รายงานวิจัยฉบับสมบูรณ์

โครงการ: การสังเคราะห์พอลิเมอร์ลอกแบบจับจำเพาะจากนาโนพอลิเมอร์ชีวภาพเพื่อประยุกต์ใช้ในการวิเคราะห์สารชีวโมเลกุลและแยกสารให้บริสุทธิ์

โดย อ.ดร.ธีรพล เปียฉ่ำ

มีนาคม 2556

รายงานวิจัยฉบับสมบูรณ์

โครงการ: การสังเคราะห์พอลิเมอร์ลอกแบบจับจำเพาะจากนาโนพอลิเมอร์ชีวภาพเพื่อประยุกต์ใช้ในการวิเคราะห์สารชีวโมเลกุลและแยกสารให้บริสุทธิ์

คณะผู้วิจัย	สังกัด
อ.ดร.ธีรพล เปียฉ่ำ	มหาวิทยาลัยมหิดล
ศ.ดร.วีระพงศ์ ปรัชชญาสิทธิกุล	มหาวิทยาลัยมหิดล

สนับสนุนโดยสำนักงานคณะกรรมการอุดมศึกษา, สำนักงานกองทุนสนับสนุนการวิจัย และ มหาวิทยาลัยมหิดล

(ความคิดเห็นในรายงานนี้เป็นของผู้วิจัย สกอ. และ สกว. ไม่จำเป็นต้องเห็นด้วยเสมอไป)

กิตติกรรมประกาศ

คณะผู้วิจัยขอขอบคุณสำนักงานคณะกรรมการอุดมศึกษา (สกอ.) และ สำนักงานสนับสนุนการวิจัย (สกว.) และมหาวิทยาลัยมหิดล ที่ได้พิจารณาให้ทุนสนับสนุนการพัฒนาศักยภาพในการทำงานวิจัยของอาจารย์รุ่นใหม่ ทำให้งานวิจัยประสบความสำเร็จตามความมุ่งหมาย

ขอขอบพระคุณท่าน ศ.ดร. วีระพงศ์ ปรัชญาสิทธิกุล ที่กรุณารับเป็นนักวิจัยที่ปรึกษาและได้ให้ความช่วยเหลือแนะนำด้านวิชาการตลอดจนการให้แรงบันดาลใจในการขับเคลื่อนงานวิจัยอย่างต่อเนื่อง

ขอขอบพระคุณ ภาคจุลชีววิทยาคลินิกและเทคโนโลยีประยุกต์ คณะเทคนิคการแพทย์ มหาวิทยาลัยมหิดล ที่เอื้อเฟื้อสถานที่อุปกรณ์ สารเคมี และเครื่องมือต่าง ๆ ตลอดจนให้การสนับสนุนการวิจัยในครั้งนี้

ธีรพล เปียฉ่ำ

Abstract (บทคัดย่อ)

Project Code: MRG5380036

Project Title: SYNTHESIS OF MOLECULARLY IMPRINTED POLYMERS-BASED NANOBIOPOLYMER FOR BIOSENSING AND SEPARATION

Investigator: Dr.Theeraphon Piacham Mahidol University

Prof.Dr.Virapong Prachayasittikul Mahidol University

E-mail Address: theeraphon.pia@mahidol.ac.th

Project Period: July 2010-March 2013

Molecularly imprinted polymers (MIPs) are macromolecular matrices that can mimic the functional properties of antibodies, receptors and enzymes while possessing higher durability. As such, these polymers are interesting materials for applications in biomimetic sensor, drug synthesis, drug delivery and separation. In this study, we prepared MIPs and molecularly imprinted nanospheres (MINs) as receptors with specific recognition properties toward tocopherol succinate (TPS) in comparison to tocopherol (TP) and tocopherol nicotinate (TPN). MIPs were synthesized using methacrylic acid (MAA) as functional monomer, ethylene glycol dimethacrylate (EGDMA) as crosslinking agent and dichloromethane or acetronitrile as porogenic solvent under thermal-induced polymerization condition. Results indicated that imprinted polymers of TPS-MIP, TP-MIP and TPN-MIP all bound specifically to their template molecules at 2 folds greater than the non-imprinted polymers. The calculated binding capacity of all MIP was approximately 2 mg per gram of polymer when using the optimal rebinding solvent EtOH:H_2O (3:2, v/v). Furthermore, the MINs toward TPS and TP were prepared by precipitation polymerization that yielded particles that are 200-400 nm in size. The binding capacities of MINs to their templates were greater than that of the non-imprinted nanospheres when using the optimal rebinding solvent EtOH:H_2O (4:1, v/v). Computer simulation was performed to provide mechanistic insights on the binding modalities of template-monomer complexes. In conclusion, we had successful prepared MIPs and MINs for binding specifically to TP and TPS. Such MIPs and MINs have great potential for industrial and medical applications, particularly for the selective separation of TP and TPS.

In this second part of study, A simple technique for generating molecularly imprinted polymer-coated on bacterial cellulose nanofiber have been prepared by immersing solvent treated-bacterial cellulose into a dilute pre-polymerization mixture solution prior to polymerization. This technique can be

easily used to combine two fascinating materials like BC nanofibers and MIPs to afford promising polymer composites that are useful for various innovative applications in biomedical, pharmaceutical and industrial sectors.

Keywords: Molecularly imprinted polymers, bacterial cellulose, tocopherol succinate, tocopherol, quercetin, nanofibers

บทคัดย่อ

รหัสโครงการ: MRG5380036

ชื่อโครงการ: การสังเคราะห์พอลิเมอร์ลอกแบบจับจำเพาะจากนาโนพอลิเมอร์ชีวภาพเพื่อประยุกต์ใช้ในการวิเคราะห์สารชีวโมเลกุลและแยกสารให้บริสุทธิ์

ชื่อนักวิจัย: อ.ดร.ธีรพล เปียฉ่ำ มหาวิทยาลัยมหิดล

ศ.ดร.วีระพงศ์ ปรัชชญาสิทธิกุล มหาวิทยาลัยมหิดล

E-mail Address: mttpc@mahidol.ac.th

ระยะเวลาโครงการ: กรกฎาคม 2553 -มีนาคม 2556

พอลิเมอร์ลอกแบบจับจำเพาะเป็นพอลิเมอร์ร่างแหที่สร้างขึ้นและทำหน้าที่เสมือนแอนติบอดี หรือรีเซฟเตอร์เทียมรวมไปถึงเอนไซม์ ซึ่งมีความคงทนค่อนข้างสูงจึงได้ถูกนำมาใช้ในงานทางด้านการวิเคราะห์หาสารที่สนใจมากมาย อาทิ เป็น เซนเซอร์ทางชีวภาพ, การสังเคราะห์ยา, การนำส่งยาและการแยกสารให้บริสุทธิ์ ในการศึกษาชิ้นนี้ได้ทำการสร้างพอลิเมอร์ลอกแบบจับจำเพาะและพอลิเมอร์ลอกแบบจับจำเพาะแบบเม็ดอนุภาคนาโนที่มีคุณสมบัติในการจับจำเพาะกับอนุพันธ์ของวิตามินอี (Tocopherol succinate) ซึ่งมีคุณสมบัติเป็นสารต้านมะเร็งและวิตามินอี (Tocopherol) และ Tocopherol nicotinate โดยใช้เทคนิคทาง molecular imprinting ด้วยการใช้ โมโนเมอร์ฟังค์ชั่นเป็น methacrylic acid, โมโนเมอร์ตัวเชื่อมเป็น ethylene glycol dimethacrylate และตัวทำละลายชนิดต่าง ๆอาทิ dichlorometane, acetonitrile, และใช้ตัวกระตุ้นปฏิกิริยาชนิด AIBN เพื่อทำให้เกิดการสานกันเป็นร่างแหและมีลอยประทับของโมเลกุลเป้าหมาย หลังจากสร้างพอลิเมอร์ลอกแบบเป็นผลสำเร็จ จะนำไปทำการทดสอบการจับจำเพาะ ซึ่งพบว่าพอลิเมอร์ลอกแบบจับจำเพาะที่ได้จากการเตรียมโดยใช้วิตามินอีอนุพันธ์และวิตามินอีเป็นโมเลกุลเป้าหมายนั้น สามารถจับ จำเพาะกับวิตามินอีอนุพันธ์และวิตามินอีตั้งต้นได้มากกว่า 2 เท่า โดยเทียบกับพอลิเมอร์ควบคุม โดยจับได้ประมาณที่ 2 mg/ g polymer ในสารละลายเอทานอลผสมกับน้ำในอัตราส่วน 3 ต่อ 2 และในส่วนพอลิเมอร์ควบคุมนั้น สามารถจับวิตามินอีได้น้อยมาก และผู้วิจัยได้เตรียมพอลิเมอร์เม็ดกลมโดยวิธี precipitation polymerization ซึ่งได้พอลิเมอร์ลอกแบบจับจำเพาะชนิดเม็ดกลม ขนาด 200-400 นาโนเมตร และทำการทดสอบการจับจำเพาะซึ่งพบว่าพอลิเมอร์ลอกแบบจับจำเพาะชนิดเม็ดกลมมีความสามารถจับจำเพาะได้ดีในสารละลายเอทานอลผสมกับน้ำในอัตราส่วน 4 ต่อ 1 นอกจากนี้ผู้วิจัยได้ทำการศึกษาและทดสอบความสัมพันธ์ระหว่างโมเลกุลเป้าหมายและโมโนเมอร์ฟังค์ชั่นโดยใช้วิธีการทางคอมพิวเตอร์ ซึ่งจากผลงานวิจัยข้างต้นในการสร้างพอลิเมอร์ลอกแบบจับจำเพาะต่อวิตามินอีอนุพันธ์และวิตามินอีนั้นจักเป็นประโยชน์ในทางการประยุกต์ใช้ในทางวิทยาศาสตร์การแพทย์เพื่อเป็นตัวนำส่งยารวมไปถึงการแยกสารจากผลิตภัณฑ์ธรรมชาติในเชิงอุตสาหกรรมต่อไป

ผู้วิจัยได้ค้นพบเทคนิคในการสร้างพอลิเมอร์ลอกแบบจับจำเพาะบนเส้นใยนาโนของเซลลูโลสจากแบคทีเรีย โดยนำเซลลูโลสจากแบคทีเรียที่มีน้ำเป็นองค์ประกอบไปทำการเปลี่ยนสภาพเป็นเมทานอลและนำไปใส่ในสารละลายเจือจางที่มีส่วนประกอบของสารเคอซิติน โมโนเมอร์ฟังค์ชั่น, โมโนเมอร์ตัวเชื่อมและตัวกระตุ้นปฏิกิริยา ได้พอลิเมอร์ลอกแบบจับจำเพาะเคลือบบนเส้นใยนาโนของเซลลูโลสจากแบคทีเรีย โดยวิธีการเป็นวิธีการที่ง่ายไม่ซับซ้อนในการสร้างพอลิเมอร์ลอกแบบจับจำเพาะเคลือบบนเส้นใยนาโนของเซลลูโลสจากแบคทีเรียซึ่งจักเป็น

ประโยชน์ในการสร้างคอมโพสิตพอลิเมอร์ที่มีคุณสมบัติการจับจำเพาะซึ่งจะเป็นประโยชน์ในการประยุกต์ใช้ทางชีวการแพทย์, เภสัชวิทยา และ ภาคอุตสาหกรรมต่อไป

กุญแจคำ: พอลิเมอร์ลอกแบบจับจำเพาะ, เซลลูโลสจากแบคทีเรีย, วิตามินอี ซักซิเนต, วิตามินอี, เคอร์ซิติน, เส้นใยนาโน

