REFERENCES

1. Xu, J.; Granger, M. C.; Chen, Q.; Strojek, J. W.; Lister, T. E. and Swain, G. M. "Boron-doped diamond thin-film electrodes." Anal. Chem. 69 (1997): 591A-597A.

2. Popa, E.; Kubota, Y.; Tryk, D. A and Fujishima, "A. Selective voltammetric and amperometric detection of uric acid with oxidized diamond film electrodes." Anal. Chem. 72 (2002): 1724-1727.

3. Terashima, C.; Rao, T. N.; Sarada, B. V.; Tryk, D. A. and Fujishima, A. "Electrochemical oxidation of chlorophenols at a boron-doped diamond electrode and their determination by high-performance liquid chromatography with amperometric detection." Anal. Chem. 74 (2002): 895-902.

4. Chailapakul, O.; Siangproh, W.; Sarada, B. V.; Terashima, C.; Rao, T. N.; Tryk, D. A. and Fujishima, "A. The electrochemical oxidation of homocysteine at boron-doped diamond electrodes with application to HPLC amperometric detection." Analyst 127 (2002) : 1164-1168.

5. Montilla, F.; Morallón, E.; Duo, I.; Comninellis, Ch. and Vázquez, J. L. "Platinum particles deposited on synthetic boron-doped diamond surface. Application to methanol oxidation." Electrochim. Acta 48 (2005): 3891-3897.

6. Wang, J. and Angnes, L. "Miniaturized glucose sensors based on electrochemical codeposition of rhodium and glucose oxidase onto carbon-fiber electrodes." Anal. Chem. 64 (1992): 456-459.

7. Wang, J.; Liu, J.; Chen, L. and Lu, F. "Highly selective membrane-free, mediator-free glucose biosensor." Anal. Chem. 66 (1994): 3600-3603.

8. Wang, J. and Chen, Q. "Enzyme microelectrode array strips for glucose and lactate." Anal. Chem. 66 (1994): 1007-1011.

9. Skoog, D. A. and Leary, J. J. Principles of Instrumental Analysis. 4th Ed. New York: Harcourt Brace College Publishers, 1992.

10. Wang, J. Analytical Electrochemistry. New York: Wiley-VCH,1994.

11. Eggins, B. Biosensors: An Introduction. New York: John Wiley & Sons, 1996.

12. Kissinger, P. T. and Heineman, W. R. Laboratory Techniques in Electroanalytical Chemistry. 2nd Ed. New York: Marcel Dekker, 1996.

13. Hupert, M.; Muck, A.; Wang, J.; Stotter, J.; Cvackova, Z.; Haymond, S.; Show, Y. and Swain, G. M. "Conductive diamond thin-films in electrochemistry." Diamond Rel. Mater. 12 (2003): 1940-1949

14. Fujishima, A.; Einaga, Y.; Rao, T. N. and Tyrk, D. A. Diamond Electrochemistry. Tokyo: Elsevier, 2005.

15. Notsu, H.; Tatsuma, T. and Fujishima, A. "Tyrosinase-modified boron-doped diamond electrodes for the determination of phenol derivatives." J. Electroanal. Chem. 523 (2002): 86-92.

16. Ivandini, T. A.; Sato, R.; Makide, Y.; Fujishima, A. and Einaga, Y. "Pt-implanted boron-doped diamond electrodes and the application for electrochemical detection of hydrogen peroxide." Diamond Rel. Mater. 14 (2005): 2133-2138.

17. Riedo, B.; Dietle, G. and Enea, O. "Growth of Pt clusters electrodeposited onto boron-doped diamond films." Thins Solid Films 488 (2005): 82-86.

18. Uchikado, R.; Rao, T. N.; Tryk, D. A. and Fujishima, A. "Metal-modified diamond electrode as an electrochemical detector for glucose." Chem. Lett. 30 (2001): 144-145.

19. DeBattisti, A.; Ferro, S. and Colle, M. D. "Electroanalysis at conductive diamond modified by noble-metal oxides." J. Phys. Chem. B 105 (2001): 1679-1682.

