References

Alexandrecu, A. T.; Ng, Y.-L.; and Dobson, C. M. Characterization of a trifluoroetahnol-induced partially folded state of α-lactalbumin. J. Mol. Biol. 235 (1994): 587-599.

Allenby, A.C.; Fletcher, J.; Schock, C.; and Tees, T. F. S. The effect of heat, pH and organic solvents on the electrical impedance and permeability of excised human skin Br. J. Derm. 81 supplement 4 (1969): 31-39.

Arshad, S. H. Allergy. Harcourt Brance and Company, China: Churchill Livingstone, 2002

Atkins, D. Allergen spotlight. ARIA 2(1) (2000): 1-2.

Bakuni, V. Alcohol-induced molten globule intermediates of proteins: Are they real folding intermediates or of pathway product? Arch. Bio. Biophys. 357 (1998): 274-284.

Bhattacharjya, S. and Balaram, P. Hexafluoroacetone hydrate as a structure modifier in proteins: Characterization of a molten globule state of hen egg-white lysozyme. Protein Science 6 (1997): 1065-1073.

Brange, J. Physical stability of proteins. In S. Frokjaer and L. Hovagaard (eds), Pharmaceutical formulation development of peptides and proteins, pp.89-112. USA: Taler&Francis Inc., 2000.

Buck, M.; Radford, S. E.; and Dobson, C. M. A partially folded state of hen egg white lysozyme in trifluoroethanol: structural characterization and implications for protein folding. Biochemistry 32 (1993): 669-678.

Bummer, P. M.; and Koppenol, S. Chemical and physical considerations in protein and peptide stability In E. J. McNally (ed), Drugs and pharmaceutical sciences: Protein formulation and delivery V.99, pp5-65. New York: Macel Dekker Inc., 2000.

Bychkova, V. E.; Pain, R. H.; and Ptitsyn, O. B. The 'molten globule' state is involved in the translocation of protein across membranes? FEBS letters 238 (1988): 231-234.

Bychkova, V. E.; Dujsekina, A. E.; Klenin, S. I.; Tiktopulo, E. I.; Uversky, V. N.; and Ptitsyn, O. B. Molten globule-like state of cytochrome c conditions stimulating those near membrane surface. Biochemistry 35 (1996): 6058-6063.

Calandrini, V.; Frioretto, D.; Onori, G.; and Santucci, A. Role of hydrophobic interactions on the stabilization of native state of globular proteins. Chem. Phys. Lett. 324 (2000): 344-348.

Chaffotte, A. F.; Guillou, Y.; and Goldberg, M. E. Kinetic resolution of peptide bond and side chain far-UV circular dichroism during the folding of hen egg white lysozyme. Biochemistry 31 (1992): 9694-9702.

Chen, L.; Hodgson, K.O.; and Doniach, S. A lysozyme folding intermediate revealed by solution X-ray scattering. J. Mol. Biol. 261 (1996): 658-671.

Chittchang, M.; Alur, H. H.; Mitra, A. K.; and Jonhson, T. P. Poly (L-lysine) as model drug macromolecule which to investigate secondary structure and microporous membrane transport, part I: physiochemical and stability studies. J. Pharm. Phrmaco. 54 (2002): 315-323.

Creighton, T. E. Review article protein folding. Biochem. J. 270 (1990): 1-16.

Demoly, P., Michel, F-B, and Bousquet, J. In vivo method for study of allergy skin tests, techniques and interpretation. In E. M. Ton and E. Charles and (eds), Allergy principles and practice, pp 430-439 USA: Mosby-Year book, INC., 1998.

Dick, I. P.; and Scott, R. C. Pig ear skin as an in vitro model for human skin permeability. J. Pharm. Pharmacol. 44 (1992): 640-645.

Dobson, C. M.; Evan, P. A.; and Radford, S. E. Understanding how proteins fold: the lysozyme story so far. TIBS 19 (1994): 31-37.

Dubey, V. K.; and Jagannadham, M. V. Differences in the unfolding of procerain induced by pH, guanidine hydrochloride, urea and temperature. Biochemistry 42 (2003): 12287-12297.

Endo, T.; and Schatz, G. Latent membrane perturbation activity of a mitochondrial precursor protein is exposed by unfolding. EMBO J. 7(4) (1988): 1153-1158.

Fink, A. L.; Calciano, L. J.; Goto, Y.; Kurotsu, T.; and Palleros, D. R. Classification of acid denaturation of proteins: Intermediates and unfolded states. Biochemistry 33 (1994): 12504-12511.

Gladwin, S. T.; and Evan, P. A. Structure of very early protein foliding intermediates: new insight through a variant of hydrogen exchange labeling. Folding and Design 1 (1996): 407-417.

Goda, S.; Takano, K.; Yamagata, Y.; Nagata, R.; Akutsu, H.; Maki, S.; Namba, K.; and Yutani, K. Amyloid protofilament formation of hen egg lysozyme in highly concentrated ethanol solution. Protein Science 9 (2000): 369-375.

Goto, Y.; Calciano, L. J.; and Fink, A. L. Acid-induced folding of proteins. Proc. Natl. Acad. Sci. 87 (1990a): 573-577.

Goto,Y.; Takahashi, N.; and Fink, A. L. Mechanism of acid-induced folding of proteins. Biochemistry 29 (1990b): 3480-3488.

Hadgraft, J. Skin deep (review article). Eur. J. Pharm. Biopharm. 58 (2004): 291-299.

Inamori, T.; Ghanem, A.-H.; Higuchi, W. I.; and Srinivasan, V. Macromolecule transport in and effective pore size of ethanol prenetrated human epidermal membrane. Int. J. Pharm. 105 (1994): 113-123.

Itzhaki, L. S.; Evan,P. A.; Dobson, C. M.; and Radford, S. E. Tertiary interactions in the folding pathway of hen lysozyme: kinetic studies using fluorescent probes. Biochemistry 33 (1994): 5212-5220.

Katayama, K.; Matsui, R.; Hatanaka, T.; and Koizumi, T. Effect of pH on skin permeation enhancement of acidic drugs by 1-menthol-ethanol system Int. J. Pharm. 226 (2001): 69-80.