ความสำคัญและที่มาของปัญหาการวิจัย

จุดแข็งของประเทศคือการที่เรามีความหลากหลายทางชีวภาพและสินค้าทางการเกษตรที่มีหลากหลายถึงกระนั้นราคาสินค้าทางการเกษตรยังคงอยู่ในระดับที่ต่ำกว่าที่ควรจะเป็น การนำสินค้าทางการเกษตรมาเพิ่มมูลค่าโดยการวิจัยและนำเทคโนโลยีที่เหมาะสมมาประยุกต์ใช้น่าจะเป็นประโยชน์ต่อเศรษฐกิจรวมไปถึงการพึ่งพาตนเองอย่างยั่งยืนต่อไป ซึ่งผลิตภัณฑ์ที่น่าสนใจในการนำมาประยุกต์ใช้หนึ่งคือ วุ้นน้ำมะพร้าว (bacterial cellulose) ซึ่งสามารถผลิตได้โดยกระบวนการหมักน้ำมะพร้าวแก่กับเชื้อแบคทีเรียที่ชื่อ *Acetobacter xylinum* ซึ่งจะได้ผลผลิตเป็น polyglucosan chain ซึ่งเป็นนาโนไฟเบอร์ (nano-fiber) และสายนาโนไฟเบอร์เหล่านี้จะสานกันเป็นร่างแหหรือเป็น nanoparticle ขึ้นอยู่กับขบวนการในการเลี้ยงเชื้อ คุณสมบัติของสายนาโนไฟเบอร์นี้สามารถนำมาประยุกต์ใช้และขึ้นรูปได้มากมาย อาทิ ตัวนำส่งยา, ผิวหนังเทียม, หลอดเลือดเทียม เป็นต้น ทิศทางหนึ่งที่น่าจะนำวุ้นน้ำมะพร้าวมาประยุกต์ใช้เนื่องจากการมีคุณสมบัติเป็นนาโนไฟเบอร์คือการนำมาผนวกกับเทคโนโลยีในการพัฒนาพอลิเมอร์ลอกแบบจับจำเพาะ(molecularly imprinted polymer) ซึ่งมีคุณสมบัติจับจำเพาะและทำหน้าที่เสมือนแอนติบอดี (artificial antibody) หรือรีเซฟเตอร์เทียม (artificial receptor) พอลิเมอร์ชนิดนี้มีความคงทนค่อนข้างสูงจึงได้ถูกนำมาใช้ในงานทางด้านการวิเคราะห์หาสารที่สนใจมากมาย ประยุกต์ใช้ในการแยกสารที่มีฤทธิ์ทางชีวภาพ (bioactive compound) ให้บริสุทธิ์ เช่น ยา, สารออกฤทธิ์จากสมุนไพรต่าง ๆรวมไปถึงชีวโมเลกุล (biomolecule) ในปัจจุบันประเทศไทยมีการศึกษาวิจัยสารที่มีฤทธิ์ทางชีวภาพจากสมุนไพรไทยมากมายและได้มีการนำเทคโนโลยีที่หลากหลายเข้ามาใช้ในการแยกหรือตรวจจับสารที่มีฤทธิ์ทางชีวภาพ เนื่องจากขบวนการตรวจจับหรือแยกสารให้บริสุทธิ์ต้องใช้เทคโนโลยีและเครื่องมือที่มีค่าใช้จ่ายสูง อาทิ supercritical fluid extraction(SFE) ดังนั้น การนำพอลิเมอร์ลอกแบบจับจำเพาะมาประยุกต์ใช้นั้นจักเป็นประโยชน์ด้วยคุณสมบัติที่พอลิเมอร์มีความคงทนค่อนข้างสูง, สามารถแยกสารได้ด้วยขั้นตอนสั้น ๆ, ค่าใช้จ่ายในการผลิตไม่สูง รวมไปถึงการนำ นาโนไฟเบอร์จาก bacterial cellulose หรือวุ้นน้ำมะพร้าวซึ่งเป็น Biocompatible biopolymer ที่สามารถผลิตได้ในราคาถูกมาประยุกต์ใช้ร่วมกันนั้น จักเป็นประโยชน์ในงานด้านการตรวจจับและแยกสารที่มีฤทธิ์ทางชีวภาพจากสมุนไพรไทยในวงกว้างและนำไปสู่การประยุกต์ ใช้ในเชิงอุตสาหกรรมสมุนไพร รวมไปถึงการนำไปใช้ในการเป็นตัวนำส่งยาแบบจำเพาะต่อไป ทั้งยังเป็นการการเพิ่มมูลค่าผลิตภัณฑ์ทางการเกษตรและนำไปสู่งานวิจัยและการประยุกต์ใช้ในเชิงลึกต่อไป

วัตถุประสงค์ของโครงการ

1. เพื่อศึกษาวิจัยออกแบบและพัฒนาพอลิเมอร์ลอกแบบจับจำเพาะโดยใช้(bacterial cellulose) เป็นส่วนประกอบและนำไปใช้ในงานแยก bioactive compound จากสมุนไพรต่าง ๆรวมไปถึงชีวโมเลกุล (biomolecule) ให้บริสุทธิ์
2. เพื่อศึกษาวิจัยออกแบบและพัฒนาพอลิเมอร์ลอกแบบจับจำเพาะโดยใช้(bacterial cellulose) เป็นส่วนประกอบไปใช้ในงานด้านการวิเคราะห์และตรวจจับ(sensor)ทางเคมีชีวภาพ (bio-chemical analysis) รวมไปถึงคุณสมบัติการเป็นตัวนำส่งยาแบบจำเพาะ (specific drug delivery)

Outputs จากโครงการ

1. Synthesis and computational investigation of molecularly imprinted nanospheres for selective recognition of α-tocopherol succinate (Submitted for publication)
2. A simple method for creating molecularly imprinted polymer-coated bacterial cellulose nanofibers (Submitted for publication)

เนื้อหางานวิจัยส่วนที่ 1

Synthesis and computational investigation of molecularly imprinted nanospheres for selective recognition of α-tocopherol succinate

1. Introduction

Significant changes to the environment and climate as a result of global warming had increased the exposure to toxic substances that may culminate in the development of pathogenic diseases [1-3]. Among these, cancer has been found to increase incidentally owing to increases of UV exposure and toxicant-induced gene mutation. The development of therapeutic agent toward cancer has predominantly focused on addressing issues pertaining to its toxicity, drug delivery properties and multidrug resistance [4, 5]. Furthermore, intense efforts have been invested in improving therapeutic approaches as to increase patient survival [6, 7].

Tocopherol succinate (TPS), a vitamin E analogue, is a promising and attractive compound with known anti-cancer activity toward several types of human cancer cell lines. Particularly, TPS can selectively induce apoptosis in malignant cells [8-11] while being non-toxic to normal cells and tissues. Structure-function relationship study of the terminal dicarboxylic moiety of tocopherol (TP) analogues have been previously investigated [12] and it was concluded that the apoptogenic activity depended on the length and charge of the ester moiety. Birringer et al. provided further insights into the structure-function relationship of vitamin E by dividing the structure into three distinct domains that are responsible for X, Y

and Z [13]. The pharmacokinetic property of TPS is similar to that of TP in which after infusion it is circulated in the blood stream by docking to lipoproteins where it subsequently targets the micro-capillary of tumor cells. In regards to its physicochemical properties, the hydrophobic nature of the molecule is responsible for the propensity of TP to bind lipoprotein and travel through the peripheral tissues followed by its sequential transfer to tumor cells. As compare to the normal tissue that exerts neutral state membrane, malignant cells possess acidic membranes in the protonated state. The inherent physicochemical property of TPS enables it to counteract this by being freely diffusible into malignant cells owing to its weak acidic nature that comprises of charged and deprotonated moieties. TPS undergoes hydrolysis and is converted to TP by nonspecific esterases from hepatocytes [14, 15].

Molecular imprinting is a technique that affords the production of synthetic receptors or so-called plastic antibodies. Such molecularly imprinted polymers (MIPs) are recognition matrices that have the ability to recognize and bind specifically to compounds of interest. MIPs are known to possess higher durability than biological receptors as it is known to possess excellent thermostability, reusable and is easy to store [16]. As such, MIP has been successfully utilized for various applications such as substitutes for biological antibodies and receptors [17], separation matrices for chromatography [18] and solid phase extraction [19], analytical sensors [20, 21], immuno assays [22], drug delivery [23, 24], enzyme inhibitor synthesis [25, 26] and enzyme mimetics [27, 28]. In recent years, molecular imprinting have been employed in the synthesis of polymers affording robust recognition properties toward several compounds of interests and several recent reviews have been published describing their potential utilizations for selective

separation of compounds [29-35]. Of particular note, is its utilization as specific drug delivery agents such as for metal-based anti-inflammatory drug [36], glycyrrhizic acid [37], hyaluronic acid [38] and 5-fluorouracil [39] among others.

Molecularly imprinted polymers are prepared in essentially three major steps: (i) formation of template-monomer complexes, cross-linking and polymerization [40]. The first step involving the self-association of template-monomer complexes in which functional monomers affording the strongest binding are typically deemed as the optimal one to use in molecular imprinting experiments. Several approaches exist for calculating the interaction energy of template-monomer complexes: (i) computational chemistry approach (i.e. involving the computation of the molecular energy of template, monomer and their complexes followed by calculating their energetic difference) [41, 42], (ii) data mining approach (i.e. involving the use of multivariate analysis methods such as neural network for correlating molecular descriptors with their experimental imprinting factor) [43, 44], molecular dynamics approach (i.e. involving the construction of atomistic model of molecularly imprinted polymer in which the cross-linked polymer contained template cavities to mimic experimental settings) [45-47]. Previously, we had successfully employed the computational chemistry approach for calculating the interaction energy of sulfonamides [48] and tocopherols [49]. The data mining approach was first introduced by us as a facile method that allows simultaneous modeling of molecular and solvent descriptors by means of quantitative structure-property relationship (QSPR) to make predictions of the imprinting factor values for template-monomer complexes of interests [43, 44]. A more extensive account on computational approaches for the rational design of molecularly

imprinted polymers has previously been reviewed [50-52]. Further information on QSPR is provided in our previous review articles [53, 54] and the utilization of such modeling approach had successfully been demonstrated on a wide range of biological activities [55-59] and chemical properties [43, 44, 60-62].

A wide range of MIPs with specific binding properties towards TP and its derivatives has been synthesized by various polymerization techniques. Faizal et al. utilized molecular imprinting membranes synthesized via phase inversion technique bearing calix[4]resorcarenes moieties that engages in multiple non-covalent interactions with TP. The imprinted membrane was reported to bind TP over 2-folds higher than the non-imprinted membranes in methanol/water (2:1, v/v) [63]. Furthermore, Faizal et al. had also employed the phase inversion imprinting technique for preparing copolymers of acrylonitrile with α-tocopherol methacrylate (TPM) monomer by means of the covalent imprinting method [64]. Moreover, Faizal et al. had also synthesized a hybrid molecular imprinting polymer for TP using pre-polymerization powders of TPM cross-linked by divinylbenzene onto a scaffold matrix of polysulfone, cellulose acetate, and nylon [65]. Puoci et al demonstrated the use of α-TP imprinted polymer as solid phase extraction sorbents of α-TP from bay leaves extract [66]. In our previous investigation we had successfully prepared bulk monoliths and nanospheres for selective recognition of TP and TP acetate (TPA). The study also provided mechanistic insights into the binding modes of TP-MAA and TPA-MAA complexes [49]. Liu et al. prepared MIP-based nano-sensing material towards TP by anchoring the MIP layer onto 1-vinyl-3-octylimidazolium ionic liquid-modified CdSe/ZnS quantum dots. Such smart material exhibited florescence quenching signal upon TP binding without the

need for inducers and derivatization [67]. TP-MIP as drug delivery devices has been investigated for their recognition characteristics in organic and aqueous media including their release property in gastrointestinal simulated fluids [68].

