูลสะเดาจากโซเดียมอัลจิเนตซึ่งเชื่อมโยงด้วยกลูตารอลอัลดีไฮด์ การควบคุมการปลดปล่อยของสารสกัดสะเดาขึ้นอยู่ปริมาณการเชื่อมโยงของกลูตารอลอัลดีไฮด์ และ จำนวนชั้นเคลือบของยางธรรมชาติ ปัจจัยที่มีผลต่อการเตรียมแคปซูลถูกศึกษา เช่น ความเข้มข้นของสารเชื่อมโยง ปริมาณสะเดา จำนวนชั้นของยางที่ใช้เคลือบ จากผล SEMชี้ให้เห็นว่าผนังของแคปซูลที่เตรียมได้มีผิวเรียบ และไม่มีรูพรุน การบวมตัวของแคปซูลลดลง เมื่อเวลาแช่ในสารเชื่อมโยงนานขึ้น

ระดับการปลดปล่อยของสารสกัดสะเดาขึ้นอยู่กับปัจจัยที่ใช้ในการเกิดปฏิกิริยาในการเชื่อมโยง และจำนวนชั้นสารเคลือบด้วยยางธรรมชาติ สภาวะที่เหมาะสมสำหรับการเตรียมแคปซูลชนิดนี้ เวลาแช่กลูตารอลอัลดีไฮด์เป็นเวลา 30 นาที และเคลือบด้วยยางธรรมชาติ 3 ชั้นสุดท้ายนี้ การเตรียมแคปซูลสะเดาจากผสมพอลิไวนิลแอลกอฮอล์ และโซเดียมอัลจิเนต โดยใช้สารกลูตารอลอัลดีไฮด์เป็นสารเชื่อมโยง ปัจจัยที่มีผลต่อการเตรียมแคปซูลถูกตรวจสอบ เช่น เวลาในการแช่ในกลูตารอลอัลดีไฮด์ อัตราส่วนระหว่างพอลิเมอร์ทั้งสอง ปริมาณสะเดาถูกตรวจสอบ โครงสร้างของพอลิเมอร์ผสมถูกตรวจสอบโดยเทคนิค FTIR และ XRD นอกจากนี้การบวมตัวของแคปซูล และการทนความร้อนของแคปซูลถูกศึกษา ผลการทดลองพบว่าความแข็งแรงของผนังของแคปซูลขึ้นอยู่กับอัตราส่วนระหว่างพอลิเมอร์ทั้งสอง และความหนาแน่นการเชื่อมโยง จากเทคนิค SEM EPMA และ AFM ชี้ให้เห็นว่าผนังของแคปซูลมีลักษณะขรุขระ และไม่มีรูพรุน การบวมตัวของแคปซูลขึ้นอยู่กับเวลาในแช่ในสารละลายในกลูตารอลอัลดีไฮด์ ระดับการปลดปล่อยของสารสกัดสะเดาจากแคปซูลขึ้นอยู่กับปริมาณการเชื่อมโยง และจำนวนชั้นในการเคลือบ นอกจากนี้ระดับของการปลดปล่อยของอะซาดิแรคตินจากแคปซูลลดลงเมื่อมีปริมาณพอลิไวนิลแอลกอฮอลล์เพิ่มขึ้น สภาวะที่เหมาะสมสำหรับการเตรียมแคปซูลชนิดนี้ อัตราส่วนของพอลิเมอร์ผสม 7:3 พอลิไวนิลแอลกอฮอล์:โซเดียมอัลจิเนต แช่ในกลูตารอลอัลดีไฮด์เป็นเวลา 30 นาที จำนวนชั้นเคลือบด้วยยางยางธรรมชาติ 3 ชั้น พฤติกรรมการปลดปล่อยของแคปซูลที่เตรียมจากทั้ง 4 วิธี สอดคล้องกับสมการจลนพลศาสตร์อย่างง่าย สุดท้ายนี้ศักยภาพเป็นไปได้ของแคปซูลสะเดาที่เตรียมได้นี้ถูกนำไปใช้งานด้านเกษตรกรรม และ กำจัดยุง

คำสำคัญ: เทคนิคการห่อหุ้ม, ยางธรรมชาติ, พอลิเมอร์ย่อยสลาย, สะเดา

Abstract

Project Code : MRG5080406

Project Title : Study the basic model of controlling release and storage of Azadirachtin via biodegradable polymer

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Project Period: July 2007-June 2009

At present, the use of huge quantities of synthetic pesticides in conventional agriculture has lead to the major environmental problems. Natural pesticides are now being developed to avoid such problems. Neem (Azadiracthin A) seed oil hereafter designated as neem Aza-A, is one such natural pesticide known to be a powerful insect antifeedant and growth-regulating substance yet limited in application because of its rapid degradation in the environment. Therefore, encapsulation of neem Aza-A within membranes to control its release and improve its stability in the environment may improve its effectiveness. The four approaches of encapsulated neem Aza-A were studied in this work. Firstly, preparation of cyclized natural as a polymer membrane coating on the obtained capsule was investigated to reduce the tackiness between capsules. In the case of the first method, the controlling release of neem Aza-A, was achieved by utilization of foam. The neem Aza-A containing beads have been prepared by changing the experimental variables such as NR coating and foam type. The SEM data indicated that the structure of the walls the beads are smooth and nonporous. At particular intervals, the remaining concentration of neem Aza-A was analyzed by HPLC or UV spectroscopy. The efficiency of encapsulated neem Aza-A in polymer foam matrix obtained from poly (vinyl alcohol) was higher than the other polymers due to it hydrophilic polymer matrix. The swelling result indicates that swelling of the polymeric beads decreases with increasing NR coating layer. For the second method, microcapsule of neem Aza-A with partially hydrolyzed poly (vinyl acetate) with 0, 40 and 87 % hydrolysis as matrix has also been prepared via spray drying technique in this study. Optimum condition of encapsulation for neem Aza-A by using spray drying was investigated. The neem Aza-A containing beads have been prepared by changing the experimental variables such as the extent of crosslinking and the amount of loading in order to optimize the process variables. The SEM data indicated that the structure of the walls the beads are smooth and nonporous. The swelling results indicated that swelling of the polymeric beads decreases with increasing exposure time to the crosslinking agent and crystalline content. It was found from the experiment that the ratio of 87% hydrolyzed poly (vinyl acetate) to water being 1:40 gave the highest concentration of neem Aza-A in the device. The degree of release of neem Aza-A was controlled by condition parameters. The photostabilization of unencapsulated and encapsulated neem Aza-A when exposed to ultraviolet light was evaluated. For the third method, controlling the release of the pesticide was achieved by utilization of glutaraldehyde-aglinate gel capsules modified by coating with a natural rubber (NR) layer. The optimization of the properties of the neem Aza-A containing beads was achieved by changing variables such as the extent of crosslinking, the amount of loading and NR layer. The SEM data indicated that the

walls of the beads are smooth and nonporous. The swelling results indicated that swelling of the polymeric beads decreased with increasing exposure time to glutaraldehyde and reduced the rate of release of the pesticide. The degree of release of neem Aza-A from capsules was controlled by their condition of formation. The optimum condition for encapsulated neem Aza-A was glutaraldehyde storage time of 30 min and 3 NR coating layers. Finally, the controlling release of neem Aza-A was achieved by utilization of glutaraldehyde-aglinate gel and poly (vinyl alcohol) capsules. The neem Aza-A-containing beads have been prepared by changing the experimental variables such as the extent of crosslinking, blend ratio and the amount of loading in order to optimize the process variables. The chemical structure of capsule wall was evaluated through FTIR, and XRD. In addition, the swelling behavior of capsule and thermal stability of capsule were investigated in this work. The strength of capsule wall depended on the poly (vinyl alcohol) in matrix and crosslinking density. SEM, EPMA and AFM data indicated that the structure of the walls the beads are rough and nonporous. The swelling results indicated that swelling of the polymeric beads decreases with increasing exposure time to the crosslinking agent. The degree of release of neem Aza-A was controlled by condition parameters including crosslinking content and NR coating layer. In addition, the degree of Aza release from capsule dramatically decreased when amount of PVA in composite blend increased. The best condition for encapsulated neem Aza-A was the ratio of polymer blend with poly (vinyl alcohol): sodium alginate 7:3, glutaraldehyde storage time of 30 min and 3 NR coating layers. The release data from four approaches have been fitted to an empirical equation to estimate the kinetic parameter. In conclusion, the obtained capsules have possible potential used in agriculture field and mosquito protection.

Keywords: Encapsulation, Natural rubber, Biopolymer, Neem

Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

I. Publication

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2. S. Riyajan,J. T. Sakdapipanich, "Encapsulated neem extract containing Azadiractin-A within hydrolyzed poly(vinyl acetate) for controlling its release and photodegradation stability" Chemical Engineering Journal, inpress 2009

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4. S. Riyajan, J. T. Sakdapipanich, "Thermal Property and Thermodynamic Parameter of Rubber Blends Containing the Cyclic Structure" Kaustschuk Gummi Kunststoffe, **62(3),** 665-670 (2009)

5. S. Riyajan, "Preparation and Characterization of Neem Capsule *Via* Glutaraldehyde Crosslinked Sodium Alginate and Poly (Vinyl Alcohol) Blend for Its Control Release" will be prepared for Carbohydrate Polymer

6. S. Riyajan, "Encapsulation of neem *via* foam techniques" will be prepared for Carbohydrate Polymer

II. Proceeding

1. Riyajan, S., Encapsulation of Azadirachtin by using Partially Hydrolyzed Poly (vinyl acetate) 33th Congress on science and Technology of Thailand (STT), 18-20, October, 2007, Walailak University, Thailand

2. Riyajan, S., Jitladda T, Charnchai, K. Encapsulation of Neem by using Glutaraldehyde Crosslinked Sodium Alginate Containing Natural Rubber, Pure and Applied International Chemistry Conference 2008 (PACCON 2008) ,Jan 30 – Feb 1, 2008, Sofitel Centara Grand Bangkok, Bangkok

3. Riyajan, S., Jitladda T. Preparation of Neem Capsule Via Glutaraldehyde Crosslinked Sodium Alginate and Poly(vinyl Alcohol) for its Control Release

Thailand Materials Science and Technology Conference (MSAT-5) Sep 16-19, 2008 Miracle Grand Conventional Hotel ,Bangkok, Thailand

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III.การนำผลงานวิจัยไปใช้ประโยชน์

- เชิงพาณิชย์

งานวิจัยนี้คาดว่าจะเตรียมร่างอนุสิทธบัตร “วิธี การเตรียม และการเก็บรักษาอะซาไดแรคตินด้วยวิธีโฟม”นอกจากนี้พยายามนำแคปซูลอะซาดิแรคตินที่ห่อหุ้มด้วยพอลิเมอร์ย่อยสลายไปใช้งานเกษตรกรรม และกำจัดยุง จึงเป็นแนวทางหนึ่งที่ถูกนำมาใช้ในการลดปริมาณการใช้สารเคมีสังเคราะห์กำจัดแมลง โดยควบคุมอัตราการปลดปล่อยของสารออกฤทธิ์ให้เป็นไปตามต้องการเพื่อลดความเป็นพิษต่อคน และ สิ่งแวดล้อม

- เชิงสาธารณะ

เกิดเครือข่ายในการทำการวิจัยระหว่าง ผู้วิจัย และ นักวิจัยพี่เลี้ยง รวมถึงนักวิจัยมหาวิทยาลัยอื่นๆ และให้คำปรึกษา แก่นักศึกษาวิทยาศาสตร์พอลิเมอร์ เคมี

- เชิงวิชาการ

ช่วยสนับสนุนการเรียนการสอน วิชาโครงงานพิเศษของนักศึกษาระดับปริญญาตรี และวิทยานิพนธ์ของนักศึกษาระดับปริญญาโท ของ สาขาวิทยาศาสตร์พอลิมอร์ คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ งานวิจัยนี้ช่วยสนับสนุนสร้างความพร้อมในการวิจัยวิทยาศาสตร์พอลิเมอร์ สร้างผลงานวิจัยที่มีคุณภาพให้ประเทศชาติ

บทที่ 1
บทนำ

1 ความสำคัญและที่มาของปัญหาที่ทำการวิจัย

ปัจจุบันประชากรของประเทศไทยมีประมาณ 62 ล้านคนความต้องการอาหารบริโภคได้ทวีสูงมากขึ้นเมื่อเทียบกับ 10-20 ปีก่อนนี้เนื้อที่ของประเทศมิได้ขยายออกไปตามจำนวนประชากรที่เพิ่มขึ้น การเกษตรของไทยได้เปลี่ยนรูปจากเกษตรกรรมแบบดั้งเดิมซึ่งมีการปลูกพืชเลี้ยงสัตว์เพื่อแบ่งกันกิน แบ่งกันใช้ในครอบครัวไปเป็นอุตสาหกรรมเกษตร คือนำผลผลิตป้อนโรงงานอุตสาหกรรมและส่งขายต่างประเทศจึงจำเป็นที่เกษตรกรซึ่งเป็นประชากรส่วนใหญ่ของประเทศจะต้องเพิ่มผลผลิตให้มากขึ้นในเนื้อที่ที่มีอยู่จำกัด การเพิ่มผลผลิตต้องอาศัยปัจจัยหลายอย่าง เช่น ปุ๋ย ค่าจ้างแรงงาน และที่สุดคือการใช้วัตถุป้องกันและกำจัดศัตรูพืช ซึ่งส่วนใหญ่เป็นวัตถุมีพิษบางครั้งเสียค่าใช้มากกว่าร้อยละ 50 การลดใช้สารเคมีจึงเป็นสิ่งจำเป็นเพื่อมิให้เป็นอันตรายต่อผู้บริโภค สินค้าเกษตรและตัวเกษตรกรเอง จำเป็นหาแนวทางแก้ปัญหา โดยใช้สารสกัดจากพืชมาใช้ทดแทนสารเคมีเพื่อกำจัดศัตรูพืช สารกำจัดแมลงและศัตรูพืชมีมากมายหลายชนิดแต่ละชนิดมีผลต่อแมลงศัตรูพืชต่างกันออกไป แต่ส่วนใหญ่มักพบว่า การควบคุมประสิทธิภาพการออกฤทธิ์ของสารกำจัดแมลงศัตรูพืชไม่ได้เป็นไปตามที่คาดหวัง จึงมักเพิ่มปริมาณการใช้สารเคมีมากขึ้นเพื่อให้ได้ประสิทธิภาพตามต้องการสาเหตุหลักเนื่องจากความไม่เสถียรภาพของสารกำจัดแมลงศัตรูพืช ซึ่งจะทำให้ประสิทธิภาพของสารกำจัดแมลงศัตรูพืชที่ใช้จริงในแปลงเกษตรต่ำกว่าเมื่อมีการทดสอบในห้องปฏิบัติการ ปัจจัยต่างๆ ที่เกี่ยวข้องกับการสลายตัวของสารกำจัดแมลงศัตรูพืชมักเกิดจากแสงแดดความร้อน ความชื้น ตลอดจนการชะล้างและการพัดพาไปของฝนและลม ซึ่งก่อให้เกิดมลภาวะที่เป็นพิษในสิ่งแวดล้อม ดังนั้นงานวิจัยนี้แก้ปัญหาดังกล่าว โดยการศึกษาการห่อหุ้ม Azadiracthin ด้วยพอลิเมอร์ย่อยสลาย เพื่อเป็นแนวทางไปใช้งานเกษตรกรรมต่อไป จึงเป็นแนวทางหนึ่งที่ถูกนำมาใช้ในการลดปริมาณการใช้สารเคมีกำจัดแมลง โดยควบคุมอัตราการปลดปล่อยของสารออกฤทธิ์ให้เป็นไปตามต้องการเพื่อลดความเป็นพิษต่อคนและสัตว์เลี้ยงลูกด้วยน้ำนม

2 วัตถุประสงค์ของโครงการ

2.1 ควบคุมการสลายตัว Azadiracthin โดย ด้วยพอลิเมอร์ย่อยสลาย

2.2 หาตัวแปรที่ใช้ในเตรียมโฟมเพื่อใช้การควบคุมการสลายตัวของ Azadirachtin

2.3 หาสารเคลือบปิดผิวแคปซูลเพื่อควบคุมการปลดปล่อยของ Azadiracthin

2.4 ศึกษาประสิทธิภาพการสลายตัว Azadiracthin ภายใต้ความร้อนและแสงแดด

2.5 ศึกษาแบบจำลองการปลดปล่อย Azadiracthin จากเมททริกซ์

3ขอบเขตของการวิจัย

งานวิจัยนี้ศึกษาตัวแปรที่ใช้ในการเตรียมแคปซูล ที่มีผลต่อการปลดปล่อยของ Azadiracthin หาสารเคลือบปิดผิวแคปซูลเพื่อควบคุมการปลดปล่อย Azadiracthin และทดสอบประสิทธิภาพการปลดปล่อย Azadiracthin จากพอลิเมอร์ที่ย่อยสลายตัวได้

1.4 ประโยชน์ที่คาดว่าได้รับ

งานวิจัยเป็นการศึกษาความรู้พื้นฐานการเตรียมเทคนิคการห่อหุ้มสารสกัดสะเดาด้วยวิธีใหม่ ประโยชน์ที่ได้ของงานวิจัยนี้คือช่วยลดปัญหาการสลายตัวของ อะซาไดแรคตินAzadiractin ซึ่งเป็นสารสกัดจากเมล็ดสะเดา (ใช้ทำหน้าที่กำจัดแมลงศัตรูพืช ซึ่งเป็นพืชเศรษฐกิจของประเทศไทย) เพราะว่าสารนี้โดนความร้อนและแสงแดดจะสลายตัวอย่างรวดเร็ว ดั้งนั้นต้องห่อหุ้มสารเหล่านี้เพื่อให้ใช้งานได้ยาวนานขึ้น จึงสามารถช่วยแก้ปัญหาการเก็บสารสะเดาอีกแนวทางหนึ่งอีกด้วยนอกจากนั้นงานวิจัยมุ่งเน้นให้ผลิตภัณฑ์ที่ได้มีมาตรฐานดีเหมาะสมต่อการนำไปใช้ในการป้องกันกำจัดศัตรูพืช เป็นการดำเนินธุรกิจเกษตรที่พึ่งพาวัตถุดิบที่มีภายในประเทศเป็นการช่วยรัฐลดอัตราการนำเข้าสารเคมีในการป้องกันกำจัดศัตรูพืชและช่วยทำให้สมดุลย์ธรรมชาติที่สูญเสียไปกลับคืนมา

ถ้างานวิจัยประสบความสำเร็จในอนาคตงานวิจัยนำแคปซูลไปใช้ในงานการออกฤทธิ์การกำจัดยุงลายซึ่งเป็นตัวก่อปัญหาไข้เลือดออกของประชากรในประเทศไทยอีกด้วย

บทที่ 2
การทบทวนเอกสารและงานวิจัยที่เกี่ยวข้อง

1 บทนำ

ประเทศไทยเป็นประเทศหนึ่งที่มีต้น สะเดาอยู่อย่างมากมาย สะเดาเป็นพันธุ์ไม้ที่น่าสนใจและมีประโยชน์หลายประการชนิดหนึ่งเพราะเป็นไม้ทนแล้งสามารถขึ้นได้โดยไม่เลือกดิน เจริญเติบโตเร็วพอสมควรสามารถใช้เหง้าปลูกได้ทนไฟแตกหน่อได้ดี สัตว์เลี้ยงไม่กิน ปัญหาเกี่ยวกับโรคแมลงทำอันตรายมีน้อยใบและดอกสามารถใช้ประกอบอาหาร ของคน เนื้อไม้เหมาะสำหรับใช้ในการก่อสร้างและใช้ทำเชื้อเพลิงและเมื่อพูดถึงสะเดาจะหมายถึงสะเดาอินเดีย แต่ในบ้านเราจะหมายถึงสะเดาไทย ส่วนสะเดาที่นำไปใช้เป็นสารฆ่าแมลงได้ดีคือ เมล็ดหรือผล เพราะมีสาร Azadirachtin สูง ส่วนในส่วนอื่น เช่น ใบ หรือเปลือก ก็มีการนำไปใช้ป้องกันกำจัดแมลงเหมือนกันแต่ให้ผลไม่ค่อยดี การนำสารสกัดสะเดาไปใช้ป้องกันกำจัดแมลงนั้น บางคนก็บอกว่าได้ผล บางคนก็บอกว่าไม่ได้ผล ทั้งนี้เนื่องจาก สาเหตุหลายประการด้วยกัน แต่ปัจจัยสำคัญที่จะใช้สารสกัดสะเดาในการป้องกันกำจัดแมลงให้ได้ผลนั้นจะต้องใช้อย่าง ถูกวิธีดังนี้

รูปที่ 1 ภาพแสดงผลของสะเดา

2 สะเดา

ผงสะเดา (Neem powder) หมายถึง สะเดาที่บดเป็นผงซึ่งได้จากการบดเมล็ดหรือผลสะเดาแห้งโดยการหว่านรอบ ต้นพืช เช่น ในนาข้าว นาแห้ว เพื่อป้องกันหนอนเจาะลำต้น เจาะผล หรือแมลงปากดูด เป็นต้น อาจผสมกับดิน เพื่อป้องกันกำจัด หนอนของด้วงหมัดกระโดด ไส้เดือนฝอย และแมลงบางชนิดที่อาศัยในดิน หรืออาจใช้ผสมกับทรายโรยตามยอดต้นพืช เพื่อ ป้องกันกำจัดหนอนเจาะยอด เป็นต้น

น้ำมันสะเดา (Neem oil) หมายถึง น้ำมันที่ได้จากการอัด หรือหีบ หรือใช้สารเคมีสกัด (เฮกเซน เบนซิน อีเธอร์) เมล็ดใน (seed kernel) เวลาใช้จำเป็นต้องผสมสาร emulsifier เพื่อให้น้ำมันรวมกับน้ำได้ หรือใช้เครื่องฉีดชนิด ULV และเท่าที่มีรายงานน้ำมันสะเดาสามารถใช้ได้ผลดีกับแมลงบางชนิดเท่านั้น เช่น ตั๊กแตน ไรแดง ด้วงทำลายเมล็ด ธัญพืชในโรงเก็บ เป็นต้น

สารสกัดสะเดา (Neem extracts) หมายถึง การนำผงสะเดาหรืออาจใช้ส่วนที่เหลือจากการสกัดน้ำมันออกแล้วก็ได้ โดยการนำมาสกัดด้วยแอลกอฮอล์หรือน้ำ จากนั้นกรองเอาน้ำยาออก ซึ่งเรียกน้ำยานี้ว่า "สารสกัดสะเดา" เวลาจะใช้ก็เติม น้ำยาจับใบลงไปด้วยเพื่อให้คงทนอยู่ได้นานขึ้น

กากสะเดา (Neem cake) เป็นส่วนที่เหลือตกค้างในผ้ากรอง นำกากสะเดาไปตากแดดหรือผสมกับกากน้ำลายเพื่อ ให้จับตัวกัน แล้วอัดให้เป็นเม็ดหรือเป็นแท่ง สามารถนำไปขายต่อไป กากสะเดายังคงมีสารฆ่าศัตรูพืชได้อีก และใช้ เป็นปุ๋ยหรือเป็นสารอาหารสัตว์ได้

จะเห็นได้ว่า ผลิตภัณฑ์สะเดาที่ใช้มีอย่างน้อย 4 ชนิดดังกล่าว ในที่นี้จะพูดถึงเฉพาะสารสกัดสะเดาเท่านั้น ซึ่งมีการใช้กันมากและมีแนวโน้มในการป้องกันกำจัดแมลงให้ผลดีกว่าชนิดอื่น สำหรับประเด็นหลักที่ทำให้การใช้ สารสกัดสะเดาได้ผลหรือไม่คือ

1. ชนิดของแมลงศัตรูพืช จากประสบการณ์ในการทดสอบประสิทธิภาพของสารสกัดสะเดาในสภาพธรรมชาติ ปรากฏ ว่า สารสกัดสะเดาให้ผลดีในการป้องกันกำจัดแมลงแต่ละชนิดแตกต่างกันไป เพื่อให้ง่ายต่อการปฏิบัติจึงแบ่งแมลงเป็น 3 กลุ่ม คือ

1.1 ใช้สารสกัดสะเดาได้ผลดี แมลงหลายชนิดที่อ่อนแอต่อสารสะกัดสะเดา เช่น หนอนกระทู้ชนิดต่าง ๆ หนอนหนังเหนียว หนอนใยผัก หนอนชอนใบ หนอนม้วนใบ หนอนบุ้ง หนอนแก้ว หนอนหัวกะโหลก เพลี้ยอ่อน เพลี้ยไก่แจ้ เป็นต้น ดังนั้นในการป้องกัน กำจัดแมลงดังกล่าวสามารถใช้สารสกัดสะเดาเพียงอย่างเดียว โดยไม่จำเป็นต้องใช้สารฆ่าแมลงสังเคราะห์ฉีดสลับในช่วงที่ แมลงระบาด

1.2 ใช้สารสกัดสะเดา สารสกัดสะเดาให้ผลปานกลางในการป้องกันกำจัดแมลงหลายชนิด เช่น หนอนเจาะ สมอฝ้าย หนอนเจาะต้นกล้าถั่ว หนอนเจาะดอกกล้วยไม้ หนอนเจาะผลมะเขือ หนอนเจาะยอดคะน้า แมลงวันทอง เพลี้ยจักจั่น เพลี้ยไก่แจ้ เพลี้ยไฟ และไรแดง เป็นต้น ในกรณีที่แมลงเหล่านี้ระบาดมาก การใช้ สารสกัดสะเดาจะไม่ได้ผล จำเป็น ต้องใช้สารฆ่าแมลงสังเคราะห์ฉีดในระยะที่แมลงระบาดสัก 1-2 ครั้ง จากนั้นจึงใช้สารสกัดสะเดาต่อไป

1.3 ใช้ สารสกัดสะเดาไม่ได้ผลหรือได้ผลค่อนข้างต่ำ แมลงต่อไปนี้อยู่ในกลุ่มที่ใช้สารสกัดสะเดาไม่ได้ผลหรือ ได้ผลน้อย คือ ด้วงปีกแข็งกัดกินใบพืช หมัดกระโดด มวนแดง มวนเขียว เป็นต้น ดังนั้นการใช้สารสกัดสะเดาจึงไม่แนะนำ กับแมลงดังกล่าว

2. คุณภาพของ สารสกัดสะเดา หมายถึง ปริมาณของสาร Azadirachtin ที่มีอยู่ในสารสกัด จากผลการทดลองพบว่าในขณะ ที่ผลิต สารสกัดสะเดาไม่ว่าจะผลิตใช้เองหรือผลิตเป็นการค้า สารสกัดสะเดาจะต้องมีสารออกฤทธิ์ (Azadirachtin) ในปริมาณมาก พอสมควร โดยเฉลี่ยไม่ต่ำกว่า 0.1%

(หรืออาจต่ำกว่าได้ในกรณีที่ผลิตใช้เอง) ถ้าผลิตใช้เองโดยใช้น้ำเป็นตัวสกัด ควรรีบใช้ให้หมดภายใน 2-3 วัน แต่ถ้าผลิตเป็นการค้า สารสกัดสะเดาสามารถใช้ป้องกันกำจัดแมลงได้นานถึง 1 ปี ในช่วงที่ทิ้งสารสกัดนี้ไว้ สาร Azadirachtin จะค่อย ๆ สลายตัว แต่ประสิทธิภาพในการป้องกันกำจัดแมลงยังคงใช้ได้ผลดีอยู่

3. วิธีการใช้ การใช้ สารสกัดสะเดาแตกต่างจากการใช้สารฆ่าแมลงสังเคราะห์ คือ จำเป็นต้องฉีดต่อเนื่องเป็น ระยะเวลาประมาณ 3 ครั้ง โดยเว้นระยะการฉีด 5-7 วัน หรือถ้าใช้ สารสกัดไปนาน ๆ ประมาณ 1 ปี อาจเว้นระยะเวลาการฉีด แต่ละครั้ง 1-2 เดือน และสิ่งที่ควรคำนึงถึงคือพยายามฉีดสารสกัดสะเดาก่อนที่แมลงจะระบาดมาก

<u>คุณสมบัติของสารสกัดสะเดาดังนี้</u>

- เป็นสารไล่แมลง ทำให้แมลงไม่เข้ามาในบริเวณที่ใช้สารสกัดสะเดา
- เป็นสารยับยั้งการกินอาหารทำให้การทำลายพืชลดลง
- เป็นสารยับยั้งการเจริญเติบโตของหนอนไข่และดักแด้ ทำให้ตัวเต็มวัยลดลง
- ระงับการสร้างสารไคติน
- รบกวนการผสมพันธ์และการสื่อสารเพื่อการผสมพันธ์ของแมลง ทำให้แม่ผีเสื้อหรือตัวเต็มวัยสร้างไข่น้อยลง และยับยั้งการวางไข่ นอกจากนี้ยังทำให้การฟักตัวของไข่น้อยลงอีกด้วย มีผลให้ตัวอ่อนหรือหนอนมีจำนวนน้อย การระบาดจึงไม่รุนแรง
- ไม่ทำลายศัตรูธรรมชาติที่เป็นประโยชน์เช่น ตัวห้ำ ตัวเบียนไม่อันตรายต่อ แมลงผสมเกสรเช่นผึ้ง ดังนั้นเมื่อใช้สารสะเดาเป็นเวลานานก็จะเกิดสมดุลที่แมลงควบคุมกันเอง ทำให้การพ่นสารสะเดาห่างขึ้นเป็นการประหยัดค่าใช้จ่ายและยังได้ผลผลิตเพิ่มมากขึ้นอีกด้วย

<u>ตัวอย่างการใช้สารสกัดสะเดา</u>

การใช้สารสกัดจากสะเดา (Azadirachta indica A. Juss) โดยเฉพาะอย่างยิ่งน้ำมันสะเดาและส่วนต่าง ๆ ของสะเดาในการป้องกันกำจัดแมลงศัตรูในโรงเก็บ พบรายงานการใช้ผลดีกับแมลงหลายชนิด เช่น Callosobruchus maculatus และ C. Chinensis

, Corcyra cephalonica Stainton, Rhyzopertha dominica (F) และ Tribolium castaneum (Herbst), Sitophilus granarias (L) และ S. oryzae ทั้งนี้ลักษณะการออกฤทธิ์ของน้ำมันสะเดา ส่วนของต้นตลอดจนสารสกัดรูปแบบต่าง ๆ ที่มีต่อแมลงและไรศัตรูพืชมีหลายลักษณะได้แก่ การเป็นสารออกฤทธิ์ไล่แมลง (Repellent) ยับยั้งการพัฒนาการเจริญเติบโต(growth inhibitor) ยับยั้งการวางไข่ (Ovicidal effect).

<u>การสกัดสารสะเดา</u>

การสกัดสารจากเมล็ดสะเดาทำได้โดยใช้สารละลายอินทรีย์ชนิดที่มีขั้ว (Polar solvents) เช่น น้ำ หรือสารละลายแอลกอฮอล์เช่น เอทธานอล (ethanol),

เมทานอล (Methanol) ฯลฯ สกัดสารออกฤทธิ์ที่มีประสิทธิภาพดีในการป้องกันและกำจัดแมลงศัตรูพืชที่มีชื่อว่า Azadirachtin ($C_{36}H_{44}O_{16}$) ซึ่งเป็นสารพวก Limonoid (Tetranortriterpenoid)

ปัญหาการเพาะปลูกอย่างหนึ่งก็คือเรื่องแมลง ซึ่งเป็นศัตรูของพืช ปัจจุบันเกษตรกรแก้ปัญหาโดยใช้สารเคมีในการแก้ปัญหาดังกล่าว แต่ทว่าสารเคมีที่ใช้นั้นตกค้างในพืชทำให้มีปัญหาต่อผู้บริโภค แต่มีเกษตรกรเป็นจำนวนน้อยที่ใช้สกัดจากธรรมชาติในการไล่แมลงเช่นสารสกัดสะเดา ทั้งนี้อาจเนื่องมาจากสมบัติสารAzadirachtinเป็นสารที่สลายตัวได้ง่ายในสภาพธรรมชาติ อุณหภูมิที่สูงเกินไปและแสงแดดซึ่งมีผลต่อแสดงความสามารถในการออกฤทธิ์ของสารสกัดที่ได้จากขบวนการผลิตและการใช้งานการเกษตรกรรมที่ต้องสัมผัสกับความร้อนและแสงแดดทำให้เกษตรกรจะต้องฉีดพ่นสารสกัดสะเดาเป็นจำที่ถี่ซึ่งส่งผลให้ค่าใช้จ่ายการเพาะปลูกพืชมีต้นทุนที่สูงขึ้น ดังนั้นการพัฒนาการพัฒนาผลิตภัณฑ์สะเดาที่ในรูปที่ทนต่อสภาวะความร้อนและแสงแดดเป็นสิ่งที่จำเป็นเพื่อให้การออกฤทธิ์ของสารสกัดสะเดาให้ยาวนาน และปัญหาการเก็บรักษาสารสกัดสะเดาก็ปัญหาอย่างหนึ่งที่ต้องการแก้ไขด้วยเพราะสารสะเดาจะเสื่อมสภาพได้เร็วมาก จาก ดังนั้นงานวิจัยนี้มุ่งเน้นหาวิธีในการควบคุมสลายตัวสารสกัดสะเดา และยืดอายุในการเก็บรักษาสารสกัดสะเดาโดยการห่อหุ้มสารสารสกัดสะเดาด้วยโฟมที่ทำมาจากยางธรรมชาติร่วมถึงโฟมสังเคราะห์ที่ใช้แล้วเพื่อที่ต้องการลดต้นทุนผลิตการผลิตภัณฑ์สารสะเดาเพื่อที่ใช้ผลิตภัณฑ์ให้สามารถเชิงการค้าได้ในอนาคต

3 โมโนลิทิก ดิไวซ์

3.1 การควบคุมการปลดปล่อยของวัตถุมีพิษ

การควบคุมการปลกปล่อย โดยที่อัตราอาจจะลดลงทีละน้อยหรือว่ามีการเพิ่มสูงขึ้นจนถึงจุดสูงสุดที่สามารถควบคุมได้ โดยการกำหนดการควบคุมการปลดปล่อยอาจเปลี่ยนแปลงได้ทั้งนี้ขึ้นอยู่กับการออกแบบ

แนวคิดและแบบแผนของการควบคุม การปลดปล่อย รวมเข้าไว้ได้หลายกลไกตลอดจนการกำหนดกฎที่ง่ายที่สุดเพื่อเป็นพื้นฐานในการพิจารณาการปลดปล่อยของวัตถุมีพิษจากส่วนห่อหุ้มที่แข็งแรงอาทิเช่น ซิลิกาเจล ไมกา และ activated charcoal แต่ที่สำคัญที่สุดของระบบ ในปัจุบันและเป็นพื้นฐานของกลไกที่ สามารถควบคุมได้เช่นการแพร่ผ่านโดยการควบคุมอัตราของสารที่ต้องห่อหุ้ม การชะของวัสดุที่ใช้สำหรับห่อหุ้ม และปฏิกิริยาเคมีที่ลดลง ได้มีผู้พยายามที่จะออกแบบกลไกสำหรับอันดับศูนย์ของการปลดปล่อยค่าคงที่ แต่เวลาที่ใช้ในระบบขึ้นอยู่กับจลนศาสตร์การปลดปล่อย

3.2 การควบคุมโดยอาศัยเยื่อกลางของการแพร่ผ่าน

กลไกการควบคุมแพร่ผ่านามารถแบ่งได้เป็น 2 ประเภท คือ reservoir systems เกี่ยวกับการรวม ตัวขอกันทั้งหมดของวัตถุมีพิษในแคปซูลภายใต้การควบคุมการแพร่ผ่านโดยเยื่อและ monolithics systems เกี่ยวกับวัตถุมีพิษที่กระจายหรือถูกละลายภายในการควบคุมของสารห่อหุ้ม เป็นการพิสูจน์

ถึงอัตราการแพร่ผ่านของระบบจากการถูกควบคุมการปลดปล่อยภายใต้กฎของ Fick อันดับขั้นของอัตราการแพร่ผ่านขึ้นอยู่กับ 5 ปัจจัย โดย 2 ปัจจัยประเมินได้ตามกลไกของมิติและเรขาคณิตและอีก 3 ปัจจัยประเมินจากวัตถุมีพิษและพอลิเมอร์ที่ทำปฏิกิริยากัน

dM/dt =(A/h)D(Cs-KCs)…….(1)

จากสมการ (1) A แทนพื้นที่ผิวของเยื่อผนัง h แทนความหนาของผนัง D แทนสัมประสิทธิ์การแพร่ของวัตถุมีพิษในพอลิเมอร์ Cs แทนความสามารถการละลายอิ่มตัวสูงสุดของวัตถุมีพิษในพอลิเมอร์ K แทนสัมประสิทธิ์การแบ่งของวัตถุมีพิษระหว่างพอลิเมอร์และตัวกลางเกี่ยวกับกลไกของสิ่งแวดล้อม Cc ในสมการ (1) เป็นความเข้มข้นของการถูกปลดปล่อยของวัตถุมีพิษในสิ่งแวดล้อม สัมประสิทธิ์ความสามารถในการละลายและการ แบ่งส่วนอาจเป็นการนำไปสู่การวิเคราะห์ทำนายในเทอมของประยุกต์ใช้ทฤษฎีทางเทอร์โมไดนามิก การเปลี่ยนแปลงอยู่เสมอของการแทรกซึมโมเลกุลของวัตถุมีพิษขณะที่ การถูกประเมินโดยสัมประสิทธิ์การแพร่ผ่านเป็นตัวแปรทางจลศาสตร์ที่ถูกควบคุมโดยขนาด รูปร่าง และความมีขั้วของการแทรกซึม และโดยโครงสร้างของตัวกลางแพร่ผ่าน

มีการปรับปรุง ระบบ reservoir system โดยการทำนายกฎของ Fick ที่ว่าถ้าวัตถุมีพิษถูกล้อมรอบภายในเยื่อผนังและถ้าความเข้มข้นของวัตถุมีพิษที่เหลืออยู่ภายในคงที่ จากนั้นสภาวะคงที่จะถูกสร้างขึ้นระหว่างอัตราการปลดปล่อยที่จะเป็นอันดับศูนย์ เช่น ค่าคงที่เป็นต้นแบบต่างๆ ของกฎของ Fick เกี่ยวกับการปรับปรุง reservoir system สำหรับกลไกที่เป็นแบบแผ่นหรือแคปซูลขนาดเล็ก

3. สรุป

สารกำจัดแมลงและศัตรูพืชมีมากมายหลายชนิดแต่ละชนิดมีผลต่อแมลงศัตรูพืชต่างกันออกไป แต่ส่วนใหญ่มักพบว่า การควบคุมประสิทธิภาพการออกฤทธิ์ของสารกำจัดแมลงศัตรูพืชไม่ได้เป็นไปตามที่คาดหวัง จึงมักเพิ่มปริมาณการใช้สารเคมีมากขึ้นเพื่อให้ได้ประสิทธิภาพตามต้องการสาเหตุหลักเนื่องจากความไม่เสถียรภาพของสารกำจัดแมลงศัตรูพืช ซึ่งจะทำให้ประสิทธิภาพของสารกำจัดแมลงศัตรูพืชที่ใช้จริงในแปลงเกษตรต่ำกว่าเมื่อมีการทดสอบในห้องปฏิบัติการ ปัจจัยต่างๆ ที่เกี่ยวข้องกับการสลายตัวของสารกำจัดแมลงศัตรูพืช มักเกิดจากแสงแดดความร้อน ความชื้น ตลอดจนการชะล้างและการพัดพาไปของฝนและลม ซึ่งก่อให้เกิดมลภาวะที่เป็นพิษในสิ่งแวดล้อม การศึกษาการเตรียม แคปซูลของ Azadiracthin-A โดยวิธี โฟมจากยางธรรมชาติเพื่อไปใช้งานเกษตรกรรม จึงเป็นแนวทางหนึ่งที่ถูกนำมาใช้ในการลดปริมาณการใช้สารกำจัดแมลงโดยควบคุมอัตราการปลดปล่อยของสารออกฤทธิ์ให้เป็นไปตามต้องการเพื่อลดความเป็นพิษต่อคนและสัตว์เลี้องลูกด้วยน้ำนม ป้องกันสารกำจัดศัตรูพืชจากแสงแดด อันก่อให้เกิดการระเหยและการเสื่อมสภาพ ช่วยป้องกันสารกำจัดศัตรูพืชในสิ่งแวดล้อม

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Chapter 3

Encapsulation of neem *via* foam techniques

ABSRACT

The use of huge quantity of synthetic pesticides with the conventional agriculture leads to some important environmental problems. Thus, natural pesticide was applied for this purposed, but it is not stable under environment. The controlling release of natural liquid pesticide "neem (Azadiracthin A) seed oil hereafter designated as neem Aza-A, was achieved by utilization of foam. The neem Aza-A containing beads have been prepared by changing the experimental variables such as NR coating and foam type. The SEM data indicated that the structure of the walls the beads are smooth and nonporous. The efficiency of encapsulated neem Aza-A in polymer foam matrix obtained from poly (vinyl alcohol) was higer than the other polymers due to it hydrophilic polymer matrix. The swelling result indicates that swelling of the polymeric beads decreases with increasing NR coating layer. At particular intervals, the remaining concentration of neem Aza-A was analyzed by HPLC.

