อนุภาคนาโนที่มีประจุจากอนุพันธ์พอลิไวนิลแอลกอฮอล์

นางสาวปารมี จิตต์จำนงค์

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CHARGED NANOPARTICLES FROM POLY(VINYL ALCOHOL) DERIVATIVES

Miss Paramee Chitchumnong

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry
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งานวิจัยนี้ได้ทำการเตรียมพอลิเมอร์ที่มีประจุลบคือ พอลิ(ไวนิลแอลกอฮอล์-โค-ไวนิลบิวเทนซัลโทน) และ พอลิ(ไวนิลแอลกอฮอล์-โค-ไวนิลโดเดคเคน-โค-ไวนิลบิวเทนซัลโทน) และพอลิเมอร์ที่มีประจุบวกคือ พอลิ(ไวนิลแอลกอฮอล์-โค-ไวนิลไดอะมิโนบิวทาเนต) และ พอลิ(ไวนิลแอลกอฮอล์-โค-ไวนิลโดเดคเคน-โค-ไวนิลไดอะมิโนบิวทาเนต) โดยทำการติด1,4-บิวเทนซัลโทนและโดเดคเคนหรือไดอะมิโนบิวทาเนตและโดเดคเคนบนพอลิ(ไวนิลแอลกอฮอล์)ด้วยปฏิกิริยาแทนที่ พอลิเมอร์ที่มีเปอร์เซ็นต์การแทนที่ของ 1,4-บิวเทนซัลโทน โดเดคเคน ไดอะมิโนบิวทาเนตต่างๆได้ถูกเตรียมขึ้น พอลิไวนิลแอลกอฮอล์ที่มีประจุลบสามารถเกิดการประกอบตัวเองขึ้นเป็นอนุภาคโดยการแลกเปลี่ยนของตัวทำละลายจากไดเมธิลซัลฟอกไซด์เป็นน้ำ จากการทดลองพบว่าการแทนที่ของหมู่ซัลโทนและโดเดคซิลบนสายโซ่พอลิไวนิลแอลกอฮอล์มีอิทธิพลต่อขนาดของอนุภาค โดยอนุภาคของพอลิ(ไวนิลแอลกอฮอล์-โค-ไวนิลบิวเทนซัลโทน)มีขนาดใหญ่ขึ้นเมื่อเปอร์เซ็นต์การแทนที่ของหมู่ซัลโทนเพิ่มขึ้น ในขณะที่อนุภาคของพอลิ(ไวนิลแอลกอฮอล์-โค-ไวนิลโดเดคเคน-โค-ไวนิลบิวเทนซัลโทน)มีขนาดเล็กลงเมื่อเปอร์เซ็นต์การแทนที่ของหมู่ซัลโทนเพิ่มขึ้น ในทางตรงกันข้ามพอลิเมอร์ที่มีประจุบวกไม่สามารถเกิดการประกอบตัวเองขึ้นเป็นรูปร่างจำเพาะได้ นอกจากนี้ได้ทำการกักเก็บสารกันแดด 2-เอทิลเฮกซิล พารา-เมททอกซีซินนาเมต (อีเอชเอ็มซี) ในอนุภาคที่เตรียมได้เพื่อทำการศึกษาประสิทธิภาพในการกักเก็บและสมบัติการปลดปล่อยแบบควบคุม

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PARAMEE CHITCHUMNONG: CHARGED NANOPARTICLES FROM POLY(VINYL ALCOHOL) DERIVATIVES. ADVISOR: ASSOC. PROF. SUPASON WANICHWECHARUNGRUANG, Ph.D., 89 pp.

Negatively charged polymers, poly(vinyl alcohol-co-vinyl butane sultone) and poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone), and positively charged polymers, poly(vinyl alcohol-co-vinyl diethylamino butanate) and poly(vinyl alcohol-co-vinyl diethylamino butanate), were synthesized by grafting poly(vinyl alcohol) with 1,4-butane sultone and dodecyl moieties or diethylamino butanate and dodecyl moieties through appropriate substitution reactions. Polymer with various sultone, dodecyl, diethylamino butanate substitution degrees were prepared. The negatively charged polymers were able to self-assemble into particles upon changing solvent from dimethyl sulfoxide to water. It was found that the degree of substitution of sultone groups and dodecyl moieties on PVA backbone affected the particle size. The size of poly(vinyl alcohol-co-vinyl butane sultone) particles increased as the sultone substitution increased while that of poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone) particles decreased as the sultone substitution increased. In contrast, none of the positively charged polymers obtained could self-assemble into any specific architecture. Encapsulate 2-ethylhexyl *p*-methoxycinnamate (EHMC) into the prepared particles were demonstrated and their release profiles were investigated.

Department: Chemistry Student's Signature Paramee Chitchumnong
Field of Study: Chemistry Advisor's Signature
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CONTENTS

LIST OF TABLES

Tables **Page**

LIST OF FIGURES

Figures **Page**

LIST OF SCHEMES

Schemes **Page**

LIST OF ABBREVIATIONS

br	broad
°C	degree Celsius
cm^{-1}	unit of wavenumber (IR)
d	doublet
DLS	dynamic light scattering
DMF	dimethyl formamide
DMSO	dimethyl sulfoxide
DMSO-d_6	deuterated dimethyl sulfoxide
DS	degree of substitution
DSC	differential scanning calorimetry
FT-IR	Fourier transform infrared spectrophotometer
g	gram
h	hour
Hz	hertz
IR	Infrared
J	coupling constant
KBr	potassium bromide
m	multiplet
mg	milligram
min	minute
mL	milliliter
mmol	millimole
mV	millivoltage
Mw	molecular weight
mW	milliwatt
nm	nanometer
NMR	nuclear magnetic resonance
ppm	parts per million
s	singlet
SEM	scanning electron microscope

t	triplet
TEM	transmission electron microscope
T_g	glass transition temperature
T_m	melting temperature
UV	ultraviolet
%	percent
δ	chemical shift
ε	molar absorptivity
λ	wavelength

CHAPTER I

INTRODUCTION

1.1 Polymeric nanoparticles

Nanotechnology is a field of applied science and technology which is the control of matter on the atomic and molecular scale. Nanotechnology is used to describe many fields of research. One of those research fields involves drug delivery which uses polymeric nanoparticles as carriers. The dimensions of polymeric nanoparticles in this field are less than 1,000 nm.

Traditional drug delivery routes are oral and intravenous administrations and both of them are still widely used today. However, both routes face problems on drug efficiency and therapeutic benefit. In oral administration via tablets, capsules, or liquids, drugs are easily degraded by proteolytic enzymes in the gastrointestinal tract. In intravenous administrations, drugs have a low bioavailability and requiring frequent injections because they are rapidly cleared from the blood circulation [1, 2, 3].

During the 1980s and 1990s, drug delivery systems were developed to give a better drug efficiency with minimum toxic side effects [4]. The early micro/nanoparticles were mostly prepared from poly(alkylcyanoacrylate). In oral delivery, microparticles were dampened by the fact that big particles could not traverse the intestinal lumen into the lymphatic system. In intravenous administrations, drug loaded nanoparticles were effectively cleared out by phagocytosis.

Another class of vehicle that has been applied for drug delivery is liposome, a spherical lipid particle. Liposomes can protect drugs from degradation, target the drug to action site and reduce the toxic side effects [5]. However, liposomes possess inherent problems such as low encapsulation efficiency, poor storage stability and rapid leakage of water-soluble drugs in the blood [6]. Polymeric nanoparticles have shown their specific advantages over liposomes because of higher storage stability and unique ability to create a controlled release. Polymeric nanoparticles also show

further advantage over microparticles when used intravenously. This is because diameters of smallest capillaries in the body are 5-6 μm. Suitable particles must be smaller than 5 μm and do not form aggregates and embolism[7].

The widely used polymers for nanoparticles are poly(lactic acid) (PLA) [8,9], poly(glycolic acid) (PGA) [10] and their co-polymers, poly(lactide-co-glycolide) (PLGA) [11-13] because of their biocompatibility and restorability through natural pathways. The nanoparticles preparation method includes emulsification solvent evaporation [14], solvent displacement [15] and interfacial phase deposition through salting out [16] or emulsification diffusion processes [17, 18].

1.2 Poly(vinyl alcohol)

Poly(vinyl alcohol) (synonyms: PVA, elvanol, ethenol homopolymer, polyviol, vinol, vinyl alcohol polymer) was first prepared by Hermann and Haehnel in 1924 by the hydrolysis of polyvinyl acetate in ethanol with potassium hydroxide. Poly(vinyl alcohol) is a water-soluble synthetic polymer, prepared by the polymerization of vinyl acetate, followed by partial or complete hydrolysis. The primary raw material used in the manufacture of poly(vinyl alcohol) is vinyl acetate monomer dissolved in methanol. The polymerization involves the presence of two proprietary catalytic agents. After polymerization, the material undergoes controlled hydrolysis with aqueous sodium hydroxide, during which the acetyl groups in the vinyl acetate are removed. Poly(vinyl alcohol) is precipitated, washed and dried to form an odorless, tasteless, white or cream-colored granular powder. The physical characteristics of poly(vinyl alcohol) vary depending on the degree of polymerization and hydrolysis. Poly(vinyl alcohol) is classified into two classes, fully hydrolyzed (A) and partially hydrolyzed (B).

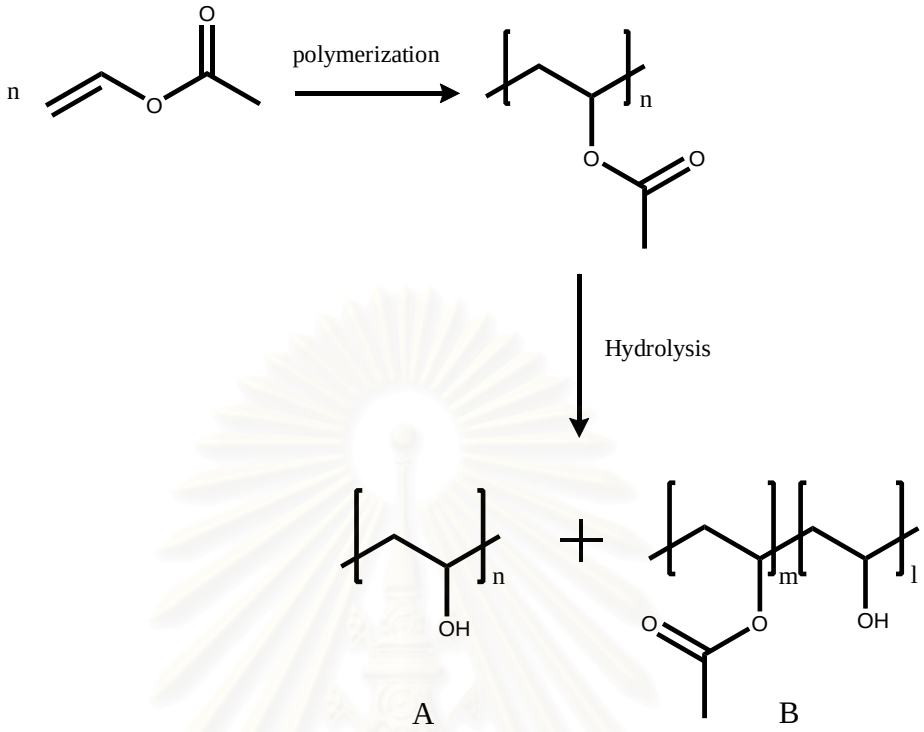


Figure 1.1 Synthesis of poly(vinyl alcohol)

Poly(vinyl alcohol) was selected as starting material in this work because of its biodegradability, biocompatibility, non-toxicity, non-carcinogenicity [19], chemical resistance and the possibility for substitution through chemical linkage to its oxy-residue [20]. Poly(vinyl alcohol) is an extremely safe chemical to both human and the environment. The United States Food and Drug Administration (US-FDA) has approved that poly(vinyl alcohol) is Substances Generally Recognized as Safe (GRAS) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has evaluated poly(vinyl alcohol) (partially hydrolyzed) as a moisture barrier film for food supplement tablets and for foods that contain inclusions or dry food with inclusions that need to be protected from moisture uptake, for example, dried fruits to be included in breakfast cereals or nuts to be included in yoghurt.

Due to its safety, poly(vinyl alcohol) has various applications in pharmaceutical, medical, cosmetic and food industries.

Poly(vinyl alcohol) is used as adhesive and thickener material in latex paints and common household white glues or in other adhesive mixtures such as remoistenable labels and seals, sizing and coating agent in paper products because it

can increase stiffness to the paper which is useful for certain applications such as tube winding, carton sealing and board lamination. In textile industries, poly(vinyl alcohol) is used as sizing and finishing agent. It can also be incorporated into a water-soluble fabric in the manufacture of degradable protective apparel, laundry bag for hospitals, rags, sponges, sheets, covers, as well as physiological hygiene products.

Poly(vinyl alcohol) is safe for cosmetic formulations. It is used as emulsifier and thickener in cosmetic products such as hairspray, shampoo, cleansing cream, cold cream, shaving cream and make-up. Poly(vinyl alcohol) is also used safely for food industries as an indirect additive, which is a coating, binder, sealing and surface finishing agent such as food package, dairy-based desserts, confectionery and cereal products and dietary supplement tablets.

Poly(vinyl alcohol) is used in a number of prepared biomedical applications including soft contact lenses, implants and artificial organs. In pharmaceutical applications, poly(vinyl alcohol) is used in preparation of jellies that dry rapidly when applied topically, transdermal patches and used as drug delivery matrices or in the form of powders add to mixtures for tablet formation. Moreover, poly(vinyl alcohol) microspheres is also used for controlled release of drugs such as oral drug and ophthalmic solutions, synthetic tear, because poly(vinyl alcohol) has a good dispersion and coating properties [21].

In the area of drug delivery, poly(vinyl alcohol) is commonly used as emulsion stabilizer in the preparation of nanoparticels by solvent evaporation [22] and dialysis method [23]. Additionally, poly(vinyl alcohol) is also used as a starting material to prepare hydrogel [19] or micelles or amphiphilic polymeric micro/nanoparticles [24-26].

1.3 Literature reviews

1.3.1 Poly(vinyl alcohol) (PVA)

Researchers involving the use of PVA as a main material to construct carriers are quite limited. The following paragraphs summarized those limited numbers of works.

In 1998, Wu and coworkers prepared poly(vinyl alcohol) hydrogel nanoparticles by a water-in-oil emulsion technology plus repeating freezing-thawing process. Application in protein/peptide drug delivery was demonstrated (Figure 1.2). The

average diameter of PVA hydrogel nanoparticles is 675.5±42.7 nm and protein drug loading efficiency is 96.2±3.8%. The particles have prolonged released characteristics [19].

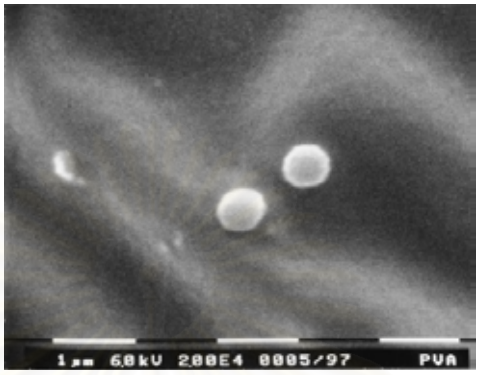


Figure 1.2 Scanning electron micrograph of poly(vinyl alcohol) hydrogel nanoparticles (this figure is taken from reference19).

In 2000, Kissel and coworkers synthesized poly(2-sulfobutyl-vinyl alcohol)-g-poly(lactide-co-glycolide), a negatively charged biodegradable polymer and the obtained polymer was prepared into nanoparticles by solvent displacement technique. The average diameter of the nanoparticles is ~100-500 nm, depending on polymer composition [27] (Figure 1.3).

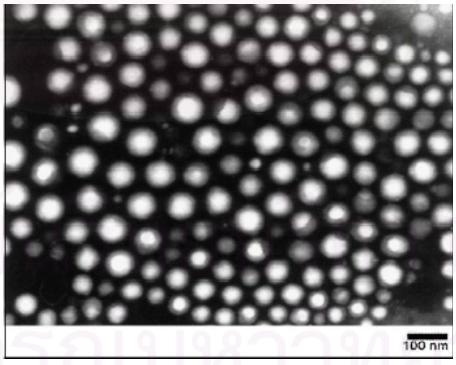


Figure 1.3 Transmission electron micrographs of poly(2-sulfobutyl-vinyl alcohol)-g-poly(lactide-co-glycolide) nanoparticles (this figure is taken from reference 27).

In 2002, Luppi and coworkers prepared poly(vinyl alcohol-co-vinyl oleate) micelles to entrap retinyl palmitate. The micelles have a high lipophilic drug loading ability, good stability in an aqueous environment and enhance retinyl palmitate transcutaneous permeation [24].

In 2004, Kissel and coworkers synthesized poly[(vinyl-3-(diethylamino)-propylcarbamate-*co*-(vinyl acetate)-*co*-(vinyl alcohol)]-graft-poly(L-lactic acid), a water-soluble branched polyester containing positively charged backbone. This polymer and insulin can interact into nanocomplexes by self-assembling (Figure 1.4). The nanocomplexe is 200-500 nm diameters with zeta-potential of +5 to +35 mV and an insulin loading of up to 98% [28].

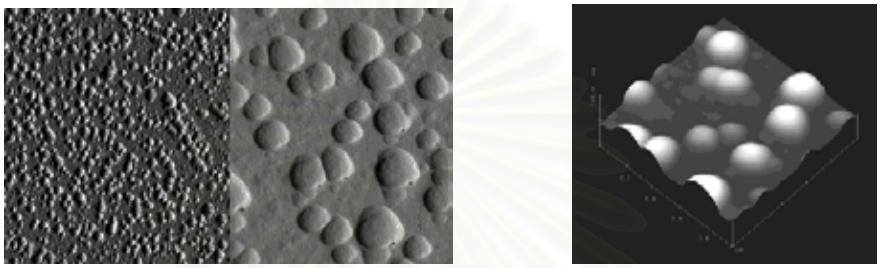


Figure 1.4 Atomic force micrograph of poly[(vinyl-3-(diethylamino)-propylcarbamate-*co*-(vinyl acetate)-*co*-(vinyl alcohol)]-graft-poly(L-lactic acid) nanoparticles (this figure is taken from reference 28).

In 2004, Kishida and coworkers prepared PVA-DNA nanoparticles by ultra high pressure technology for gene delivery (Figure 1.5). The average of the nanoparticles was 200 nm. The driving force of nanoparticles formation is hydrogen bonding between DNA and PVA. Experiments on gene loading, cytotoxicity and cellular uptake indicated that PVA-DNA nanoparticles were a good DNA carrier [29].

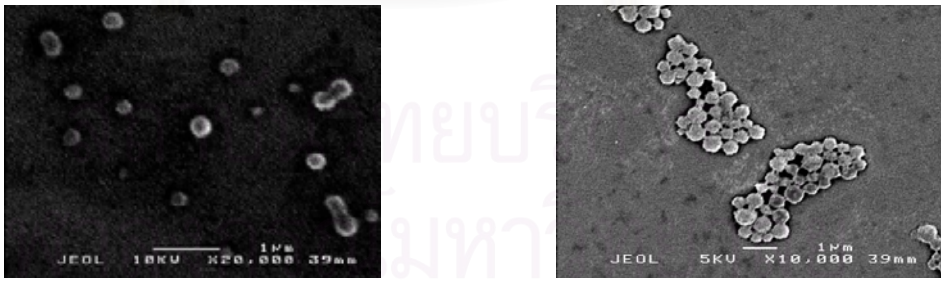


Figure 1.5 Scanning electron micrograph of PVA-DNA nanoparticles (this figure is taken from reference 29).

In 2005, Kissel and coworkers synthesized amine-modified poly(vinyl alcohol) by a two step reaction using carbonyl diimidazole activated diamines. The obtained amine-modified poly(vinyl alcohol) could complex with DNA through both electrostatic and hydrophobic interactions [30] (Figure 1.6).

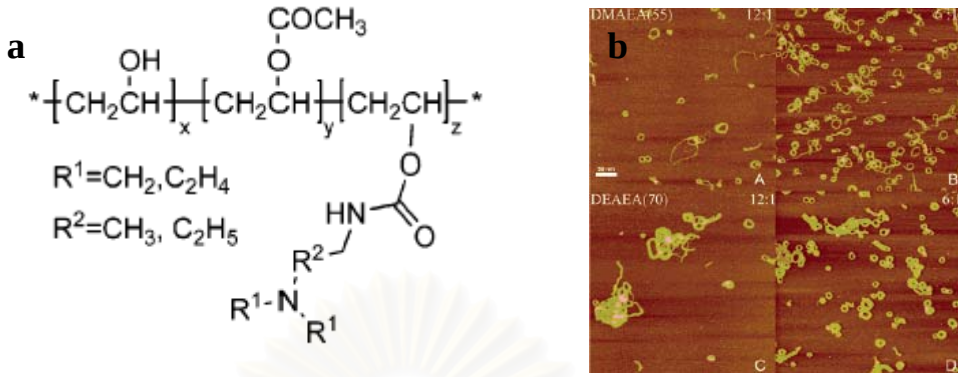


Figure 1.6 The structure of amine-modified poly(vinyl alcohol) (a) and atomic force micrograph of amine-modified poly(vinyl alcohol)-DNA nanocomplexes (b) (this figure is taken from reference 30).

In 2006, Kissel and coworkers synthesized poly[vinyl-3-(dialkylamino)alkylcarbamate-*co*-vinyl acetate-*co*-vinyl alcohol]-graft-poly(D,L-lactide-*co*-glycolide) by a ring opening polymerization. The obtained polymer was used as a drug delivery system. Drug could be entrapped into this charge-modified poly(vinyl alcohol) through electrostatic interactions of the amine group [31] (Figure 1.7).

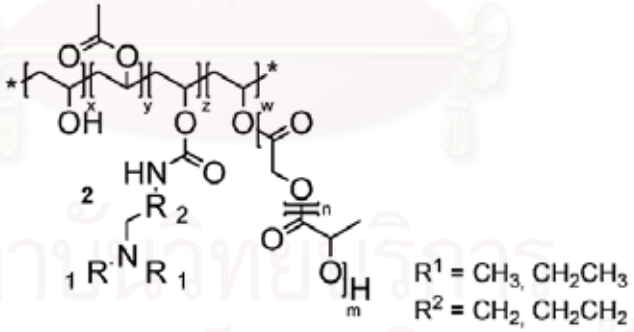


Figure 1.7 The structure of poly[vinyl-3-(dialkylamino)alkylcarbamate-*co*-vinyl acetate-*co*-vinyl alcohol]-graft-poly(D,L-lactide-*co*-glycolide) (this figure is taken from reference 31).

In 2007, Reverchon and coworkers prepared poly(vinyl alcohol) particles by Supercritical AntiSolvent technique using Gas AntiSolvent (GAS); the solution is put in the vessel then the antisolvent is loaded into the vessel with the outlet valve closed

and precipitation is obtained from the expanded liquid. Finally, pure antisolvent is fed in order to wash away the solvent, and semi-continuous Supercritical AntiSolvent process (SAS), which is characterized by the continuous delivery of solvent and antisolvent in the precipitator. The particles from GAS technique were submicro- and microparticles (0.4 and 2 μm diameter) (Figure 1.8) depending on the pressurization rate. Various particulate shapes were obtained, e.g., spherical (nanoparticles and microparticles), balloons (more than tenth of microns) and filaments (more than hundreds of microns) [32].

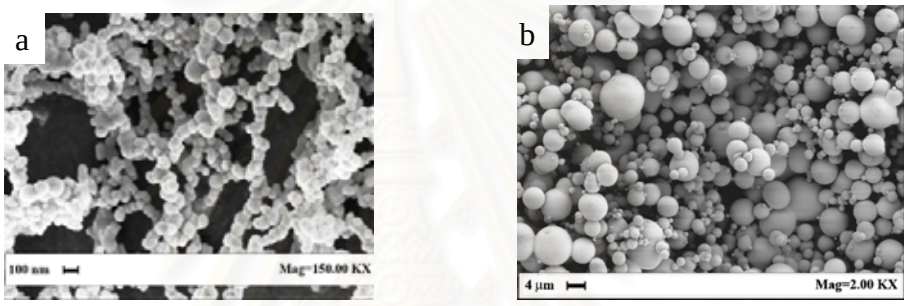
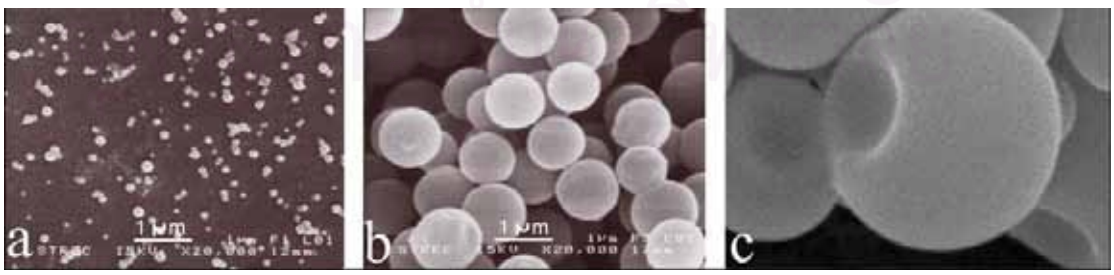
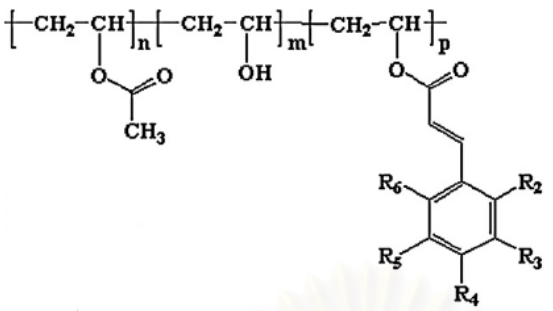


Figure 1.8 Scanning electron micrograph of PVA particles by SAS at 150 bar (a) at 120 bar (b) (this figure is taken from reference 32).

In 2008, Wanichwecharungruang and coworkers prepared micro/nanoparticles of various poly(vinylalcohol-co-vinylcinnamate) derivatives by dialysis method. Self-assembly of the obtained poly(vinylalcohol-co-vinylcinnamate) derivatives gave both spherical micelle nanoparticles and hollow reverse micellar microparticles of uniform sizes [33] (Figure 1.9).





d

poly(vinylalcohol-co-vinylcinnamate) : R_2-R_6 = H
poly(vinylalcohol-co-vinyl-4-methoxycinnamate) : R_2, R_3, R_5 = H, R_4 = OCH_3
poly(vinylalcohol-co-vinyl-2,4-dimethoxycinnamate) : R_3, R_5 = H, R_2, R_4 = OCH_3
poly(vinylalcohol-co-vinyl-2,4,5-trimethoxycinnamate) : R_3 = H, R_2, R_4, R_5 = OCH_3

Figure 1.9 Scanning electron micrograph of poly(vinylalcohol-co-vinyl-4-methoxycinnamate) particles by dialyzing against water (micellar particles, a) and hexane (reverse micellar particles, b and c) and the structure of poly(vinylalcohol-co-vinylcinnamate) derivatives (this figure is taken from reference 33).

1.3.2 Charged nanoparticles

Not very many charged polymers were prepared and fabricated into carriers. The following paragraph summarized these limited research works.

In 1990, Brouwer and coworkers prepared permanently charged poly(styrene-cationic comonomer) latices by emulsion copolymerization of styrene and *N*-trimethyl *N*-ethylmethacrylate ammonium salts (TMA) [34] (Figure 1.10).

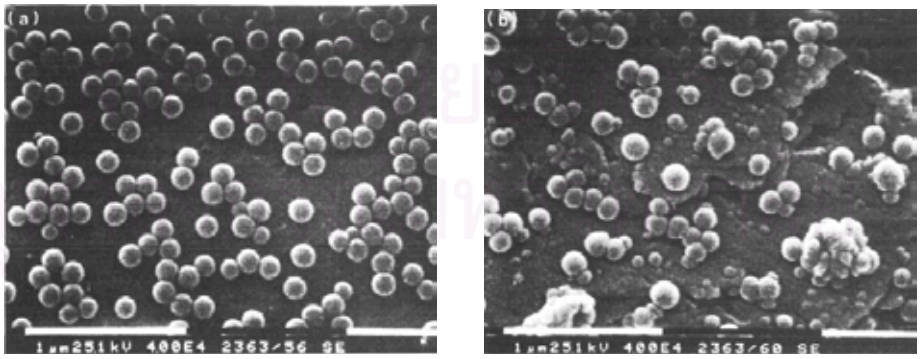


Figure 1.10 Scanning electron micrograph of styrene/TMA copolymer particles; (a) 80/01 styrene/TMA copolymer latex; (b) 80/20 styrene/TMA copolymer latex (this figure is taken from reference 34).

In 2008, Chono and coworkers have developed a nanoparticle formulation [liposomes-protamine-hyaluronic acid nanoparticle (LPH-NP)] for systemically delivering siRNA into the tumor. LPH-NP was prepared by self-assembling process. This process was prepared by cationic liposomes coated on negatively charged complex (mixing of siRNA and hyaluronic acid). The particle size, zeta potential and siRNA encapsulation efficiency of the formulation were approximately 115 nm, +25 mV and 90%, respectively [35] (Figure 1.11).

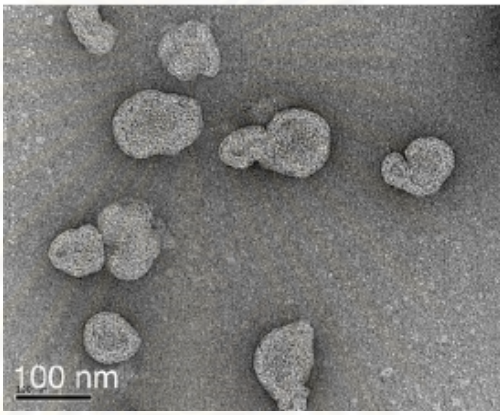


Figure 1.11 Transmission electron micrographs of optimal LPD-NP (this figure is taken from reference 35).

