

REFERENCES

- (1) Lim, C.; Sandman, D. J.; Sukwattanasinitt, M. Topological polymerization of *tert*-butylcalix[4]arenes containing diynes. *Macromolecules*, 41 (2008) 675-681.
- (2) Tajima, K.; Aida, T. Controlled polymerizations with constrained geometries *Chem. Commun.* 24 (2000) 2399-2412.
- (3) He, J. A.; Yang, K.; Kumar, J.; Tripathy, S. K. Electrostatic Self-Assembly of Polydiacetylene Nanocrystals: Nonlinear optical properties and chain orientation *J. Phys. Chem. B.* 103 (1999) 11050-11056.
- (4) Wegner, G. Z., Polymerisation von derivaten des 2,4-hexadiin-1,6-diols im kristallin zustand *Naturforsch.* 24b (1969) 824-832.
- (5) Baughman, R. H.; Yee, K. C. Solid-state polymerization of linear and cyclic acetylenes *J. Polym. Sci. Macromol. Rev.* 13 (1978) 219-239.
- (6) Enkelmann, V. Structural aspects of the topochemical polymerization of diacetylenes *Adv. Polym. Sci.* 63 (1984) 91-136.
- (7) Reppy, M.A.; Pindzola, B.A. Biosensing with polydiacetylene materials: structures, optical properties and applications *Chem. Commun.* (2007) 4137-4338.
- (8) Yoon, J.; Chae, S.; Kim, J. Colorimetric sensors for volatile organic compounds (VOCs) based on conjugated polymer-embedded electrospun fibers *J. Am. Chem. Soc.* 129 (2007) 3038-3039.
- (9) Mino, N.; Tamura, H.; Ogawa, K. Photoreactivity of 10,12-pentacosadiynoic acid monolayers and color transitions of the polymerized monolayers on an aqueous subphase *Langmuir* 8 (1992) 594-598.
- (10) Stanish, I.; Santos, J.P.; Singh, A. One-step, chemisorbed immobilization of highly stable, polydiacetylene phospholipid vesicles onto gold films *J. Am. Chem. Soc.* 123 (2001) 1008-1009.
- (11) Kim, J.M.; Ji, E.K.; Woo, S.M.; Lee, H.; Ahn, D.J. Immobilized polydiacetylene vesicles on solid substrates for use as chemosensors *Adv. Mater.* 15 (2003) 1118-1121.

- (12) Shim, H.Y.; Lee, S.H.; Ahn, D.J.; Ahn, K.D.; Kim, J.M. Micropatterning of diacetylenic liposomes on glass surface *Mater. Sci. Eng. C-Biomimetic Supramol. Syst.* 24 (2004) 157-161.
- (13) Decher, G.; Hong, J.D.; Schmitt, Buildup of ultrathin multilayer films by a self-assembly process: *J. Thin Solid Films* 210 (1992), 831-835.
- (14) Decher, G. Fuzzy Nanoassemblies: toward layered polymeric multicomposites *Science* 277 (1997) 1232-1237.
- (15) Saremi, F.; Tieke, B.; Jordan, G.; Rammensee, W. Organized multilayers of polydiacetylenes prepared by electrostatic self-assembly *Supramol. Sci.* 4 (1997) 471-477.
- (16) Su, Y.L. Assembly of polydiacetylene vesicles on solid substrates *J. Colloid Interface Sci.* 292 (2005) 271-276.
- (17) Kim, J.; Kim, J.M.; Ahn, D.J. Layer-by-layer deposition of polydiacetylene vesicles and linear poly(sulfonates) *Macromol. Res.* 14 (2006) 478-482.
- (18) Eckhardt, H.; Boudreaux, D. S.; Chance, R. R., Effects of substituent-induced strain on the electronic structure of polydiacetylenes *J. Chem. Phys.* 85 (1986) 4116-4119.
- (19) Fujita, N. Sakamoto, Y., Shirakawa, M. Ojima, M. Fuji, A. Ozaki, M. Shinkai, Seiji. Polydiacetylene nanofibers created in low-molecular-weight gels by post modification: control of blue and red phases by odd-even effect in alkyl chains *J. Am. Chem. Soc.* 129 (2007) 4134-4135.
- (20) Schott, M. The colors of polydiacetylenes: a commentary *J. Phys. Chem. B* 110 (2006) 15864-15868.
- (21) Dobrosavljevic, V.; Stratt, R. M., The role of conformational disorder in the electronic structure of conjugated polymers: Substituted polydiacetylenes *Phys. Rev. B* 35 (1987) 2781-2794.
- (22) Dautel, O. J.; Robitzer, M.; Le' re-Porte, J. P.; Serein-Spirau, F.; Moreau, J. L, Self-organized ureido substituted diacetylenic organogel. photopolymerization of one-dimensional supramolecular assemblies to give conjugated nanofibers *J. Am. Chem. Soc.* 128 (2006) 16213-16223.
- (23) Dei, S.; Matsumoto, A.; Matsumoto A. Thermochromism of polydiacetylenes in the solid state and in solution by the self-organization of polymer chains containing no polar group *Macromolecule* 41 (2008) 2467-2473.

- (24) Nallicheri, R. A.; Rubner, M. F., Investigations of the mechanochromic behavior of poly(urethane diacetylene) segmented copolymers *Macromolecules* 24 (1991) 517-525.
- (25) Chance, R. R., Chromism in polydiacetylene solutions and crystals *Macromolecules* 13 (1980) 396-398.
- (26) Chance, R. R.; Patel, G. N.; Witt, J. D. Thermal effects on the optical properties of single crystals and solution-cast films of urethane substituted polydiacetylenes *J. Chem. Phys.* 71 (1979) 206-211.
- (27) Tachibana, H.; Hosaka, N.; Tokura, Y., Effect of alkyl chain length on thermochromic phase transition in urethane-substituted polydiacetylene crystals *Polymer* 42 (2001) 8311-8314.
- (28) Okada, S.; Peng, S.; Spevak, W.; Charych, D. Color and chromism of polydiacetylene vesicles *Acc. Chem. Res.* 31 (1998) 229-239.
- (29) Ahn, D. J.; Chae, E. H.; Lee, G. S; Shim, H. Y.; Chang, T. E.; Ahn, K. D.; Kim, J. M., Colorimetric reversibility of polydiacetylene supramolecules having enhanced hydrogen-bonding under thermal and pH stimuli *J. Am. Chem. Soc.* 125 (2003) 8976-8977.
- (30) Kim, J. Lee, J.S. Choi, H. Sohn, D., Ahn, D.J. Rational design and in-situ FTIR analyses of colorimetrically reversible polydiacetylene supramolecules *Macromolecules* 38 (2005) 9366-9376.
- (31) Lee, S.B.; Koepsel, R.R.; Russell, A.J. Surface dispersion and hardening of self-assembled diacetylene nanotubes *Nano Lett.* 11 (2005) 2202-2206.
- (32) Gu, Y.; Cao, W.; Zhu, L.; Chen, D.; Jiang, M. Polymer mortar assisted self-assembly of nanocrystalline polydiacetylene bricks showing reversible thermochromism *Macromolecules* 41 (2008) 2299-2303.
- (33) Muller, H.; Eckhardt, C.J. Stress induced change of electronic structure in a polydiacetylene crystal *Mol. Cryst. Liq. Cryst.* 45 (1978) 313-318.
- (34) Kew, S.J.; Hall, E. H. pH response of carboxy-terminated colorimetric polydiacetylene vesicles *Anal. Chem.* 78 (2006) 2331-2338.
- (35) Cheng, Q.; Stevens, C.; Charge-induced chromatic transition of amino acid-derivatized polydiacetylene liposomes *Langmuir* 14 (1998) 1974-1976.
- (36) Jonas, U.; Shah, K.; Norvez, S.; Charych, D.H. Reversible color switching and unusual solution polymerization of hydrazide-modified diacetylene lipids *J. Am. Chem. Soc.* 121 (1999) 4580-4588.

- (37) Song, J.; Cheng, Q.; Zhu, S.; Stevens, R.C. Smart materials for biosensing devices: cell-mimicking supramolecular assemblies and colorimetric detection of pathogenic agents *Biomed. Microdevices* 4 (2002) 213-218.
- (38) Sun, C.; Zhang, Y.; Fan, Yi.; Li, Yajun.; Li, Jinghong. Mannose-Escherichia coli interaction in the presence of metal cations studied in vitro by colorimetric polydiacetylene/glycolipid liposomes *J. Inorg. Biochem.* 98 (2004) 925-930.
- (39) Su, Y.L.; Li, J.R.; Jiang, L.; Cao, J. Biosensor signal amplification of vesicles functionalized with glycolipid for colorimetric detection of Escherichia coli *J. Colloid Interface Sci.* 284 (2005) 114-119.
- (40) Charych, D.H.; Nagy, J.O.; Spevak, W.; Bednarski, M.D. Direct colorimetric detection of a receptor-ligand interaction by a polymerized bilayer assembly *Science* 261 (1993) 585-587.
- (41) Charych, D.; Cheng, Q.; Reichert, A.; Kuziemko, G.; Stroh, M.; Nagy, J.O.; Spevak, W.; Stevens, R. A litmus test for molecular recognition using artificial membranes *Chemistry & Biology* 3 (1996) 113-120.
- (42) Katz, M.; Ben-Shlush, I.; Kolusheva, S.; Jelinek, R. Rapid colorimetric screening of drug interaction and penetration through lipid barriers *Pharm. Res.* (2006) 1-9.
- (43) Kolusheva, S.; Boyer, L.; Jelinek, R. A Colorimetric assay for rapid screening of antimicrobial peptides *Nat. Biotechnol.* 18 (2000) 225-227.
- (44) Ma, G.; Cheng, Q. Vesicular polydiacetylene sensor for colorimetric signaling of bacterial pore-forming toxin *Langmuir* 21 (2005) 6123-6126.
- (45) Kolusheva, S.; Zadmard, R.; Schrader, T.; Jelinek, R. Color fingerprinting of proteins by calixarenes embedded in lipid/polydiacetylene vesicles *J. Am. Chem. Soc.* 128 (2006) 13592-13598.
- (46) Kim, J.; Lee, J.S.; Lee, J.S.; Woo, S.Y.; Ahn, D.J. Unique effects of cyclodextrins on the formation and colorimetric transition of polydiacetylene vesicles *Macromol. Chem. Phys.* 206 (2005) 2299-2306.
- (47) Kolusheva, S.; Shahal, T.; Jelinek, R. Cation-selective color sensors composed of Ionophore-phospholipid-polydiacetylene mixed vesicles *J. Am. Chem. Soc.* 122 (2000) 776-780.
- (48) Sasaki, D.Y.; Waggoner, T.A.; Last, J.A.; Alam, T.M. Crown ether functionalized lipid membranes: Lead ion recognition and molecular reorganization *Langmuir* 18 (2002) 3714-3721.