Katamari, Y. O.; Konno, T.; Kataoka, M.; and Akasaka, K. The methanol-induced transition and the expanded helical conformation in hen lysozyme. Protein Sciences 7 (1998): 681-688.

Kato, S.; Shimamoto, N.; and Utiyama. H. Identification and characterization of the direct folding process of hen egg white lysozyme. Biochemistry. 21 (1982): 38-43.

Kelly, S. M.; and Price, N. C. The application of circular dichroism to studies of protein folding and unfolding (review article). Biochim. Biophys. Acta 1338 (1997): 161-185.

Konermann, L.; and Douglas, D. J. Acid-induced unfolding of cytochrome c at different methanol concentrations: Electrospray ionization mass spectrometry specifically monitors changes in the tertiary structure. Biochemistry 36 (1997): 12296-12302.

Koshiba, T.; Yao, M.; Kobashigawa, Y.; Demura, M.; Nakagawa, A.; Tanaka, I.; Kuwajima, K.; and Nitta, K. Structure and thermodynamics of the extraordinarily stable molten globule state of canine milk lysozyme. Biochemistry 39 (2000): 3248-3257.

Kjellman, N.-I.; and Björkstén, B. Natural history and prevention of food hypersensitivity. In D. D. Metalfe, H. A. Sampson, and R. A. Simon (eds) Food allergy adverse reaction to foods and food additive, pp 445-459. USA: Black well Science., 1997.

Lehmann, M. S.; Mason, S. A.; and McIntyre, G. J. Study of ethanol-lysozyme interaction using neutron diffraction. Biochemistry 24 (1985): 5862-5869.

Leduc, V.; Demeulemester, C.; Polack, B.; Guizard, C.; Le Guern, L.; and Peltre, G. Immunochemical detection of egg-white antigens and allergens in meat products. Allergy 54 (1999): 464-472.

Mattos, C.; and Ringe, D. Protein in organic solvents. Cur. Op. Struct. Biol. 11 (2001): 761-764.

Matulis, D.; Baumann, C. G.; Bloomfield, V. A.; and V. A., Lovrien, R. E. 1-anilino-8-naphthalene sulfonate as a protein conformational tightening agent. Biopolymer 49 (1999): 451-458.

Matulis, D.; and Loverien, R. 1-anilino-8-naphthalene sulfonate anion protein binding depends primarily on ion pair formation. Biophysic. J. 74 (1998): 422-429.

Megrab, N. A.; Williams, A. C.; and Barry, B. W. Oestradiol permeation across human skin, silastic and snake skin membranes: the effects of ethanol/water co-solvent systems. Int. J. Pharm. 116 (1995): 101-112.

Morgan, C. J.; Miranker, A.; and Dobson, C. M. Characterization of collapsed states in the early states of the refolding of hen lysozyme. Biochemistry 37 (1998): 8473-8480.

Moser, K.; Kriwet, K.; Naik, A.; Kalia, Y. N.; and Guy, R. H. Passive skin penetration enhancement and its quantification in vitro. Eur. J. Pharm. Biolpharm 52 (2001): 103-112.

Nargaad, A.; Skov, P. S.; and Bindslev-Jensen, C. Egg and milk allergy in adults: comparison between fresh food and commercial allergen extracts in skin prick test and histamine release from basophils. Clin. Exp. Allergy 22 (1992): 940-947.

Nölting B., Protein folding kinetics. Germanny: Springer-Verlag Berlin Heidelberg, 1999.

Nelson, D. L. and Cox, M. M. The three-dimensional structure of proteins. In Linger principles of biochemistry pp. 159-202, Worth Publisher., 2000.

Oas, T. G.; and Kim, P. S. A peptide model of a protein folding intermediate. Nature 336 (1988): 42-48

Ohgushi, M.; and Wada, A. 'Molten-globule state': a compact form of globular protein with mobile side chains. FEBS letters 164 (1983): 21-24

Pelton, J. T.; and Mclean, L. R. Spectroscopic method for analysis of protein secondary structure. Anal. Biochem. 277 (2000): 167-176.

Peng, Z.; and Kim, P. S. A protein dissection study of a molten globule. Biochemistry 33 (1994): 2136-2141.

Pillai, O.; Nair, V.; Panchagnula, R. Transdermal iontophoresis of insulin: IV. Influence of chemical enhancers. Int. J. Pharm. 269 (2004): 109-120.

Privilov, P. L. Intermediate states in protein folding (review article). J. Mol. Biol. 258 (1996): 707-725.

Ptitsyn, O. B.; Pain, R. H.; Semisotonov, G.V.; Zerovnik, E.; and Razgulyaev, O. I. Evidence for a molten globule state as general intermediate in protein folding. FEBS letters 262 (1990): 20-24.

Ptitsyn, O. B.; and Uversky, V. N. The molten globule is a third thermodynamical state of protein molecules. FEBS letters 342 (1994): 15-18.

Radford, S. E.; Dobson, C. M.; and Evans, P. A. The folding of hen lysozyme involves partially structured intermediates and multiple pathways. Nature 385 (1992): 302-307.

Rothman, J. E.; and Kornberg, R. D. An unfolding story of protein translocation. Nature 322 (1986): 209-210.

Rothwarf, D. M.; and Scheraga, H. A. Role of non-native aromatic and hydrophobic interactions in the folding of hen egg white lysozyme. Biochemistry 35 (1996): 13797-13807.

Rupley, J. A.; Gratton, E.; and Careri, G. Water and globular protein. TIBS. (1983): 18-22.

Sampson, H. A. Adverse reactions to foods. In E. M. Ton and E. Charles and (eds), Allergy principles and practice, pp 1162-1181. USA: Mosby-Year book, INC.1998.

Schmook, F. P.; Meingassner, J. G.; and Billich, A. Comparison of human skin or epidermis models with human and animal skin in in vitro percutaneous absorption. Int. J. Pharm 215 (2001): 51-56.