1. Introduction

The definition of encapsulation is a process in which thin films, generally of polymeric material is applied to little solid particles, liquid or gases droplets [1]. This method is used to trap active components and release them under controlled conditions. The reactive agents have been encapsulated in the agriculture industry, fertilizer and pesticide. The techniques for preparation of capsule are spray dryer, coaservation, emulsion and interfacial polymerization [1]. The foams are the two major classes of cellular solids [2-6]. Two-dimensional cellular solids with regular array of cells are known as honeycombs. The cells of a honeycomb could be hexagonal, square, triangular, or any other shape. Foams, on the other hand are three-dimensional cellular solids, in which cells have random orientation. The foams are

further classified in two groups, i.e. open-cell and closed-cell . In the case of open-cell foams, the solid material is distributed as little beams, which form the cell struts, whereas, the closed-cell foams posses solid distributed as little plates which form the cell faces. The cellular structures can be fabricated out of metals, plastics, ceramics, glasses, etc for a wide variety of engineering applications, e.g. polymeric and glass foams for thermal insulation.

The improved granular formulation of neem seed extract containing neem Aza-A have enhanced storage stability, and the ability to gradually release neem Aza-A for application in the plant rhizosphere [7-8]. It was found that the best formulation contained inert particulate matter as a carrier, at least one lipophilic substance as a deactivator/binder, optional colorant and neem seed extract containing neem Aza-A. The invention also required the development of a method for the preparation of the formulation by coating the carrier with a lipophilic substance, subsequently impregnating the coated carrier with neem seed extract followed by an optional coating with a colorant and finally a lipophilic substance, by spraying and drying at a temperature below 50^{o}C.

T.M. Aminabbavi. and co-worker studied the encapsulation of a natural liquid pesticide using sodium alginate (Na-Alg) as a controlled release (CR) polymer after crosslinking with glutaraldehyde (GA) [10]. They found that the swelling of the polymeric beads decreased with increasing exposure time to the crosslinking agent. However, no significant variation in swelling was observed with different amounts of neem Aza-A loading. In addition, the rate of neem Aza-A from beads released was very fast. Thus, this work tries to apply the natural rubber coating on sodium alginate capsule. The sodium alginate Na–Alg has been used as a control release matrix material in medicine [17-19], membrane [20-22] and agriculture [10, 23-24] after crosslinking it with calcium chloride. Alginates polysaccharides are known to be haemocompatible and do not accumulate in any organs of the human body. It has been reported that glutaraldehyde (GA) solution and alginate can react together by coacervation due to the chemical reaction between hydroxyl groups of Na–Alg and GA

In the work presented here we test the feasibility of encapsulating neem Aza-A in a matrix made from foam. To the best of our knowledge, this is the first of its kind

of study wherein the effect of natural rubber coating capsule on release of neem Aza-A from modified capsules. The optimum condition and releasing of neem Aza-A from capsule were investigated

2. Experiment

2.1. Materials

Neem seed kernels were purchased from local Thailand; neem Aza-A extract was prepared according to the procedure as following. Neem seed kernels (5g) had their cortex removed then crushed into small pieces, deoiled by grinding in light petroleum (200 mL) and filtered. The grinding and filtering were repeated twice more. The deoiled neem seed powder was stirred in 200 mL of methanol for 2 h and filtered at room temperature. The meal was reextrated with two further portions of methanol. The combined methanol filtrates were concentrated to approximately 50 mL, the aqueous methanol solution was extracted three times with an equal volume of n-hexane (each was 50 mL) followed by 3×50 ml of dichloromethane (Fluka Company). The methanol-water layer was discarded and the dichlormethane layers were combined and dried over $MgSO_4$ (Fluka Company) and then evaporated to dryness. Two grams of the product were dissolved in eight mL of hexane during stirring. The liquid was separated into two layers using a separating funnel. The process was repeated by addition of a further 8 mL of ether. The methanol layer was evaporated and the residue was dissolved in 2 mL dichloromethane and then treated with 10 mL n-hexane and 10 mL ether, according to the above-mentioned process. The final yield of 65.0% Aza-A was 0.8 g from 1 kg of neem seeds. The sodium alginate, glutaraldehyde (25% w/v) solution and AR grade methanol samples were all purchased from Fluka agent, Thailand. Concentrated NR latex used in this study is high ammonia latex received from Jana company, Co., Ltd. (Songkla Thailand).

2.2. Methods

2.2.1. Preparation of capsule beads and efficiency of entrapment

Foam structures having the dimensions of (a) 0.5*0.5*0.5 cm^3, (b) 0.5*1.0*1.0 cm^3, (c) 0.5*1.0*2.0 cm^3 and (d) 0.5*2.0*2.0 cm with square cells were prepared. The prepared foams were added in neem containing Azadirachtin-A 7,000 ppm

solution. Then, the foam absorbed neem for 10 min at ambient temperature. The mixture foam was left from neem solution and then dried at 40^{o}C until weight of this sample with constant. The masses of the beads were taken at definite intervals of time until the constant mass was achieved. All the mass measurements were done on a Mettler single pan balance (Model AB 204, Mettler). In order to obtain reproducible results, experiments were conducted in triplicate, and the average values were used for the calculation and plotting of the data vs. time.

2.2.2Coating of capsule with NR

Concentrated NR latex used in this study is high ammonia latex received from Jana company, Co., Ltd. (Songkhla Thailand).

% TSC of latex is defined as the percentage by weight of the concentrated latex which is non-volatile at a definite temperature in an open atmosphere. The %TSC of concentrated NR latex in this study was determined by using method described in ASTM D107688 as shown in equation (1).

%TSC = (W/Wt)*100……(1)

Where, W = weight of dry NR sample (g)

Wt = weight of NR latex sample (g)

%DRC of latex is defined as the percentage by weight of the concentrated latex which is precipitated by acetic acid. The %DRC of concentrated NR latex was determined (equation 2) by using method described in ASTM D1076-88 .

%DRC = (Wx/Wt) *100……(2)

Where, W = weight of dry NR coagulum (g)

Wt = weight of NR latex sample (g)

The 5 g of dried natural rubber were dissolved in toluene (50 ml) in beaker (250 ml). The capsules (5 g) were dipped to the toluene solution and dried at room temperature. So, the dry capsule mixed with neem Aza-A (7000 ppm) were dipped into a toluene solution of natural rubber (5% w/w).Then, the coating capsules were dried at 30 ^{o}C for 24 h. Multiple coatings were prepared by the immersion of the single-coated neem capsules into a natural rubber with 30%DRC. Thereafter, the procedure was the same as during the preparation of single-coated neem capsules.

The third-coated neem capsules were derived by the dipping of double-coated neem capsules into a natural rubber with 30%DRC and then the same methodology as that given above mention. Fourth-coatings were prepared by the immersion of the third-coated neem capsules into a natural rubber with 60 DRC and dried at 60^{o}C until its weight was constant.

2.2.3. Bead size measurement

Five samples of the completely dried beads from different formulations were selected and their sizes were measured by using a micrometer screw gauge (Sargent, USA) with an accuracy of ±0.01 mm.

2.2.4. Swelling study of the individual beads

Swelling property of the beads was subjected to a measurement of swelling ratio in aqueous medium as a function of time. The bead samples exposed to GA at different time at 26±2^{O}C were selected and incubated with distilled water in a watch glass. The mass of all bead samples was taken at different interval period times and the average value was calculated. During this process, care should be exercised while it was handed of the swollen beads so as to avoid any weight loss due to breaking or erosion of the beads. All the mass measurements of the swollen beads were taken on a Mettler single pan balance and having accuracy up to fifth decimal. The percentage swelling ratio of bead was calculated as in equation 3.

$$\%\text{Swelling ratio} = \frac{(\text{Wet weight-dry weight})*100}{\text{dry weight}} \quad \ldots\ldots (3)$$

2.2.5. Scanning electron microscope (SEM) and Confocal scanning laser microscopy

The aim of SEM study is to obtain a topographical characterization of beads. The sample was deposited on brass hold and sputtered with gold. SEM photographs were taken with JSM 6400 Scanning Microscope (Japan) at the required magnification at room temperature. Confocal scanning laser microscopy photographs of this capsule were studied.

2.2.6 Releasing study

Experiments were performed in triplicate in order to minimize the variation error. The cumulatively release of neem Aza-A from capsule beads was estimated from.

The release results were investigated by using an empirical equation to estimate the value of n as follows (equation 4)

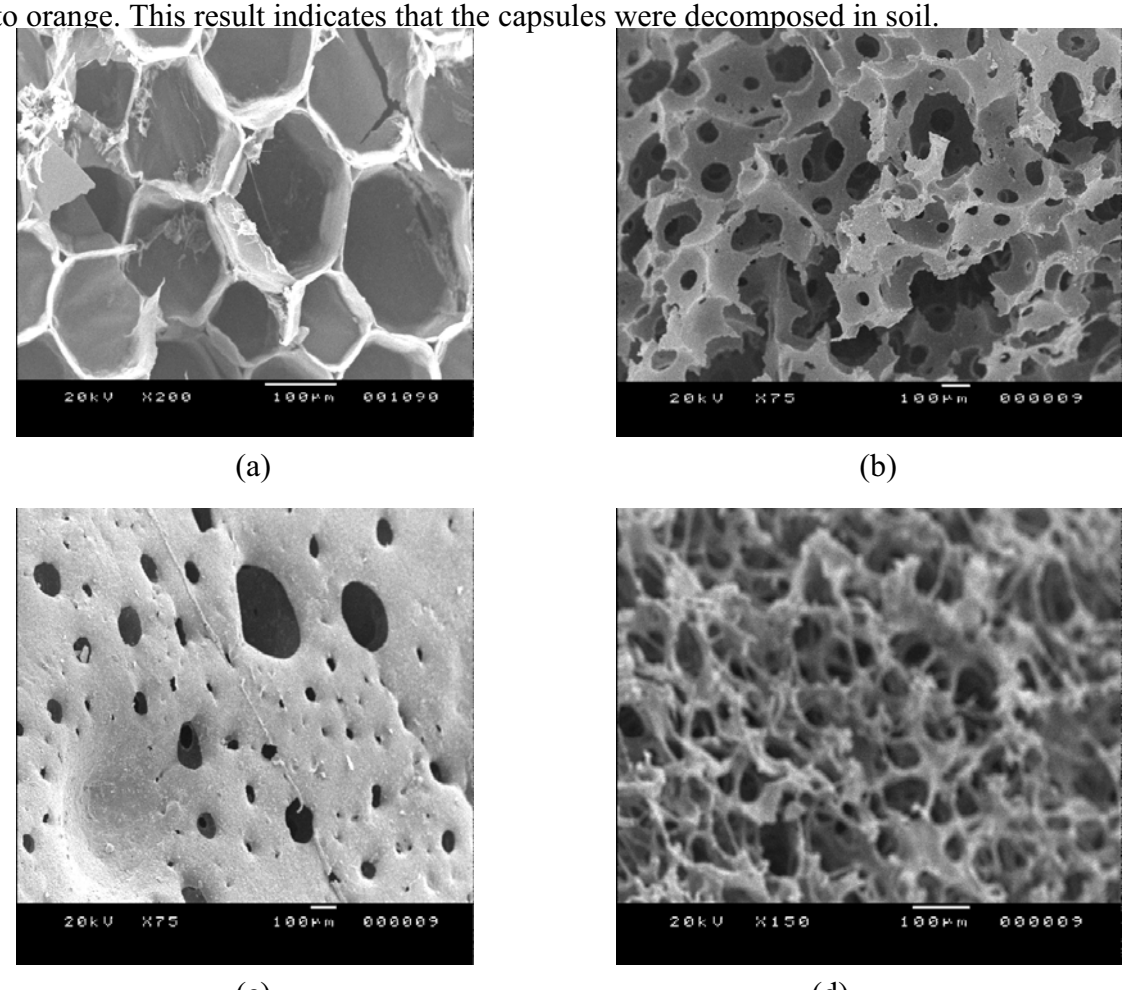
$$M_t/M_\infty = Kt^n \text{ or } \log (M_t/M_\infty) = \log (K) + n\log(t) \quad \text{.....(4)}$$

Where M_t/M_∞ is the released fraction at time t, n is the release exponent, and K is the release factor. From the slope and intercept of the plot of log (M_t/M_∞) against log (t), kinetic parameters n was calculated.

3. Results and discussion

3.1 Morphology of capsule obtained from different types

Figure 1 represents the SEM micrographs of capsules from different types. It was found that the pore size of capsule obtained from surgar cane was the lagest comparing to other samples. The average pore size of capsule obtained from sugar cane foam, natural rubber foam, polyurethane and poly (vinyl alcohol) foam was 150, 90, 70 and 45 micron, respectively. The capsule obtained from surgar cane was buried in soil for 1 month. The color of capsule changed from yellow into orange as shown in Figure 2. Figure1 represents the SEM micrographs of capsules from different types. It was found that the pore size of capsule obtained from surgar cane was the lagest comparing to other samples. The average pore size of capsule obtained from sugar cane foam, natural rubber foam, polyurethane and poly (vinyl alcohol) foam was 150, 90, 70 and 45 micron, respectively. The capsule obtained from surgar cane was buried in soil for 1 month. The appearance of capsule changed from white into orange. This result indicates that the capsules were decomposed in soil.



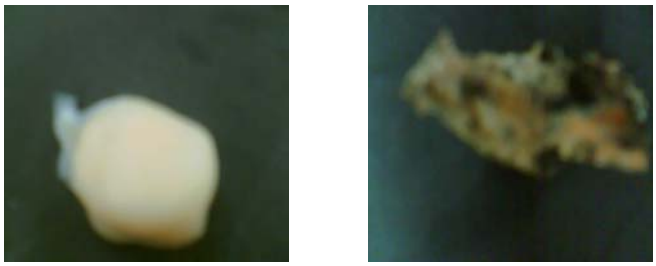
(a) (b)

(c) (d)

Figure 1 SEM micrographs of capsules obtained from (a) sugar cane foam (b) natural rubber foam (c) polyurethane and (d) poly(vinyl alcohol) foam

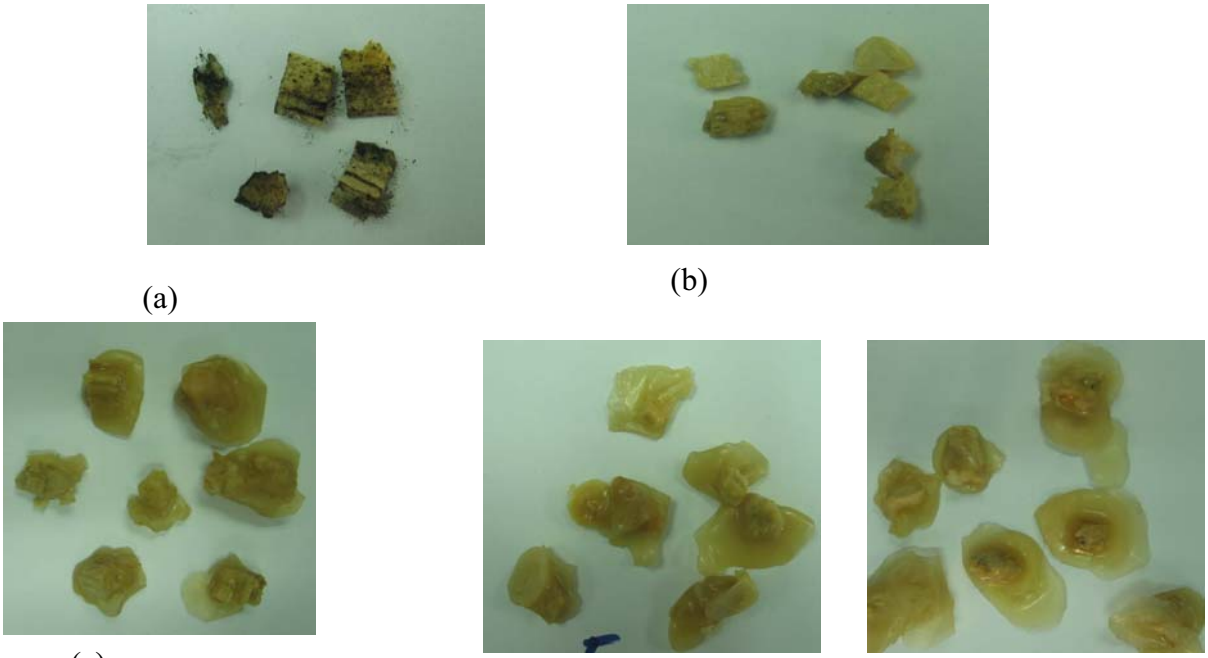
Figure 1 represents the SEM micrographs of capsules from different types. It was found that the pore size of capsule obtained from sugar cane was the largest comparing to other samples. The average pore size of capsule obtained from sugar

cane foam, natural rubber foam, polyurethane and poly (vinyl alcohol) foam was 150, 90, 70 and 45 micron, respectively. The capsule obtained from sugar cane was buried in soil for 1 month. The color of capsule changed from white into the yellow.



(a) (b)

Figure 2 Optical microscope of capsule obtained from surgar cane (a) before bury in soil and (b) bury in soil for 2 months

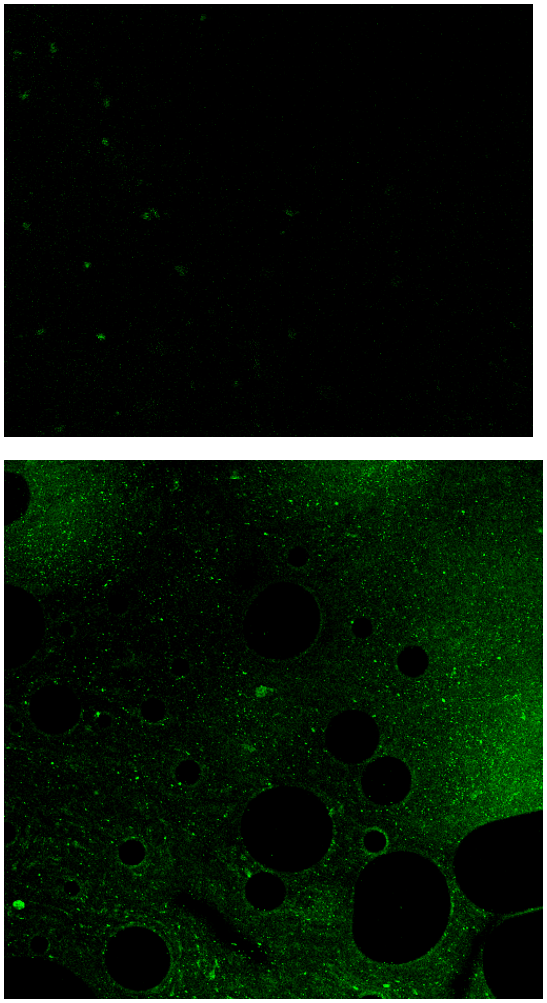


(a) (b)

(c) (d) (e)

Figure 3 The Optical microscopy of sugar cane capsule coated with (a) 0 (b) 1 layer (c) 2 layer (d) 3 layers and (e) 4 layers

Figure 3 shows the optical microscopy of sugar cane capsule coated with different NR layer coating. The pore of sugar capsule was coated NR coating. The diameter of capsule increased with NR coating layer.



(a)

(b)

Figure 4 Confocal scanning laser microscopy of (a) NR sheet and (b) neem dispersed NR matrix

Confocal laser scanning microscopy using activity probes reveals electron transport processes typical of neem as shown in **Figure 4.** The result shows the neem homogeneously dispersed in NR matrix.

The cyclized NR having 90% degree of cyclization was applied to help the reduction in tackiness of capsule.

3.2 Swelling ratio of capsule study

The swelling of any capsule in a solvent depends upon the diffusion coefficient of the solvent, the relaxation rate of the amorphous regions of the polymer chain and its NR coating layer. **Figure 5** depicts the swelling ratio of neem Aza-A capsules prepared from different NR coating layer in a water medium and storage time over

400 h. It is clear that the swelling ratio of capsules obtained from 1 NR coating was lower than that capsule NR coating 3 or 5 layer.

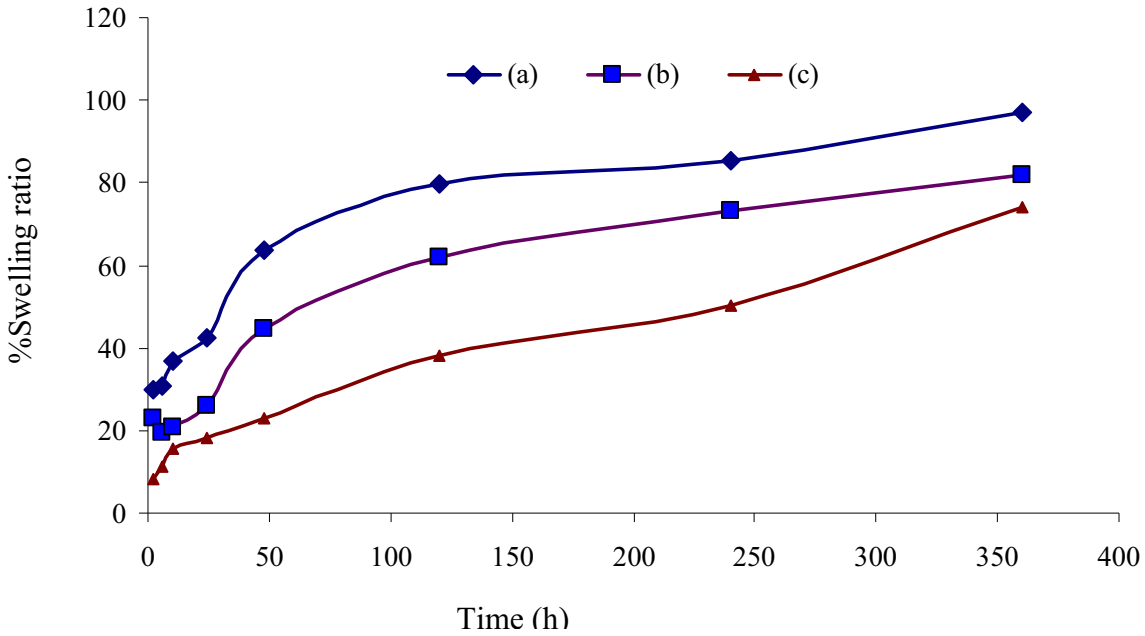


Figure 5 Swelling ratio of surgar cane capsule coated with (a) 1, (b) 3, and (c)5 NR coating layer having 50% DRC

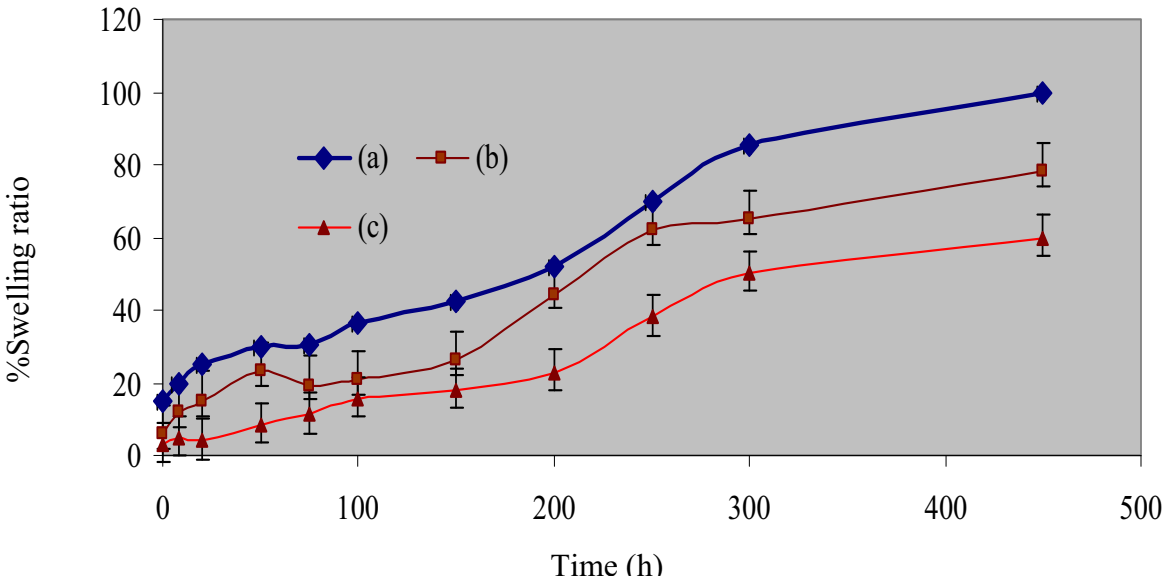


Figure 6 Swelling ratio of NR foam capsule coated with (a) 1, (b) 3, and (c)5 NR coating layer having 50% DRC

The swelling ratio of NR foam capsule coated with NR latex is shown in Figure 3.6. It was found that the swelling ratio of capsule was proportional to time and NR coating layer. The beads obtained from capsule with 1 NR coating 1 layer show a maximum uptake of water during the 200 hour. The beads derived from

3 NR coating layers and 5 NR coating layers absorbed lower water than the beads obtained from 1 NR coating layer due to more wall thickness. Equilibrium swelling ratio was achieved in 350 h for 1 NR coating layer.

3.3 Releasing study

Figure 7 shows the calibration curve of Azadirachtin-A observed from HPLC and the regression of this system was 0.9993.

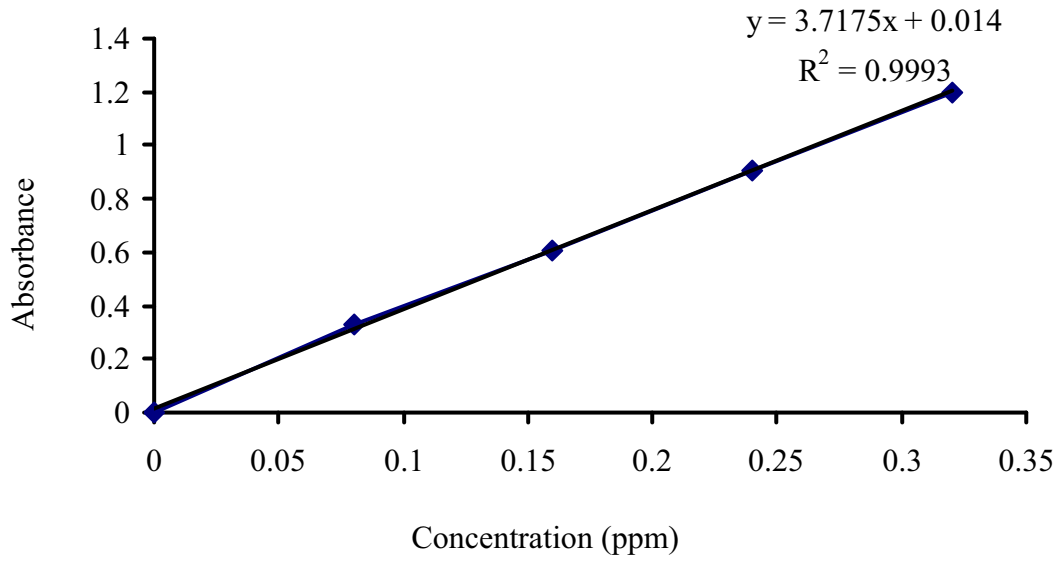


Figure 7 The Calibration curve of neem Azadirachtin-A observed from HPLC

Figure 8 represents the DRC on %cumulative neem release from NR foam capsule with dimension of 0.5*1.0*2.0 cm^3 coated with 2 NR coating layer. It was found that capsule, the %cumulative neem release from NR foam capsule decreased with increasing %DRC NR coating. The release rate of neem Aza-A from microcapsules obtained from 10%, 20%, 30% was high during the first 10, 15, 25 h, respectively followed by a slow release. Release of neem Aza-A from the 10%, 20%, 30% DRC NR foam capsules was found to be almost complete within about 15, 20, 100 h, respectively. This result indicates that higher DRC NR coating on capsule leaded to decreasing cumulative of neem from NR foam. In the case of capsules obtained from 50 and 60% NR DRC the release rate of neem Aza-A from the capsules was high during the first 180 h and 220 h, respectively followed by a slow release.

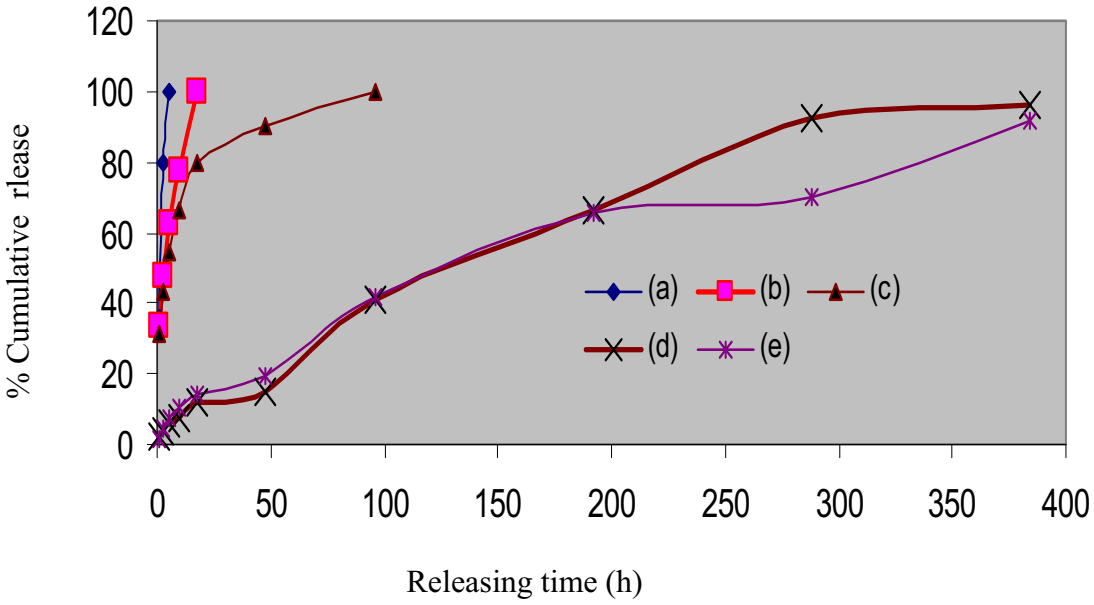


Figure 8 The effect of DRC on %cumulative neem release from NR foam capsule with dimension of 0.5*1.0*2.0 cm^3 coated with 2 NR coating layer with (a)10, (b) 20, (c) 30, (d) 50 and (e) 60 in aqueous medium

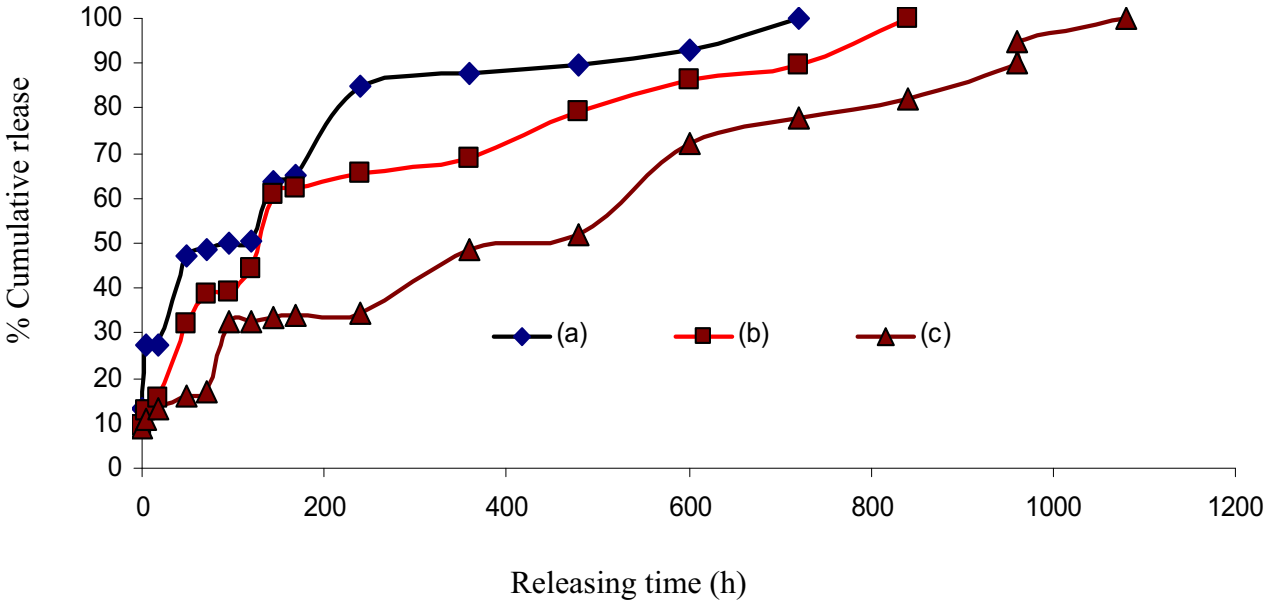


Figure 9 The effect of NR layer coating on %cumulative neem release from NR foam capsule with dimension of 0.5*1.0*2.0 cm^3 coated with with (a) 1, (b) 3, (c) 5, NR coating layer in aqueous medium

Figure 9 shows the effect of NR layer coating on % cumulative neem release from NR foam capsule coated NR layered. It is clear that the % cumulative neem release from NR foam capsule increased as a function of releasing time and NR coating layers.