In 2008, Sayin and coworkers prepared nanoparticulate systems by using two different charged chitosan derivatives, *N*-trimethyl chitosan (TMC, polycationic), and mono-*N*-carboxymethyl chitosan (MCC, polyampholytic) for mucosal immunization. Nanoparticles were prepared using ionic gelation method and loaded with tetanus toxoid (TT). Nanoparticles had a high loading efficacy (>90% m/m) and particle size was in the range of 40–400 nm. MCC had a negative surface charge and TMC and chitosan had a positive surface charge [36] (Figure 1.12)

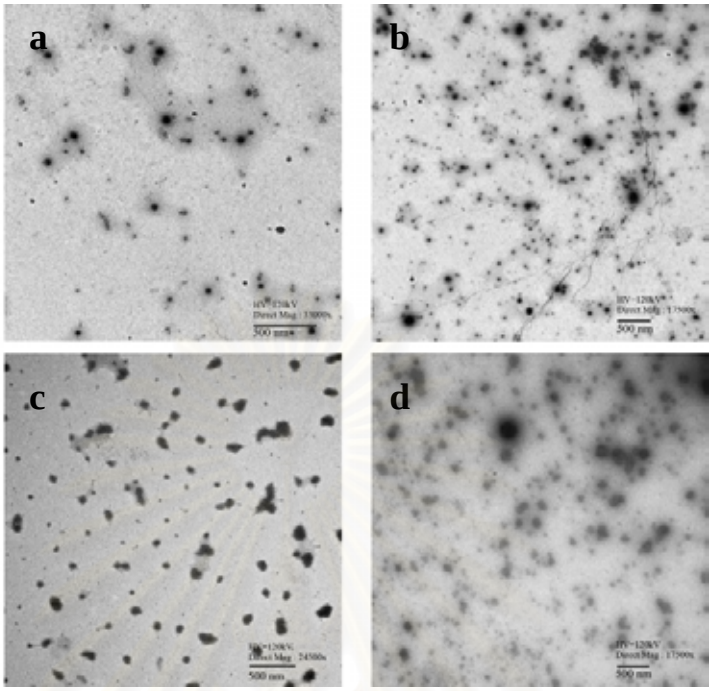


Figure 1.12 Transmission electron micrographs of MCC nanoparticles: (a) Empty, and (b) TT loaded, (c) TT loaded chitosan nanoparticles and (d) TT loaded TMC nanoparticles (this figure is taken from reference 36).

1.4 Research goal

The objectives of this research can be summarized as follows:

1. To synthesized poly(vinyl alcohol-co-vinyl butane sultone) and poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone) with: various degrees of substitution of 1,4-butane sultone and dodecyl moities.
2. To synthesized poly(vinyl alcohol-co-vinyl diethylamino butanate) and poly(vinyl alcohol-co-vinyl dodecane-co- vinyl diethylamino butanate) with: various degrees of substitution of diethylamine and dodecyl moities.
3. To carry out the self-assembly of the obtained PVA derivatives via dialysis method.

CHAPTER II

EXPERIMENTALS

2.1 Materials and Chemicals

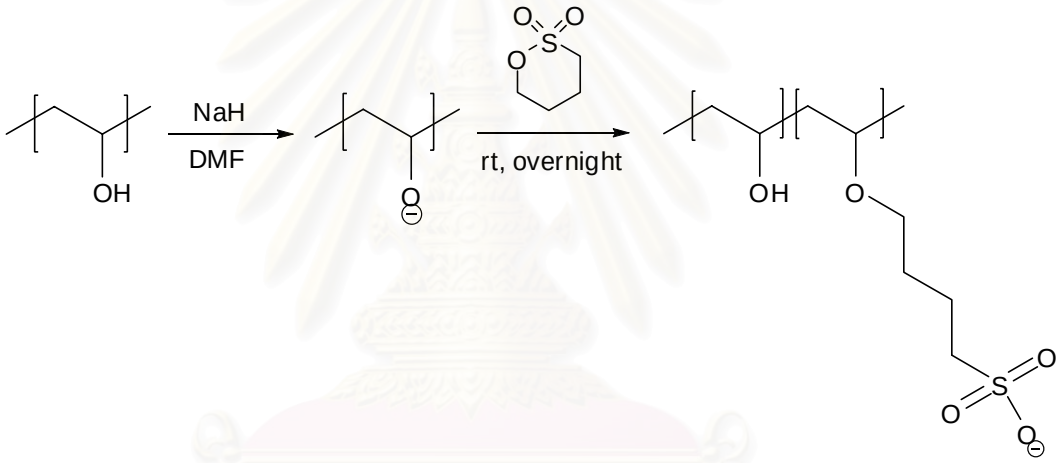
Poly(vinyl alcohol), Mw 124,000–186,000, 87– 89% deacetylated (PV(OH) 124,000) and dialysis membranes with molecular weight cutoff of 7374 Daltons, 76 mm flat width, were purchased from Aldrich Chemical Company (Steinheim, Germany). CelluSep T4 dialysis tube with molecular weight cutoff of 12,000–14,000, 75 mm flat width, 17.9 mL/cm volume capacity was purchased from Membrane Filtration Products, Seguin, TX, USA). 1,4-Butane sultone; 1-hydoxybenzotriazole (HOBt); 1-(3-dimethylaminopropyl)-3-ethylcarboiimide (EDCI) and succinic anhydride were purchased from Acros Organics (Geel, Belgium). 1-Bromododecane and sodium hydride were purchased from Fluka (Buchs, Switzerland). Diethylamine was purchased from Scharlau Chemie (Barcelona, Spain). 4-Dimethylaminopyridine (DMAP), ethanol and all deuterated solvents were purchased from Merck (Damstadt, Germany). Dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO) were purchased from Labscan (Dublin, Ireland). 2-Ethylhexyl *p*-methoxycinnamate (EHMC) and sodium dodecyl sulfate were obtained from Institute of Beauty and Health Sciences Co., Ltd. (Bangkok, Thailand).

2.2 Instruments and Equiments

The ^{1}H and ^{13}C NMR analyses were performed using a Varian Mercury spectrometer, which operated at 400.00 MHz for ^{1}H and 100.00 MHz for ^{13}C nuclei (Varian Company, Palo Alto, CA, USA) in deuterated chloroform ($CDCl_3$) or deuterated dimethylsulfoxide (DMSO-d6) or deuterium oxide (D_2O) with tetramethysilane (TMS) as an internal reference. UV Absorption spectra were acquired with a UV 2500 UV–vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan) using a quartz cell with 1 cm pathlength. FT-IR spectra were obtained using a Nicolet Fourier transform Infrared spectrophotometer: Impact 410 (Nicolet Instruments Technologies, Inc., Madison, WI, USA). Thermogram of each sample was acquired at 25-220 ^{o}C under nitrogen with a scanning rate of 10 ^{o}C/min using differential scanning calorimetry:

DSC 204 (Netzsch Group, Selb, Germany). Transmission electron micrographs (TEM) were obtained using JEM-2100 (Jeol, Ltd., Japan) with an accelerating votage of 100-120 kV in conjunction with selected area electron diffraction (SAED). The particle size and zeta potential were acquired by Mastersizer S and Zetasizer nanoseries (Mulvern Instruments, Worcestershire, UK), respectively, equipped with a He-Ne laser beam at 632.8 nm (scattering angle of 173^{o}. The particle suspension was freezedried using Freeze-Dry/Shell Freeze System Model 7753501 (Labconco Corp., Kansus, MI, USA)

2.3 Synthesis of Poly(vinyl alcohol-co-vinyl butane sultone) (PVA-BS)



Scheme 2.1 Synthetic pathway of poly(vinyl alcohol-co-vinyl butane sultone) (**PVA-BS**)

Poly(vinyl alcohol-co-vinyl butane sultone) were synthesized by reacting PVA with 1,4-butane sultone under anhydrous condition using NaH as a catalyst. PVA (0.44 g, 10 mmol monomeric units) were dissolved in 45 mL of heated anhydrous DMF. Then, NaH and 1,4-butane sultone (see Table 2.1) were added. The mixture was stirred overnight at room temperature. The substituted polymer was purified by dialysing against distilled water using the dialysis cellulose membrane tube (MWCO 07374, 76 mm flat width, Aldrich Chemical Company, Steinheim, Germany). Dry products were obtained by freeze-drying.

Table 2.1 Condition used during the syntheses of **PVA-BS**

Compounds	Amounts of 1,4-butane sultone
PVA-BS1	1 mL, 10 mmol
PVA-BS2	0.5 mL, 5 mmol
PVA-BS3	0.2 mL, 2 mmol
PVA-BS4	0.1 mL, 1 mmol

PVA-BS1: Light orange solid. DS: 0.194. Yield: 76%. Tg: 104.4 ^{o}C. FT-IR (KBr, cm^{-1}): 3426 (s, br, OH stretching), 2945, 2867 (s, –C–H stretching), 1330 (s, SO_3^- stretching), 1442 (m, $–CH_2–$ bending), 1378 (m, C–O–H bending), 1048 (s, C–O–H stretching) and 1200, 1100 (s, C–O–C stretching). ^{1}H NMR (400 MHz, D_2O, δ, ppm): 4.0–3.2 (br, 4H, –C**H**–OH and 3H, –C**H**–O–C**H**$_2$–), 2.7 (t, *J*= 7.6, 14.4 Hz, 2H, –C**H**$_2$–SO_3^-) and 1.0-1.8 (br, 10H,–CH–C**H**$_2$–CH– of PVA backbone and 4H, O–CH_2–C**H**$_2$–C**H**$_2$–CH_2–SO_3^-).

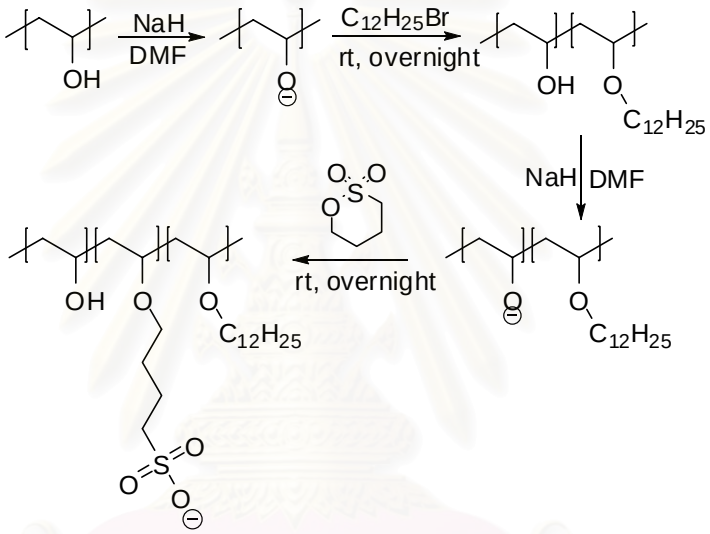
PVA-BS2: Light orange solid. DS: 0.066. Yield: 85%. Tg: 83.7 ^{o}C. FT-IR (KBr, cm^{-1}): 3394 (s, br, OH stretching), 2933, 2863 (s, –C–H stretching), 1330, 1144 (s, SO_3^- stretching), 1430 (m, $–CH_2–$bending), 1375 (m, C–O–H bending) 1046 (s, C–O–H stretching) and 1196, 1096 (s, C–O–C stretching). ^{1}H NMR (400 MHz, D_2O, δ, ppm): 4.0–3.2 (br, 14H, –C**H**–OH and 3H, –C**H**–O–C**H**$_2$–), 2.7 (t, *J*= 7.6, 15.2 Hz, 2H, –C**H**$_2$–SO_3^-) and 1.0-1.8 (br, 28H, –CH–C**H**$_2$–CH– of PVA backbone and 4H, O–CH_2–C**H**$_2$–C**H**$_2$–CH_2–SO_3^-).

PVA-BS3: Light orange solid. DS: 0.036. Yield: 94%. Tg: 81.7 ^{o}C. FT-IR (KBr, cm^{-1}): 3381 (s, br, OH stretching), 2936, 2854 (s, –C–H stretching), 1330, 1147 (s, SO_3^- stretching), 1433 (m, $–CH_2–$ bending), 1375 (m, C–O–H bending) 1040 (s, C–O–H, stretching) and 1199, 1099 (s, C–O–C stretching). ^{1}H NMR (400 MHz, D_2O, δ, ppm): 4.0–3.2 (br, 27H, –C**H**–OH and 3H, –C**H**–O–C**H**$_2$–), 2.7 (t, *J*= 7.4, 14.8 Hz, 2H, –C**H**$_2$–SO_3^-) and 1.0-1.8 (br, 54H, –CH–C**H**$_2$–CH– of PVA backbone and 4H, O–CH_2–C**H**$_2$–C**H**$_2$–CH_2–SO_3^-).

PVA-BS4: Light orange solid. DS: 0.013. Yield: 81%. Tg: 76.9 ^{o}C. FT-IR (KBr, cm^{-1}): 3372 (s, br, OH stretching), 2927, 2850 (s, –C–H stretching), 1330, 1144 (s, SO_3^- stretching), 1436 (m, $–CH_2–$ bending), 1375 (m, C–O–H bending) 1037 (s, C–

O–H stretching) and 1196, 1095 (s, C–O–C stretching). ^{1}H NMR (400 MHz, D_2O, δ, ppm): 4.0–3.2 (br, 76H, –C**H**–OH and 3H, –C**H**–O–C**H**2–), 2.7 (t, *J*= 7.8, 16 Hz, 2H, –C**H**$_2$–SO_3^-) and 1.0-1.8 (br, 152H, –CH–C**H**$_2$–CH– of PVA backbone and 4H, O–CH_2–C**H**$_2$–C**H**$_2$–CH_2–SO_3^-).

2.4 Synthesis of Poly(vinyl alcohol-co-vinyl dodecane (PVA-12C) and Poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone) (PVA-12C-BS)



Scheme 2.2 Synthetic pathway of poly(vinyl alcohol-co-dodecane-co-vinyl butane sultone) (**PVA-12C-BS**)

Poly(vinyl alcohol-co-vinyl dodecane (**PVA-12C**) and poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone) (**PVA-12C-BS**) were prepared at various ratios of vinyl alcohol : vinyl dodecane and vinyl alcohol : vinyl dodecane : vinyl butane sultone.

Poly(vinyl alcohol-co-dodecane-co-vinyl butane sultone) was prepared in two steps; dodecane grafting and butane sultone grafting.

First, poly(vinyl alcohol-co-vinyl dodecane) (**PVA-12C**) was prepared by reacting PVA with 1-bromododecane under anhydrous condition using NaH as a catalyst. PVA (0.44 g, 10 mmol monomeric units) was dissolved in 45 mL of heated anhydrous DMF. Then, NaH and 1-bromododecane (see Table 2.2) were added. The mixture was stirred overnight at room temperature. The obtained polymer (**PVA-12C**)

was purified by dialysing against distilled water. The products were dried by freeze-drying.

Second, poly(vinyl alcohol-co-dodecane-co-vinyl butane sultone) (**PVA-12C-BS**) was prepared by reacting **PVA-12C** with butane sultone. Appropriate **PVA-12C** (0.5 g) was dissolved in 45 mL of heated anhydrous DMF. Then, NaH and 1,4-butane sultone (see Table 2.2) were added. The mixture was stirred overnight at room temperature. The substituted polymer (**PVA-12C-BS**) was purified by dialysing against distilled water. Dry products were obtained by freeze-drying.

Table 2.2 Condition used during the syntheses of **PVA-12C-BS**

Compounds	Amounts of 1-bromododecane	Amounts of 1,4-butane sultone
PVA-12C1	0.50 mL, 2 mmol	-
PVA-12C2	0.12 mL, 0.5 mmol	-
PVA-12C1-BS1	-	2 mL, 20 mmol
PVA-12C1-BS2	-	1 mL, 10 mmol
PVA-12C1-BS3	-	0.5 mL, 5 mmol
PVA-12C1-BS4	-	0.2 mL, 2 mmol
PVA-12C2-BS1	-	1 mL, 10 mmol
PVA-12C2-BS2	-	0.5 mL, 5 mmol
PVA-12C2-BS3	-	0.2 mL, 2 mmol

PVA-12C1-BS1: Light orange solid. DS of dodecyl group: 0.033. DS of butane sultone: 0.135. Yield: 101%. Tg: 81.3 ^{o}C. FT-IR (KBr, cm^{-1}): 3393 (s, br, OH stretching), 2930, 2854 (s, –C–H stretching), 1333 (s, SO_3^- stretching), 1455 (m, –CH2– bending), 1375 (m, C–O–H bending) 1043 (s, C–O–H stretching) and 1193, 1095 (s, C–O–C stretching). ^{1}H NMR (400 MHz, D_2O, δ, ppm): 4.2-4.9 (br, 9H, –O**H**), 4.0–3.2 (br, 9H, –C**H**–OH and 1H, –C**H**–O–C**H**$_2$–), 2.7 (t, *J*=7.2, 14.4 Hz, 3H, –C**H**$_2$–SO_3^-), 0.8-1.8 (br, 7H, –C**H**$_2$– of dodecyl moiety, 6H, –C**H**$_2$–C**H**$_2$– and 22H, –CH–C**H**$_2$–CH– of PVA backbone) and 0.7 (t, *J*=8, 16 Hz, 1H, –CH_2–C**H**$_3$)

PVA-12C1-BS2: Light orange solid. DS of dodecyl group: 0.033. DS of butane sultone: 0.037. Yield: 96%. Tg: 71.6 ^{o}C. FT-IR (KBr, cm^{-1}): 3384 (s, br, OH

stretching), 2924, 2854 (s, –C–H stretching), 1330, 1141 (s, SO_3^- stretching), 1455 (m, –CH2– bending), 1375 (m, C–O–H bending) 1043 (s, C–O–H stretching) and 1196, 1095 (s, C–O–C stretching). ^{1}H NMR (400 MHz, D_2O, δ, ppm): 4.5 (br, 13H, –O**H**), 4.0–3.2 (br, 13H, –C**H**–OH and 1H, –C**H**–O–C**H**$_2$–), 2.7 (t, *J*=6.8, 13.6 Hz, 1H, –C**H**$_2$–SO_3^-), 0.8-1.7 (br, 10H, –C**H**$_2$– of dodecyl moiety , 2H, –C**H**$_2$–C**H**$_2$– and 27H, –CH–C**H**$_2$–CH– of PVA backbone) and 0.7 (t, *J*=6.8, 13.6 Hz, 1H, –CH_2–C**H**$_3$)

PVA-12C1-BS3: Light orange solid. DS of dodecyl group: 0.033. DS of butane sultone: 0.028. Yield: 94%. Tg: 70.6 ^{o}C. FT-IR (KBr, cm^{-1}): 3378 (s, br, OH stretching), 2924, 2854 (s, –C–H stretching), 1330, 1144 (s, SO_3^- stretching), 1446 (m, –CH2– bending), 1375 (m, C–O–H bending) 1040 (s, C–O–H stretching) and 1199, 1098 (s, C–O–C stretching). ^{1}H NMR (400 MHz, D_2O-, δ, ppm): 4.5 (br, 17H, –O**H**), 4.0–3.2 (br, 17H, –C**H**–OH and 2H, –C**H**–O–C**H**$_2$–), 2.7 (t, *J*=7.4, 14.8 Hz, 1H, –C**H**$_2$–SO_3^-), 0.8-1.7 (br, 13H, –C**H**2– of dodecyl moiety, 2H, –C**H**$_2$–C**H**$_2$– and 36H,–CH–C**H**$_2$–CH– of PVA backbone) and 0.7 (t, *J*=6.8, 13.6 Hz, 2H, –CH_2–C**H**$_3$)

PVA-12C1-BS4: Light orange solid. DS of dodecyl group: 0.033. DS of butane sultone: 0.022. Yield: 100%. Tg: 69.0 ^{o}C. FT-IR (KBr, cm^{-1}): 3378 (s, br, OH stretching), 2921, 2848 (s, –C–H stretching), 1333, 1141 (s, SO_3^- stretching), 1449 (m, –CH2– bending), 1375 (m, C–O–H bending) 1040 (s, C–O–H stretching) and 1193, 1095 (s, C–O–C stretching). ^{1}H NMR (400 MHz, D_2O, δ, ppm): 4.5 (br, 21H, –O**H**), 4.2–3.2 (br, 21H, –C**H**–OH and 2H, –C**H**–O–C**H**$_2$–), 2.7 (t, *J*=7.2, 14.4 Hz, 1H, –C**H**$_2$–SO_3^-), 0.8-1.9 (br, 16H, –C**H**$_2$– of dodecyl moiety, 2H, –C**H**$_2$–C**H**$_2$– and 45H, –CH–C**H**$_2$–CH– of PVA backbone) and 0.7 (t, *J*=7.2, 14.4 Hz, H, 2H, –CH_2–C**H**$_3$)

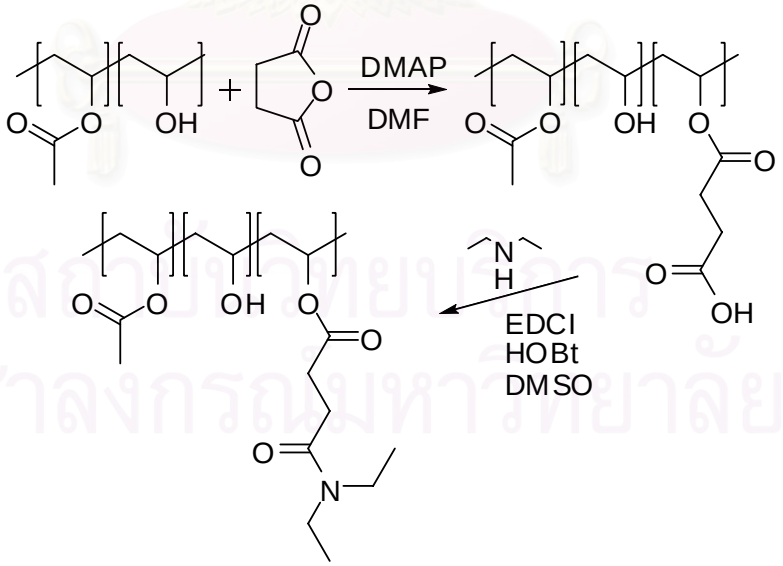
PVA-12C2-BS1: Light orange solid. DS of dodecyl group: 0.021. DS of butane sultone: 0.036. Yield: 107%. Tg: 80.3 ^{o}C. FT-IR (KBr, cm^{-1}): 3369 (s, br, OH stretching), 2918, 2851 (s, –C–H stretching), 1330, 1144 (s, SO_3^- stretching), 1439 (m, –CH2– bending), 1375 (m, C–O–H bending) 1040 (s, C–O–H stretching) and 1199, 1092 (s, C–O–C stretching). ^{1}H NMR (400 MHz, D_2O, δ, ppm): 4.5 (br, 15H, –O**H**), 4.0–3.2 (br, 15H, –C**H**–OH and 1H, –C**H**–O–C**H**$_2$–), 2.7 (t, *J*= 8, 16.4 Hz, 1H, –C**H**$_2$–SO_3^-), 0.8-1.8 (br, 7H, –C**H**$_2$– of dodecyl moiety, 2H, –C**H**$_2$–C**H**$_2$– and 32H, –CH–C**H**$_2$–CH– of PVA backbone) and 0.7 (t, *J*= 8.2, 20.4 Hz, 1H, –CH_2–C**H**$_3$)

PVA-12C2-BS2: Light orange solid. DS of dodecyl group: 0.021. DS of butane sultone: 0.027. Yield: 110%. Tg: 76.9 ^{o}C. FT-IR (KBr, cm^{-1}): 3381 (s, br, OH

stretching), 2924, 2854 (s, –C–H stretching), 1330, 1144 (s, SO_3^- stretching), 1443 (m, –CH2– bending), 1375 (m, C–O–H bending) 1043 (s, C–O–H stretching) and 1196, 1095 (s, C–O–C stretching). ^{1}H NMR (400 MHz, D_2O, δ, ppm): 4.5 (br, 18H, –O**H**), 4.0–3.2 (br, 18H, –C**H**–OH and 1H, –C**H**–O–C**H**$_2$–), 2.7 (t, *J*= 7.8, 15.6 Hz, 1H, –C**H**$_2$–SO_3^-), 0.8-1.8 (br, 8H, –C**H**$_2$– of dodecyl moiety, 2H, –C**H**$_2$–C**H**$_2$– and 37H, –CH–C**H**$_2$–CH– of PVA backbone) and 0.7 (t, *J*= 7.4, 14.8 Hz, 1H, –CH_2–C**H**$_3$)

PVA-12C2-BS3: Light orange solid. DS of dodecyl group: 0.021. DS of butane sultone: 0.025. Yield: 106%. Tg: 78.3 ^{o}C. FT-IR (KBr, cm^{-1}): 3369 (s, br, OH stretching), 2918, 2851 (s, –C–H stretching), 1330, 1144 (s, SO_3^- stretching), 1439 (m, –CH2– bending), 1375 (m, C–O–H bending) 1040 (s, C–O–H stretching) and 1199, 1092 (s, C–O–C stretching). ^{1}H NMR (400 MHz, D_2O, δ, ppm): 4.5 (br, 19H, –O**H**), 4.0–3.2 (br,19H, –C**H**–OH and 1H, –C**H**–O–C**H**$_2$–), 2.7 (t, *J*= 6.6, 13.2 Hz, 1H, –C**H**$_2$–SO_3^-), 0.8-1.8 (br, 8H, –C**H**$_2$– of dodecyl moiety, 2H, –C**H**$_2$–C**H**$_2$– and 40H, –CH–C**H**$_2$–CH– of PVA backbone) and 0.7 (t, *J*= 7.8, 15.6 Hz, 1H, –CH_2–C**H**$_3$)

2.5 Synthesis of poly(vinyl alcohol-co-vinyl diethylamino butanate) (PVA-SA-EA)



Scheme 2.3 Synthetic pathway of poly(vinyl alcohol-co-vinyl diethylamino butanate) (**PVA-SA-EA**)

Poly(vinyl alcohol-co-vinyl diethylamino butanate) (**PVA-SA-EA**) was prepared in two steps; succinic anhydride grafting and amine substitution.

First, PVA was reacted with succinic anhydride using DMAP as a catalyst. PVA (0.44 g, 10 mmol monomeric units) were dissolved in 45 mL of heated anhydrous DMF. Then, DMAP was added. The mixture was stirred for 5 minutes and succinic anhydride (see Table 2.3) was added. The mixture was stirred overnight at room temperature. The obtained polymer was dialyzed against aqueous 0.5% (v/v) HCl solution and then against distilled water. Dry products were obtained by freeze-drying.

Second, the obtained polymer (**PVA-SA**) (0.5 g) was dissolved in 100 mL of heated DMSO. Then, diethylamine and HOBt (see Table 2.3) were added. The mixture was cooled down to -4 ^{O}C and EDCI (see Table 2.3) was added. The reaction mixture was stirred overnight at room temperature. The substituted polymer (**PVA-EA**) was purified by dialysis against distilled water. Dry products were obtained by freeze-drying.

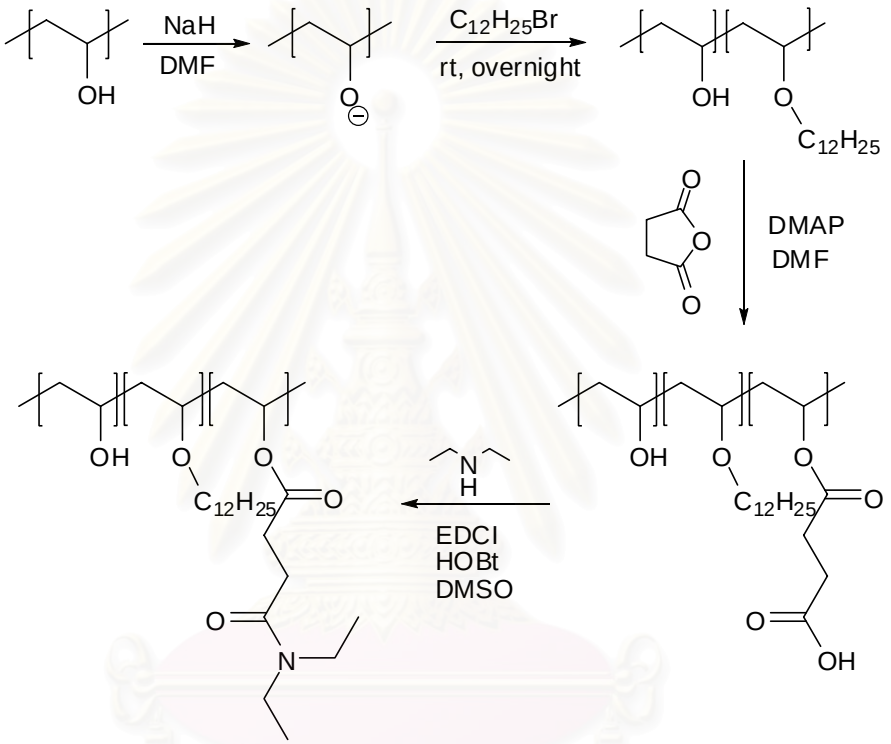
Table 2.3 Condition used during the syntheses of **PVA-SA-EA**

Compounds	Amounts of succinic anhydride	Amounts of DMAP	Amounts of diethylamine	Amounts of HOBt	Amounts of EDCI
PVA-SA1	0.30 g, 3 mmol	0.36 g, 3 mmol	-	-	-
PVA-SA2	0.20 g, 2 mmol	0.24 g, 2 mmol	-	-	-
PVA-SA3	0.10 g, 1 mmol	0.12 g, 1 mmol	-	-	-
PVA-SA3-EA	-	-	0.21 mL, 2 mmol	0.54 g, 4 mmol	0.77 g, 4 mmol

PVA-SA3-EA: yellow solid. DS: 0.084. Yield: 94%. ^{1}H NMR (400 MHz, D_2O, δ, ppm): 3.6-4.0 (br, 10H, –C**H**–OH, 1H, –C**H**–$OCOCH_3$ and 1H, –C**H**–$OCOCH_2$), 3.3 (br, 4H, N–C**H**$_2$–CH_3), 2.6 (br, 4H, –C**H**$_2$–CO), 1.9 (s, 4H, C**H**$_3$–CO), 1.6 (br, 24H, –

CH–C**H**$_2$–CH– of PVA backbone), 1.0 (t, *J*= 6.4, 12.8 Hz, 3H, N–CH$_2$–C**H**$_3$) and 0.9 (t, *J*= 6.8, 13.6 Hz, 3H, N–CH$_2$–C**H**$_3$)

2.6 Synthesis of poly(vinyl alcohol-co-vinyl dodecane-co-vinyl diethylamino butanate) (PVA-12C-SA-EA)



Scheme 2.4 Synthetic pathway of poly(vinyl alcohol-co-vinyl dodecane-co-vinyl diethylamino butanate) (**PVA-12C-SA-EA**)

Poly(vinyl alcohol-co-vinyl dodecane-co-vinyl diethylamino butanate) (**PVA-12C-SA-EA**) was prepared in three steps; dodecane grafting, succinic anhydride grafting and amine substitution.

