REFERENCES

Alving, C.R. and Matyas, G.R. (2005) The grand challenge for the future. In S.H.E. Kaufmann, and P.H. Lambert (Eds.), Design and selection of vaccine adjuvants: principles and practice (pp. 99-118). Marryland: Birkhäuser Basel.

Alpar, H.O., Somavarapu, S., Atuah, K.N., and Bramwell, V.W. (2005) Biodegradable mucoadhesive particulates for nasal and pulmonary antigen and DNA delivery. Advanced Drug Delivery Reviews, 57, 411-430.

Basomba, A., Tabar, A.I., Rojas, D.H., Garcia, B.E., Alalmar, R., Olaguibel, J.M., Prado, J.M., Martin, S., and Rico, P. (2002) Allergen vaccination with a liposome-encapsulated extract of dermatophagoides pteronyssinus: a randomized, double-blind, placebo-controlled trial in asthmatic patients. Allergy Clinical Immunology, 109, 943-948.

Butterfield, J.H., Gleich, G.J., Yunginger, J.W., Zimmermann, E.M., and Reed, C.E. (1981) Immunotherapy with short ragweed fraction A:D-glutamic acid: D-lysine polymer in ragweed hay fever. Journal of Allergy and Clinical Immunology, 67, 272-278.

Calvo, P., Remunan-Lo´pez, J.L., and Alonso, V.J. (1997) Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carriers. Journal of Applied Polymer Science, 63, 125-132.

Cao, G. and Liu, D. (2008) Template-based synthesis of nanorod, nanowire, and nanotube arrays. Advances in Colloid and Interface Science, 136, 45-46.

Chen, R., Khormaee, S., Eccleston, M.E., and Slater, N.K.H. (2009) The role of hydrophobic amino acid grafts in the enhancement of membrane-disruptive activity of pH-responsive pseudo-peptides. Biomaterials, 10, 1954-1961.

Chew, J.L., Wolfowicz, C.B., Mao, H.Q., Leong, K.W., and Chua, K.Y. (2003) Chitosan nanoparticles containing plasmid DNA encoding house dust mite allergen, Der p 1 for oral vaccination in mice. Vaccine, 21, 2720-2729.

Corrado, O.J., Pastorello, E., Ollier, S., Cresswell, L., Zanussi, C., Ortolani, C., Incorvaia, A., Fugazza, A., Lovely, J.R., and Harris, R.I. (1989) A double-blind study of hyposensitization with an alginate conjugated extract of

D.pteronyssinus (Conjuvac) in patients with perennial rhinitis: 1. Clinical aspects. Allergy, 44, 108-115.

Davda, J., De, S., Zhou, W., and Labhasetwar, V. (2001) Biodegradable nanospheres: therapeutic applications, Biomedical Polymers and Polymer Therapeutics (pp 19-31). New York: Plenum Publishers.

Fangkangwanwong, J., Akashi, M., Kida, T., and Chirachanchai, S. (2006) One-pot synthesis in aqueous system for water-soluble chitosan-graft-poly(ethylene glycol) methyl ether. Biopolymers, 82, 580-586.

Fangkangwanwong, J., Akashi, M., Kida,T., and Chirachanchai, S. (2006) Chitosan-hydroxybenzotriazole aqueous solution: a novel water-based system for chitosan functionalization. Macromolecular Rapid Communications, 27, 1039-1046.

Ferna´ndez-Urrusuno, R., Calvo, R.P., Remunan-Lo´pez, C., Vila-Jato, J.L., and Alonso, M.J. (1999) Enhancement of nasal absorption of insulin using chitosan nanoparticles. Pharmaceutical Research, 16, 1576-1581.

Gao, Y., Zhang, Z., Chen, L., Gu, W., and Li, Y. (2009) Chitosan N-betainates/DNA self-assembly nanoparticles for gene delivery: in vitro uptake and transfection efficiency. International Journal of Pharmaceutics, online publishing.