Semisotnov, G. V.; Rodionova, N. A.; Razgulyaev, O. I.; Uversky, V. N.; Gripas', A. F.; and Gilmashin. Study of the 'molten globule' intermediate state in protein folding by a hydrophobic fluorescent probe. Biopolymer 31 (1991): 119-128.

Sporik, R.; Hill, D. J.; and Hosking, C. S. Specific of allergen skin testing in predicting positive open food challenges to milk, egg and peanut in children. Clin. Exp. Allergy 30 (2000): 1540-1546.

Sreerama, N. and Woody, R. W. Circular dichroism of peptides and proteins. In N. Berova, K. Nakanishi, and R. W. Woody (eds), Circular dichroism: principles and applications, pp 601-620. USA: John Wiley & Sons Inc., 2000

Suhonen, T. M.; Bouwstra, J. A.; and Urtti. A. Chemical enhancement of percutaneous absorption in relation to stratum corneum structural alteration. J. Control. Release. 59 (1999): 149-161.

Tanaka, S.; Oda, Y.; Ataka, M.; Onuma, K.; Fujiwara, S.; and Yonezawa, Y. Denaturation and aggregation of hen egg white lysozyme in aqueous ethanol solution studied by dynamic light scattering. Biopolymers 59 (2001): 370-379.

Thornton, J. M. and Barlow, D. J. Peptide and protein structure. In R. C. Hider and D. J. Barlow (eds), Polypeptide and protein drugs, prediction, characterization and formulation, pp 1-30. London: Ellis Horwood series in pharmaceutical technology, Department of pharmacy King's college, 1991.

Timasheff, S. N. Protein-solvent interactions and protein conformation. Acc. Chem. Res. 3 (1970): 62-68.

Turner, P. C., McLennan, A. G., Bates, A. D. and White, M. R. H. Molecular Biology. UK: School of biological sciences, University of Liverpool, 1998.

Van der Goot, F. G.; González-Mañas, J. M.; Lakey, J. H.; and Pattus, F. A 'molten-globule' membrane insertion intermediate of the pore-forming domain of colicin A. Nature 354 (1991): 408-410.

Van der Goot, F. G.; J., Lakey, J. H.; and Pattus, F. The molten globule intermediate for protein insertion or translocation through membranes. Trends. Cell. Bio. 2 (1992): 343-348.

Watson, J. D.; Baker, T. A.; Bell, S. P.; Gann, A.; Levine, M.; Losick, M.; and Losick, R. Weak and strong bonds determine macromolecular structure. In Molecular Biology of the Gene (fifth edition), pp. 69-92. Benjamin Cammings: Pearson education INC., 2004.

Yuksel, N.; Kanik, A. E.; and Baykara, T. Comparison of in vitro dissolution profile by ANOVA-based, model dependent and -independent methods. Int. J. Pharm 209 (2000): 57-67.

Web sites

Allergen Data Collection: Hen's Egg White (Gallus domesticus)[Online] Available from: http://www.food-allegens.de/[2004, November 12]

Circular Dichroism Spectroscopy[Online] Available from: http://www.isa.au.dk[2005, October 2]

Circular Dichroism[Online] Available from: http://www.ap-lab.com/circulardichroism.htm[2005, October 2]

Food allergies[Online] Available from: www.allergyasthmatherapy.com/Conditions/Food-Allergies.htm[2005, October 15]

Protein Data Bank[Online] Available from: http://www.rcsb.org/pdb/[2006, March 2]

SDS-PAGE analysis of lysozyme[Online] Available from: http://faculty.mansfield.edu/bganong/index.cfm[2004, December 14]

Structural and functional properties of the amino acids[Online] Available from: http://www.fst.rdg.ac.uk/courses/fs916/index.htm[2005, October 21]

APPENDICES

APPENDIX A

Validation of CD technique

Analytical method validation is a process to evaluate the suitable and consistent method for application. The parameters which were determined in this study were precision and linearity.

1. Precision

The precision of this experiment was expressed as the percentages of coefficient of variation (% CV) in Table 8 were 8.48 and 7.54 at 222 and 289 nm, respectively. The low % CV indicated the good precision of this technique.

Table 8 The data precision of lysozyme dissolved in water

No. sample	The CD intensity of lysozyme Molar ellipticity (deg.cm^2/decimol)	
	At 222 nm	At 289 nm
1	-7.31 X 10^3	73.86
2	-7.10 X 10^3	64.85
3	-7.21 X 10^3	66.03
4	-6.45 X 10^3	69.27
5	-5.88 X 10^3	76.70
6	-6.40 X 10^3	77.26
Average	-6.72 X 10^3	71.33
Standard deviation	0.57 X 10^3	5.38
% CV	8.48	7.54

2. Linearity

Figures 55 and 56, shows the linearity curve of concentrations of lysozyme which dissolved in water versus ellipticity (mdeg) at 222 and 289 nm, respectively. The regression coefficients (R^2) for standard curve were 0.9999 and 0.9989 for 222 and 289 nm, respectively. These results illustrate a good linearity.

Table 9 Linearity data of concentrations of lysozyme dissolved in water versus ellipticity (mdeg) at 222 nm

Concentration of lysozyme (mg/ml)	Ellipticity (mdeg)
0.02	1.4668
0.05	3.1387
0.1	6.5777
0.5	33.1316
1	67.5045

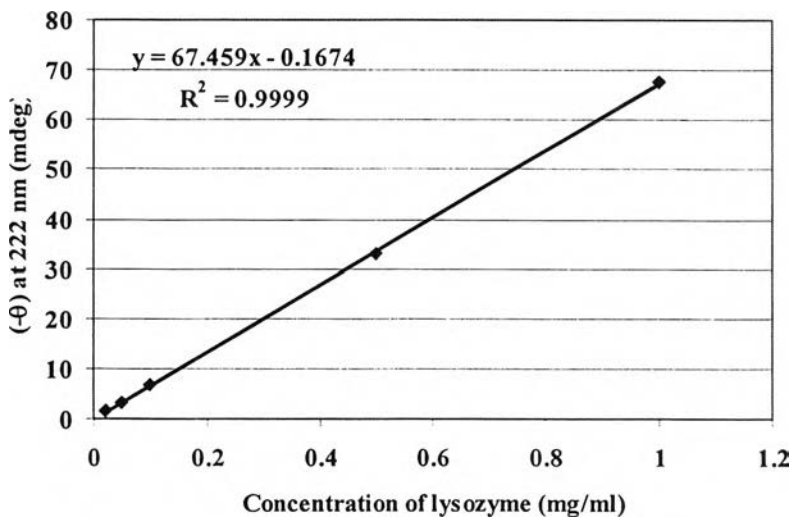


Figure 60 Linearity curve of concentrations of lysozyme dissolved in water versus ellipticity (mdeg) at 222 nm