The natural rubber film is very strong, rigid and hard to swell, so the diffusion through this coating is the rate limiting step for swelling and neem-A release. The release was prolonged by additional natural rubber layers on the capsule surface. The neem Aza-A cumulatively release of capsule derived from 200, 400 and 500 in aqueous medium was 82, 85 and 95%, respectively and when NR coated on capsule increase from 1 to be 3 layers, the neem Aza-A cumulatively release of capsule stored in at the same condition was 55, 68 and 82 %, respectively. It is to be noted that with an increase in NR coating, the capsule matrix becomes more dense resulting in a decrease in the rate of diffusion of neem Aza-A through the swollen beads, especially beads with fourth- NR coating.

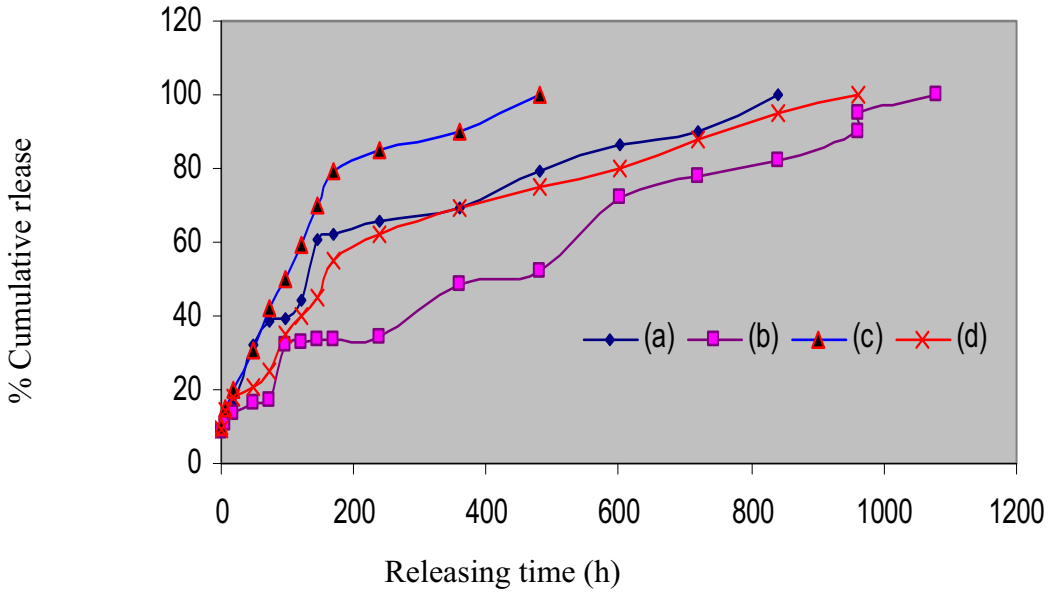


Figure 10 The effect of NR layer coating and cyclized NR on %cumulative neem release from NR foam capsule with dimension of 0.5*1.0*2.0 cm^3 coated with without cyclized (a)3, (b) 5 and with cyclized NR (c) 3 and 5 NR coating layer in aqueous medium

Figure 10 represents the effect of NR layer coating and cyclized NR on %cumulative neem release from NR foam capsule with dimension of 0.5*1.0*2.0 cm^3 coated with NR layered. It was found that the %cumulative neem release from NR foam capsule in presence of cyclized NR was lower than that sample without cyclized NR due to low adhesion force between foam wall and polymer coating.

The neem Aza-A cumulatively release of capsule coating 3 NR layer containing cyclized NR derived from 200, 400 and 500 in aqueous medium was 82, 90 and 100%, respectively and when NR coated on capsule without cyclized NR, the neem Aza-A cumulatively release of capsule stored in at the same condition was 55, 68 and 82 %, respectively. Results of a study of the effect of NR foam size on its rate of release in distilled water from capsules with (a) 0.5*0.5*0.5 cm, (b) 0.5*1.0*1.0 cm, (c) 0.5*1.0*2.0 cm and 0.5*2.0*2.0 cm is shown in **Figure11**. It is clear that the release rate of neem Aza-A from the microcapsules was proportional to the release time. The release rate of neem Aza-A from microcapsules obtained from 0.5*1.0*1.0 was high during the first 50 h followed by a slow release. Release of neem Aza-A from the 0.5*1.0*1.0 NR capsules was found to be almost complete within about 200 h. This result indicates that high amounts of neem Aza-A was present on the surface of the capsules. In the case of capsules obtained from 0.5*1.0*2.0 and 0.5*2.0*2.0 the release rate of neem Aza-A from the microcapsules was high during the first 200 h and 300 h, respectively followed by a slow release. Finally, release of neem Aza-A from the capsules obtained from 0.5*1.0*2.0 and 0.5*2.0*2.0 was found to be almost complete within about > 600 and >800 h, respectively. This result indicates that neem Aza-A was entrapped in the NR foam. This could be explained by the amount of neem Aza-A released being dependent on its hydrophilicity and crosslinking density in the polymer matrix.

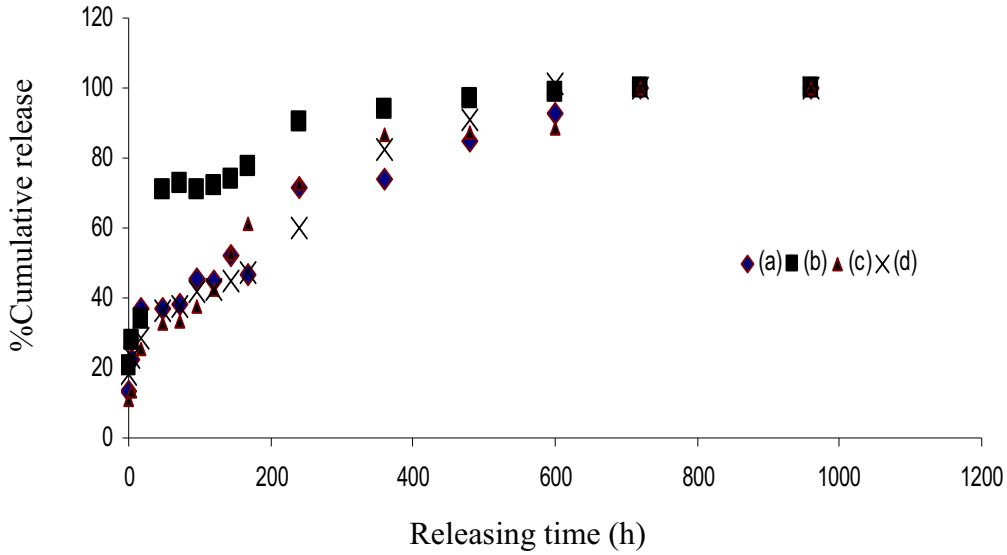


Figure 11 The effect of capsule size on %cumulative neem release from natural rubber foam capsule in aqueous medium (a) 0.5*0.5*0.5 cm, (b) 0.5*1.0*1.0 cm, (c) 0.5*1.0*2.0 cm and 0.5*2.0*2.0 cm coated with 3 NR coating layers

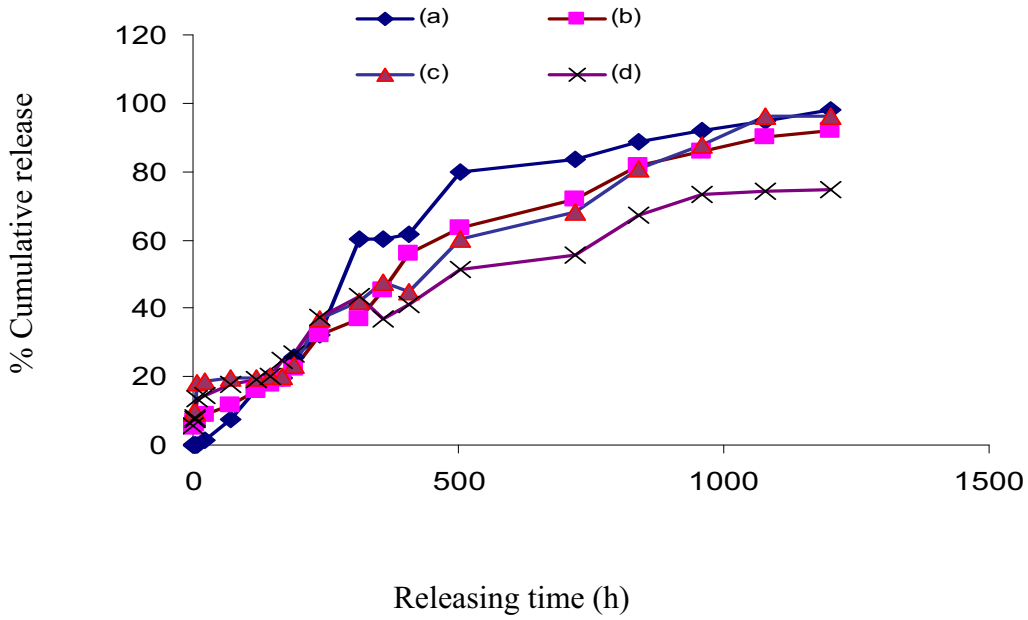


Figure 12 The effect of capsule size on %cumulative neem release from poly (vinyl alcohol) foam capsule in aqueous medium (a) 0.5*0.5*0.5 cm^3 , (b) 0.5*1.0*1.0 cm^3, (c) 0.5*1.0*2.0 cm^3 and 0.5*2.0*2.0 cm^3 coated with 3 NR layered

The effect of the particle size of the capsule obtained from poly (vinyl alcohol) on releasing neem Aza-A from the capsule in aqueous medium is depicted in **Figure 12.** It is clear that the % cumulative neem Aza-A of the capsule with 0.5*0.5*0.5 cm^3 diameter was higher than that of the other sample due to the higher surface area, leading to a greater amount of neem Aza-A diffusing in the aqueous phase. Release of neem Aza-A from the 0.5*1.0*1.0 NR capsules was found to be almost complete within about 1,200 h. In the case of capsules obtained from 0.5*1.0*2.0 and 0.5*2.0*2.0 cm the release rate of neem Aza-A from the microcapsules was high during the first 800 h and 1,200 h. followed by a slow release. The neem release rate of capsule obtained from PVA foam was lower than that of capsule derived from NR foam due to smaller pore size and higher polar between polymer matrix and neem Aza-A.

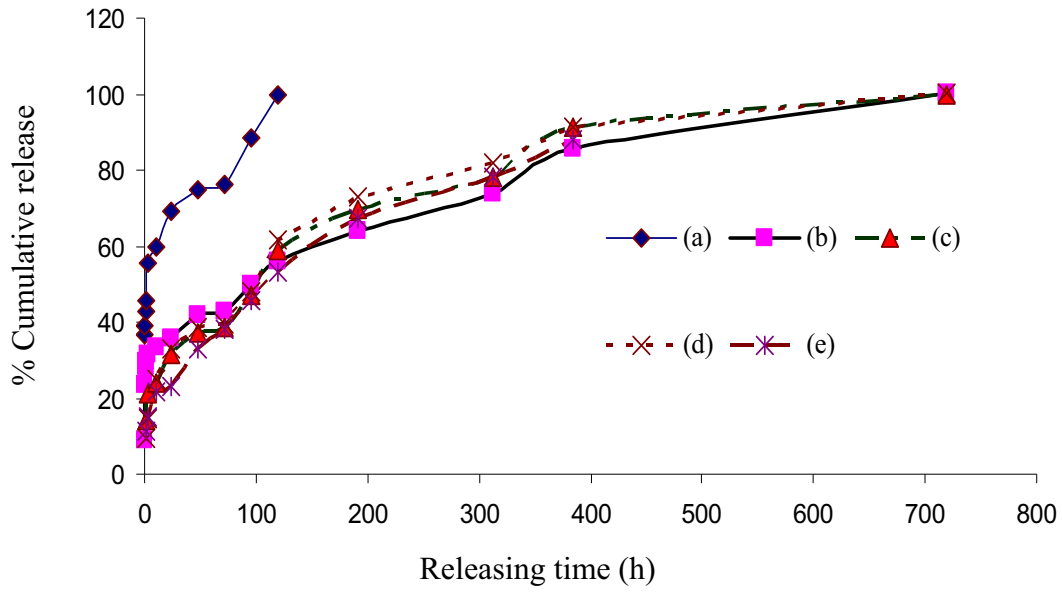


Figure 13 The effect of capsule size on %cumulative neem release from poly (vinyl alcohol) foam capsule in aqueous medium (a) 0.5*0.5*0.5 cm^3 , (b) 0.5*1.0*1.0 cm^3, (c) 0.5*1.0*2.0 cm^3 and (d) 0.5*2.0*2.0 cm^3 and (e) 1*2.0*2.0 cm^3 coated with 3 NR layered

Figure 13 represents the effect of capsule size on %cumulative neem release from poly (vinyl alcohol) foam capsule in aqueous medium (a) 0.5*0.5*0.5 cm^3 , (b)

0.5*1.0*1.0 cm^3, (c) 0.5*1.0*2.0 cm^3 and (d) 0.5*2.0*2.0 cm^3 and (e) 1*2.0*2.0 cm^3 coated with 3 NR layered

Aza-A release from the beads were subjected to a number of physical and chemical parameters including those related directly to the release medium, the release conditions (temperature) and those resulting from change in the characteristics of the controlling release device (beads). The effect of degree of capsule size on the kinetics of Aza-A release is depicted in **Figure 13. I**t is found that the higher the particle size the higher the release rate. The release rate of Aza-A beads at dimension of 0.5*0.5*0.5 cm^3 have shown 100% release in the 120 hour, whereas the Aza-loaded beads with dimension of 0.5*1.0*1.0 cm^3 have shown 100% at 700 hour.

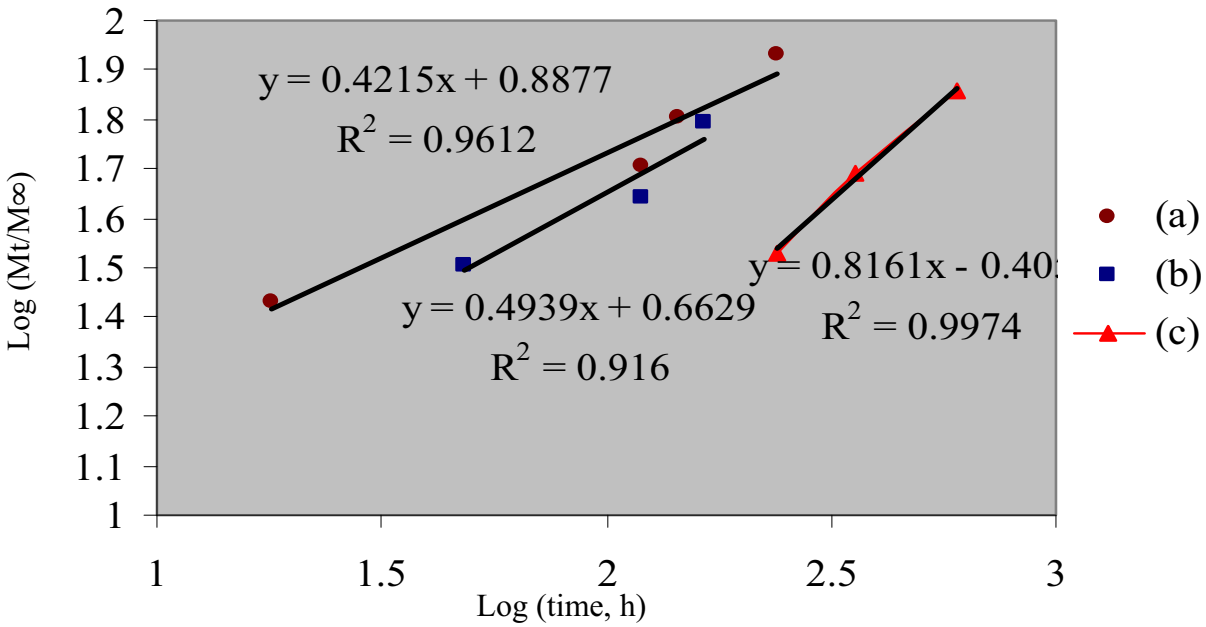


Figure 14 Fitting of release kinetics of neem Aza-A from NR foam capsule with coated natural rubber coated NR at (a) 1, (b) 3 and (c) 5 layere by the power law

The n value of neem Aza-A coated with NR is represented in **Figure 14**. It was found that the n value of this sample obtained from1, 3 and 5 layers was 0.4211, 0.4939 and 0.8161, respectively at regression of 0.9612, 0.916 and 0.9974,respectively.Thus, the neem Aza-A release mechanism of beaded coated with NR was Fickian diffusion.

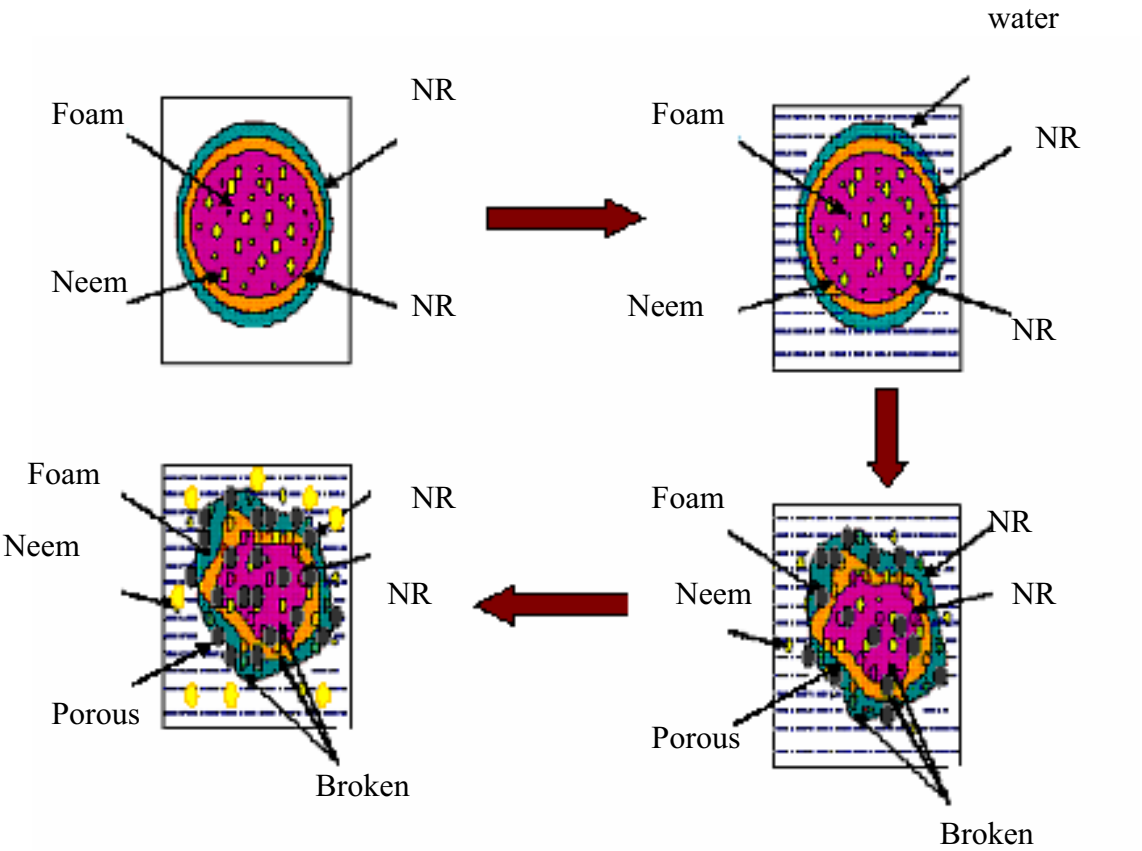


Figure 15 Possible model of neem release from NR foam in water

The release of the neem Aza-A from the polymer matrix has been schematically presented in **Figure 15.** At the same time, there are cases where the polymer matrix exhibits swelling with no significant limitations. The variety of factors affecting the rate of the diffusion transfer of a solvent, including (a) the polymer transition from glassy to rubberlike state; (b) relaxation transitions on the surface and in the bulk of a sample; (c) dependence of the diffusion mobility of water on its concentration in the polymer;(d) expansion of the sample, reaching several tens or even a few hundreds percentages with respect to the initial dimensions, requires development of a complicated multiparametric model of the water transport in polymer. The difference of osmotic pressure between inside and outside of capsule increased leads to destroy the wall polymer of capsule.

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4.Conclusion

The successfully prepared the capsule for the controlling release of natural liquid pesticide “neem (Azadiracthin A) seed oil was applied by utilization of foam. The degree for controlling release of the neem Aza-A containing beads depended on changing the experimental variables such as NR coating and foam type. The structure of the walls the beads are smooth and nonporous observed by SEM. The efficiency of encapsulated neem Aza-A in polymer foam matrix obtained from poly(vinyl alcohol) was higer than the other polymers due to it hydrophilic polymer matrix. The swelling result indicates that swelling of the polymeric beads decreases with increasing NR coating layer.

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Chapter 4

Encapsulated neem extract containing Azadiractin-A *within* hydrolyzed poly(vinyl acetate) for controlling its release and photodegradation stability

ABSTRACT

Neem extract containing Azadiractin-A (neem Aza-A), encased in microcapsules, in a matrix of partially hydrolyzed poly (vinyl acetate) crosslinked with glutaralaldehyde 5% w/v and 0.05 % hydrochloric acid was prepared via a spray drying technique. The photostabilization of unencapsulated and encapsulated neem Aza-A when exposed to ultraviolet light was evaluated. Neem Aza-A solutions and neem Aza-A microcapsules were applied onto the surface of glass slides. At particular intervals, the remaining concentration of neem Aza-A was measured by HPLC. When the ratio of 87% hydrolyzed polyvinyl acetate to water was 1:40 this produced the highest concentration of neem Aza-A after exposure to UV. The degree of swelling ratio of microcapsule depended on the crosslinking density and the crystalline content. The neem Aza-A release was measured in water at 25 °C and the effects of different matrices and other parameters such as polymer-water ratios and particle size were studied. The release mechanism of the neem Aza-A from the microspheres was also investigated.

Key words**: encapsulation; neem; poly (vinyl acetate); controlled release**, **poly (vinyl alcohol)**

1. Introduction. The use of conventional broad-spectrum synthetic insecticides is in decline due to public concern and regulatory demands for the use of selective and environmentally benign pest control products. Consequently, in recent years, research has been increasingly focused on the development of natural insecticides originating from plants, because it is believed that they are innocuous. Currently, some attention is being given to the use of neem-based botanical insecticides [1-2]. Neem (Azadiractin indica A.Juss.) is a tree belonging to the Meliaceae family and is widely distributed in South Asia, South-East Asia, and some other tropical areas [1-2]. The

major insecticide from neem seed kernels is the tetranortriterpinoid, Azadiractin-A (Aza-A). Aza-A, is a powerful deterrent to insect feeding and a growth-regulating substance, that shows considerable promise as an insecticide [3]. It can suppress at least 200 species of insect pests belonging to different orders, associated with agriculture and storage, causes negligible hazard to nontarget organisms including humans but it has a short environmental persistence [2]. Its short environmental persistence is due to the presence of sensitive moieties such as p-electrons, ester linkages, and an epoxide ring [3]. However, the major problem is its sensitivity to photodegradation so it is rapidly lost in sunlight. This limits its use in agriculture because an insecticide should persist long enough to cause the death of the insect pest. Many researchers have attempted to stabilize Aza-A. Microencapsulation has been used to try to solve this problem. Microencapsulation encloses the sensitive ingredients within a coating or wall material [4]. The wall material protects the sensitive ingredient (or core) against adverse reactions, prevents the loss of volatile ingredients, and can control the rate of release of the ingredient. T.Wei-Hong and co-worker [4] reported that mixing Aza-A with UV light absorbers can enhance its photostability. The addition of ferulic acid, gallic acid, and rutin provided a moderate degree of photostabilization of Aza-A. In addition, numerous investigators have concentrated on the encapsulation of neem into urea formaldehyde crosslinked starch (UF-St), guar gum (UF-GG) and UF-(St + GG) [5-6], lipophilic substances [7], and sodium alginate (Na-Alg)[8]. In this work, neem had been encapsulated in partially hydrolyzed poly(vinyl acetate) (or poly(vinyl alcohol)) by spray drying. Here, we report on the effect of glutaraldehyde crosslinked poly(vinyl alcohol) type with 0, 40 and 87% hydrolysed poly(vinyl acetate) on the efficiency of encapsulating neem Aza-A. This is the first report on the quality of capsules of neem Aza A encapsulated in glutaraldehyde crosslinked poly(vinyl alcohol). In addition, this is the first report of the photodegradation of neem Aza A in capsules obtained using different %age hydrolysed poly(vinyl acetate) types. Poly (vinyl alcohol) has been used as a polymer matrix for encapsulation of the reactive agents [9-12] because it is a biodegradable polymer and cheap to make. Spray drying is used for producing pharmaceutical powders for inhalation, etc [13-19]. The production method has a major effect on the physical properties of the capsules such as flowability, hygroscopicity and dissolution. Variations in the production methods cause variations of the physical state (amorphous versus crystalline), particle size and composition at

the particle surface. In addition, the biological activity of compounds enclosed in the particles is influenced by the drying process. In some particular cases of spray drying, several impose stresses that can destabilize proteins and peptides, such as high pressure, high shear and immense air–liquid interfaces during atomisation, heating and dehydration [16]. In the process, the sensitive ingredient is mixed or homogenized in a solution containing wall material in which it forms a stable emulsion. The emulsion is then fed into a spray dryer where it is converted to a dried particle. However, spray drying provides the possibility of creating particles with the active protein in one step, which are then suitable for inhalation [16]. To the best of our knowledge, this study is the first of its kind in which the partially hydrolysed poly(vinyl acetate) beads containing neem Aza-A are prepared by the spray drying technique. In the work presented here we test the feasibility of encapsulating neem Aza-A in a matrix made from poly(vinyl alcohol) to produce a product with good end-use properties. The efficiency of encapsulating neem Aza-A were also studied. The photodegradation and release of neem Aza-A in the capsules was also evaluated.

2. Materials and methods

2.1 Materials

Neem seed kernels were purchased locally in Thailand; Aza-A extract was prepared according to the procedure given in section 2.2 below. Polymers used in this experiment were, 1) poly (vinyl alcohol) (PVA) (Fluka) with an 87% hydrolyzed poly(vinyl acetate), 2) poly (vinyl alcohol) (PVA) (Fluka) with a 40% hydrolyzed poly(vinyl acetate) all prepared in our laboratory 3) poly(vinyl acetate) (Aldrich). Water was prepared with a Milli-Q Plus water purification system (Millipore). Methanol and glutaraldehyde was purchased from Fluka Company. All other solvents and chemicals were of analytical grade.

2.2Preparation of neem Aza-A solution

Neem seed kernels (5g) had their cortex removed then crushed into small pieces, deoiled by grinding in light petroleum (200 mL) and filtered. The grinding and filtering were repeated twice more. The deoiled neem seed powder was stirred in 200 mL of methanol for 2 h and filtered at room temperature. The meal was reextrated

with two further portions of methanol. The combined methanol filtrates were concentrated to approximately 50 mL, the aqueous methanol solution was extracted three times with an equal volume of n-hexane (each was 50 mL) followed by 3×50 ml of dichloromethane (Fluka Company). The methanol-water layer was discarded and the dichlormethane layers were combined and dried over $MgSO_4$ (Fluka Company) and then evaporated to dryness. Two grams of the product were dissolved in eight mL of hexane during stirring. The liquid was separated into two layers using a separating funnel. The process was repeated by addition of a further 8 mL of ether. The methanol layer was evaporated and the residue was dissolved in 2 mL dichloromethane and then treated with 10 mL n-hexane and 10 mL ether, according to the above-mentioned process. The final yield of 65.0% Aza-A was 0.8 g from 1 kg of neem seeds.

2.3Capsules preparation

The ratios of polymer with 0, 40% and 87% hydrolyzed poly(vinyl acetate) to distilled water containing glutaraldehyde 5% w/v and 0.1 % hydrochloric acid, are shown in **Table 1**, were prepared for the encapsulation of the neem Aza-A product. Suitable amounts of the neem Aza-A product in solution were added to the polymer solutions in water to obtain mixtures of the neem Aza-A solution: polymer in the proportion of 10:5 (w/w). Microcapsules were obtained by spraying the solutions through a mini Buchi-191spray dryer equipped with a 0.7 mm nozzle at 206 kPa. The microparticles were collected and stored under vacuum at room temperature for 48 h.

2.4 Measurement of diameter

The diameters of beads were measured using an optical microscope (OM, and scanning electron microscope (SEM, (JMS-5800 LV, JEOL). The capsule particles were collected at the outlet of the channel without being assembled. Average diameters were calculated over samples of at least 40 bead particles.

2.5 Swelling study

Capsule samples were weighed and immersed in Millipore water for a period of over 35 h at 32 ºC. The samples were then dried in an oven at 50 ºC for 24 h and weighed

until a constant weight was achieved. The degree of swelling ratio was estimated from this equation (1)

$$\text{Swelling ratio} = (W_2-W_1)/ W_1 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where W_1 = the original weight of the sample

W_2= the weight of swollen sample

This experiment was repeated three times.

2.6 Irradiation experiments

Solutions of neem Aza-A extract in methanol were applied to the surface of glass slides using a pipette and the methanol was evaporated at room temperature, leaving the slide with a thin layer of neem Aza-A. These slides were exposed to UV light under a UV lamp (UV B CLEO 15W, T.S.T. Supplies & Trading Co., LTD) (254 nm, at a distance of 10cm). At intervals, two slides were removed and rinsed with methanol (total 2 mL) and then analyzed for neem Aza-A using HPLC (Bio-Rad Laboratories). The total period of the test was 44 h. The irradiation experiment was repeated three times.

2.7 Recovery of neem Aza-A

Solutions (50 μL, containing 206 μg of neem Aza-A extract without UV light absorbers in methanol were applied onto the surface of glass slides. After the methanol was evaporated, slides were rinsed with 2 mL of methanol. The residual neem Aza-A was estimated by HPLC (Bio-Rad Laboratories) and this experiment was repeated three times.

2.8 The encapsulation yield (EY)

The EY was calculated as the ratio of the mass of the microcapsules obtained at the end of the process and the mass of the initial substances added including neem Aza-A.

2.9 Encapsulation efficiency (EE)

The EE was calculated as the ratio between the initial mass of neem Aza-A used for encapsulation and its mass in the final product. About 20 mg of exactly

weighed microcapsule sample was extracted in distilled water to form a homogeneous solution. The total neem Aza-A in the solution was extracted for 48 h with a 50/50 MeOH/H_2O mixture and its mass was determined by HPLC (Bio-Rad Laboratories).

2.10 Release of neem Aza-A from capsules

Approximately 8 % of the capsule mass containing the neem Aza-A product were used. The release study was performed in distilled water. An 8 mg sample of the capsule was dispersed in 500 mL of the release water medium at 25 °C. The supernatant was collected after certain time intervals to determine the amount of the neem Aza-A product released, as determined by HPLC (Bio-Rad Laboratories). This experiment was repeated three times.

2.11 Infrared spectroscopy (IR) and X-ray diffraction

IR was performed on the hydrolyzed poly(vinyl acetate) with 0, 40% and 87% hydrolyzed polyvinyl acetate, in the range of 400-4000 cm^{-1}, using KBr pellets in a Shimadzu FTIR-8300 spectrometer.

The crystallinity of the polymer matrix was observed by X-ray diffractometry performed on a X' Pert MPD, Philips X-ray diffractometer under the following conditions: Nickel filtered Cu Kα radiation (λ=0.15406 nm) at a current of 25 mA and a voltage of 35 kV. The scanning rate was 4°/min in the angle range of 8-80° (2θ).

3. Results and discussion

3.1 FTIR and XRD study

Results of the FITR analysis of the 0, 40%, 87% hydrolyzed poly(vinyl acetate) are shown in **Figure 1.** The broad OH peak of the 40% hydrolyzed poly(vinyl acetate) was small compared to that of the 87% hydrolyzed poly(vinyl acetate), and the intensity of the carbonyl peak was much higher and comparable to that in the non hydrolyzed poly(vinyl acetate). Thus, the higher intensity peaks in the 40% hydrolyzed poly(vinyl acetate) were comparable to the non hydrolyzed poly(vinyl acetate), while the low intensity peaks are comparable to those in the 87% hydrolyzed poly(vinyl acetate).

The diffraction pattern of capsules having 0% hydrolyzed polyvinyl acetate (Figure 2) shows two peaks, one of high intensity at 19.8^{o} which corresponds to (110) reflection [21]and one of low intensity at 39.7^{o} resulting from the crystalline phase. Also, there is a broad region under these peaks ranging from roughly 5 to 80^{o} that is related to the predominant amorphous phase. The diffraction patterns of capsules from the 40% or 87% hydrolyzed poly(vinyl acetate) exhibited two major peaks characteristic of a crystalline polymer at 19.45^{o} (strong), and 40.89^{o}(weak). The capsule with 87% hydrolysed poly (vinyl acetate) had the highest crystalline region compared to the other samples due to its microstructure with a low amount of vinyl acetate. A broad peak centered at 2θ = 19.51◦ can be associated with the amorphous behaviour of pure PVA. This peak corresponds to (1 1 0) reflection. All the diffraction patterns included three peaks, at 2θ =11.49◦, 17.48◦, and 41.34◦ that corresponded to the crystalline phase of PVA [14].