First, poly(vinyl alcohol-co-vinyl dodecane) was prepared by reacting PVA with 1-bromododecane under anhydrous condition using NaH as a catalyst. PVA (0.44 g, 10 mmol monomeric units) were dissolved in 45 mL of heated anhydrous DMF. Then, NaH (0.24 g, 10 mmol) and 1-bromododecane (0.50 mL, 2 mmol) were added. The mixture was stirred overnight at room temperature. The obtained polymer

was separated by dialysing against distilled water and the products (**PVA-12C1**) were freeze-dried.

Second, **PVA-12C1** (0.5 g) was reacted with succinic anhydride using DMAP as a catalyst. **PVA-12C1** was dissolved in 45 mL of heated anhydrous DMF. Then, DAMP (see Table 2.4) was added. The mixture was stirred for 5 minutes and succinic anhydride (see Table 2.4) was added. The mixture was stirred overnight at room temperature. The reaction mixture was dialysed against aqueous 0.5% (v/v) HCl solution and distilled water, respectively. Dry products were obtained by freeze-drying.

Each obtained polymer (**PVA-12C1-SA**) was dissolved in 100 mL of heated DMSO. Then, diethylamine and HOBt (see Table 2.4) were added. The mixture was cooled down to -4 ^{O}C and EDCI was added. The reaction mixture was stirred overnight at room temperature. The substituted polymer (**PVA-12C1-SA-EA**) was purified by dialysis against distilled water. Dry products were obtained by freeze-drying.

Table 2.4 Condition used during the syntheses of **PVA-12C-SA-EA**

Compounds	Amounts of succinic anhydride	Amounts of DMAP	Amounts of diethylamine	Amounts of HOBt	Amounts of EDCI
PVA-12C1-SA-EA1	0.25 g, 2.5 mmol	0.31 g, 2.5 mmol	0.10 mL, 1 mmol	0.13 g, 1 mmol	0.19 g, 1 mmol
PVA-12C1-SA-EA2	0.50 g, 5 mmol	0.61 g, 5 mmol	0.21 mL, 2 mmol	0.27 g, 2 mmol	0.38 g, 2 mmol

PVA-12C1-SA-EA1: yellow solid. DS of dodecyl group: 0.033. DS of diethylamine: 0.068. Yield: 86%. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.4 (br, 13H, –O**H**), 3.4-3.9 (br, 16H, –C**H**–OH, –C**H**–O–C**H**$_2$– and –C**H**–OCOCH$_2$), 1.3(br, 39H, –CH–C**H**$_2$–CH– of PVA backbone and –C**H**$_2$– of dodecyl moiety), 1.0 (t, *J*= 6.4, 12.8 Hz, 3H, N–CH$_2$–C**H**$_3$), 0.9 (t, *J*= 6.8, 13.6 Hz, 3H, N–CH$_2$–C**H**$_3$) and 0.8 (br, 2H, –CH$_2$–C**H**$_3$ of dodecyl moiety)

PVA-12C1-SA-EA2: yellow solid. DS of dodecyl group: 0.033. DS of diethylamine: 0.105. Yield: 84%. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.5 (br, 8H, –O**H**), 3.5-3.9 (br, 10H, –C**H**–OH, –C**H**–O–C**H**$_2$– and –C**H**–OCCH$_2$), 1.5 (br, 26H, –CH–C**H**$_2$–CH– of PVA backbone and –C**H**$_2$– of dodecyl moiety), 1.0 (br, 3H, N–CH$_2$–C**H**$_3$), 0.9 (br, 3H, N–CH$_2$–C**H**$_3$) and 0.8 (br, 1H, –CH$_2$–C**H**$_3$ of dodecyl moiety)

2.7 Preparation of nanoparticles

Nanoparticles were prepared from the synthesized polymers using solvent displacement technique. Forty milligrams of polymer samples were dissolved in 10 mL of DMSO (4000 ppm) and the solution was transferred into a dialysis membrane tube and dialyzed against 1000 mL deionized water (Milli-Q®) at room temperature. Water was changed at 3, 8, 15, 20, 25, 30 and 40 h. After dialysis, a volume of the obtained colloidal suspension inside the dialysis tube normally increased from its starting polymeric solution to approximately 20 mL. The suspension was diluted with water to make the final volume of 50 mL before being subjected to TEM, AFM and dynamic light scattering analyses.

2.8 2-Ethylhexyl *p*-methoxycinnamate (EHMC) encapsulation

Twenty milligrams of polymer sample (**PVA-BS3,** and **PVA-12C2-BS1)** and 20 milligrams of EHMC were dissolved in 5 mL of DMSO. The solutions (4000 ppm) were placed into a dialysis membrane tube and immersed into 500 mL deionized water. Dialysis was carried out at room temperature; aliquots of solution outside the tube were withdrawn at 24, 48 and 72 h, for EHMC quantitative using UV/VIS spectrophotometry with an aid of a calibration curve constructed from EHMC standard solutions in ethanol. Percentage of encapsulation efficiency (%EE) were determined using equation (1) and the % drug loading were calculated using equation (2)

$$\%EE = \left[\frac{\text{amount of EHMC used - amount of EHMC found in the medium}}{\text{amount of EHMC used}} \right] \text{x } 100 \quad (1)$$

$$\% \text{ drug loading} = \left[\frac{\text{weight of drug in nanoparticles}}{\text{weight of nanoparticles}} \right] \times 100 \qquad (2)$$

2.9 Release of 2-Ethylhexyl *p*-methoxycinnamate (EHMC)

Releases of EHMC from EHMC-loaded nanoparticles were measured using a dialysis method [37]. Four milligram of **PVA-BS3** and **PVA-12C2-BS1** and four milligram of EHMC were dissolved in 1 mL of DMSO (4000 ppm). The solutions were placed into a regenerated dialysis membrane tube (CelluSep T4, regenerated cellulose tubular membrane, MWCO 12,000–14,000, 45 mm flat width, 6.42 mL/cm volume capacity, Membrane Filtration Products, Seguin, TX, USA) and dialyzed against 1000 mL deionized water (Milli-Q®) at room temperature to formed EHMC-loaded nanoparticles. Then the obtained EHMC-loaded nanoparticle suspension were immersed into 100 mL release medium (30% (v/v) ethanol in water). Dialysis was carried out at room temperature, and 3 mL of the medium were withdrawn at 1, 2, 4, 6, 8, 10, 12, 24, and 48h with the same volume of fresh medium added for every withdrawal. The amount of EHMC in each withdrawn aliquots was determined by UV/VIS spectrophotometry using a calibration curve constructed from EHMC standard solutions in ethanol.

As for comparison, aqueous suspension of EHMC in water was prepared using sodium dodecyl sulfate (SDS) similar procedure as described in 2.8 except that the polymer was replaced with SDS. Release profile of EHMC from this oil in water emulsion was these acquired using similar procedure to that of the release of EHMC from polymer nanospheres.

CHAPTER III

RESULTS AND DISCUSSION

3.1 Synthesis of poly(vinyl alcohol-co-vinyl butane sultone) (PVA-BS)

Grafting of 1,4-butane sultone (BS) onto PVA (Mw 124,000-186,000), (PVA, 124,000 Daltons), could be carried out successfully by substitution reaction of 1,4-butane soltone on PVA backbone (hydroxyl functionalities). Grafting sultone onto the polymer chain means putting on some negative charges onto the polymer. Higher sultone substitution thus corresponds to a more negatively charged polymer.

PVA (124,000 Daltons) was reacted with 1,4-butane sultone at various mole ratios (Table 3.1). Structures of the products were characterized by ^{1}H-NMR and IR.

^{1}H-NMR spectrum of PVA in D_2O shows resonances of the backbone methylene protons at 1.32-1.88 ppm and the methine protons attached to the –OH and the $–OCOCH_3$ at 3.88 and 3.83 ppm, respectively (Figure 3.1). The $–CH_3$ protons of the acetate group resonance at 1.95 ppm while the resonance of the hydroxyl protons is at the same position as that of D_2O, i.e., broad peak at 4.64 ppm. The ^{1}H spectra of the synthesized poly(vinyl alcohol-co-vinyl butane sultone) in D_2O show the described peaks with additional resonance at 2.7 from the methane protons attached to sultone group. Degree of substitution of each product (l, m and p; see Table 3.1) was obtained from the integration of ^{1}H-NMR spectrum peaks at 1.0-1.8 (–CH–C**H**$_2$–CH– of PVA backbone and –C**H**$_2$–CH_2– SO_3^-) and 2.7 ppm (–C**H**$_2$–SO_3^-). It should be noted here that the ^{1}H-NMR spectra obtained using the long delay time between pulses (25 s for 45 ^{O} pluses) were similar to the spectra obtained using normal delay time (1 s for 45 ^{O} pluses). Structure, degree of substitution and glass transition temperature (T_g) of the obtained poly(vinyl alcohol-co-vinyl butane sultone) are shown in Table 3.1.

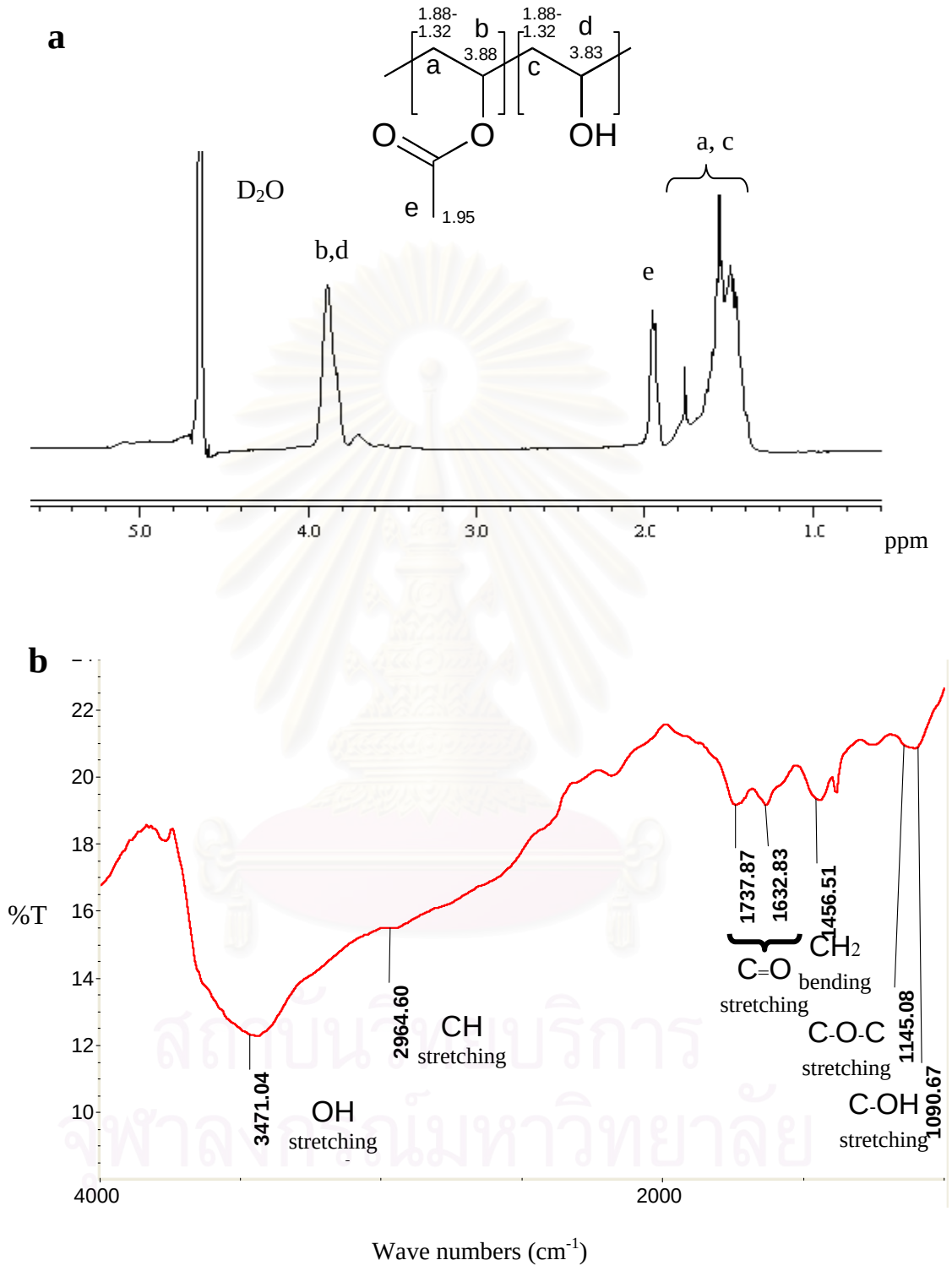


Figure 3.1 ^{1}H-NMR (a) and IR (b) spectrum of PVA.

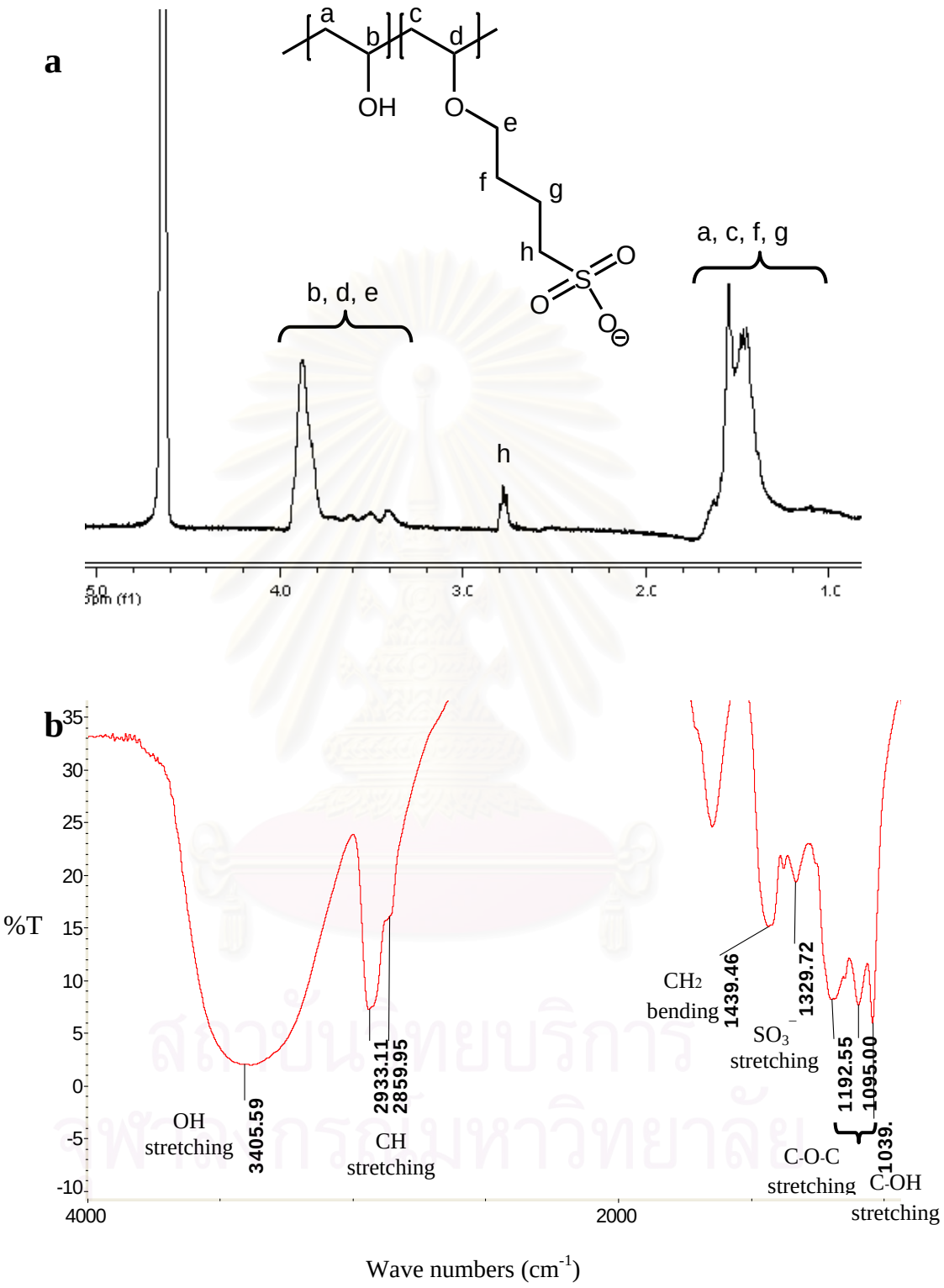
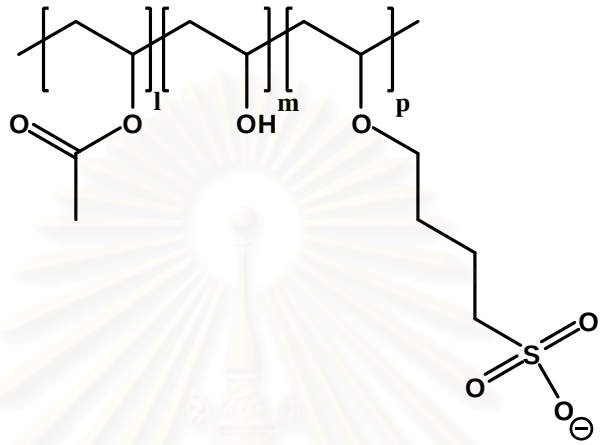


Figure 3.2 ^{1}H-NMR (a) and IR (b) spectrum of **PVA-BS2**

Table 3.1 Structure and physicochemical properties of the poly(vinyl alcohol-co-vinyl butane sultone)



Compound	Mole ratio[a] PVA:BS	Product characterization				
		l	m	p (DS)	T_g[b] (^oC)	T_m[c] (^oC)
PVA[d]	-	0.11	0.89	-	-	191.5
PVA-BS1	1:1	-	0.806	0.194	104.4	-
PVA-BS2	1:0.5	-	0.934	0.066	83.7	-
PVA-BS3	1:0.2	-	0.964	0.036	81.7	-
PVA-BS4	1:0.1	-	0.987	0.013	76.9	-

[a] used in the reaction (mole OH:mole BS)

[b] glass transition temperature as obtained from DSC analysis

[c] melting temperature as obtained from DSC analysis

[d] Mw 124,000-186,000

In this work, the prepared poly(vinyl alcohol-co-vinyl butane sultone) with the highest substitution degree (most negatively charged product) was **PVA-BS1** which possesses % sultone substitution of ~19% (DS = 0.194). **PVA-BS1** can easily be dissolved in water, thus self-assembly into nanoparticles in water could not be induced. The structures of the obtain poly(vinyl alcohol-co-vinyl butane sultone) (**PVA-BS1, PVA-BS2**, **PVA-BS3** and **PVA-BS4**) were confirmed by IR spectra in

which SO_3^- stretching at ~1330 and 1145 cm^{-1} could be clearly observed (Figure 3.2). Thus, it can be concluded that negative charges were successfully grafted onto PVA.

The thermogram of PVA (124,000 Daltons) showed melting temperature (T_m) at 191.5 ^OC (Table 3.1, Appendix **A16**). The **PVA-BS1**, **PVA-BS2**, **PVA-BS3** and **PVA-BS4** polymer possess glass transition temperature at 104.4, 83.7, 81.7 and 76.9 ^OC, respectively, with no melting characteristic (Appendix **A17-A20**). This indicates that the thermal property of PVA changed upon the grafting of sultone group onto its backbone. T_g seems to decrease with decreasing sultone substitution degree. Disappear of T_m with an appearance of T_g in the grafted product indicated that crystallinity in the PVA is decreased upon butane sultone grafting due to some disruption of intramolecular H-bonding.

Then, the there obtained water insoluble polymers **(PVA-BS2**, **PVA-BS3, PVA-BS4**) were induced into nanoparticles by solvent displacement technique (at 800 ppm) using DMSO as a solvent and water as an anti-solvent. When DMSO was displaced by water, the negative charges (sultone groups) are probably arranging themselves out ward to give maximum interaction with the hydrophilic water while the PVA backbone are probably trying to be away from water (Figure 3.3).

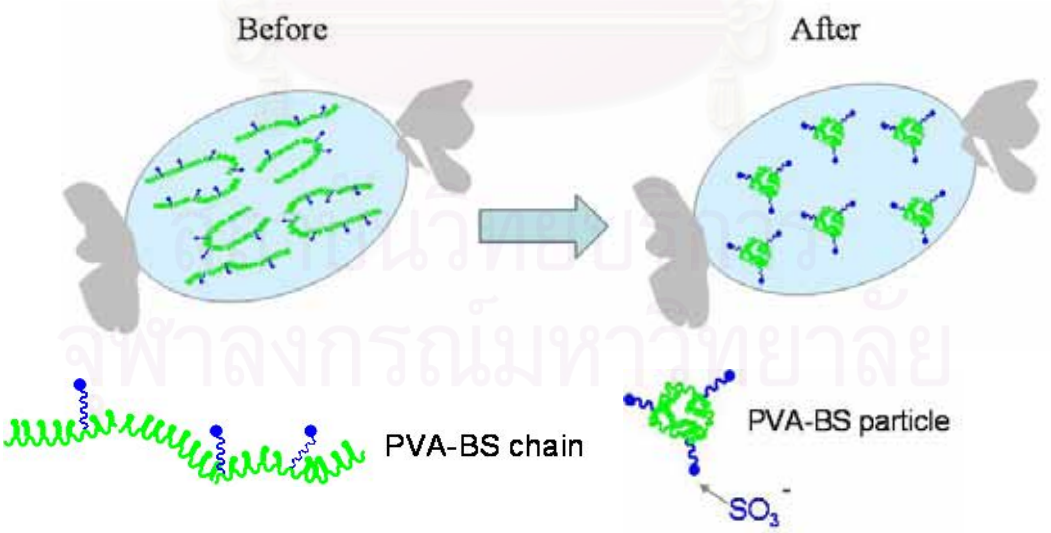


Figure 3.3 Particle formations by solvent displacement technique

This results in spherical micelles with negative surfaces. Spherical nanoparticles were observed by TEM (Figure 3.4)

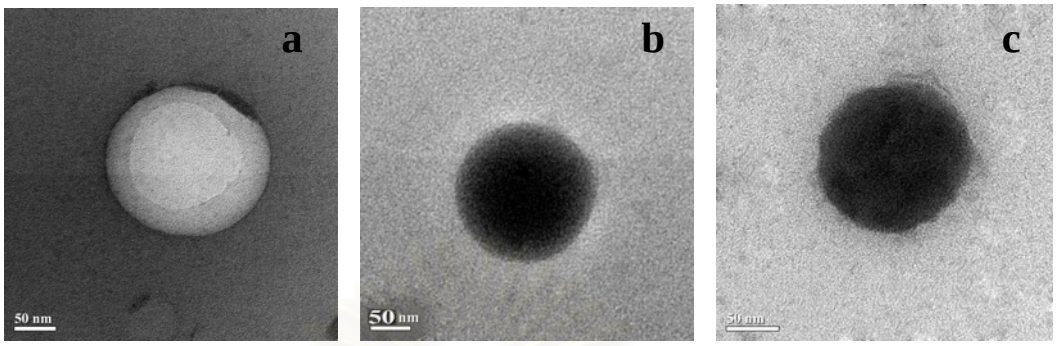


Figure 3.4 TEM photographs of **PVA-BS2** (a), **PVA-BS3** (b) and **PVA-BS4** (c) nanoparticles

The average hydrodynamic diameters determined by dynamic light scattering (DLS) method for **PVA-BS2**, **PVA-BS3** and **PVA-BS4** were 401 ± 3 nm, 320 ± 12 nm and 164 ± 0.4 nm, respectively. The particle sizes of poly(vinyl alcohol-co-vinyl butane sultone) increased with the increasing substitution degree of butane sultone onto PVA backbone (Figure 3.6-b). In this case, it is possible that polymers with more negative charge could hold more water molecules on their surfaces thus making their hydrodynamic size bigger (Figure 3.5). In other words, it is possible that with more negative charges on the polymer chains, the polymer become more hydrated and thus bigger particles were obtained (Figure 3.6). The fact that the least sultone substituted polymer gave particles with highest zeta-potential value (Figure 3.6-c) confirmed that these particles must be small and possess the least surface area.

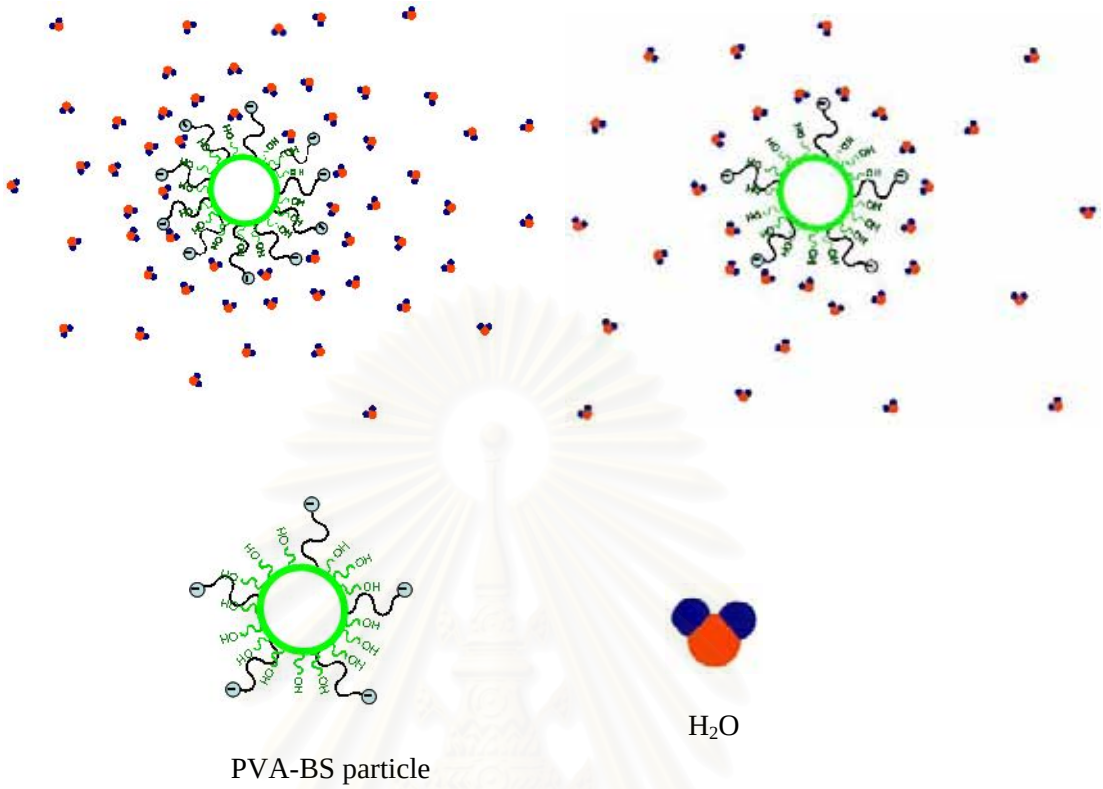


Figure 3.5 Schematic representations of **PVA-BS** particles in water.

From Figure 3.6-a, the situation is consistent with broad size distributions of **PVA-BS2** and **PVA-BS3**. In the other words, broad size distributions were probably a result of both hydration and probably ion-pair formation around the negative charges of the grafted sultone moieties.

The TEM size of **PVA-BS2**, **PVA-BS3** and **PVA-BS4** are comparable (Figure 3.4) while there hydrodynamic sizes (from DLS) are quite different and much bigger. This probably is a result of dry (TEM image) and fully hydrated (DLS measurement) particles. Since **PVA-BS2** is the highest sultone substituted polymer, it should be most hydrated in aqueous suspension (during DLS measurement). The DLS follows such hypothesis well.

Zeta-potential is a useful indicator of surface charge property and can be employed as an index to the stability of the nanoparticles. In most circumstances, the higher the absolute value of the zeta-potential of the particles, the larger amount of charge on their surface. These might result in stronger repellent interactions among the particles, and hence, higher stability of the suspension. It should be noted here that

the values of < -30 mV or > + 30 mV were usually used as an indicator of particle stability [38]. Zeta-potentials of poly(vinyl alcohol-co-vinyl butane sultone) nanoparticles were negative, indicating negative charge on the particles surfaces when the particles were dispersed in water. This probably is attributed to the presence of the grafted sultone groups. As show in Table 3.2, the most stable aqueous colloid among the three colloidal products is **PVA-BS4**, the product with the highest negative zeta-potential value (-28.7 mV). This value is close to -30 mV, and this means that **PVA-BS4** particles can stably disperse in water. Visual observation of **PVA-BS4** colloid also indicates good stability (no precipitate formed).

Table 3.2 The hydrodynamic diameters and Zeta-potential of nanoparticulate suspensions prepared from poly(vinyl alcohol-co-vinyl butane sultone) with DS 0.066 (**PVA-BS2**), DS 0.036 (**PVA-BS3**) and DS 0.013 (**PVA-BS4**), respectively, determined from dynamic light scattering technique.