Gref, R., Minamitake, Y., Peracchia, M.T., Trebetskoy, V.S., Torchilin V.P., and Langer, R. (1994) Biodegradable long-circulating polymeric nanospheres. Science, 263, 1600-1603.

Hoffmann, C.T., Jakob T., and Behrendt, H. (2009) Determinants of allergenicity. Journal of Allergy and Clinical Immunology, 123, 558-566.

Huggins, J.L. and Looney, R.J. (2004) Allergen immunotherapy. American Family Physician, 70, 689-696.

Hui, C.Y. and Min, H.H. (2009) The effect of the molecular weight of chitosan nanoparticles and its application on drug delivery. Microchemical Journal, 92, 87-91.

Ishii, H., Minegishi, M., Lavitpichayawong, B., and Mitani, T. (1994) Synthesis of chitosan-amino acid conjugates and their use in heavy metal uptake. Macromolecules, 17, 21-23.

Kersten, G.F. and Crommelin, D.J. (1995) Liposomes and ISCOMS as vaccine formulations. Biochemical and Biophysical Research Communications, 1241, 117-138.

Kim, H., Gihm, S.H., Park, C.R., Lee, K.Y., Kim, T.W., Kwon, I.C., Chung, H., and Jeong, S.Y. (2001) Structural characteristics of size-controlled self-aggregates of deoxycholic acid-modified chitosan and their application as a DNA delivery carrier carrier. Bioconjugate Chemistry, 12, 932-938.

Kroll, A., Pillukat, M.H., and Hahn, D. (2009) Current in vitro methods in nanoparticle risk assessment: limitations and challenges. European Journal of Pharmaceutics and Biopharmaceutics, 72, 370–377.

Kumar, M., Long, X., Behera, A.K., Hellermann, G.R., Lockey, R.F., and Mohapatra, S.S. (2003) Chitosan IFN-gamma-pDNA nanoparticle (CIN) therapy for allergic asthma. Genetic Vaccines, 1, 3.

Lee, K.Y., Jo, W.H., Kwon, I.C., Kim Y.H., and Jeong, S.Y. (1998) Structural determination and interior polarity of self-aggregates prepared from deoxycholic acid-modified chitosan in water. Macromolecules, 31, 378-383.

Mallapragada, S.K. and Narasimhan, B. (2008) Immunomodulatory biomaterials. International Journal of Pharmaceutics, 364, 265–271.

Nagamoto, T., Hattori, Y., Takayama, K., and Maitani, Y. (2004) Novel chitosan particles and chitosan-coated emulsions inducing immune response via intranasal vaccine delivery. Pharmaceutical Research, 21, 671-674.

Nam, H.Y., Kwon, S.M., Chung, H., Lee, S.Y., Kwon, S.H., Jeon, H., Kim, Y., Park, J.H., Kim, J., Her, S., Oh, Y.K., Kwon, I.C., Kim, K., and Jeong, S.Y. (2009) Cellular uptake mechanism and intracellular fate of hydrophobically modified glycol chitosan nanoparticles. Journal of Controlled Release, 135, 259-267.

Ortolani, C., Pastorello, E.A., Incorvaia, C., Ispana, M., Farioli, L., Zara, C., Prevattoni V., and Zanussi, C. (1994) A double-blind, placebo-controlled study of immunotherapy with an alginate conjugated extract of parietaria judaica in patients with parietaria hay fever. European Journal of Allergy and Clinical Immunology, 49, 13-21.

Park, J.S., Han, T.H., Lee, K.Y., Han, S.S., Hwang, J.J., Moon, D.H., Kim, S.Y., and Cho, Y.W. (2006) N-acetyl histidine-conjugated glycol chitosan self-assembled nanoparticles for intracytoplasmic delivery of drugs: endocytosis, exocytosis and drug release. Journal of Controlled Release, 115, 37-45.