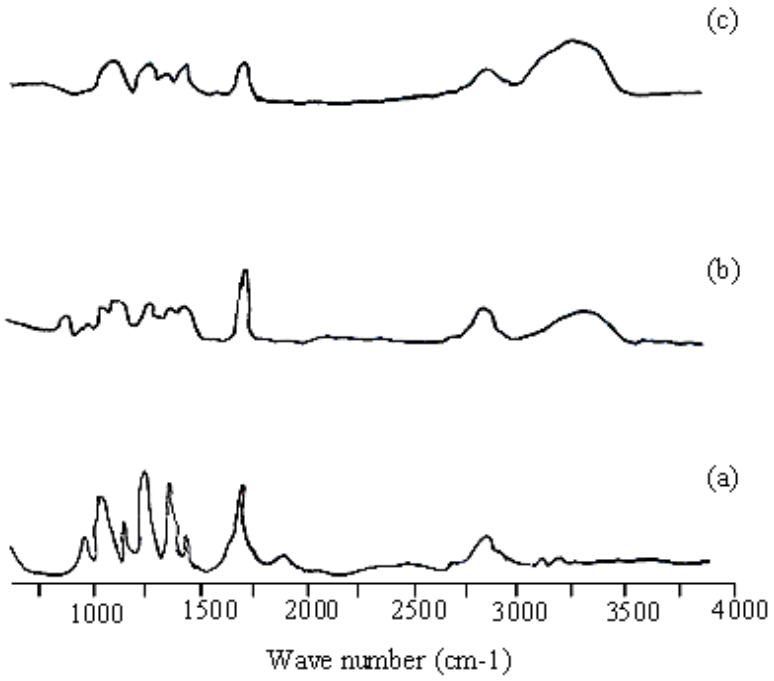


Figure1. Spectra of (a) 0, (b) 40, and (c) 87% hydrolyzed poly(vinyl acetate) observed by FTIR

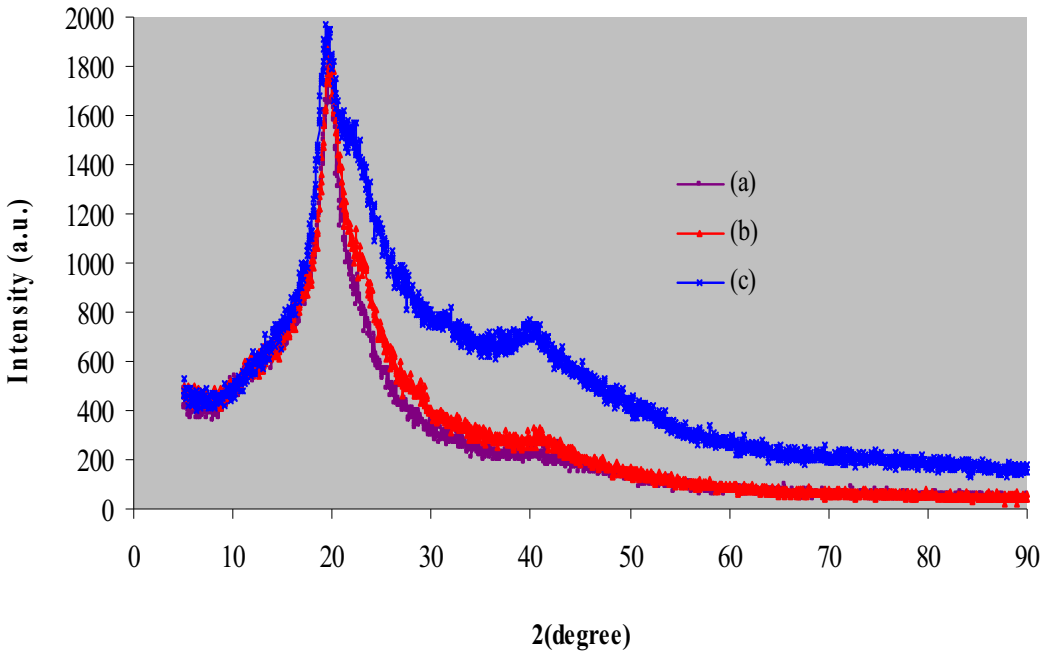


Figure 2. X-ray diffraction patterns of (a) 0, (b) 40, and (c) 87% hydrolyzed poly(vinyl acetate) films.

3.2 Morphology of microcapsule, Encapsulation yield and encapsulation efficiency

The particle size of the capsule beads was analyzed using both an optical microscope (OM) and SEM as shown in **Table 1**. **Figure 3** illustrates OM micrographs of capsules obtained from (a) 0% (b) 40% and (c) 87% hydrolysed poly (vinyl acetate). It is obvious that the morphology of capsules show little change and overall the average particle size of the capsules was 12 microns

The SEM photomicrographs of the microcapsules are shown in **Figure 4.** The average diameter of capsules obtained from the 0, 40 and 87% hydrolysates of poly (vinyl acetate) were 10, 9 and 8 micron at a ratio between polymer and distilled water, 1:35, respectively. Some aggregates of capsules obtained from three types were observed. The particle sizes of capsule observed from OM were larger than that of capsule analyzed from SEM due to shrinkage of the capsule after SEM sample preparation.

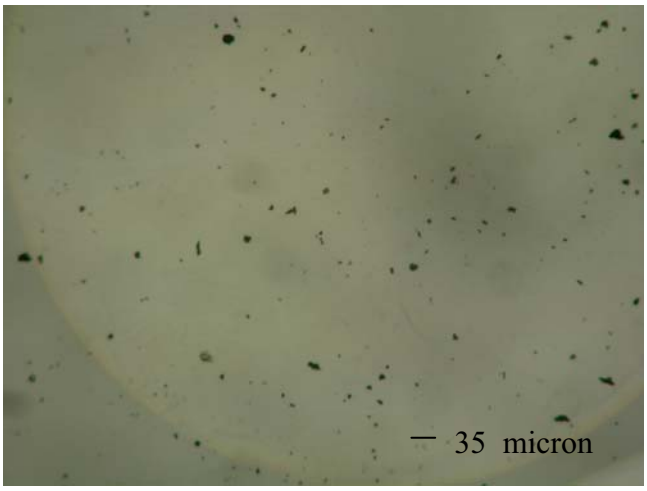
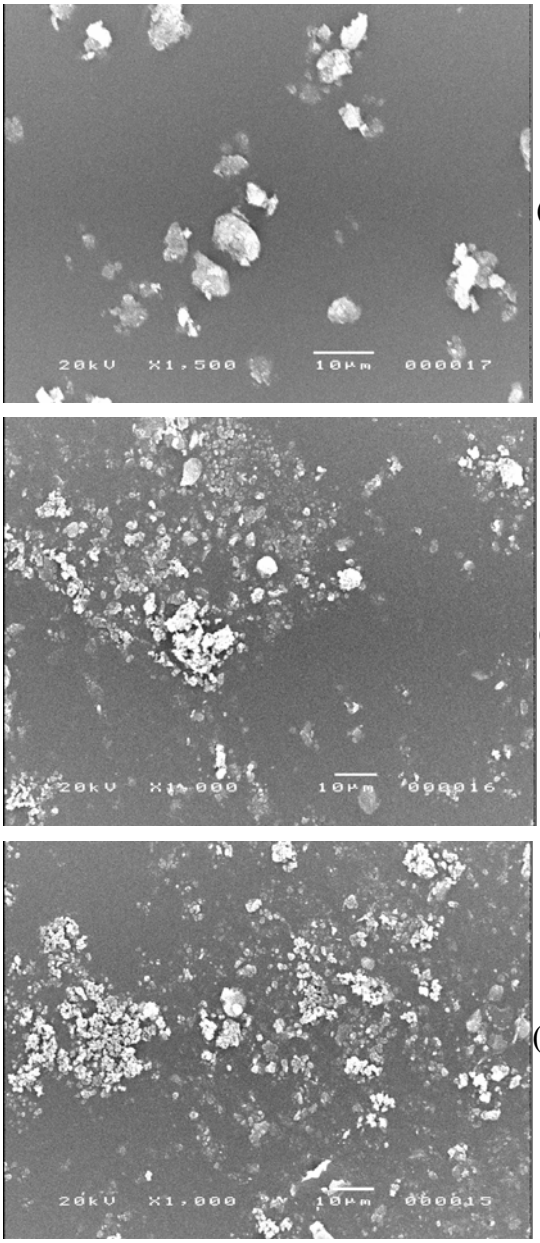


Figure 3 Optical micrography of microcapsule with 87% hydrolyzed poly(vinyl acetate)



(a)

(b)

(c)

Figure 4 SEM micrographs of capsules obtained from a PVA: distilled water ratio of (1:35) and a %age hydrolysis of poly(vinyl acetate) of (a) 0, (b) 40 and (c) 87%

The ratio between water and polymer, and %age hydrolyzed poly(vinyl acetate) on encapsulation yield (EY), and encapsulation efficiency (EE) were investigated (**Table. 1**.) When the ratios of water and non hydrolyzed poly(vinyl acetate) decreased from 1/20, 1/35 and 1/40, the encapsulation yields were about 85, 95 and 96%, respectively. In the case of the encapsulation efficiency, the result showed similar trends to the efficiency yield. The encapsulation efficiency of capsules was 75, 78, and 79 when the ratio between non hydrolyzed poly(vinyl acetate) and distilled water was 75, 78, and 79%, respectively. When the degree of %age hydrolyzed poly(vinyl acetate) was increased from 0 to 40%, the efficiency yield was 86, 94, and 96% at 1/20, 1/35 and 1/40 (40% hydrolyzed poly(vinyl acetate)/distilled water), respectively, whereas the encapsulation efficiency of this system was 76, 78, and 80% when the ratio of 40% hydrolyzed poly(vinyl acetate)/water were 1/20, 1/35 and 1/40, respectively. In the case of the 87% hydrolyzed poly(vinyl acetate), the efficiency yield was 84, 96, and 97 when the ratios between 87% hydrolyzed poly(vinyl acetate) and distilled water were1/20, 1/35, and 1/40, respectively. The encapsulation efficiency of this system was 79, 80 and 81 when the ratios of 87% hydrolyzed poly(vinyl acetate) and distilled water were 1/20, 1/35, and 1/40, respectively.

Table.1 Particle size, efficiency of yield and efficiency of encapsulation obtained at different ratios between polymer and distilled water containing glutaraldehyde 5% w/v and 0.1 % hydrochloric acid

Ratio between polymer and distilled water	Average paticle size(μm) (±S.D) observed by OM	Average particle size(μm) (±%S.D) observed by SEM	%Efficiency of yield (±%S.D)	% Efficiency of encapsulation (±%S.D)
0% hydrolyzed polyvinyl acetate				
1:20	15(2)	11(3)	85(2)	75(4)
1:35	14(3)	10(3)	95(2)	78(4)
1:40	14(2)	8(2)	96(2)	79(2)
40% hydrolyzed polyvinyl acetate				
1:20	15(2)	10(2)	86(3)	76(2)
1:35	14(3)	9(3)	94(2)	78(2)
1:40	15(2)	8 (2)	96(3)	80(3)
87% hydrolyzed polyvinyl acetate				
1:20	16(4)	12(3)	84(4)	79(3)
1:35	14(2)	8(3)	96(2)	80(5)
1:40	15(3)	9(3)	97(2)	81(2)

The ratio between water and polymer, and %age hydrolyzed poly(vinyl acetate) on encapsulation yield (EY), and encapsulation efficiency (EE) were investigated (**Table 1**.) When the ratios of water and non hydrolyzed poly(vinyl acetate) decreased from 1/20, 1/35 and 1/40, the encapsulation yields were about 85, 95 and 96%, respectively. In the case of the encapsulation efficiency, the result showed a similar trend to the efficiency yield. The encapsulation efficiency of capsules was 75, 78, and 79 when the ratio between non hydrolyzed poly(vinyl acetate) and distilled water was 75, 78, and 79%, respectively. When the degree of %age hydrolyzed poly(vinyl acetate) was increased from 0 to be 40%, the efficiency yield was 86, 94, and 96% at 1/20, 1/35 and 1/40 (40% hydrolyzed poly(vinyl acetate)/distilled water), respectively, whereas the encapsulation efficiency of this system was 76, 78, and 80% when the ratio of 40% hydrolyzed poly(vinyl acetate)/water were 1/20, 1/35 and 1/40, respectively. In the case of the 87% hydrolyzed poly(vinyl acetate), the efficiency yield was 84, 96, and 97 when ratios between 87% hydrolyzed poly(vinyl acetate) and distilled water were1/20, 1/35, and 1/40, respectively. The encapsulation efficiency of this system was 79, 80 and 81 when the ratios of 87% hydrolyzed poly(vinyl acetate) and distilled water were 1/20, 1/35, and 1/40, respectively.

3.3 Swelling study

The swelling of any capsule in a solvent depends upon the diffusion coefficient of the solvent, the relaxation rate of the amorphous regions of the polymer chain and its degree of crystallinity and crosslinking density in the polymer matrix. **Figure 5** depicts the swelling ratio of neem Aza-A capsules prepared from different %age hydrolyzates of poly(vinyl acetate) and crosslinking densities in a water medium and storage time over 35 h. It is clear that the swelling ratio of capsules obtained from the 0% hydrolysed poly(vinyl acetate) was lower than that capsule having 40 and 87% hydrolyzed poly(vinyl acetate) due to lower crosslinking density occurring from the hydroxyl groups in polymer and glutaraldehyde and the lower crystallinity in the sample observed from XRD.

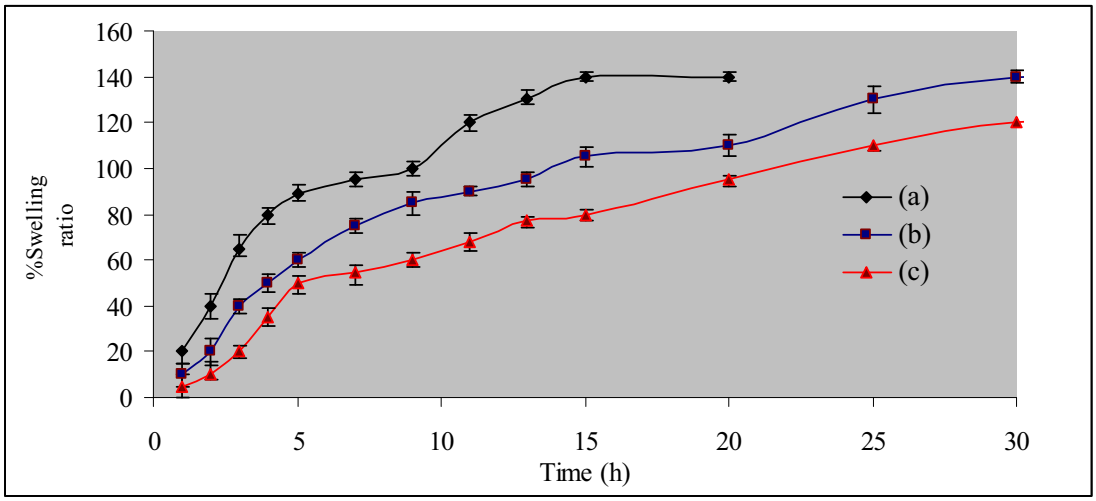


Figure 5 Swelling ratio of capsule with (a) 0, (b) 40, and (c) 87% hydrolyzed poly(vinyl acetate)

3.4 Release of neem Aza-A from the capsule

The Neem Aza-A was dispersed evenly throughout the matrix of the capsule and was unable to diffuse to any significant extent within the matrix. However when the polymer matrix was placed in a thermodynamically compatible medium, the hydrolyzed poly(vinyl acetate) swelled owing to absorption of the medium, then the neem Aza-A in the swollen part diffused out of the polymer matrix. The release of the neem Aza-A from the polymer matrix has been schematically described in **Figure 6**. In hydrophilic membranes, there usually are both geometric and mechanical reactions with respect to swelling [19]. At the same time, there are cases where the polymer matrix exhibits swelling with no significant limitations. The variety of factors affecting the rate of the diffusion transfer of a solvent, including (a) the polymer transition from glassy to rubberlike state; (b) relaxation transitions on the surface and in the bulk of a sample; (c) dependence of the diffusion mobility of water on its concentration in the polymer;(d) expansion of the sample, reaching several tens or even a few hundreds percentages with respect to the initial dimensions, requires development of a complicated multiparametric model of the water transport in polymers.

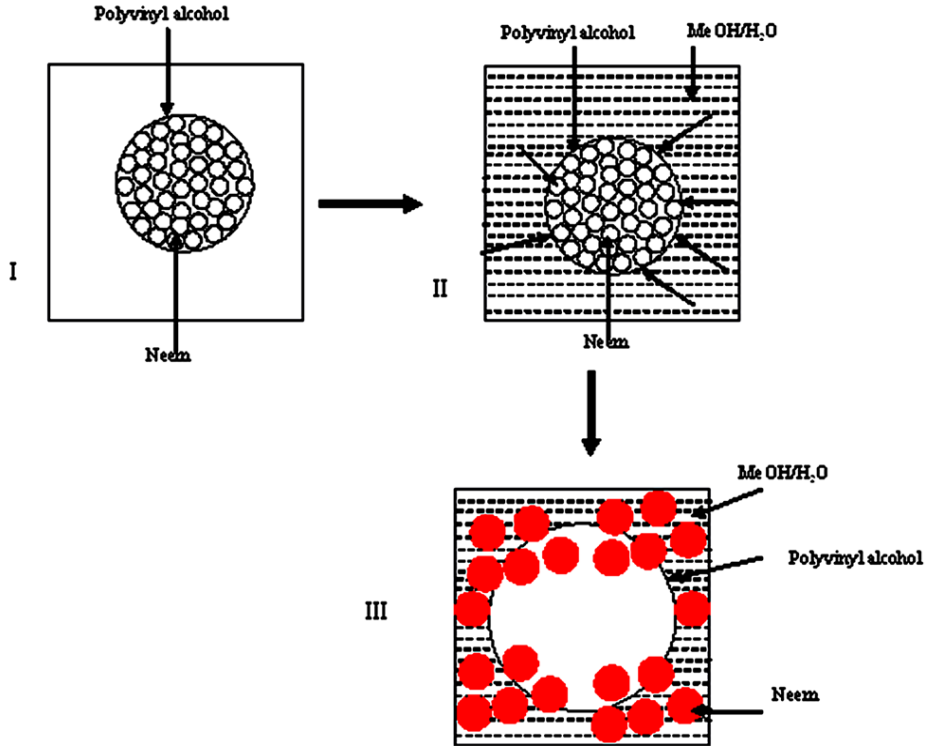


Figure 6 Schematic representation of a swelling type controlled release system of neem containing Aza-A (I) neem Aza-A entrapped in poly (vinyl alcohol) (II) neem containing Aza-A entrapped in poly (vinyl alcohol) in a thermodynamically stable system with H_2O diffusing into the polymer matrix. (III) neem Aza-A released into the H_2O system on swelling of the polymer matrix

Results of a study of the effect of the neem Aza-A concentration on its rate of release in distilled water from capsules with 0, 40 and 87% hydrolyzed poly(vinyl acetate) is shown in **Figure 7** . It is clear that the release rate of neem Aza-A from the microcapsules was proportional to the release time. The release rate of neem Aza-A from microcapsules obtained from the non hydrolyzed poly(vinyl acetate) was high

during the first 10 h followed by a slow release. Release of neem Aza-A from the non hydrolyzed poly(vinyl acetate) microcapsules was found to be almost complete within about 15 h. This result indicates that high amounts of neem Aza-A was present on the surface of the capsules. In the case of capsules obtained from the 40 and 87% hydrolyzed poly(vinyl acetate), the release rate of neem Aza-A from the microcapsules was high during the first 15 h. followed by a slow release. Finally, release of neem Aza-A from the microcapsules obtained from 40% and 87% hydrolyzed poly(vinyl acetate) was found to be almost complete within about 25 and 30 h, respectively. This result indicates that neem Aza-A was entrapped in the polymer matrix crosslinked with glutaraldehyde. This could be explained by the amount of neem Aza-A released being dependent on its hydrophilicity and crosslinking density in the polymer matrix.

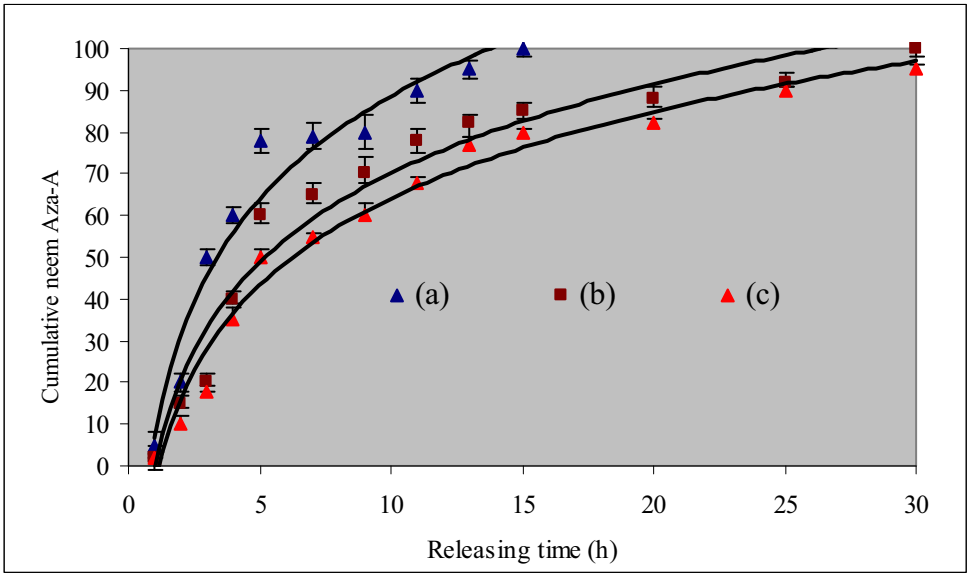


Figure 7 Relationship between the release rate of neem Aza-A and the release time from microcapsules obtained from (a) 0% hydrolyzed poly(vinyl acetate), (b) 40% hydrolyzed poly(vinyl acetate), and (c) 87% hydrolyzed poly(vinyl acetate)

The effect of the particle size of the capsule on releasing neem Aza-A from the capsule in aqueous medium is depicted in **Figure 8**. It is clear that the % cumulative neem Aza-A of the capsule with 8 micron diameter was higher than that of sample having a 12 micron diameter due to the higher surface area, leading to a greater amount of neem Aza-A diffusing in the aqueous phase.

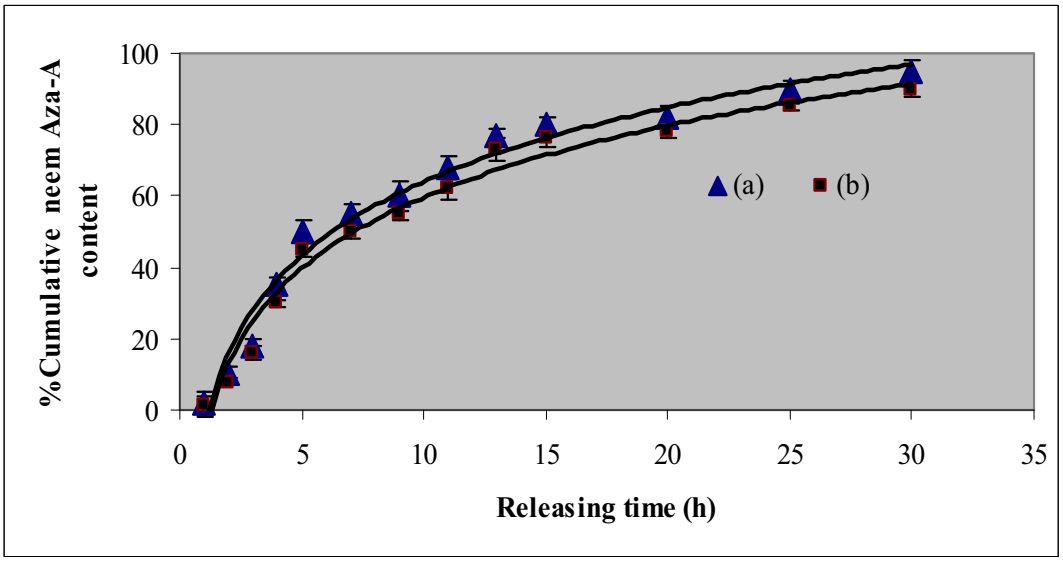


Figure 8 Relationship between the release rate of neem Aza-A and release time from microcapsules obtained from 87% hydrolyzed poly(vinyl acetate) having diameters of (a) 8 and (b) 12 microns

3.5 Photodegradation of unencapsulated and encapsulated neem Aza-A.

The stability of neem Aza-A subjected to UV irradiation is reported in terms of the %age residual neem Aza-A (**Figure 9).** It was found that the rate of degradation of unencapsulated neem Aza-A was much swifter than that of the encapsulated neem Aza-A. The rate of neem Aza-A degradation reduced rapidly from the time of initiation and became constant after 30 h of UV irradiation. When the neem oil was irradiated for 10 and 30 h, the residual neem Aza-A was 50 and 19%, respectively. This results correspond to those from the work of K. M. S. Sundaram and J. Curry [20] . They studied the photostabilization of neem-based azadirachtin insecticide (AZ-A) applied onto a glass surface in the presence of three UV absorbers, 2,4-dihydroxybenzophenone (Uvinul M-41)0, UM), 4-aminobenzoic acid (PABA) and Fluorescent brightener-28 (FB-28), a stilbene disulfonic acid derivative. It was found that for effective photostabilization, both AZ-A and the UV absorber must have matching UV spectra with a similar λmax. The mechanism of photostabilization was likely due to either energy transfer from AZ-A to the UV absorber and/or competitive absorption of UV photons by the absorber. Photoinstability of AZ-A in the presence of FB-28 was due to energy transfer from the activated FB-28 to AZ-A molecules.

Based on the UV spectral data, UV protectants can be selected and matched to stabilize UV-labile pesticides.

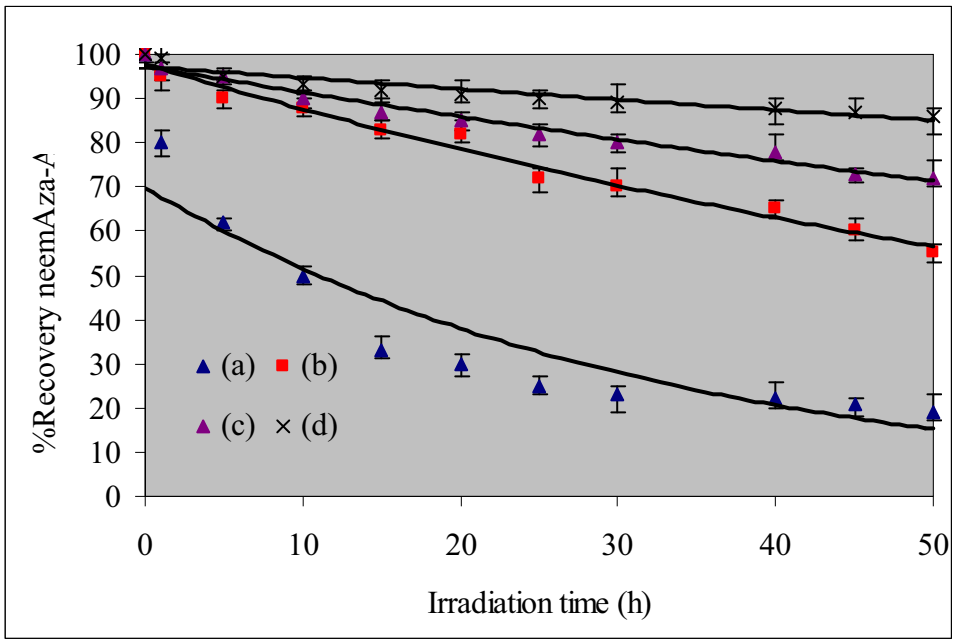


Figure 9 Recovery of neem Aza-A after UV irradiation of (a) unencapsulated neem Aza-A , and encapsulated neem Aza-A in (b) 0% hydrolyzed poly(vinyl acetate), (c) 40% hydrolyzed poly(vinyl acetate), and (d) 87% hydrolyzed poly(vinyl acetate)

The employed material matrix for encapsulation of neem Aza-A was 0, 40, and 87% hydrolyzed poly(vinyl acetate). These results show that the efficiency of thermal stability for encapsulated neem Aza-A obtained from the 87% hydrolyzed poly(vinyl acetate) was higher than that of other samples. The residual neem Aza-A for encapsulated neem Aza-A obtained from the non hydrolyzed poly(vinyl acetate) was 85 and 78% after 10 and 30 h of UV irradiation time. In the case of 40% hydrolyzed poly(vinyl acetate), the residual neem Aza-A was 88 and 82% after 10 and 30 h of UV irradiation time, respectively. When the degree of hydrolysis of poly(vinyl acetate) was increased from 40 to 87% and 90%, the residual neem Aza-A was 95% and 90% after 10 and 30 h of UV irradiation time respectively.

The rate constant values for photodegradation (in $hour^{-1}$) are given in **Table 2.** The photodegradation of unencapsulated or encapsulated neem Aza-A under ultraviolet irradiation were investigated. The rate constant values of unencapsulated neem Aza-A under ultraviolet irradiation were found to be higher than that of

encapsulated neem Aza-A. The rate constants of unencapsulated and encapsulated neem Aza-A derived for the non hydrolyzed poly(vinyl acetate) were 0.014616 and 0.014616 h^{-1}, respectively. When the percentage of hydrolyzed poly(vinyl acetate) was increased from 0 to be 40 or 87%, the rate constants for the encapsulated neem Aza-A were found to be 0.005447, and 0.002782 h^{-1}, respectively. This confirms that the neem Aza-A was entrapped and partially protected within the hydrolyzed poly(vinyl acetate) matrix.

Table 2 Rate constant values of unencapsulated or encapsulated neem Aza-A for photodegradation.

Sample Name	Rate constant value of unencapsulated or encapsulated neem Aza-A for photodegradation (k, h^{-1})
Unencapsulated neem containing Aza-A	0.014616
Encapsulated neem containing Aza-A obtained % hydrolyzed polyvinyl acetate	
0	0.008287
40	0.005447
87	0.002782

The aim of this part of the investigation was to compare the efficiency of photodegradation between the encapsulated and unencapsulated neem Aza-A. This estimation was based on defining the efficiency of the photodegradation data, as the irradiation time (in hour units) needed to reduce the amount of neem Aza-A to 30% of the initial value under accelerated conditions is given in **Table. 3**. It is clear that the efficiency of photodegradation of unencapsulated neem Aza-A was 3 h. In the case of encapsulated neem Aza-A, it was found that the efficiency of photodegradation of encapsulated neem Aza-A was lower than that of unencapsulated neem Aza-A. The photodegradation of encapsulated neem Aza-A obtained from 0% and 40% hydrolyzed polyvinyl acetate was 30 h and 50 h, respectively. When the degree of

hydrolyze poly(vinyl acetate) was increased from 40 to 87%, the photodegradation of encapsulated neem Aza-A was above 50 h. This indicates that the encapsulated neem Aza-A helped to improve its stability to photodegradation.

Table 3 Efficiency of photodegraded encapsulated and unencapsulated neem Aza-A under accelerating condition

Sample Name	Efficency of photodegradation* (h)
Unencapsulated neem Aza-A	3
Encapsulated neem Aza-A obtained from % different hydrolyzed polyvinyl acetate	
0	30
40	50
87	>50

4. Conclusions

The encapsulation of neem Aza-A was successfully carried out for the first time in 0%, 40 and 87% hydrolyzed poly (vinyl acetate) capsules, that produced a slow release of neem Aza-A that is suitable for application in the agricultural industry. It can be concluded that neem Aza-A is highly photolabile in the presence of UV light. The encapsulation did improve the stability of neem Aza-A under conditions that facilitated photodegradation. The swelling of the capsules decreased with the increasing degree of hydrolysis of the glutaraldehyde crosslinked vinyl acetate. The capsules obtained from the 87% hydrolyzed poly(vinyl acetate) gave the highest photodegradative stability of neem Aza-A. Therefore, this method can be used to enhance the storage of neem Aza-A under conditions that allow for photodegradation. In the present study, the use of hydrolyzed poly(vinyl acetate) for the controlled release of neem Aza-A results in increased photodegradation stability with an extended shelf life of the neem Aza-A.

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Chapter 5

Development of Neem Capsule *Via* Glutaraldehyde Crosslinked Sodium Alginate Capsules with Natural Rubber Coating its for its Control Release

ABSTRACT

Nowadays, the use of huge quantity of synthetic pesticides with the conventional agriculture leads to some important environmental problems. Thus, natural pesticide was applied for this purposed, but it is not stable under environment. Therefore, the encapsulation of natural pesticides was obtained by membranes to control the diffusion of water and the release of the water-soluble active agent and the stability of natural pesticides under environment. The controlling release of natural liquid pesticide “neem (Azadiracthin A) seed oil hereafter designated as neem Aza-A, was achieved by utilization of glutaraldehyde-aglinate gel capsules modified with coated natural rubber layer. The neem Aza-A containing beads have been prepared by changing the experimental variables such as the extent of crosslinking and the amount of loading in order to optimize the process variables. The SEM data indicated that the structure of the walls the beads are smooth and nonporous. The swelling result indicates that swelling of the polymeric beads decreases with increasing exposure time to the crosslinking agent. At particular intervals, the remaining concentration of neem Aza-A was analyzed by HPLC. The release data have been fitted to an empirical equation to estimate the kinetic parameter. The degree of release of neem Aza-A from capsules was controlled by condition parameters.

Keywords: Neem, Encapsulation, Natural rubber, Coaservation

1. Introduction

The use of synthetic pesticides in the world began in the 1930s and became widespread after World War II. By 1950, pesticide was found to increase farm yield far beyond pre-World War II levels [1]. Farmers depend heavily on synthetic pesticides to control insects in their crops. Today, it is one of the most commonly used methods in controlling insects but it is relatively high in toxicity and have high environmental impact. Thus, natural pesticide is applied to solve this problem due to no residue toxic chemical reagent on environment.

Neem (Azadirachtin indica A.Juss.), a tree, belongs to the Meliaceae family and is widely distributed in South Asia, South-East Asia, and some other tropical areas [2-7]. The neem seed kernels contains Azadiracthin-A (Aza-A), which is the major insecticidal tetranortriterpinoid. Neem Aza-A is a powerful insect antifeedant and growth-regulating substance, exhibiting considerable promise as an insecticide [2]. It can control at least 200 species of agriculture and storage insect pest belonging to different orders, but it has short environmental persistence, and causes negligible hazard to nontarget organisms including humans. Its short environmental persistence is due to the presence of sensitive moieties such as *p*-electrons, ester linkages, and epoxide ring. Thus, Aza-A is highly photolabile, either breaking down or isomerizing under sunlight. However, the photodegradation of Aza-A in sunlight is the major problem limiting its use in agriculture because the insecticide should persist long enough to cause the death of the insect. Many works solve this problem. The controlled stability of neem containing Aza-A can be done the two major approaches such as addition of antioxidant in neem solution and encapsulation of neem by polymer metric. For example, the addition of UV light absorbers can enhance the photostability of Aza-A [4]. The addition of ferulic acid, gallic acid, and rutin provided a moderate degree of photostabilization of Aza-A [4].The present patent invention relates to an improved granular formulation of neem seed extract containing neem Aza-A having enhanced storage stability [8], and the ability for gradual release of neem Aza-A for application to plant rhizosphere. The formulation consist of inert particulate as a carrier at least one lipophilic substance as a deactivator/binder, optional colorant and neem seed extract containing neem Aza-A [8]. The formulation provides the gradual release of neem Aza-A and effectively at the point of application.

This patent also relates to a process for the preparation of the formulation by coating the carrier with a lipophilic substance, subsequently impregnating the coated carrier with neem seed extract followed by optional coating with a colorant and finally lipophilic a substance, such as by spraying and drying at a temperature below 50^{o}C [8].

The second method is microencapsulation which packages the sensitive ingredients within a coating or wall material [9-16]. The wall material protects the sensitive ingredient (or core) against adverse reactions, prevents the loss of volatile ingredients, and can control the rate of release of the ingredient. In addition, microencapsulation can convert liquids into free-flowing powders, so that they can be more easily handled.

Preparation of the polymeric granules containing 20, 35 and 50% (w/w) of the natural liquid pesticide viz., Azadirachta Indica A. Juss. (neem) seed oil (NSO) was reported by T.M. Aminabhavi and co-worker [5]. The polymer matrices used for encapsulation were urea formaldehyde crosslinked starch (UF-St), guar gum (UF-GG) and UF-(St + GG)[5]. They found that the release of the active ingredient depended on the type of the matrix and its swelling ability. The percentage loading of NSO with different matrices and their density exerted an influence on the release data. Then, they had studied the release kinetics and encapsulation efficiency of urea formaldehyde (UF) crosslinked matrices of St, GG, and St + GG for the controlled release of the solid (chlorpyrifos) and liquid (neem seed oil) pesticides [6] in 2001. They indicated that variable release rates were related to the polymer type and especially the pesticide type.