Molecularly imprinted polymers with binding specificity towards the apoptogenic TPS have not yet been reported. Therefore, this study aims to synthesize bulk monoliths and nanospheres via precipitation polymerization for further application as TPS delivery matrices. Computer simulation was also employed to provide pertinent insights on the binding modalities of the investigated template-monomer complexes in order to understand the origins of the observed binding performance.

2. Materials and Methods

2.1. Reagents

Tocopherol (TP), tocopherol nicotinate (TPN), tocopherol succinate (TPS), methacrylic acid (MAA), ethyleneglycol dimethylacrylate (EDMA) and azobis-isobutyronitrile (AIBN) were purchased from Sigma-Aldrich. All solvents were of analytical or HPLC grade.

2.2. Preparation of molecularly imprinted polymers

Molecularly imprinted polymers were synthesized using TP, TPN and TPS as template molecules (Figure 1). To 10 mL of DCM, 0.5 mmol of template, 8 mmol of MAA as functional monomer, 50 mmol of EDMA as cross-linking monomer, and 202 mg of AIBN were added. The pre-polymerization mixture was purged with argon for 10 min and screw-capped in a 20 mL borosilicate tube. Thermal-induced polymerization was performed in a pre-heated water bath at

60°C for 18 h. The obtained monolithic polymer was ground by a mechanical mortar to produce particles of varying sizes. Sedimentation in acetone was performed to separate and collect particles with diameters around 10-25 μm. Templates were eluted from the polymer by using acetic acid:methanol (15%, v/v). Spectrophotometric examinations were performed in order to determine the amount of remaining templates. Non-imprinted polymers (NIPs) were prepared in the same manner with the omission of templates.

2.3. Preparation of molecularly imprinted polymers nanospheres

Molecularly imprinted polymers nanospheres (MINs) toward TP and TPS were prepared according to Ye and co-worker [69]. Briefly, 80 mL of pre-polymerization mixture in acetonitrile contained 0.5 mmol of template, 8 mmol of MAA, 50 mmol EDMA and AIBN. The pre-polymerization mixture was purged with argon for 15 min prior to subjecting it to thermal-induced polymerization at 60°C for 24 h. Templates were then eluted from the obtained nanospheres using acetic acid:methanol (15%, v/v) via Soxhlet extraction. The non-imprinted polymers nanospheres (NINs) were prepared in the absence of template molecules.

2.4. Scanning electron microscopy

Particle sizes of imprinted and non-imprinted nanospheres were determined from scanning electron microscope (HITACHI S-3400). Briefly, the nanospheres were mounted on metallic studs via double-sided conductive tape and subsequently applying gold ion coating using sputter coater (Bal-tec SCD 050) for 90 s under vacuum at current intensity of 60 mA and scanning accelerating voltage of 15 kV.

2.5. Binding analysis

Binding analysis was performed by incubating a fixed amount of template (0.1 mg/mL) against various amounts of polymers in a 1 mL microfuge tube on a rocking table at room temperature for 12 h. Supernatants were taken after centrifugation at 12,000 rpm for 5 min prior to determining the amount of template bound via spectrophotometry.

2.6. Computer simulation

Chemical structures of template molecules (i.e. TP, TPN and TPS) and functional monomer MAA were drawn into MarvinSketch [70] and were subsequently converted to the Gaussian input file format using Babel [71]. Full geometry optimizations with no symmetry constrains were then performed initially at the semi-empirical AM1 level in order to afford good starting structures for subsequent refinement at the density functional theory level using Becke's three-parameter Lee-Yang-Parr (B3LYP) [72, 73] and the 6-31G(d) basis set. Single point energy calculation was then performed on these optimized structures using the B3LYP functional and 6-311++G(d,p) basis set. All computational chemistry calculations were performed using Gaussian 09 [74].

Template-monomer complexes were then constructed by using the optimized structures of template and monomer. The functional monomer MAA was placed near each of the oxygen and nitrogen atoms of the template molecule to give rise to several possible template-monomer complexes. These structures were then subjected to full geometry optimizations. Finally, the total energy of the template molecules, functional monomers and complexes were

parsed from the Gaussian output files using in-house developed scripts. The interaction energy was then computed using the following equation:

$$\Delta E = E_{template-monomer} - E_{template} - E_{monomer} \quad (1)$$

where ΔE, $E_{template-monomer}$, $E_{template}$ and $E_{monomer}$ represents the interaction energy, energy of template-monomer complexes, energy of template molecule and energy of functional monomer, respectively.

3. Result and discussion

3.1. Molecular imprinting of tocopherols

Molecularly imprinted polymers toward TP, TPN and TPS have been successfully prepared. Results indicated that TP-MIP, TPN-MIP and TPS-MIP in DCM displayed very low binding capacity toward their respective template analytes. Such findings were in accordance with the results from our previous investigations [49]. In order to resolve this situation, appropriate solvents for template rebinding were investigated using various amounts of aqueous-ethanol mixtures. It was found that the optimum ratio for the ethanol-aqueous mixture was 3:2, v/v. Such aqueous mixtures ratio displayed good rebinding and selective recognition for TP and its analogs as compared to the control non-imprinting polymer (NIP) as shown in Figure 2. It should be noted that these MIPs were synthesized via thermal-induced polymerization. TP-MIP displayed recognition property greater than the MIP prepared from the previous report [49], which was also synthesized by thermal-induced polymerization. Such binding properties may be attributed to differences in the template and monomer content ratio and the

porogenic solvent used [66]. Particularly, results revealed that TP-MIP, TPS-MIP and TPN-MIP all bound its respective template molecules with 2-fold greater binding than the NIP at 10 mg polymer. The binding capacity for all MIPs was approximately 2 mg/g of polymer.

It was observed that NIP could bind TP and TPN rather non-specifically even when the polymer concentration was increased. On the other hand, NIP was shown to have low binding capacity towards TPS. It can be seen from Figure 2 that 80 mg of TPS-MIP exhibited high affinity towards TPS as deduced from a binding capacity of greater than 92% in comparison to 13% binding by NIP at template concentration of 0.1 mg/mL while TPN-MIP, TPS-NIP, TP-MIP and TP-NIP provided binding performances of 78.2%, 55.2%, 92% and 55%, respectively. It can be seen that TPS-MIP provided over 7 folds higher % binding when compared to their control NIP while TPN-MIP and TP-MIP afforded roughly 1.5-folds higher % binding than the NIP. Such specific binding of TPS may be attributed to their inherent physicochemical properties in which the succinic moiety of TPS possesses both hydrogen bond donating and accepting properties. This may consequently lead to strong binding of TPS to MAA, which also contain hydrogen bond donating and accepting capacities. Such strong template-monomer interaction of TPS and MAA may potentially give rise to a rather defined binding cavity. On the other hand, the rather non-specific binding of MIP and NIP to their respective templates TP and TPN may be attributed to the long chain aliphatic moiety as well as the one-point monomer interactions at hydroxyl group of TP and pyridine nitrogen of TPN.

In light of the non-specific binding properties as afforded by TP-MIP and TPN-MIP, a second round of polymer synthesis was

performed to produce molecularly imprinted nanospheres toward TP and TPS using the precipitation polymerization technique. Sizes and morphology of the obtained nanospheres were determined by SEM (Figure 3) to have uniform shape and size in the range of 200-400 nm, which may be suitable for future drug delivery applications. The binding capacities of TP-MIN, TPS-MIN and NIN were investigated by means of batch analysis in acetonitrile. It was observed that 80 mg of TPS-MIN and NIN afforded binding capacities of 47% and 19%, respectively, while TP-MIN and NIN had binding performances of 35% and 22%, respectively (Figure 4). Such results indicated that TPS-MIN could bind to its template more specific than that of TP-MIN.

To further enhance the binding performance of TPS-MIN, the binding solvents were subjected to optimization studies in which the aqueous content in organic solvent was varied. 40 mg of TPS-MIN were incubated with 0.1 mg/mL of TPS in different solvent mixtures including EtOH:H_2O (4:1, v/v), EtOH:H_2O (3:2, v/v) and MeCN:H_2O (1:1, v/v), which afforded % binding of 43%, 22% and 17%, respectively for TPS-MIN while % binding of 10%, 7% and 8%, respectively, for TPS-NIN. The results indicated that EtOH:H_2O using a ratio of 4:1, v/v augmented the binding specificity of TPS-MIN by 4-folds higher than the control NIN.

3.2. Computer simulation of tocopherol-imprinted polymers

Mechanistic insights into the origin of the observed binding specificities for the tocopherol-imprinted polymers were deduced from computational chemistry calculations. Computational chemistry had been demonstrated to be useful in elucidating the physicochemical properties of chemical entities that is pertinent for

understanding the origins of the investigated biological activities and chemical properties [28, 75-79]. First, the template and functional monomer were subjected to an initial geometry optimization at AM1 level followed by a refined optimization at DFT level using B3LYP/6-31G(d) and finally subjected to a single point energy calculation at B3LYP/6-311++G(d,p) level. Second, the optimized structures of template and functional monomer were used for subsequent geometry optimizations of template-monomer complexes by iteratively placing the functional monomer at each of the possible functional moiety of the template molecule. This resulted in several possible complexes and their corresponding interaction energy, which were obtained by calculating the energetic difference of the template, functional monomer and their complexes.

It can be seen from Table 1 that TP-MAA complexes revealed interaction energies (in order of increasing values) of -46.462, -31.612, -30.683, -28.122 and -16.199 kJ mol^{-1} for TP-MAA complexes 1, 3, 2, 4 and 5, respectively, where its binding modalities are correspondingly shown in Figures 6a, 6c, 6b, 6d and 6e, respectively. It was observed from TP-MAA complex 1 that the hydroxyl group emanating from the benzopyran core structure acted as both hydrogen bond donor and acceptor by interacting with the two complementary functional moieties (i.e. hydroxyl and carbonyl groups) of MAA. Such two-point interaction with MAA accounted for the high interaction energy of TP-MAA complex 1. The remaining complexes essentially provided one-point interaction and consequently had lower interaction energy than that of TP-MAA complex 1.

The interaction energies for TPN-MAA complexes were -54.083, -41.588 and -23.627 kJ mol^{-1} for TPN-MAA complexes 2, 1 and 3, respectively, and its structures are correspondingly depicted in

Figures 7b, 7a and 7c, respectively. As TPN could only provide hydrogen bond accepting capacity, its interaction with MAA were essentially one-point interactions. It was observed from TPN-MAA complex 2 that the nitrogen atom from the pyridine ring afforded the highest interaction energy while one-point interaction with oxygen atoms at various positions of TPN provided lower interaction energy.

Interaction energies of -76.378, -42.562, -41.882, -37.031 and -32.917 kJ mol^{-1} were observed for TPS-MAA complexes 1, 4, 2, 3 and 5, respectively, and its structures are correspondingly shown in Figures 8a, 8d, 8b, 8c and 8e, respectively. TPS-MAA complex 1 was found to afford the highest interaction energy of -76.378 and this could be attributed to the two-point interaction of MAA with the succinate moiety of TPS in which the carboxylic group provided both hydrogen bond donating and accepting capacities. The bond distances of interacting atoms at the site of this two-point interaction were 1.67 and 1.68 Å. Such distances were found to be the least of the investigated conformers thereby corroborating the observed high interaction energy.