Compound	DS	Hydrodynamic diameters (nm) (Average size ± SD)	Zeta-potential (mV)
PVA-BS2	0.066	401 ± 3	-20.6 ± 0.3
PVA-BS3	0.036	320 ± 12	-11.6 ± 1.6
PVA-BS4	0.013	164 ± 0.4	-28.7 ± 0.2

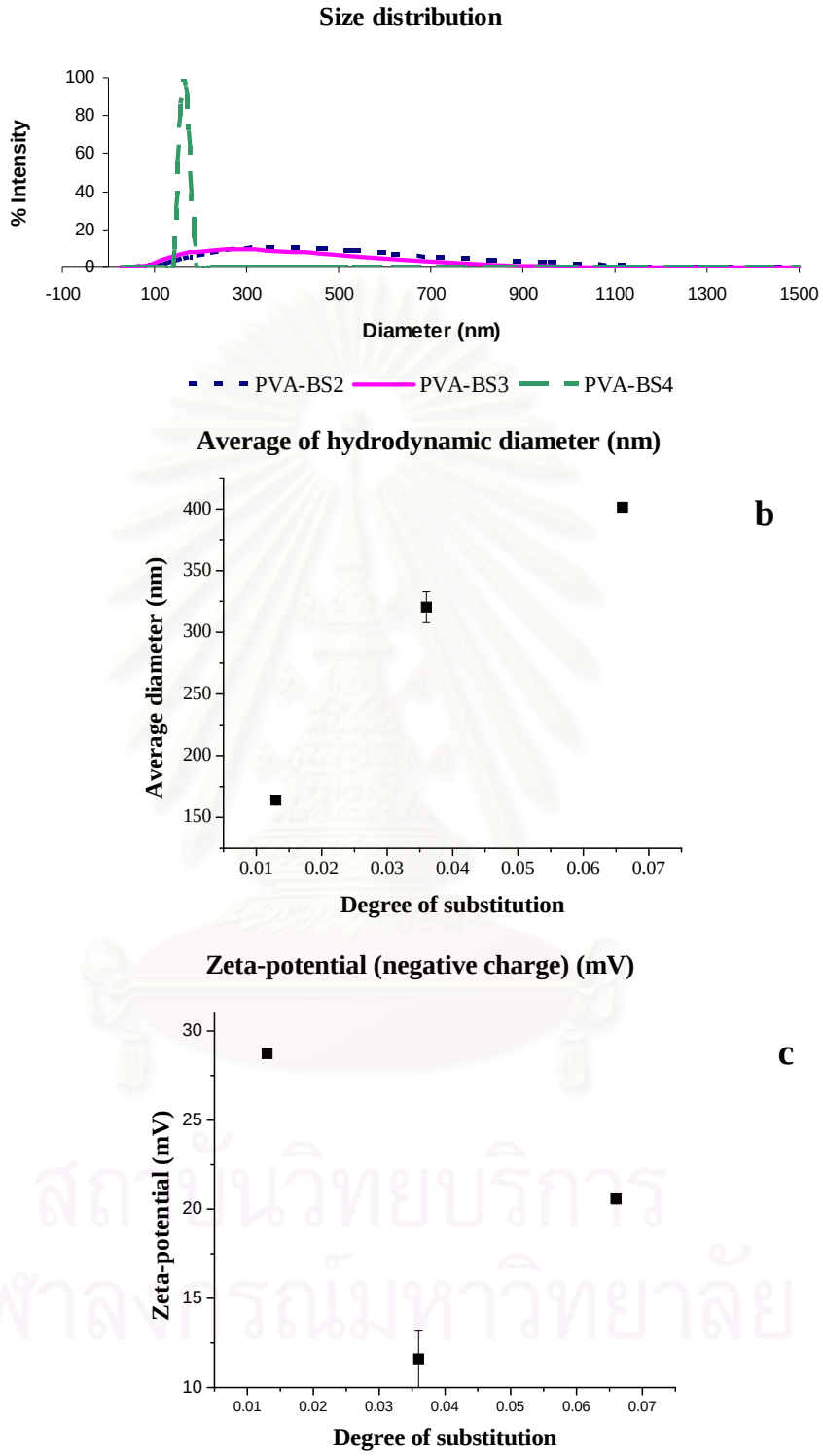


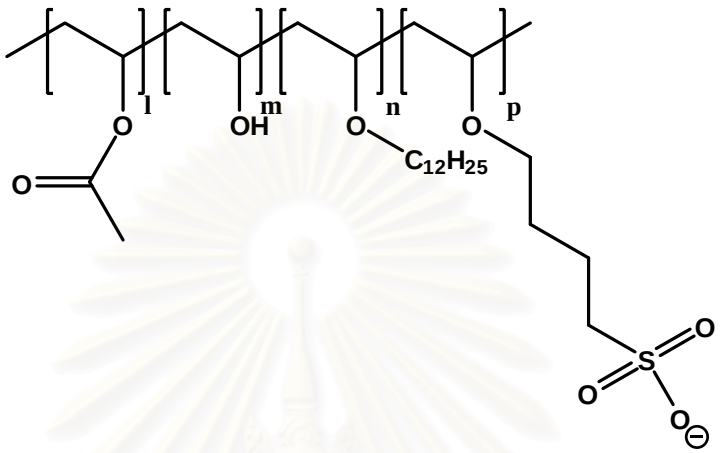
Figure 3.6 Size distribution (a), Average of hydrodynamic diameter (b) and Zeta-potential (c) of nanoparticles prepared from **PVA-BS** with various degrees of sultone substitution. Average numbers are shown with the error represent standard deviation of at least three data points.

3.2 Synthesis of Poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone) (PVA-12C-BS)

Grafting of 1-bromododecane (12C) and 1,4-butane sultone (BS) onto PVA (Mw 124,000-186,000) (PVA, 124,000 Daltons) could be carried out successfully by substitution reaction of 1-bromododecane and 1,4-butane sultone on PVA backbone (hydroxyl functionalities). Introducing dodecane onto the polymer chain means putting on some hydrophobic moieties onto the polymer. While introducing sultone onto the polymer chain means putting on some negatively charges onto the polymer. The ratio of hydroxyl group/dodecane group/sultone group varies with the degree of dodecane and sultone substitution, and this ratio governs the hydrophobic/hydrophilic characteristic of the obtained polymers.

Structures of the products were characterized by ^{1}H-NMR and IR (Appendix **A6**-**A12**). The ^{1}H-NMR spectra of the synthesized poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone) in D_2O are similar to poly(vinyl alcohol-co-vinyl butane sultone) except that the former shows the resonance at 0.7 ppm from terminal methyl protons of dodecane chain. Degree of substitution (l, n, m and p; see Table 3.3) was obtained from the ^{1}H-NMR spectrum of each product using integration of peaks at 0.8-1.8 ppm (–C**H**$_2$– of dodecane chain, –C**H**$_2$–C**H**$_2$– of sultone chain and–CH–C**H**$_2$–CH– of PVA backbone), 0.7 ppm (–CH$_2$–C**H**$_3$) and 2.7 ppm (–C**H**$_2$–SO_3^-). Degree of substitution and glass transition temperature (T_g) of the obtained poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone) derivatives are shown in Table 3.3.

Table 3.3 Structure and thermal characteristics of the poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone)



Compound	Mole ratio[a] PVA-12C:BS	Product characterization					
		l	n	m	p(DS)	T_g[b] (^oC)	T_m[c] (^OC)
PVA[d]	-	0.11	-	0.89	-	-	191.5
PVA-12C1-BS1	1:2	-	0.033	0.832	0.135	81.3	-
PVA-12C1-BS2	1:1	-	0.033	0.930	0.037	71.6	-
PVA-12C1-BS3	1:0.5	-	0.033	0.939	0.028	70.6	-
PVA-12C1-BS4	1:0.2	-	0.033	0.945	0.022	69.0	-
PVA-12C2-BS1	1:1	-	0.021	0.943	0.036	80.3	-
PVA-12C2-BS2	1:0.5	-	0.021	0.952	0.027	76.9	-
PVA-12C2-BS3	1:0.2	-	0.021	0.954	0.025	78.3	-

[a] used in the reaction (mole OH:mole BS)

[b] glass transition temperature as obtained from DSC analysis

[c] melting temperature as obtained from DSC analysis

[d] Mw 124,000-186,000

The thermogram of PVA (124,000 Daltons) showed melting temperature (T_m) at 191.5 ^{O}C. The **PVA-12C1-BS1**, **PVA-12C1-BS2**, **PVA-12C1-BS3**, **PVA-12C1-BS4**, **PVA-12C2-BS1**, **PVA-12C2-BS2**, and **PVA-12C2-BS3** polymer possess glass transition temperature (T_g) at 81.3, 71.6, 70.6, 69.0, 80.3, 76.9 and 78.3 ^{O}C, respectively with no melting characteristic (Table 3.3, Appendix **A21**-**A27**). This indicates that the thermal property of PVA changes with grafting of dodecane moiety and sultone group. The polymer becomes less crystalline (no Tm) upon grafting. Comparing **PVA-12C1-BS1** with **PVA-12C1-BS2** and **PVA-12C2-BS1** with **PVA-12C2-BS2**, it can be concluded that at similar dodecane substitution degree (n), higher sultone substitution degree (p) results in higher T_g values of the obtained polymers. In addition, comparing between **PVA-12C1-BS2** and **PVA-12C2-BS1**; **PVA-12C1-BS3** and **PVA-12C2-BS2**, it is clear that at similar sultone substitution degree (p), higher dodecane substitution degree (n) results in lower T_g values of the obtained polymers.

The shapes of all the obtained self-assembled polymers were spherical as observed by TEM (Figure 3.7). TEM pictures show the self-assembled particulates as white spheres against darker background, however, the **PVA-12C2-BS3** shows dark sphere against lighter background. This agrees with the fact that **PVA-12C2-BS3** is prepared from the lowest sultone substituted polymer. The less negative charge-spheres, thus responses to the high voltage electrons in a lesser degree comparing to spheres with more sultone substitution, and thus these spheres appeared darker.

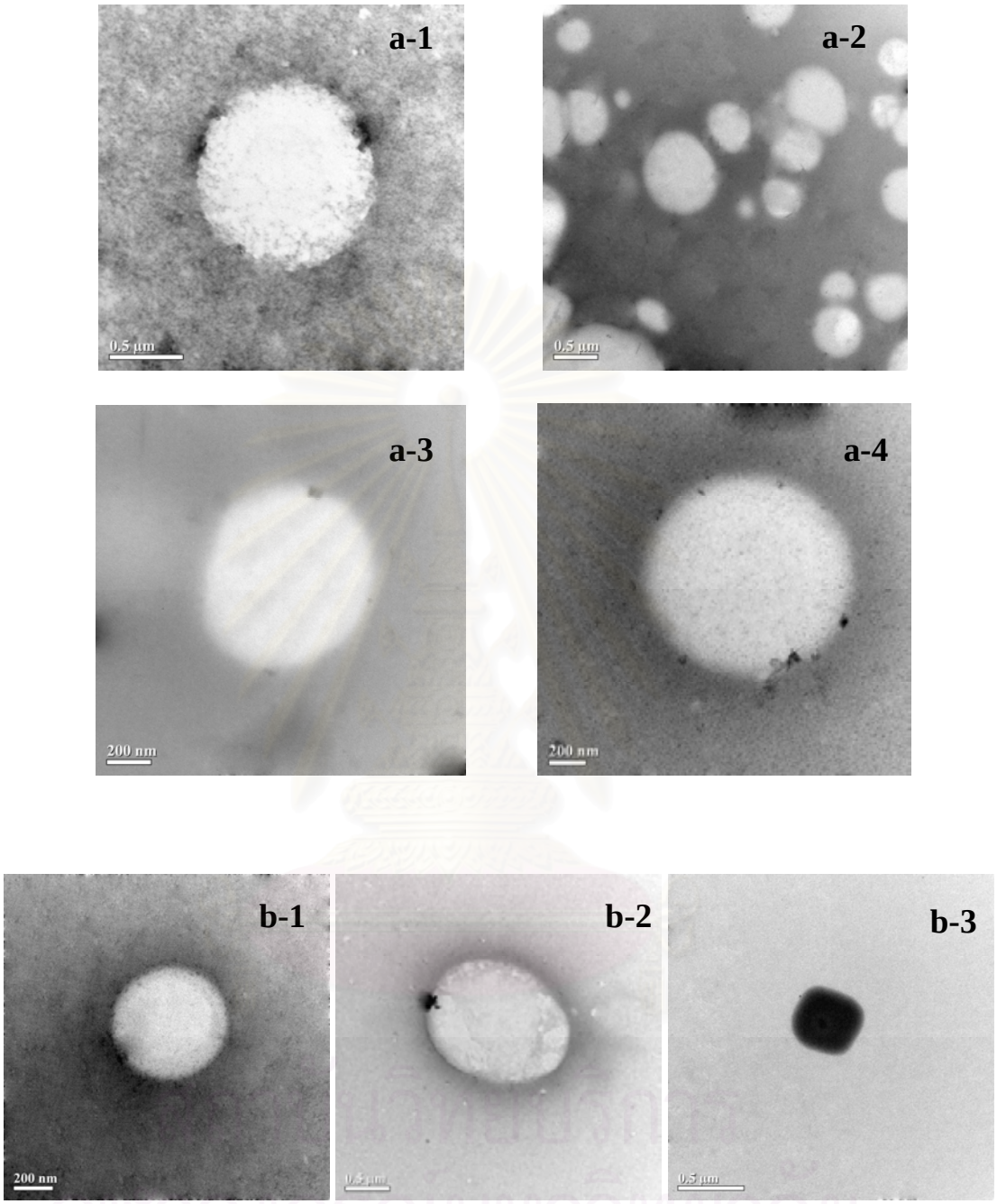


Figure 3.7 TEM photographs of **PVA-12C1-BS1** (a-1), **PVA-12C1-BS2** (a-2), **PVA-12C1-BS3** (a-3), **PVA-12C1-BS4** (a-4), **PVA-12C2-BS1** (b-1), **PVA-12C2-BS2** (b-2) and **PVA-12C2-BS3** (b-3).

As shown in Table 3.4, the average hydrodynamic diameters of **PVA-12C1-BS1**, **PVA-12C1-BS2**, **PVA-12C1-BS3**, **PVA-12C1-BS4**, **PVA-12C2-BS1**, **PVA-12C2-BS2** and **PVA-12C2-BS3**, were 144 ± 4 nm, 264 ± 2 nm, 761 ± 7 nm, 710 ± 7 nm, 455 ± 2 nm, 608 ± 1 nm and 774 ± 3 nm, respectively. The particle sizes of the obtained self-assembled polymers increased when the butane sultone substitution degree was decreased. Explanation on this is that more negative charge polymers form into smaller particles in order to increase the surface area as to minimize the repulsion of the negative charges at the particles' surfaces. The particle sizes of the obtained self-assembled polymers increased when the dodecyl moieties substitution degree was decreased. This can be explained by a good the hydrophobic interaction of the dodecyl moieties inside the particles.

The zeta-potential of the obtained particles are comparable. All particles are stable aqueous colloid as demonstrated by their zeta-potential values of about -29 mV to -36 mV.

Table 3.4 The hydrodynamic diameters and Zeta-potential of nanoparticulate suspensions prepared from poly(vinyl alcohol-co-vinyl butane sultone) with DS 0.135 (**PVA-12C1-BS1**), DS 0.037 (**PVA-12C1-BS2**), DS 0.028 (**PVA-12C1-BS3**), DS 0.022 (**PVA-12C1-BS4**), DS 0.036 (**PVA-12C2-BS1**), DS 0.027 (**PVA-12C2-BS2**) and DS 0.025 (**PVA-12C2-BS3**), determined from dynamic light scattering technique.

Compound	DS of 12C	DS of BS	Hydrodynamic diameters (nm) (Average size ± SD)	Zeta-potential (mV)
PVA-12C1-BS1	0.033	0.135	144 ± 4	-35.6 ± 0.8
PVA-12C1-BS2	0.033	0.037	264 ± 2	-31.6 ± 0.3
PVA-12C1-BS3	0.033	0.028	761 ± 7	-31.6 ± 0.08
PVA-12C1-BS4	0.033	0.022	710 ± 7	-32.6 ± 0.4
PVA-12C2-BS1	0.021	0.036	455 ± 2	-24.5 ± 0.07
PVA-12C2-BS2	0.021	0.027	608 ± 1	-29.9 ± 0.04
PVA-12C2-BS3	0.021	0.025	774 ± 3	-30.8 ± 0.4

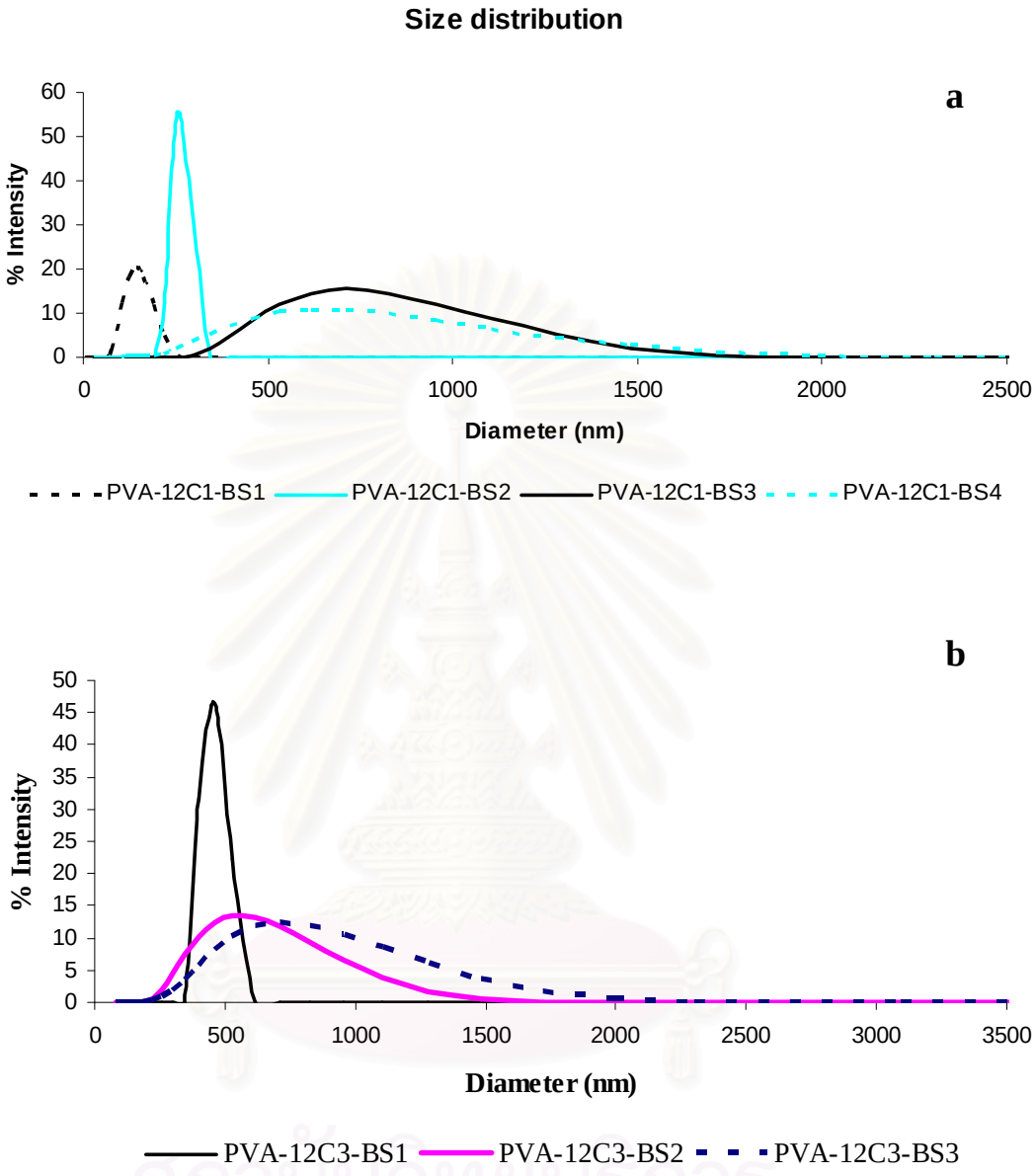


Figure 3.8 Size distributions of nanoparticles prepared from **PVA-12C1-BS** (a) and **PVA-12C2-BS** (b) with various degrees of sultone substitution.

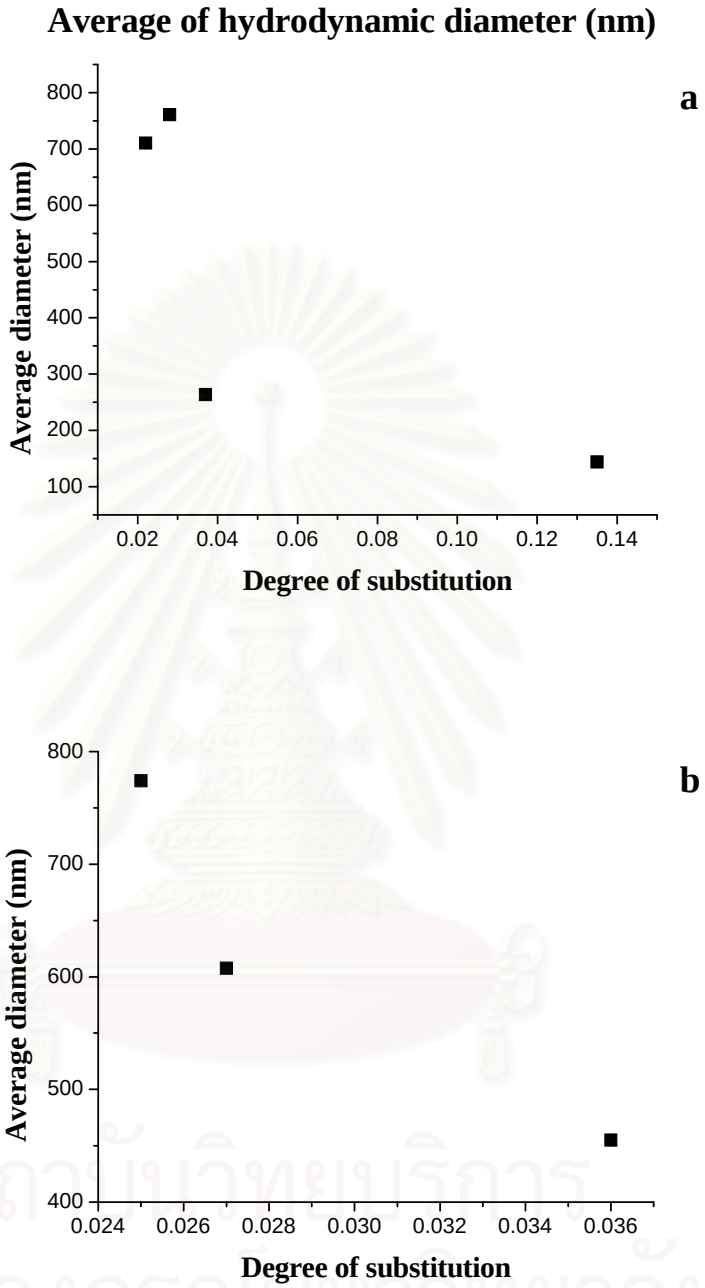


Figure 3.9 Average of hydrodynamic diameter of nanoparticles prepared from **PVA-12C1-BS** (DS of 12C = 0.033) (a) and **PVA-12C2-BS** (DS of 12C = 0.021) (b) with various degrees of sultone substitution. Average numbers are shown with the error represent standard deviation of at least three data points.

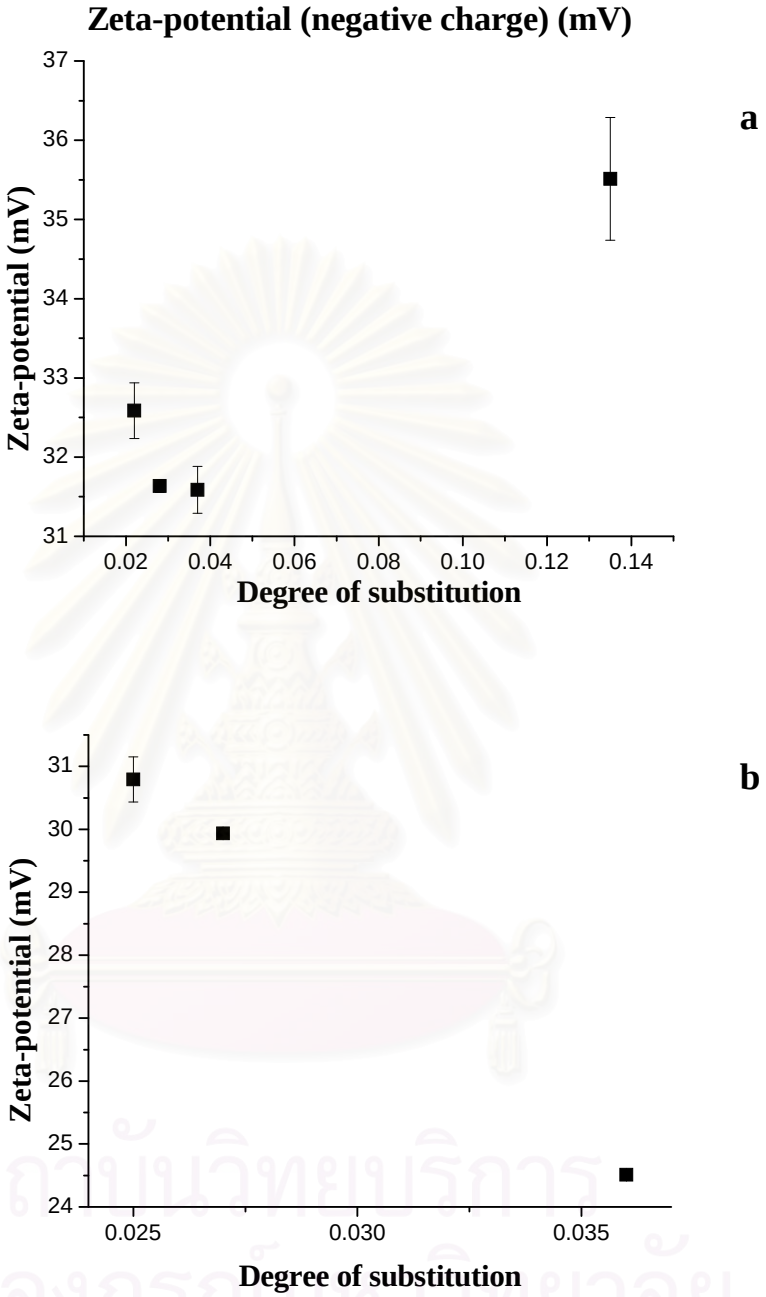
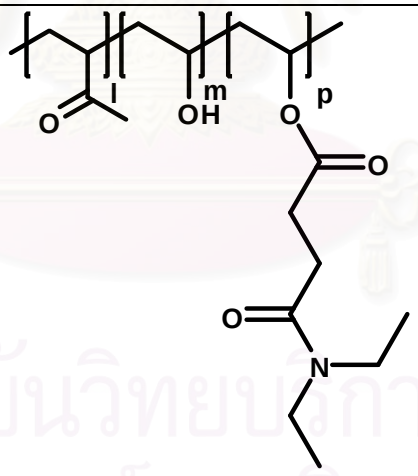


Figure 3.10 Zeta-potential of nanoparticles prepared from **PVA-12C1-BS** (a), and **PVA-12C2-BS** (b) with various degrees of sultone substitution. Average numbers are shown with the error represent standard deviation of at least three data points.

3.3 Synthesis of poly(vinyl alcohol-co-vinyl diethylamino butanate) (PVA-SA-EA)

Grafting of positive charge onto PVA (Mw 124,000-186,000), could be done successfully by reacting PVA with succinic anhydride and diethylamine. The structure of the product was characterized by ^{1}H-NMR (Appendix **A13**). The ^{1}H-NMR spectra of the synthesized poly(vinyl alcohol-co-vinyl diethylamino butanate) in D_2O show the resonance of terminal methyl protons of the anime group at 0.9 and 1.0 ppm and $–CH_2–$ protons of amine group at 3.2 and 3.3 ppm. Degree of substitution (l, m and p in table 3.5) was obtained from the ^{1}H-NMR spectrum of each product using the ratio of peak area at 1.3-1.9 ppm ($–CH–C\mathbf{H}_2–CH–$ of PVA backbone) and 0.9-1 ppm ($–CH_2–C\mathbf{H}_3$ of amine).

Table 3.5 Structure and thermal characteristics of the poly(vinyl alcohol-co-vinyl diethylamino butanate)



Compound	Mole ratio[a] PVA:EA	Product characterization			
		l	m	p or DS	T_m [b] (^{O}C)
PVA[c]	-	0.11	0.89	-	191.5
PVA-SA3-EA	1:1	0.11	0.81	0.084	-

[a] used in the reaction (mole OH:mole EA)

[b] melting temperature as obtained from DSC analysis

[c] Mw 124,000-186,000

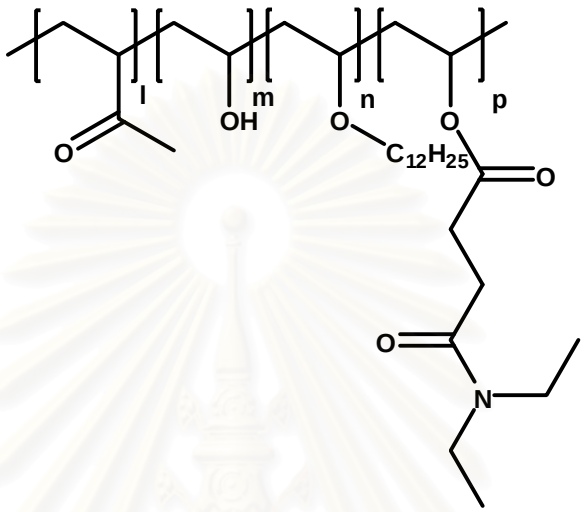
The obtained polymer **PVA-SA3-EA** can be dissolved in DMSO. Self-assembling induced by solvent displacement (displacing DMF with water), however, did not give any nanoparticulate.

3.4 Synthesis of poly(vinyl alcohol-co-vinyl dodecane-co-vinyl diethylamino butanate) (PVA-12C-SA-EA)

PVA (Mw 124,000-186,000), (PVA, 124,000 Daltons) was reacted with 1-bromododecane, succinic anhydride and diethylamine**.** The structure of the product was characterized by ^{1}H-NMR (Appendix **A14**-**A15**). The ^{1}H spectra of the synthesized poly(vinyl alcohol-co-vinyl diethylamino butanate) in DMSO-d6 show the resonances of the terminal methyl protons of the anime group at 0.9 and 1.0 ppm and terminal methyl protons of dodecane chain at 0.8 ppm. Degree of substitution (l, m, n and p in table 3.6) was obtain from the ^{1}H-NMR spectrum of each product using the ratio of peak area at 1.0-1.8 ppm (–C**H**$_2$– of dodecane chain and–CH–C**H**$_2$–CH– of PVA backbone) and 0.9-1 ppm (–CH$_2$–C**H**$_3$ of amine).

The obtained polymers (**PVA-12C-SA-EA**) were subjected to nanoparticulate formation by solvent displacement (displacing DMF with water). Coagulation into plastic-like solid (Figure 3.11) was observed during the solvent displacement. Thus it was concluded that the polymer could not self-assemble into nanoparticulate.