20. Okumura, K.; Nakagawa, K.; Shimamura, T. Ikenaga, N.; Nishitani-Gamo, T. Kobayashi, T. and Suzuki, T. "Direct formation of acetaldehyde from ethane using carbon dioxide as a novel oxidant over oxidized diamond-supported catalysts." J. Phys. Chem. B 107 (2003): 13419-13424.

21. Růžička, J. and Hansen, E. H. Flow injection analysis. 2 nd Ed. New York: JohnWiley & Sons, 1998.

22. Fifield, F. W. and Kealey, D. Principles and practical of analytical chemistry. 4th Ed. Glasgow: Blackie Academic & Professional, 1995.

23. Croubels, S. K.; Vanoosthuyze, K. E. I. and Preteghem, C. H. V. "Use of metal chelate affinity chromatography and membrane-based ion-exchange as clean-up procedure for trace residue analysis of tetracyclines in animal tissues and egg." J. Chromatogr. B 690 (1997): 173-179.

24. Katz, S. E. and Fassbender, C. A. "Microbiological Assay with increased sensitivity for chlortetracycline in eggs" J. Agric. Food. Chem. 18 (1970): 1165-1167.

25. Nouw, J.; Egmond, H. van; Smulders, I.; Loeffen, G.; Schouten, J. and Stegeman, H. "A microbiological assay system for assessment of raw milk exceeding EU maximum residue levels." International Diary Journal 9 (1996): 85-90.

26. Kurittu, J.; Lönnberg, S.; Virta, M. and Karp, M. "A group-specific microbiological test for the detection of tetracyclines residues in raw milk." J. Agric. Food. Chem. 48 (2000): 3372-3373.

27. Kohn, K. W. "Determination of tetracyclines by extraction of fluorescent complexes: Application to biological materials." Anal. Chem. 33 (1961): 862-866.

28. Ibsen, K. H.; Saunders, R. L. and Urist, M. R. "Fluorometric determination of oxytetracycline in biological material." Anal. Biochem. 5 (1963): 505-514.

29. Georges, J. and Ghazarian, S. "Study of europium-sensitized fluorescence of tetracycline in a micellar solution of Triton X-100 by fluorescence and thermal lens spectrometry." Anal. Chim. Acta 276 (1993): 401-419.

30. Croubels, S.; Peteghem, C. V. and Baeyens, W. "Sensitive spectrofluorimetry determination of tetracycline residues in bovine milk." Analyst 119 (1994): 2713-2716.

31. Han, H.; He, Z. and Zeng, Y.'e "Chemiluminescence determination of tetracyclines using a Tris(2'2-bipyridine)ruthenium(II) and potassium permanganate system." Anal. Sci. 15 (1999): 467-470.

32. Pena, A.; Palilis, L. P.; Lino, C. M.; Irena Silveira, M. and Calokerinos, A. C. "Determination of tetracycline and its major degradation products by chemilumninescence." Anal. Chim. Acta 405 (2000): 51-56.

33. Lau, C.; Lu, J.and Kai, M. "Chemiluminescence determination of tetracycline based on radical production in a basic acetonitrile-hydrogen peroxide reaction." Anal. Chim. Acta 503 (2004): 235-239.

34. Akwarthan, A.A.and Townshend, A. "Determination of tetracycline by flow injection with chemiluminescence detection." Anal. Chim. Acta 205(1988): 261-265.

35. Zheng, X.; Mei, Y and Zhang, Z. "Flow-injection chemiluminescence determination of tetracyclines with in situ electrogenerated bromine as the oxidant." Anal. Chim. Acta 440 (2001): 143-149.

36. Sryropoulos, A. B.; Calokerinos, A. C. "Continuous-flow chemiluminescence determination of some tetracycline." Anal. Chim. Acta 255 (1991): 403-411.

37. Townshend, A.; Ruengsitagoon, W.; Thongpoon, C. and Liawruangrath, S. "Flow injection chemiluminescence determination of tetracycline." Anal. Chim. Acta 541 (2005): 103-109.

38. Oka, H. "Improvement of chemical analysis of antibiotics: XXIII. Identification of residual tetracyclines in bovine tissues by electrospray high-performance liquid chromatography-tandem mass spectrometry." J. Chromatogr. B 693 (1997): 337-344.