The computer simulation suggested that template-monomer complexes providing the strongest binding were TPS-MAA > TPN-MAA > TP-MAA where the highest interaction energy were -76.378, -54.083 and -46.462, respectively. The high interaction energy afforded by TPS-MAA complex is in good agreement with the experimental finding that TPS-MIP also provided the best binding performance of up to 7-folds higher than the control NIP for bulk monoliths while 3-folds higher binding than the control NIP were observed for the uniformly-sized TPS-MIN.

4. Conclusion

Everyday we are constantly bombarded by a wide range of environmental factors that predisposes us to the development of cancer. Tocopherol succinate is a vitamin E derivative that has been shown to possess promising anti-cancer activity. In this work, molecularly imprinted polymers with binding specificity toward tocopherol and derivatives were prepared by bulk polymerization and precipitation polymerization. TPS-imprinted polymers prepared by both methods were found to have higher specificity and selectivity than the other derivatives as deduced from 7- and 3-folds higher % binding for bulk and uniformly-sized particles, respectively. Nanospheres prepared by precipitation polymerization afforded polymers with uniform size (~200-400 nm) and shape, which are suitable for further applications in drug delivery efforts.

Figure Legends

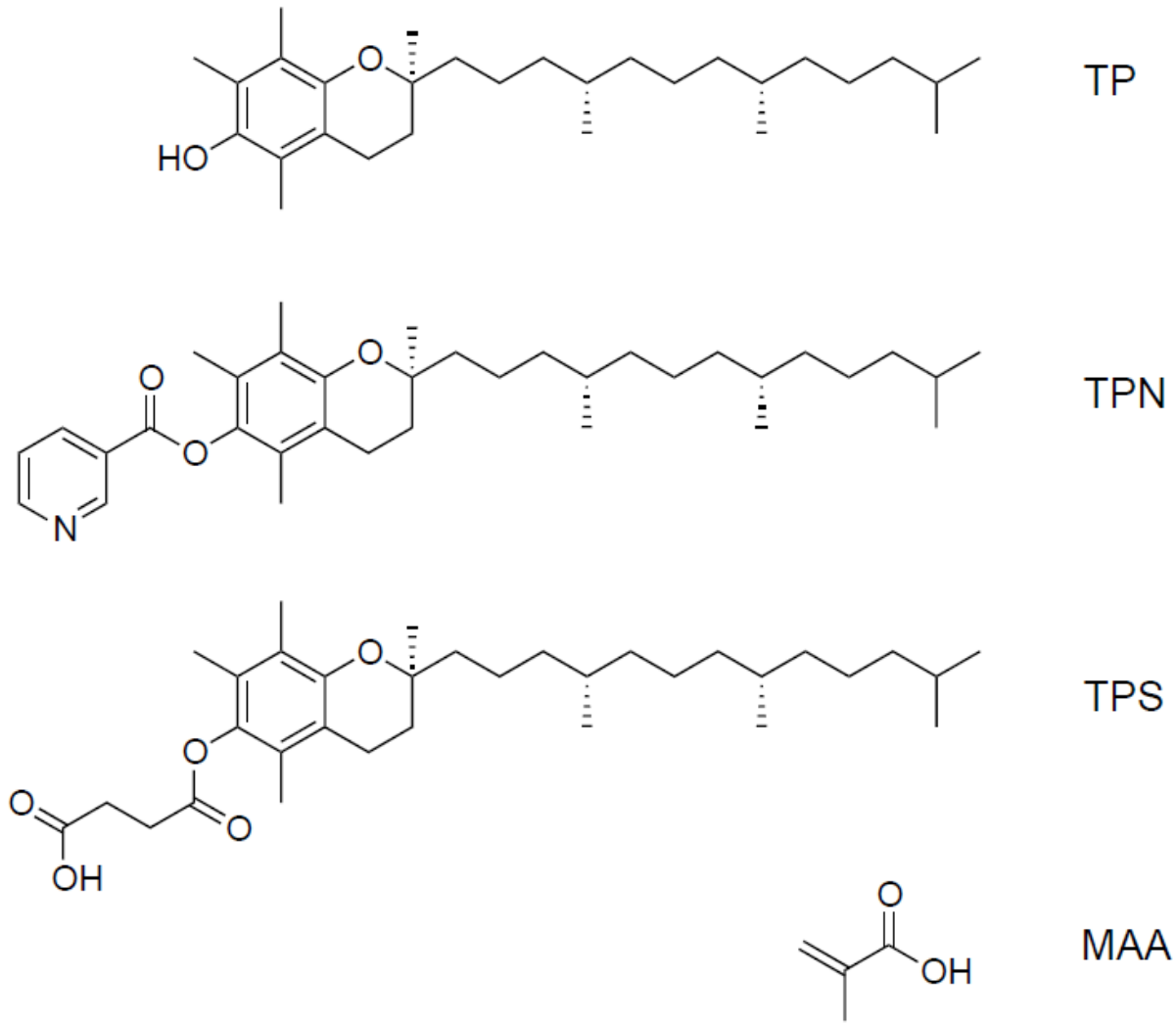

Fig. 1. Chemical structures of tocopherol (TP), tocopherol nicotinate (TPN) and tocopherol succinate (TPS).

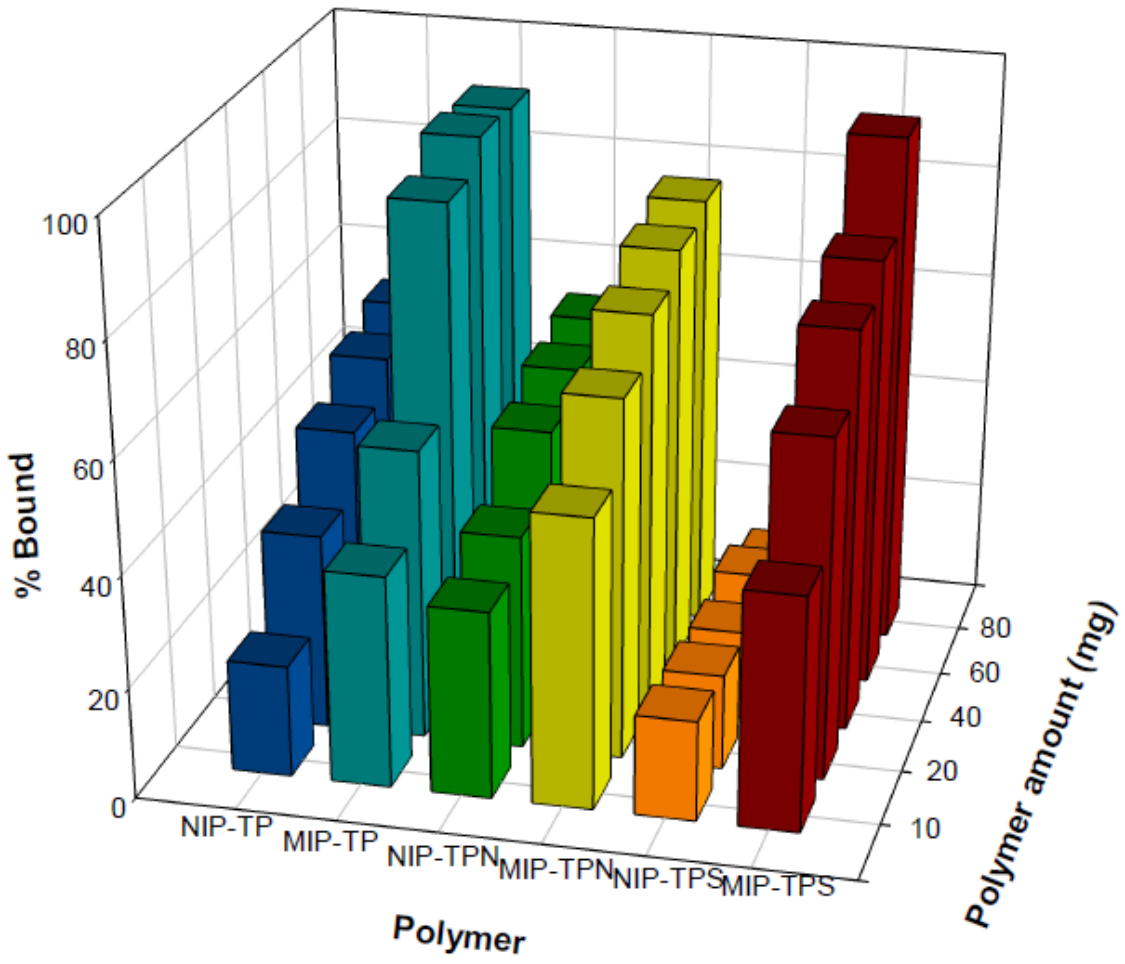

Fig. 2. Rebinding experiment of TP-MIP, TPS-MIP and TPN-MIP toward their template.

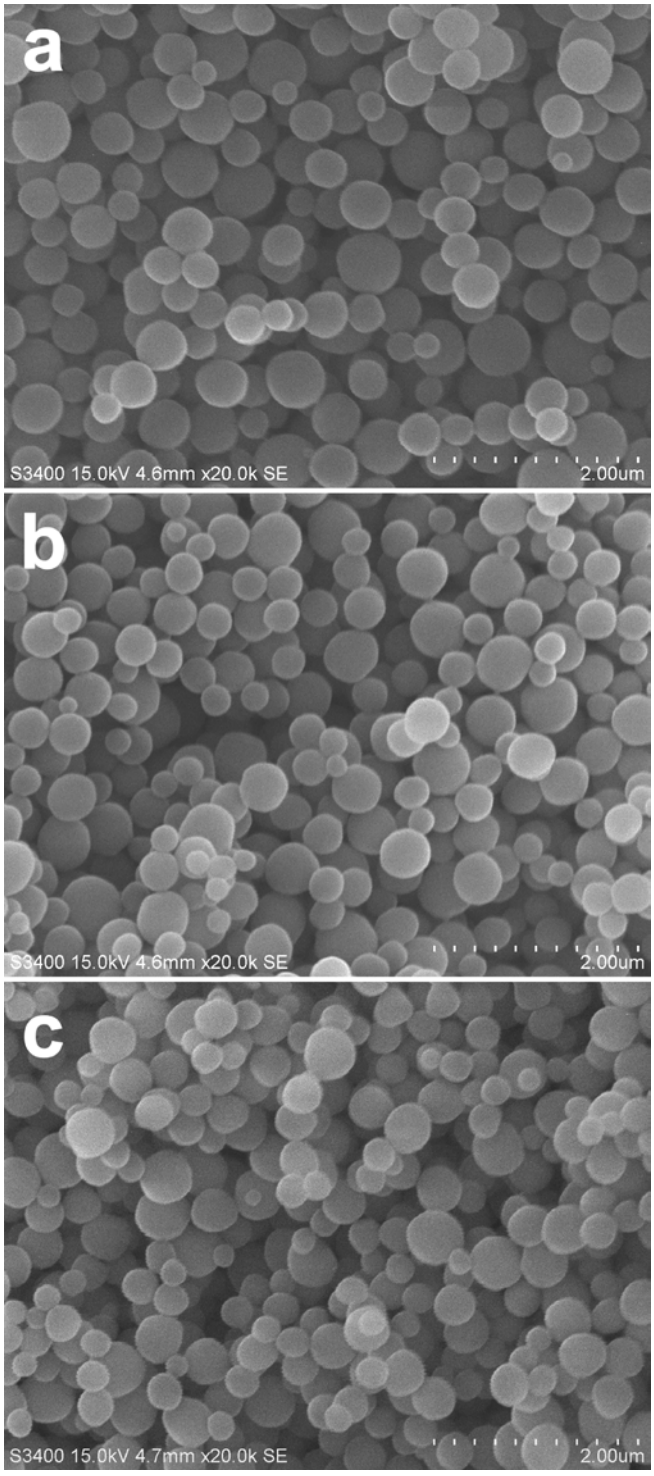

Fig. 3. Scanning electron micrograph of TPS-MIN (a), TP-MIN (b) and NIN (c).