Phongying, S., Aiba, S.I., and Chirachanchai, S. (2007) Direct chitosan nanoscaffold formation via chitin whiskers. Polymer, 48, 393-400.

Prashanth, K.V.H. and Tharanathan, R.N. (2007) Chitin/chitosan: modifications and their unlimited application potential-an overview. Trends in Food Science & Technology, 18, 117-131.

Rolland, J.M., Gardner, L.M., and O'Hehir, R.E. (2009) Allergen-related approaches to immunotherapy. Pharmacology & Therapeutics, 121, 273-284.

Ruszczak, Z. and Friess, W. (2003) Collagen as a carrier for on-site delivery of antibacterial drugs. Advanced Drug Delivery Reviews. 55, 1679-1698.

Sano, A., Maeda, M., Nagahara, S., Ochiya, T., Honma, K., Itoh, H., Miyata, T., and Fujioka, K. (2003) Atelocollagen for protein and gene delivery. Advanced Drug Delivery Reviews, 55, 1651-1677.

Scholl, I., Nitulescu, G.B., and Jarolim, E.J. (2005) Review of novel particulate antigen delivery systems with focus on treatment of type I allergy. Journal of Controlled Release, 104, 1-27.

Seferian, P.G. and Martinez, M.L. (2001) Immune stimulating activity of two new chitosan containing adjuvant formulations. Vaccine, 19, 661-668.

Sheng, Y., Liu, C., Yuan, Y., Tao, X., Yang, F., Shan, X., Zhou, H., and Xu, F. (2009) Long-circulating polymeric nanoparticles bearing a combinatorial coating of PEG and water-soluble chitosan. Biomaterials, 30, 2340-2348.

Singh, R. and Jr, J.W.L. (2009) Nanoparticle-based targeted drug delivery. Experimental and Molecular Pathology, 86, 215–223.

Suri, S. "ABC's of allergies. CSA discovery guides." CSA. August 2006. 14 March 2009 <http://www.csa.com/discoveryguides/allergy/reviewF.php>

Sutane, R. 14 March 2009 <http://www.nhrbc.org/HIV_vaccine/paper16.2.html>

Szebeni, J. (2001) Complement activation-related psudoallergy caused by liposomes, micellar carriers of intravenous drugs, and radiocontrast agents. Critical Reviews, 18, 567-606.

Vilaa, A., Sa´ncheza, A., Janesa, K., Behrensb, I., and Kisselb, T. (2004) Low molecular weight chitosan nanoparticles as new carriers for nasal vaccine delivery in mice. European Journal of Pharmaceutics and Biopharmaceutics, 57, 123–131.

Wing, F.L. and Marie Chia, M.L. (2009) Nucleic acid delivery with chitosan and its derivatives. Journal of Controlled Release, 134, 158-168.

Wiesman, Z., Dom, N.B., Sharvit, E., Grinberg, S., Linder, C. Heldman, E., and Zacca, M. (2007) Novel cationic vesicle platform derived from vernonia oil for efficient delivery of DNA through plant cuticle membranes. Journal of Biotechnology, 130, 85-94.

Xie, Y., Gong, Y.F., Zhou, N.J., Chen, J., Zhou, X.J., Lu, N.H., and Wang, C.W. (2005) The role of local immune response in gastric mucosa in the protection induced by Helicobacter pylori vaccine with chitosan as adjuvant. Zhonghua Yi Xue Za Zhi, 85, 2629-2635.

Yi, S.S., Noh, J.M., and Lee, Y.S. (2009) Amino acid modified chitosan beads: improved polymer supports for immobilization of lipase from Candida rugosa. Journal of Molecular Catalysis B: Enzymatic, 57, 123-129.

Ying, W.C., Tzu, H.W., and Wen, C.L. (2009) Chitosan coating for the protection if amino acids that entrapped within hydrogenated fat. Food Hydrocolloids, 23, 1057-1061.