Table 3.6 Structure and thermal characteristics of the poly(vinyl alcohol-co-vinyl diethylamino butanate)



Compound	Mole ratio[a] PVA:EA	Product characterization				
		l	n	m	p(DS)	T_m [c] (^OC)
PVA[d]	-	0.11	-	0.89	-	191.5
PVA-12C1-SA-EA1	1:0.1	-	0.036	0.88	0.069	-
PVA-12C1-SA-EA2	1:0.2	-	0.036	0.86	0.105	-

[a] used in the reaction (mole OH:mole EA)

[b] melting temperature as obtained from DSC analysis

[c] Mw 124,000-186,000

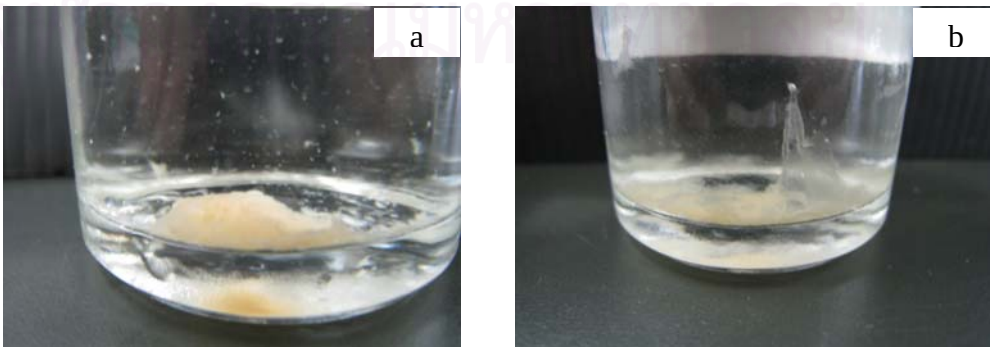


Figure 3.11 Pictures of **PVA-12C1-SA-EA1** (a) and **PVA-12C1-SA-EA2** (b)

3.5 2-Ethylhexyl *p*-methoxycinnamate (EHMC) encapsulation

The encapsulations of EHMC into **PVA-BS3** and **PVA-12C2-BS1** nanoparticles were carried out by performing the dialysis of polymer solution containing EHMC (20 milligrams of polymer sample (**PVA-BS3** or **PVA-12C2-BS1**) in 5 mL DMSO in a presence of 20 milligrams of EHMC, against water. Encapsulation efficiency was determined by quantitating amounts of EHMC in the dialysate using UV/VIS spectrophotometry with the aid of calibration curve. The % encapsulation efficiency (%EE) was calculated using equation (1)

$$\%EE = \left[\frac{\text{amount of EHMC used - amount of EHMC found in the medium}}{\text{amount of EHMC used}} \right] \times 100 \quad (1)$$

The amount of EHMC loaded into nanoparticles was determined by quantitating amounts of EHMC in the dialysate using UV/VIS spectrophotometry at 310 nm with the aid of calibration curve (see Appendix B). The % drug loading was calculated using equation (2)

$$\%\ \text{drug loading} = \left[\frac{\text{weight of drug in nanoparticles}}{\text{weight of nanoparticles and drug}} \right] \times 100 \quad (2)$$

Table 3.7 Drug loading (%loading) and encapsulation efficiency percentages (%EE) of EHMC- encapsulated **PVA-BS3** and **PVA-12C2-BS1** nanoparticles.

Compound	%EE	%loading
PVA-BS3	99.83	49.92
PVA-12C2-BS1	99.80	49.90

As shown in Table 3.7, the % EE of the processes for EHMC encapsulation into **PVA-BS3** and **PVA-12C2-BS1** nanoparticles are ~99% (see Appendix B for calculation) at the drug loading percentage of ~50% (see Appendix B for calculation). That means these obtained polymers have a high capacity to entrap EHMC.

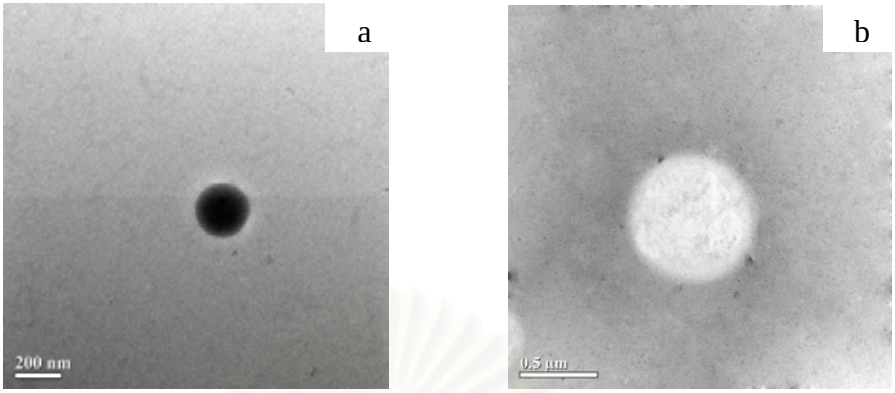


Figure 3.12 TEM photographs of (a) unencapsulated **PVA-BS3** nanoparticles and (b) EHMC-encapsulated **PVA-BS3** nanoparticles at 800 ppm.

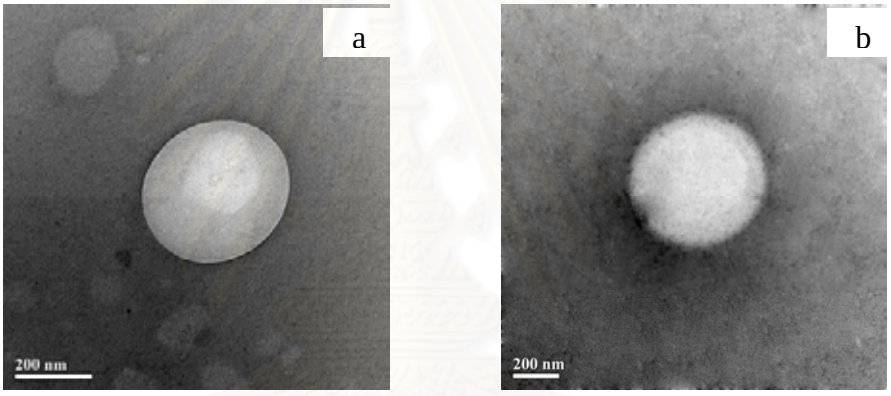


Figure 3.13 TEM photographs of (a) unencapsulated **PVA-12C2-BS1** nanoparticles and (b) EHMC-encapsulated **PVA-12C2-BS1** nanoparticles at 800 ppm.

It should be noted here that the obtained particles were subjected to both TEM and SEM analysis but only TEM pictures are shown here because all particles either bursted or melted during the SEM acquisitions. The bursting may result from the present of charged groups along the polymer chains which help conducting high voltage electrons and thus leads to particle deformation, melting or bursting.

The hydrodynamic diameters, determined by dynamic light scattering (DLS) method, of unencapsulated **PVA-BS3** and the 49.92% EHMC-encapsulated **PVA-BS3** were 320 ± 12 nm and 152 ± 6 nm, respectively. The fact that the size of EHMC-encapsulated **PVA-BS3** particles is smaller than that of unencapsulated particles implies a stronger hydrophobic interaction at particles' cores in the presence of EHMC. This agrees well with previous results in which the polymer with higher

substitution degree of dodecyl moieties gave smaller particles (P.38). Self-assembly of unencapsulated **PVA-12C2-BS1** gave particles with size distribution at 455 ± 2 nm while the 49.90% EHMC-encapsulated **PVA-12C2-BS1** particles are 2146 ± 8 nm. It is obvious that size of EHMC-encapsulated **PVA-BS3** particles was much bigger than the unencapsulated particles. It is possible that particles core of **PVA-12C2-BS1** has already been optimally filled with dodecyl moieties and the presence of EHMC may disrupt such optimal thus resulting is much bigger particles. Interestingly, the zeta-potential value of **PVA-BS3** was significantly improved by EHMC-encapsulation. It is possible that EHMC at the particles' core help pushing out all the charges to the particle's surfaces.

Table 3.8 The hydrodynamic diameters and Zeta-potential of nanoparticulate suspensions prepared from EHMC-encapsulated **PVA-BS3**, EHMC-encapsulated **PVA-12C1-BS2** and EHMC-encapsulated **PVA-12C2-BS1**, determined from dynamic light scattering technique.

Compound	Hydrodynamic diameters (nm) (Average size ± SD)	Zeta-potential (mV)
unencapsulated **PVA-BS3**	320 ± 12	-11.6 ± 1.6
EHMC-encapsulated **PVA-BS3**	152 ± 6	-24.7 ± 0.2
unencapsulated **PVA-12C2-BS1**	455 ± 2	-24.5 ± 0.07
EHMC-encapsulated **PVA-12C2-BS1**	2146 ± 8	-31.6 ± 0.3

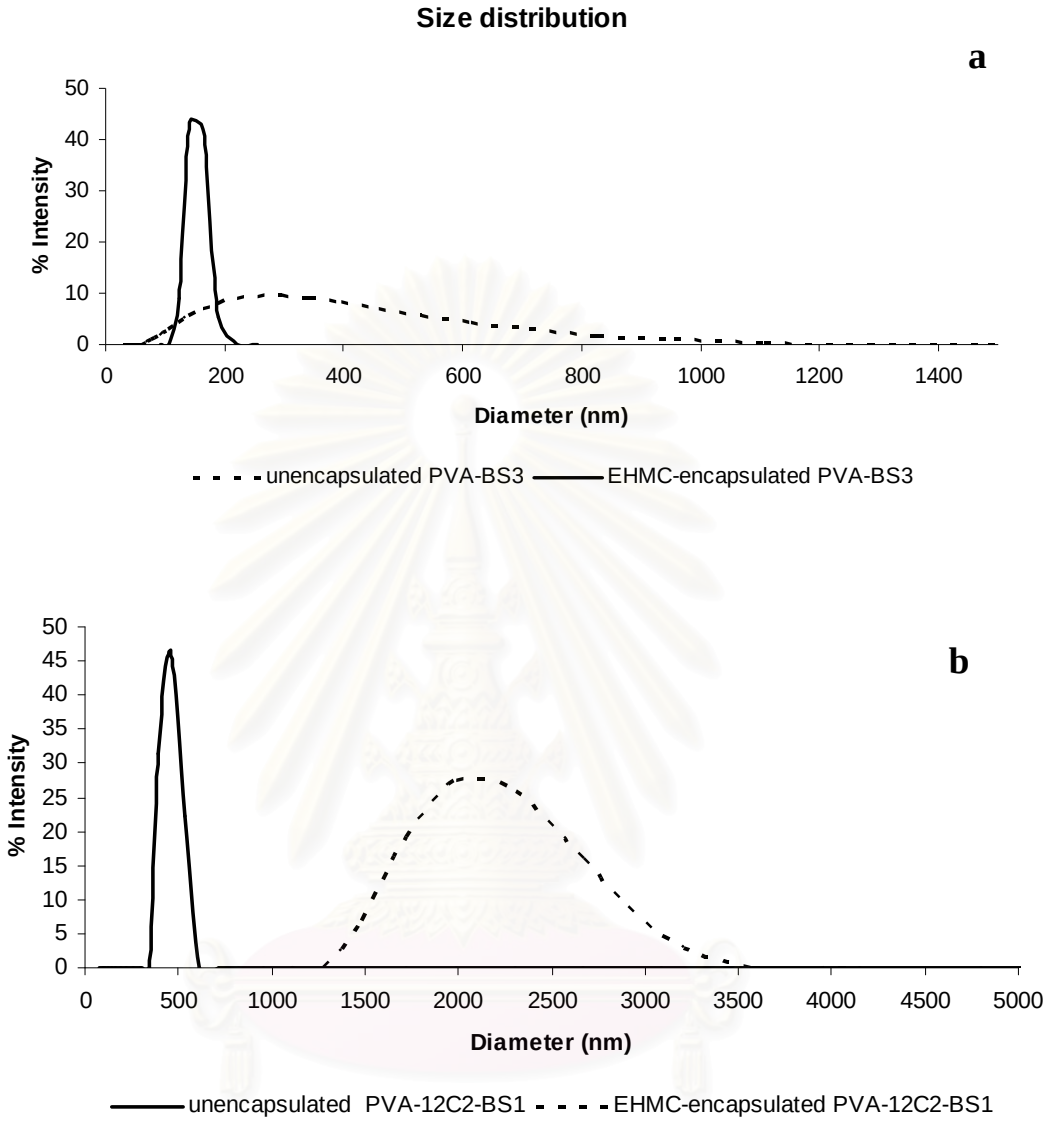


Figure 3.14 Size distributions of nanoparticles prepared from unencapsulated and EHMC encapsulated **PVA-BS3** (a) and **PVA-12C2-BS1** (b)

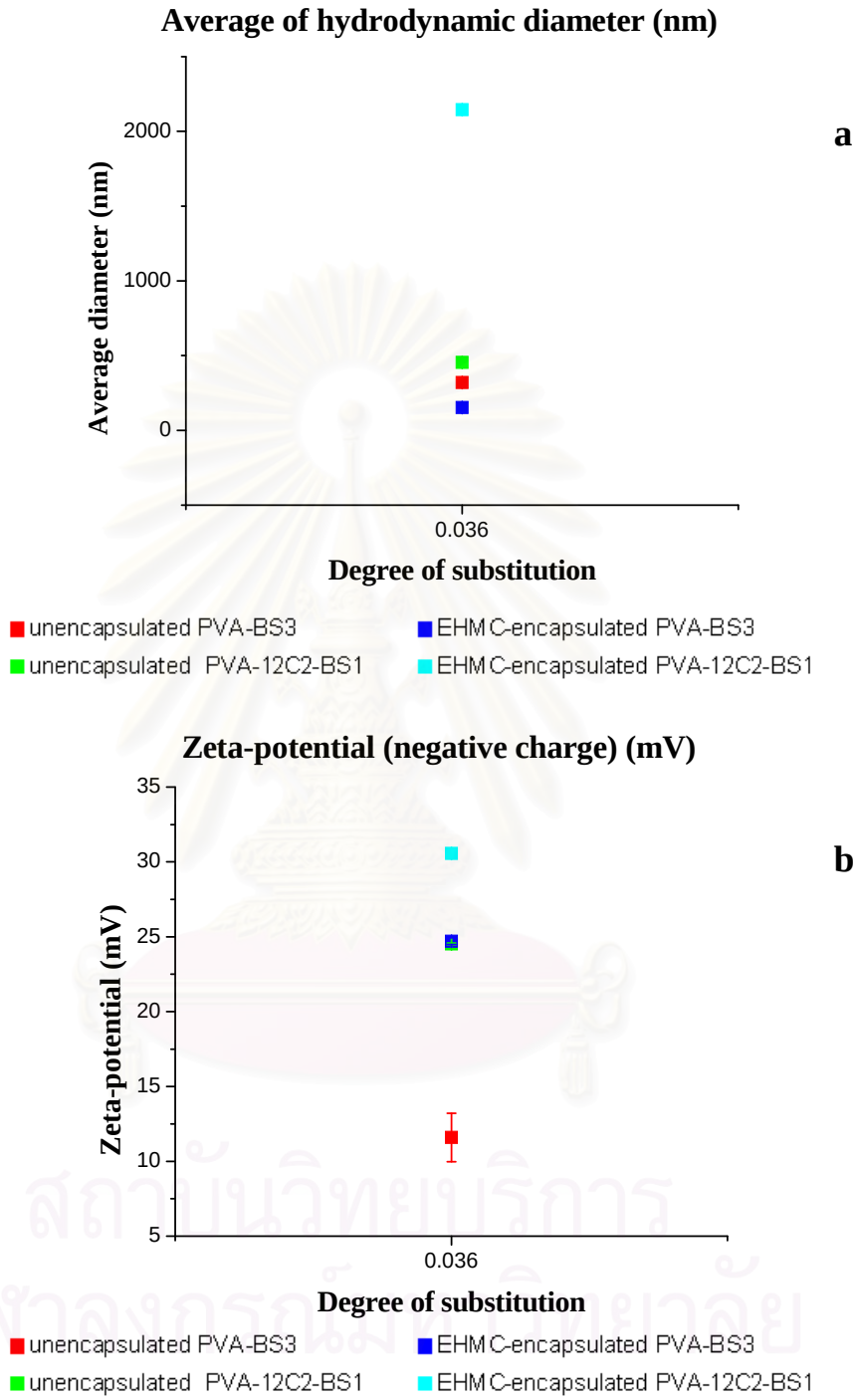


Figure 3.15 Average of hydrodynamic diameter (a) and Zeta-potential (b) of nanoparticles prepared from unencapsulated and EHMC-encapsulated **PVA-BS3** and **PVA-12C2-BS1**. Average numbers are shown with the error represent standard deviation of at least three data points.

3.6 Release of 2-Ethylhexyl *p*-methoxycinnamate (EHMC)

A dialysis method was used to perform the in *vitro* release test of EHMC-loaded **PVA-BS3**, EHMC-loaded **PVA-12C2-BS1** and EHMC-loaded SDS nanoparticles. During the experiments, the medium (3 mL) were withdrawn to determine the amounts of EHMC released from the nanoparticles using UV/VIS spectrophotometry at 310 nm with the aid of calibration curve. Figure 3.4 shows the release curves of EHMC-loaded **PVA-BS3**, EHMC-loaded **PVA-12C2-BS1** and EHMC-loaded SDS nanoparticles. EHMC-loaded **PVA-BS3** and EHMC-loaded **PVA-12C2-BS1** slowly released EHMC. The release rates were comparable and Mann-Whitney test indicates that the polymers have a good release property for EHMC or hydrophobic compounds.

The release rates of EHMC from **PVA-BS3**, **PVA-12C2-BS1** and SDS nanoparticles were slow (Table 3.8).

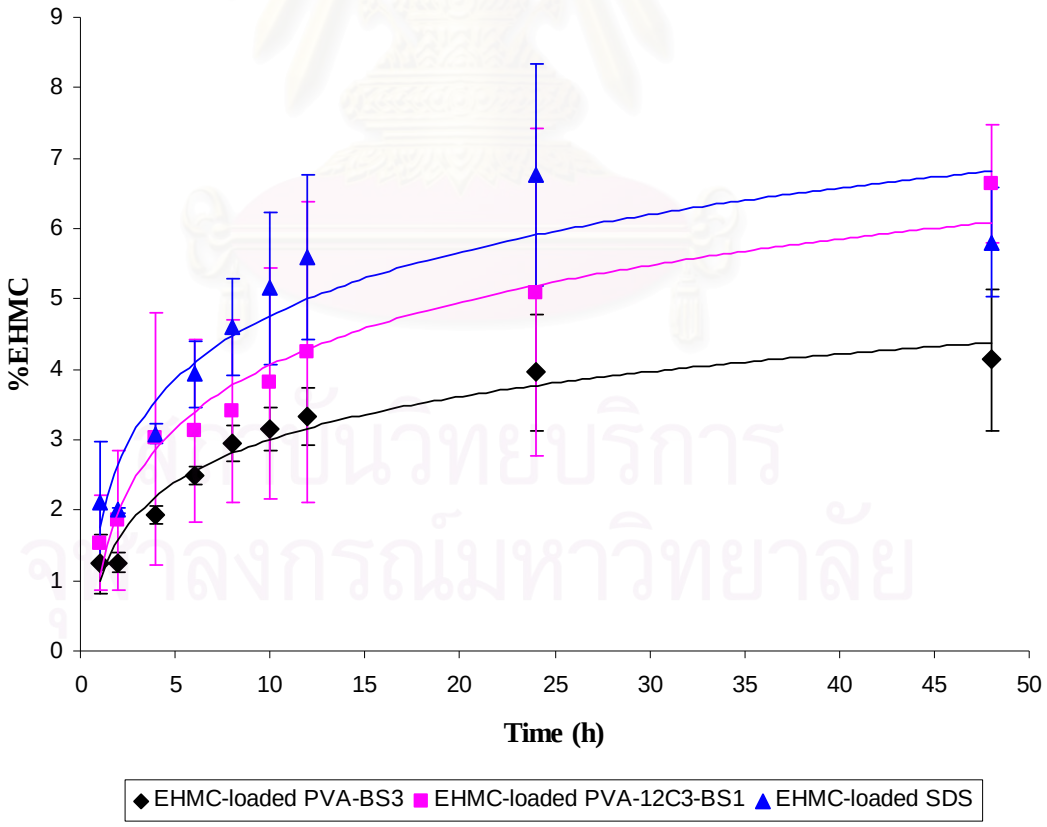


Figure 3.16 *In vitro* release profile of EHMC from **PVA-BS3**, **PVA-12C2-BS1**and SDS nanoparticles for 48 h.

Table 3.9 The percentage of EHMC released tolal and release rate of EHMC from **PVA-BS3**, **PVA-12C2-BS1** and SDS nanoparticles.

Time (h)	% total released			Released rate (mg/h)		
	PVA-BS3	**PVA-12C2-BS1**	SDS	**PVA-BS3**	**PVA-12C2-BS1**	SDS
0	0	0	0	0	0	0
1	1.24	1.53	2.11	0.050	0.061	0.085
2	1.26	1.85	2.00	0.025	0.037	0.040
4	1.936	3.02	3.08	0.019	0.030	0.031
6	2.50	3.13	3.93	0.017	0.021	0.026
8	2.95	3.40	4.61	0.015	0.017	0.023
10	3.16	3.81	5.15	0.013	0.015	0.021
12	3.33	4.25	5.59	0.011	0.014	0.019
24	3.96	5.10	6.75	0.007	0.009	0.011
48	4.13	6.63	5.80	0.003	0.006	0.005

CHAPTER IV

CONCLUSION

In this research, negatively charged polymers, poly(vinyl alcohol-co-vinyl butane sultone) and poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone), and positively charged polymers, poly(vinyl alcohol-co-vinyl diethylamino butanate) and poly(vinyl alcohol-co-vinyl diethylamino butanate), could be synthesized successfully. Negative charge (1,4-butane sultone) and dodecyl moieties (1-bromododecane) were grafted onto PVA chain by substitution reaction and positive charge (diethylamine) could be done successfully by reacting PVA with succinic anhydride and diethylamine. The negatively charged polymers can be induced into particle using dialysis method. On the other hand, the positively charged polymers can not be induced into particle.

The relationships between sizes/shape of particles and chemical structures of the PVA derivatives were investigated. It was found that the degree of substitution of sultone groups and dodecyl moieties on PVA backbone affected the particle size. The particle size of poly(vinyl alcohol-co-vinyl butane sultone) decreased with decreased sultone substitution degree, but the particle size of poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone) decreased with increased sultone substitution degree. Poly(vinyl alcohol-co-vinyl butane sultone) (DS of BS = 0.036) and poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone) (DS of 12C = 0.021, DS of BS = 0.036) were chosen as representative polymeric spheres to study 2-ethylhexyl *p*-methoxycinnamate encapsulations and release. It was found that the obtained polymer could effectively encapsulate EHMC (%EE = 99%) at 50% loading.

This work demonstrated that poly(vinyl alcohol-co-vinyl butane sultone) and poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone) which are negatively charged poly(vinyl alcohol) can be induced into nanoparticles.

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APPENDICES

APPENDICES A

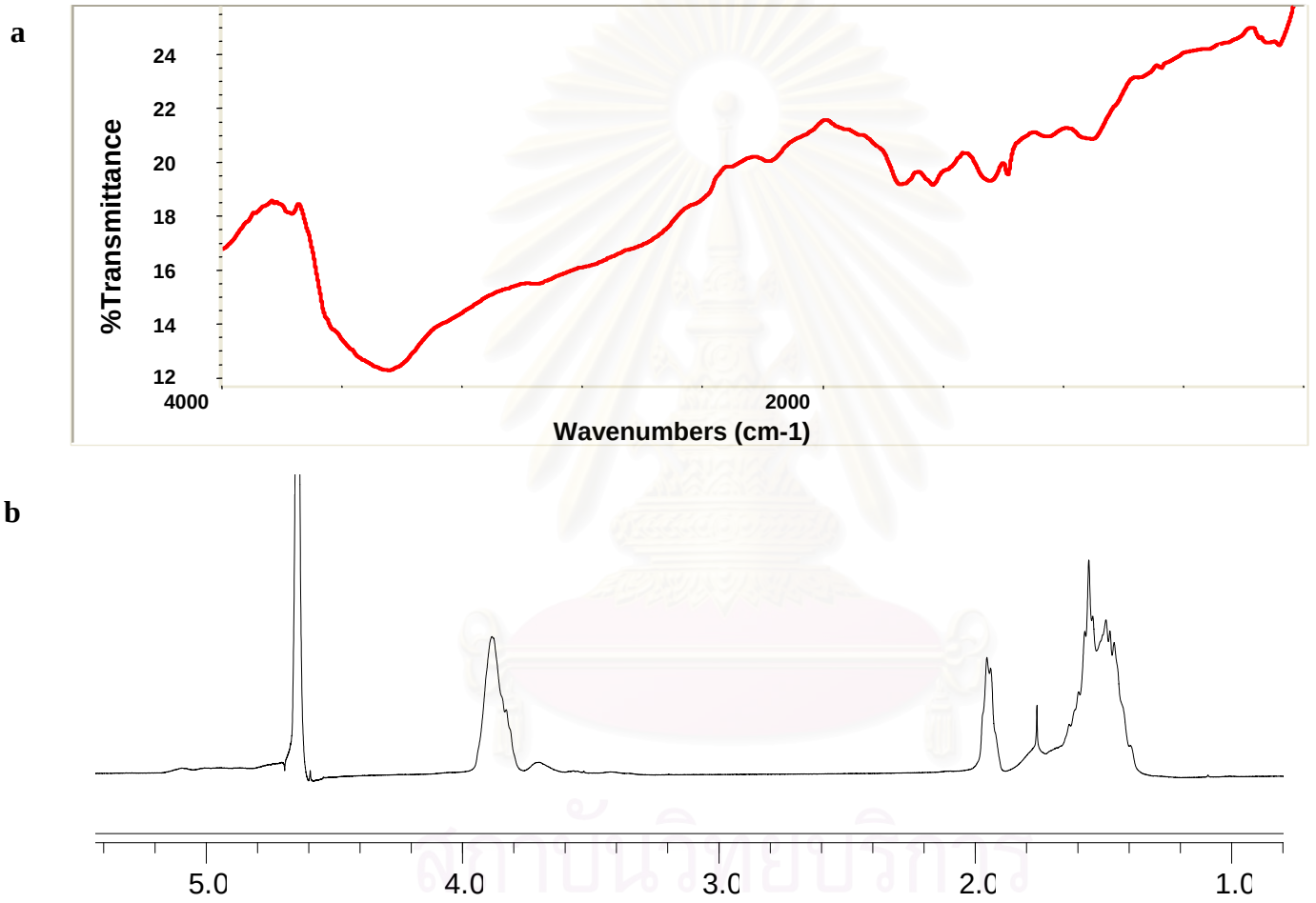


Figure A1 IR (a) and ^{1}H-NMR spectrum in D_2O (b) of Poly(vinyl alcohol) (PVA, 124,000 Daltons)

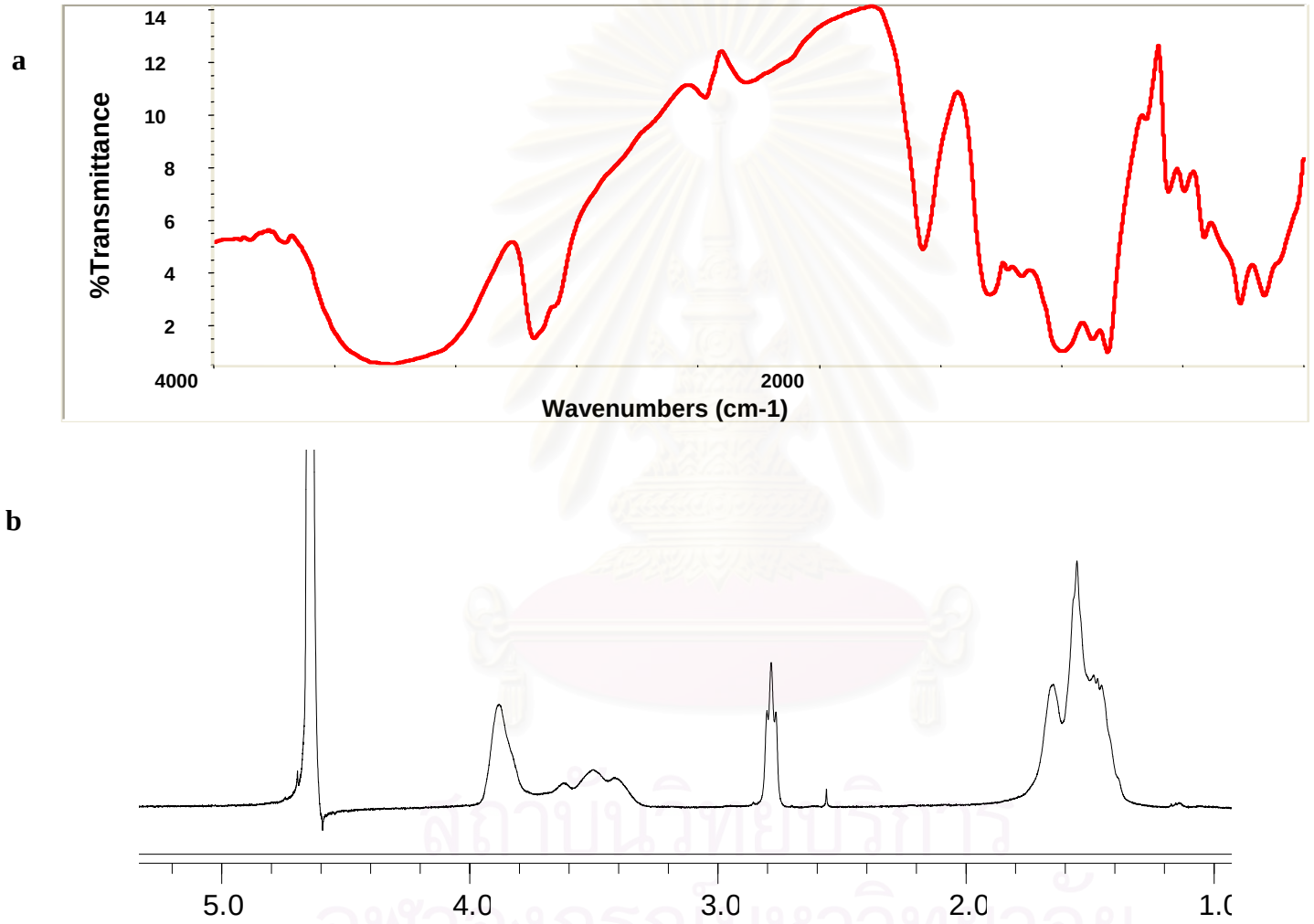
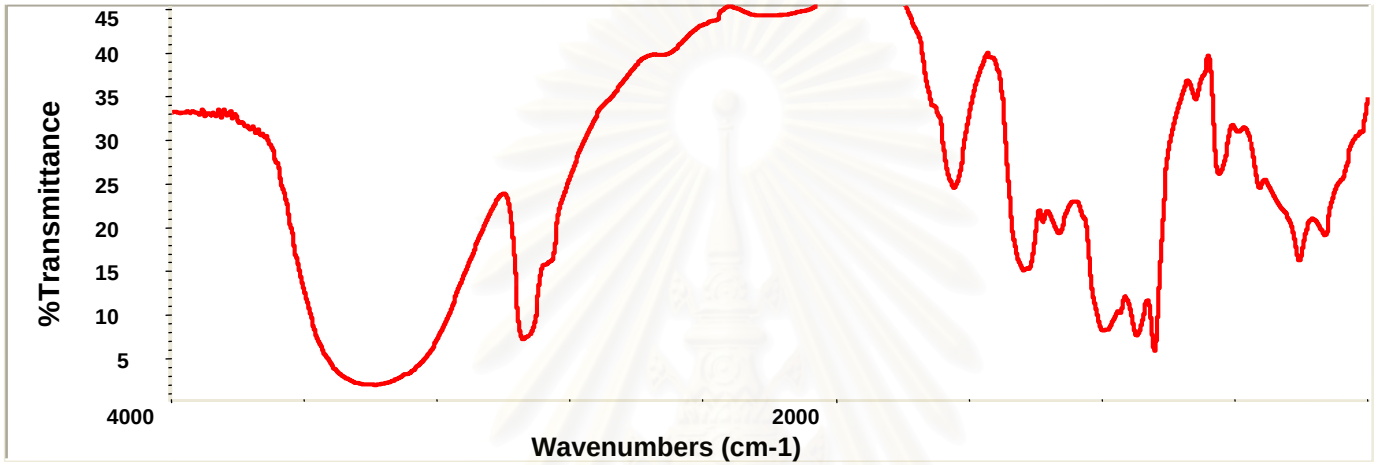


