

REFERENCES

- Akiyoshi, K. and Sunmoto, J. (1996) Supramolecular assembly of hydrophobized Polysaccharides. Supramolecular Science, 3, 157-163.
- Berry, C. and Curtis, A.S.G. (2003) Functionalization of magnetic nanoparticles for applications in biomedicine. Journal of Physics D: Applied Physics, 36, R198-R206.
- Borchard, G. and Junginger, H.E. (2001) Modern drug delivery applications of chitosan. Advanced Drug Delivery Reviews, 52(2), 103.
- Canalle, L.A., van Berkel, S.S., de Haan, L.T. and van Hest, J.C.M. (2009) Copper-free clickable coatings. Advanced Functional Materials, 19, 3464-3470.
- Charles, S.W. Magnetic fluids (ferrofluids). In: Dormann, JL., Fiorani, D., editors. (1992) Magnetic properties of fine particles. North Holland: Elsevier, 267-374.
- Denizot, B., Tanguy, G., Hindre, F., Rump, E., Jeune, J. and Jallet, P. (1999) Phosphorycholine coating of iron oxide nanoparticles. Journal of Colloid and Interface Science, 209(1), 66-71.
- Dirks, A.J., Cornelissen, J.J.L.M., van Delft, F.L., van Hest, J.C.M., Nolte, R.J.M., Rowan, A.E. and Ruje, F.P.J.T. (2007) "Click" chemistry in (Bio) materials Science. QSAR & Combinatorial Sciecnce, 26, 1200.
- Domszy, J.G. and Roberts, G.A.F. (1985) Evaluation of infrared spectroscopic techniques for analyzing chitosan. Macromolecule Chemistry, 186:1671-1677.
- Dresco, P.A., Zaitsev, V.S., Gambino, R.J. and Chu, B. (1999) Preparation and properties of magnetite and polymer magnetite nanoparticles. Langmuir, 15, 1945.
- Fournier, D., Hoogenboom, R. and Schubert, U.S. (2007) Clicking polymers: a straightforward approach to novel molecular architectures. Chemical Society Reviews, 36, 1369.
- Gupta, A.K. and Gupta, M. (2005) Synthesis and surface engineering of iron oxidenanoparticles for biomedical applications. Biomaterials, 26, 3995-4021.
- Gupta, A.K. and Wells, S. (2004) Surface modified superparamagnetic nanoparticles

- for drug delivery: preparation, characterization and cytotoxicity studies. IEEE Trans Nanobiosci, 3(1), 66-73.
- Gupta, A.K. and Curtis, A.S.G. (2004) Lactoferrin and ceruloplasmin derivatized superparamagnetic iron oxide nanoparticles for targeting cell surface receptors. Biomaterials, 25(15), 3029-3040.
- Hildebrandt, B., Wust, P., Ahlers, O., Dieing, A., Sreenivasa, G., Kerner, T., Felix, R. and Riess, H. (2002) The cellular and molecular basis of hyperthermia. Critical Review Oncology/ Hematol, 43, 33–56.
- Himo, F., Lovell, T., Hilgraf, R., Rostovtsev, V.V., Noddleman, L., Sharpless, K.B. and Fokin, V.V. (2005) Copper(I)-catalyzed synthesis of azoles. DFT Study Predicts Unprecedented Reactivity and Intermediates. Journal of American Society, 127, 210-216.
- Jeong, Y., Nah, J-W., Na, K., Cho, C.S. and Kim, S.H. (1999) Self assembling nanospheres of hydrophobized pullulans in water. Drug Development and Industry Pharmacy, 917-927.
- Karlsen, J. and Skaugrud, O. (1991) Excipient properties of chitosan. Manufacturers Chemicals, 62, 18-19.
- Kawai, N., Futakuchi, M., Yoshida, T., Ito, A., Sato, S., Taku, N., Honda, H., Shirai, T. and Kohri, K. (2008) Effect of heat therapy using magnetic nanoparticles conjugated with cationic liposomes on prostate tumor in bone. Prostate, 68, 784-792.
- Kim, E.H., Ahn, Y. and Lee, H.S. (2007) Biomedical applications of superparamagnetic iron oxide nanoparticles encapsulated within chitosan. Journal of Alloys and Compounds, 43(4), 633-636.
- Kolb, H. C., Finn, M. G. and Sharpless, K. B. (2001) Click chemistry: diverse chemical function from a few good reactions. Angewandte Chemie International Edition, 40 (11), 2004–2021.
- Krasinski, A., Fokin, V.V. and Sharpless, K.B. (2004) Direct synthesis of 1,5-disubstituted-4-magnesio-1,2,3-triazoles, revisited. Organic Letters, 6,1237-1240.
- Li, J.K., Wang, N. and Wu, X.E. (1997) A novel biodegradable system based on