Table 10 Linearity data of concentrations of lysozyme dissolved in water versus ellipticity (mdeg) at 289 nm

Concentration of lysozyme (mg/ml)	Ellipticity (mdeg)
0.02	0.1331
0.05	0.3292
0.1	0.6620
0.5	3.4664
1	6.5133

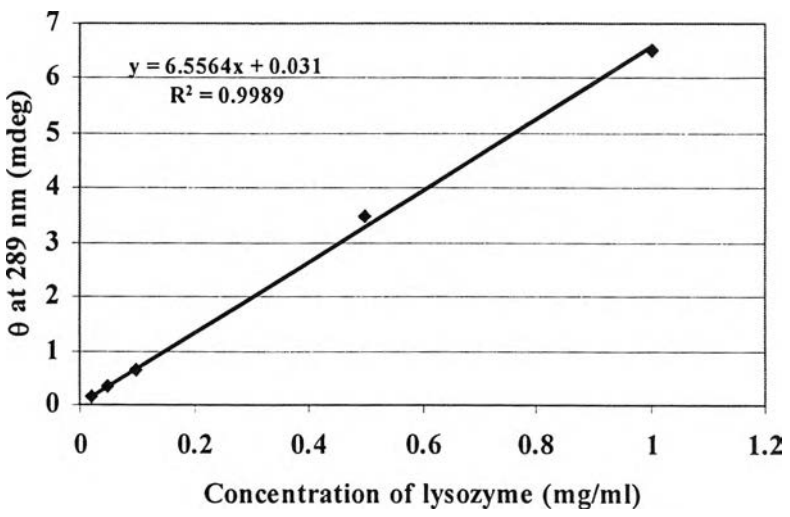
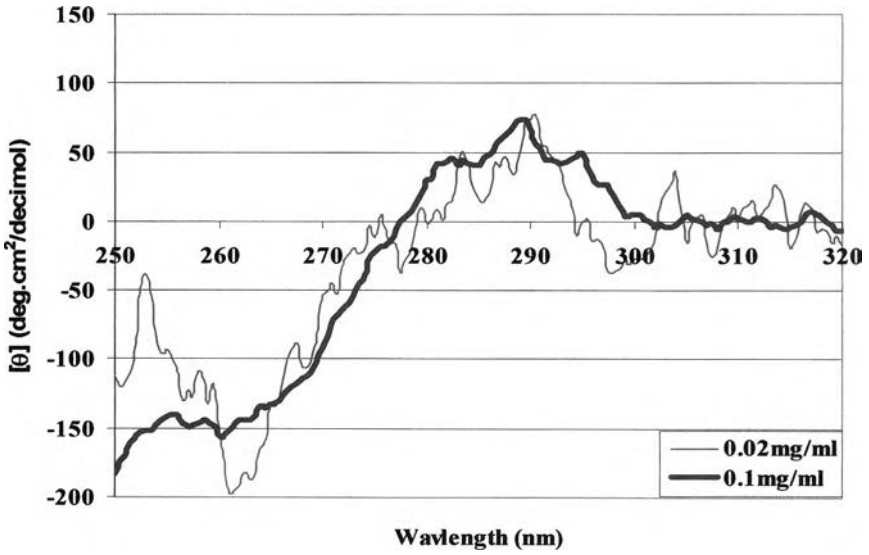
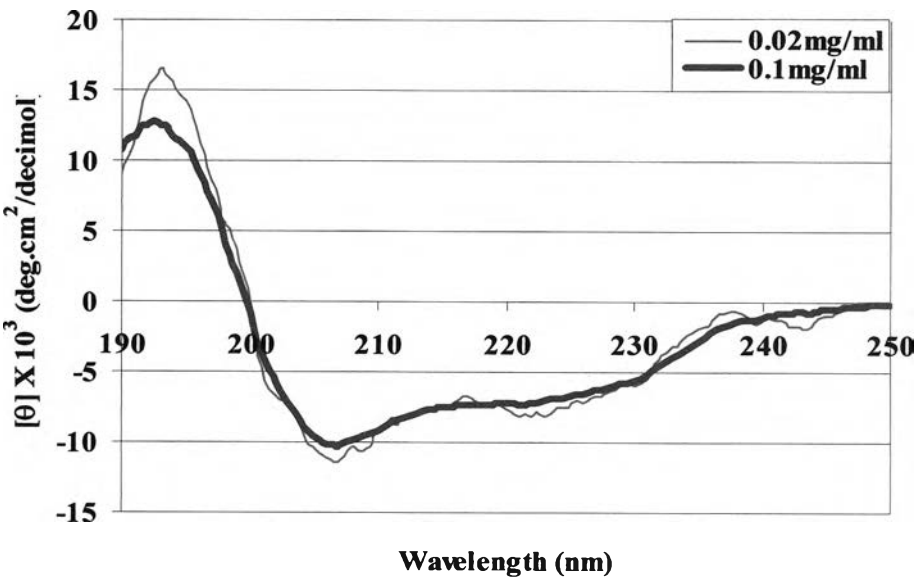


Figure 61 Linearity curve of concentrations of lysozyme dissolved in water versus ellipticity (mdeg) at 289 nm



A



B

Figure 62 The CD spectra of native lysozyme dissolved in water at 0.02 mg/ml in the far-UV (A) and near-UV (B) regions