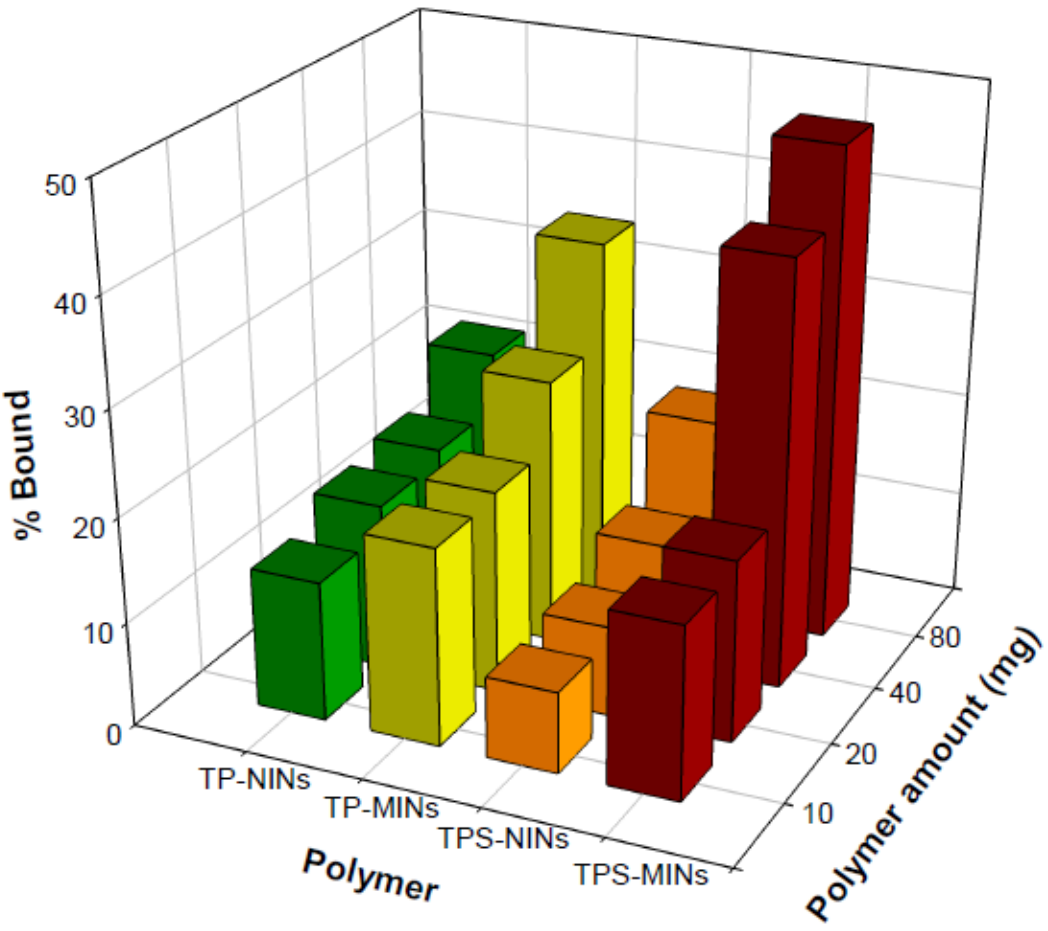

Fig. 4. Rebinding experiment of TPS-MIN, TP-MIN and NIN toward TPS and TP.

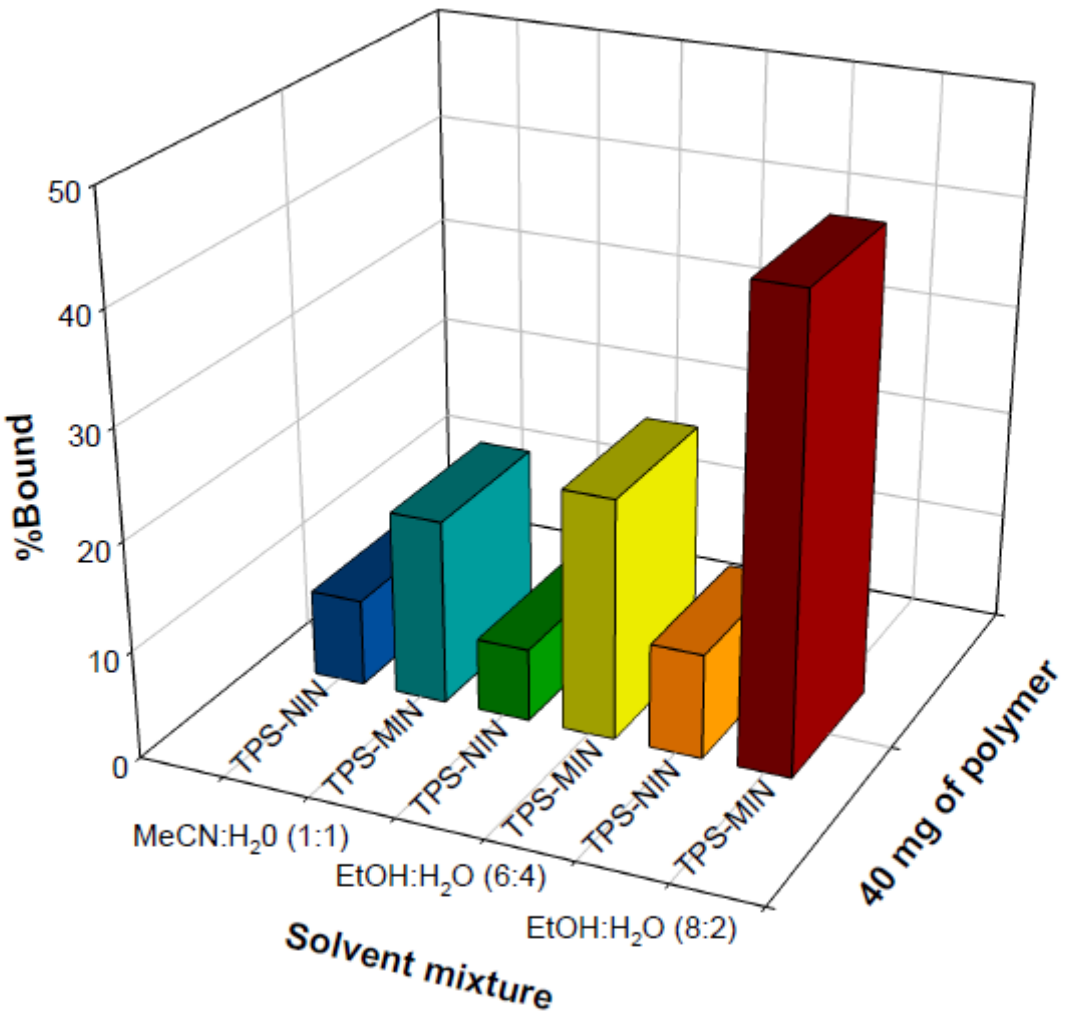

Fig. 5. Optimization of binding solvent of TPS-MIN.

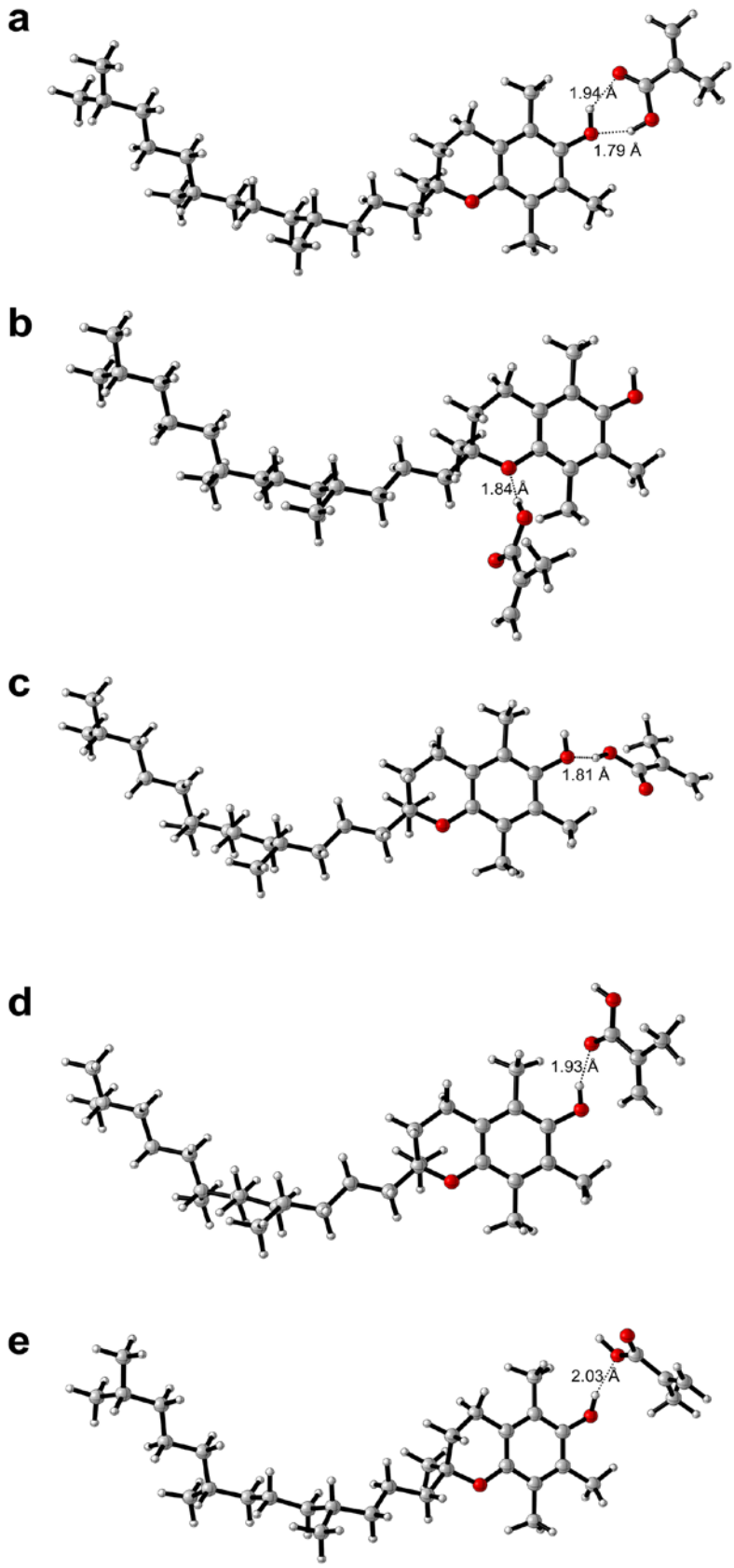

Fig. 6. Possible binding modalities as obtained from computational chemistry calculations for TP-MAA complexes 1-5 (a-e).