39. Nakazawa, H.; Ino, S.; K.; Kato, K.; Watanabe, T.; Ito, Y. and Oka, H. "Simultaneous determination of residual tetracyclines in foods by high-performance liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry." J. Chromatogr. B 732(1999); 55-64.

40. Iwaki, K.; Okumura, N. and Yamazaki, M. "Rapid determination of tetracycline antibiotics in serum by reverse-phase high-performance liquid chromatography with fluorescence detection." J. Chromatogr. 619 (1993): 319-323.

41. Sczesny, S; Nau, H.and Hamscher, G. "Residue analysis of tetracyclines and their metabolites in eggs. and in the environment by HPLC coupled with microbiological and tandem mass spectrometry." J. Agric. Food. Chem. 51 (2003): 697-703.

42. Cooper, A. D.; Stubbings, G. W. F.; Kelly, M.; Tarbin, J. A.; Farrington, W. H. H. and Shearer, G. "Improved method for the on-line metal chelate affinity chromatography-high-performance liquid chromatographic determination of tetracycline antibiotics in animal products." J. Chromatogr. A 812 (1998): 321-326.

43. Fletouris, D. J.; Psomas, J. E. and Botsoglou, N. A. "Trace analysis of oxytetracycline and tetracycline in milk by high-performance liquid chromatography." J. Agric. Food Chem. 38 (1990) 1913-1917.

44. Furusawa, N. "Rapid liquid chromatographic determination of oxytetracycline in milk." J. Chromatogr. A 839 (1999): 247-251.

45. Brillants, S.; Tanasomwang, V.; Thongrod, S. and Dachanatanwitaya, N. "Oxytetracycline residues in giant freshwater prawn (Macrobrachium rosenbergii)." J. Agric. Food Chem. 49 (2001): 4995-4999.

46. Aoyama, R. G.; McErlane, K. M.; Erber, H.; Kittis, D. D. and Burt, H. M. "High-performance liquid chromatographic analysis of oxytetracycline in Chinook salmon following a administration of medicated feed." J. Chromatogr. 588 (1991): 181-186.

47. Jacobsen, A. M.; Halling-Sørensen, B.; Ingerslev, F. and Hansen, S. H. "Simultaneous extraction of tetracycline, macrolide and sulfonamide antiobiotics from agricultural soils using pressurized liquid extraction,

followed by solid phase extraction and liquid chromatography-tandem mass spectrometry." J.Chromatogr. A 1038 (2004): 157-170.

48. Carson, M. C.; Ngoh, M. A. and Hadley, S. W. "Confirmation of multiple tetracycline residues in milk and oxytetracycline in shrimp by liquid chromatograph-particle beam mass spectrometry." J. Chromatogr. B 712 (1998): 113-128.

49. Oka, H.; Ikai, Y.; Kawamura, N.; Uno, K.; Yamada, M.; Harada, K.-I. and Suzuki, M. "Improvement of chemical analysis of antibiotics : XII. Simultaneous analysis of seven tetracyclines in honey." J. Chromatogr. 400 (1987): 253-261.

50. Oka, H.; Ikai, Y.; Kawamura, N.; Uno, K.; Yamada, M..; Harada, K.-I.; Uchiyama, M.; Asukabe, H. and Suzuki, M. "Improvement of chemical analysis of antibiotics : X. Determination of eight tetracyclines using thin-layer and high-performance liquid chromatography." J. Chromatogr. 393 (1987): 285-296.

51. Naidong, W.; Hauglustain, C.; Roets, E. and Hoogmartens, J. "Assay and purity control of oxytetracycline and doxycycline by thin-layer chromatography-a comparison with liquid chromatography." J. Pharm. Biomed. Anal. 8 (1990) 891-898.

52. Bossuyt, R.; Renterghem, R. V. and Waes, G. "Identification of antibiotic residues in milk by thin-layer chromatography." J. Chromatogr. 124 (1976): 37-42.

53. E. Ragazzi, E. and Veronese, G. "Simple method for the quantitative analysis of tetracyclines by direct fluorimetry after thin-layer chromatography on cellulose plates." J. Chromatogr. 132 (1977): 105-114.