The improved granular formulation of neem seed extract containing neem Aza-A have enhanced storage stability, and the ability to gradually release neem Aza-A for application in the plant rhizosphere [8]. It was found that the best formulation contained inert particulate matter as a carrier, at least one lipophilic substance as a deactivator/binder, optional colorant and neem seed extract containing neem Aza-A. The invention also required the development of a method for the preparation of the formulation by coating the carrier with a lipophilic substance, subsequently impregnating the coated carrier with neem seed extract followed by an optional

coating with a colorant and finally a lipophilic substance, by spraying and drying at a temperature below 50^{o}C.

T.M. Aminabbavi. and co-worker studied the encapsulation of a natural liquid pesticide using sodium alginate (Na-Alg) as a controlled release (CR) polymer after crosslinking with glutaraldehyde (GA) [10]. They found that the swelling of the polymeric beads decreased with increasing exposure time to the crosslinking agent. However, no significant variation in swelling was observed with different amounts of neem Aza-A loading. In addition, the rate of neem Aza-A from beads released was very fast. Thus, this work tries to apply the natural rubber coating on sodium alginate capsule. The sodium alginate Na–Alg has been used as a control release matrix material in medicine [17-19], membrane [20-22] and agriculture [10, 23-24] after crosslinking it with calcium chloride. Alginates polysaccharides are known to be haemocompatible and do not accumulate in any organs of the human body. It has been reported that glutaraldehyde (GA) solution and alginate can react together by coacervation due to the chemical reaction between hydroxyl groups of Na–Alg and GA

In the work presented here we test the feasibility of encapsulating neem Aza-A in a matrix made from sodium alginate to produce a product with good end-use properties. To the best of our knowledge, this is the first of its kind of study wherein the effect of natural rubber coating capsule on release of neem Aza-A from modified capsules. The effect of physically hydrophobic on alginate bead was also investigated. The optimum condition and releasing of neem Aza-A from capsule were investigated.

2. Methodology

2.1. Materials

Neem seed kernels were purchased from local Thailand; neem Aza-A extract was prepared according to the procedure given in detail [1] the sodium alginate, glutaraldehyde (25% w/v) solution and AR grade methanol samples were all purchased from Fluka agent, Thailand. Concentrated NR latex used in this study is high ammonia latex received from Jana company, Co., Ltd. (Songkhla Thailand)

2.2. Methods

2.2.1. Preparation of capsule beads and efficiency of entrapment

A 4% sodium alginate solution in distilled water was prepared by heating mental. After complete cooling the amounts of neem Aza-A (7000 ppm) was added and mixed thoroughly using a magnetic stirrer. The polymer solution containing neem Aza-A was added dropwise into methanol containing 1% glutaraldehyde and 1% of 1 N HCl, using a 25-ml hypodermic syringe (0.8 mm diameter) with constant stirring. The beads formed were removed from methanol at a selected time interval say 10, 20 and 30 min. The beads were washed with water and then dried. The efficiency of entrapment was calculated as the ratio between the initial mass of neem Aza-A to be encapsulated and its mass in the final product. About 20 mg of exactly weighed microcapsule sample was extracted in distilled water to form a homogeneous solution. The total neem Aza-A in the solution was extracted for 48 h with a 50/50 MeOH/H_2O mixture and its mass was determined by HPLC (PerkinElmer LC).

2.2.2. Drying rate study of the beads

A 3 samples of the beads formed after crosslinking with GA were selected for the drying study and were allowed to dry in an oven (VELP) maintained at 31± 2°C (the initial mass of the beads should be nearly equal). The masses of the beads were taken at definite intervals of time until the constant mass was achieved. All the mass measurements were done on a Mettler single pan balance (Model AB 204, Mettler). In order to obtain reproducible results, experiments were conducted in triplicate, and the average values were used for the calculation and plotting of the data vs. time.

2.2.3 Coating of capsule with NR

Concentrated NR latex used in this study is high ammonia latex received from Jana company, Co., Ltd. (Songkla Thailand).

% TSC of latex is defined as the percentage by weight of the concentrated latex which is non-volatile at a definite temperature in an open atmosphere. The %TSC of concentrated NR latex in this study was determined by using method described in ASTM D107688 as shown in equation (1).

%TSC = (W/Wt)*100……(1)

Where, W = weight of dry NR sample (g)

Wt = weight of NR latex sample (g)

%DRC of latex is defined as the percentage by weight of the concentrated latex which is precipitated by acetic acid. The %DRC of concentrated NR latex was determined (equation 2) by using method described in ASTM D1076-88 .

%DRC = (Wx/Wt) *100……(2)

Where, W = weight of dry NR coagulum (g)

Wt = weight of NR latex sample (g)

The 5 g of dried natural rubber were dissolved in toluene (50 ml) in beaker (250 ml). The capsules (5 g) were dipped to the toluene solution and dried at room temperature.

So, the dry capsules of crosslinked sodium alginate mixed with neem Aza-A (7000 ppm) were dipped into a toluene solution of natural rubber (5% w/w).Then, the coating capsules were dried at 30 ^{o}C for 24 h. Multiple coatings were prepared by the immersion of the single-coated neem capsules into a natural rubber with 30%DRC. Thereafter, the procedure was the same as during the preparation of single-coated neem capsules. The third-coated neem capsules were derived by the dipping of double-coated neem capsules into a natural rubber with 30%DRC and then the same methodology as that given above mention. Fourth-coatings were prepared by the immersion of the third-coated neem capsules into a natural rubber with 60 DRC and dried at 60^{o}C until its weight was constant.

2.2.4. Bead size measurement

Five samples of the completely dried beads from different formulations were selected and their sizes were measured by using a micrometer screw gauge (Sargent, USA) with an accuracy of ±0.01 mm.

2.2.5. Swelling study of the individual beads

Swelling property of the beads was subjected to a measurement of swelling ratio in aqueous medium as a function of time. The bead samples exposed to GA at different time at 26±2^{O}C were selected and incubated with distilled water in a watch glass. The mass of all bead samples was taken at different interval period times and the average value was calculated. During this process, care should be exercised while it was handed of the swollen beads so as to avoid any weight loss due to breaking or

erosion of the beads. All the mass measurements of the swollen beads were taken on a Mettler single pan balance and having accuracy up to fifth decimal. The percentage swelling ratio of bead was calculated as in equation 3.

$$\%\text{Swelling ratio} = \frac{(\text{Wet weight-dry weight})*100}{\text{dry weight}} \quad \ldots\ldots (3)$$

2.2.6. Fourier transforms infrared (FTIR) measurements

Fourier transforms infrared; FTIR (Bruker, EQUINOX 55) spectral data were obtained to detect any chemical interactions between neem and sodium alginate and sodium alginate alone.

2.2.7. Scanning electron microscope (SEM)

The aim of SEM study is to obtain a topographical characterization of beads. The sample was deposited on brass hold and sputtered with gold. SEM photographs were taken with JSM 6400 Scanning Microscope (Japan) at the required magnification at room temperature.

2.2.8 Thermogravimetric Analysis (TGA)

Thermal studies were conducted with Mettler-Toledo instrument (TGA/SDTA 851) analysis the heating rate for the thermogravimetric analysis of sodium alginate alone and capsule beads was 30^{o}C/min. A small amount (1-3 mg) of sample was taken for the analysis and the samples heated from 30 to 800^{o}C at in nitrogen. The TGA and DTG curves are drawn for each sample.

2.2.9 Content uniformity, Dissolution and Releasing studies

Beads were evaluated for the neem content and this was done by refluxing a known mass of the beads with 100 ml of methanol at 65°C. Refluxing was continued for 1 h to ensure complete extraction of neem from the beads. Then the absorbance of methanol containing the extracted amount of neem was taken at a wavelength of 211nm in a HPLC (PerkinElmer LC) using pure methanol as a blank.

The dissolution study was done in 250-ml conical flasks containing the dissolution media (0.1% Tween-80 solution in distilled water) with the closer caps which were kept in an incubator (WTB Binder, Germany) maintained at 35°C. Two to three beads

weighing about 10 mg were taken in the dissolution media. At definite intervals of time, the conical flasks were shaken well and a 10-ml aliquot was taken for the analysis of neem Aza-A using HPLC (PerkinElmer LC) at 211 nm. Experiments were performed in triplicate in order to minimize the variation error. The cumulatively release of neem Aza-A from capsule beads was estimated from.

The release results were investigated by using an empirical equation to estimate the value of n as follows (equation 4)

$$Mt/M\infty = Kt^n \text{ or } \log (Mt/M\infty) = \log (K) + n\log(t) \quad \text{.....(4)}$$

Where $Mt/M\infty$ is the released fraction at time t, n is the release exponent, and K is the release factor. From the slope and intercept of the plot of log ($Mt/M\infty$) against log (t), kinetic parameters n was calculated.

3. Results and discussion

The definition of encapsulation is a process in which thin films, generally of polymeric material is applied to little solid particles, liquid or gases droplets. This method is used to trap active components and release them under controlled conditions. The reactive agents have been encapsulated in the agriculture industry, fertilizer and pesticide. This work is focused on the development of a new neem encapsulation using a sodium alginate matrix through coaservation and coated with natural rubber. Alginate is extracted from brown seaweed; it has free carboxylic groups which react with divalent captions, mainly calcium, to form stable gels. Neem contains major Azadirachtin-A, a powerful insect antifeedant and growth-regulating substance with exceptional environmental characteristics. However, the use of azadirachtin-based neem pesticides may be limited by the acid and base sensitivity of the compound and its susceptibility to photodegradation due to presence of light-absorbing moieties 2, 3. Therefore, the aim of this paper is to apply the encapsulation techniques of solving this problem. The encapsulation of neem Aza-A by sodium alginate as called capsules and the efficiency of neem Aza-A encapsulation were improved to coat the capsule with natural rubber. The characterization of resulting capsules is the thermal property and release study. The crosslinking between sodium alginate and glutaral aldehyde are shown in **Figure1**. Sodium alginate can be crosslinked with glutaraldehyde GA , the chemical reaction between hydroxyl groups

of sodium alginate and GA was confirmed by fourier transform infrared FTIR measurements as shown in **Figure 2**. The crosslinking ability of alginate as a function of alginate composition and length of the molecule has been used here to crosslink it with GA.

Table1 Particle size, efficiency of yield and efficiency of encapsulation obtained at different ratios between polymer and distilled water containing glutaraldehyde 5% w/v and 0.1 % hydrochloric aci

Time of exposure to 5% GA (min)	% Neem Aza loading	Bead diameter (mm)	% Entrapment efficiency
10	5	1.11±0.12	91.2±1.2
	10	1.25±0.12	89.1±1.3
	20	1.28±0.14	85.**2**± 1.1
20	5	1.28±0.22	89.**7**±1.3
	10	1.32±0.13	88.8±1.4
	20	1.48±0.12	83.7±1.4
30	5	1.35±0.22	80.**6**±1.5
	10	1.43±0.15	78.4±1.4
	20	1.45±0.17	73.6±1.6

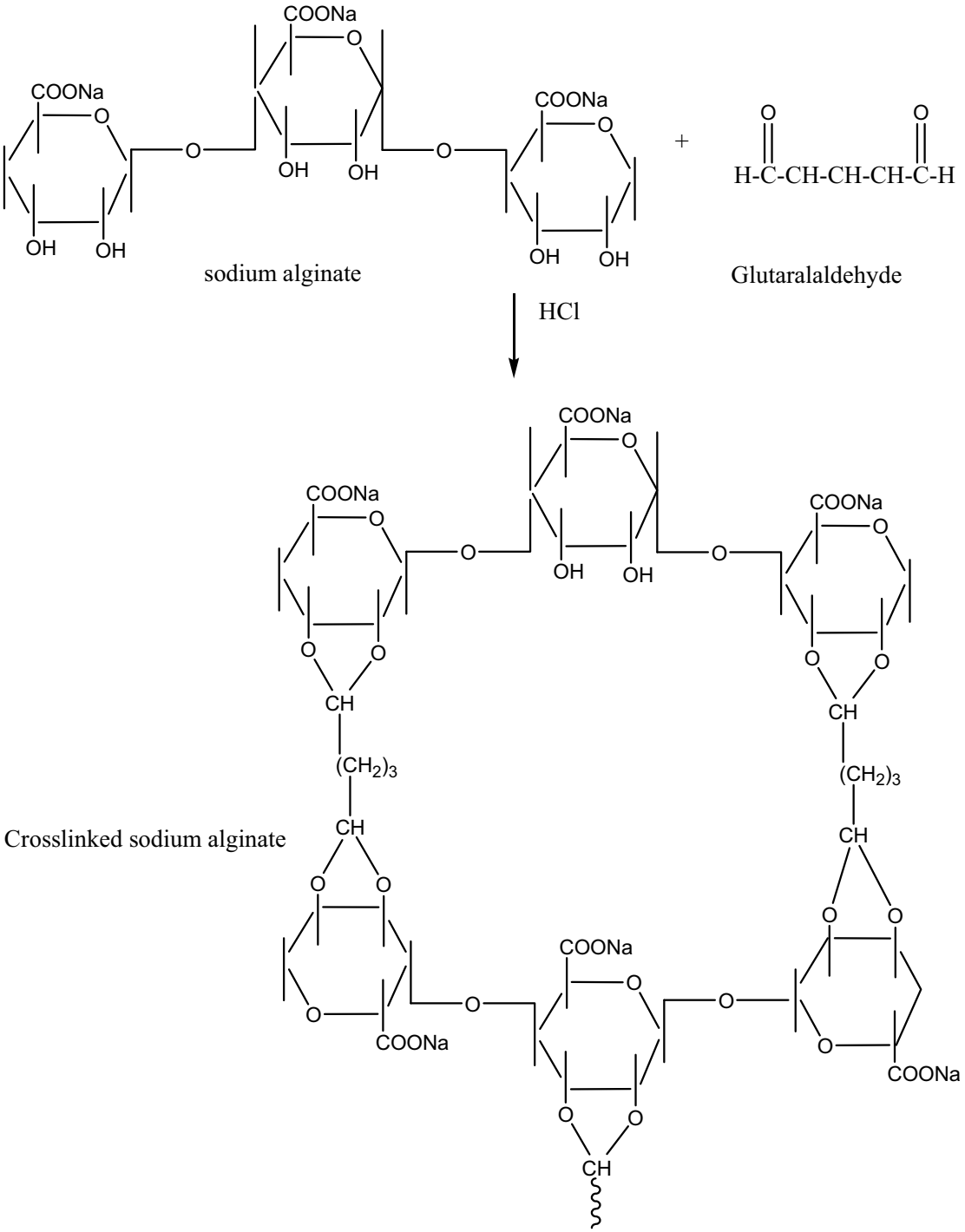


Figure 1 Reaction between sodium alginate and gluraldehyde by hydrolic acid as catalyst

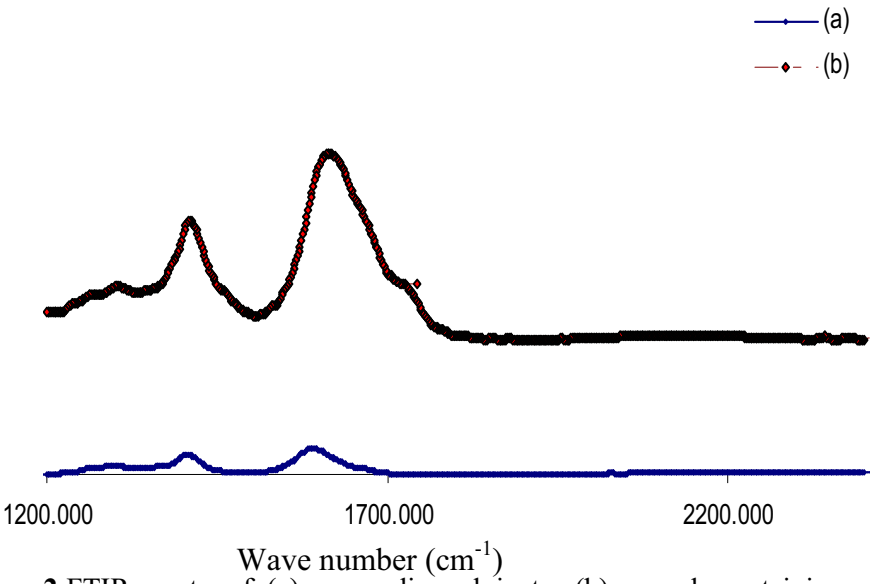


Figure 2 FTIR spectra of (a) pure sodium alginate , (b) capsule containing neem Aza-A

3.1Preparation of neem capsules

The encapsulation of neem (as called capsule) was prepared by sodium alginate as a controlled release polymer after crosslinking with glutaraldehyde, and then the capsule was coated with natural rubber solution. The optimum condition for encapsulation of neem such as storage time in glutaraldehyde solution was investigated. In order to optimize the drying conditions, some samples of the beads with different extent of crosslinking were selected such that the initial weight is nearly equal.

The percentage entrapment efficiency was varied by varying exposure time periods to the crosslinking agent. It was found that the percentage entrapment efficiency decreased drastically with a decrease in aqueous medium. Beads produced in 5.0% HCl in methanol at 298^{o}C exposed for10 min showed the highest entrapment efficiency i.e., 91.2% and the lowest entrapment efficiency i.e., 73.6% was observed for 0.5% HCl content in methanol at 298^{o}C exposed for 30 min as shown in **Table 1**. Neem Aza-A is soluble in aqueous media and hence, an increase in the percentage entrapment efficiency was observed with an decreasing storage time period in

glutaraldehyde containing HCl as a catalyst due to the increased release of the neem Aza-A from the matrix at longer time of exposure.

3.2 Drying study of capsule

Results of drying is presented in **Figure 3** indicate that the beads with longer time of exposure to the crosslinking agent exhibit higher drying rates than the beads exposed to shorter time to glutaraldehyde. The beads exposed for 10 min dried quickly (i.e., within 40 h) when compared to beads exposed to the crosslinking agent for 30 min (i.e., 72 h), while an intermediary drying time (i.e. 60 h) was required by the beads exposed to crosslinking for 20 min. This may be due to an increased rigidity of the polymer formed after a longer exposure time to the crosslinking agent thereby showing a decreased desorption rate of liquid from the beads.

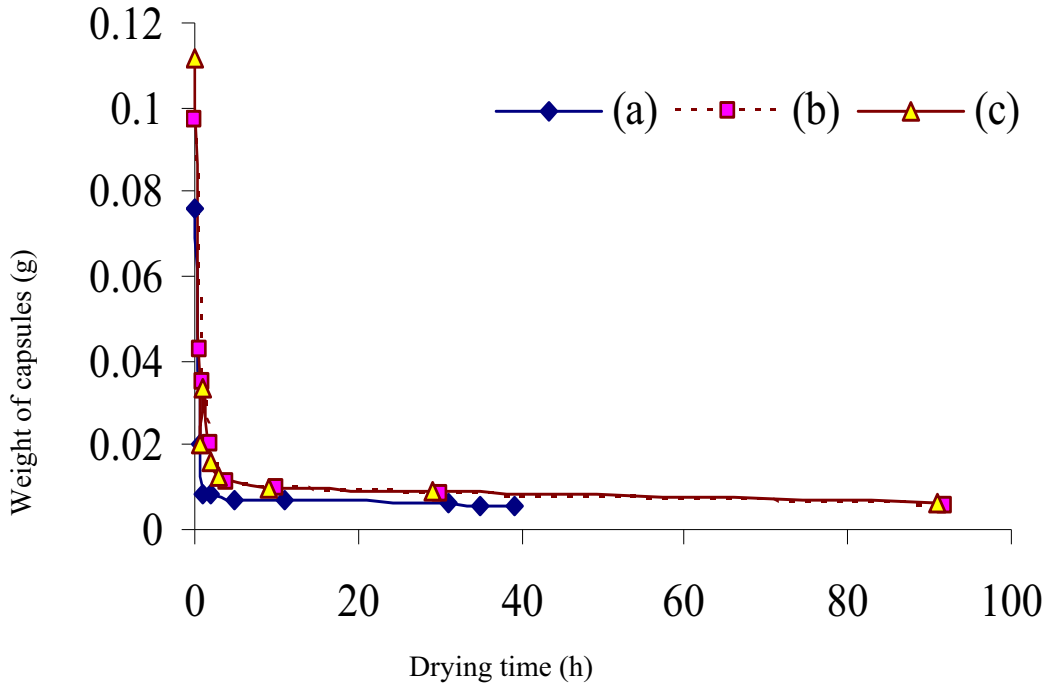


Figure 3 Effect of crosslinking on drying of beads at (a) 10, (b) 20 and (c) 30 min exposured to glutaldehyde solution.

3.3 Swelling ratio of capsule

Figure 4 reveals the effect of crosslinking on percentage of swelling ratio by beads at various exposure times to glutaraldehyde. It was found that all the beads show a maximum amount of water absorption during the first hour, but beads formed

by exposing for only 10 min to the crosslinking agent absorb more water than the beads formed by exposing for 20 and 30 min. The particles produced in this work were analyzed for their sizes using the light scattering method. Aza-A release from the beads were subjected to a number of physical and chemical parameters including those related directly to the release medium, the release conditions (temperature) and those resulting from change in the characteristics of the controlling release device (beads).

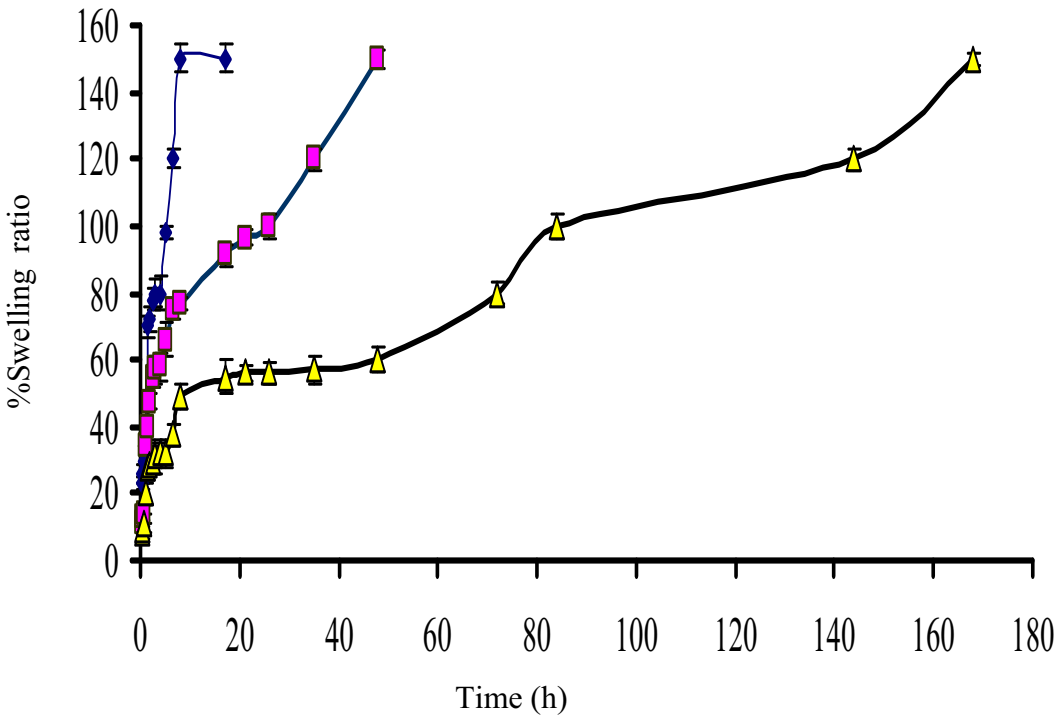


Figure 4 Effect of crosslinking on percentage of swelling ratio by beads at (a) 10, (b) 20 and (c) 30 min exposure to glutaraldehyde.

Figure 5 exhibit the effect of natural rubber layer on percentage of swelling by beads prepared at 30 min storage time of glutaldehyde solution. From the results reported in section, it can be said that the structure of capsules with influence the release rate of neem Aza-A should be improved by coating the capsule with NR. Diffusion in polymers is an important mechanism in pharmacy for the controlled release of drugs [9]. Diffusion in polymeric systems is passive, if the driving force is purely a brownian molecular motion, but diffusion can also be activated by external effects, either by the influence of the release medium by swelling or biodegradation, or by the effects of physical forces as electrical, osmotic or convective forces. The

fundamental of diffusion is based on Fick's laws which describe the macroscopic transport of molecules by a concentration gradient [9].

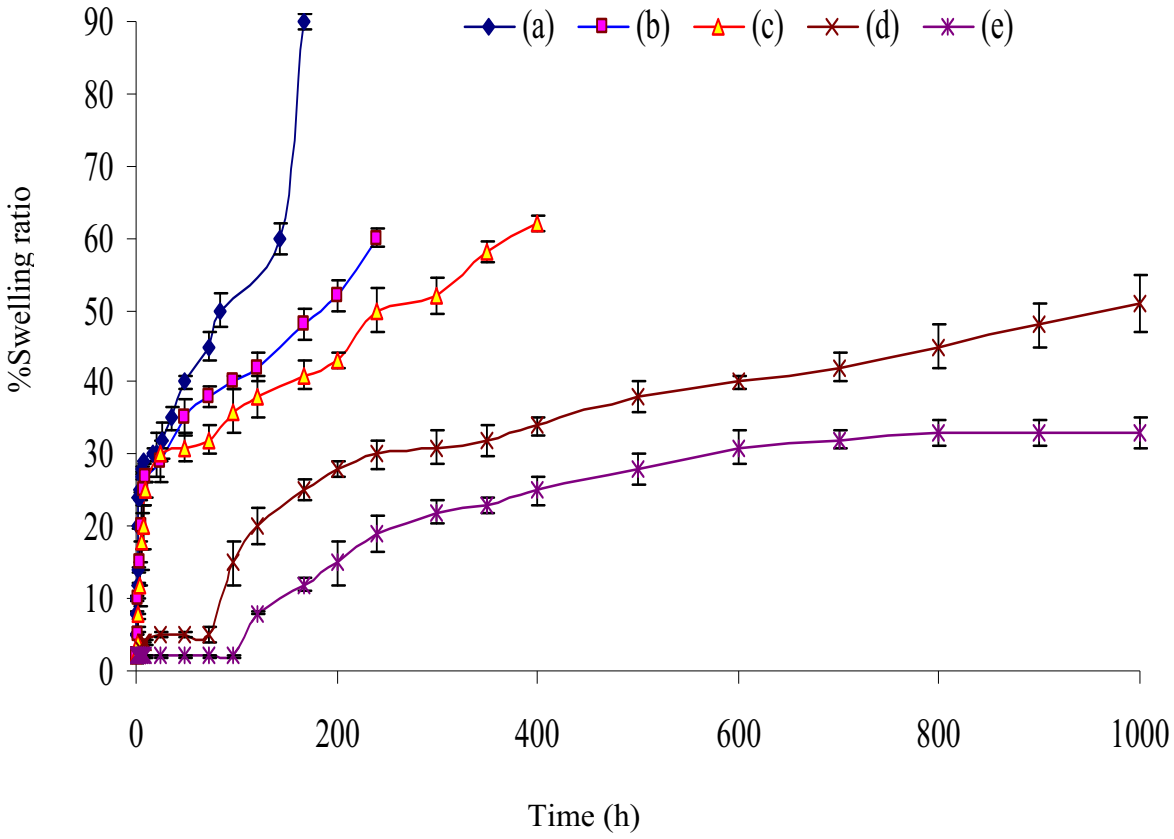


Figure 5 Effect of NR layer on percentage of water uptake by beads at (a) 1 (b) 2 and (c) 3 NR layer

The suitable coating material must be nonreactive, essentially immiscible with the material being encapsulated and capable of being rapidly hardened to form a film. Natural rubber was selected to be a coating agent of neem Aza A-sodium alginate capsules to provide an adequate barrier wall. So, the dry capsules of crosslinked sodium alginate mixed with neem Aza-A (7000 ppm) were dipped into a toluene solution of natural rubber (5% w/w).Then, the coating capsules were dried at 30 ^{o}C for 24 h. Multiple coatings were prepared by the immersion of the single-coated neem Aza-A capsules into a natural rubber with 30 DRC. Thereafter, the procedure was the same as during the preparation of single-coated neem Aza-A capsules. The

third-coated neem Aza-A capsules were derived by the dipping of double-coated neem Aza-A capsules into a NR with 30%DRC and then the same methodology as that given above mention. Fourth-coatings were prepared by the immersion of the third-coated neem Aza-A capsules into a natural rubber with 30% DRC and dried at 60^{o}C until its weight was constant. It is clear that the rate of swelling decreased dramatically after coating neem Aza-A capsule with NR compared with that of the bead without coating. When NR layer on capsules increased, the swelling ratio of these resulting capsules dramatically decreased, especially capsule bead obtained from four-coated NR. The swelling ratio of neem Aza-A obtained from first-coated neem Aza-A in aqueous medium at 2, 24, 72 and 240 h of storage neem Aza-A was 10, 29, 38 and 60%, respectively. When the NR-coated on capsules increased from 1 to be 3 layers, the swelling ratio of neem Aza-A obtained from first-coated neem Aza-A in aqueous medium at 2, 24, 72 and 240 h of storage neem Aza-A was 2, 5, 5 and 30%, respectively.

3.4 Morphology of capsules

The particles produced in this work were analyzed for their sizes using the light scattering method. The SEM photographs of capsule were shown in **Figure 6** and it is clear that the particles are egg in shape. The mean particle size was 0.14 mm observed by both OM and SEM. After the capsules were coated with NR, their diameter was drastically increased from 0.14 mm to be 3 mm and more smooth on surface of casule was also observed.

a b

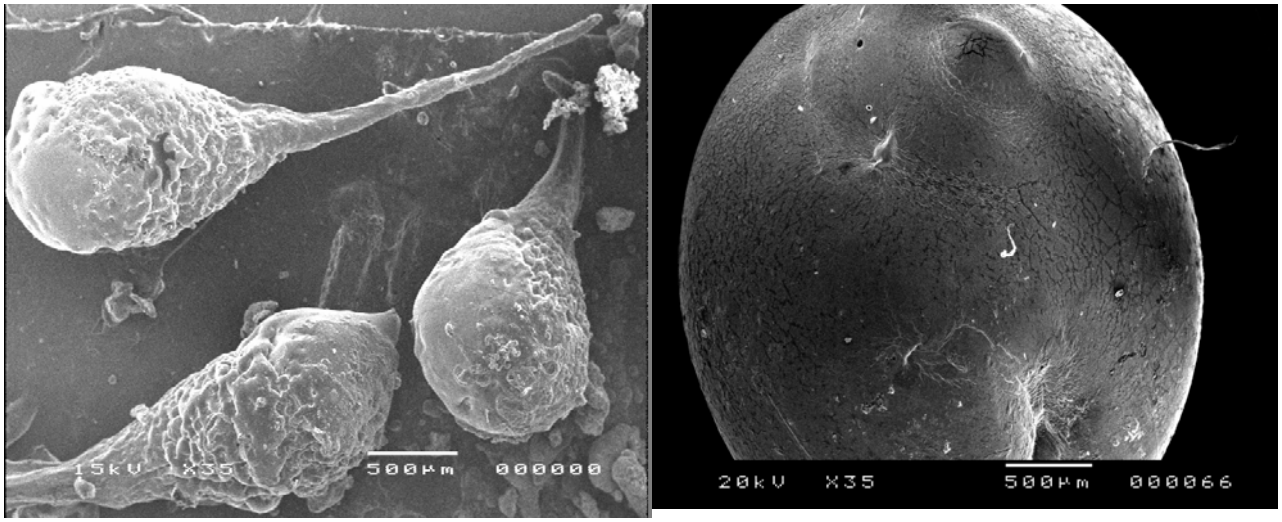


Figure 6 Scanning electron microscopic photographs of capsule beads (a) without and (b) with NR coating from 30 min of storage time in glutaraldehyde solution

3.5 Release rate study of sodium alginate capsule

Aza-A release from the beads were subjected to a number of physical and chemical parameters including those related directly to the release medium, the release conditions (temperature) and those resulting from change in the characteristics of the controlling release device (beads). The effect of degree of crosslinking of sodium alginate beads on the kinetics of Aza-A release is depicted in **Figure 7**. It is found that the higher the exposure time to glutaraldehyde the higher the release rate. The release rate of Aza-A beads at 10 min with exposure to glutaraldehyde have shown 100% release in the 5 hour, whereas the Aza-loaded beads with exposure to glutaraldehyde have shown 100% at 10 hour, but Aza-A-loaded beads with exposure to glutaraldehyde have shown 100% release at 25 hour. To observe the effect of the extent of crosslinking on the release kinetics of the beads exposed to the crosslinking agent, exposure to glutaraldehyde at 30 min were selected for NR coating.

The n value is an empirical parameter characterizing the release mechanism [10]. On the basis of the diffusion exponent, an n value of 0.5 indicates the nutrient release mechanism approaches to a Fickian diffusion controlled release, whereas n equal to 1.0 indicates the nutrient release mechanism approaches to zero-order release.

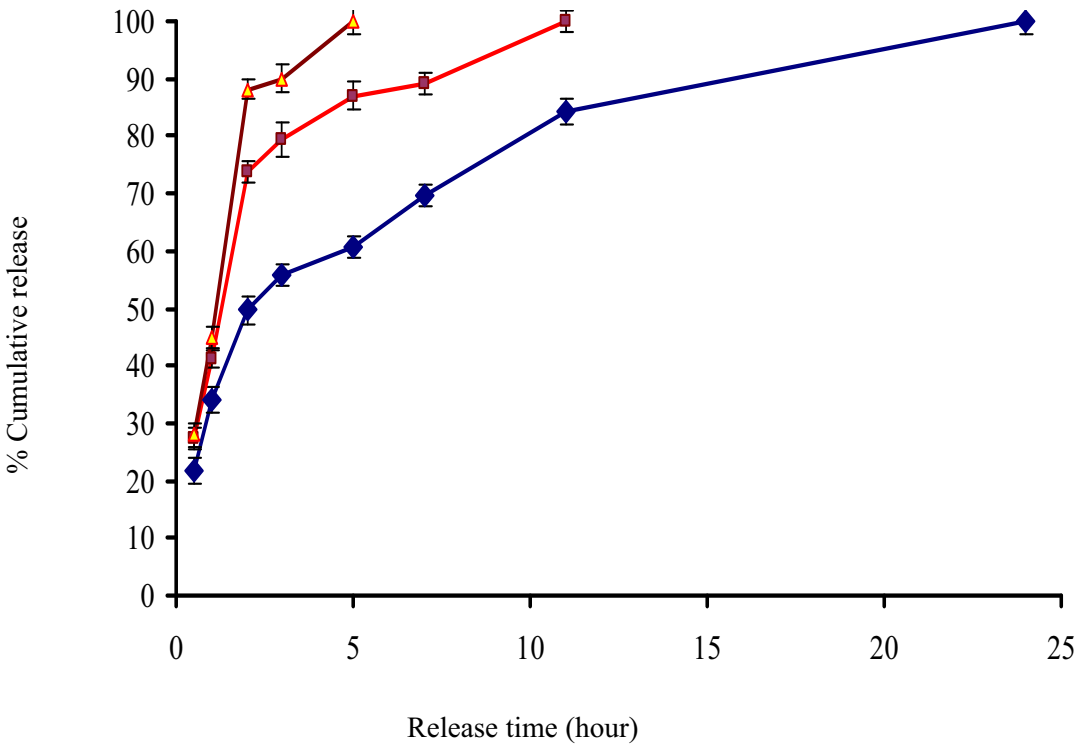


Figure 7 Effect of crosslinking on the release neem for capsules (I) uncoated natural rubber at (a) 10, (b) 20 and 30 min in glutaraldehyde without coating NR

The n value from 0.5 to 1.0 is a reactive agent release mechanism for non-Fickian diffusion or chain relaxation control release. From the plot of log (Mt/M∞) against log(t), release exponent (n) has been calculated as shown **Figure 8**. It was found that the n value is in the range from 0.3515 to 0.6258. The n value of sample obtained from 30 min storage time in glutaraldehyde solution indicating that the release in this system deviate from Fickian diffusion controlled release. The n of capsule beads obtained from 10 and 20 min of storage time was 0.599 and 0.6258, respectively, indicating that these system exhibit non-Fickian diffusion.