- (49) Pincus, J.L.; Jin, C.; Huang, W.; Jacobs, H.K.; Gopalan, A.S.; Song, Y.; Shelnut, J.A.; Sasaki, D.Y. Selective fluorescence detection of divalent and trivalent metal ions with functionalized lipid membranes *J. Mater. Chem.* 15 (2005) 2938-2945.
- (50) Waggoner, T.A.; Last, J.A.; Kotula, P.G.; Sasaki, D.Y. Self-assembled columns of stacked lipid bilayers mediated by metal ion recognition *J. Am. Chem. Soc.* 123 (2001) 496-497.
- (51) Boonyiseng, J. Preparation of thin films containing thermochromic polydiacetylene vesicle *Thesis Chulalongkorn university* (2006).
- (52) Pigos, J.M.; Zhu, Z.; Musfeldt, J.L. Optical properties of a supramolecular assembly containing polydiacetylene *Chem. Mater.* 11 (1999) 3275-3278.
- (53) Rubner, M.F.; Sandman, D.J.; Yelazquez, C. On the structural origin of the thermochromic behavior of urethane-substituted poly(diacetylenes) *Macromolecules* 20 (1987) 1296-1300
- (54) Carpick, R.W.; Sasaki, D.Y.; Marcus, M.S.; Eriksson, M.A.; Burns, A.R. Polydiacetylene films: a review of recent investigations into chromogenic transitions and nanomechanical properties *J. Phys. Condens. Matter.* 16 (2004) 679-697.
- (55) Lee, D.; Sahoo, S. K.; Cholli, A. L.; Sandman, D. J. Structural aspects of the thermochromic transition in urethane-substituted polydiacetylenes *Macromolecules* 35 (2002) 4347-4355
- (56) Wang, X.; Whitten, J. E.; Sandman, D. J. Ultraviolet photoelectron spectroscopy study of the thermochromic phase transition in urethane-substituted polydiacetylenes *J. Chem. Phys.* 126 (2007) 184905/1-184905/5.
- (57) Guo, C. X.; Boullanger, P.; Liu, T.; Jiang, L. Size effect of polydiacetylene vesicles functionalized with glycolipids on their colorimetric detection ability *J. Phys. Chem. B* 109 (2005) 18765-18771.
- (58) Reichert, A.; Nagy, J. O.; Spevak, W.; Charych, D. Polydiacetylene liposomes functionalized with Sialic acid bind and colorimetrically detect influenza virus *J. Am. Chem. Soc.* 117 (1995) 829-830.
- (59) Charych, D.; Cheng, Q.; Reichert, A.; Kuziemko, G.; Stroh, M.; Nagy, J. O.; Spevak, W.; Stevens, R. C. A 'litmus test' for molecular recognition using artificial membranes *Chem. Biol.* 3 (1996) 113-120.

- (60) Kolusheva, S.; Shahal, T.; Jelinek, R. Cation-selective color sensors composed of ionophore-phospholipid-polydiacetylene mixed vesicles *J. Am. Chem. Soc.* 122 (2000) 776-780.
- (61) Ma, G.; Cheng, Q. Vesicular polydiacetylene sensor for colorimetric signaling of bacterial pore-forming toxin *Langmuir* 21 (2005) 6123-6126.
- (62) Wang, C.; Ma, Z.; Su, Z. Facile method to detect oligonucleotides with functionalized polydiacetylene vesicles. *Sens. Actuators, B* 113 (2006) 510-515.
- (63) Ribi, H.O. U.S. Patent 6 607 744 B1 (2003).
- (64) Ribi, H.O. U.S. Patent 6 787 108 (2004).
- (65) Ribi, H.O. U.S. Patent 6 866 863 B2 (2005).
- (66) Hays, D.S. U.S. Patent Application 20 050 101 794 A1 (2005).
- (67) Sasaki, D.Y.; Carpick, R.W.; Burns, A.R. High molecular orientation in mono- and trilayer polydiacetylene films imaged by atomic force microscopy *J. Colloid Interface Sci.* 229 (2000) 490-496.
- (68) Hub, H.H.; Hupfer, B.; Koch, H.; Ringsdorf, H. Polymerizable phospholipid analogues-new stable biomembrane and cell models *Angew. Chem. Int. Ed. Engl.* 19 (1980) 938-940.
- (69) Akimoto, A.; Dorn, K.; Gros, L.; Ringsdorf, H.; Schupp, H. Polymerized surfactana vesicles: novel membrane mimetic systems *Angew. Chem. Int. Ed. Engl.* 20 (1981) 90-91.
- (70) Hupfer, B.; Ringsdorf, H.; Schupp, H. Polyreaction oriented systems 21-polymeric phospholipid monolayers *Makromol. Chem.* 182 (1981) 247-253.
- (71) Gaboriaud, F.; Golan, R.; Volinsky, R.; Berman, A.; Jelinek, R. Organization and structural properties of langmuir films of conjugated polydiacetylene and phospholipids *Langmuir* 17 (2001) 3651-3657.
- (72) Ahn, D.; Chae, E.; Lee, G.; Shim, H.; Chang, T.; Ahn, K.; Kim, J. Colorimetric reversibility of polydiacetylene supramolecules having enhanced hydrogen-bonding under thermal and pH stimuli *J. Am. Chem. Soc.* 125 (2003) 8976-8977.
- (73) Decher, G.; Lehr, B.; Lowack, K.; Lvov, Y.; Schmitt, J. New nanocomposite films for biosensors: layer-by-layer adsorbed films of polyelectrolytes, proteins or DNA *Biosens. Bioelectron.* 9 (1994) 677-684.

- (74) Lvov, Y.; Ariga, K.; Kunitake, T. Assembly of multicomponent protein films by means of electrostatic layer-by-layer adsorption *J. Am. Chem. Soc.* 117 (1995) 6117-6123.
- (75) Anzai, J.; Kobayashi, Y.; Nakanura, N.; Nishimura, M.; Hoshi, T. Layer-by-layer construction of multilayer thin films composed of avidin and biotin-labeled poly(amine)s *Langmuir* 15 (1999) 221-226.
- (76) Emoto, K.; Iijima, M.; Nagasaki, Y.; Kataoka, K. Functionality of polymeric micelle hydrogels with organized three-dimensional architecture on surfaces *J. Am. Chem. Soc.* 122 (2000) 2653-2654.
- (77) Mendelsohn, J.D.; Barrett, C.J.; Chan, V.V.; Pal, A.J.; Mayes, A.M.; Rubner, M.F. Fabrication of microporous thin films from polyelectrolyte multilayers *Langmuir* 16 (2000) 5017-5023.
- (78) Constantine, C.A.; Gattus-Asfura, K.M.; Mello, S.V.; Crespo, G.; Rastogi, V.; Cheng, T.C.; DeFrank, J.J.; Leblanc, R.M. Layer-by-layer biosensor assembly incorporating functionalized quantum dots *Langmuir* 19 (2003) 9863-9867.
- (79) Saremi, F.; Tieke, B.; Jordan, G.; Rammensee, W. Organized multilayers of polydiacetylenes prepared by electrostatic self-assembly *Supramol. Sci.* 4 (1997) 471-477.
- (80) Katagiri, K.; Hamasaki, R.; Ariga, K.; Kikuchi, J. Layered paving of vesicular nanoparticles formed with cerasome as a bioinspired organic-inorganic hybrid *J. Am. Chem. Soc.* 124 (2002) 7892-7893.
- (81) Michael, M.; Vautier, D.; Voegel, J.C.; Schaaf, P.; Ball, V. Layer by layer self-assembled polyelectrolyte multilayers with embedded phospholipid vesicles *Langmuir* 20 (2004) 4835-4839.
- (82) Michael, M.; Izqueirido, A.; Decher, G.; Voegel, J.C.; Schaaf, P.; Ball, V. Layer by layer self-assembled polyelectrolyte multilayers with embedded phospholipid vesicles obtained by spraying: integrity of the vesicles *Langmuir* 21 (2005) 7854-7859.
- (83) Chance, R.R.; Baughman, R.H.; Muller, H.C.; Eckhardt, J. Thermochromism in a polydiacetylene crystal *J. Chem. Phys.* 6 (1977) 3616-3618.
- (84) Toutianoush, A.; Krasemann, L.; Tieke, B. Polyelectrolyte multilayer membranes for pervaporation separation of alcohol/water mixtures *Colloids Surf. A* 98 (2002) 881-889.

- (85) Krasemann, L.; Toutianoush, A.; Tieke, B. Self-assembled polyelectrolyte multilayer membranes with highly improved pervaporation separation of ethanol/water mixtures *J. Membr. Sci.* 181 (2001) 221-228.
- (86) Dubas, S.T.; Iamsamai, C.; Potiyaraj, P. Optical alcohol sensor based on dye-chitosan polyelectrolyte multilayers *Sens. Actuators B Chem.* 113 (2006) 370-375.
- (87) Lasic, D. D.; Needham, D. The "Stealth" liposome: A prototypical biomaterial. *Chem. Rev.* 95 (1995) 2601-2628.
- (88) Gupta, V.K.; Jain, A.K.; Kumari, P. PVC-based membranes of *N,N'*dibenzyl-1,4,10,13-tetraoxa-7-16-diazacyclooctadecane as Pb(II)-selective sensor *Sensors and Actuators B* 120 (2006) 259-265.
- (89) Kim, L.M.; Lee, Y.B.; Chae, S.K.; Ahn, D.J. Patterned color and fluorescent images with polydiacetylene supramolecules embedded in poly(vinyl alcohol) films *Adv. Funct. Mater.* 16 (2006) 2103-2109.