Yoksan, R. and Akashi, M. (2009) Low molecular weight chitosan-g-L-phenylalanine: preparation, characterization, and complex formation with DNA. Carbohydrate Polymers, 75, 95–103.

Yoksan, R., Akashi, M., Hiwatari, K.I., and Chirachanchai, S. (2003). Controlled hydrophobic/hydrophilicity of chitosan for spheres without specific processing technique. Biopolymers, 69, 386-390.

Yoksan, R. and Chirachanchai, S. (2008) Amphiphilic chitosan nanosphere: studies on formation, toxicity, and guest molecule incorporation. Bioorganic & Medicinal Chemistry, 16, 2687-2696.

APPENDICES

Appendix A Calculation of Molecular Weight (M_W)

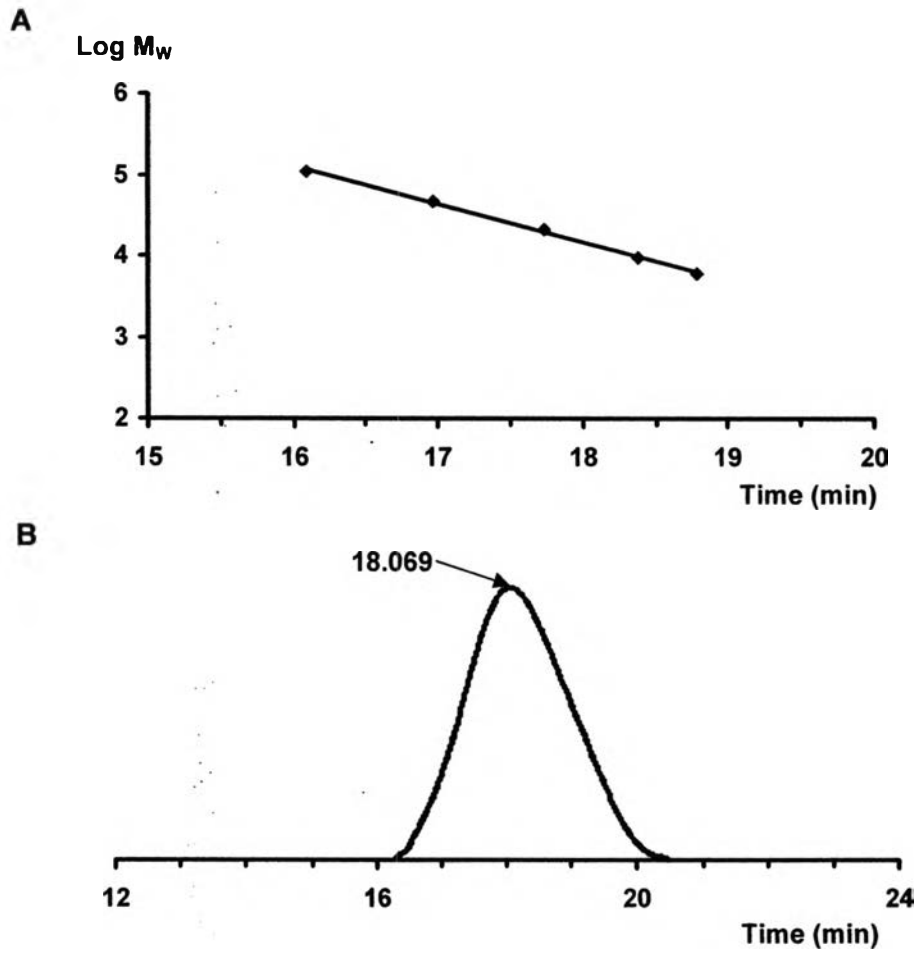

Figure A. (A) Pullulan standard curve by GPC technique (column: TSK $GMPW_{XL}$ 7.8 mm × 30.0 cm combined with TSK $GMPW_{XL}$ 7.8 mm × 30.0 cm, Eluent: $0.5MCH_3COOH/0.5MCH_3COONa$, Temperature column: 30°C) Log M_W = -0.4696x + 12.618, R^2 = 0.996; and (B) retention time of low molecular weight chitosan (**LCS**) by GPC technique.