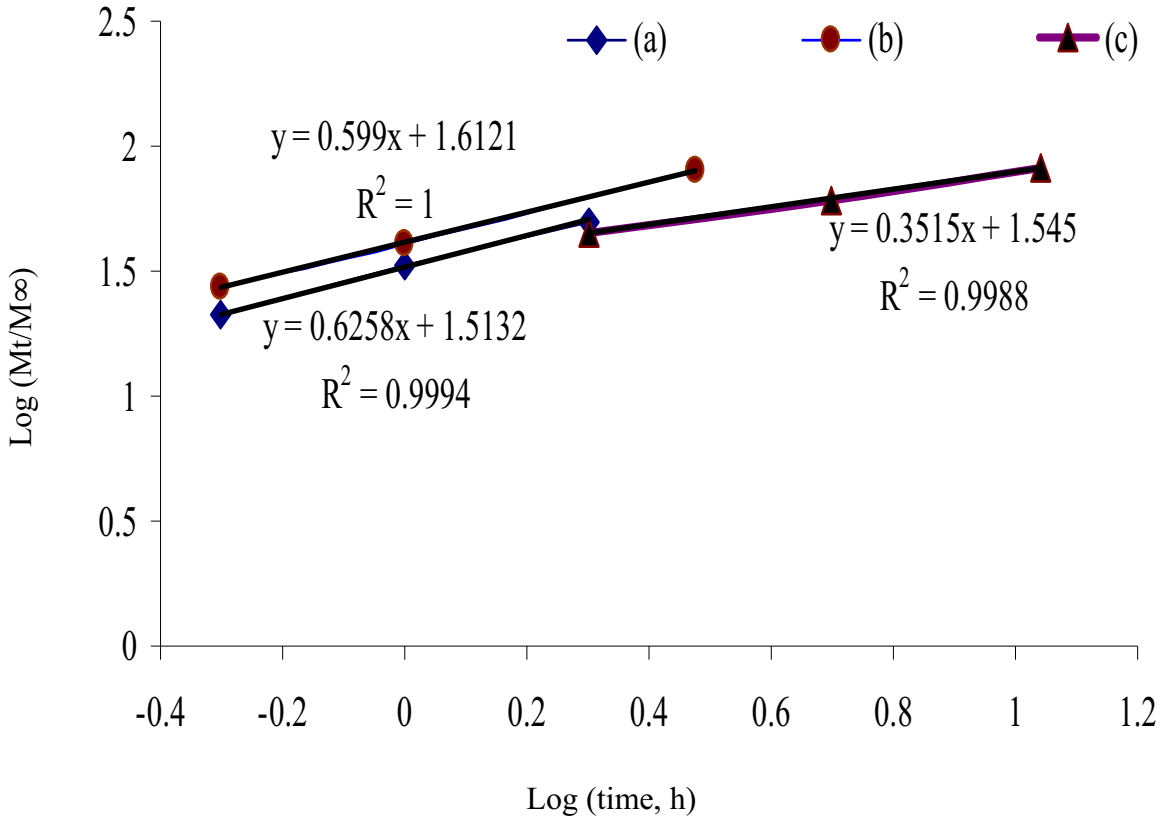


Figure 8 Fitting of release kinetics of neem Aza-A from capsule with uncoated natural rubber at (a) 10, (b) 20 and 30 min in glutaraldehyde without coating NR by the power law

The effect of release rate of Aza-A from the beads coated with different layers of natural rubber and exposed for 30 min to glutaraldehyde are presented in **Figure 9**. The release profile from neem Aza-A without NR coating is also shown for comparison. It is obvious that the neem Aza-A release rate is reduced significantly by NR coating, which is consistent with the results of the swelling study. The natural rubber film is very strong, rigid and hard to swell, so the diffusion through this coating is the rate limiting step for swelling and neem-A release. The release was prolonged by additional natural rubber layers on the capsule surface. The neem Aza-A cumulatively release of capsule derived from 2, 24, 72 and 240 in aqueous medium was 31, 69, 81 and 100%, respectively and when NR coated on capsule increase from 1 to be 3 layers, the neem Aza-A cumulatively release of capsule stored in at the

same condition was 8, 29, 36 and 60%, respectively. It is to be noted that with an increase in NR coating, the capsule matrix becomes more dense resulting in a decrease in the rate of diffusion of neem Aza-A through the swollen beads, especially beads with fourth- NR coating. The n value of neem Aza-A coated with NR is represented in **Table 2**, estimated from **Figure 10**. It was found that the n value of this sample obtained from 0, 1, 2 and 3 layers was 0.3515, 0.3766, 0.4476 and 0.3497, respevtively at regression of 0.9988, 0.9875, 0.9870 and 0.9991,respectively.Thus, the neem Aza-A release mechanism of beaded coated with NR was Fickian diffusion.

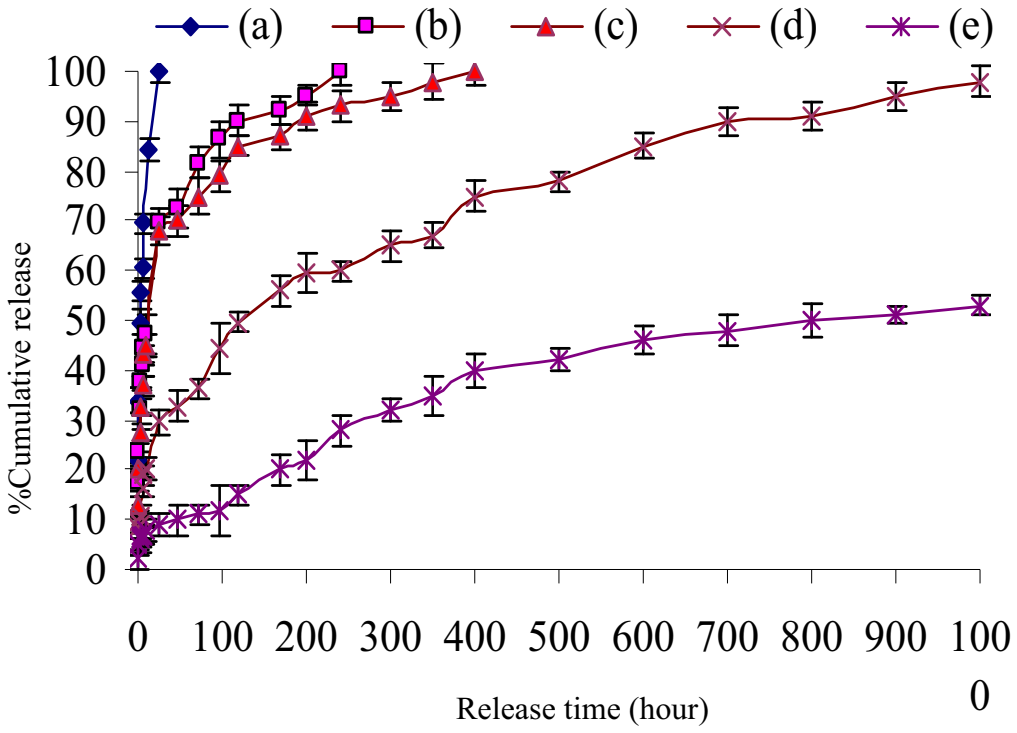


Figure 9 Effect of NR coating on the release neem Aza-A for capsules coated with natural rubber (a) 0 (b) 1 (c) 2, (d) 3 and 4 layer of NR

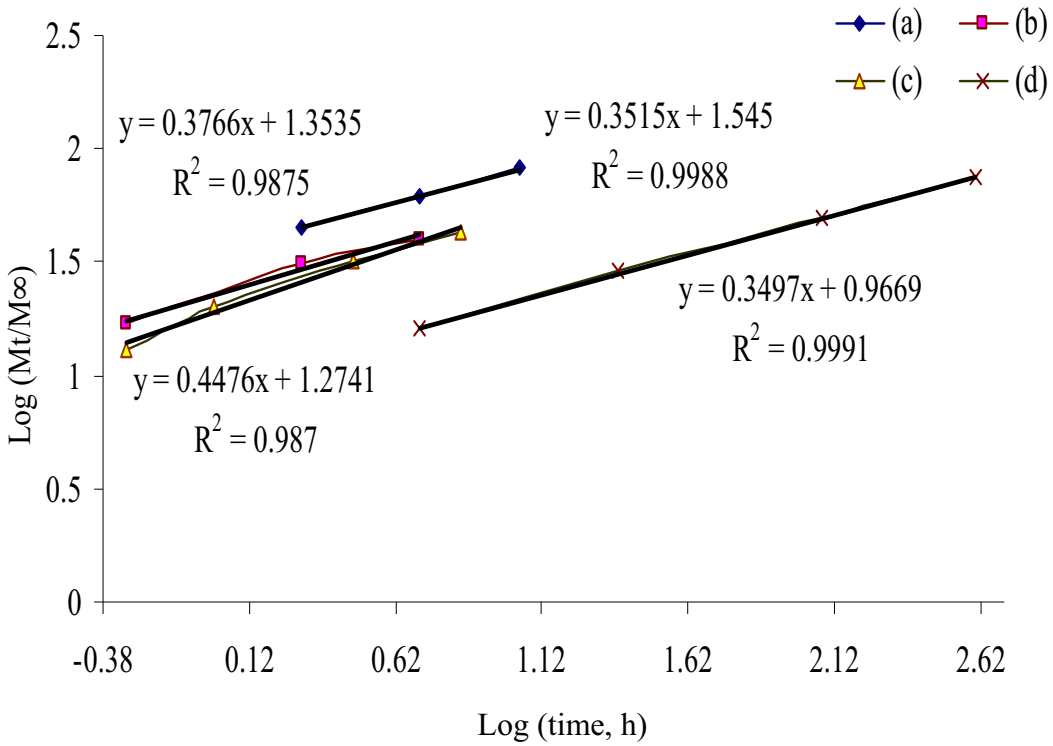


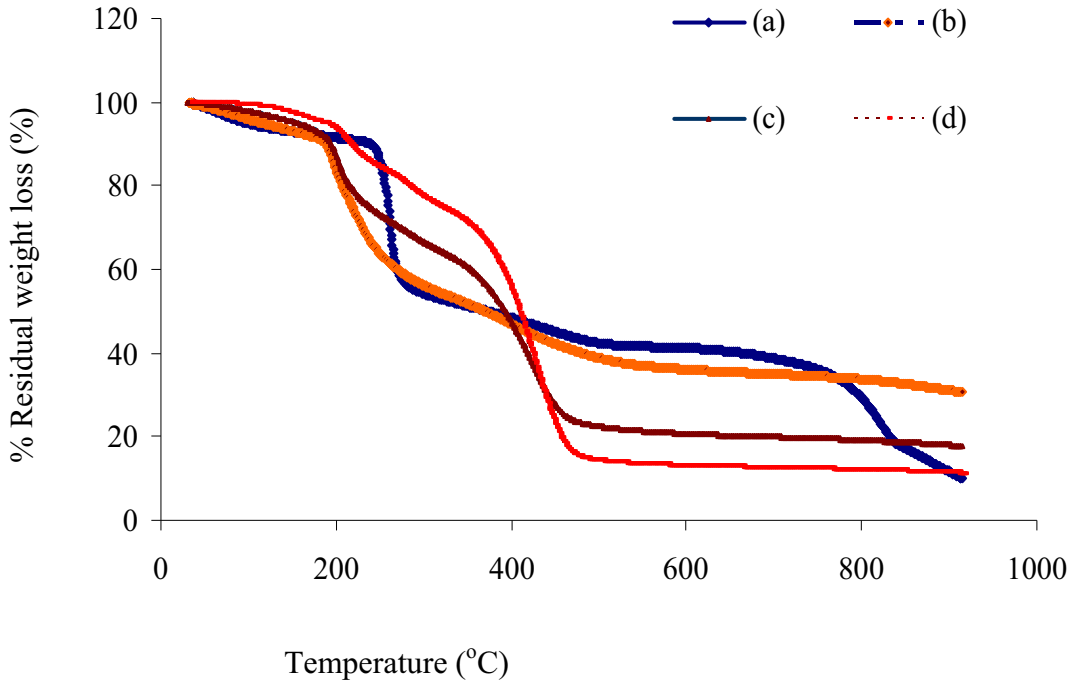
Figure10 Fitting of release kinetics of neem Aza-A from capsule with uncoated natural rubber (a) and coated NR at (b) 1, (c) 2 and (d) 3 layer in glutaraldehyde by the power law

Table 2 The results of n and r calculated from Eq. (10)

Storage time in glutaralaldehyde	NR coating number	n	R^2
10	0	0.6258	0.9994
20	0	0.599	1.000
30	0	0.3515	0.9988
30	1	0.3766	0.9875
30	2	0.4476	0.9870
30	3	0.3497	0.9991

3.6 Thermal stability of capsules

The increase in NR layer on capsule was further confirmed by TGA analysis. The weight loss before 400^{o}C in the TGA curves was attributed to the thermal degradation of polymer coating from capsules, from which the polymer content in the capsules particle. The TGA and derivative thermogravimetry (DTG) curves for the pyrolysis of capsules are shown in **Figure 11**, respectively. It was found that pure sodium alginate began to degage at 100^{o}C and the residue left was about 0% at 450^{o}C and reaches to maximum at 243^{o}C. The natural rubber shows better thermal stability than that of sodium alginate alone. For neem Aza-A capsules, the degradation of neem Aza-A experienced a comparatively long time and wide temperature range until 450^{o}C. In the case of capsule NR coating, below 250^{o}C there is no degradation. In the temperature range of 250–400^{o}C about 85% of the material is degraded. During this stage weight loss and volatilization of degradation products take place rapidly. Beyond 420^{o}C the weight loss is about 6–7%. The weight loss contents of capsule with NR coating at from 320 to 450^{o}C increased with NR coating layers on capsules.



II

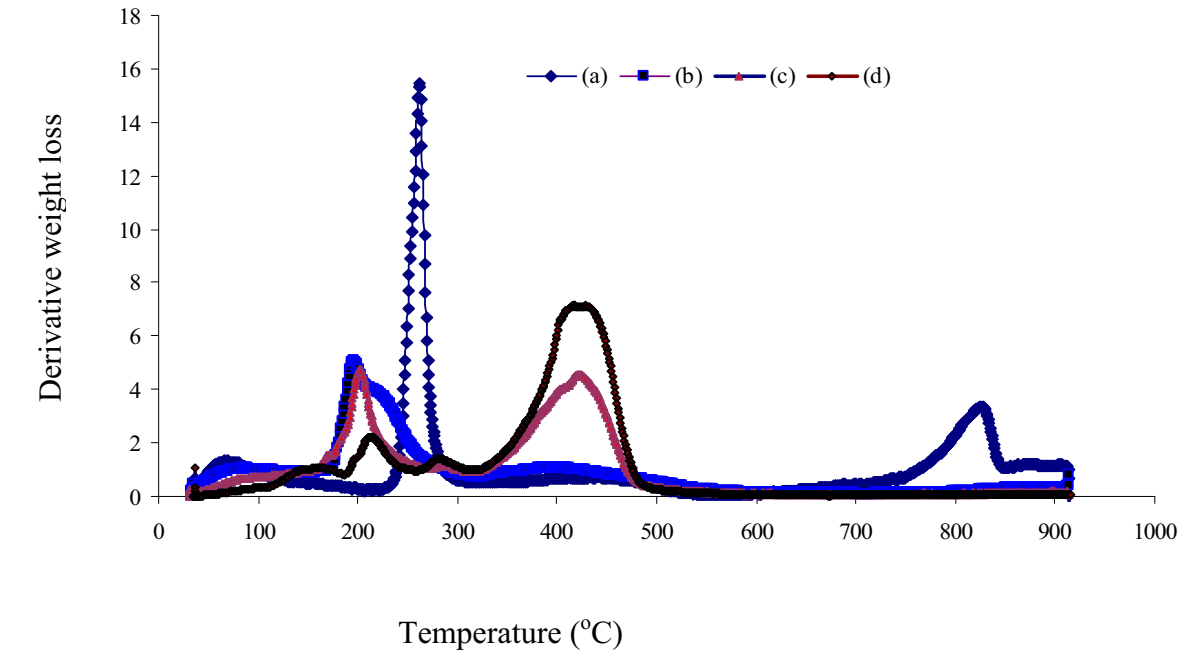


Figure 11 Thermal stability of sodium alginate alone (a) and capsule with at (b) 0, (c) 1, (d) 2, and (c) 3 layer NR coating observed by TGA, I (TGA) and II (DTGA)

4. Conclusion

Experimental results suggest that neem Aza-A can be successfully encapsulated into the sodium-alginated containing glutaraldehyde and then it was coated with NR matrix. After the capsules using sodium alginate matrix were coated with NR, the surface capsules was quite roughness. NR coating the neem Aza-A-sodium alginate capsules had a pronounced effect on slow release neem Aza-A. The NR could be used as an adequate barrier of the capsules to give better distribution of urea in sodium alginate matrix and consequently more efficient release of neem Aza-A as compared to the uncoated capsules. The rate of neem Aza-A release decreased with the increase in dipping time in NR latex. The rate of release of neem Aza-A from capsule in aqueous medium depends on exposure times to glutaraldehyde and a number of layers of NR on its surface capsule.

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Chapter 6

Preparation and Characterization of Neem Capsule *Via* Glutaraldehyde Crosslinked Sodium Alginate and Poly (Vinyl Alcohol) Blend for Its Control Release

ABSTRACT

Recently, the use of huge quantity of synthetic pesticides with the conventional agriculture leads to some important environmental problems. Thus, natural pesticide was applied for this purposed, but it is not stable under environment. Therefore, the encapsulation of natural pesticides was obtained by membranes to control the diffusion of water and the release of the water-soluble active agent and the stability of natural pesticides under environment. The controlling release of natural liquid pesticide “neem (Azadiracthin A) seed oil hereafter designated as Aza, was achieved by utilization of glutaraldehyde-aglinate gel and poly (vinyl alcohol) capsules. The Aza-containing beads have been prepared by changing the experimental variables such as the extent of crosslinking, blend ratio and the amount of loading in order to optimize the process variables. The chemical structure of capsule wall was evaluated through FTIR, and XRD. In addition, the swelling behavior of capsule and thermal stability of capsule were investigated in this work. The strength of capsule wall depended on the poly(vinyl alcohol) in matrix and crosslinking density. SEM, EPMA and AFM data indicated that the structure of the walls the beads are rough and nonporous. The swelling results indicated that swelling of the polymeric beads decreases with increasing exposure time to the crosslinking agent. At particular intervals, the remaining concentration of Aza was analyzed by HPLC. The release data have been fitted to an empirical equation to estimate the kinetic parameter. The degree of release of Aza was controlled by condition parameters.

1. Introduction

The possible application of biosemi-interpenetrating polymer network are applied in medicine, biosensor, sensitized solar cell, antimicrobial materials, photonics, tissue engineering, nano composites, catalyst and membranes due to low toxicity and biocompatibility [1-3]. In addition, its advantages are capability of undergoing first-order ionic strength and electric field. The polymer, used to produce semi-interpenetrating, are poly (vinyl alcohol), polystyrene, polymethacrylate and chitosan [2]. The use of polysaccharide as biopolymer has recently gained great important in view of low toxicity and high biocompatibility. The definition of encapsulation is a process in which thin films, generally of polymeric material based on biopolymer is applied to little solid particles, liquid or gases droplets [4]. This method is used to trap active components and release them under controlled conditions. The reactive agents have been encapsulated in the agriculture industry, fertilizer and pesticide [5]. This work is focused on the development of a new neem encapsulation using a sodium alginate matrix through coaservation and coated with natural rubber. Alginate is extracted from brown seaweed; it has free carboxylic groups which react with divalent cations, mainly calcium, to form stable gels. The use of conventional broad-spectrum synthetic insecticides is rapidly declining due to public concern and regulatory demands for selective and environmentally benign pest control the development of natural insecticides originating from plants, which are believed to be innocuous [3]. Currently, attention is being focused on the use of neem-based botanical insecticide. Neem (Azadirachtin indica A.Juss.) is a tree that belongs to the Meliaceae family and is widely distributed in South Asia, South-East Asia, and some other tropical areas. In the present paper we have studied controlling the release onset time of neem from the glutaralaldehyde alginate gel bead and polymer blend between sodium alginate and polyvinyl alcohol. The effect of physically hydrophobic on alginate bead and polymer blend bead was also investigated. Alginate made from brown algae which is an anionic linear polysacaride consisted of 1,4-linked β-D-mannuronates residues and 1,4-linked α-l-guluronates in different proportions [4] The advantages of alginate are hydrophiic, biocompatible, and relatively economical. Thus, it has been widely used in medical application. PVA are widely used in medical material because PVA posses some useful properties such as non-toxicity biocompatibility, high hydrophilicity and chemical and mechanical resistance. Two biodegradable polymers are applied use a

polymeric metric for preparing neem Aza-A capsule. In 2000, they had studied the release kinetics and encapsulation efficiency of urea formaldehyde (UF) crosslinked matrices of starch (St), guar gum (GG), and starch + guar gum (St + GG) for the controlled release of the solid (chlorpyrifos) and liquid (neem seed oil) pesticides [6]. They observed that variable release rates were related to the polymer type and especially the pesticide type. It was possible to slow the release rates of pesticides using cheap available materials such as starch and guar gum. G.M. Chandrasekaran invented an improved granular formulation of neem seed extract containing Aza-A having enhanced storage stability, and the ability to gradually release Aza-A for application in the plant rhizosphere[7]. It was found that the best formulation contained inert particulate matter as a carrier, at least one lipophilic substance as a deactivator/binder, optional colorant and neem seed extract containing Aza-A. The formulation allowed for an effective gradual release of Aza-A at the point of application. The invention also required the development of a method for the preparation of the formulation by coating the carrier with a lipophilic substance, subsequently impregnating the coated carrier with neem seed extract followed by an optional coating with a colorant and finally a lipophilic substance, such as by spraying and drying at a temperature below 50^{o}C. T.M. Aminabbavi. and co-worker studied the encapsulation of a natural liquid pesticide “neem (Azadirachta Indica A. Juss.) seed oil” hereafter designated as NSO, using sodium alginate (Na-Alg) as a controlled release (CR) polymer after crosslinking with glutaraldehyde (GA) [8]. They found that the swelling of the polymeric beads decreased with increasing exposure time to the crosslinking agent. However, no significant variation in swelling was observed with different amounts of NSO loading. In order to understand the crosslinkability and its effect on the NSO release patterns of the beads, an attempt was made to calculate the molar mass between crosslinks using the Flory–Rehner equation. The release data have been fitted to an empirical equation to estimate the kinetic parameters. In this work, the neem had been encapsulated in the partially hydrolyzed polyvinyl acetated (or poly (vinyl alcohol)) by spray drying. Polyvinyl alcohol has been used as a polymer matrix for encapsulation of the reactive agents [9-14]. Spray drying is used for producing pharmaceutical powders for inhalation, etc [15-21]. The production method has a major effect on the physical properties, such as flowability, hygroscopicity and dissolution, due to variation of the physical state (amorphous versus crystalline), particle size and composition at the particle surface. In addition,

the biological activity of proteins, etc. included in the particles is influenced by the drying process. In the particular case of spray drying, several processes impose stresses that can destabilize proteins and peptides, such as high pressure, high shear and immense air–liquid interfaces during atomisation, heating and dehydration [18]. In this process, the sensitive ingredient was mixed or homogenized in a solution containing wall material to form a stable emulsion. The emulsion was then fed into a spray dryer where it was converted to a dried particle. However, spray drying provides the possibility of creating particles with the active protein in one step, which are suitable for inhalation [18]. In a similar way to stabilizing proteins during freeze-drying, it was found that addition of disaccharides (such as sucrose and trehalose) or amino acids (such as arginine hydrochloride) to the liquid formulation increased the stability during spray drying and subsequent storage. In this process, the sensitive ingredient was mixed or homogenized in a solution containing wall material to form a stable emulsion. The emulsion is then fed into a spray dryer where it is converted to a dried particle. The preparation of the neem-Aza A capsule based on sodium alginate and Poly (vinyl alcohol) (PVA) using as the crosslinking agent have not been found in the literature reviews.

To the best of our knowledge, this is the first of its kind of study wherein the two biodegradable polymer (PVA and sodium alginate) beads containing Aza are being prepared by coaservation technique. In the work presented here we test the feasibility of encapsulating Aza in a matrix made from poly (vinyl alcohol) and sodium alginate to produce a product with good end-use properties.

2. Methodology

2.1Materials

Neem seed kernels were purchased from local Thailand. A 4% sodium alginate solution in distilled water was prepared by gentle heating. After complete cooling different amounts of neem were added and mixed thoroughly using a magnetic stirrer. The polymer solution containing neem (sodium alginate alone or mixture of 75% sodium alginate and 25% polyvinyl alcohol) was added dropwise into methanol containing 1% glutaraldehyde and 1% of 1 N HCl, using a 25-ml hypodermic syringe (1 mm diameter) with constant stirring. The beads formed were removed from

methanol at a selected time interval say 10, 20 and 30 min. The beads were washed with water and then dried. At particular intervals, the remaining concentration of Aza was analyzed by HPLC. About 20 mg exactly weighed microcapsule sample was extracted in distilled water to form homogeneous solution for 48 h. The total Aza-A in the solution was extracted by mixture between 50/50 MeOH/H_2O and its mass was determined by a HPLC. The morphology of capsule was investigated by SEM. Sodium alginate, glutaraldehyde (25% w/v) solution and AR grade methanol samples were all purchased from Fluka agent, Thailand. The concentrated NR latex used in this study is a high ammonia latex received from Jana company, Co., Ltd. (Songkla Thailand)

2.2 Methods

2.2.1 Preparation of capsule beads and efficiency of entrapment

A 4% sodium alginate solution in distilled water was prepared in a heating mantle. After complete cooling neem Aza-A (7000 ppm) was added at different concentrations and mixed thoroughly using a magnetic stirrer. The polymer solution containing neem Aza-A was added dropwise into methanol containing 1% glutaraldehyde and 1% of 1 N HCl, using a 25-ml hypodermic syringe (0.8 mm diameter) with constant stirring. The beads formed were removed from methanol after 10, 20 and 30 min. The beads were washed with water and then dried. The efficiency of entrapment was calculated as the ratio between the initial mass of neem Aza-A to be encapsulated and its mass in the final product. About 20 mg of the exactly weighed microcapsule sample was mixed in distilled water (250 ml) to form a homogeneous suspension. The total neem Aza-A in the solution was extracted with a 50/50 MeOH/H_2O mixture for 48 h and the amount was determined by HPLC (PerkinElmer LC).

2.2.2 Drying rate study of the beads

3 equal samples of the beads formed after crosslinking with GA were selected for the drying study and were allowed to dry in an oven (VELP) maintained at 31± 2°C The bead weights were taken at definite time intervals until constant weights were achieved. All the weight measurements were made on a Mettler single pan balance (Model AB 204, Mettler). In order to obtain reproducible results, experiments were

conducted in triplicate, and the average values were used for the calculation and plotting of the data vs. time.

2.2.3 Bead size measurement

Five samples of the completely dried beads from the different formulations were selected and their sizes were measured using a micrometer screw gauge (Sargent, USA) with an accuracy of ±0.01 mm.

2.2.4 Swelling study of the individual beads

The swelling property of the beads was subjected to a measurement of their swelling ratio in an aqueous medium as a function of time. The bead samples exposed to GA for different times at 26±2^{O}C were selected and incubated with distilled water in a watch glass. The mass of all bead samples was taken at measured time intervals and the average value was calculated. During this process, care was exercised during handling of the swollen beads to avoid any weight loss due to breaking or erosion of the beads. All the weight measurements of the swollen beads were taken on a Mettler single pan balance with accuracy up to the fifth decimal. The percentage swelling ratio of the beads was calculated as in equation 1.

$$\%\text{Swelling ratio} = \frac{(\text{Wet weight-dry weight})*100}{\text{dry weight}} \quad \ldots\ldots (1)$$

2.2.5 Fourier transformed infrared (FTIR) measurements

Fourier transformed infrared; FTIR (Bruker, EQUINOX 55) spectral data were obtained to detect any chemical interactions between neem and the sodium alginate and sodium alginate alone.

2.2.6 Scanning electron microscope (SEM)

The aim of the SEM study was to obtain a topographical characterization of the beads. The sample was deposited on a brass hold and sputtered with gold. SEM photographs were taken with a JSM 6400 Scanning Microscope (Japan) at the required magnification at room temperature. +

2.2.7Electron probe microanalysis (EPMA)

The capsule specimen was analyzed with a fully shielded CAMECA-SXR-50 electron probe microanalysis (EPMA) device equipped with four spectrometers and operated at 20 keV. Secondary electron (SE) imaging was conducted on the specimens.

2.2.8 Atomic force microscope (AFM)

An Atomic Force Microscope - Multimode Nanoscope IIIA from Digital Instruments, Santa Barbara, USA, was employed to investigate the topology, surface roughness of capsule , in contact mode. The specimen was probed with silicon nitride (Si3N4) tip at spring constant of 0.12 N/m. Tip velocity of 5 mm/s was used. The height and deflection images were recorded with the resolution of 512 lines.

2.2.9 Thermogravimetric Analysis (TGA)

Thermal studies were conducted with a Mettler-Toledo instrument (TGA/SDTA 851). The heating rate for thermogravimetric analysis of sodium alginate alone and the capsule beads was 30^{o}C/min. A small amount (1-3 mg) of sample was taken for the analysis and the samples heated from 30 to 800^{o}C in nitrogen. The TGA and DTG curves have been drawn for each sample.

2.2.10 Content uniformity, Dissolution and Releasing studies

Beads were evaluated for their neem content by refluxing a known mass of the beads with 100 ml of methanol at 65°C. Refluxing was continued for 1 h to ensure complete extraction of neem from the beads. The absorbance of the methanol solution containing the extracted neem was measured at 211nm in a HPLC (PerkinElmer LC) using pure methanol as a blank.

The dissolution study was carried out in 250-ml conical flasks containing the dissolution medium (0.1% Tween-80 solution in distilled water) with closure caps and kept at 35°C in an incubator (WTB Binder, Germany). Two or three beads weighing about 10 mg were taken added to the dissolution medium. At definite time intervals of time, the conical flasks were well shaken well and a 10-ml aliquot was taken for the analysis of neem Aza-A using HPLC (PerkinElmer LC) at 211 nm. Experiments were performed in triplicate in order to minimize the variation error. The cumulative release of neem Aza-A from the capsule beads was estimated using an empirical equation to estimate the value of n as follows (equation 2)

$$Mt/M\infty = Kt^n \text{ or } \log (Mt/M\infty) = \log (K) + n\log(t) \quad \text{.....(2)}$$

Where $Mt/M\infty$ is the released fraction at time t, n is the release exponent, and K is the release factor. From the slope and intercept of the plot of log ($Mt/M\infty$) against log (t), the kinetic parameter n was calculated.

3. Results and discussion

3.1Preparation of Aza-A neem capsule obtained from blend between sodium alginate and poly(vinyl alcohol)

The encapsulation of neem (as called capsule) was obtained by using sodium alginate as a controlled release polymer after crosslinking with glutaraldehyde, and then the capsule was coated **NR.** Capsules were prepared using sodium alginate and PVA solutions at 5% (w/v) concentrations identified in **Table 1**. The average diameter of the capsules ranged from 1.2 to 1.4 mm. As shown in **Table 1**, the EE, expressed as the percentage of Aza entrapped, decreased with an increase in concentration of neem, going from 84% for the 2.5 % neem down to 72% for the 5 g of neem. It must be pointed out that the concentration of neem had also a marked influence on the neem loss from the capsules. Capsules formed using mixture between 5 g alginate solution and 5 g PVA solution and 5 g neem were very weak and loose, whereas capsules prepared with the solution at mixture between 5 g alginate solution and 5 g PVA solution and 5 g neem were more compact and had an appropriate mechanical resistance. The appearance of all capsules were spherical shape, exception at sample obtained from mixture between 2.5 g alginate solution and 7.5 g PVA solution and 2 g neem and capsule derived from10 g PVA solution and 2 g neem due its rheology of solution.

Table 1 Concentration of alginate and PVA solution, glutaral aldehyde concentration, EE and neem content of capsules and appearance and particle size of capsule
Interaction between PVA and sodium alginate by FTIR and XRD

run	5%PVA (g)	5%NaAg (g)	Neem Aza-A (g)	GA	Appearance	Particle size (μm)	EE
1	5	5	1	5	Spherical	1.26±0.02	84
2	5	5	2.5	5	Spherical	1.27±0.02	76
3	5	5	5	5	Spherical	1.25±0.02	72
4	5	5	2.5	2.5	Spherical	1.30±0.03	76
5	5	5	2.5	7.5	Spherical	1.28±0.02	77
6	2.5	7.5	2.5	5	Egg-shaped	1.36±0.03	76
7	7.5	2.5	2.5	5	Spherical	1.46±0.03	82
8	10	0	2.5	5	-	-	-
9	0	10	2.5	5	Egg-shaped	1.28±0.02	76

The semi-interpenetrating polymer network of sodium alginate-polyvinyl alcohol containing Aza capsule was prepared by the solution method. The influence The semi-interpenetrating polymer network of sodium alginate-polyvinyl alcohol containing Aza capsule was prepared by the solution method. The influence of the glutaraldehyde storage time on the properties of the polymer blend was studied. The presence of a large number of hydroxyl groups in PVA resulting from capsule and strong hydrogen bonding (may be both the intermolecular and intramolecular types in polymer metric), will affect the solubility of PVA in water. The FT-IR spectrum of PVA alone, sodium alginate alone, the capsule is shown in **Figure 2.** Glutaraldehyde treatment of PVA produces intermediate heat stability. The ester linkage of the semi-interpenetrating sample is confirmed by FTIR.

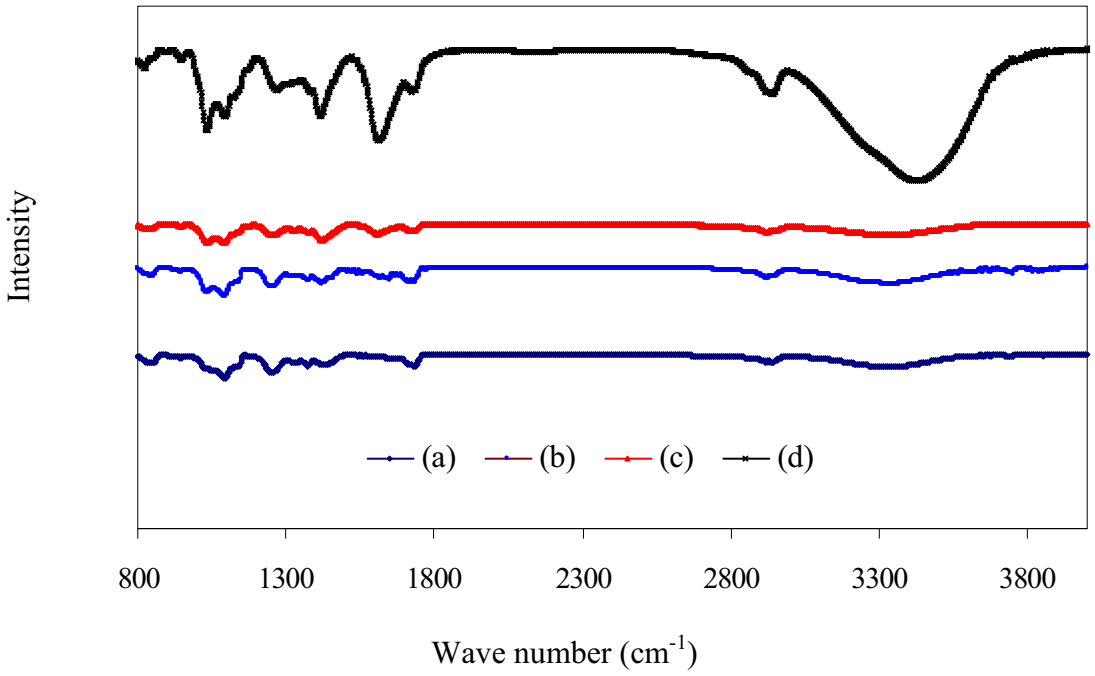


Figure1 FTIR spectra of (a) PVA capsule alone (b) 75/25 PVA/NaAg, capsule (c) 50/50 PVA/NaAg, capsule (d) 25/75 PVA/NaAg, capsule

The main peaks of PVA alone shown at 1327 and 843, and 1087 cm^{-1} are attributed to C-H bending and C-O stretching, respectively. The changes of the characteristic spectra peaks reflect the chemical interactions when two or more substances are blended. In the typical spectrum of the semi-IPN sample sheet, the characteristic peak at 1729 cm^{-1} was shifted to 1730 cm^{-1}. This document indicates that there are hydrogen bonded interactions between the hydroxyl groups, carbonyl groups of PVA and carbonyl groups of glutaldehyde.