APPENDICES

APPENDIX A

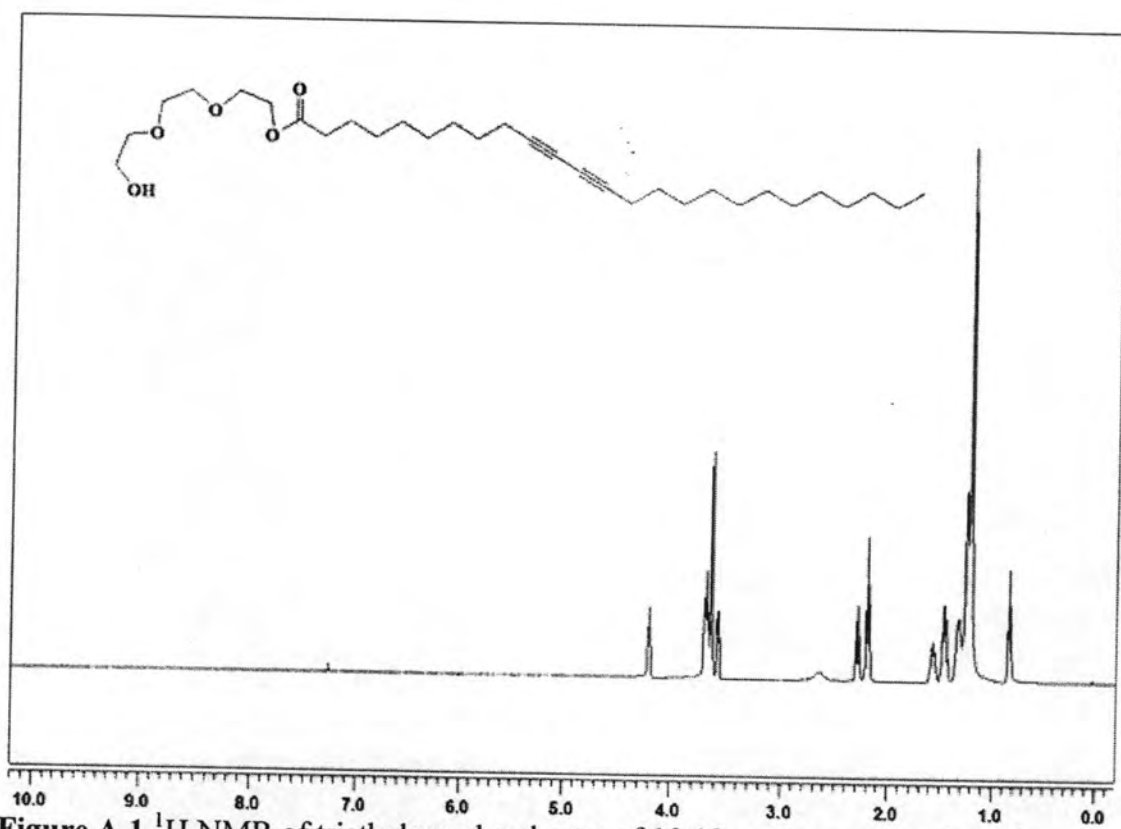


Figure A.1 ^1H NMR of triethylene glycol ester of 10,12-pentacosadiynoic acid

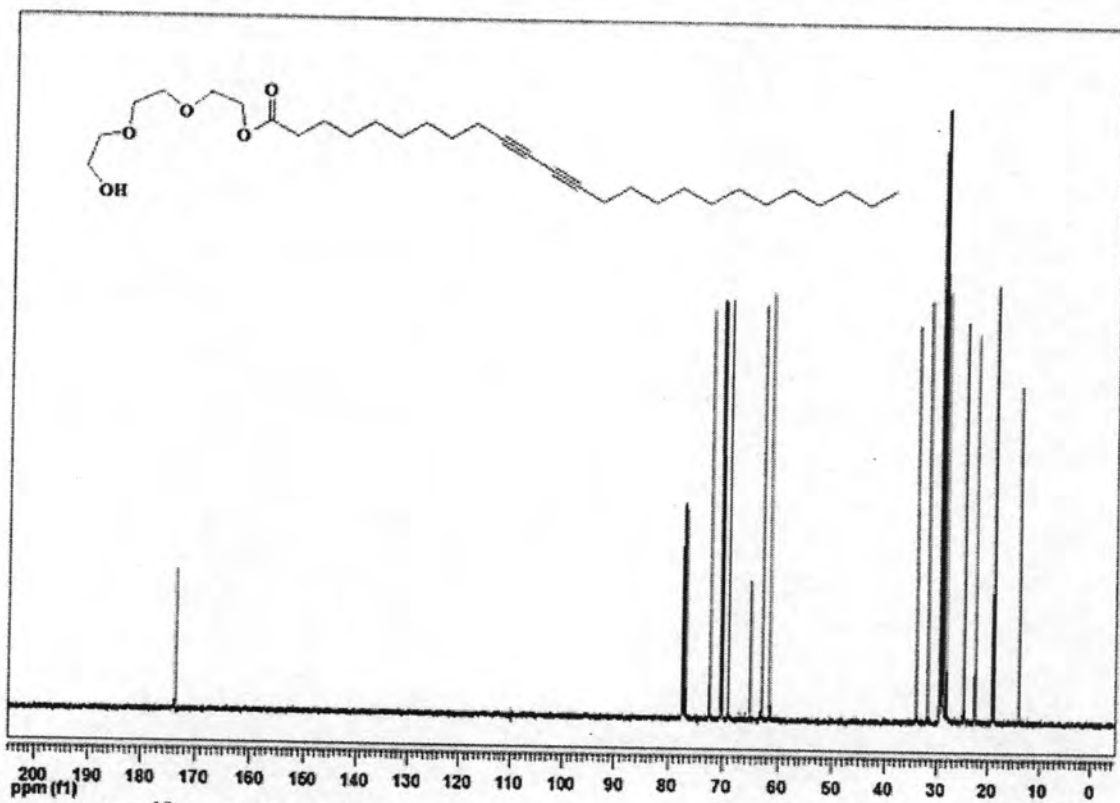


Figure A.2 ^{13}C NMR of triethylene glycol ester of 10,12-pentacosadiynoic acid

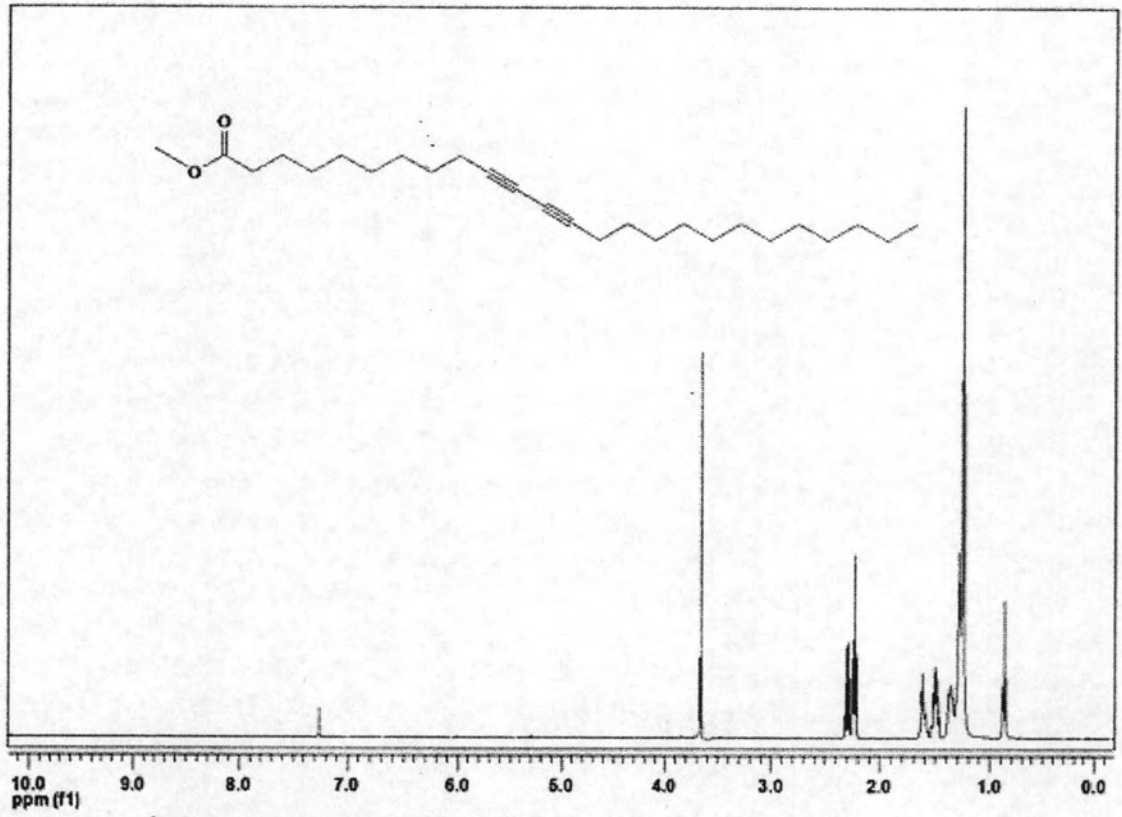


Figure A.3 ¹H NMR of methyl 10,12-pentacosadiynoate

APPENDIX B



Figure B.1 Winning the first runner up in Thailand innovation awards 2007 for innovation product and business plan. (from left to right : Mr. Toedtoon Champaiboon, Miss Sansanee Boonchit, Miss Jasuma Boonyiseng, Mr. Anupat Potisatityuenyong and Dr. Suchinda Chotipanich). The right picture is two winning cups.

APPENDIX C**REPRINT**

- (1) Potisatityuenyong, A.; Tumcharern, G.; Dubas, S.T.; Sukwattanasinitt, M. *J. Colloid. Interface Sci.* 304 (2006) 45-51.
- (2) Potisatityuenyong, A.; Rojanathanes, R.; Tumcharern, G.; Sukwattanasinitt, M. *J. Langmuir* 24 (2008) 4461-4463.



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Layer-by-layer assembly of intact polydiacetylene vesicles with retained chromic properties

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Abstract

Photopolymerized vesicles of 10,12-pentacosadiynoic acid (PPCDA) were successfully assembled into polyelectrolyte multilayer (PEM) thin films using either chitosan or poly(ethylenimine) (PEI) as a polycation. The PEM films assembled from chitosan or PEI polycation retained the blue color of PPCDA vesicles. For the blue films, an increase in absorbance at 635 nm as a function of the number of deposited layers of PPCDA vesicles was observed, confirming the uniform layer-by-layer deposition process. The spherical structures of PPCDA vesicles, as well as their important colorimetric responses to solvent (ethanol), pH, and temperature, are retained in the PEM film. Compared to the vesicles dispersed in water, the PEM films are much more stable to aging. Layer-by-layer assembly thus provides a convenient means to prepare colorimetric sensing devices, with extended shelf life, from polydiacetylene vesicles.