- gelatin nanoparticles and poly(lactic-co-glycolic acid) microspheres for protein and peptide drug delivery. *Journal of Pharmaceutical Science*, 86(8), 917-927.
- Lutz, J.F. and Börner, H.G. (2008) Modification of polysulfones by click chemistry amphiphilic graft copolymers and their protein adsorption and cell adhesion properties. *Progress in Polymer Science-Elsevier*, 33, 1.
- Lutz, J.F. (2008) Click chemistry beyond metal-catalyzed cycloaddition. *Angewandte Chemie International Edition*, 120, 2212 and 47, 2182.
- Mao, S., Sun, W. and Kissel, T. (2010) Chitosan –based formulations for delivery of DNA and siRNA. *Advanced Drug Delivery Reviews*, 62, 12-27.
- Massart, R. (1981) Preparation of aqueous magnetic liquids in alkaline and acidic Media. *IEEE Transactions on Magnetics*, 17, 2, 1247–1248.
- Massia, S.P., Stark, J. and Letbetter D.S. (2000) Surface immobilized dextran limits cell adhesion and spreading. *Biomaterials*, 21, 2253-2261.
- Mincheva, R., Stoilova, O., Penchev, H., Ruskov, T., Spirov, I., Manolova, N. and Rashkov, I. (2008) Synthesis of polymer-stabilized magnetic nanoparticles and fabrication of nanocomposite fibers thereof using electrospinning. *European Polymer Journal*, 44, 615-627.
- Nishimura, S., Kohgo, O., Kurita, K. and Kuzuhara, H. (1991) Chemospecific manipulations of a rigid polysaccharide: syntheses of novel chitosan derivatives with excellent solubility in common organic solvents by regioselective chemical modifications. *Macromolecules*, 24(17), 4745-4748.
- Olsvik, O., Popovic, T., Skjerve, E., Cudjoe, K.S., Hornes, E., Ugelstad, J. and Uhlen, M. (1994) Magnetic separation techniques in diagnostic microbiology. *Clinical Microbiology Reviews*, 7, 43-54.
- Opsteen, J.A.,and van Hest, J.C.M. (2007) Applications of polymer bioconjugates. *Macromolecular Engineering*, 4, 2645-2687.
- Pankhurst, Q.A., Connolly, J., Jones, S.K. and Dobson, J. (2003) Applications of magnetic nanoparticles in biomedicine. *Journal of Physics D: Applied Physics*, 36, R167.
- Powell, R. and Gannon, F. (2002) Purification of DNA by phenol extraction and