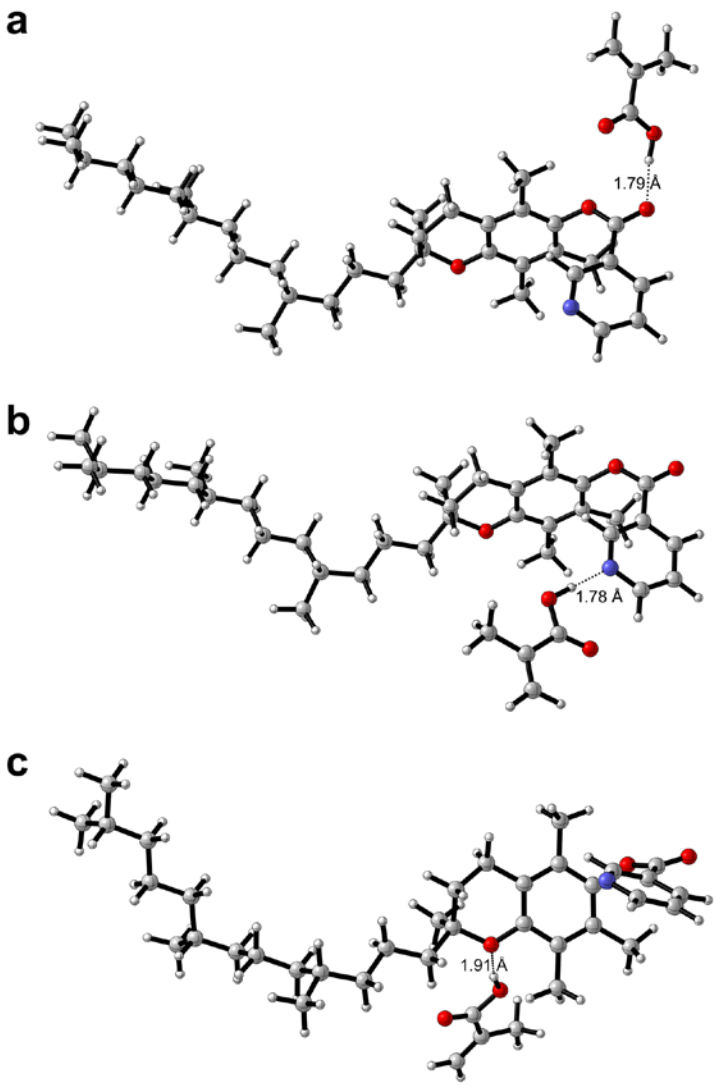

Fig. 7. Possible binding modalities as obtained from computational chemistry calculations for TPN-MAA complexes 1-3 (a-c).

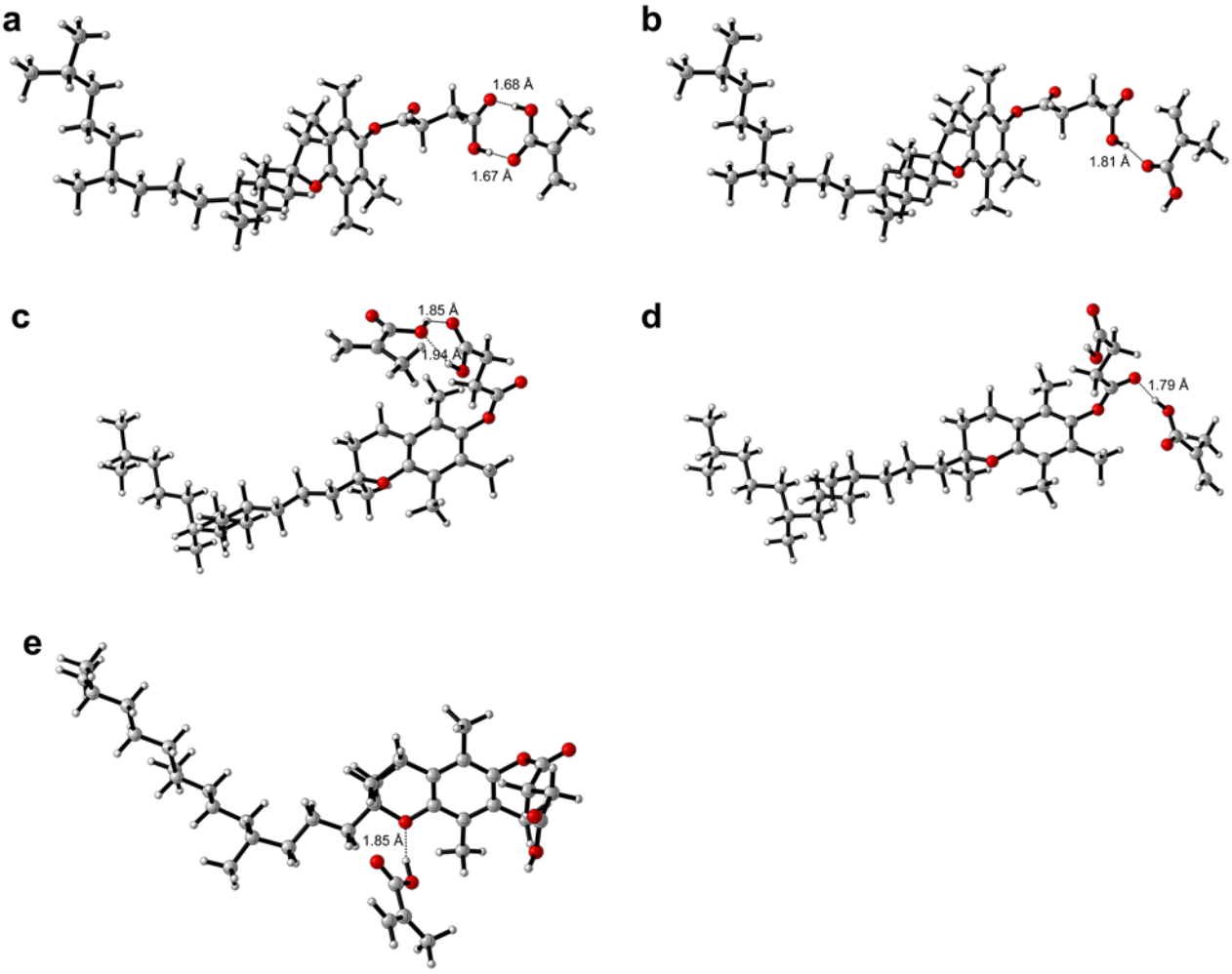

Fig. 8. Possible binding modalities as obtained from computational chemistry calculations for TPS-MAA complexes 1-5 (a-e).

References

[1] P.R. Hunter, J. Appl. Microbiol. 94 (2003) 37S-46S.
[2] G. Dapul-Hidalgo, L. Bielory, Ann. Allergy Asthma Immunol. 109 (2012) 166-172.
[3] P. Thomas, A. Swaminathan, R.M. Lucas, Global Change Biol. 18 (2012) 2392-2405.
[4] E.A. Dubikovskaya, S.H. Thorne, T.H. Pillow, C.H. Contag, P.A. Wender, Proc. Natl. Acad. Sci. USA 105 (2008) 12128-12133.
[5] A. Abraham, N. Sridhar, A. Veeranjaneyulu, Indian J. Pharmacol. 32 (2000) 256-268.
[6] R.B. Campbell, B. Ying, G.M. Kuesters, R. Hemphill, J. Pharm. Sci. 98 (2009) 411-429.
[7] D. Bechet, P. Couleaud, C. Frochot, M.L. Viriot, F. Guillemin, M. Barberi-Heyob, Trends Biotechnol. 26 (2008) 612-621.
[8] C. Constantinou, A. Papas, A.I. Constantinou, Int. J. Cancer 123 (2008) 739-752.
[9] M. Shanker, B. Gopalan, S. Patel, D. Bocangel, S. Chada, R. Ramesh, Cancer Lett. 254 (2007) 217-226.
[10] Y. Zhao, J. Neuzil, K. Wu, Mol. Nutr. Food Res. 53 (2009) 129-139.
[11] J. Neuzil, Brit. J. Cancer 89 (2003) 1822-1826.
[12] K. Kogure, S. Hama, M. Kisaki, H. Takemasa, A. Tokumura, I. Suzuki, K. Fukuzawa, Biochim. Biophys. Acta, Gen. Subj. 1672 (2004) 93-99.
[13] M. Birringer, J.H. EyTina, B.A. Salvatore, J. Neuzil, Brit. J. Cancer 88 (2003) 1948-1955.
[14] J.H. Wu, K.D. Croft, Mol. Aspects Med. 28 (2007) 437-452.
[15] J. Neuzil, H. Massa, Biochem. Biophys. Res. Commun. 327 (2005) 1024-1027.
[16] H. Bagheri, H. Piri-Moghadam, M. Naderi, Trends Anal. Chem. 34 (2012) 126-138.
[17] L. Ye, K. Mosbach, Chem. Mat. 20 (2008) 859-868.

[18] X. Wei, A. Samadi, S.M. Husson, Sep. Sci. Technol. 40 (2005) 109-129.
[19] V. Pichon, K. Haupt, J. Liq. Chromatol. Rel. Technol. 29 (2006) 989-1023.
[20] X.A. Ton, V. Acha, K. Haupt, B. Tse Sum Bui, Biosens. Bioelectron. 36 (2012) 22-28.
[21] T. Piacham, A. Josell, H. Arwin, V. Prachayasittikul, L. Ye, Anal. Chim. Acta 536 (2005) 191-196.
[22] M.C. Moreno-Bondi, M.E. Benito-Peña, J.L. Urraca, G. Orellana, Topics Curr. Chem. 325 (2012) 111-164.
[23] F. Puoci, G. Cirillo, M. Curcio, O.I. Parisi, F. Iemma, N. Picci, Exp. Opin. Drug Discov. 8 (2011) 1379-1393.
[24] D. Cunliffe, A. Kirby, C. Alexander, Adv. Drug Deliv. Rev. 57 (2005) 1836-1853.
[25] Y. Yu, L. Ye, K. Haupt, K. Mosbach, Angew. Chem. Int. Ed. 41 (2002) 4459-4463.
[26] H. Zhang, T. Piacham, M. Drew, M. Patek, K. Mosbach, L. Ye, J. Am. Chem. Soc. 128 (2006) 4178-4179.
[27] T. Piacham, C. Isarankura Na Ayudhya, V. Prachayasittikul, L. Bulow, L. Ye, Chem. Commun. (2003) 1254-1255.
[28] T. Piacham, C. Isarankura-Na-Ayudhya, C. Nantasenamat, S. Yainoy, L. Ye, L. Bulow, V. Prachayasittikul, Biochem. Biophys. Res. Commun. 341 (2006) 925-930.
[29] L. Chen, B. Li, Anal. Meth. 4 (2012) 2613-2621.
[30] W.J. Cheong, F. Ali, J.H. Choi, J.O. Lee, K. Yune Sung, Talanta 106 (2013) 45-59.
[31] W.J. Cheong, S.H. Yang, F. Ali, J. Sep. Sci. 36 (2013) 609-628.
[32] Y. Hu, J. Pan, K. Zhang, H. Lian, G. Li, Trends Anal. Chem. 43 (2013) 37-52.
[33] A. Murray, B. Örmeci, Environ. Sci. Pollut. Res. 19 (2012) 3820-3830.
[34] P.S. Sharma, F. D'Souza, W. Kutner, Trends Anal. Chem. 34 (2012) 59-76.