3.2XRD results

The XRD scans of the semi-interpenetrating polymer network between sodium alginate and PVA, are shown in **Figure 2**. The observed PVA alone spectrum reveals a semi-crystallinine feature. It is vital to note that there are two halos cited at 19.5 and 40.5^{o}. The first one has a clear crystalline peak at scattering angles 2θ = 19.5^{o} and 40.5^{o}. The first one has a clear crystalline peak at a scattering angle 2θ =19.5^{o} that corresponds to a (101) spacing. The second halo has a low intensity and broad shape

and corresponds to noncrystalline zones within the crystalline polymer matrix. After adding glutaraldehyde in the semi-interpenetrating sample sheet, the intensity of the diffraction peak at 19 ^{o} for PVA becomes gradually flat and broad. This phenomenon is due to the significant hydrogen bonding interactions among PVA, sodium alginate and glutaraldehyde molecules and covalent bond between PVA and glutaraldehyde. In other word, the addition of glutaraldehyde in the semi-interpenetrating sample improves the compatibility between PVA and sodium alginate.

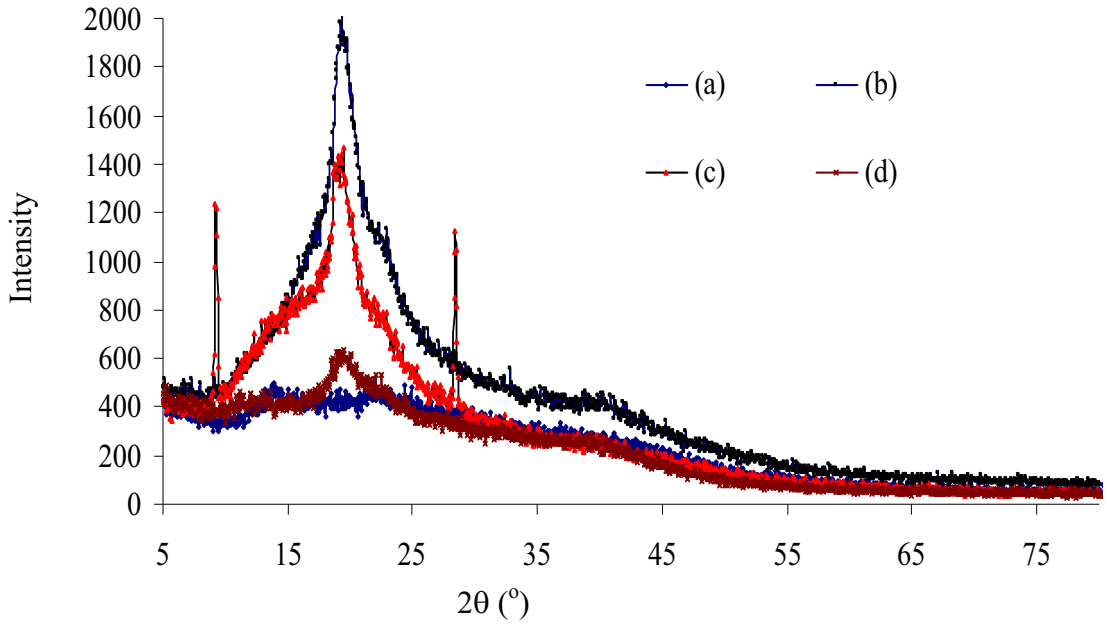


Figure 2 XRD spectra of (a) PVA capsule alone (b) 75/25 PVA/NaAg, capsule (c) 50/50 PVA/NaAg, capsule (d) 25/75 PVA/NaAg, capsule

3.3 Swelling behavior of capsule

The effect of PVA/Sodium alginate blend ratio on the percentage of swelling ratio in water medium is shown in **Figure 3**. The beads obtained from sodium alginate alone show a maximum uptake of water during the fifth hour. The beads derived from PVA and sodium alginate absorbed lower water than the beads obtained from sodium alginate alone due to crystallization of PVA. Equilibrium swelling ratio was achieved in 180 h for sodium alginate alone. The particles produced in this work were analyzed for their sizes using the light scattering method. Aza-A release from the

capsule was measured after subjecting them to a number of physical and chemical parameters including those related directly to the release medium and those resulting from changes to the characteristics of the structures controlling release (beads).

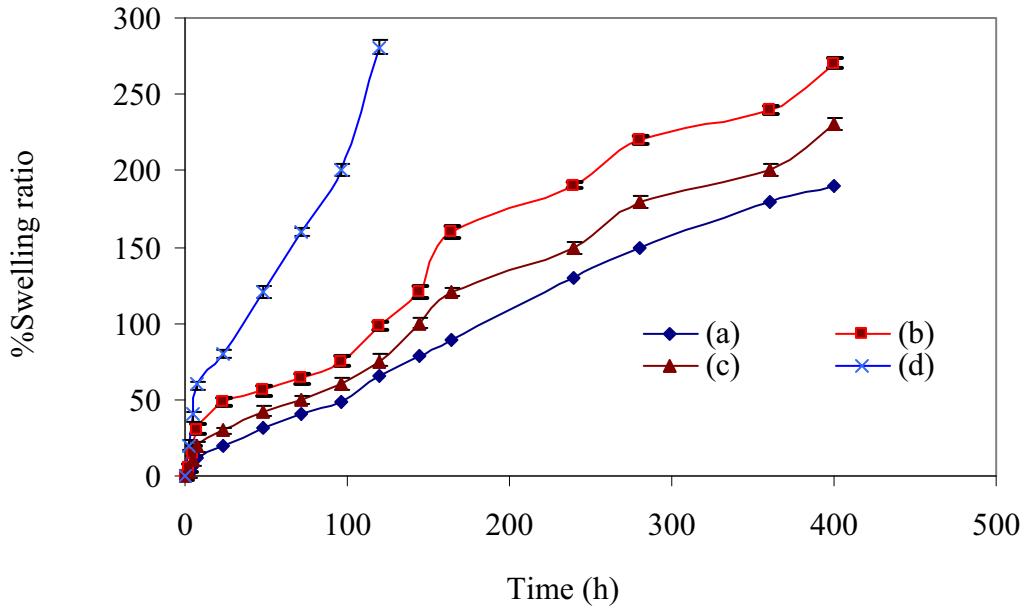


Figure 3 Swelling ratio of capsule (a) PVA capsule alone (b) 75/25 PVA/NaAg, capsule (c) 50/50 PVA/NaAg, capsule (d) 25/75 PVA/NaAg, capsule

3.4Thermal study behaviour

DSC results

DSC experiment was studied to understand the thermal behavior of the capsules and these results are illustrated in **Figure 4**. Temperature of the end point of endotherm peak shifted to 10 ^{o}C with the addition of sodium alginate in capsule matrix. Tg value of PVA polymer used in this study was found to be 60^{o}C, whereas the value for blend PVA/NaAg 75/25 was found to be 70^{o}C, while Tg of PVA/NaAg 50/50 was not observed. Tg of this polymer blend (PVA/NaAg; 75/25) was affected by interaction between them.

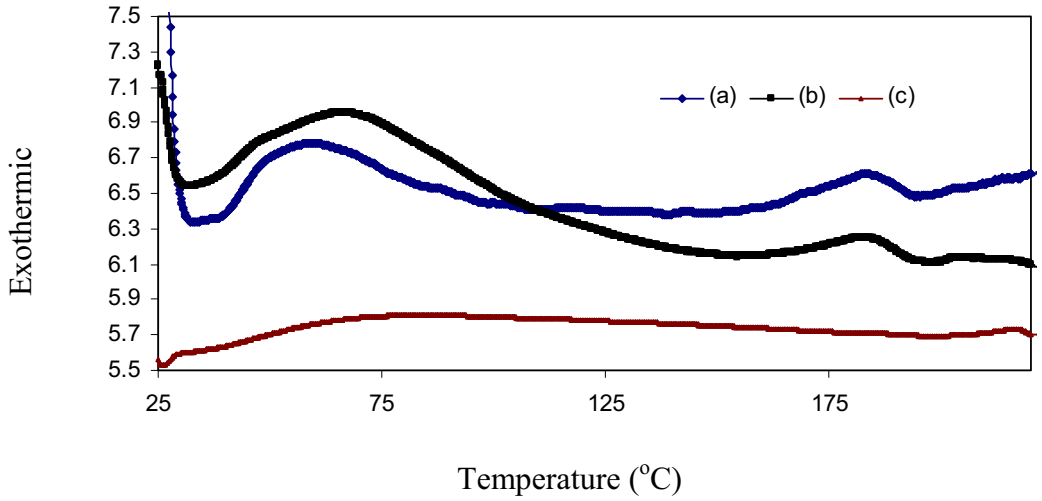


Figure 4 DSC of (a) PVA capsule alone (b) 75/25 PVA/NaAg, capsule and (c) 50/50 PVA/NaAg, capsul

TGA study

The objective of this TGA is to investigate the variation and to understand the thermal behavior and the capsule polymer structure modifications. The TGA and DTGA measurements of capsule in the presence of glutaraldehyde are shown in **Figure 5** and **Figure 6,** respectively**.**

From the thermogravimetric analysis and observations of the temperature maximum, a better heat stability for the glutaldehyde cross-linked PVA was observed. Three temperature regions can be identified over which most of the weight change occurs in the capsule. The first weight loss occurs between 90 and 120°C which correspond to the removal of water. The second weight loss occurs between 300°C and 400 °C and corresponds to the side chain decomposition of PVA molecule. Third degradation between 400 and 550°C corresponds to the decomposition of the PVA main chain.

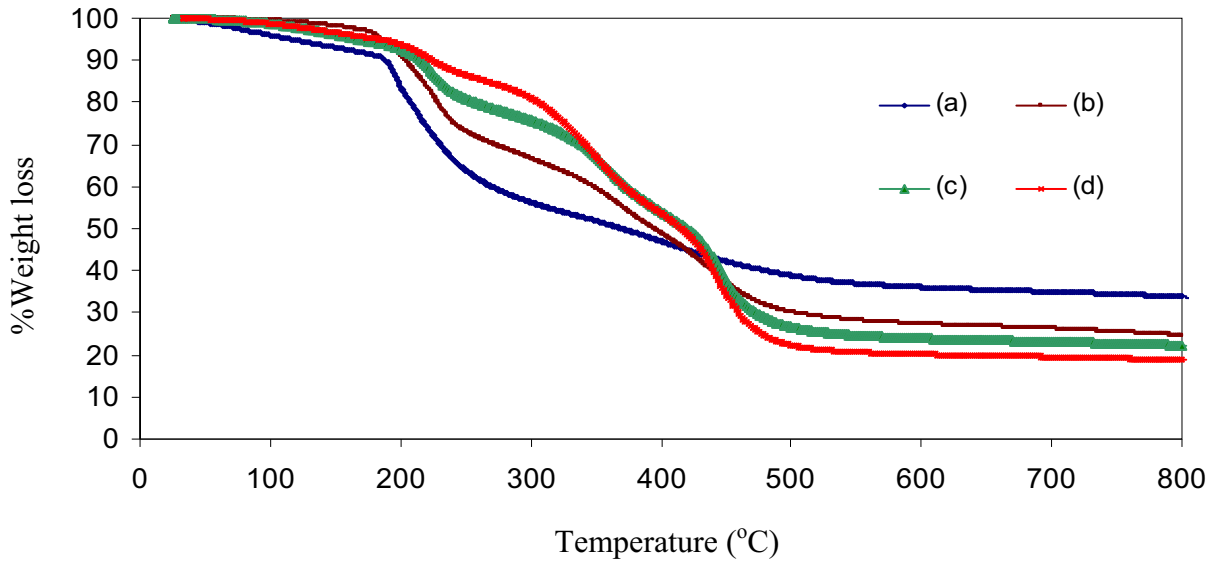


Figure 5 TGA of (a)PVA capsule alone (b) 75/25 PVA/NaAg, capsule (c) 50/50 PVA/NaAg, capsule (d) 25/75 PVA/NaAg, capsule

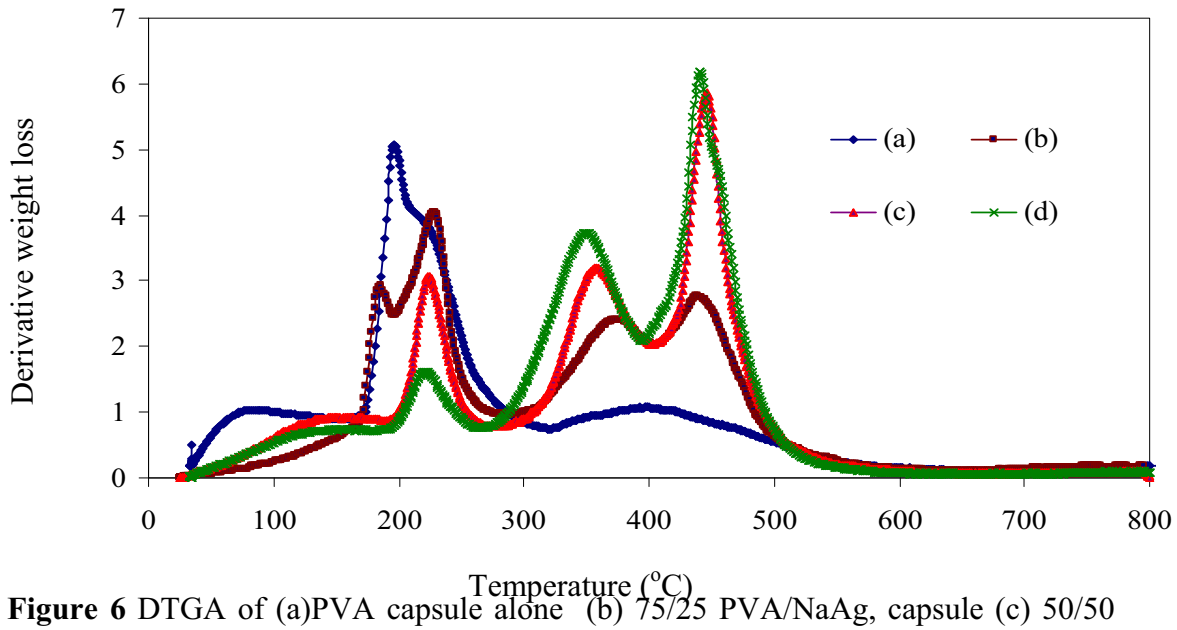


Figure 6 DTGA of (a)PVA capsule alone (b) 75/25 PVA/NaAg, capsule (c) 50/50 PVA/NaAg, capsule (d) 25/75 PVA/NaAg, capsule

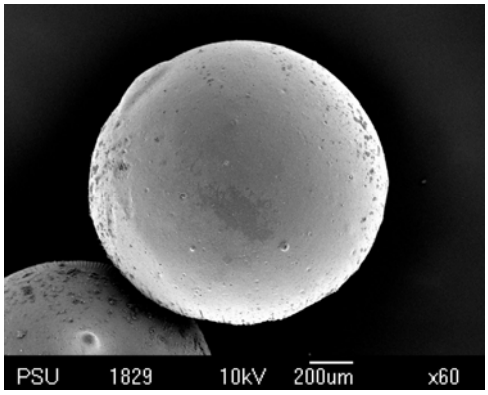
3.5 SEM, EMPA and AFM study

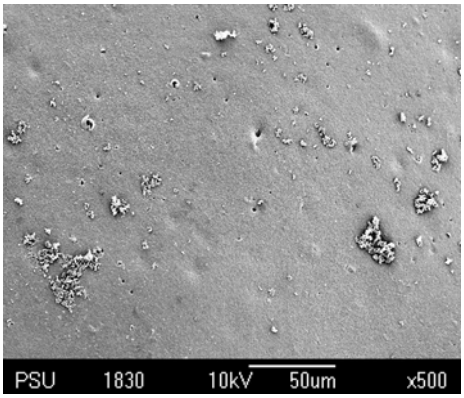
SEM photographs of a several beads taken are shown in **Figure 7**. Since it is seen from the **Figure 7**, all sample beads are almost of spherical shape capsule obtained

from showed egg-like shape. EMPA was applied to investigate the polymer blend matrix from capsule. We investigated the spatial distribution of the PVA components on the surface of the capsule. The hydroxyl groups of the PVA-containing capsules were reacted with glutaraldehyde , and their distribution was analyzed by EPMA (**Figure 8**). We found that the surface distribution of PVA in the capsule sample was uniform. The different colors depend on the amounts of the element atoms in the sample, in which the highest element atom shows up as white and then decreases as following red orange, yellow, green, blue and violet. The surface PVA in the capsule sample was localized in high-density islands of ca. 25 to 30 micron diameter. Atomic force microscopy (AFM) is a vital tool that can be applied to directly study the morphology of capsule. **Figure 9** shows the two-dimensional images of capsule with sodium alginate/PVA. The amplitude parameters analysis shows that the roughness average is only 250 nm. These results suggest that the surface of starch/PVA blend films became level and roghness due to the polymer blend ratio and chemical reaction between PVA or sodium alginate and glutaral aldehyde.

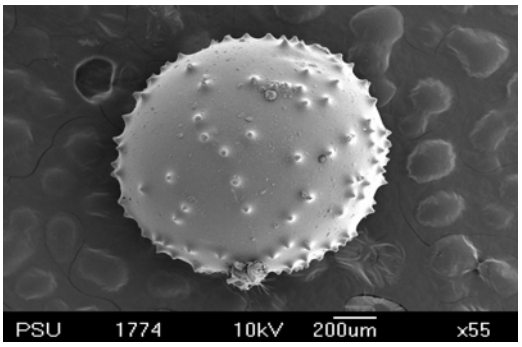
Expand region

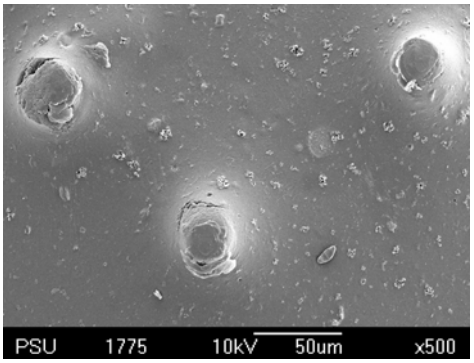
(a)



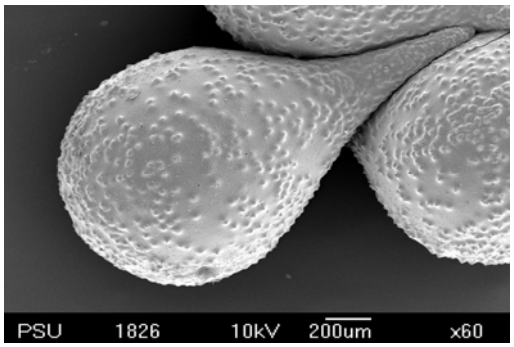


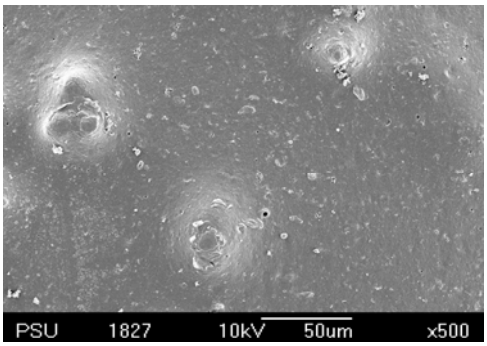
(b)





(c)





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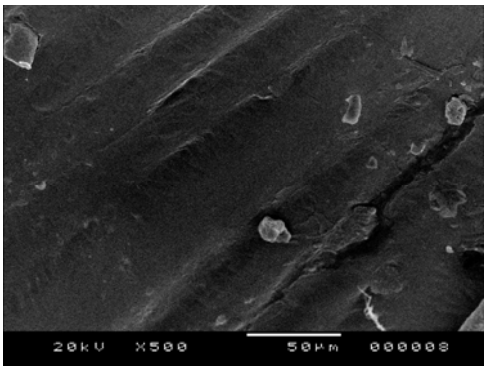
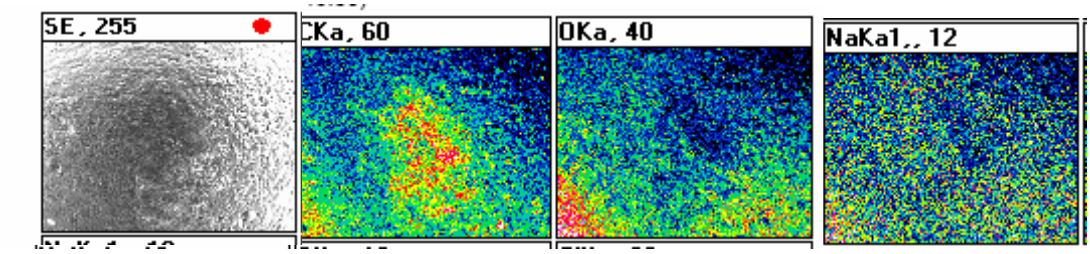
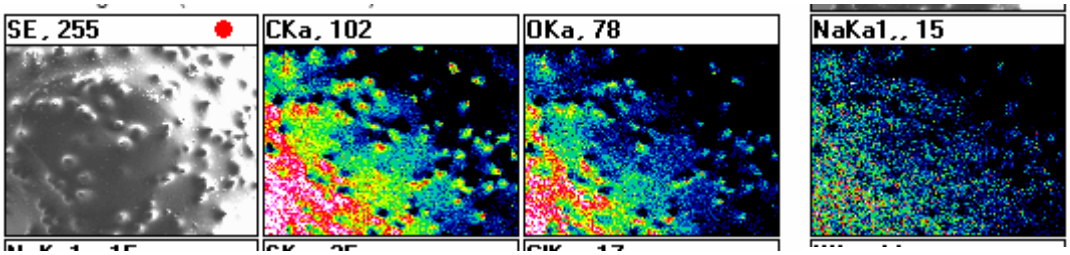


Figure 7 Scanning electron micrographs of (a) 75/25 PVA/NaAg, capsule (b) 50/50 PVA/NaAg, capsule (c) 25/75 PVA/NaAg, capsule and (d) 100 NaAg

(a)



(b)



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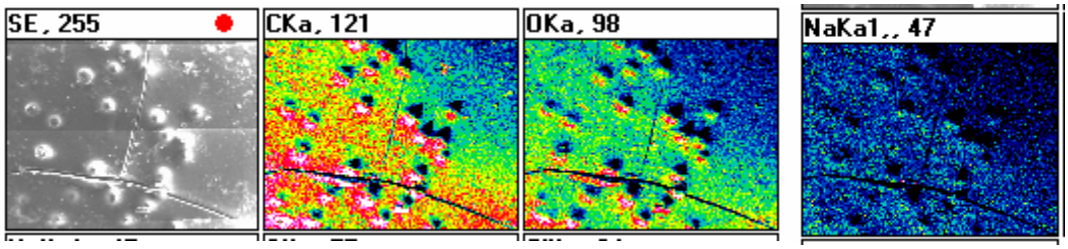


(d)

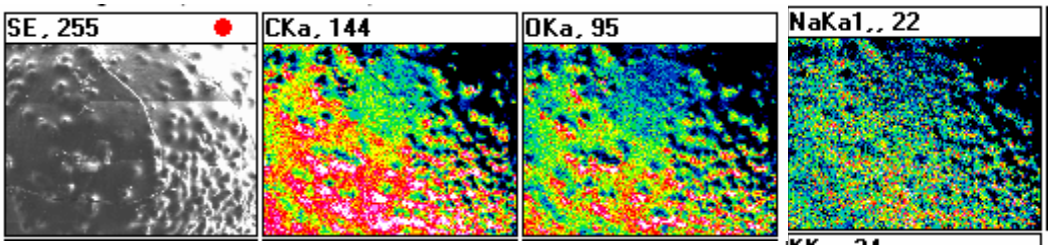
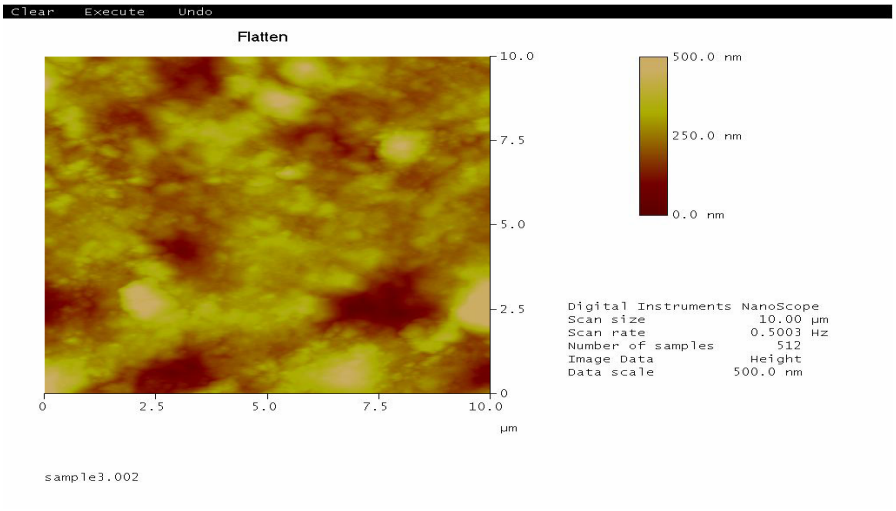
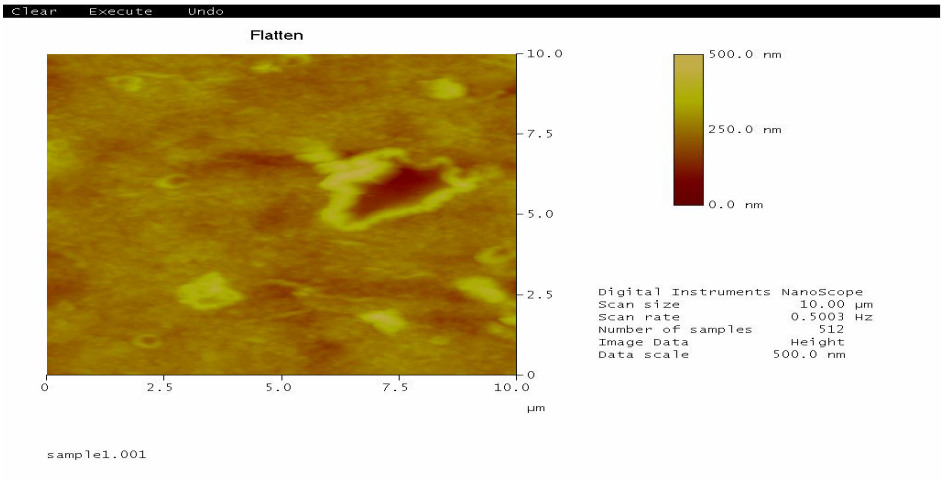


Figure 8 EPMA micrographs of (a) 75/25 PVA/NaAg, capsule (b) 50/50 PVA/NaAg, capsule (c) 25/75 PVA/NaAg, capsule and (d) sodium alginate

(a)



(b)



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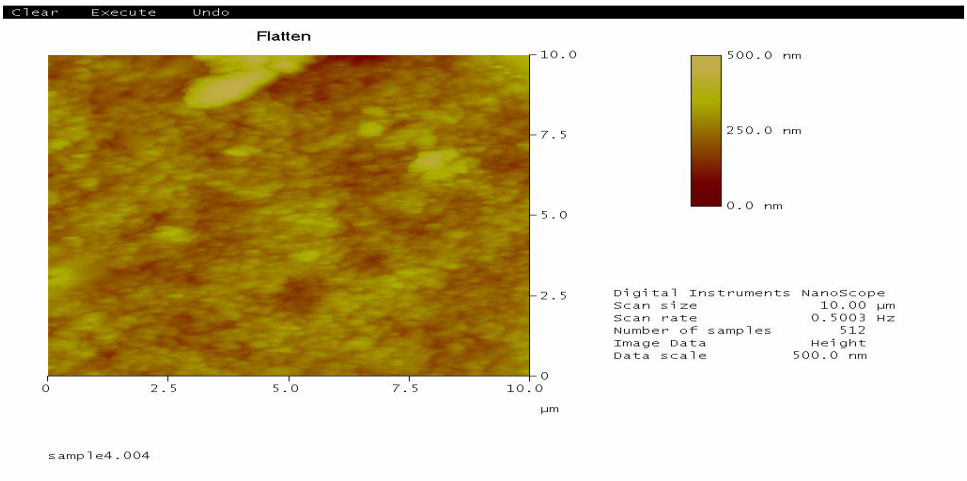

Figure 9 Scanning electron micrographs of (a) 75/25 PVA/NaAg, capsule (b) 50/50 PVA/NaAg, capsule (c) 25/75 PVA/NaAg, capsule

In **Figure 9** (a, b,c) the topographic images derived from atomic force microscopy of capsule obtained different polymer blend ratios between PVA and sodium alginate. It is clear that the morphology of capsule obtained from sodium alginate alone (Figure 9 (c) was more smooth than that of the capsule obtained from blend PVA and sodium alginate (Figure 9 (b) (c)).

3.7Releasing study

The effect of neem content in capsule on % cumulative releasing of neem is shown in **Figure 10**. **Figures 10** exhibits that % cumulative releasing from the 50/50 PVA/NaAg beads with 1 g is much higher than that leads in presence of 2.5 and 5.0 g. The cumulative releasing of neem for the beads increased as a function of releasing time. The maximum cumulative releasing of neem for the beads obtained from 1, 2.5 and 5 g of neem was 100, 81 and 70% at 450 h of releasing time. When the neem content in beads increases from 1 g to 2.5 g, % cumulative releasing of neem beads increases. Higher neem content might be lead to the easier penetration liquid through leads and then faster neem diffusion occurs from the beads. The other explanation is that while neem content in the beads increases, loose structure in the polymeric beads has formed in which high loading (1 g) is quicker than that of lower loading due to possibility of formation of a large pore volume, which might enhance the neem release.

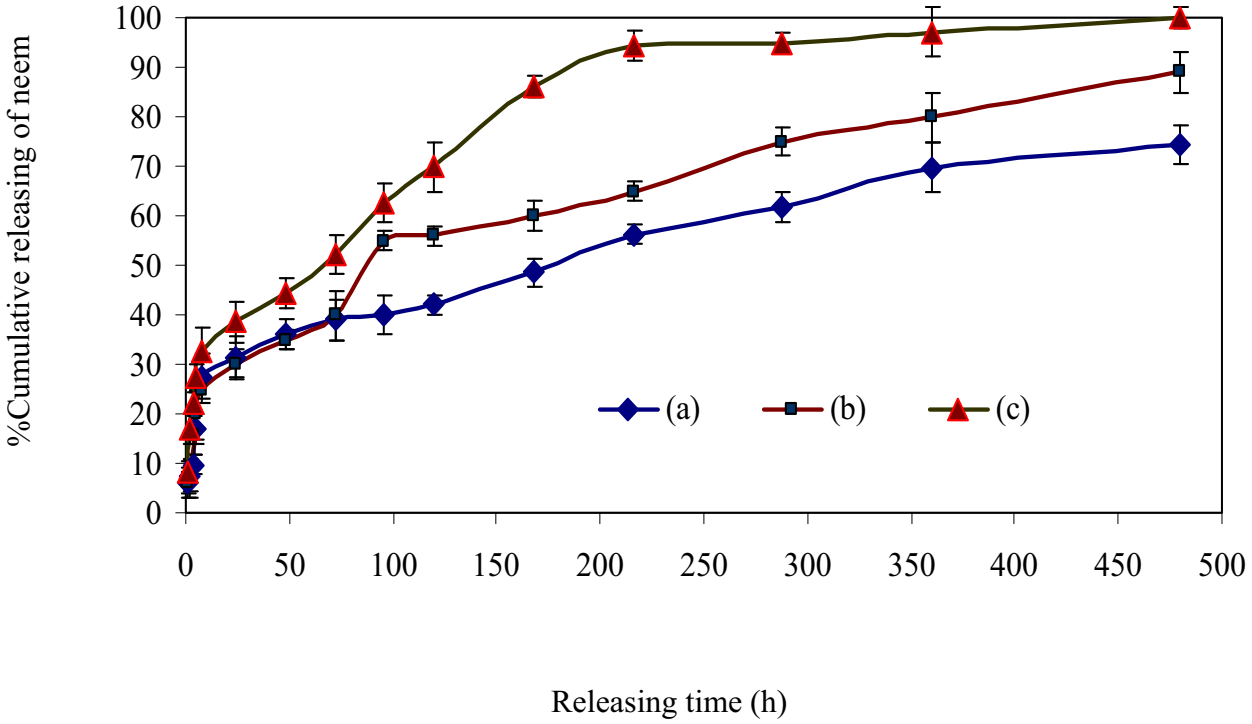

Figure10 Effect of neem content on the release neem for capsules obtained from PVA /NaAg 50/50 (a) 1 g, (b) 2.5 g and (c) 10 g

Neem release from the beads was subjected to a number of physical and chemical parameters including those related directly to the release medium, and those resulting from the change in the characteristics of the beads. One of the most effective ways to change release rate of beads is to change cross-link density of the matrix by employing varying concentrations of the cross-linking agent. The effect of exposure time to GA on the release rate of neem has been investigated at GA concentration namely 2.5, 5 and 7.5 %w/ v at 30 min of exposure time. The result is shown in **Figure 11**, which clearly indicates that with increasing GA concentration (2.5-7.5% w/v), the release rate decreases as a function of glutaraldehyde concentration. The maximum neem release from the 2.5 % w/v of glutaraldehyde PVA/NaAg beads, which were prepared with a glutaraldehyde of 2.5 % w/v, was found to be 100% at 480 h.The observed decreases in the cumulative release are due to the fact that increasing concentration of GA result in an increase in cross-link density of the bead which gives rise to a compact network of the polymer. Consequently, the free volume

reduces and penetration of water molecules and diffusion of neem molecules become difficult. These results were also supported by swelling measurements. Similar results were reported by many other workers. Kulkarni and coworkers [35] studied controlled release of diclofenac sodium from cross-linked alginate beads. They have reported that when the exposure to GA increased from 5 to 10 min at 25 °C and 40 °C, DS release significantly decreased. To understand the release of neem from cross-linked PVA/NaAg beads in aqueous release study was carried out at 32 °C. Figure 12 displays the cumulative neem release of beads in different PVA/NaAlg ratios (with 0/10, 2.5/7.5, 5/5, 7.5/2.5, neem 2.5 g , and 5% GA concentration). From the Figures 12, it is observed that release rate of neem is much higher for the NaAg beads than for the PVA/NaAg beads. The highest cumulative neem release obtained at the end of 480 h was 100% for NaAlg beads, which have2.5 g of neem. On the other hand; the least cumulative neem release obtained was found to be 62% with 7.5/2.5 PVA/NaAlg beads, which have 2.5 of neem. When the amount of PVA is increased in the PVA/NaAlg beads from 0/10 to 7.5/2.5, decreases in the neem release are also observed as it is seen from the figures. Alginate is a natural water-soluble polymer and contains hydroxyl and carboxyl groups, which impart hydrophilicity to the molecule. On the other hand, PVA is virtually a linear polymer with a small hydrated volume compared to alginate and thus PVA produces a compact network of macromolecular chains in the blend beads. Therefore, penetration of liquid molecules through PVA/NaAlg beads and then diffusion of drug to external medium is difficult compared to the NaAlg beads.