- ethanol precipitation. Oxford University Press, 1-2.
- Randy, D. P., Sara, P., Margriet, J., Van, B., Heidi, V. den, R., Kristien, B., Wim, L., Jules, M., Gustaaf, B. and Guido, M. (2007) Silane ligand exchange to make hydrophobic superparamagnetic nanoparticles water-dispersible. Chemistry of Materials, 19, 1821-1831.
- Reimers, G.W. and Khalafalla, S.E. (1972) Preparing magnetic fluids by a peptizing method. US Bureau Mines Tech Rep. 59.
- Rostovtsev, V.V., Green, L.G., Fokin, V.V. and Sharpless, K.B. (2002) A stepwise Huisgen cycloaddition process: copper(I)-catalyzed regioselective ligation of azides and terminal alkynes. Angewandte Chemie International Edition, 41, 2596-2599.
- Šafařík, I. and Šafaříková, M. (2004) Magnetic techniques for the isolation and purification of proteins and peptides. BioMagnetic Research and Technology, 2, 7.
- Savant, V. and Torres, J.A. (1995) Two food applications of biopolymers: edible coatings controlling microbial surface spoilage and chitosan use to recover proteins from aqueous processing wastes. American Chitoscience Society, 1, 1-4.
- Schwick, H.G. and Heide, K. (1969) Immunochemical and immunology of collagen and gelatin. Bibliotheca Haematologica, 111-125.
- Sun, J., Zhou, S., Hou, P., Yang, Y., Weng, J., Li, X. and Li, M. (2006) Synthesis and characterization of biocompatible Fe₃O₄ nanoparticles. Journal of Biomedical Materials Research Part A, DOI: 10.1002/jbm.a.30909.
- TornØe, C.W., Christensen, C. and Meldal, M. (2002) Peptidotriazoles on solid phase: [1,2,3-triazoles by regiospecific copper(I)-catalyzed 1,3-dipolar cycloadditions of terminal alkynes to azides. Journal of Organic Chemistry, 67, 3057-3064.
- Vladimir, S., Zaitsev, V., Dmitry, S., filimonov, I., Gambino, R.J., Chu, B. and Presnyakov, A. (1999) Physical and chemical properties of magnetite and magnetite-polymer nanoparticles and their colloidal dispersions. Journal of Colloid and Interface Science, 212, 49.
- Wust, P., Hildebrandt, B., Sreenivasa, G., Rau, B., Gellermann, J., Riess, H., Felix,

- R. and Schlag P.M. (2002) Hyperthermia in combined treatment of cancer. *Lancet Oncology*, 3, 487–497.
- Yoksan, R., Akashi, M., Hiwatari, K-I. and Chirachanchai, S. (2003) Controlled hydrophobic/hydrophilicity of chitosan for sphere without specific processing technique. *Biopolymers*, 69(3), 386-390.
- Zampano, G., Bertoldo, M. and Gardeli, F. (2010) Defined chitosan-based networks by C-6-Azide-alkyne “click” reaction. *Reactive and Functional Polymers*, 70, 272-281.
- Zhang, L., He, R. and Gu, H. (2006) Colloidal dispersions of monodisperse magnetite nanoparticles modified with poly (ethylene glycol). *Applied Surface Science*, 253, 2611-2617.

APPENDICES

Appendix A Calculation of degree of deacetylation of chitosan (DD)