From Figure A:	M_w of LCS ~ 13000 Da.
From GPC Software for CLASS-VP:	M_w of LCS ~ 15000 Da.
	M_n of LCS ~ 8000 Da.
	PDI ~ 1.9

Appendix B Calculation of Degree of Substitution (DS)

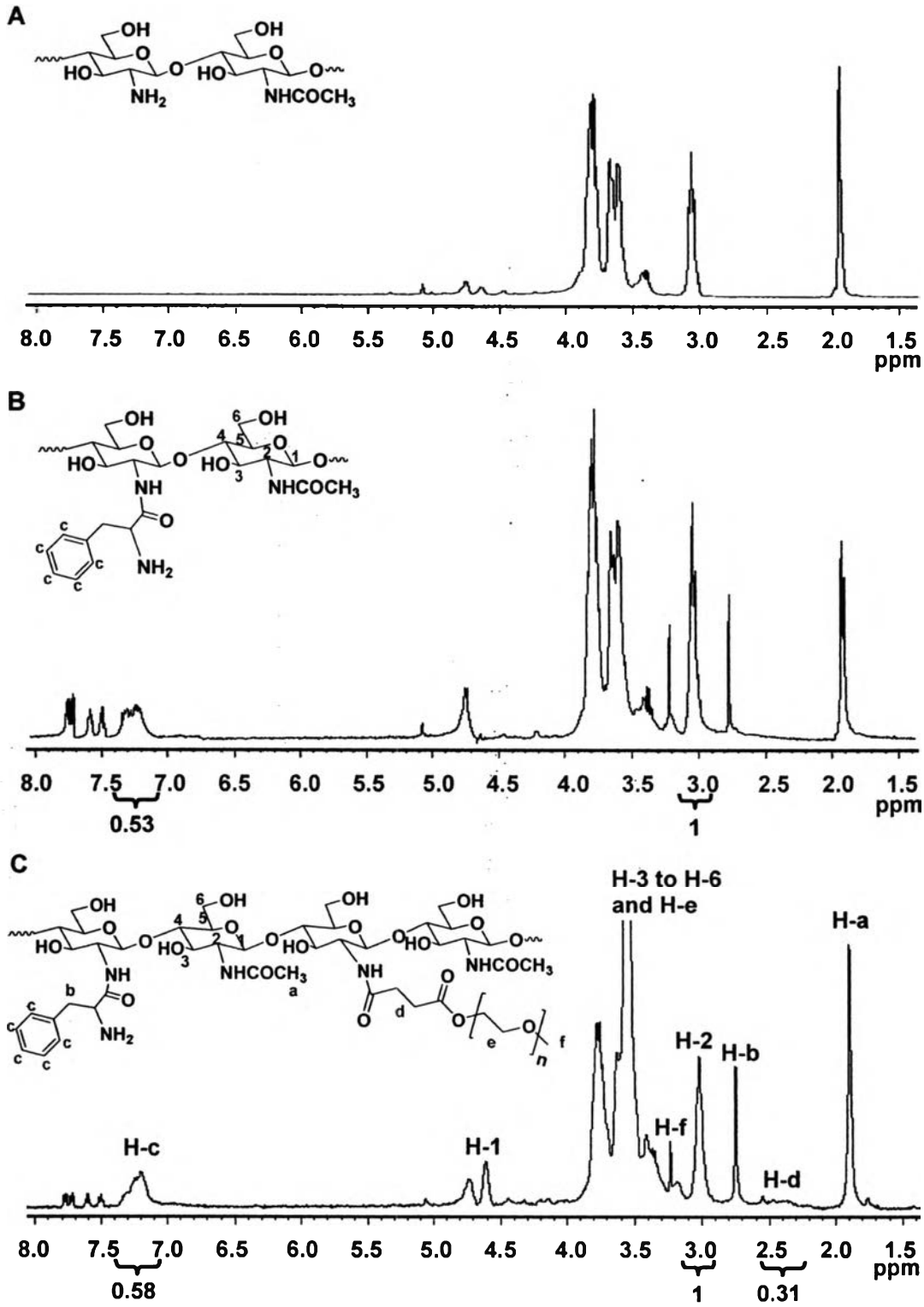

Figure B. ^{1}H NMR spectra of (A) **LCS**; (B) **LCS-Phe**; and (C) **LCS-Phe-mPEG** in 2% CD_3COOD/D_2O.

From Figure B (B): % DS of Phe $= \frac{\int(H\text{-}c)/5}{\int(H\text{-}2)} \times 100$

$= \frac{0.53/5}{1} \times 100$