APPENDIX B

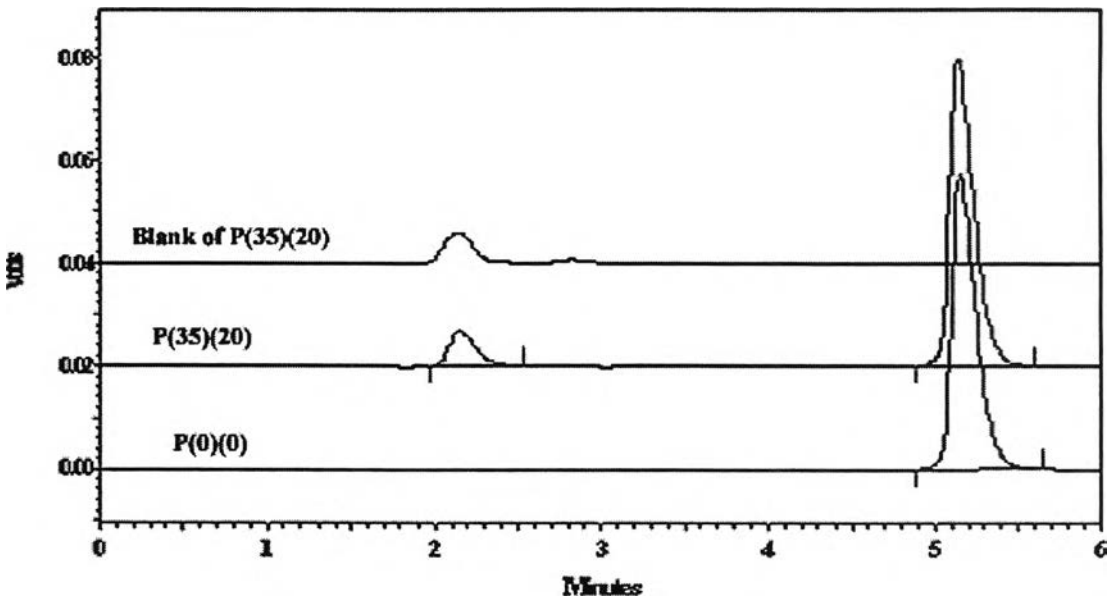


Figure 63 HPLC chromatogram of propranolol HCl after exposure to water and L(35)(20) solution, and L(35)(20) solution without propranolol HCl at 257 nm

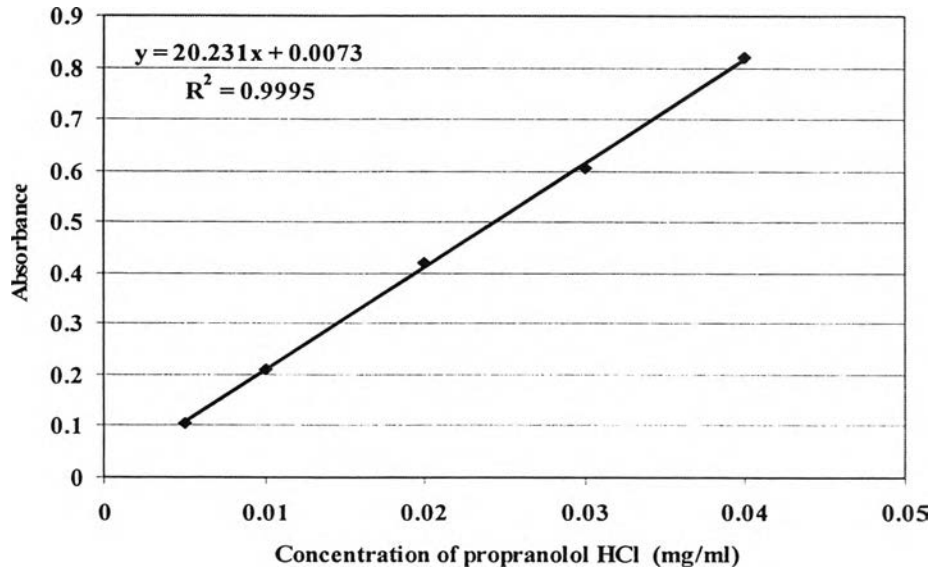
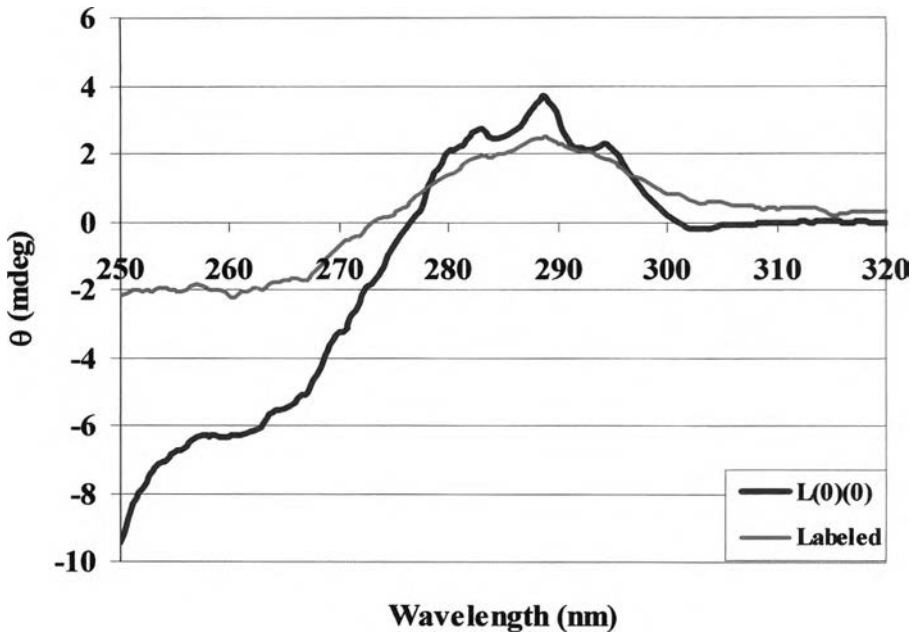
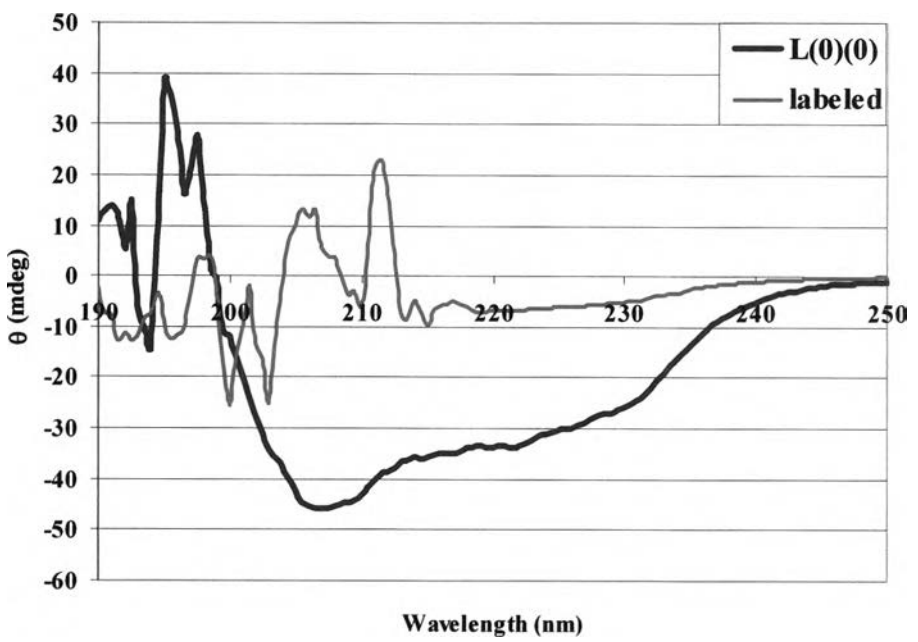