Figure A2 IR (a) and ^{1}H-NMR spectrum in D_2O (b) of poly(vinyl alcohol-co-vinyl butane sultone), DS 0.194 (**PVA-BS1**)

a



b

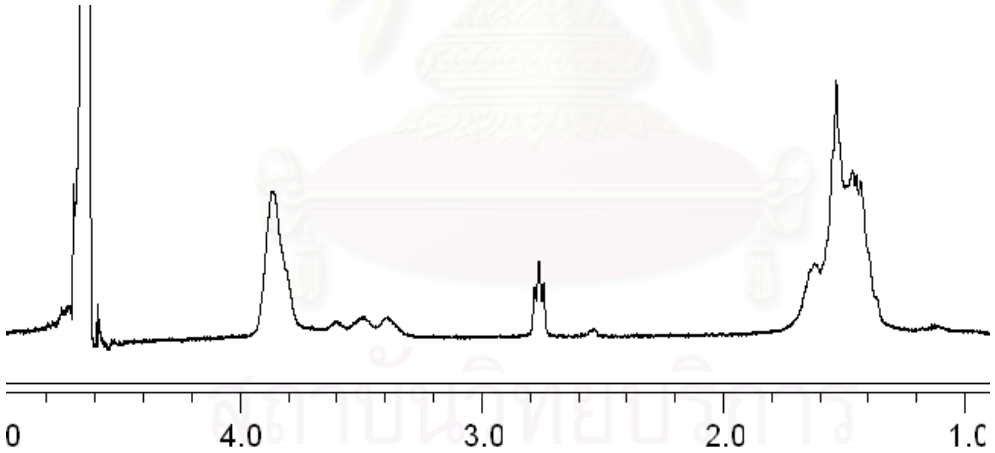
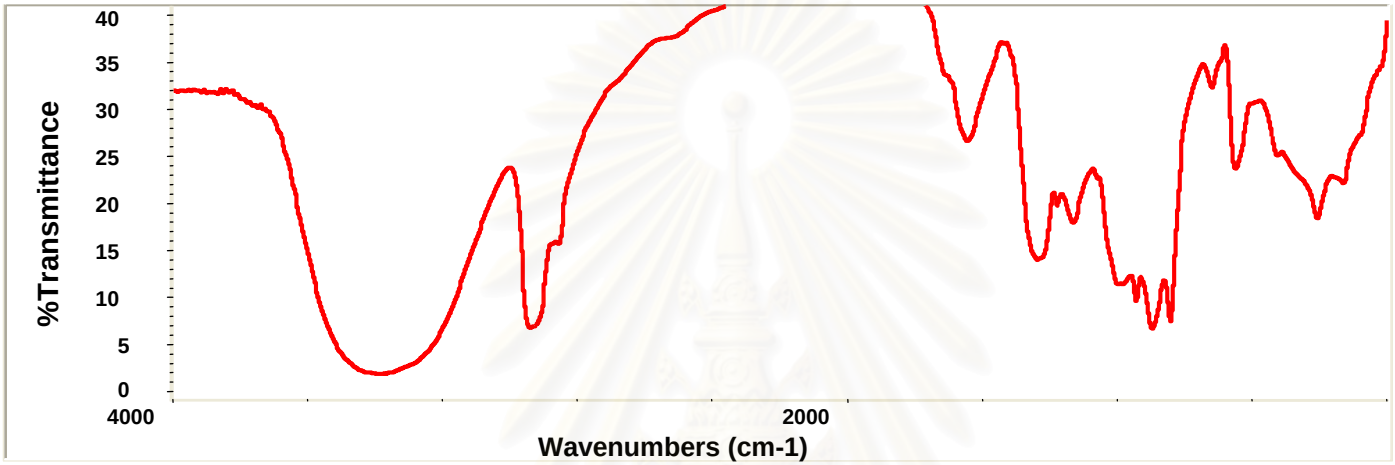


Figure A3 IR (a) and ^{1}H-NMR spectrum in D_2O (b) of poly(vinyl alcohol-co-vinyl butane sultone), DS 0.066 (**PVA-BS2**)

a



b

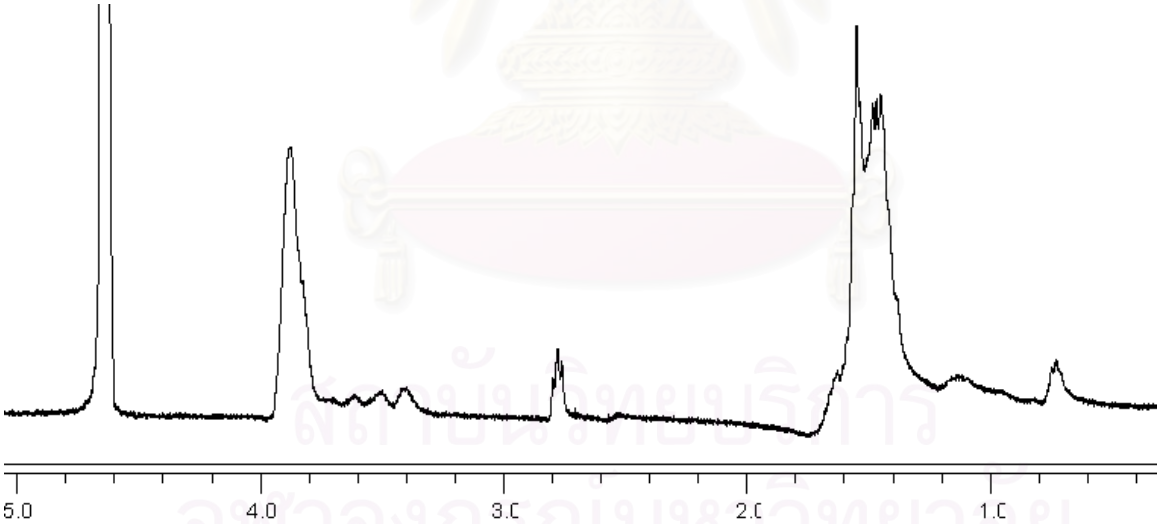
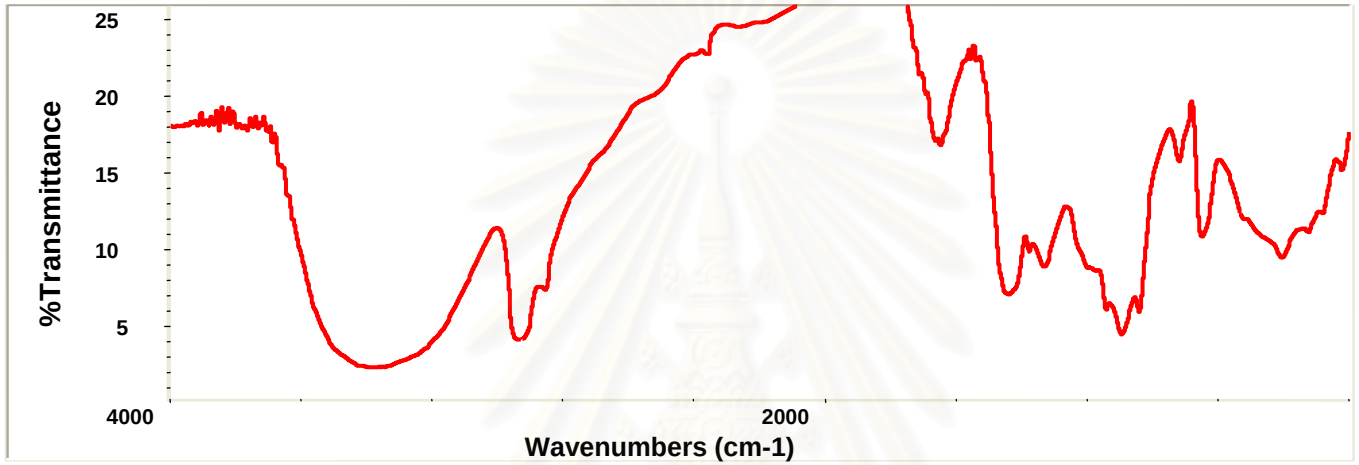


Figure A4 IR (a) and ^{1}H-NMR spectrum in D_2O (b) of poly(vinyl alcohol-co-vinyl butane sultone), DS 0.036 (**PVA-BS3**)

a



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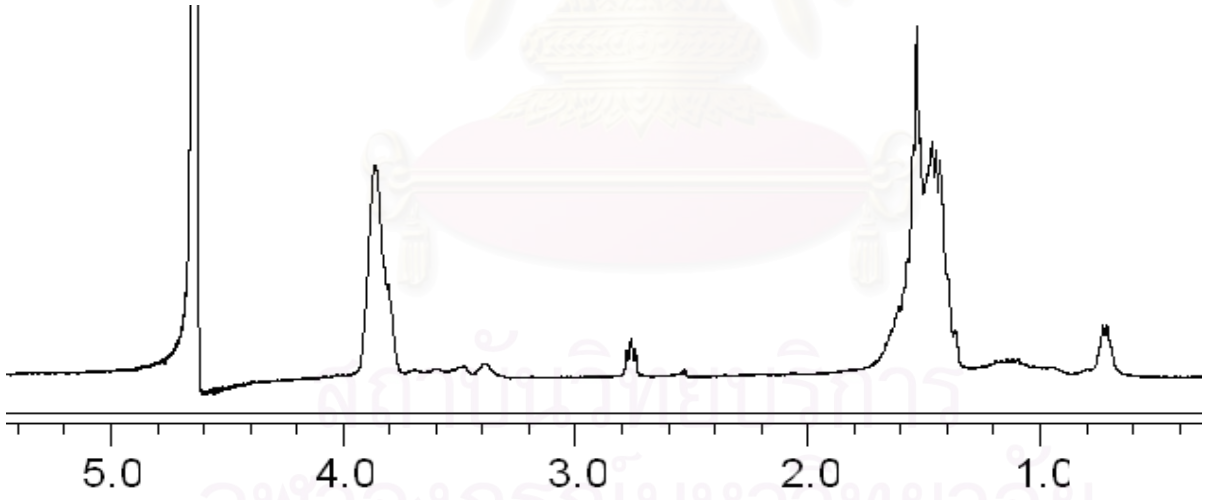
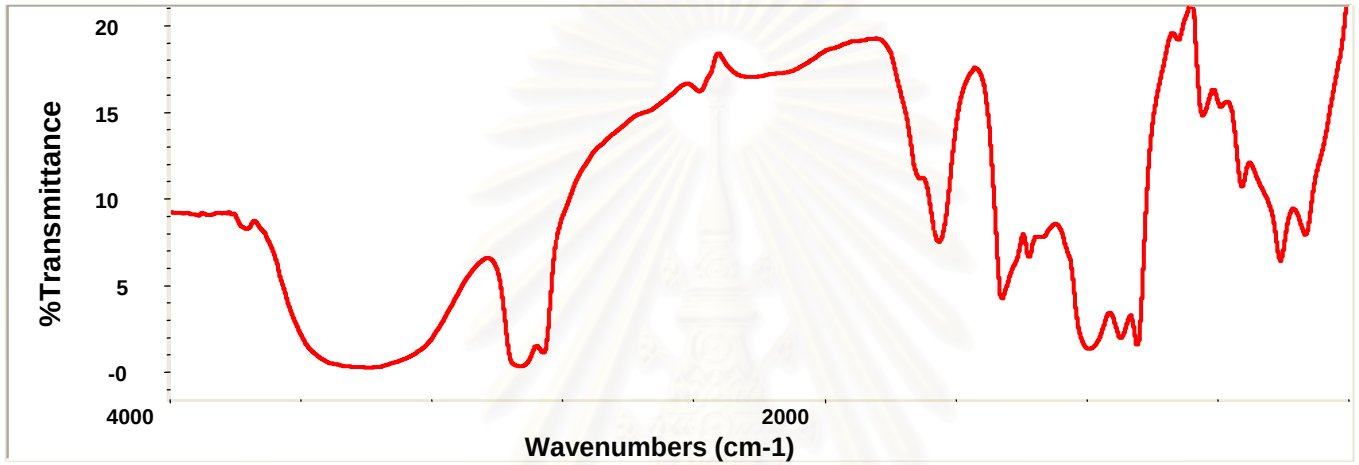


Figure A5 IR (a) and ^{1}H-NMR spectrum in D_2O (b) of poly(vinyl alcohol-co-vinyl butane sultone), DS 0.013 (**PVA-BS4**)

a



b

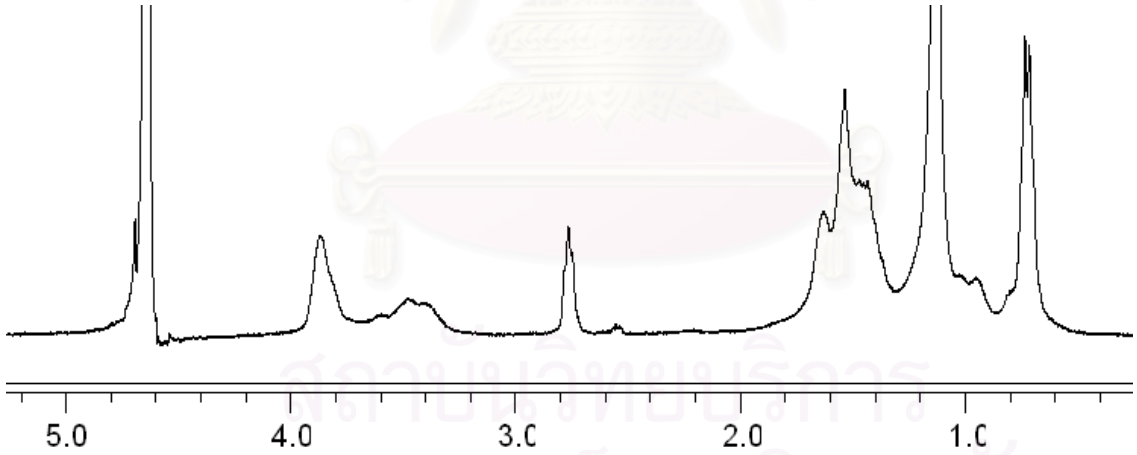


Figure A6 IR (a) and ^{1}H-NMR spectrum in D_2O (b) of poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone), DS of 12C: 0.033, DS of BS: 0.135 (**PVA-12C1-BS1**)

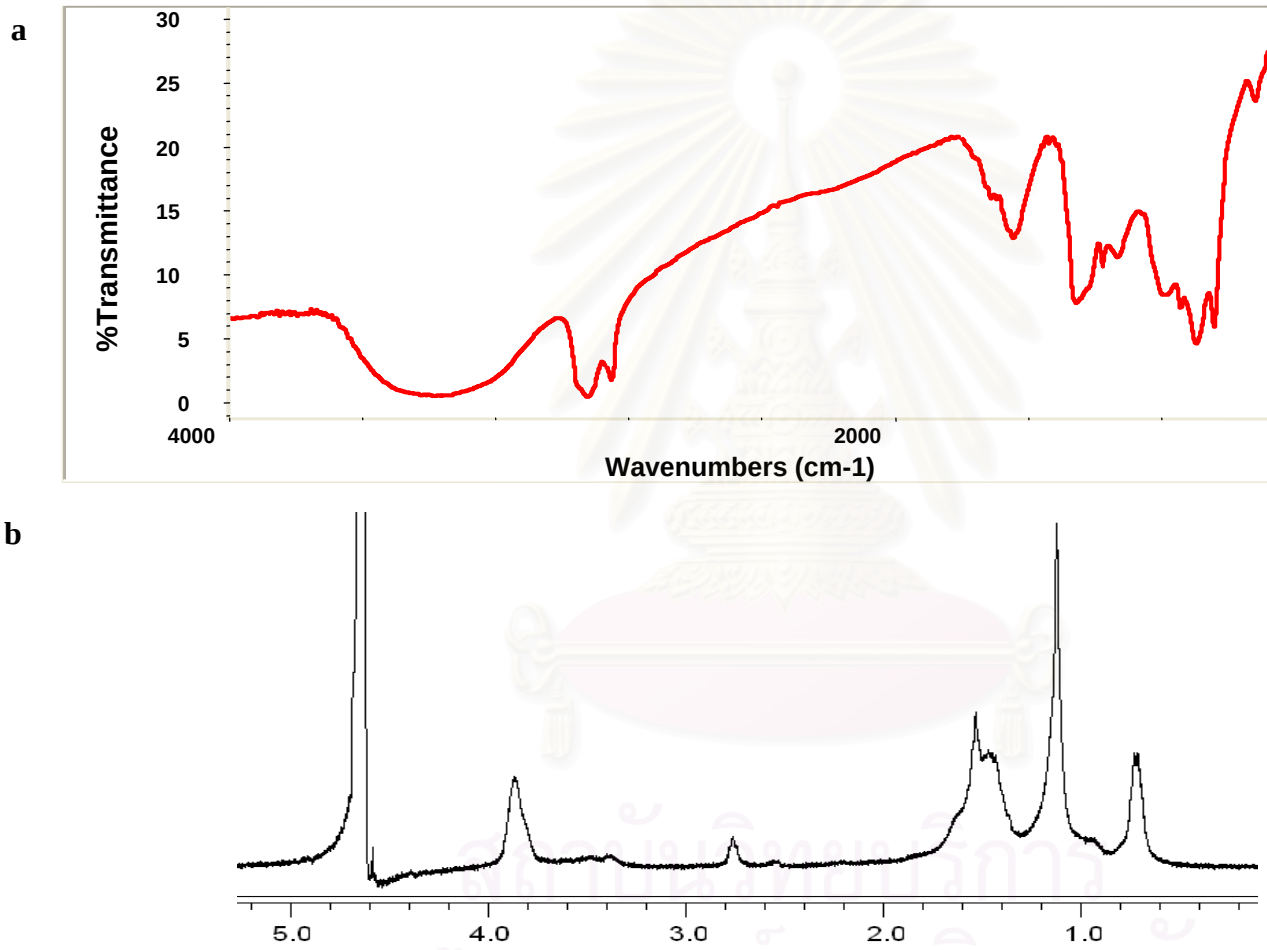
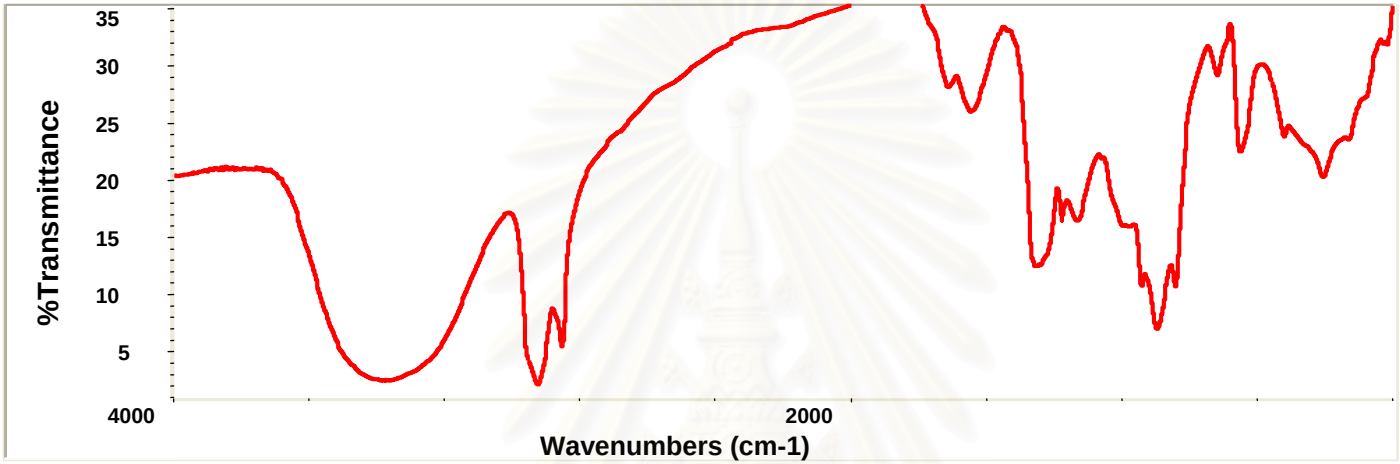


Figure A7 IR (a) and ^{1}H-NMR spectrum in D_2O (b) of poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone), DS of 12C: 0.033, DS of BS: 0.037 (**PVA-12C1-BS2**)

a



b

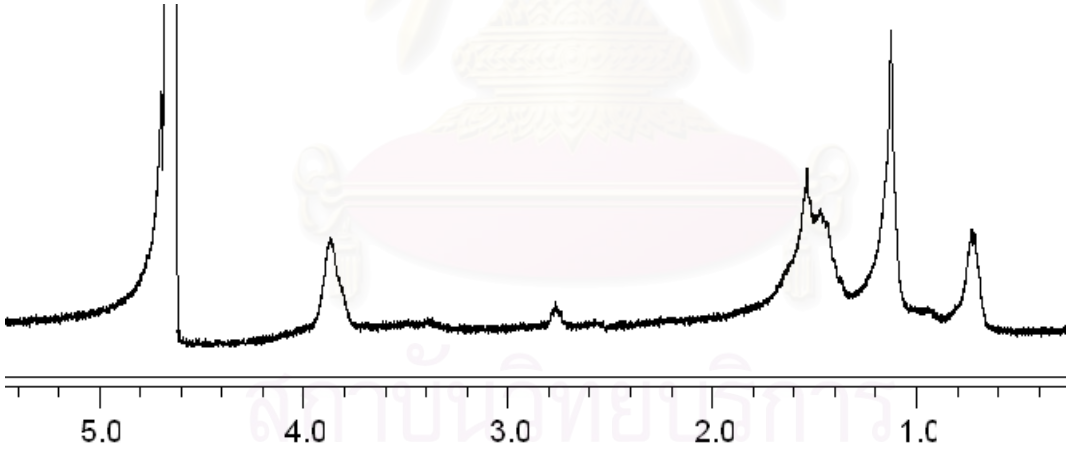
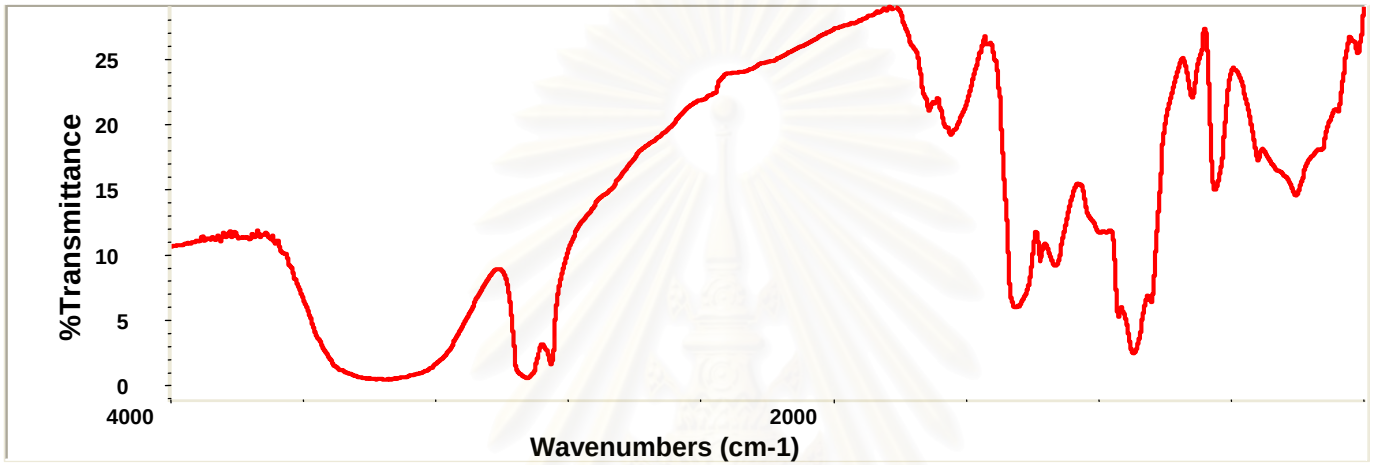


Figure A8 IR (a) and ^{1}H-NMR spectrum in D_2O (b) of poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone), DS of 12C: 0.033, DS of BS: 0.028 (**PVA-12C1-BS3**)

a



b

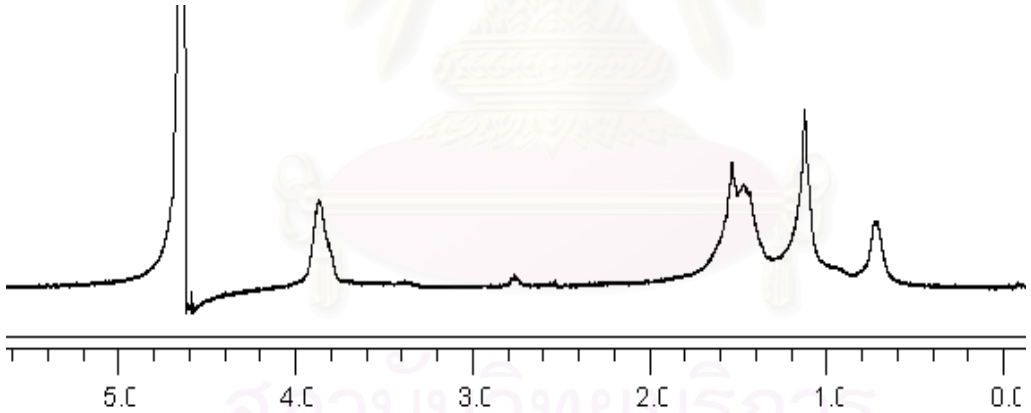


Figure A9 IR (a) and ^{1}H-NMR spectrum in D_2O (b) of poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone), DS of 12C: 0.033, DS of BS: 0.022 (**PVA-12C1-BS4**)

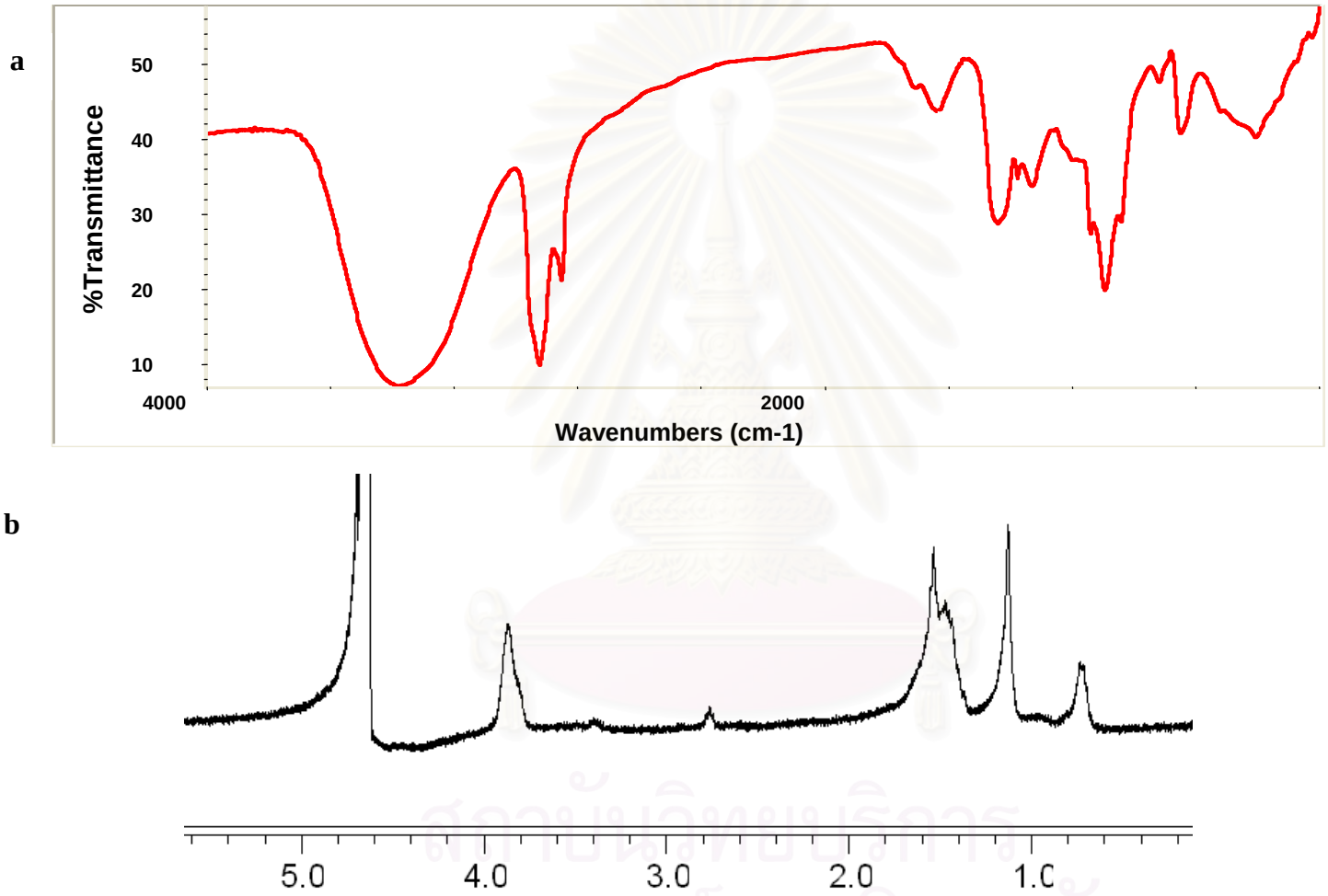
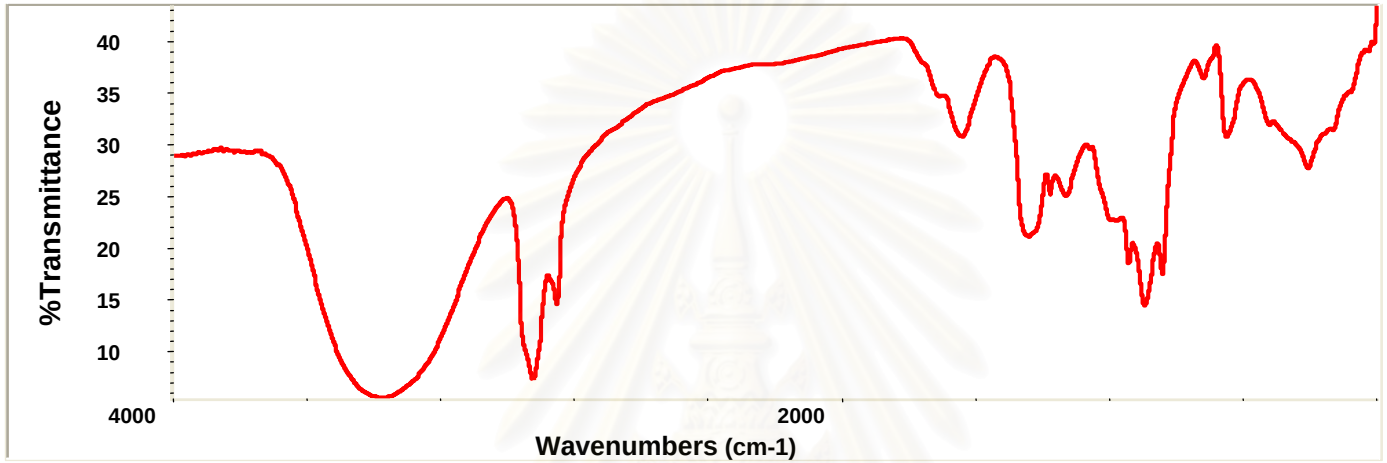