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Keywords: Adsorption; Colorimetry; Multilayer film; Polydiacetylene; Sensor; Vesicle

1. Introduction

Polydiacetylenes [1] are an interesting class of ene-yne conjugated polymers that exhibit dramatic color change from blue to red upon exposure to various stimuli including light (photochromism) [2–4], heat (thermochromism) [5–9], mechanical stress (mechanochromism) [10–12], solvents (solvatochromism) [13,14], and binding of specific biological agents (biochromism) [15–17]. Polydiacetylenes can be prepared by topochemical polymerization of monomeric diacetylenes in numerous forms: bulk solids [18,19], self-assembled films [20,21], and vesicles suspended in liquids [22,23]. Aqueous suspended polydiacetylene vesicles are generally produced from photopolymerization of vesicles formed from monomeric diacetylene lipids. The resulting nanospherical vesicles or liposomes of polydiacetylenes homogeneously dispersed in aqueous

media have been successfully used as colorimetric sensors [24–32].

Immobilization of polydiacetylene vesicles into thin films would enhance their storage stability and user-friendliness. Despite long and extensive research on polydiacetylene vesicles, reports on the fabrication of films containing these materials have just come to light in recent years. Monomeric diacetylene vesicles have been covalently fixed onto functionalized glass substrates followed by photopolymerization to form blue-phase polydiacetylene monolayer films [33,34]. This approach is useful when monolayer deposition is desired, but the fabrication of multilayer films with sufficient visible color by this technique is not generally practical. The transfer of self-assembled polydiacetylene Langmuir–Blodgett films to flat substrates is somewhat less complicated, but is not suitable for the assembly of vesicles that have spherical hydrophilic surfaces.

A little more than a decade ago, the polyelectrolyte multilayers (PEM) technique developed by Decher and co-workers emerged as an alternative method for the preparation of mul-

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tilayer thin films [35–41]. Although based on a very simple adsorption process, PEM assembly has proven to be a very powerful method for immobilizing charged species onto a substrate. In this technique, oppositely charged polyelectrolytes (polyanions and polycations) are assembled into thin films by sequential dipping of a substrate into polyelectrolyte solutions followed by rinsing steps. The electrostatic interaction between the adsorbed layer on the substrate and the oppositely charged polyelectrolytes in the solution leads to the adsorption of another polyelectrolyte layer and the reversal of the surface charge, allowing the deposition of the next layer. The PEM technique is well suited for the preparation of thin films on objects with various types of surface and shape. The thickness of the film can also be easily controlled by choosing the appropriate number of layers deposited, which is convenient for preparation of colorimetric sensing devices detectable by naked eyes. Early use of the PEM technique to assemble diacetylene monomers or polydiacetylene chains with polyallylamine (PAH) resulted in thin films with an irreversible red color, which excluded them from being colorimetric sensors [42]. It is important to emphasize here that in order for polydiacetylene vesicles to be usable in sensing applications, the characteristic blue color of the vesicles must be maintained during the film preparation process.

Decher et al. have recently demonstrated that the assembly of phospholipid vesicles in PEM thin films was possible by dipping or spraying the vesicles onto a substrate [43–45]. They also demonstrated that the phospholipid vesicles conserved their spherical shape and could be used as bioreservoirs for drug release. The deposition of unpolymerized vesicles onto thin films that were later photopolymerized has recently been reported [46]; however, no clear evidence indicates whether the spherical structure of the vesicles remained intact. To the best of our knowledge, the direct assembly of prepolymerized blue vesicles retaining blue color has not yet been successful. The aim of this article is to report our findings on the importance of the choice of polycationic polymers for assembly to preserve the blue color and the spherical structure of the polydiacetylene vesicles. The chromic responses of the PEM film to various stimuli such as ethanol content, pH, and temperature in comparison with that of vesicles dispersed in water will also be presented.

2. Materials and methods

2.1. Materials

The diacetylenic monomer 10,12-pentacosadiynoic acid (97%) was purchased from Fluka. Linear poly(ethylenimine) (PEI) was purchased from Aldrich. Chitosan was purchased from Seafresh Chitosan (Lab) Company, Thailand. The viscosity-averaged molecular weight (M_v) was 8.0×10^5 , and the degree of deacetylation determined by colloidal titration was 84%. Diethyl ether, analytical grade, was purchased from Lab-Scan, Thailand. Deionized MilliQ water with a resistance of 18.1 M Ω was used in all experiments. The solvents and chemicals were

used as received without further purification unless specified otherwise.

2.2. Preparation of PPCDA vesicles

The diacetylene monomer 10,12-pentacosadiynoic acid (PCDA) was dissolved in diethyl ether and filtered to remove any contaminating polymerized materials. The filtrate was dried under a rotary evaporator in the dark to produce a thin film of lipid monomer inside the flask. Deionized water was added to provide a 0.5 mM aqueous lipid suspension and was sonicated at 75–80 °C for 30 min. The vesicle suspension was allowed to cool to room temperature and then kept at 4 °C overnight. The vesicle suspension was irradiated by UV irradiation for 5 min to complete the topopolymerization. Following the polymerization, the solution was filtered through a 0.45- μ m PTFE syringe filter to remove any undesired lipid aggregates formed during the preparation. The resulting filtrate appeared as a transparent deep blue solution of poly(10,12-pentacosadiynoic acid) (PPCDA) vesicles.

2.3. Layer-by-layer deposition of the vesicles

Glass slide substrates were cleansed of organic contaminants by 15 min dipping in an oxidizing “piranha” solution prepared by mixing a 2:1 volume ratio of concentrated sulfuric acid with hydrogen peroxide solution (30%). The sample was then rinsed thoroughly with MilliQ water. The clean substrates were dipped into the polycationic solution (0.1% w/w for chitosan, 10 mM for PEI) at pH 3 (adjusted by 2 M HCl) for 5 min. After three rinsing steps in MilliQ water, the glass substrate was then immersed in PPCDA vesicles solution (0.5 mM of initial monomer) for 5 min. The deposition was carried out by repeating these adsorption cycles as many times as needed. A UV–vis spectrophotometer (Varian Cary 100 Bio) was used to confirm the layer-by-layer deposition process by monitoring the increase in absorbance at 640 nm as a function of the number of deposited layers.

2.4. Evaluation of the colorimetric responses

The colorimetric response (%CR) was used to semiquantify the blue-to-red transition. The %CR can be expressed as

$$\%CR = \frac{100 \times (PB_0 - PB)}{PB_0}$$

The PB is the percent blue calculated from $A_{635}/(A_{540} + A_{635})$, where A_{540} , A_{635} are the absorbance of the red ($\lambda = 540$) and blue ($\lambda = 635$) phases of PPCDA vesicles, respectively. The initial PB_0 value was determined before exposure to any stimuli. The completeness of color transition was determined as the first saturation point in the plot of %CR against the quantity of each stimulant.

2.5. Evaluation of the ethanol, pH, and temperature responses

The PEM film was prepared directly on a glass substrate, specially cut to fit the UV–vis spectrophotometer cuvette. In a

typical experiment, the coated substrate was introduced into a cuvette filled with water and allowed to stand for 5 min before the absorbance spectrum was measured to obtain the PB_0 value. The procedure was repeated after the solution was replaced with a solution having a higher ethanol content or higher pH or the temperature was increased. In every case, the system was allowed to stand for 5 min before the new spectrum was recorded. While one parameter, e.g., ethanol content, was varied, the other two parameters, e.g., pH and temperature, were controlled. The specific values for controlled parameters are presented, along with the results for each test.

2.6. Atomic force microscopy

The AFM images were obtained by Multimode SPA400 (Seiko, Japan) in semicontact mode (dynamic mode) using a SI-DF20 cantilever. The image of the vesicles was measured on an air-dried sample of PPCDA vesicles prepared from a drop of diluted solution on a freshly cleaned glass slide. For the imaging of the PEM film containing vesicles, a freshly prepared film sample on a clean glass substrate was air-dried overnight prior to the measurement.

3. Results and discussion

Two types of polycationic polymers, chitosan and poly(ethylenimine) (PEI), were studied for layer-by-layer assembly with poly(10,12-pentacosadiynoic acid (PPCDA) vesicles (Fig. 1). A clean glass substrate was alternately dipped into a polycationic electrolyte and a freshly prepared blue PPCDA

vesicle solution. The average size of PPCDA vesicles used in the layer-by-layer assembly was estimated from the vesicle solution using dynamic light scattering (DLS) and dry samples cast on glass slides using tunneling electron microscopy (TEM) and atomic force microscopy (AFM). The submicrometer-sized vesicles had an average diameter of 60 nm obtained from the light-scattering data and TEM image (in the supporting document), which agreed well with that observed from the AFM image (Fig. 2a). The spherical vesicles were also observed in the AFM image of chitosan/vesicles PEM film (Fig. 2b). The AFM image clearly shows, for the first time, that the intact polydiacetylene vesicles could be assembled into a PEM film.

The visual appearance of the resulting chitosan/vesicles and PEI/vesicles PEM films was very smooth and uniform, with a deep blue color intensifying as a function of the number of deposited layers. The blue films immediately and irreversibly turned red when they were dipped into 95% ethanol. The visible absorption spectra of the blue and red films showed the maximum absorption at 635 and 540 nm, respectively (Fig. 3). This irreversible color switch is probably related to the irrecoverable perturbation of the intramolecular hydrogen bonds between the carboxylic head groups of the polydiacetylene side chains, which reduced the conjugation length or relieved the mechanical stress on the polydiacetylene backbone [1,5].