¹H NMR spectroscopy

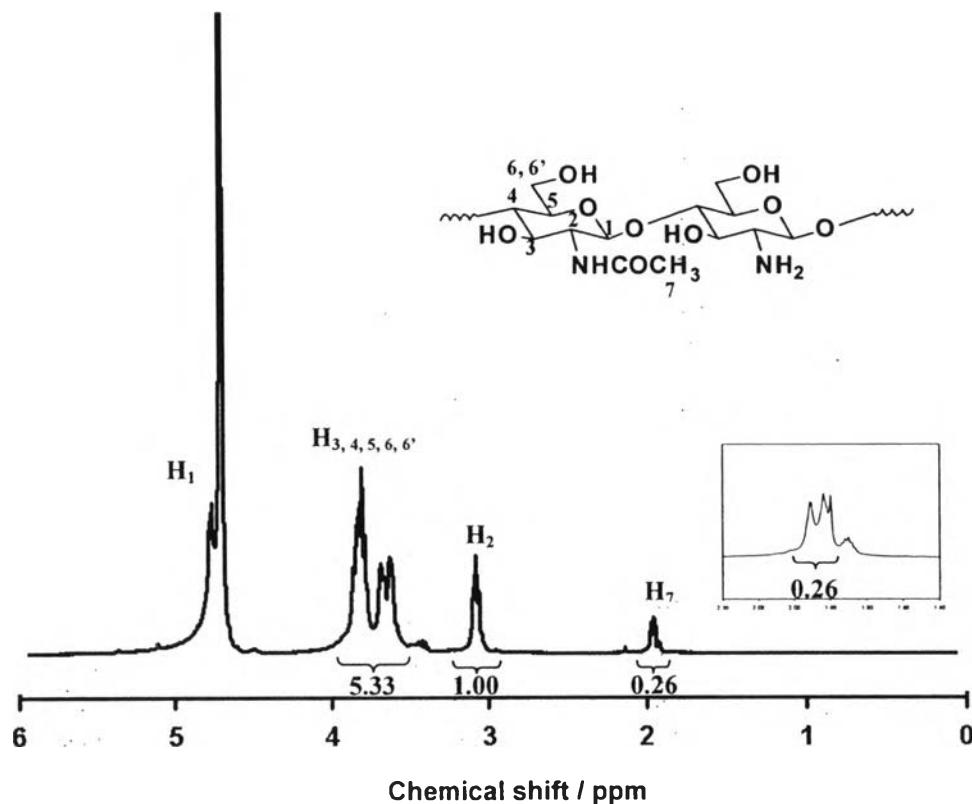


Figure A ¹H NMR spectrum of low molecular weight chitosan (LCS) in 2% $\text{CD}_3\text{COOD}/\text{D}_2\text{O}$.

$$\begin{aligned} \text{From Figure A; } \text{DD} &= 1 - \left\{ \left[\frac{1}{3} I_{\text{H}7} \Big/ \frac{1}{6} I_{\text{H}2-\text{H}6} \right] \right\} \times 100 \\ &= (1 - (0.26/3)/((5.33+1.00)/6)) \times 100 \\ &= 91 \end{aligned}$$

FTIR spectroscopy

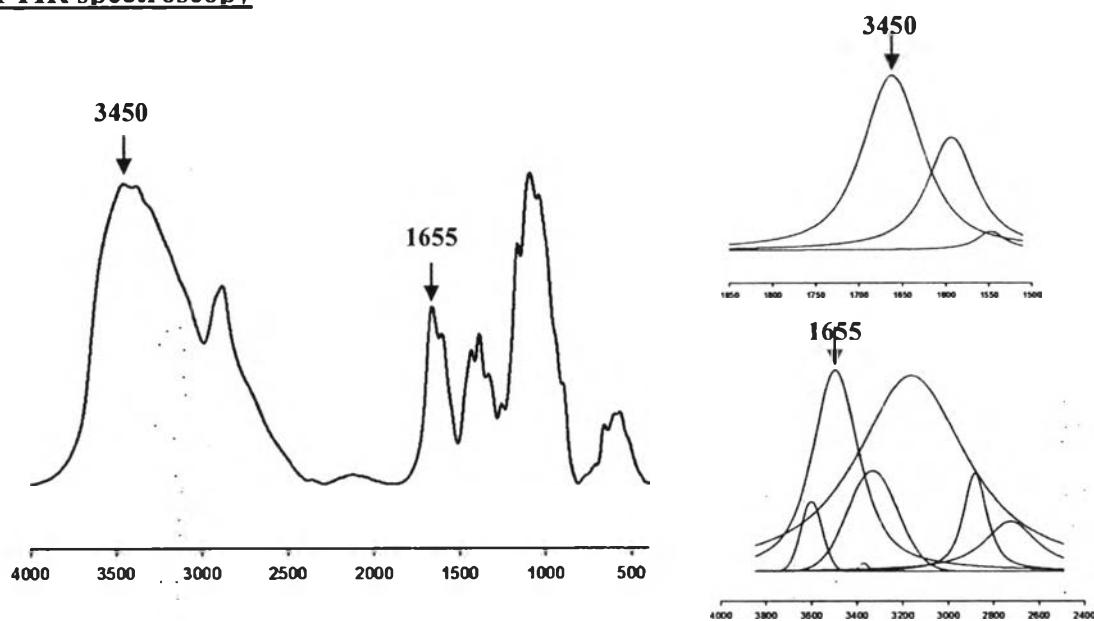


Figure B FTIR spectrum of low molecular weight chitosan (LCS).