Figure A10 IR (a) and ^{1}H-NMR spectrum in D_2O (b) of poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone), DS of 12C: 0.021, DS of BS: 0.036 (**PVA-12C2-BS1**)

a



b

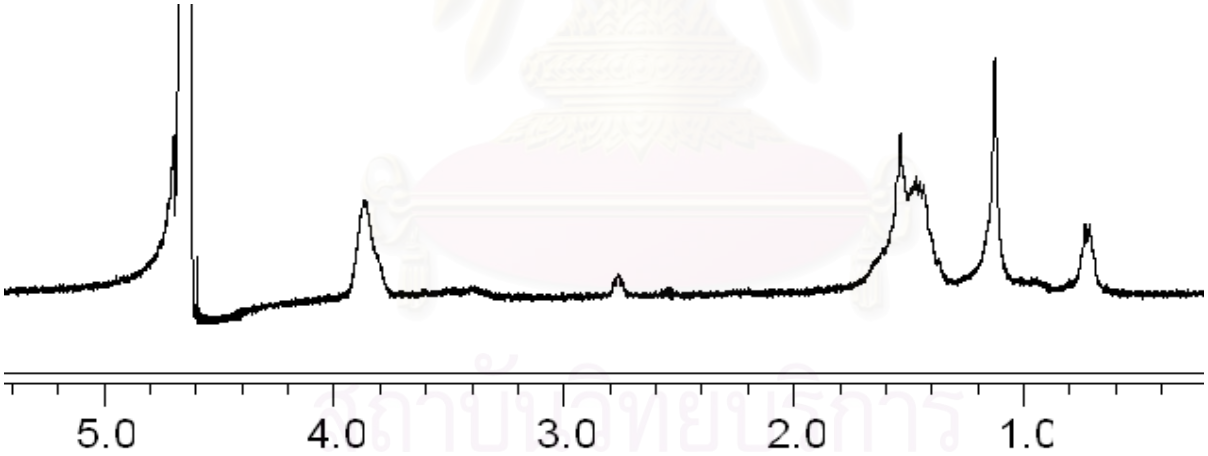
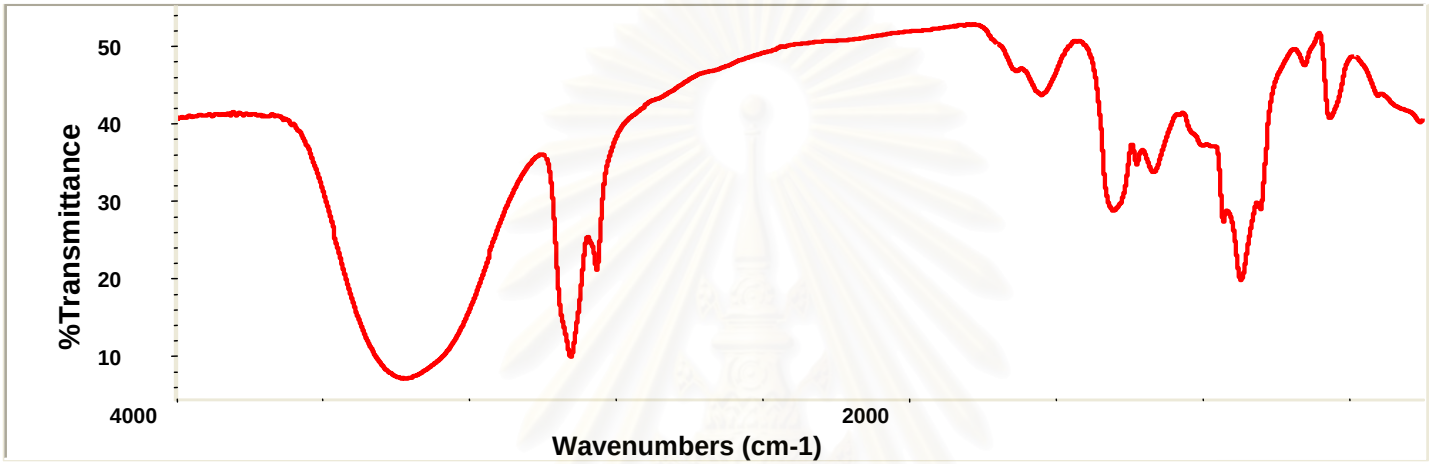


Figure A11 IR (a) and ^{1}H-NMR spectrum in D_2O (b) of poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone), DS of 12C: 0.021, DS of BS: 0.027 (**PVA-12C2-BS2**)

a



b

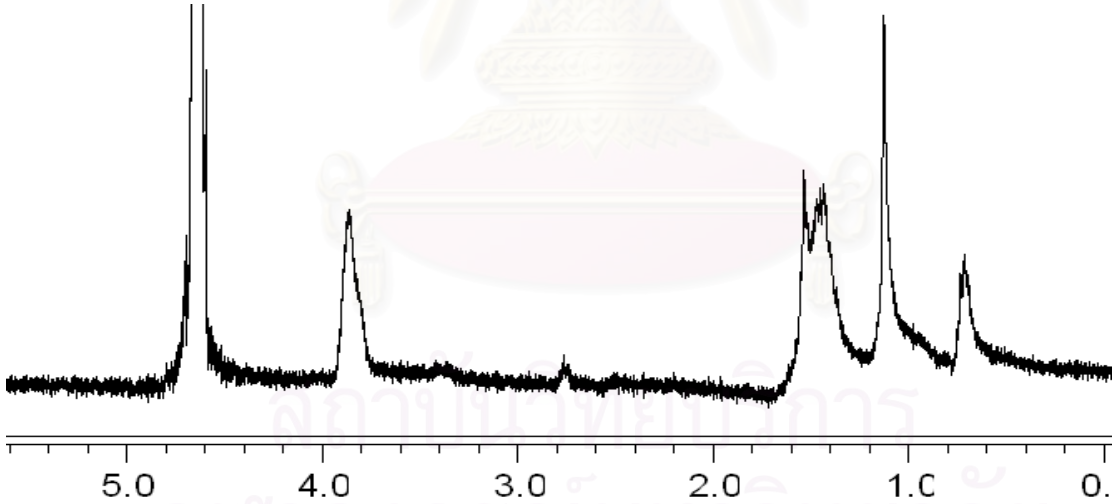


Figure A12 IR (a) and ^{1}H-NMR spectrum in D_2O (b) of poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone), DS of 12C: 0.021, DS of BS: 0.025 (**PVA-12C2-BS3**)

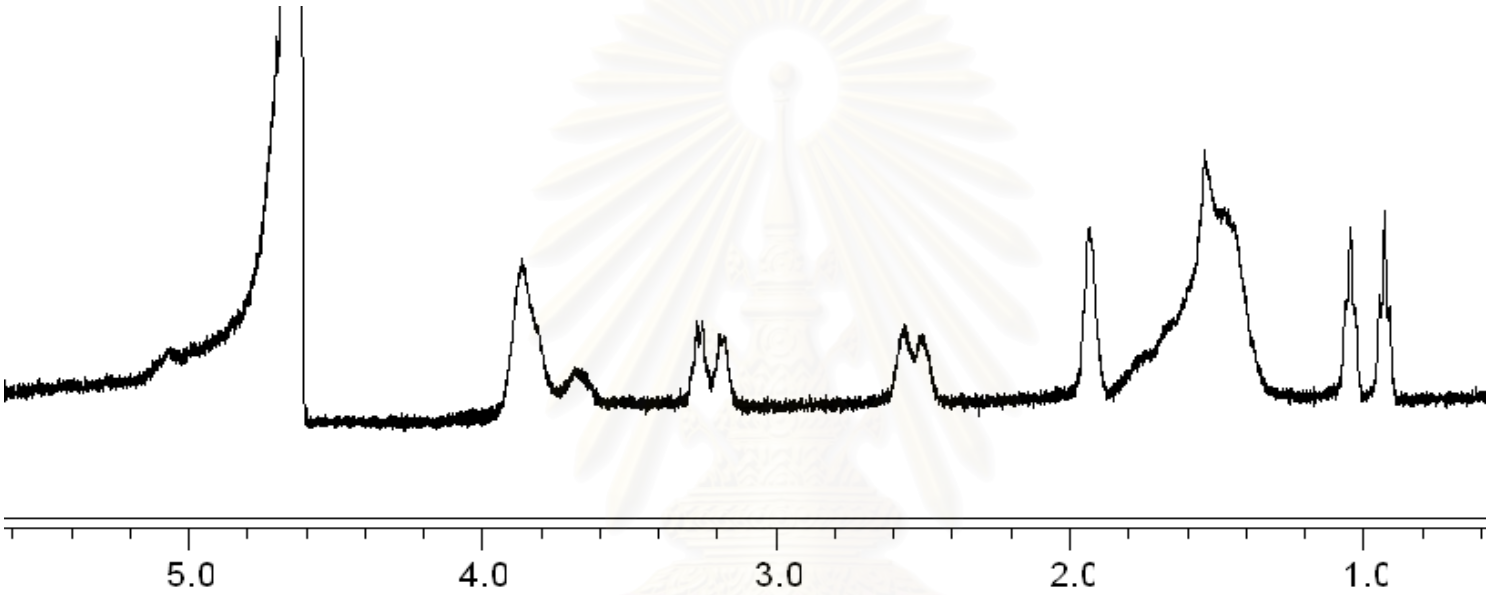


Figure A13 ^{1}H-NMR spectrum in D_2O of poly(vinyl alcohol-co-vinyl diethylamino butanate), DS 0.084 (**PVA-SA-EA3**)

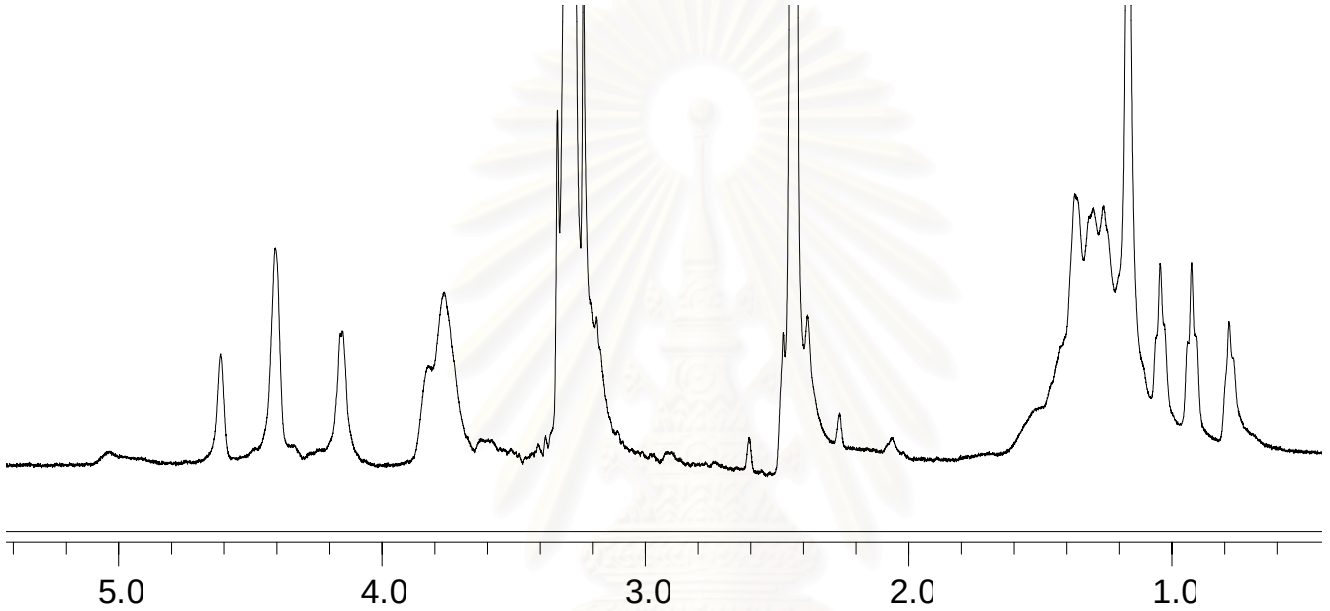


Figure A14 ^{1}H-NMR spectrum in DMSO-d_6 of poly(vinyl alcohol-co-vinyl dodecane-co-vinyl diethylamino butanate), DS of 12C: 0.033. DS of EA: 0.068. (**PVA-12C1-SA-EA1**)

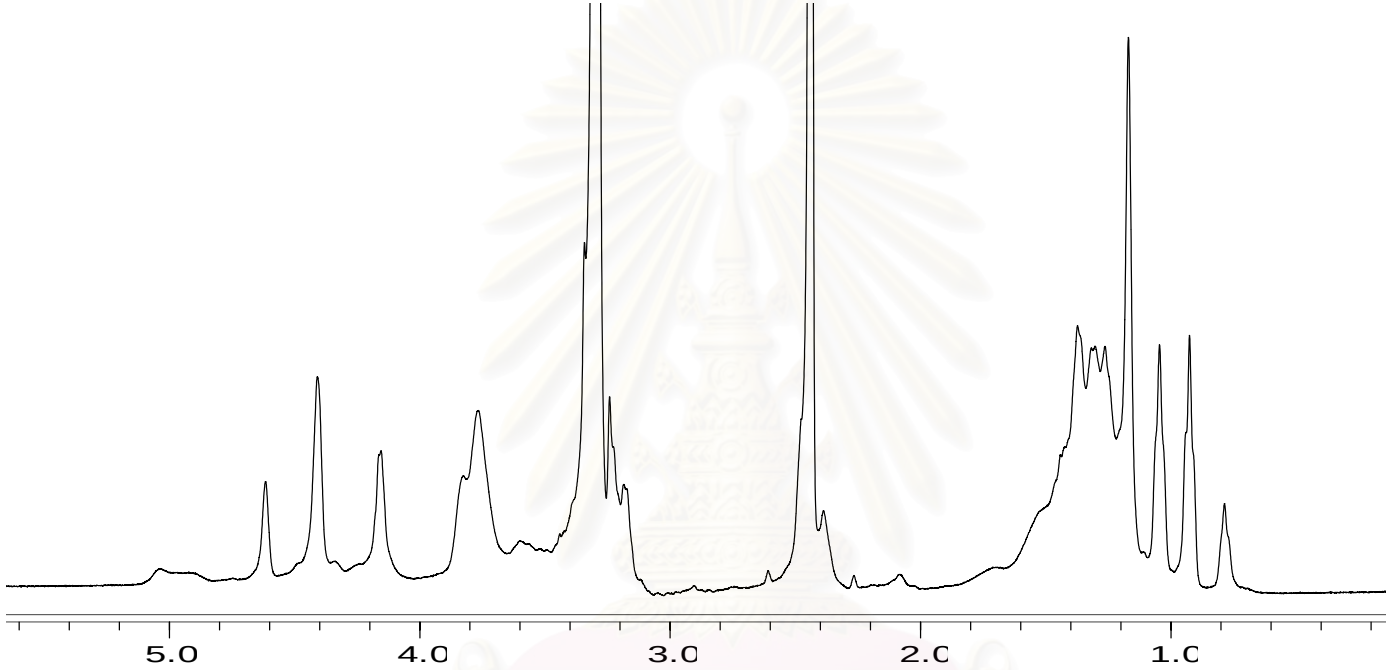


Figure A15 ^{1}H-NMR spectrum in DMSO-d_6 of poly(vinyl alcohol-co-vinyl dodecane-co-vinyl diethylamino butanate), DS of 12C: 0.033. DS of EA: 0.105. (**PVA-12C1-SA-EA2**)

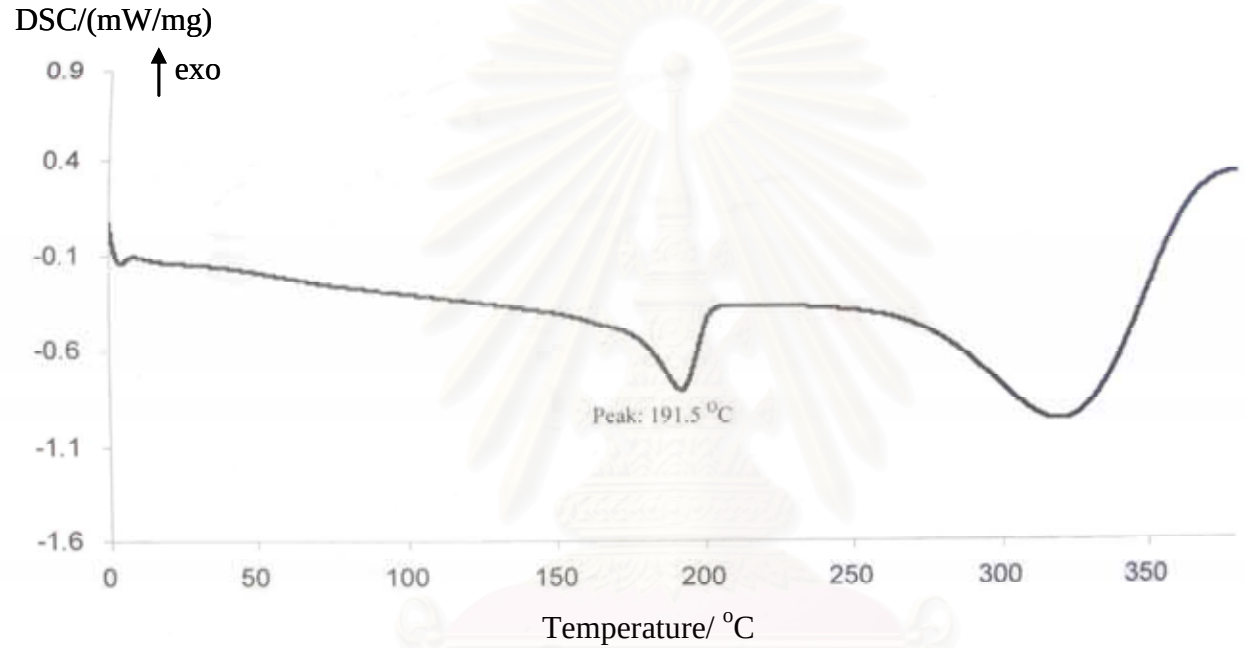


Figure A16 Thermogram of Poly(vinyl alcohol) (PVA, 124,000 Daltons)

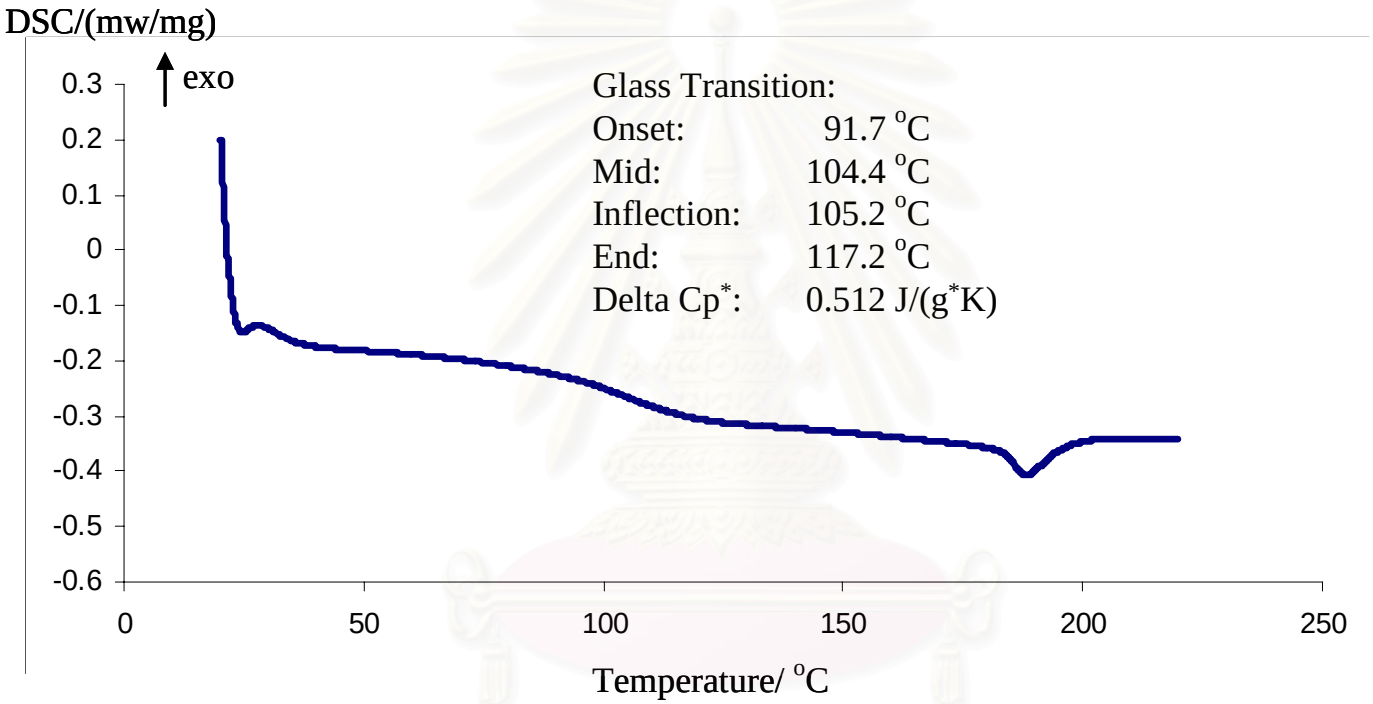


Figure A17 Thermogram of poly(vinyl alcohol-co-vinyl butane sultone), DS 0.194 (**PVA-BS1**)

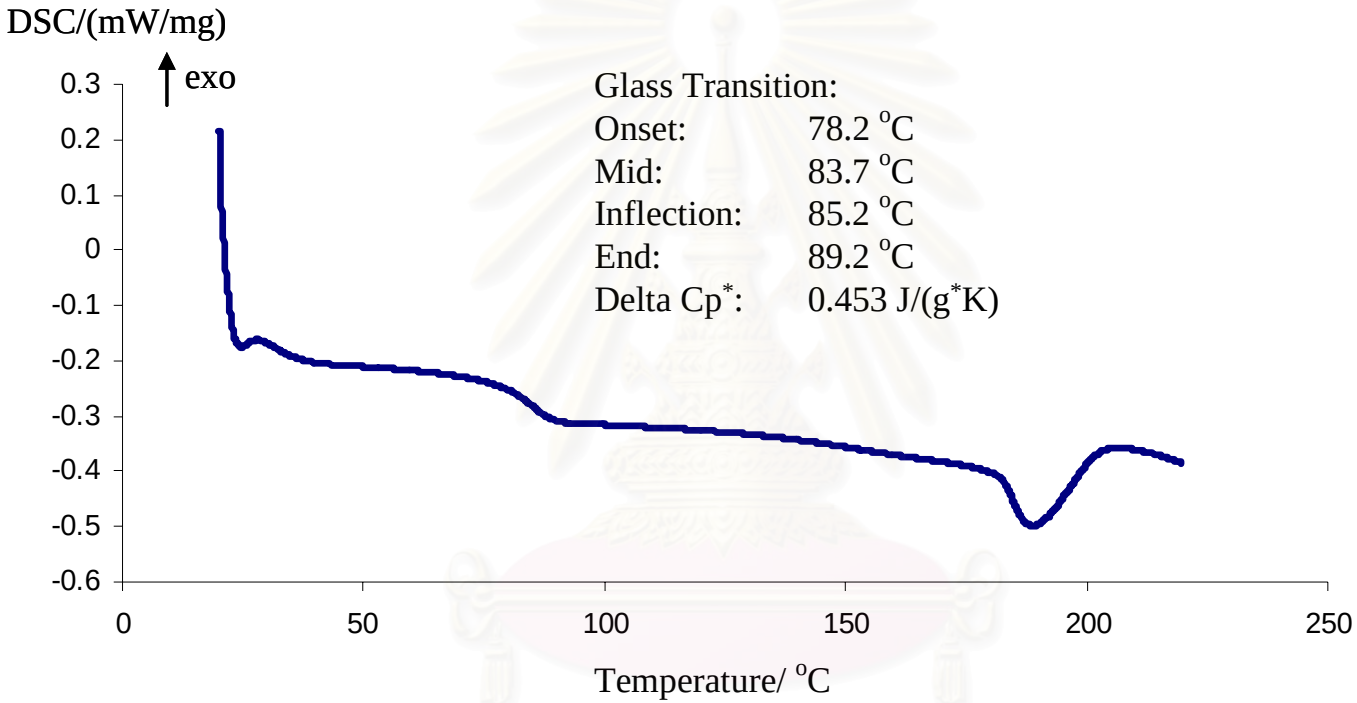


Figure A18 Thermogram of poly(vinyl alcohol-co-vinyl butane sultone), DS 0.066 (**PVA-BS2**)

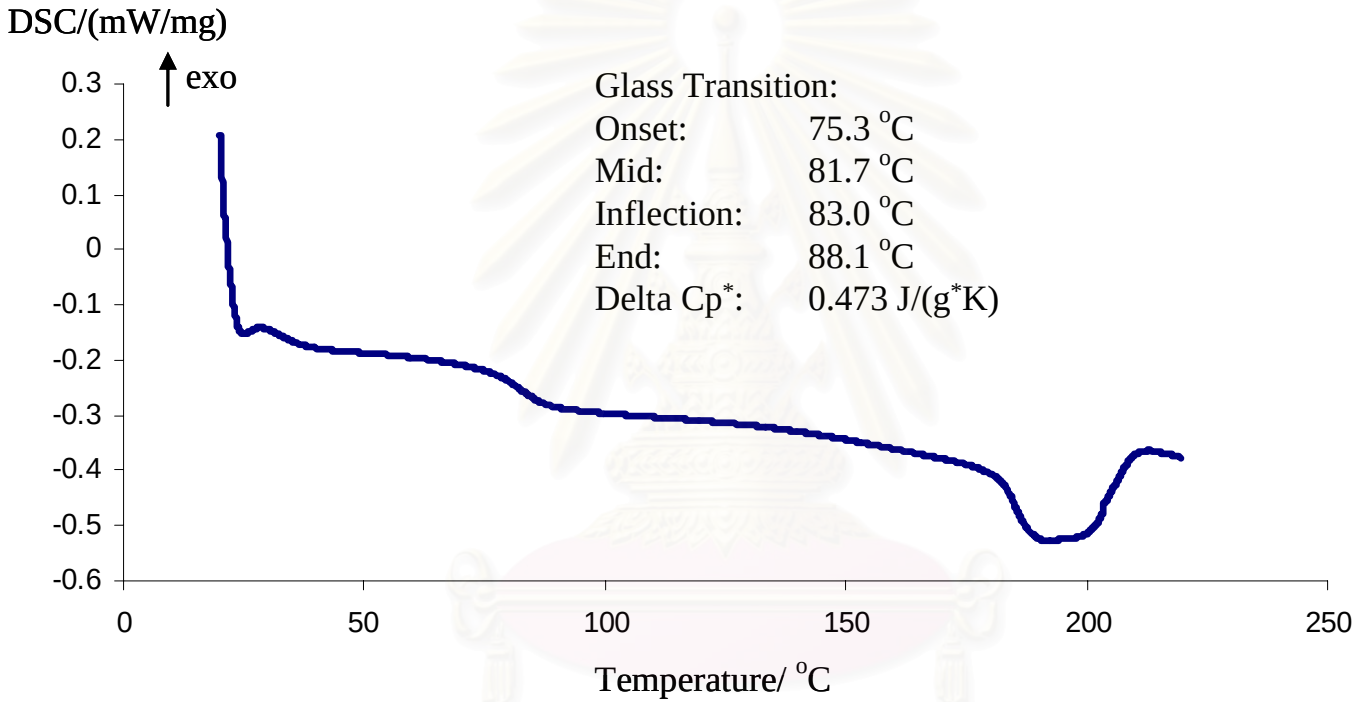


Figure A19 Thermogram of poly(vinyl alcohol-co-vinyl butane sultone), DS 0.036 (**PVA-BS3**)