UV–vis absorbance at 635 nm was employed to monitor the layer-by-layer deposition of chitosan or PEI with PPCDA vesicles. Linear relationships between the number of layers and the absorbance were obtained for both polycationic electrolytes (Fig. 4), signifying a well-defined layer-by-layer deposition

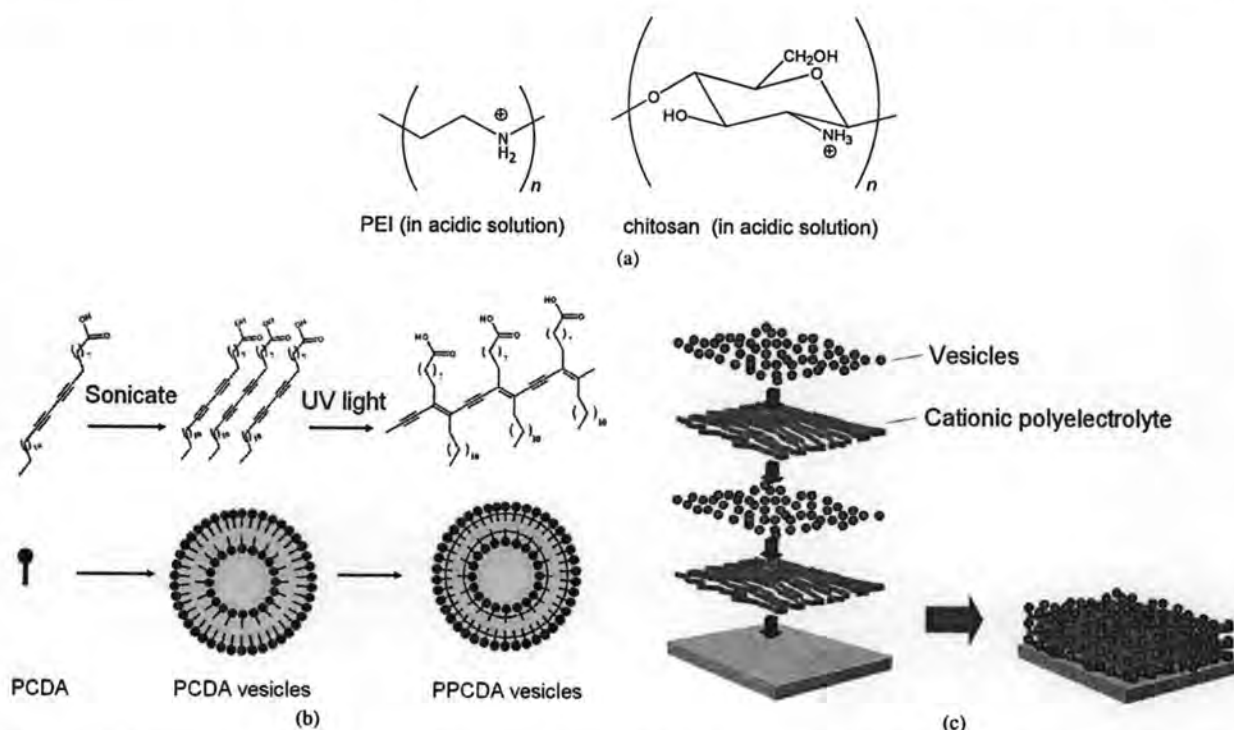


Fig. 1. (a) Chemical structures of polycationic polymers, (b) schematic preparation of PCDA vesicles, and (c) illustration of layer-by-layer assembly on glass substrate.

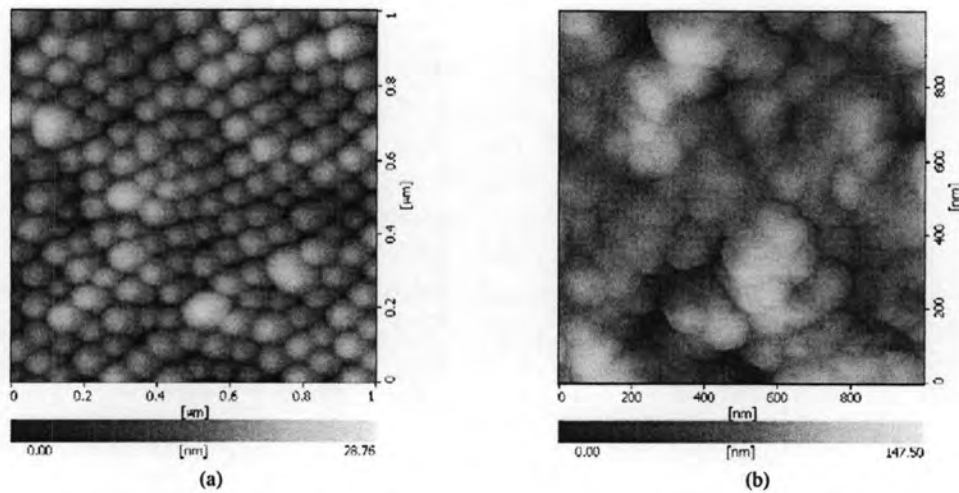


Fig. 2. AFM images of (a) PPCDA vesicles dried on a glass slide and (b) chitosan/PPCDA vesicles PEM film (20 layers).

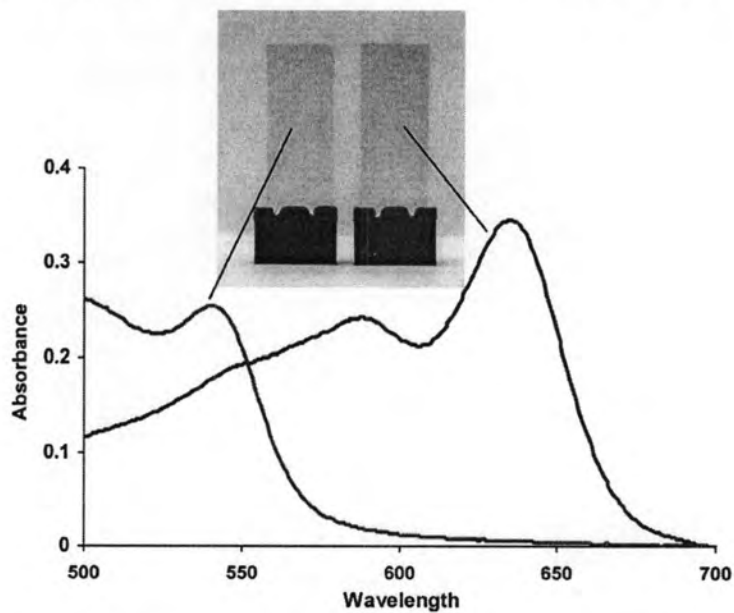


Fig. 3. Absorption spectra and picture of typical blue and red chitosan/PPCDA vesicles PEM films (20 layers) on glass substrates before (blue) and after (red) the films were dipped into 95% ethanol. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

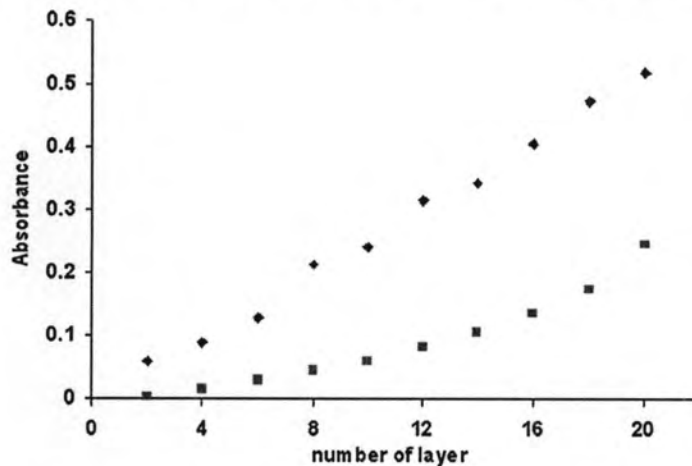


Fig. 4. UV-vis absorbance at 640 nm of PEM films prepared from chitosan/PPCDA vesicles (◆) and PEI/PPCDA vesicles (■). The experiments were carried out at pH 3 and 25 °C.

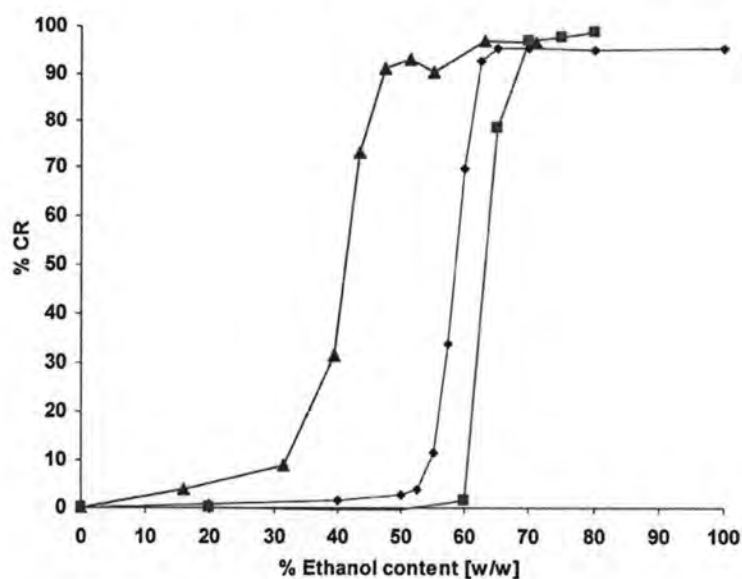


Fig. 5. The colorimetric response (%CR) as a function of EtOH content for PPCDA vesicles dispersed in water (▲), chitosan/PPCDA vesicle PEM film (◆), and PEI/PPCDA vesicles PEM film (■). The experiments were carried out at pH 6 and 25 °C.