$= 10.6$

From Figure B (C): % DS of Phe $= \frac{\int(H\text{-}c)/5}{\int(H\text{-}2)} \times 100$

$= \frac{0.58/5}{1} \times 100$

$= 11.6$

% DS of mPEG-COOH $= \frac{\int(H\text{-}d)/4}{\int(H\text{-}2)} \times 100$

$= \frac{0.31/4}{1} \times 100$

$= 7.5$

Appendix C Size and Morphology

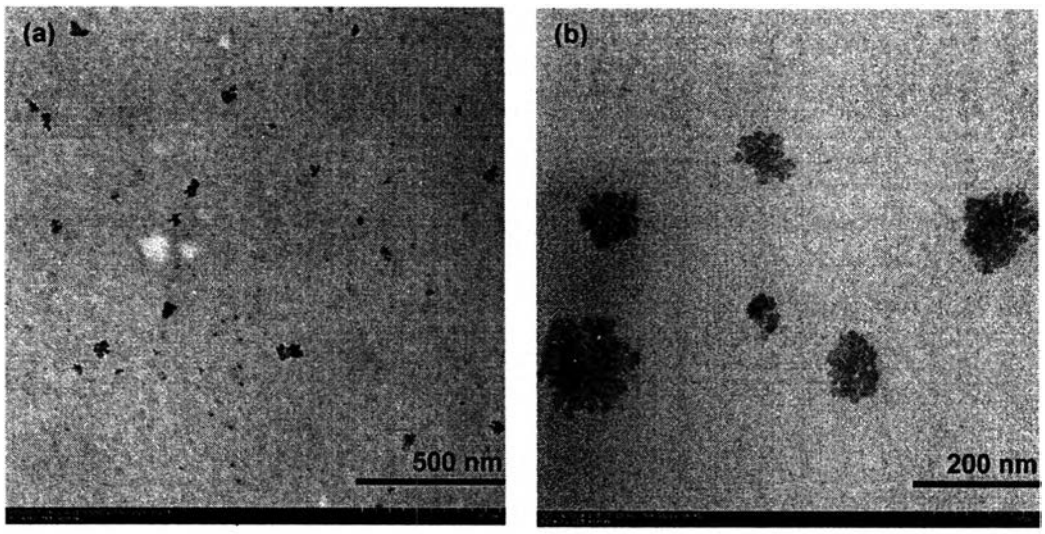

Figure C1. TEM micrographs of **2** at (a) 10,000× magnification, and (b) 20,000× magnification.