Figure 64 Calibration curve of propranolol HCl in water using spectrophotometer at 289 nm



A



B

Figure 65 The CD spectra of labeled lysozyme dissolved in water in the far-UV (A) and near-UV (B) regions.

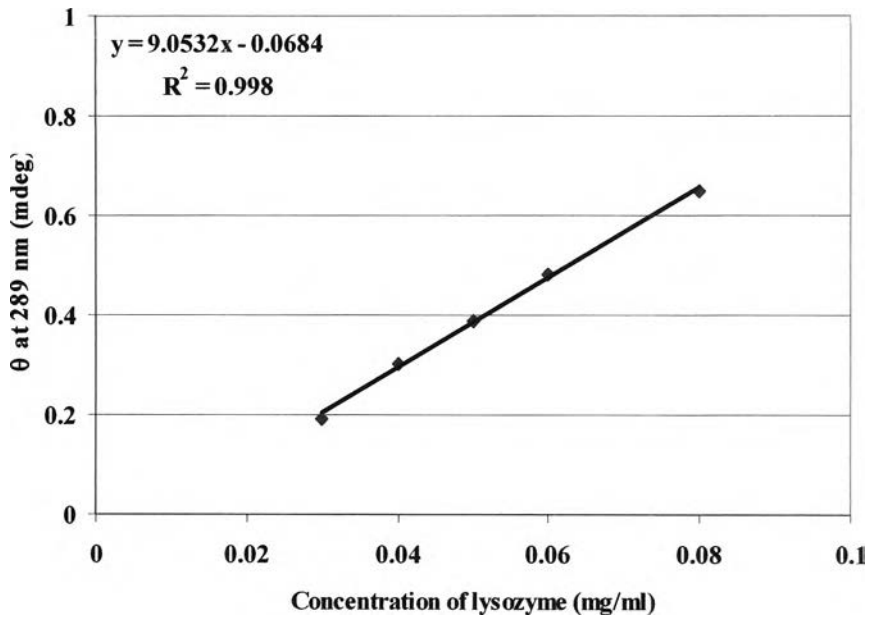


Figure 66 Calibration curve of lysozyme dissolved in water using CD intensities at 289 nm

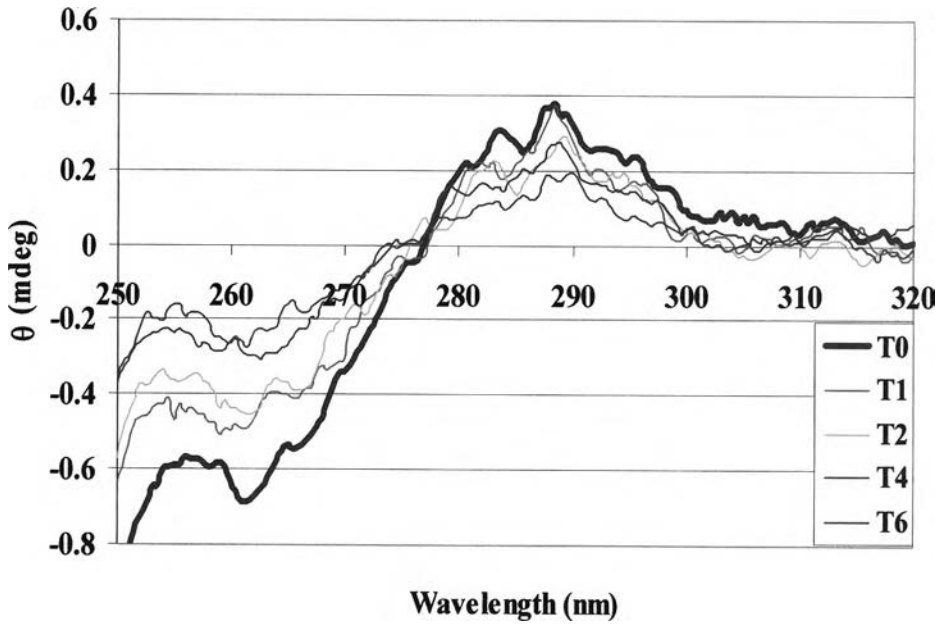


Figure 67 The CD spectra of lysozyme remaining in the donor compartment which dissolved in water at various times in the near-UV region.