[35] G. Vasapollo, R.D. Sole, L. Mergola, M.R. Lazzoi, A. Scardino, S. Scorrano, G. Mele, Int. J. Mol. Sci. 12 (2011) 5908-5945.
[36] V.S. Sumi, R. Kala, R.S. Praveen, T. Prasada Rao, Int. J. Pharm. 349 (2008) 30-37.
[37] G. Cirillo, O.I. Parisi, M. Curcio, F. Puoci, F. Iemma, U.G. Spizzirri, N. Picci, J. Pharm. Pharmacol. 62 (2010) 577-582.
[38] M. Ali, M.E. Byrne, Pharm. Res. 26 (2009) 714-726.
[39] F. Puoci, F. Iemma, G. Cirillo, N. Picci, P. Matricardi, F. Alhaique, Molecules 12 (2007) 805-814.
[40] M. Komiyama, T. Takeuchi, T. Mukawa, H. Asanumi, Molecular Imprinting—From Fundamentals to Applications, Wiley-VCH, Weinheim, 2003.
[41] S. Subrahmanyam, S.A. Piletsky, E.V. Piletska, B. Chen, K. Karim, A.P.F. Turner, Biosens. Bioelectron. 16 (2001) 631-637.
[42] S.A. Piletsky, K. Karim, E.V. Piletska, C.J. Day, K.W. Freebairn, C. Legge, A.P.F. Turner, Analyst 126 (2001) 1826-1830.
[43] C. Nantasenamat, C. Isarankura-Na-Ayudhya, T. Naenna, V. Prachayasittikul, Biosens. Bioelectron. 22 (2007) 3309-3317.
[44] C. Nantasenamat, T. Naenna, C. Isarankura Na-Ayudhya, V. Prachayasittikul, J. Comput. Aid. Mol. Des. 19 (2005) 509-524.
[45] C. Dong, X. Li, Z. Guo, J. Qi, Anal. Chim. Acta 647 (2009) 117-124.
[46] Y. Lv, Z. Lin, T. Tan, W. Feng, P. Qin, C. Li, Sens. Actuators, B 133 (2008) 15-23.
[47] C. Herdes, L. Sarkisov, Langmuir 25 (2009) 5352-5359.
[48] C. Isarankura-Na-Ayudhya, C. Nantasenamat, P. Buraparuangsang, T. Piacham, L. Ye, L. Bülow, V. Prachayasittikul, Molecules 13 (2008) 3077-3091.
[49] T. Piacham, C. Nantasenamat, T. Suksrichavalit, C. Puttipanyalears, T. Pissawong, S. Maneewas, C. Isarankura-Na-Ayudhya, V. Prachayasittikul, Molecules 14 (2009) 2985-3002.
[50] L. Levi, V. Raim, S. Srebnik, J. Mol. Recognit. 24 (2011) 883-891.

[51] I.A. Nicholls, H.S. Andersson, C. Charlton, H. Henschel, B.C.G. Karlsson, J.G. Karlsson, J. O'Mahony, A.M. Rosengren, K.J. Rosengren, S. Wikman, Biosens. Bioelectron. 25 (2009) 543-552.
[52] I.A. Nicholls, H.S. Andersson, K. Golker, H. Henschel, B.C.G. Karlsson, G.D. Olsson, A.M. Rosengren, S. Shoravi, S. Suriyanarayanan, J.G. Wiklander, S. Wikman, Anal. Bioanal. Chem. 400 (2011) 1771-1786.
[53] C. Nantasenamat, C. Isarankura-Na-Ayudhya, T. Naenna, V. Prachayasittikul, EXCLI J. 8 (2009) 74-88.
[54] C. Nantasenamat, C. Isarankura-Na-Ayudhya, V. Prachayasittikul, Exp. Opin. Drug Discov. 5 (2010) 633-654.
[55] P. Mandi, C. Nantasenamat, K. Srungboonmee, C. Isarankura-Na-Ayudhya, V. Prachayasittikul, EXCLI J 11 (2012) 453-467.
[56] R. Pingaew, P. Tongraung, A. Worachartcheewan, C. Nantasenamat, S. Prachayasittikul, S. Ruchirawat, V. Prachayasittikul, Med. Chem. Res. (2012) 1-14.
[57] C. Thippakorn, T. Suksrichavalit, C. Nantasenamat, T. Tantimongcolwat, C. Isarankura-Na-Ayudhya, T. Naenna, V. Prachayasittikul, Molecules 14 (2009) 1869-1888.
[58] A. Worachartcheewan, C. Nantasenamat, C. Isarankura-Na-Ayudhya, S. Prachayasittikul, V. Prachayasittikul, Chemometr. Intell. Lab. Syst. 109 (2011) 207-216.
[59] A. Worachartcheewan, C. Nantasenamat, T. Naenna, C. Isarankura-Na-Ayudhya, V. Prachayasittikul, Eur. J. Med. Chem. 44 (2009) 1664-1673.
[60] C. Nantasenamat, C. Isarankura-Na-Ayudhya, T. Naenna, V. Prachayasittikul, J Mol Graph Model 27 (2008) 188-196.
[61] C. Nantasenamat, C. Isarankura-Na-Ayudhya, N. Tansila, T. Naenna, V. Prachayasittikul, J. Comput. Chem. 28 (2007) 1275-1289.
[62] C. Nantasenamat, K. Srungboonmee, S. Jamsak, N. Tansila, C. Isarankura-Na-Ayudhya, V. Prachayasittikul, Chemometr. Intell. Lab. Syst. 120 (2013) 42-52.

[63] C.K.M. Faizal, Y. Kikuchi, Molecular imprinting membrane having calix[4]resorcarenes moieties for selective separation of α-tocopherol, 2nd International Conference on Chemical, Biological and Environmental Engineering (2010) 274-276.
[64] C.K.M. Faizal, Y. Hoshina, T. Kobayashi, J. Memb. Sci. 322 (2008) 503-511.
[65] C.K.M. Faizal, T. Kobayashi, Polym. Eng. Sci. 48 (2008) 1085-1093.
[66] F. Puoci, G. Cirillo, M. Curcio, F. Iemma, U.G. Spizzirri, N. Picci, Anal. Chim. Acta 593 (2007) 164-170.
[67] H. Liu, G. Fang, C. Li, M. Pan, C. Liu, C. Fan, S. Wang, J. Mat. Chem. 22 (2012) 19882-19887.
[68] F. Puoci, G. Cirillo, M. Curcio, F. Iemma, O.I. Parisi, M. Castiglione, N. Picci, Drug Deliv. 15 (2008) 253-258.
[69] L. Ye, P.A.G. Cormack, K. Mosbach, Anal. Commun. 36 (1999) 35-38.
[70] ChemAxon Ltd., MarvinSketch, Version 5.11.4, Budapest, Hungary.
[71] OpenEye Scientific Software, Babel, Version 3.3, Santa Fe, NM.
[72] A.D. Becke, J. Chem. Phys. 98 (1993) 1372-1377.
[73] C. Lee, W. Yang, R.G. Parr, Phys. Rev. B 37 (1988) 785-789.
[74] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery, J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L.

Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski, D.J. Fox, Gaussian 09, Revision A.1, Wallingford, Connecticut.
[75] C. Nantasenamat, H. Li, C. Isarankura-Na-Ayudhya, V. Prachayasittikul, Chemometr. Intell. Lab. Syst. 116 (2012) 128-136.
[76] S. Prachayasittikul, O. Wongsawatkul, A. Worachartcheewan, C. Nantasenamat, S. Ruchirawat, V. Prachayasittikul, Molecules 15 (2010) 198-214.
[77] V. Prachayasittikul, C. Isarankura-Na-Ayudhya, T. Tantimongcolwat, C. Nantasenamat, H.J. Galla, Acta Biochim. Biophys. Sin. 39 (2007) 901-913.
[78] T. Suksrichavalit, S. Prachayasittikul, C. Nantasenamat, C. Isarankura-Na-Ayudhya, V. Prachayasittikul, Eur. J. Med. Chem. 44 (2009) 3259-3265.
[79] T. Suksrichavalit, S. Prachayasittikul, T. Piacham, C. Isarankura-Na-Ayudhya, C. Nantasenamat, V. Prachayasittikul, Molecules 13 (2008) 3040-3056.

เนื้อหางานวิจัยส่วนที่ 2

A simple method for creating molecularly imprinted polymer-coated bacterial cellulose nanofibers

A simple technique for generating molecularly imprinted polymer-coated on bacterial cellulose nanofiber have been prepared by immersing solvent treated-bacterial cellulose into a dilute pre-polymerization mixture solution prior to polymerization.

Bacterial cellulose (BC) is an attractive biomaterial that provides superior properties as follows: high purity network structure (free of hemicelluloses and lignin), high crystallinity (80-90%), ultra-fine and biocompatible nano-sized fibers.[1] It has previously been used to produce soft tissue, artificial blood vessel, and artificial skin.[2,3] It is thus a promising scaffold for tissue engineering.[4] This nanocellulose has become increasingly important and opens up an innovative pool for further exploitation in biomaterial development.[5] Previous attempts to modify BC before (*in situ* modification) and after (post modification) biosynthesis have been investigated under mild and specific conditions.[6, 7] Nanocellulose polymer composites have been produced using acrylates and methacrylates as monomers and methacrylate crosslinkers.[8,9] Thus, formation of a network of synthetic polymer (SP) inside the BC scaffolds have yielded collagen-like BC-SP. Among the different synthetic strategies available, molecular imprinting is the most forefront technique for the production of nano- and micro-structured materials with pre-designed molecular recognition capabilities. Molecularly imprinted

polymers (MIPs) are tailor-made artificial receptors that are capable of binding specifically to template molecule of interest by co-polymerizing with complementary functional monomers in porogenic solvent. Such MIPs behave in many ways like antibodies and enzymes but with greater durability and stability as it can withstand high temperature, harsh solvents, and high pressure.[10] MIPs can be produced in several formats such as m monolith, nanosphere, nanofiber or thin film to suit a wide span of applications as biosensors[11], bioassay[12], nanoreactors[13], solid phase extraction[14] and so on.

Molecular recognition may appear as a simple concept of two molecules coming together to interact; however, designing such system involves great complexity as to how to engineer such a structure that could maintain both stability and specificity. The beauty of MIPs is that it relies on the simple concept of self-assembly of monomeric constituents in the presence of template molecule. Molecular imprinting has thus emerged as a rapidly evolving technology with great potentials for health applications, particularly for the molecular detection of biological and chemical agents of biomedical importance.[15] Synthetic MIPs nanofibers have mostly been prepared using electrospinning methods that afforded the entrapment of MIP particles in electrospun nanofibers.[16]

In this study, a simple technique for the synthesis of MIP-coated BC nanofibers have been prepared. Briefly, BC was synthesized using Acetobacter xylinum with coconut water as medium. BC was obtained in the water-swollen state and the BC pellicle was cut into small pieces, subsequently treated with boiling 0.1 N NaOH for 45 minutes, and washed with distilled water until neutral pH was obtained. Prior to polymerization, the BC were subjected to solvent exchange from water to methanol that was performed to remove

water from the BC scaffold. BC was then immersed in excess methanol repeatedly for 6 times during the course of 7 days. The pre-polymerization mixture was prepared by incubating the solvent treated-BC (1g) with quercetin (169 mg), 4-vinylpyridine (0.214 mL), ethylene glycol dimethacrylate (2.360 mL) and azobisisobutylonitrile (50 mg) in excess methanol solution (75 mL) for 48 hours. Subsequently, thermal-induced polymerization was initiated at 60 ◦C for 72 hours. The control polymer was obtained in the omission of template molecule.

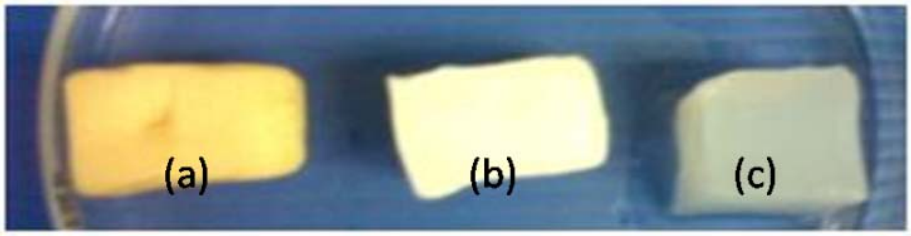

Fig. 1 Quercetin-imprinted polymer coated Bacterial Cellulose (QIP-BC) (a), Non-imprinted polymer coated Bacterial cellulose (NIP-BC) (b), Bacterial cellulose (c).