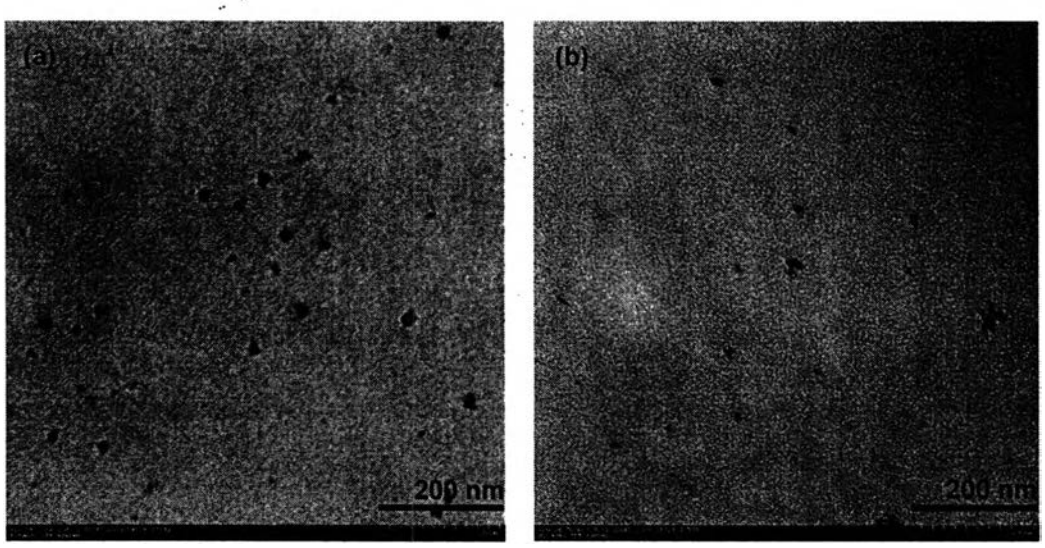

Figure C2. TEM micrographs of **LCS-Phe-mPEG** having molar ratio of LCS:Phe:mPEG (a) 1:0.5:0.1, and (b) 1:1.5:0.1 (20,000× magnification).

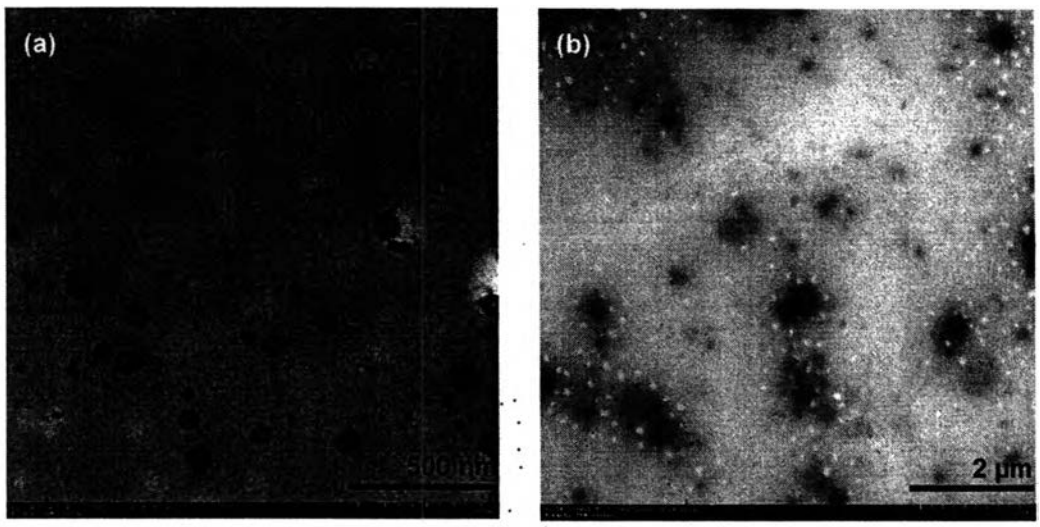

Figure C3. TEM micrographs of **LCS-Phe-mPEG** having molar ratio of LCS:Phe:mPEG (a) 1:2.0:0.1 (10,000× magnification), and (b) 1:2.5:0.1 (2,000× magnification).