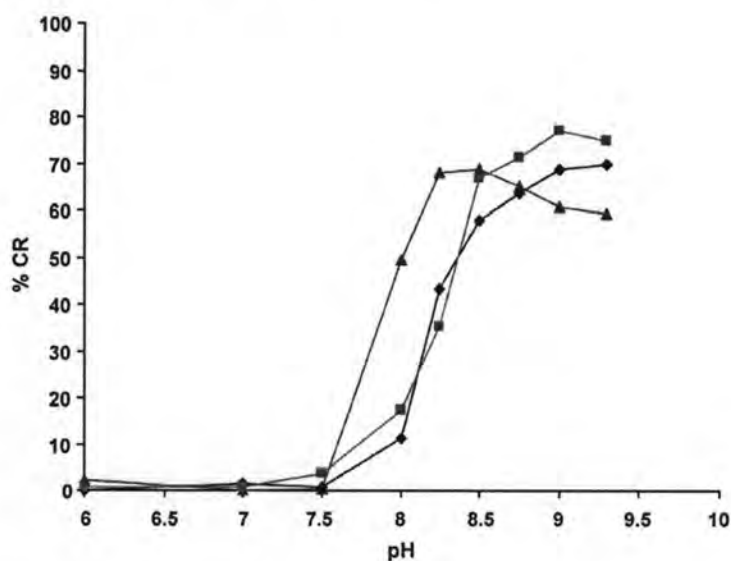


Fig. 6. The change of the colorimetric response (%CR) as a function of pH for PPCDA vesicles dispersed in water (▲), chitosan/PPCDA vesicle PEM film (◆) and PEI/PPCDA vesicles PEM film (■). The experiments were carried out in water at 25 °C.

system. The steeper slope of the films prepared from chitosan compared to those prepared from PEI indicated better thickness growth in each layer of the vesicle deposition. It is also important to note here that great care had to be taken to obtain a smooth blue film in the assembly of the vesicles with PEI. The pH range suitable for the deposition of PEI was narrower ($\text{pH} = 3.0 \pm 0.2$) than that for chitosan ($\text{pH} = 3.5 \pm 0.5$), and if the PEI/vesicle film was allowed to dry during the deposition process, the film would gradually turn red, as has recently been reported [46]. The hydrophilicity and the ability to form hydrogen bonds of the hydroxyl groups in chitosan probably operate cooperatively and favorably with the coulombic interaction to enhance the vesicle deposition and stabilize the vesicles.

For the PEM films containing vesicles to be used as colorimetric sensors, the chromic properties of PPCDA vesicles must be conserved in the films. Solvatochromism, pH sensing, and thermochromism of the films were assessed in comparison with those of vesicles dispersed in water to demonstrate the essential chromic properties of PPCDA vesicles of the PEM films. In a solvatochromism study, exposing the PEM films to the aqueous solution containing increasing percentages of ethanol resulted in an irreversible color switch from blue to red and an increase of %CR. The color transitions for the chitosan/vesicle and PEI/vesicle films were completed when the aqueous solution contained ethanol 62 and 68% (w/w), respectively (Fig. 5). The color change of the PPCDA vesicles dispersed in the aqueous

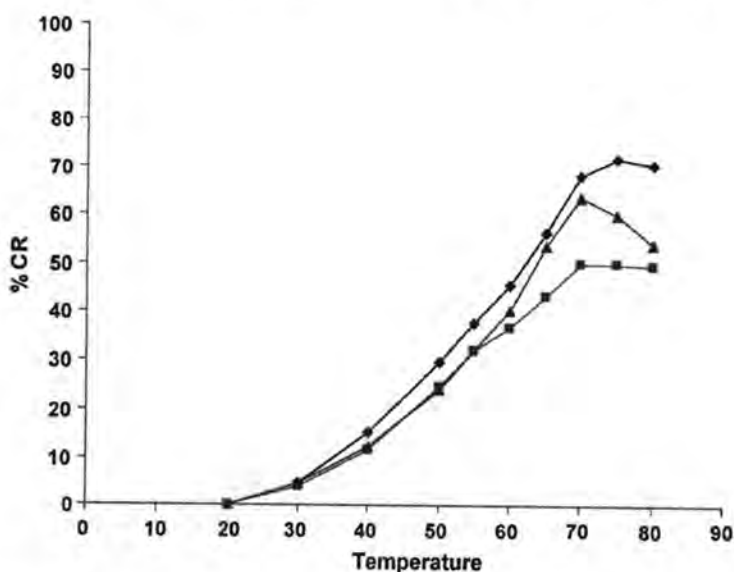


Fig. 7. The change of the colorimetric response (%CR) as a function of temperature for PPCDA vesicles dispersed in water (▲), chitosan/PPCDA vesicle PEM film (◆), and (c) PEI/PPCDA vesicles PEM film (■). The experiments were carried out in water at pH 6.

media was, however, completed at significantly lower ethanol content (47% w/w). As the electrostatic nature of the PEM films tends to repel less hydrophilic solvents, the shift in the colorimetric response of the vesicles embedded in the PEM films compared to the vesicles dispersed in water is not surprising [47–49]. The drastic change in %CR of the PEM films concurs with the sharp color change from blue to red, easily observed by naked eyes.

Another important stimulus known to trigger the color change of the blue phase polydiacetylenes is pH. The response of the PEM films containing PPCDA vesicles to the surrounding pH was evaluated in comparison to that of PPCDA vesicles dispersed in aqueous media. While the color transition of the free vesicles was completed at pH 8.3 (Fig. 6), the chitosan/vesicle and PEI/vesicle PEM films showed slightly higher transition pH (~9.0). This difference probably results from the lower acidity of the PPCDA vesicles in the highly charged environment of the PEM film. Secondary intermolecular interactions or even the molecular weight of the polyelectrolytes could also explain this color shift difference. Further work is being pursued to understand what factors influence the vesicle color switch as a function of the polycations used in the PEM assembly.

Last, the thermochromic properties of the immobilized PPCDA vesicles in the PEM films were studied with a variable-temperature UV–vis spectrometer. The %CR of the PEM films and the vesicles dispersed in aqueous media increased almost linearly with the temperature in the range of 25–70 °C and the color transition was completed around 68 °C (Fig. 7). Notably, the %CR of the vesicles dispersed in water dropped after the color transition was completed. The decrease in %CR was observed along with a decrease of both blue and red absorbances, suggesting aggregation and precipitation of the vesicles at high temperature. At high temperature, the PEI/vesicle film showed also significantly lower %CR than that of chitosan/vesicle films

corresponding to the opaque film observed in the PEI/vesicle film. These results suggest another favorable feature of chitosan/vesicle PEM films.

PPCDA vesicles dispersed in water generally aggregate and precipitate within a month. This short shelf life is one of the major obstacles to the use of the vesicles in real applications. However, the blue-phase PEM films prepared in this study can be kept in water for over a year without significant change in their appearance. This greater storage stability of the films certainly increases the practical value of the vesicles in real applications as sensing devices.

4. Summary

Layer-by-layer deposition was an effective method for immobilizing polydiacetylene vesicles into thin films with preserved colorimetric sensing properties such as solvatochromism, pH sensing, and thermochromism. The color intensity of the films can be controlled through the number of vesicle layers deposited by this technique. With chitosan, PEM films containing polydiacetylene vesicles can be conveniently prepared. The vesicles embedded in the films have excellent storage stability, as no significant change in their appearance and chromic properties occurred over a year. The films thus have potential applications as Yes/No logic labels to indicate whether sensitive products such as fresh food, medicine, or beverages have been exposed to undesirable conditions during their storage.

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Supplementary material

The online version of this article contains additional supplementary material.

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References

- [1] D. Bloor, R.R. Chance, *Polydiacetylenes: Synthesis, Structure, and Electronic Properties*, NATO ASI Series, Kluwer Academic Publishers, USA, 1985.
- [2] B. Tieke, G. Lieser, G.J. Wagner, *Polym. Sci. A* 17 (1979) 1631.
- [3] J. Olmsted, M.J. Strand, *Phys. Chem.* 87 (1983) 4790.
- [4] R.W. Carpick, D.Y. Sasaki, A.R. Burns, *Langmuir* 16 (2000) 1270.
- [5] R.R. Chance, R.H. Baughman, H. Muller, C.J.J. Eckhardt, *Chem. Phys.* 67 (1977) 3616.
- [6] M. Wenzel, G.H. Atkinson, *J. Am. Chem. Soc.* 111 (1989) 6123.
- [7] G.H. Beckham, M.F. Rubner, *Macromolecules* 26 (1993) 5198.
- [8] A. Lio, A. Reichert, D.J. Ahn, J.O. Nagy, M. Salmeron, D.H. Charych, *Langmuir* 13 (1997) 6524.
- [9] D.C. Lee, S.K. Sahoo, A.L. Cholli, D.J. Sandman, *Macromolecules* 35 (2002) 4347.
- [10] H. Muller, C.J. Eckhardt, *Mol. Cryst. Liq. Cryst.* 45 (1978) 313.
- [11] Y. Tomioka, N. Tanaka, S. Imazeki, *J. Chem. Phys.* 91 (1989) 5694.
- [12] R.A. Nallicheri, M.F. Rubner, *Macromolecules* 24 (1991) 517.
- [13] Q. Cheng, R.C. Stevens, *Langmuir* 14 (1998) 5594.
- [14] U. Jonas, K. Shah, S. Norvez, D.H. Charych, *J. Am. Chem. Soc.* 121 (1999) 4580.
- [15] D.H. Charych, J.O. Nagy, W. Spevak, M.D. Bednarski, *Science* 261 (1993) 585.
- [16] A. Reichert, J.O. Nagy, W. Spevak, D. Charych, *J. Am. Chem. Soc.* 117 (1995) 829.
- [17] D. Charych, *Chem. Biol.* 3 (1996) 113.
- [18] D.H. Charych, U. Jonas, U.S. Patent 6 306 598 (2001).
- [19] Y. Jo, T. Inoue, K. Takada, U.S. Patent 6 277 652 (2001).
- [20] S. Hankin, D.J. Sandman, E.A. Yost, T.J. Stark, *Synth. Met.* 49 (1993) 281.
- [21] W.H. Kim, B. Bihari, R. Moody, N.B. Kodali, J. Kumar, S.K. Tripathy, *Macromolecules* 27 (1994) 1819.
- [22] M. Sukwattanasinitt, D.C. Lee, M. Kim, X. Wang, L.L. Li, K. Yang, J. Kumar, S.K. Tipathy, D.J. Sandman, *Macromolecules* 32 (1999) 7361.
- [23] W. Spevak, J.O. Nagy, D.H. Charych, *Adv. Mater.* 7 (1995) 79.
- [24] H.O. Ribi, U.S. Patent 6 607 744 B1 (2003).
- [25] H.O. Ribi, U.S. Patent 6 787 108 (2004).
- [26] H.O. Ribi, U.S. Patent 6 866 863 B2 (2005).
- [27] D.S. Hays, U.S. Patent Application 20 050 101 794 A1 (2005).
- [28] D.Y. Sasaki, R.W. Carpick, A.R. Burns, *J. Colloid Interface Sci.* 229 (2000) 490.
- [29] H.H. Hub, B. Hupfer, H. Koch, H. Ringdorf, *Angew. Chem. Int. Ed. Engl.* 19 (1980) 938.
- [30] A. Akimoto, K. Dorn, L. Gros, H. Ringdorf, H. Schupp, *Angew. Chem. Int. Ed. Engl.* 20 (1981) 90.
- [31] B. Hupfer, H. Ringsdorf, H. Schupp, *Makromol. Chem.* 182 (1981) 247.
- [32] S. Okada, S. Peng, W. Spevak, D. Charych, *Acc. Chem. Res.* 31 (1998) 229.
- [33] F. Gaboriaud, R. Golan, R. Volinsky, A. Berman, R. Jelinek, *Langmuir* 17 (2001) 3651.
- [34] D. Ahn, E. Chae, G. Lee, H. Shim, T. Chang, K. Ahn, J. Kim, *J. Am. Chem. Soc.* 125 (2003) 8976.
- [35] G. Decher, B. Lehr, K. Lowack, Y. Lvov, Schmitt, *J. Biosens. Bioelectron.* 9 (1994) 677.
- [36] Y. Lvov, K. Ariga, T. Kunitake, *J. Am. Chem. Soc.* 117 (1995) 6117.
- [37] G. Decher, *Science* 277 (1997) 1232.
- [38] J. Anzai, Y. Kobayashi, N. Nakanura, M. Nishimura, T. Hoshi, *Langmuir* 15 (1999) 221.
- [39] K. Emoto, M. Iijima, Y. Nagasaki, K. Kataoka, *J. Am. Chem. Soc.* 122 (2000) 2653.
- [40] J.D. Mendelsohn, C.J. Barrett, V.V. Chan, A.J. Pal, A.M. Mayes, M.F. Rubner, *Langmuir* 16 (2000) 5017.
- [41] C.A. Constantine, K.M. Gattus-Asfura, S.V. Mello, G. Crespo, V. Rastogi, T.C. Cheng, J.J. DeFrank, R.M. Leblanc, *Langmuir* 19 (2003) 9863.
- [42] F. Saremi, B. Tieke, G. Jordan, W. Rammensee, *Supramol. Sci.* 4 (1997) 471.
- [43] K. Katagiri, R. Hamasaki, K. Ariga, J. Kikuchi, *J. Am. Chem. Soc.* 124 (2002) 7892.
- [44] M. Michael, D. Vautier, J.C. Voegel, P. Schaaf, V. Ball, *Langmuir* 20 (2004) 4835.
- [45] M. Michael, A. Izquierdo, G. Decher, J.C. Voegel, P. Schaaf, V. Ball, *Langmuir* 21 (2005) 7854.
- [46] Y. Su, *J. Colloid Interface Sci.* 292 (2005) 271.
- [47] A. Toutianoush, L. Krasemann, B. Tieke, *Colloids Surf. A* 98 (2002) 881.
- [48] L. Krasemann, A. Toutianoush, B. Tieke, *J. Membr. Sci.* 181 (2001) 221.
- [49] S.T. Dubas, C. Iamsamai, P. Potiyaraj, *Sens. Actuators B Chem.* 113 (2006) 370.