After polymerization, the quercetin-imprinted polymer coated bacterial cellulose (QIP-BC) nanofibers, non-imprinted polymer coated bacterial cellulose (NIP-BC) nanofibers (Fig. 1), quercetin-imprinted nanospheres (QIN) and non-imprinted microspheres (NIM) were obtained. The template of both types of polymer were subjected to removal from the polymer using methanol containing 15% acetic acid until no template could be detected from the washing solution via spectrometric analysis.

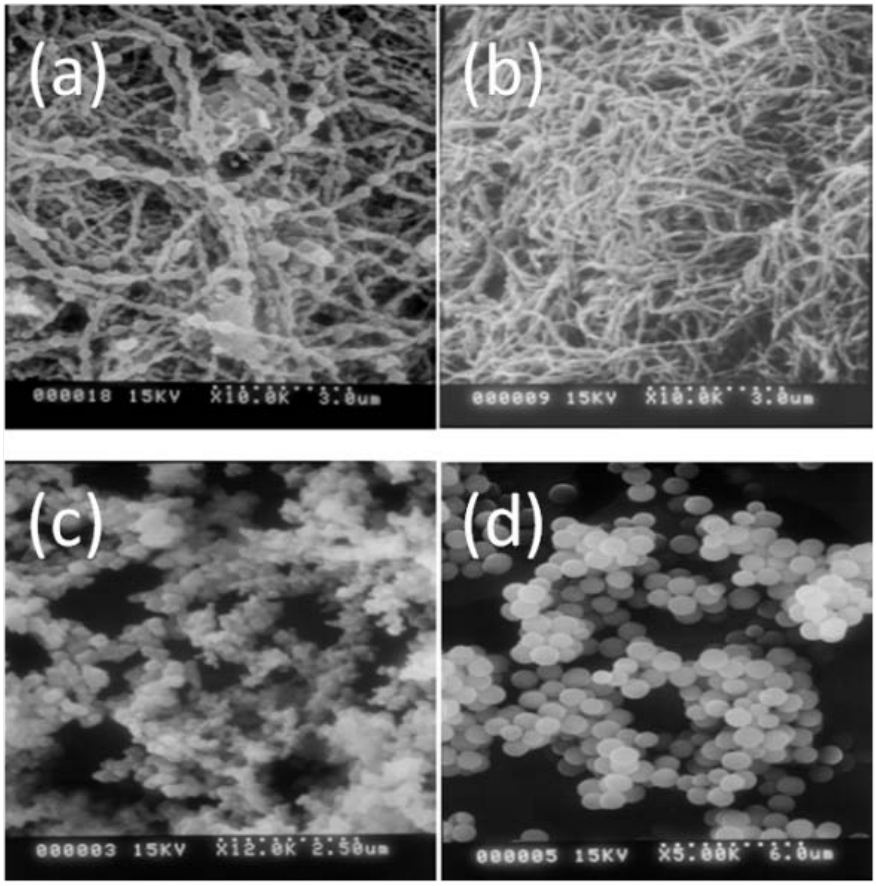

Fig. 2 Scanning electron microscopy (SEM) images of QIP-BC nanofibers (a), NIP-BC nanofibers (b), QIN (c), NIM (d).

Fig. 2 shows scanning electron microscopic (SEM) images of QIP-BC nanofibers, NIP-BC nanofibers, QIN and NIM that were formed in the excess of methanol. The QIP-BC nanofibers shows discrete nanoparticles encapsulated along BC nanofibers with diameter of 200-300 nm while the NIP-BC, coated polymer exhibited a more uniformly-shaped structure throughout the nanofiber (150-250 nm). QIN and NIM possessed diameters of 250 nm and 1 μm, respectively. NIM were shown to be larger and exhibited more uniformity than QIN.

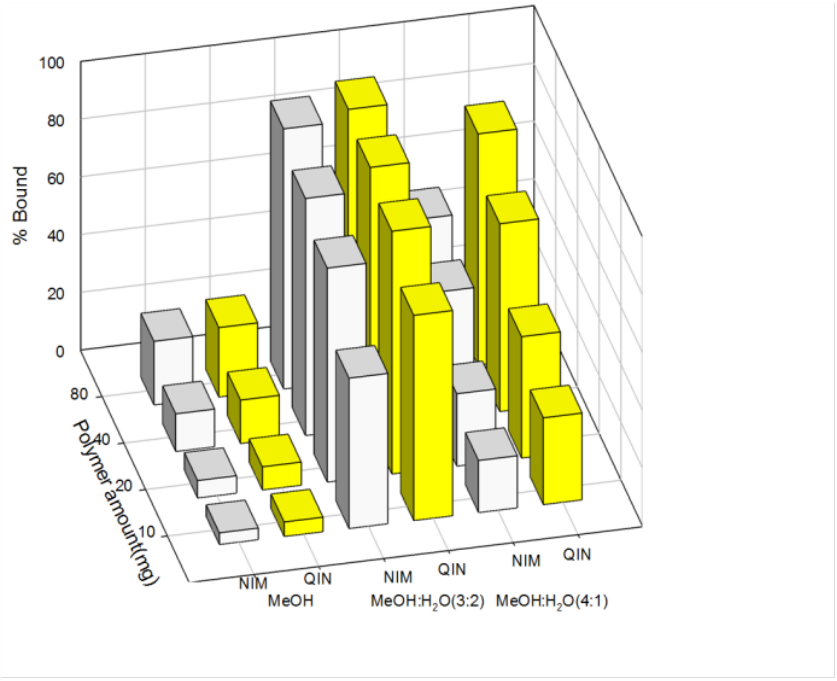

Fig. 3 Rebinding experiment of QIN and NIM toward their template with optimization of binding solvent

The binding performance of QIN and NIM were determined by incubation with 0.1 mg/ml of quercetin in different solvent mixtures including MeOH, MeOH:H_2O (3:2, v/v) and MeOH:H_2O (4:1, v/v). The results indicated that using MeOH as rebinding solvent exhibited low binding efficiency toward the template molecule. Upon addition of H_2O in the rebinding solvent, MeOH:H_2O (3:2, v/v), it was observed that an increase in non-specific interaction were achieved for both QIN and NIM. The MeOH:H_2O (4:1, v/v) appeared to be the best rebinding solvent, which afforded 80% and 53% binding to QIN and NIM, respectively, when using 80 mg of polymer (Fig. 3).

The binding properties of MIP-BC and NIP-BC were studied by incubating various amounts of nanofibers in MeOH:H_2O (4:1, v/v) using templates concentration of 0.1 mg/ml for 48 h. Spectrometric measurement of templates were evaluated from supernatant at 255 nm.

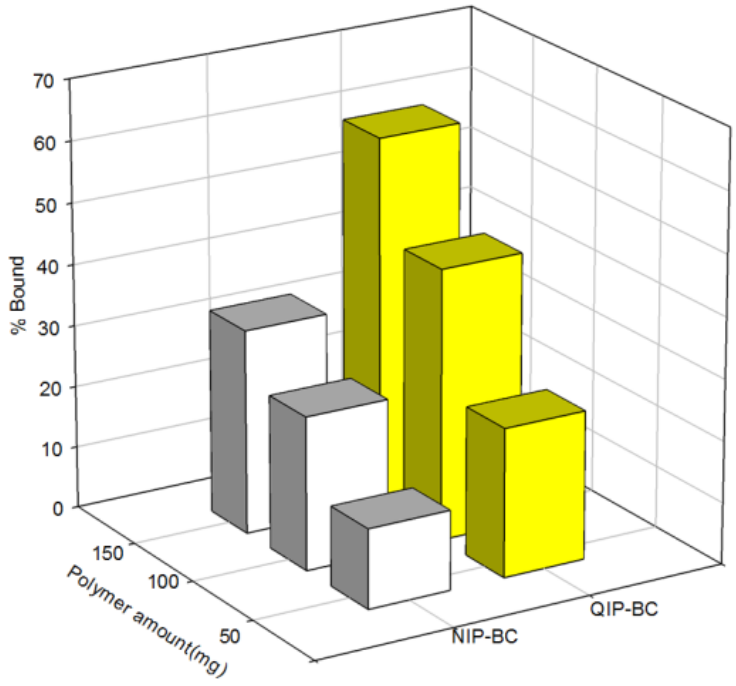

Fig. 4 Rebinding experiment of QIP-BC nanofibers and NIP-BC nanofibers

It was observed that 150 mg of QIP-BC and NIP-BC afforded binding capacities of 60% and 33%, respectively (Fig. 4). Such results showed that QIP-BC nanofibers exhibited higher recognition ability for quercetin than NIP-BC nanofibers for 2 times.

In summary, we have developed a simple method for the preparation of molecularly imprinted polymer-coated bacterial cellulose nanofibers. Solvent-exchanged bacterial celluloses were immersed in dilute pre-polymerization mixture prior to thermal-induced polymerization. This technique can be easily used to combine two fascinating materials like BC nanofibers and MIPs to afford promising polymer composites that are useful for various innovative applications in biomedical, pharmaceutical and industrial sectors.

References

1.P. Gatenholm and D. Klemm, *MRS Bulletin*, 2010, **35**, 208-213.

2.F. K. Andrade, R. Costa, L. Domingues, R. Soares and M. Gama, *Acta Biomaterialia*, 2010, **6**, 4034-4041.

3.L. Fu, J. Zhang and G. Yang, *Carbohydrate Polymers*, 2013, **92**, 1432-1442.

4.W. Jing, Y. Chunxi, W. Yizao, L. Honglin, H. Fang, D. Kerong and H. Yuan, *Soft Materials*, 2013, **11**, 173-180.

5.F. Kramer, D. Klemm, D. Schumann, N. Heßler, F. Wesarg, W. Fried and D. Stadermann, *Macromolecular Symposia*, 2006, **244**, 136-148.

6.H. C. Huang, L. C. Chen, S. B. Lin and H. H. Chen, *Carbohydrate Polymers*, 2011, **83**, 979-987.

7.P. Chen, S. Y. Cho and H. J. Jin, *Macromolecular Research*, 2010, **18**, 309-320.

8.Y. J. Choi, Y. Ahn, M. S. Kang, H. K. Jun, I. S. Kim and S. H. Moon, *Journal of Chemical Technology and Biotechnology*, 2004, **79**, 79-84.

9.R. Hobzova, M. Duskova-Smrckova, J. Michalek, E. Karpushkin and P. Gatenholm, *Polymer International*, 2012, **61**, 1193-1201.

10. L. Chen, S. Xu and J. Li, *Chemical Society Reviews*, 2011, **40**, 2922-2942.

11. T. Piacham, A. Josell, H. Arwin, V. Prachayasittikul and L. Ye, *Analytica Chimica Acta*, 2005, **536**, 191-196.

12. L. Ye, P. A. G. Cormack and K. Mosbach, *Analytical Communications*, 1999, **36**, 35-38.

13. H. Zhang, T. Piacham, M. Drew, M. Patek, K. Mosbach and L. Ye, *Journal of the American Chemical Society*, 2006, **128**, 4178-4179.

14. F. Augusto, L. W. Hantao, N. G. S. Mogollón and S. C. G. N. Braga, *TrAC - Trends in Analytical Chemistry*, 2013, **43**, 14-23.
15. L. Ye and K. Mosbach, *Chemistry of Materials*, 2008, **20**, 859-868.
16. K. Yoshimatsu, L. Ye, P. Stenlund and I. S. Chronakis, *Chemical Communications*, 2008, 2022-2024.