Appendix D pH and Nanosphere Size

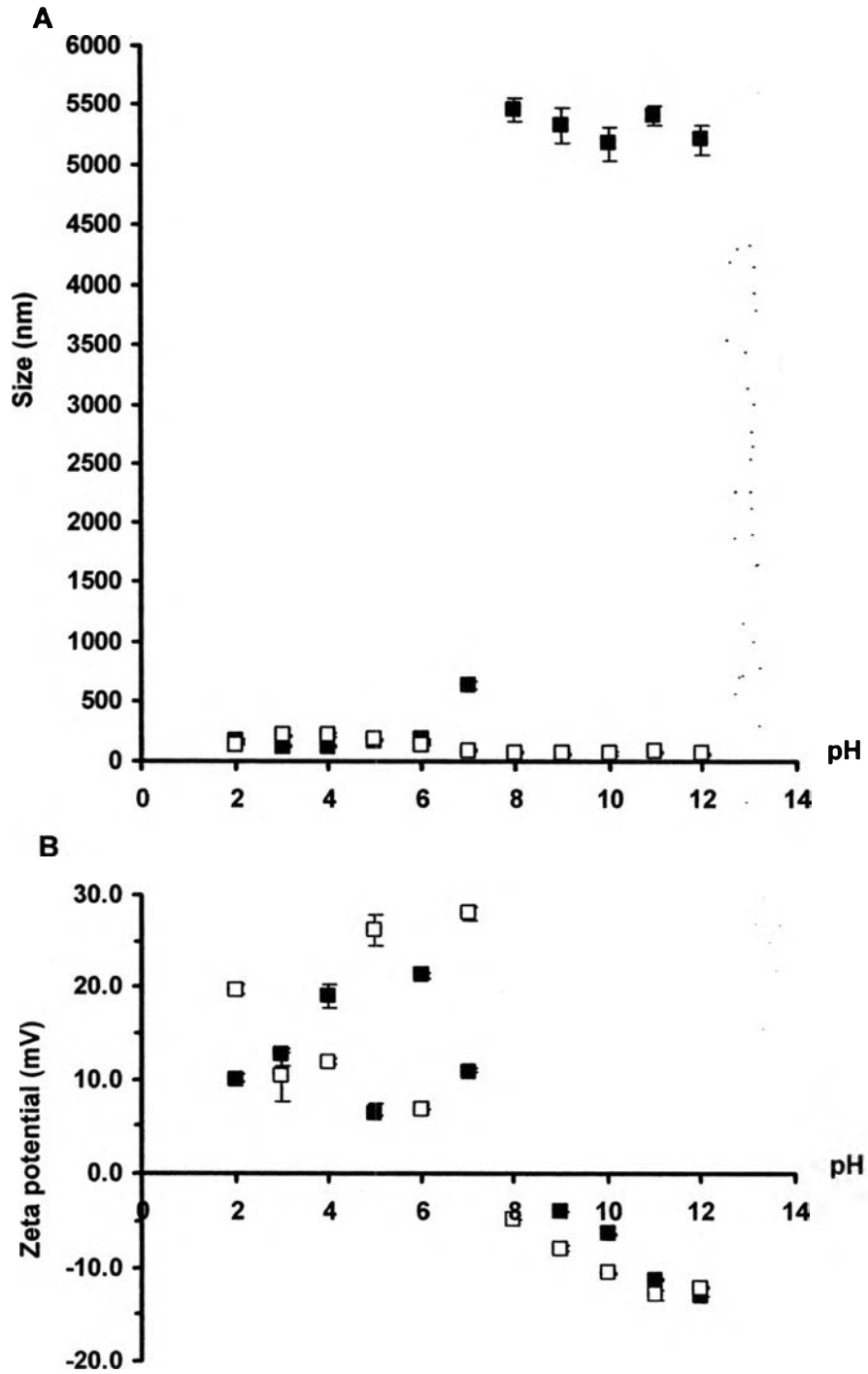

Figure D. (A) Size; and (B) zeta potential of **LCS** (■), and **2** (□) dispersed in 2-12 pH solution (1.5 mg/ml). Results are means ± SD (n=3).

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