From Figure B; DD = 100 - $[(A_{1655} / A_{3450}) \times 100 / 1.33]$

$$= 100 - [(56.12391 / 211.1925) \times 100 / 1.33]$$

$$= 80$$

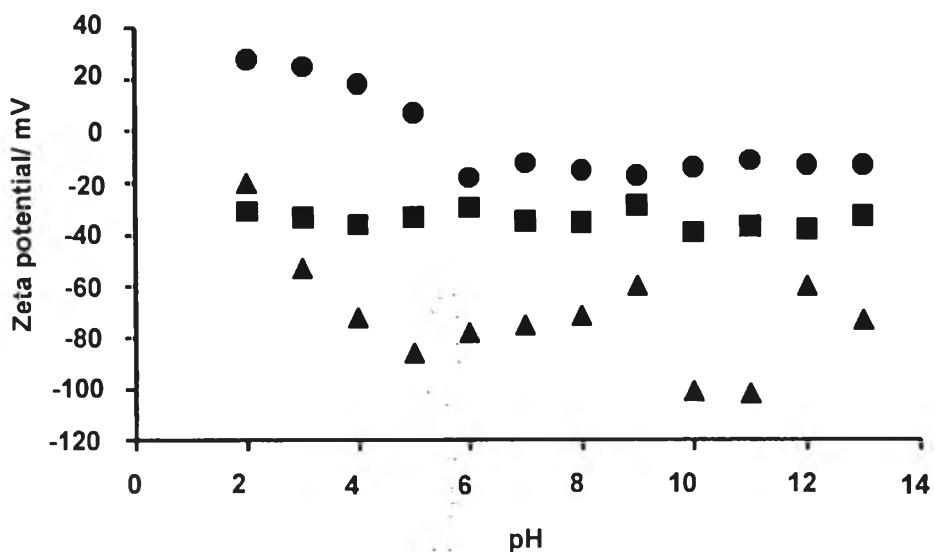
Appendix B Zeta potential of materials

Figure C Zeta potential of chitosan-magnetite nanoparticles via direct conjugation (●), Dynabeads (■), and chitosan-magnetite via 'click' chemistry (▲) in various pH of PBS buffer solution.

Appendix C Cytotoxicity by MTT assay

Cells: Mouse fibroblast L929 passage 21, 10000 cells in 96 well plates

Exposed time: 24 hours

Concentration ($\mu\text{g/ml}$)	% Viability				
	16	8	4	2	1
Cell control	100	100	100	100	100
Uncoated-magnetite particles	2.67	2.83	60.79	91.35	89.94
LCS	3.12	5.4	42.35	54.12	70.14
Azide-magnetite	3.25	3.02	36.87	84.95	85.84
Alkyne-phthaloyl-chitosan	2.89	5.09	37.84	58.63	73.82
Phthaloylchitosan-magnetite particles	2.97	4.6	41.53	49.75	67
Chitosan-magnetite particles	2.84	4.5	36.4	42.57	50.18

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Proceedings:

1. Phuangsawai, O., and Chirachanchai, S. (2011) Synthesis and characterization of chitosan-magnetite nanoparticles via “click” chemistry. Proceedings of The 2nd National Research Symposium on Petroleum, Petrochemicals, and Advanced Materials and The 17th PPC Symposium on Petroleum, Petrochemicals, and Polymers. Bangkok, Thailand.

Presentations:

1. Phuangsawai, O., and Rukachaisirikul, V. Chemical constituents form the leaves of *Tithonia Diversifolia*. (2009, March 20-21). Poster and oral presentations at the 4th Conference on Science and Technology for youths. Bangkok International trade and Exhibition center (BITEC), Bangkok, Thailand.
2. Phuangsawai, O., and Chirachanchai. S. Chitosan-magnetite Nanoparticles via Click Chemistry. (2011, January 10-11). Poster presentation at the 1st International Conference on Big Ideas in Molecular Materials, Singapore, Singapore.
3. Phuangsawai, O., and Chirachanchai, S. Synthesis and Characterization of Chitosan-magnetite Nanoparticles via “Click” chemistry. (2011, April 28). Poster presentation at The 2nd Research Symposium on Petroleum, Petrochemicals, and Advanced Materials and The 17th PPC Symposium on Petroleum, Petrochemicals, and Polymers at Queen Sirikit National Convention Center, Bangkok, Thailand.