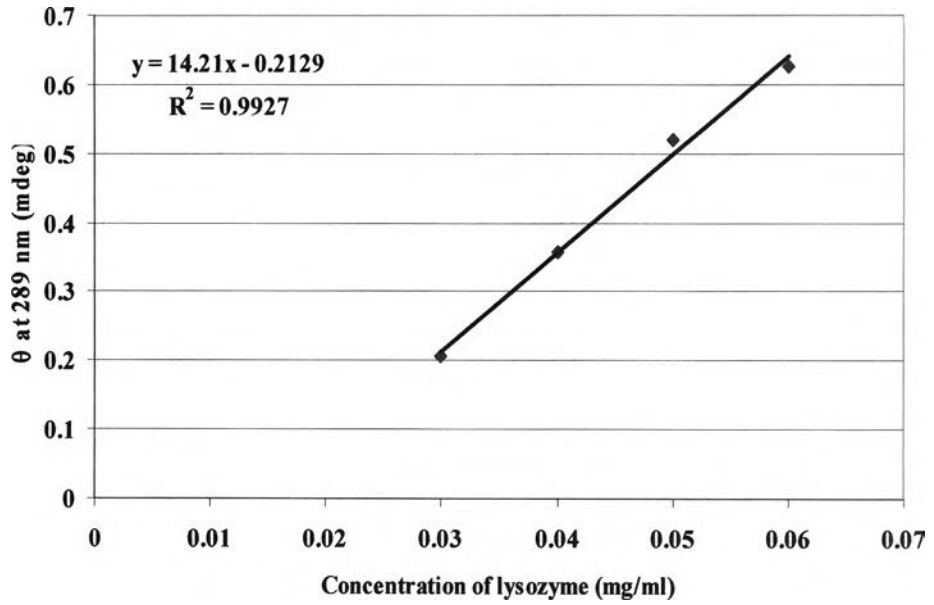


Figure 68 Calibration curve of lysozyme dissolved in L(35)(0) solution using CD intensities at 289 nm

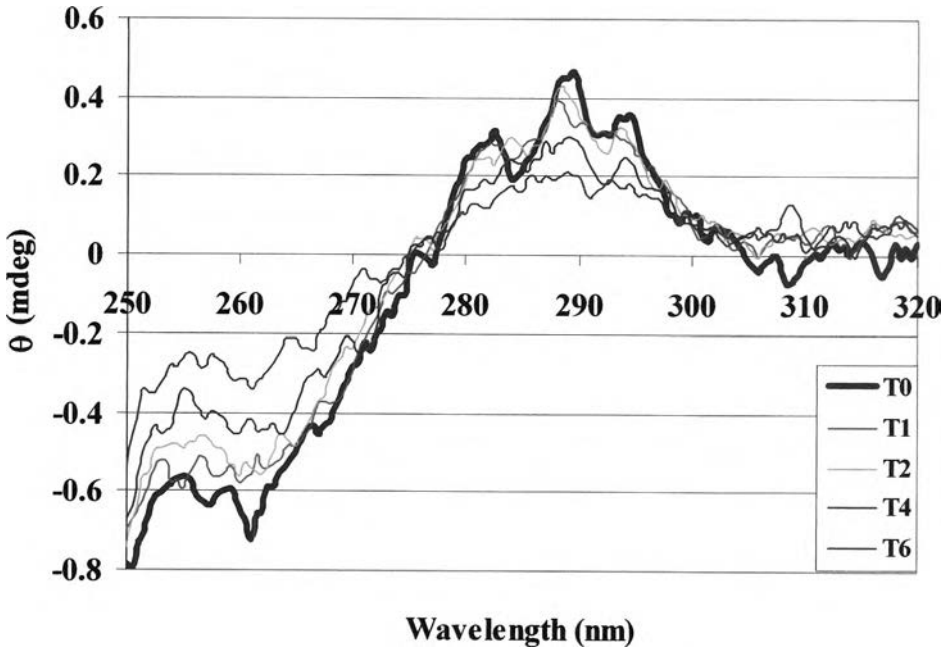


Figure 69 The CD spectra of lysozyme remaining in the donor compartment which dissolved in L(35)(0) solution at various times in the near-UV region

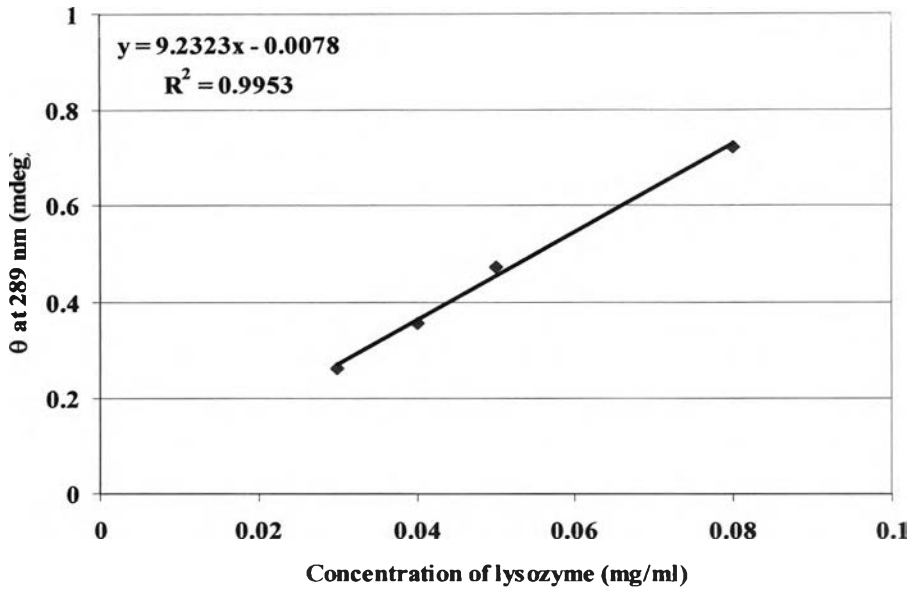


Figure 70 Calibration curve of lysozyme dissolved in L(80)(0) solution using CD intensities at 289 nm