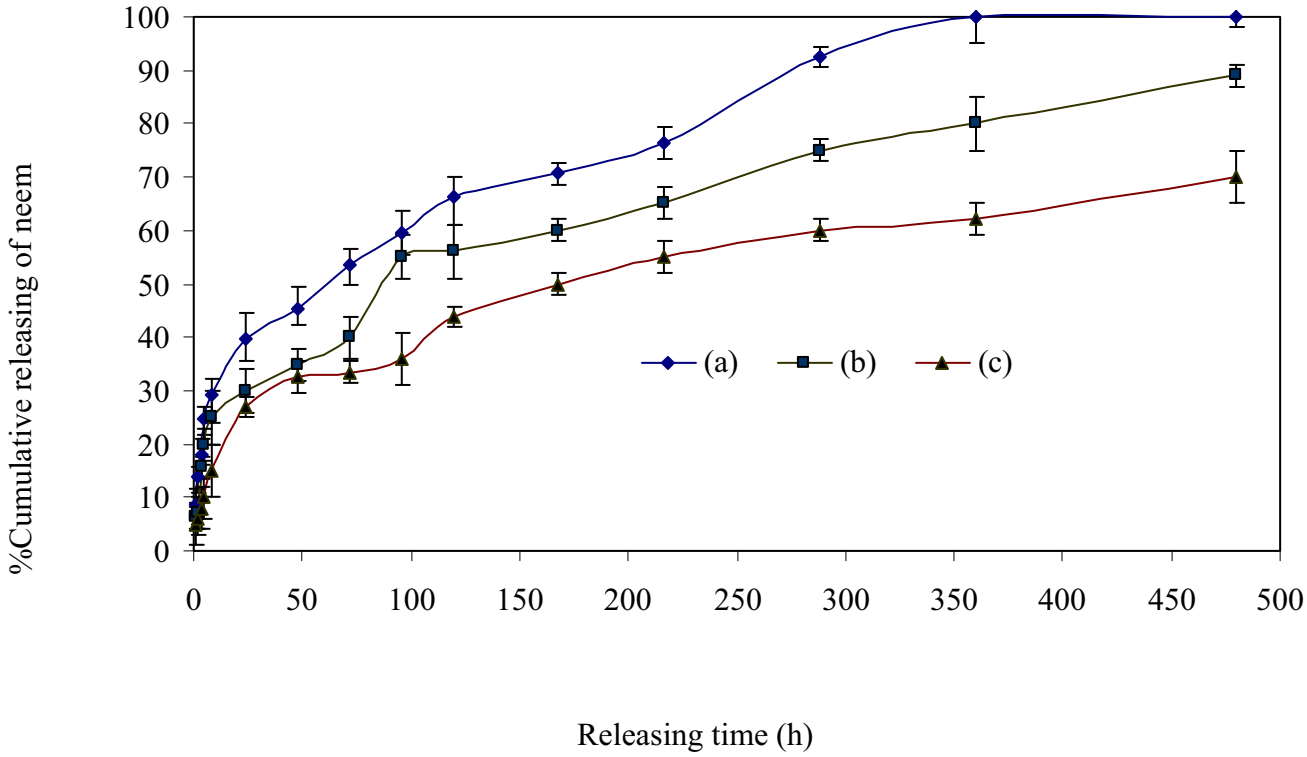

Figure 11 Effect of glutaraldehyde on the release neem Aza-A for 50/50 PVA/NaAg, capsule (a) 2.5, 5.0 and 7.5 % w/w glutaraldehyde

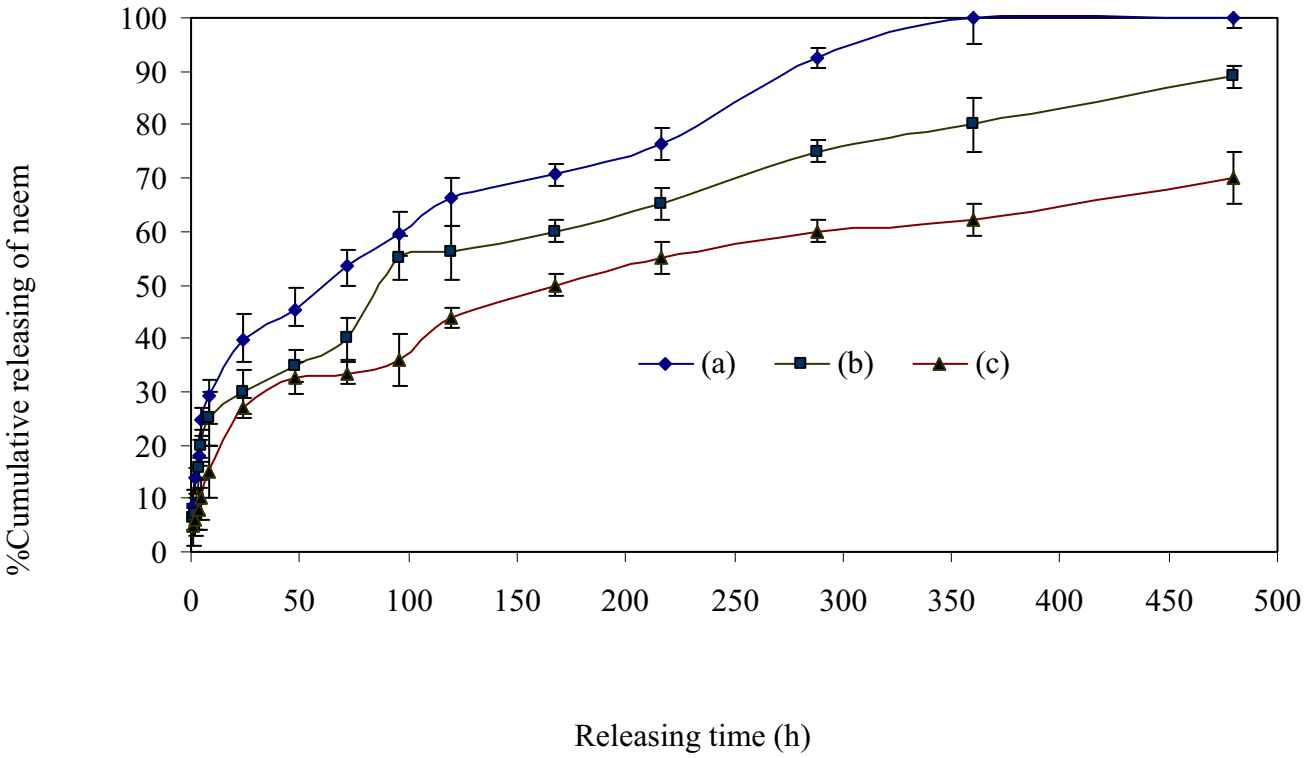

Figure 12 Effect of polymer blend ration on the release neem Aza-A for capsules a) 75/25 PVA/NaAg, capsule (b) 50/50 PVA/NaAg, capsule (c) 25/75 PVA/NaAg, capsule

3.8Releasing mechanism

The n value is an empirical parameter characterizing the release mechanism [10]. On the basis of the diffusion exponent, an n value of 0.5 indicates the nutrient release mechanism approaches to a Fickian diffusion controlled release, whereas when n is equal to 1.0 this indicates the nutrient release mechanism approaches to a zero-order release. An n value from 0.5 to 1.0 indicates a reactive agent release mechanism for a non-Fickian diffusion or a chain relaxation controlled release. From the plot of log (M_t/M_∞) against log(t), release exponent (n) has been calculated (Figure5). The n value is in the range from 0.2995 to 0.4062. The n value with 0.3289 of sample obtained from 75/25 PVA/sodium alginate for 30 min storage time in glutaraldehyde

solution indicates that the release in this system deviates from a Fickian diffusion controlled release. The n of capsule beads obtained from 50/50, 25/75 PVA/sodium alginate at storage time was 0.2909 and 0.4062, respectively, indicating that these systems exhibited non-Fickian diffusion. These results deduced that the n value of this system depends on blend ratio leading to different crosslinking density contents and different neem release mechanism patterns in the sample.

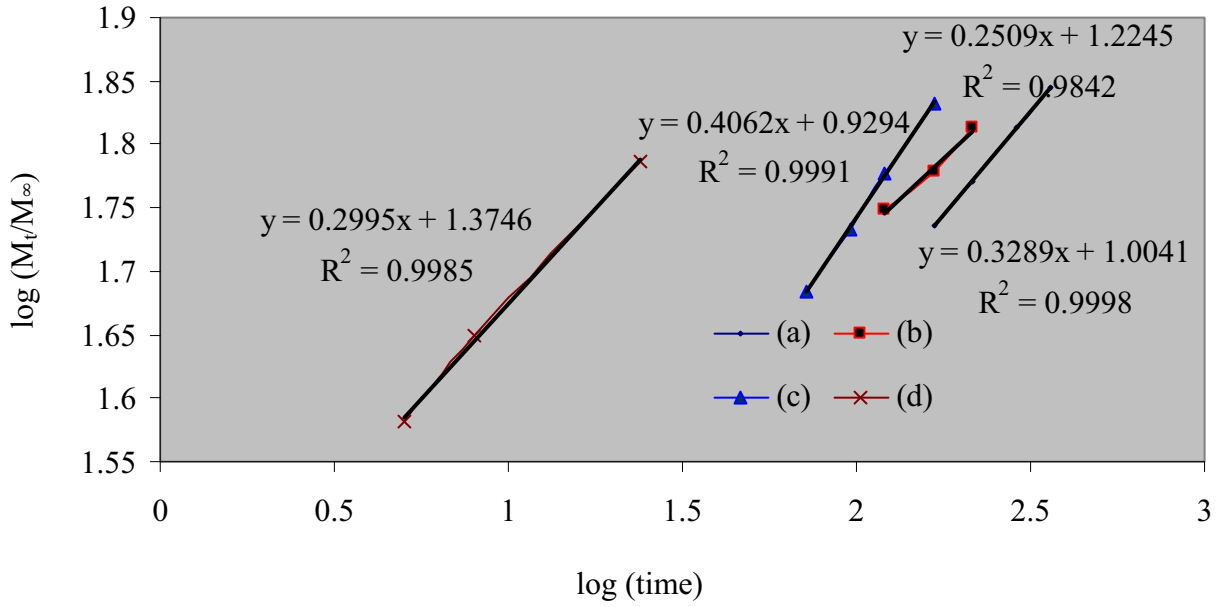

Figure 13 Relationship between log (M_t/M_∞) and log(time)

4. Conclusions

The neem can be successfully encapsulated into the sodium-alginated and polyvinyl alcohol containing glutaraldehyde matrix for coaservation method. High amount of PVA in capsules had a pronounced effect on slow release neem. The high glutaraldehyde concentration in capsule could be used as an adequate barrier of the capsules to give high amount crosslinking density in matrix and consequently more efficient release of neem as compared to the capsules obtained from low concentration of glutaraldehyde. The rate of release of neem in aqueous medium depends on concentration to glutaralaldehyde and type of polymer.

Acknowledgement

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Chapter 7

Preparation of cyclized natural rubber as coating reagent for Aza capsule products

ABSTRACT

The property of natural rubber (NR) is more interesting, excepting for some properties need to improve. The cyclization reaction is an interesting reaction for modified NR to thermoplastic elastomer. The kinetic cyclization of deproteinized natural rubber (DPNR) or purified NR latex effectively performed in latex phase by using trimethylsilyl-trifluoromethane sulphonate or trimethyl silyl triflate (TMSOTF) was studied at various temperatures and times. The cyclized products were confirmed by NMR spectroscopies including one and two dimensional NMR. The kinetic of cyclization was investigated. It was found that the degree of cyclization in DPNR was as a function of cyclization conditions. The rate constant (k) was 200 (s^{-1}) at 100^{o}C based on 95% of regression and the activation energy of cyclization in DPNR latex was 147.66 kJ/mol. Some thermodynamic parameters: enthalpy, entropy and free energy activation of 144.6 kJ/mole, -269.11J/mole and 246.9 kJ/mole, respectively were obtained for the cyclization of DPNR. The kinetic and thermodynamic parameters of cyclization obtained from this study indicate that an increase in the process temperature would increase the rate of cyclization formation.

1.Introduction

The field polymer science has been interested in preparing the cyclized rubber from natural rubber (NR), polyisoprene, styrene-butadiene rubber and polybutadiene since 1950s [1-18]. Cyclized products are used in the formulation of adhesives, paintings, inks and also in the compounding of NR to improve its mechanical characteristics [1-3]. NR and some unsaturated synthetic rubber can be transformed to cyclic structure or cyclized product by acidic reagents. Catalysts [1-15] for preparing cyclized NR were organic sulfonic acids and sulfonyl chlorides. sulfuric acid halides of amphoteric metals, in particular, titanium tetrachloride, antimony chloride, ferric chloride, and stannic chloride. Nowadays, there are still some new cyclized rubbers being synthesized, new catalyst, new cyclization methods, and

applications coming forth and a lot of patents being issued for which the cyclized rubbers as important components in the formulations. For example, Wang and co-worker [5] studied the controlled cyclization of styrene-butadiene rubber by using the aid of cationic catalyst system based on diethylaluminium chloride ($AlEt_2Cl$) and benzyl chloride ($C_6H_5CH_2Cl$) and by working in xylene solution at high temperature. Another example, Riyajan and co-worker studied the partially cyclized NR from the protein-removed NR latex or deproteinized natural rubber (DPNR) latex using sulfuric acid. It was found that the highest degree of cyclization was achieved due to low protein content in DPNR [16].

Trimethyl silyl triflate (TMSOTF) was used as a new catalyst for preparation of cyclization of DPNR in latex state [17]. This catalyst is expected to enhance solubility of cyclized NR derived from latex form. The kinetic of cyclization in DPNR in presence of TMSOTF was not investigated. Therefore, it would be studied in this work. Effects of reaction time and temperature on the degree of cyclization were studied to get the information for the kinetic of cyclization in DPNR latex. For example for kinetic of cyclization in NR latex, Gordon [19] has reported results for the kinetics of the cyclization reaction using sulphuric acid as the cyclizing reagent. The progress of the reaction was followed dilatometrically. Typical curves were reported by Gordon [19] for the variation of extent of reaction with reaction time. It was found that the occurrence of an initial period of reduced rate was attributed to the need for the sulphuric acid to diffuse into the rubber particles before the maximum rate of reaction time could be achieved. According to Gordon, the rate cyclization correlates well with the Hammett acidity function, Ho, for the aqueous phase of the reaction system. The function is defined as

$$Ho = -\log \frac{a_{H^+} a_B}{a_{BH^+}} + \frac{\log C_B}{C_{BH^+}}$$

Where the as denote acitivities, the CS concentrations, and subscripts B, BH^+ refer respectively to a Bronsted base and its conjugate acid. The first term on the right-hand side of equation is equal to pK_a for the conjugate acid. Gordon regards this correlation between Ho and the rate of cyclization as precluding a chain mechanism for the reaction, suggesting instead a mechanism which involves proton transfer.

The aim of this work is to study the kinetic of cyclization in the partially cyclized DPNR from the protein-removed NR latex or DPNR latex using TMSOTF as a catalyst. In addition, the thermodynamic parameters such as enthalpy, entropy and free energy activation were also investigated. The degree of cyclization was estimated by NMR technique.

2.Experimental

2.1 Materials and chemicals

High-ammonia natural rubber (HANR) latex was provided from Thai Rubber Latex Co.Ltd (Thailand). Terric 320, a surfactant composed of higher fatty alcohol ethoxylate, was supplied from The East Asiatic (Thailand) Public Co. DPNR latex was produced from HANR latex by enzymatic deproteinization. HANR latex was diluted to 30% dry rubber content (DRC) and incubated with 0.05% (w/v) proteolytic enzyme (KaO, KP 3939) and 1% (w/v) sodium dodecyl sulfate (SDS) at 37^{o}C for 24 h, followed by centrifugation at 13,000 rpm for 30 min. The cream fraction was re-dispersed with 1% (w/v) SDS to make 10% DRC and centrifuged twice. A part of DPNR latex was coagulated with methanol and dried under vacuum to get solid rubber. The obtained DPNR latex was used as starting materials for preparing cyclized DPNR.

2.2 Preparation of cyclized DPNR

Cyclization of DPNR in latex form was carried out by using DPNR latex of 50% DRC as a starting material. DPNR latex was mixed with 2% (w/v), Terric 320, using magnetic stirrer. The reaction was carried out by the addition of 34% w/w TMSOTF under various temperatures and times. The resulting cyclized rubber was coagulated by acetone and purified by reprecipitation with toluene/methanol, followed by drying *under vacuum* at 50^{o}C until getting a constant weight. The other similar reactions were carried out by varying dry rubber content of DPNR latex.

3.3 Measurements

^{1}H-NMR spectra and COSY were observed using $CDCl_3$ as a solvent on a BRUKER DPX-300 at 75 MHz. The degree of cyclization was estimated by NMR technique [15-18, 20].

3.Results and disscusion

3.1 Effect of time and temperature affecting on degree of cyclization

After the cyclized DPNR were prepared by using TMSOTF, its degree of cyclization was estimated by using NMR techniques. It was found that the degree of cyclization depends on temperature and time as shown in Table 1. Generally, degree of cyclization increase with temperature. The degree of cyclization of DPNR with 34% (w/w) TMSOTF at 75, 85, 95 and 100^{o}C was 0.15, 0.80, 0.97 and 0.97, respectively at 9 h (32400 sec) of reaction time. When the degree of cyclization is 1, it means that 100% degree of cyclization was observed. When the cyclization temperature was higher than 95^{o}C, the reaction between the reactive sites on DPNR molecules would be more pronounced. This result indicates that at high temperature was favor to the cyclization of DPNR with TMSOTF. The optimum temperature was 90 -100^{o}C for preparation of cyclized DPNR in this system.

The degree of cyclization of DPNR increased as reaction time increased. The cationic cyclization reaction is gradually increased, thereby needing several hours. It is clear that TMSOTF was an effective catalyst to accelerate the cyclization of DPNR latex, by an observing the cyclized product obtained within 1.5 h (5,400 sec) of reaction time at 95^{o}C. The largest part of the cyclization occurred within 5 h (18,000 sec), and the highest degree of cyclization value was observed after 9 h of reaction time at 95^{o}C.

Table 1 Effect of temperature and time on the degree of cyclization (x) in cyclized DPNR using 34% (w/v) of TMSOTF

Reaction time (second)	Degree of cyclization (x)			
	Temperature (^{o}C)			
	75	85	95	100
1800	0	0	0.06	0.05
3600	0	0	0.07	0.05
5400	0	0	0.10	0.10
7200	0	0	0.24	0.24
10800	0	0	0.74	0.74
14400	0	0.03	0.92	0.92
17720	0.02	0.36	0.94	0.94
25200	0.07	0.60	0.96	0.96
32400	0.15	0.80	0.97	0.97

Table 2 Relationship between gel content and degree of cyclization of the cyclized DPNR

Degree of cyclization(%)	Gel content (%)
7	1± 0.1
10	2 ±0.2
12	1±0.5
76	2±0.2
86	1±0.2

3.2Gel content

The cyclized DPNR was dissolved in distilled toluene to make 0.1% (w/v) solution. The rubber solution was kept in dark, without stirring for one week at room temperature. The solution was centrifuged at 8,000 rpm for 20 min to separate the insoluble or gel fractions. The gel fraction was coagulated with methanol and dried

under vacuum until getting a constant weight. The percentage of gel content was calculated by following Equation (1):

% Gel content = (weight of gel/ weight of original rubber) ×100 (1)

Table 2 shows the relationship between degree of cyclization and gel content of the cyclized DPNR resulting rubber. It is clear that low gel content (1-2%) was observed at all samples. This result reflect that small amount of crosslink was occurred.

Table 2

3.3 NMR spectroscopy

The structure of cyclized DPNR was confirmed DPNR by using both one and two dimensional NMR. It was found that the unsaturation methylene protons show singlet resonance signal at 5.16 ppm as shown in **Figure 1.** The signal at 2.10 ppm may be attributed to the methylene protons and the singlet resonance signal of the methyl proton appears at 1.70 ppm. The methyl protons attached to saturated carbons are observed as a triplet signal centered at 0.85 ppm. A centered signal at 0.85 ppm is assignable to the methyl protons attached to the first cyclic unit in the cyclized sequence. A centered signal at 1.30 ppm is due to the methylene protons attached to saturated carbons. These signals increased in intensity in return for the decrease of the methylene proton centered signal at 2.00 ppm, which is due to the methylene protons of original isoprene units. It is known that the methyl protons in the *cis*-1,4 and *trans*-1,4-isoprene units show a centered signal at 1.68 and 1.61 ppm, respectively, in $CDCl_3$. A new centered signal at 1.61 ppm is assignable to the methyl protons in the *trans*-1,4-isoprene units, which formed by isomerization of *cis*-1,4 isoprene units [15-17].

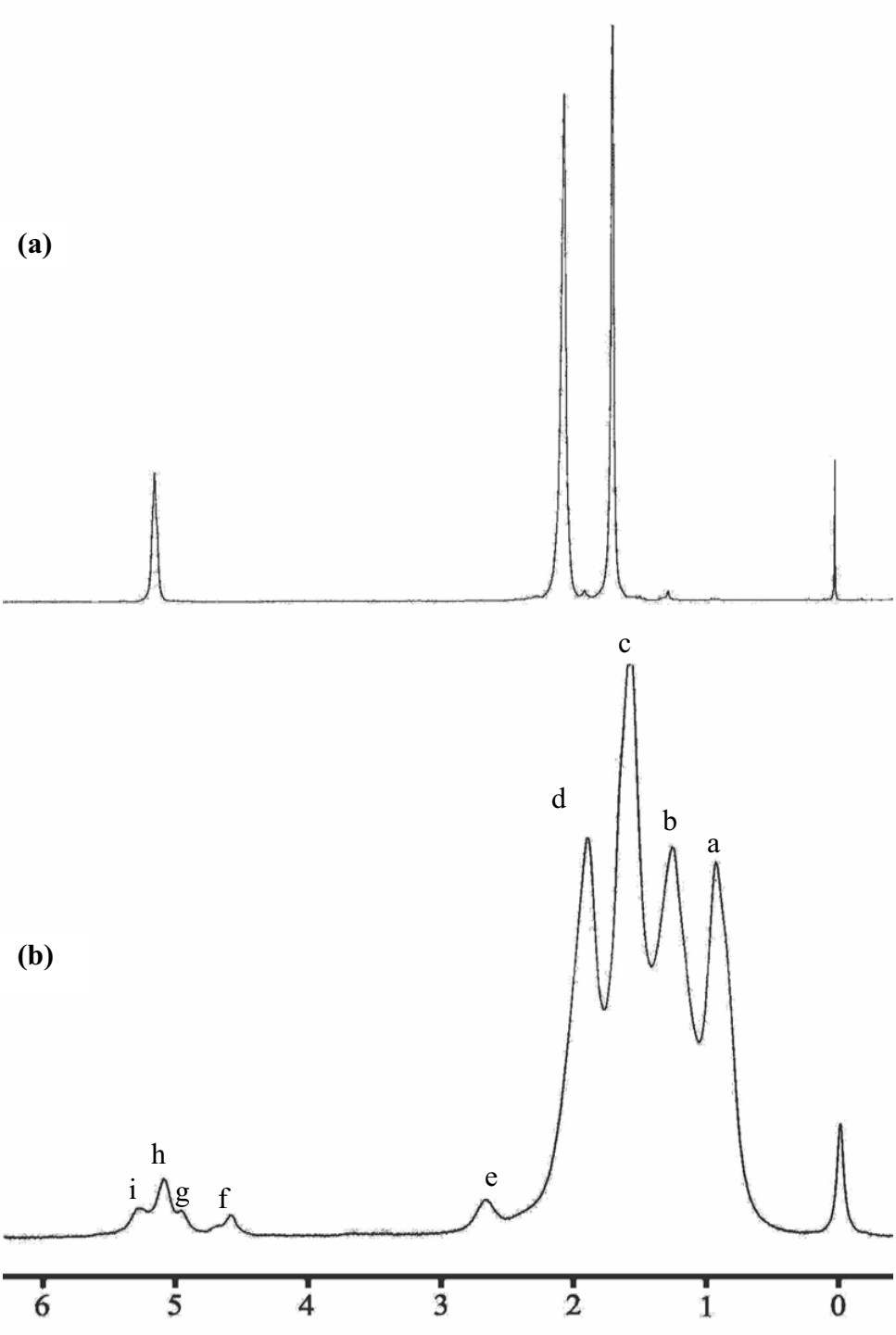

Figure 1. ^{1}H-NMR spectra of cyclized DPNR obtained (a) 0 and (b) 80% degree of cyclization

These chemical shifts agree with those reported previously by Patterson et al [18], with the exception that the use of higher magnetic fields (200 or 300 MHz), could allow the detection of 3,4-polyisoprene and exocyclic structures.

The structure of cyclized DPNR was again confirmed by COSY. In order to establish various connectivities in the cyclized rubber structure, the COSY ^{1}H-^{1}H NMR spectra were recorded as shown in **Figure. 2**. In the COSY spectrum apart from the direct coupling relayed couplings were also seen. *Cis*-1,4- and *trans*-1,4-polyisoprenes exhibited three signals at 5.1, 2.1 and 1.6 ppm, which were assigned to olefin, methylene, and methyl protons, respectively.

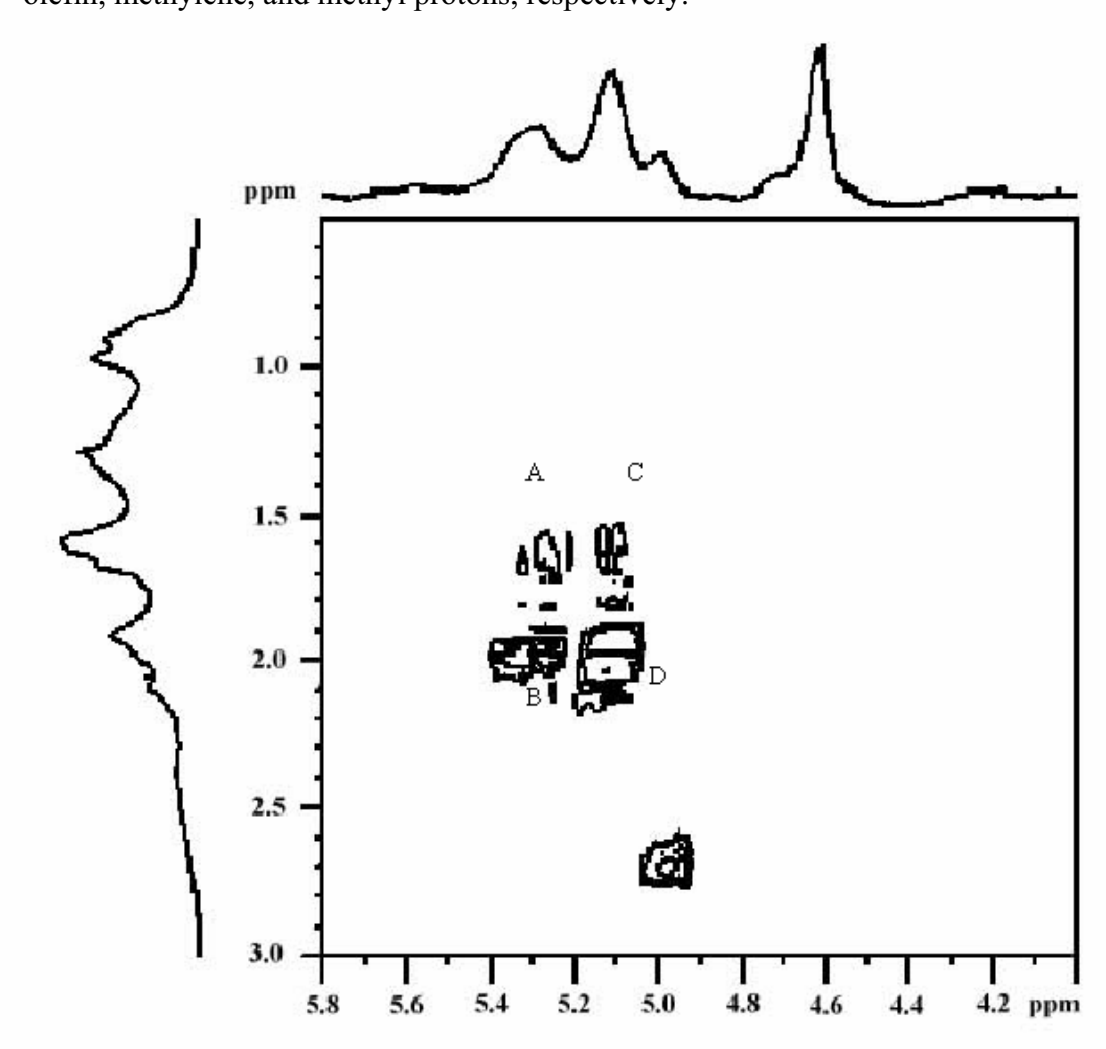

Figure 2. 2D-COSY expanded spectrum in olefinic region of cyclized DPNR with 80% degree of cyclization, observed at 300 MHz

Figure 2 represents the expanded region COSY of the cyclized DPNR with 80% degree of cyclization. It was found that many interactions among methyl, methylene and methine protons. For example, the cross-peak at 1.65/2.0 ppm was observed due to interaction between methyl group and methylene in cyclization product. The peak marked A near the middle of grid indicates that the connectivity between proton at 1.62 ppm and proton at 5.30 ppm. In addition, the peak B marked, signal proton at 5.30 ppm cross-peak with signal proton at 2.0 ppm due to interaction between methine and *trans*-methylene protons was observed. The peak C marked carbon shows the cross-peak between the signal proton at 5.16 ppm and signal proton at 1.65 ppm due to the coupling between methine proton of polyisoprene and *trans*-conformation methylene in polyisoprene chain. The correlation between the signal proton at 5.16 ppm and signal proton at 2.0 ppm attribute to interaction between methine and *cis*-conformation methylene was shown in peak D. In peak E, the interaction between methine at 4.90 ppm and methylene at 2.70 ppm.

3.4 Kinetic Parameters of Cyclization

After the cyclized DPNR were prepared by using TMSOTF, its degree of cyclization was estimated by using NMR techniques. It was found that the degree of cyclization depends on temperature and time. Then, the kinetic data of this system was investigated, as following.

The kinetic equation describing the cyclization process can be obtained from degree of conversion in cyclized DPNR and time. If the process is considered as a first order reaction, can be expressed by Equation (2), as follow:

$$\ln (1-x) = kt \qquad (2)$$

Where x is degree of cyclization. t is time (second). k is the cyclization kinetic constant. Because the rate of the first state associated to the rate of conversion of cyclization reaction, it reflects the characteristics of the main forward reaction kinetic.

Plots of ln (1-x) vs time, where x is the degree of conversion of the cyclization of DPNR, show that the cyclization reaction follows first order kinetics as shown in **Figure 3**. Linear regression analysis using the least-squares method was used to fit the data. The kinetic rate constants, k, derived from the slopes of each reaction isotherm,

are listed in **Table 3**. Theory and practice show that the cyclization rate depends on the reciprocal temperature.

Table 3

Temperature(^{o}C)	Rate constant $k\times10^{3}(s^{-1})$	Regression coefficiency
75	0.005	0.96
85	0.04	0.94
95	0.06	0.95
100	0.20	0.96

Kinetic data of the cyclization of DPNR

Therefore, it is possible to use the Arrhenius equation to express this dependence, according to Equation (3):

$$k = A\times\exp\ \frac{-Ea}{RT} \qquad (3)$$

Take ln in Equation (3); it give Equation (4)

$$\ln k = \ln A - \frac{Ea}{R}\times\frac{1}{T} \qquad (4)$$

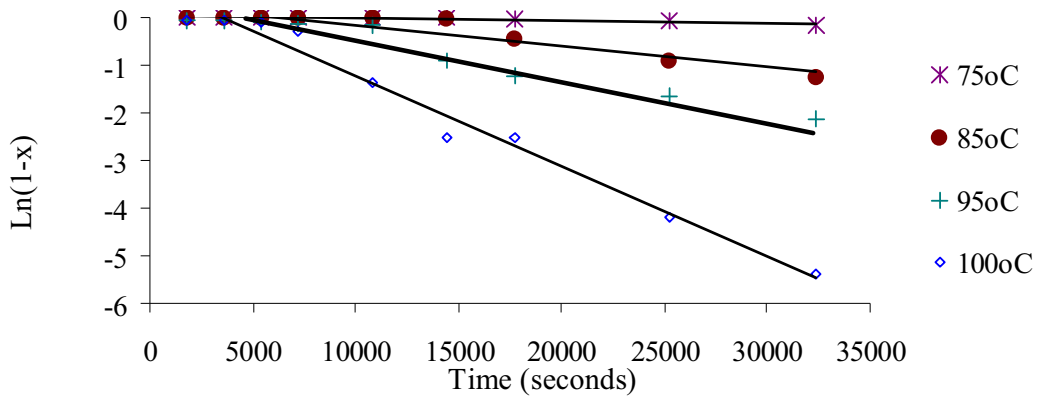

Figure 3. Kinetic plot of ln(1-x) vs time for cyclized DPNR, x is degree of cyclization

From the Arrhenius plot, ln k vs 1/T, where T is the reaction temperature, the activation energy of this type of cyclization reaction was determined as shown in **Figure 4.** The activation energy was calculated to be 147.66 kJ/ mol for cyclized DPNR, indicating the cyclization reaction rate is temperature dependent.

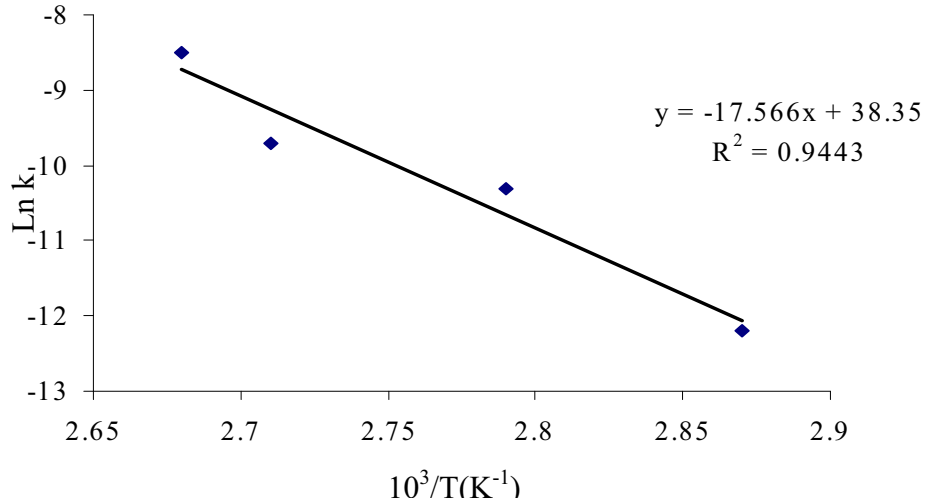

Figure 4. Arrhenius plot of the cyclization reaction of DPNR

3.5 Thermodynamics of cyclization of DPNR

The enthalpy of activation, ΔH, was calculated, using the equation (5),

$$\Delta H = E_a - RT \qquad (5)$$

to be 144.6 kJ/mol

The average entropy of activation, ΔS, and free energy of activation, ΔF, were obtained using the relationship (Equation (6)):

$$k = \frac{RT}{Nh} e^{\Delta S/R} e^{-Ea/RT} \qquad (6)$$

Here k, rate constant; R, gas constant; T, absolute temperature; N, Avogadro constant; and h, Plank constant.

The average values of the thermodynamic parameters were found to be ΔS= -269.1 J/mole and ΔF= 246.9 kJ/mole, which was calculated by this Equation (7).

$$\Delta F = \Delta H - T\Delta S \qquad (7)$$

4.Conclusion

The results from this study show that the cyclized DPNR by TMSOTF in latex could be carried out at above 95^{o}C with higher degree of cyclization. The cyclized structure was confirmed by NMR techniques including one and two dimensional NMR. The kinetic and thermodynamic parameters of cyclization obtained indicate that an increase in the process temperature would increase the rate cyclization formation. The rate constant for cyclized DPNR was found to be of the order of 2.0×10^{-5} l /moles and activation energy of cyclization of 146.77 kJ/mole. In addition, the enthalpy, entropy and free energy activation of 144.6 kJ/mole, 269.1 J/mole, and 246.9 kJ/ mole, respectively were derived from cyclization of cyclized DPNR

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