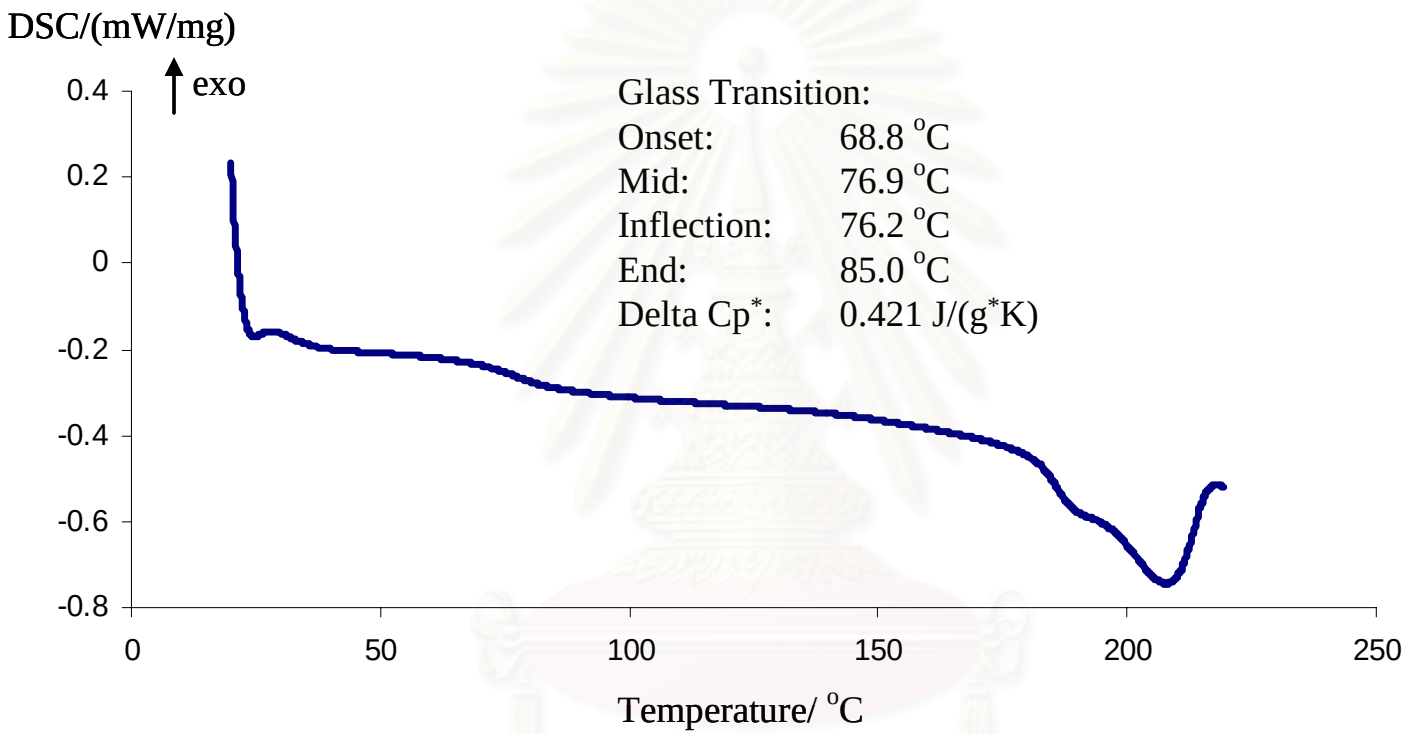


Figure A20 Thermogram of poly(vinyl alcohol-co-vinyl butane sultone), DS 0.013 (**PVA-BS4**)

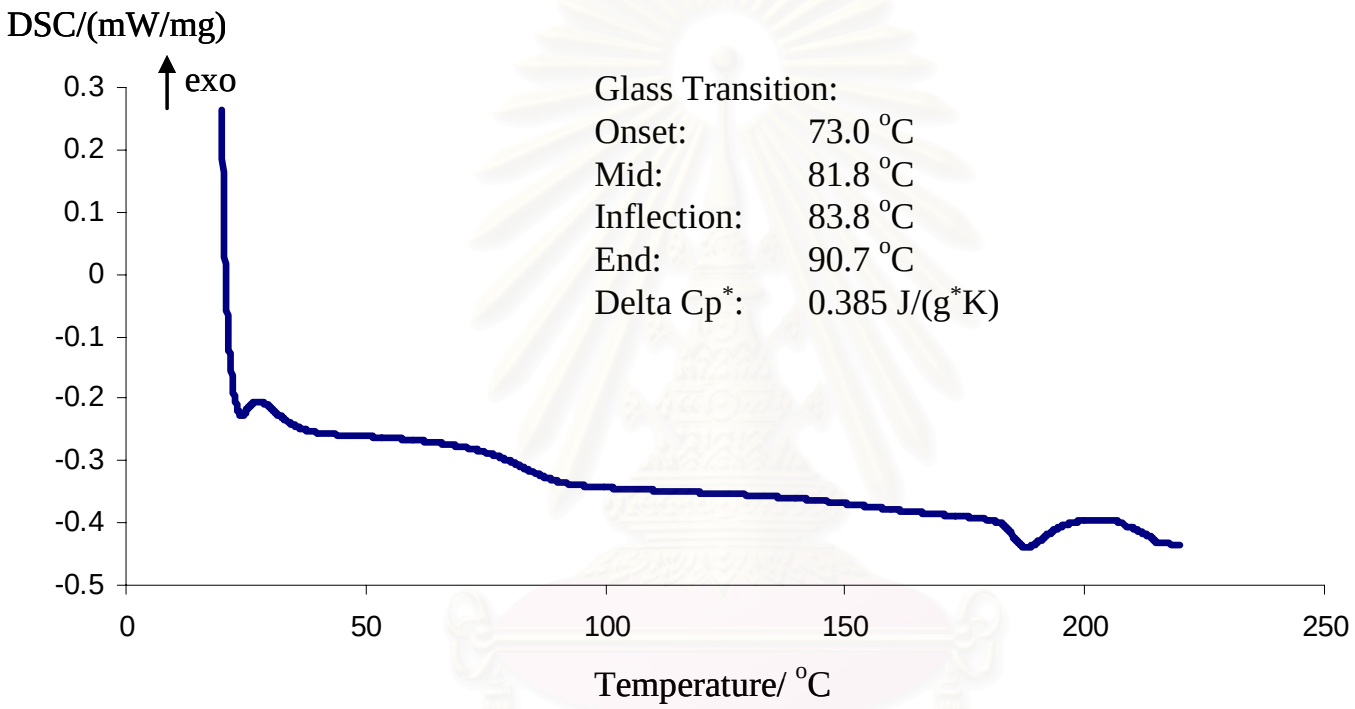


Figure A21 Thermogram of poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone), DS of 12C: 0.033, DS of BS: 0.135 (**PVA-12C1-BS1**)

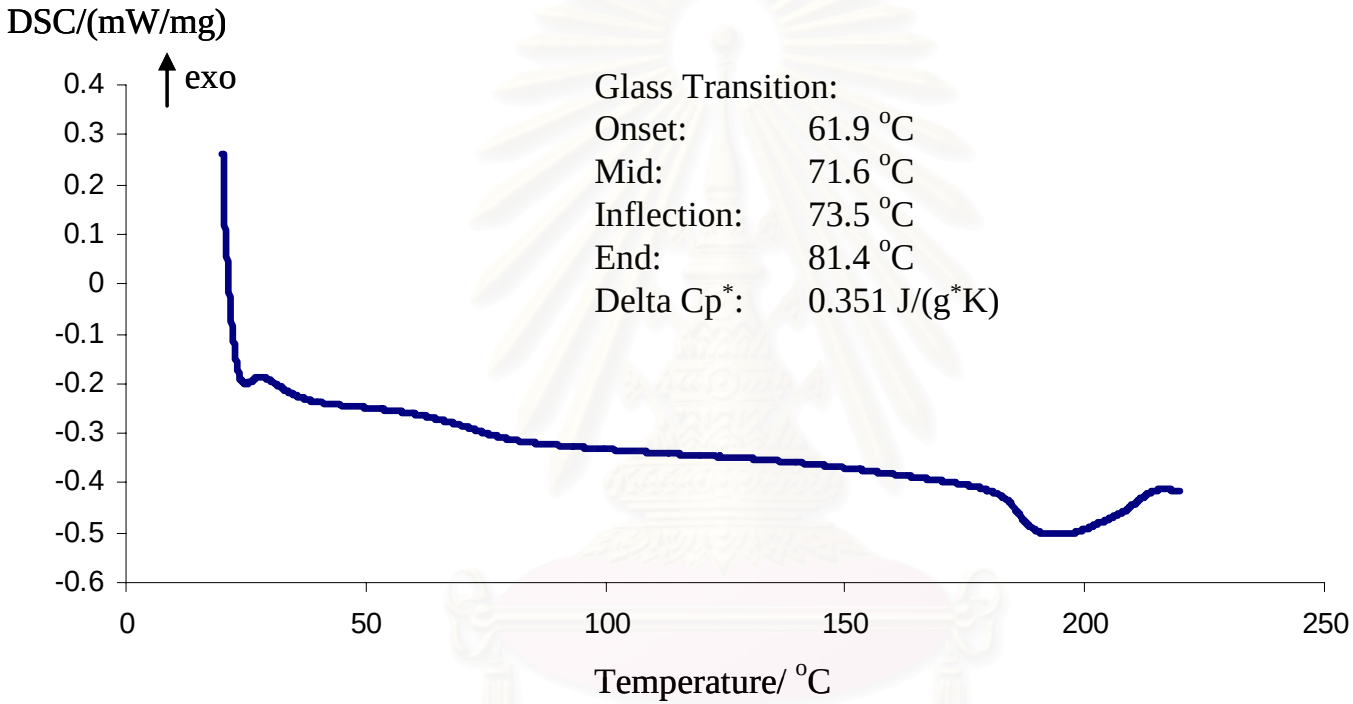


Figure A22 Thermogram of poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone), DS of 12C: 0.033, DS of BS: 0.037 (**PVA-12C1-BS2**)

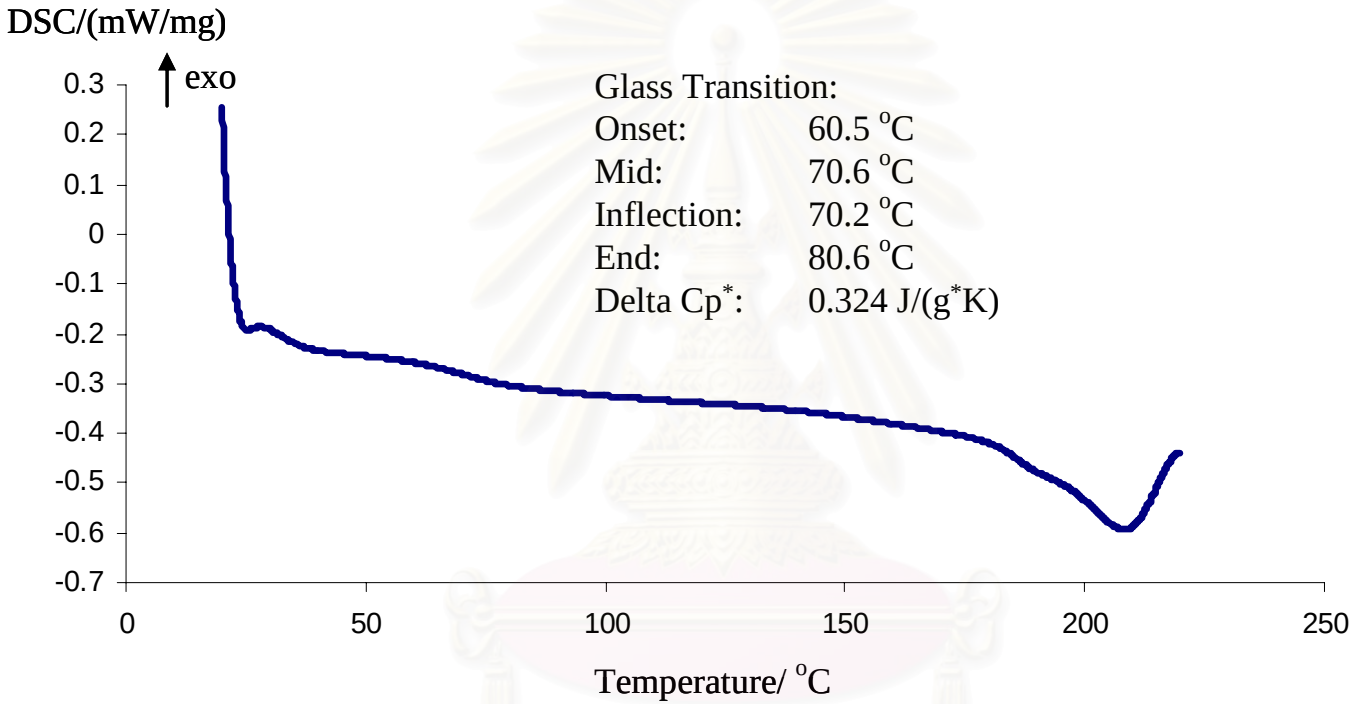


Figure A23 Thermogram of poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone), DS of 12C: 0.033, DS of BS: 0.028 (**PVA-12C1-BS3**)

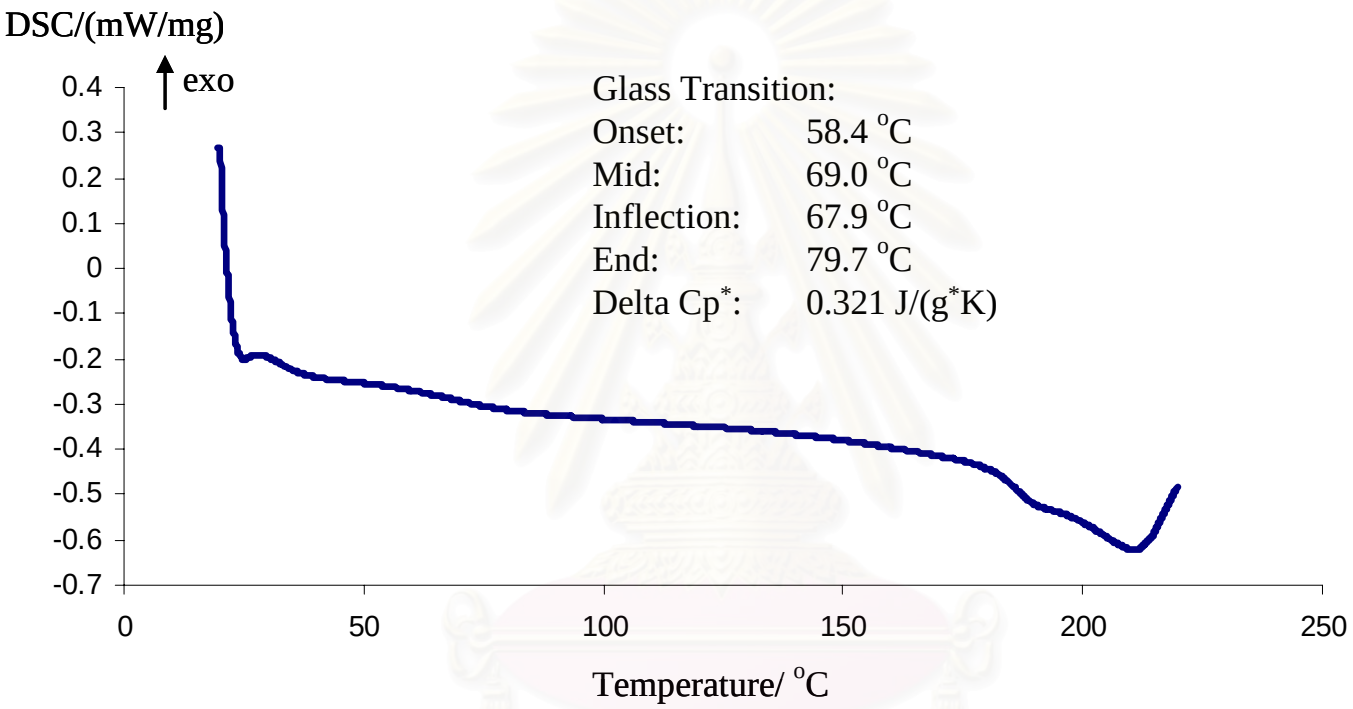


Figure A24 Thermogram of poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone), DS of 12C: 0.033, DS of BS: 0.022 (**PVA-12C1-BS4**)

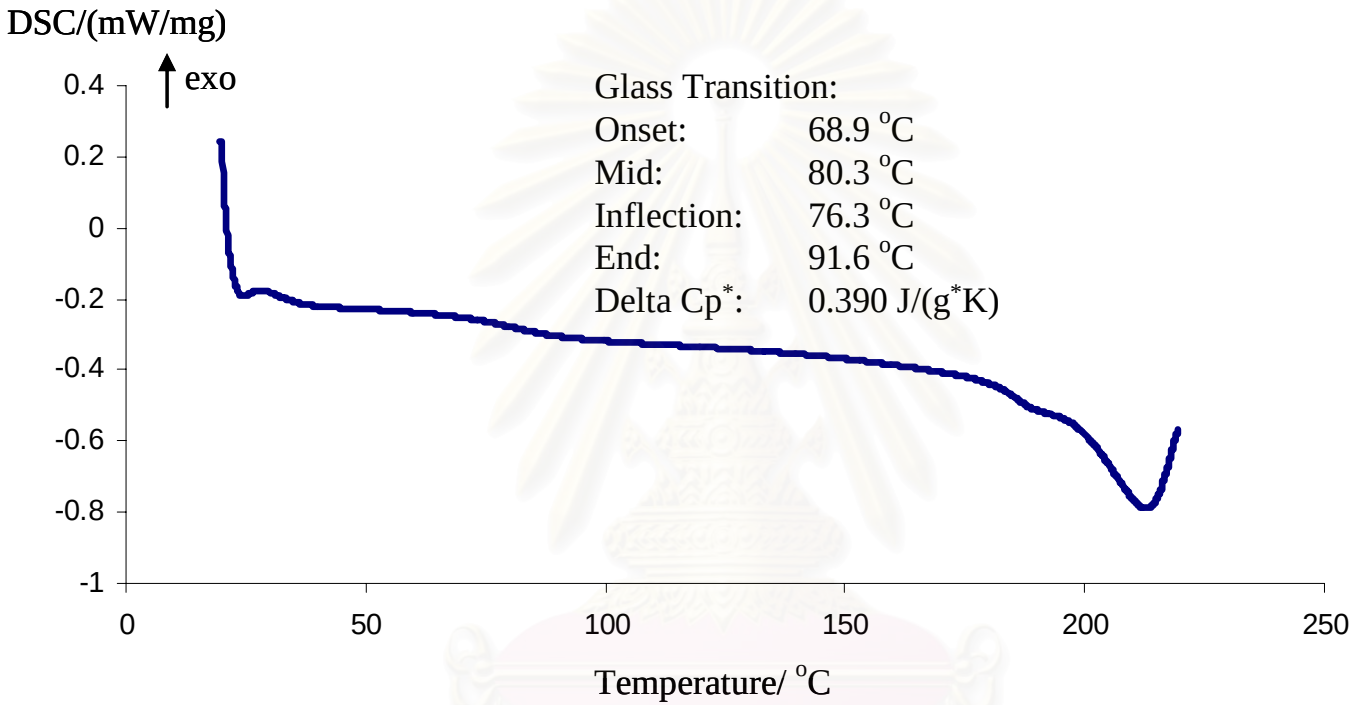


Figure A25 Thermogram of poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone), DS of 12C: 0.021, DS of BS: 0.036 (**PVA-12C2-BS1**)

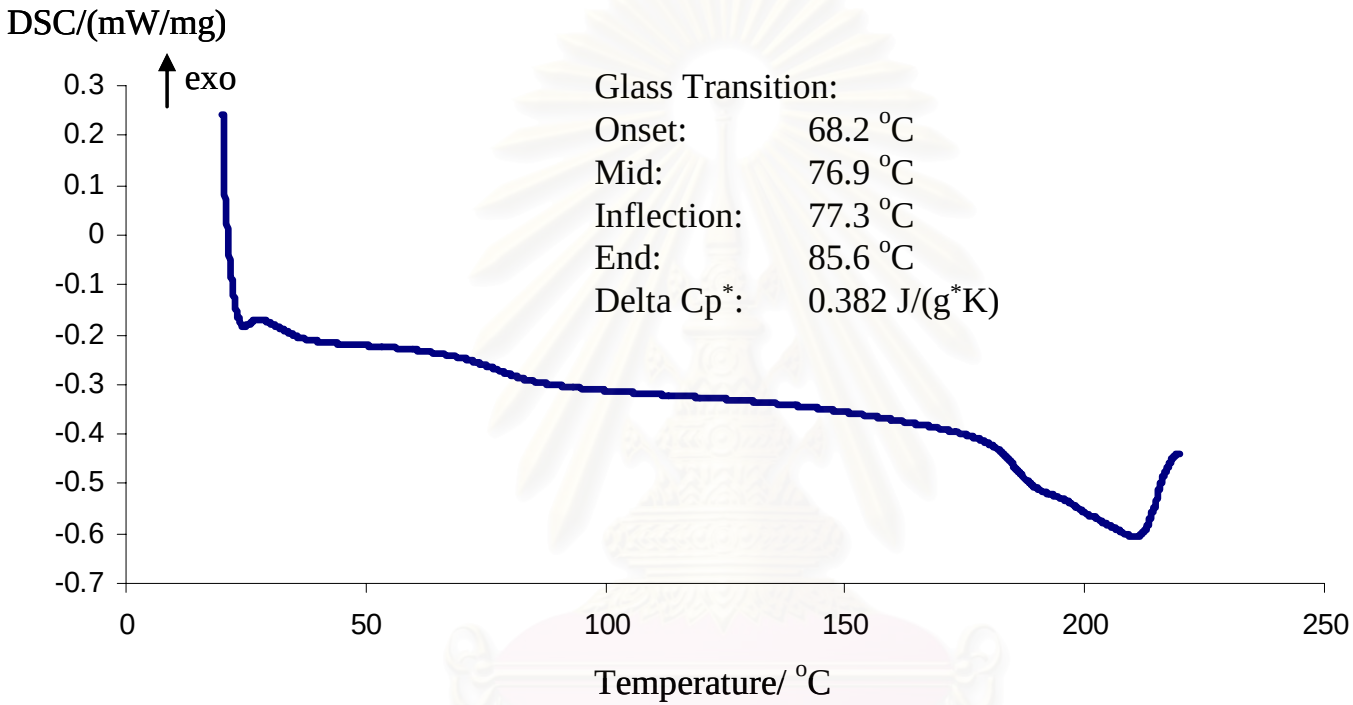


Figure A26 Thermogram of poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone), DS of 12C: 0.021, DS of BS: 0.027 (**PVA-12C2-BS2**)

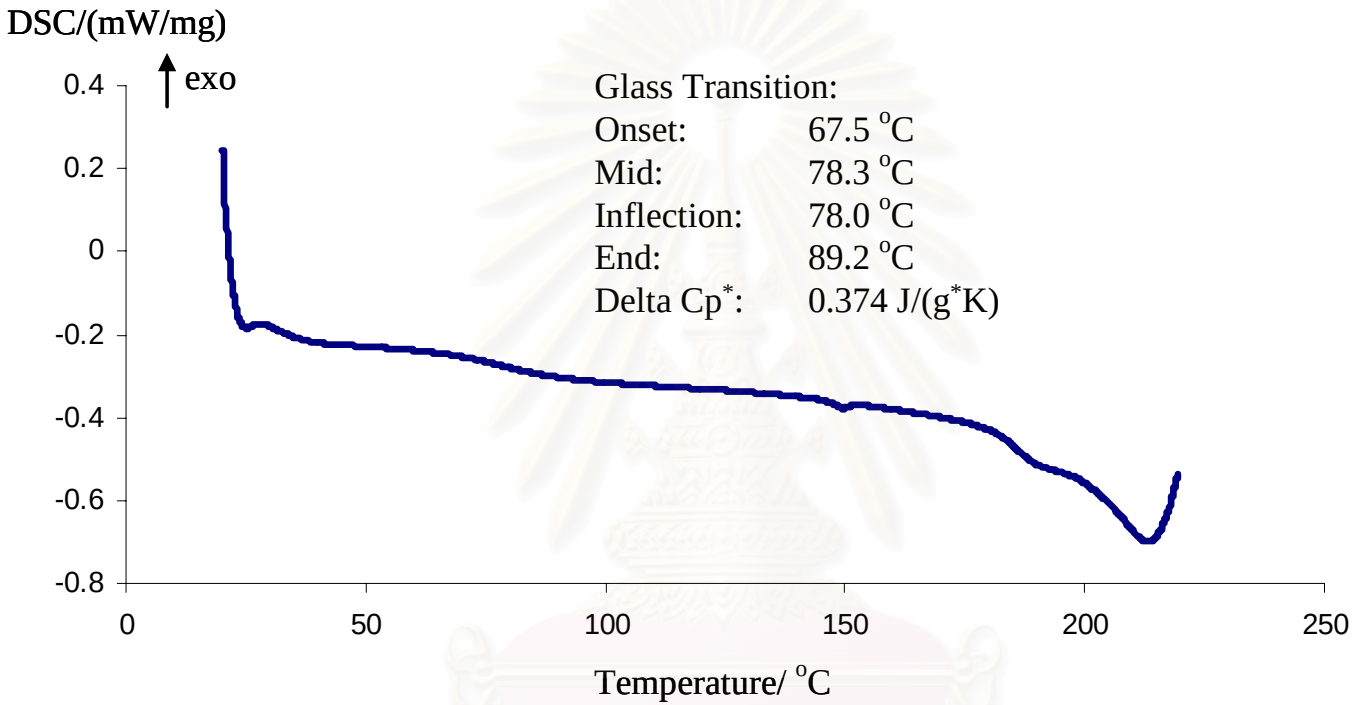
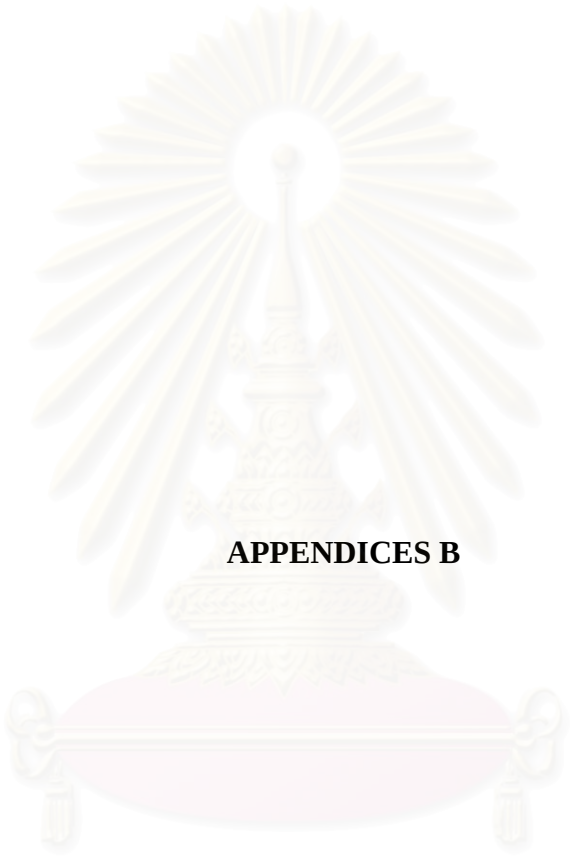


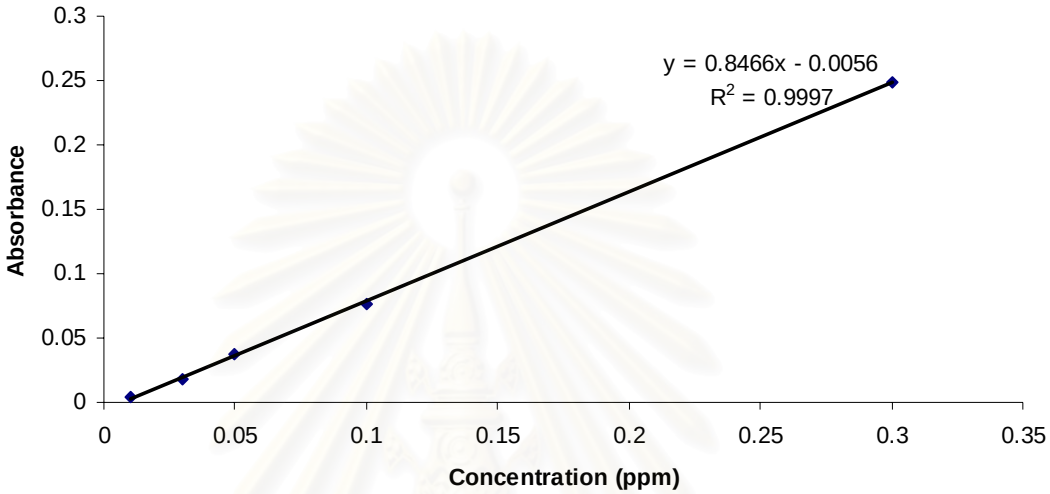
Figure A27 Thermogram of poly(vinyl alcohol-co-vinyl dodecane-co-vinyl butane sultone), DS of 12C: 0.021, DS of BS: 0.025 (**PVA-12C2-BS3**)



APPENDICES B

Calculation of % encapsulation efficiency (%EE) and % drug loading of 2-Ethylhexyl *p*-methoxycinnamate (EHMC)

Calibration curve of 2-Ethylhexyl *p*-methoxycinnamate



1. % encapsulation efficiency (%EE)

$$\%EE = \left[\frac{\text{amount of EHMC used - amount of EHMC found in the medium}}{\text{amount of EHMC used}}\right] \times 100$$

PVA-BS3: EHMC used = 20 mg

EHMC found in medium = 0.040 mg

$$\%EE = \left[\frac{20 - 0.034}{20}\right] \times 100$$

$$= 99.83\ \%$$

PVA-12C2-BS1: EHMC used = 20 mg

EHMC found in medium = 0.034 mg

$$\%EE = \left[\frac{20 - 0.040}{20}\right] \times 100$$

$$= 90.80\ \%$$

2. % drug loading

$$\% \text{ drug loading} = \left[\frac{\text{weight of drug in nanoparticles}}{\text{weight of nanoparticles}} \right] \times 100$$

PVA-BS3: EHMC in nanoparticles = 19.97 mg

EHMC used = 20 mg

PVA-BS3 used = 20 mg

$$\% \text{ drug loading} = \left[\frac{19.97}{20 + 20} \right] \times 100$$

$$= 49.92\ \%$$

PVA-12C2-BS1: EHMC in nanoparticles = 19.96 mg

EHMC used = 20 mg

PVA-12C2-BS1 used = 20 mg

$$\% \text{ drug loading} = \left[\frac{19.96}{20 + 20} \right] \times 100$$

$$= 49.90\ \%$$

VITA

Ms. Paramee Chitchumnong was born on August 2, 1983 in Bangkok, Thailand. She got a Bachelor's Degree of Science in Chemistry from Chulalongkorn University in 2005. After that, she has been a graduate student in a Master's Degree in Organic Chemistry at Chulalongkorn University in 2006-2008. During she was studying in Chulalongkorn University, she got the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund). Moreover, she had presented "Charged Nanoparticles from Poly(vinyl alcohol) Derivatives" in Pure and Applied Chemistry International Conference (PACCON 2008) and "Negatively Charge Poly(vinyl alcohol) Derivatives Nanoparticles" in 5th International Symposium on Advanced Materials in Asia-Pacific Rim (5thISAMAP) by poster presentation. The latter got the scholarships from National Center of Excellence, for Petrochemicals, and Advanced Materials (NCE-PPAM) and the Graduate School, Chulalongkorn University.

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