Electronic Absorption Spectroscopy Probed Side-Chain Movement in Chromic Transitions of Polydiacetylene Vesicles

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Thermochromism, solvatochromism, and alkalinochromism of a poly-10,12-pentacosadiynoic acid (poly(PCDA)) vesicle solution are studied by electronic absorption spectroscopy. The spectroscopic profiles reveal different sequences of side-chain movement during the chromic transitions. The gradual hypsochromic shift and reversibility of the purple solution at low temperature in the thermochromic transition indicates that the transition starts with reversible conformational alteration of methylene side chains leading to metastable purple vesicles. Further heating to 80 °C or higher eventually causes the hydrogen bonds at the carboxylic head groups to break and turns the vesicle solution to red. The irreversibility of the red vesicles indicates that it is the most thermodynamically stable form. In the ethanolochromism and alkalinochromism, the processes are however induced at the vesicle–media interface, directly bringing about the hydrogen bond breaking. The purple solutions observed in the ethanolochromism and alkalinochromism cannot reverse back to the blue one. The absorption spectra clearly demonstrate that they are mixtures of the blue and red vesicles.

Polydiacetylene is a class of conjugated polymers derived from a 1,4-addition polymerization of the corresponding diacetylene monomer. Owing to their unique color change between blue and red forms, various polydiacetylenes have been developed as colorimetric sensing materials for chemical and biological systems. Recently, poly-10,12-pentacosadiynoic acid, or poly(PCDA) for short, has attracted much attention because it can be prepared in a vesicle form homogeneously dispersed in water that is suitable for biological sensing applications.^{1–10}

Under an external stimulation, for example, adding an organic solvent, increasing pH, and raising temperature, poly(PCDA) undergoes a drastic color transition from blue to red irreversibly. Optical absorption of blue and red forms of poly(PCDA) is a consequence of a π – π^* transition of electrons within the linear π -conjugated polymer backbone. The absorption maxima of blue and red forms of poly(PCDA) vesicle solutions are generally observed near 635 and 540 nm, respectively.¹¹ Although it is widely believed that these transitions, so-called solvatochromism,¹² alkalinochromism,^{13–15} and thermochrom-

ism,^{16–21} are related to the conformational change of the polymeric side chain, their mechanisms have not yet been clearly justified. Understanding the detailed mechanisms of these transitions will advance us into logical design of suitable sensing materials for different applications.

To gain insight into the mechanism of these transitions, we carefully prepared poly(PCDA) vesicles with a reproducible size distribution (Figure 1) and examined the change of their electronic absorption spectra in the course of solvatochromic, alkalinochromic, and thermochromic transitions. The color transitions induced by these three types of simulations appeared very similarly as the color change from blue to purple and to red. The spectroscopic profiles of these transitions were however interestingly different. To our surprise, the difference in these spectroscopic data has not yet been properly addressed in the literature despite the long-known chromism properties of the polydiacetylenes.

In the solvatochromism study, the gradual addition of absolute ethanol into an aqueous solution of blue poly(PCDA) vesicles (0.1 mM lipid) caused the absorbance at 635 nm to decrease while the absorbance at 540 nm increased (Figure 2a). In a 45–50% (v/v) ethanol solution, the vesicle appeared as a purple color. The vesicle solution turned completely red when the medium contained more than 55% (v/v) of ethanol. In the case of alkalinochromism, a careful titration of the poly(PCDA) vesicle solution with NaOH (1 M) solution showed a very similar change

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(1) Charych, D.; Nagy, J. O.; Spevak, M. D.; Bednarski, M. D. *Science* **1993**, *261*, 585–588.

(2) Reichert, A.; Nagy, J. O.; Spevak, W.; Charych, D. *J. Am. Chem. Soc.* **1995**, *117*, 829–830.

(3) Charych, D.; Cheng, Q.; Reichert, A.; Kuziemko, G.; Stroh, M.; Nagy, J. O.; Spevak, W.; Stevens, R. C. *Chem. Biol.* **1996**, *3*, 113–120.

(4) Pan, J. J.; Charych, D. *Langmuir* **1997**, *13*, 1365–1367.

(5) Kolusheva, S.; Shahal, T.; Jelinek, R. *J. Am. Chem. Soc.* **2000**, *122*, 776–780.

(6) Kolusheva, S.; Kafri, R.; Katz, M.; Jelinek, R. *J. Am. Chem. Soc.* **2001**, *123*, 417–422.

(7) Katz, M.; Tsubery, H.; Kolusheva, S.; Shames, A.; Fridkin, M.; Jelinek, R. *Biochem. J.* **2003**, *375*, 405–413.

(8) Rangin, M.; Basu, A. *J. Am. Chem. Soc.* **2004**, *126*, 5038–5039.

(9) Guo, C. X.; Boullanger, P.; Liu, T.; Jiang, L. *J. Phys. Chem. B* **2005**, *109*, 18765–18771.

(10) Potisatityuenyong, A.; Tumcharern, G.; Dubas, S. T.; Sukwattanasinitt, M. *J. Colloid Interface Sci.* **2006**, *304*, 45–51.

(11) Okada, S.; Peng, S.; Spevak, W.; Charych, D. *Acc. Chem. Res.* **1998**, *31*, 229–239.

(12) Su, Y. L.; Li, J. R.; Jiang, L. *Colloid Surf. B* **2004**, *11*, 2202–2206.

(13) Kew, S. J.; Hall, E. A. *Anal. Chem.* **2006**, *78*, 2231–2238.

(14) Cheng, Q.; Yamamoto, M.; Stevens, R. C. *Langmuir* **2000**, *16*, 5333–5342.

(15) Reppy, M. A.; Pindzola, B. A. *Chem. Commun.* **2007**, *42*, 4317–4338.

(16) Deckert, A. A.; Fallon, L.; Kierman, L.; Cashin, C.; Perrone, A.; Encalade, T. *Langmuir* **1994**, *10*, 1948–1954.

(17) Deckert, A. A.; Home, J. C.; Valentine, B.; Kernan, L.; Fallon, L. *Langmuir* **1995**, *11*, 643–649.

(18) Carpick, R. W.; Mayer, T. M.; Sasaki, D. Y.; Burns, A. R. *Langmuir* **2000**, *16*, 4639–4647.

(19) Kim, J. M.; Lee, J. S.; Choi, H.; Solin, D.; Ahn, D. J. *Macromolecules* **2005**, *38*, 9366–9376.

(20) Dautel, O. J.; Robitzer, M.; Re-Porte, J. L.; Serein-Spirau, F. O.; Moreau, J. L. *J. Am. Chem. Soc.* **2006**, *128*, 16213–16223.

(21) Schott, M. *J. Phys. Chem. B* **2006**, *110*, 15864–15868.

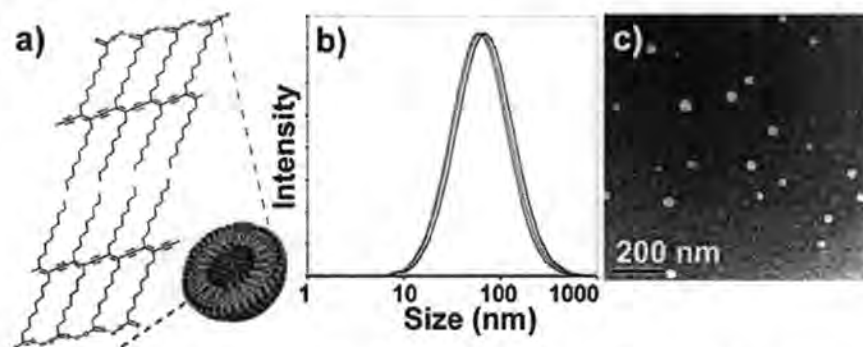


Figure 1. (a) Chemical structure of poly(PCDA) vesicles, (b) dynamic light scattering spectra of three batches of the vesicles, and (c) transmission electron microscopic image.