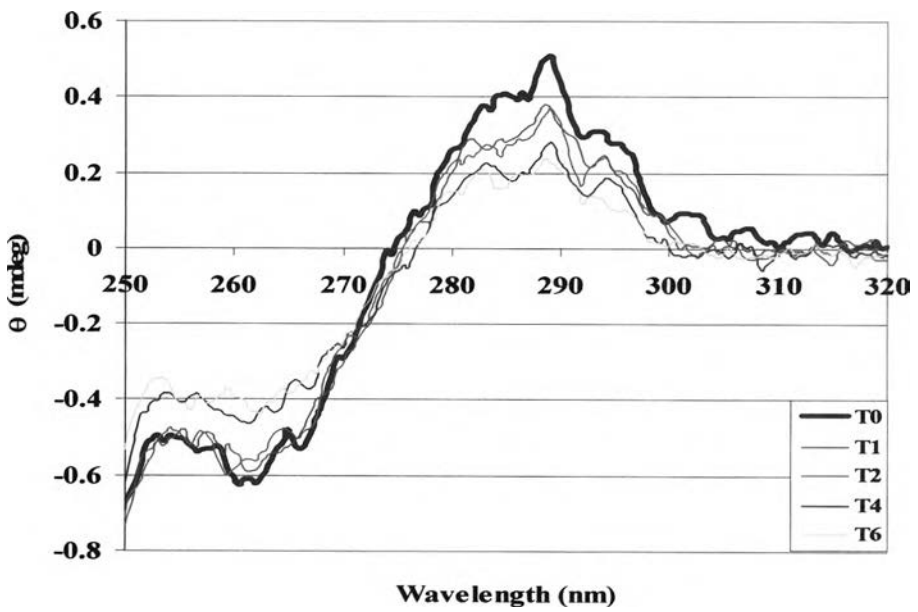


Figure 71 The CD spectra of lysozyme remaining in the donor compartment which dissolved in L(80)(0) solution at various times in the near-UV region

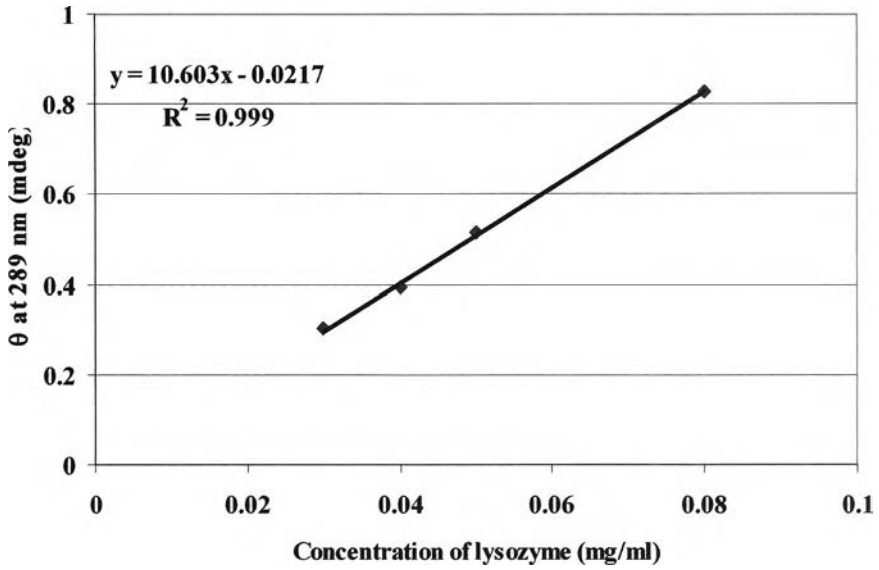


Figure 72 Calibration curve of lysozyme dissolved in L(35)(20) solution using CD intensities at 289 nm

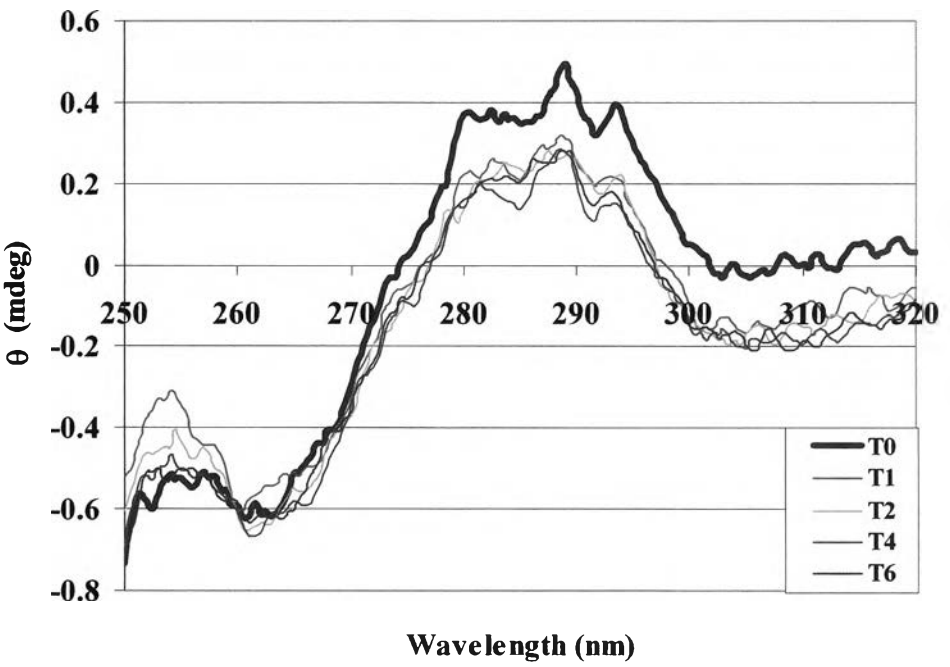


Figure 73 The CD spectra of lysozyme remaining in the donor compartment which dissolved in L(35)(20) solution at various times in the near-UV region

VITA

Wiriyaporn Sirikun was born in Singburi, Thailand, on June 28th, 1978. She received Bachelor of Science in Pharmacy degree in 2002 from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand. She presented a poster titled "Organic solvent and acid induced conformational modification of model peptide; lysozyme" at "China International Conference on Nanoscience and Technology" Beijing, China on June 9-11, 2005.