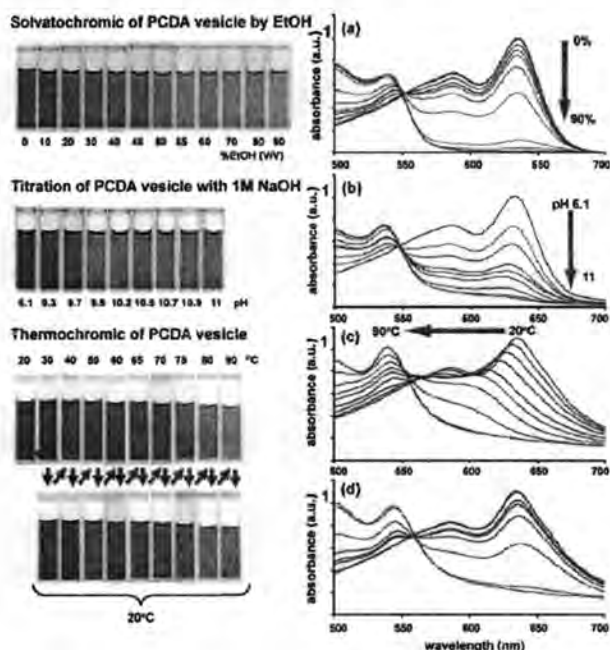


Figure 2. Absorption spectra of a poly(PCDA) vesicle aqueous solution (a) in the presence of various concentrations of ethanol, (b) during titration with NaOH with (c) heating and (d) cooling to 20 °C.

of the absorption spectra, the increase of the absorbance at 540 nm at the expense of the absorbance at 635 nm (Figure 2b). The vesicle solution was purple when the pH was 9.7–10.5 and became red at pHs higher than 10.7. A single isobestic point at 550 nm was observed during both solvatochromic and alkalinochromic transitions, implying quantitative conversion between these two forms, blue and red, of the vesicles.

Thermochromic transition was studied by gentle heating of the poly(PCDA) vesicle solution from 20 to 90 °C. The absorption spectrum was recorded 10 min after the temperature had reached the specified temperature. Unlike in the solvatochromism and alkalinochromism, the thermochromic transition showed hypsochromic shift of the λ_{max} (Figure 2c). For example, at 60 °C, the vesicle solution adopted a purple color with the maximum absorption at 612 nm. At 80 °C or higher, the color of the vesicle solution completely changed to red with the absorption maximum at 540 nm. The hypsochromic shift in the thermochromism is most likely associated with the microscopic movement of the methylene side chain to relieve part of the strain present in the ene-yne conjugated backbone, resulting in valence band lowering and $\pi-\pi^*$ gap widening.^{22–26} This process is however driven mainly by the entropic gain rather than the enthalpic gain from

the strain relief as the transitions from the blue to purple colors were essentially reversible. Evidently, the hypsochromic shift of the absorption spectra, recorded during the heating and cooling cycles, returned to almost its original λ_{max} by cooling the solution to 20 °C after being heated up to 65 °C (Figure 2d). The purple vesicles observed in the thermochromism are thus a series of thermodynamically unstable transition forms. At 80 °C or higher, the thermal energy is not only enough to disturb the hydrophobic interaction but also enough to break the hydrogen bonding between the carboxylic head groups (Figure 4), relieving all of the backbone strain and turning all of the vesicles into the red form. The relief of backbone strain was evidenced by the shift of the peaks in the Raman spectra to lower energy upon the blue to red transition, corresponding to the increase of the bond length. The double and the triple bond-stretching peaks shifted from 1475 and 2103 to 1510 and 2129 cm^{-1} , respectively (Figure 3). The red vesicles appeared to lack the reversibility as there was no recovery of the absorption at 635 nm observed in the spectra after heating the vesicle solution to 80 °C or higher (Figure 2d), indicating that the red form is a thermodynamically stable form. In the red form, not only the entropic gain but also significant enthalpy gain through the backbone strain relieving also contribute to overcoming the enthalpy lost due to hydrogen bond breaking. Upon cooling, new sets of inter- and intrachain hydrogen bonds are formed without reinstating the original backbone strain to foster the stabilization of the red form (Figure 4). It is of significance to note that the absorption spectra between 65 and 75 °C contain a little band at 540 nm, indicating that some of the vesicles already turned into the red form at these temperatures. The vesicles with smaller sizes were noted to have slightly higher sensitivity to stimulants.⁹ The size of the vesicles prepared in this work were Gaussian-like-distributed with an average diameter and polydispersity of 60 nm and 0.47, respectively (Figure 1b). It is thus likely that the smaller vesicles turned red at the temperature lower than 80 °C. It is thus important to control the size distribution of polydiacetylene vesicles to ensure reproducible results when applying them in sensing applications, and sharper responses should be obtained with vesicles having a narrower size distribution.

Although the purple color was also observed in the solvatochromism and alkalinochromism, the gradual shift of λ_{max} to a shorter wavelength was not. There was also no reversibility of

(22) Rubner, M. F.; Sandman, D. J.; Velazquez, C. *Macromolecules* **1987**, *20*, 1296–1300.

(23) Dobrosavljevic, V.; Stratt, R. M. *Phys. Rev. B* **1987**, *35*, 2781–2794.

(24) Carpick, R. W.; Sasaki, D. Y.; Marcus, M. S.; Eriksson, M. A.; Burns, A. R. *J. Phys.: Condens. Matter* **2004**, *16*, 679–697.

(25) Lee, D.; Sahoo, S. K.; Chollí, A. L.; Sandman, D. J. *Macromolecules* **2002**, *35*, 4347–4355.

(26) Wang, X.; Whitten, J. E.; Sandman, D. J. *J. Chem. Phys.* **2007**, *126*, 184905/1–184905/5.

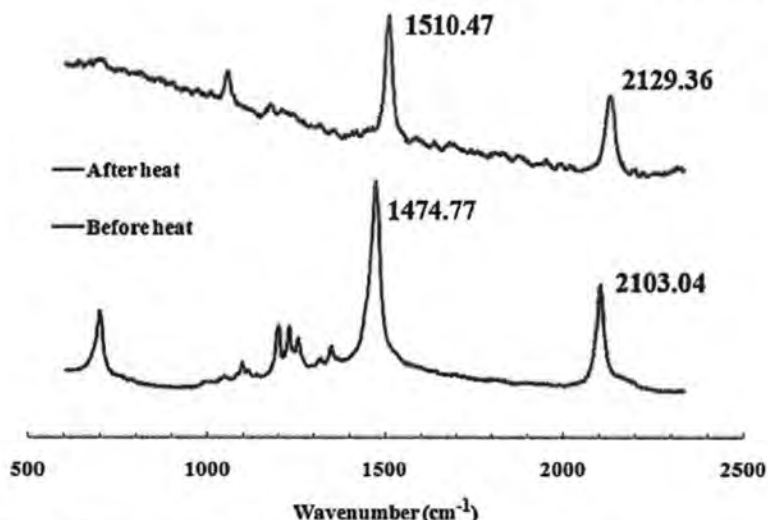


Figure 3. FT-Raman spectra of poly(PCDA) vesicles before and after being heated to 90 °C.

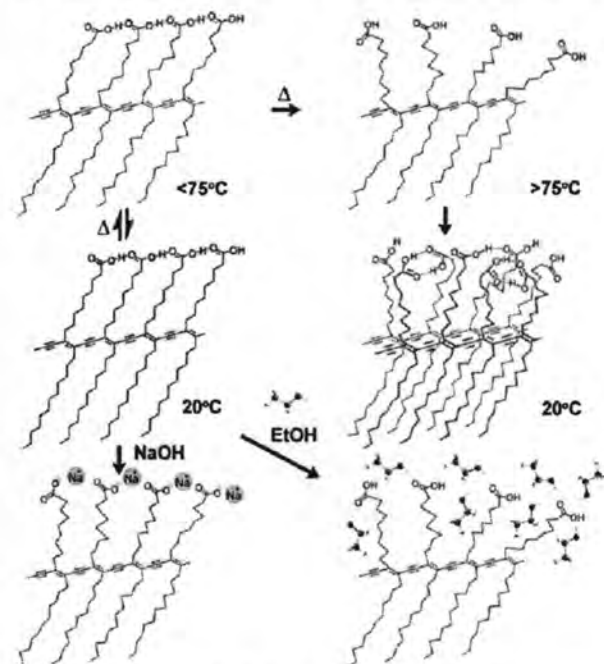


Figure 4. Proposed side-chain movement in the chromic transitions of poly(PCDA) vesicles.

the purple vesicles back to blue in the solvatochromism and alalinochromism. The purple colors observed in the solvatochromism and alalinochromism are thus actually the combination of the blue and red vesicles. As the vesicles interact with the solvent molecules or hydroxide ions at their interface, it is most likely that the molecular process in solvatochromism and alalinochromism started immediately by the hydrogen bond

breaking leading to the utmost side-chain disarray and relief of backbone strain (Figure 4). In the biochromism of polydiacetylene reported in a number of literature studies,^{1–3,5,27,28} the transition of the absorption spectra are very similar to those observed in solvatochromism and alalinochromism. It is thus reasonable to propose that the mechanism for biochromism initiated by the interface interaction resembles the solvatochromism and alalinochromism more than the thermochromism.

In conclusion, we have demonstrated that the electronic absorption spectroscopy can be used to probe the sequence of side-chain movement leading to the chromic transitions of poly(PCDA) vesicles. The thermochromic transition starts with gradual conformational alteration of the methylene chains, which turn the blue vesicles into the thermally unstable and reversible purple form. Excess heating results in hydrogen bond breaking at the carboxylic head group, turning the vesicles into the stable and irreversible red form. On the other hand, the solvatochromic and alalinochromic transitions begin with the interface interaction leading directly to hydrogen bond disruption and the irreversible red vesicles. The techniques and interpretation presented in this work should also be general for other types of chromism of various forms of polydiacetylene assemblies.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at <http://pubs.acs.org>. LA800354Q

(27) Ma, G.; Cheng, Q. *Langmuir* **2005**, *21*, 6123–6126.

(28) Wang, C.; Ma, Z.; Su, Z. *Sens. Actuators, B* **2006**, *113*, 510–515.

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