54. Omer, A. I. H.; Kariem, E. A. G. and Salama, R. B. "Separation and semi-quantitative determination of tetracycline degradation products in tetracycline hydrochloride powders and capsules by thin-layer chromatography." J. Chromatogr. 260 (1981): 486-489.

55. Radecka, C.; Wilson, W. L. "Direct densitometic determination of tetracycline in pharmaceutical preparations." J. Chromatogr. 57 (1971) 297-302.

56. Oka, H.; Ikai, Y.; Hayakawa, J.; Masuda, K.; Harada, K.; Suzuki, M.; Martz, V. and MacNeil, J. D. "Improvement of chemical analysis of antibiotics. 18. Identification of residual tetracyclines in bovine tissues by TLC/FABMS with a sample condensation technique." J. Agric. Food Chem. 41 (1993): 410-415.

57. Tsuji, K. and Robertson, J. H. "Formation of Trimethylsilly derivatives of tetracyclines for separation and quantitation by gas-liquid chromatography." Anal. Chem. 45 (1973): 2136-2140.

58. Chen, C.-L. and Gu, X.; Determination of tetracycline residues in bovine milk, serum, and urine by capillary electrophoresis." J. AOAC Int. 78 (1995): 1369-1377.

59. Tjønelund, J. and Hansen, S. H. "Use of metal complexation in non-aqueous capillary electrophoresis systems for the separation and improved detection of tetracyclines." J. Chromatogr. A 778 (1997) 235-243.

60. Nozal, L.; Simonet, B. M.; Arce, L.; Ríos, A. and Valcárcel, M. "Use of basic amphiprotic organic solvents containing neutral-surfactant aggregates as pseudostationary phas in non-aqueous capillary electrophoresis." Anal. Chim. Acta 560 (2006): 69-76.

61. Khan, N. H.; Roets, E.; Hoogmatens, J. and Vanderhaeghe, H. "Quantitative analysis of chlortetracycline and related substances by high-performance liquid chromatography." J. Pharm. Biomed. Anal. 7 (1989): 339-353.

62. Harmansson, J. "Rapid determination of tetracycline and lumecycline in human plasma and urine using high-performance liquid chromatography." J. Chromatogr. 232 (1982): 385-393.

63. Oka, H.; Uno, K.; Harada, K.-I.; Yasaka, K. and Suzuki, M. " Improvement of chemical analysis of antibiotics: V. A simple method for the analysis of tetracyclines using reversed-phase high-performance liquid chromatography." J. Chromatogr. 298 (1984): 435-443.

64. Oka, H.; Ikai, Y.; Kawamura, N.; Uno, K.; Yamada, M.; Harada, K.-I.; Uchiyama, M.; Asukabe, H.; Mori, Y. and Suzuki, M. "Improvement of chemical analysis of antibiotics : IX. A simple method for residual tetracyclines analysis in honey using a tandem cartridge clean-up system." J. Chromatogr. 389 (1987): 417-426.

65. Iversen, B.; Aanesrud, A.; Kolstad, A. K. "Determination of oxytetracycline in plasma from rainbow trout using high-performance liquid chromatography with ultraviolet detection." J. Chromatogr. 493 (1989): 217-221.

66. Fedeniuk, R. W.; Ramamurthi, S. and McCurdy, A. R. "Application of reversed-phase liquid chromatography and prepacked C_{18} cartridges for the analysis of oxytetracycline and related compounds." J. Chromatogr. B 677 (1996): 291-297.

67. Agasøster,T. and Rasmussen, K. E. "Fully automated high-performance liquid chromatographic analysis of whole blood and plasma samples using on-line dialysis as sample preparation : Determination of oxytetracycline in bovine and salmon whole blood and plasma." J. Chromatogr. 570 (1991): 91-107

68. Skúlason, S.; Ingólfsson, E. and Kristmundsdóttir, T. "Development of a simple HPLC method for separation of doxycycline and its degradation products." J. Pharm. Biomed. Anal. 33 (2003): 667-672.

69. Monser, L. and Darghouth, F. "Rapid liquid chromatographic method for simultaneous determination of tetracyclines antibiotics and 6-Epi-doxycycline in pharmaceutical products using porous graphitic carbon column." J. Pharm. Biomed. Anal. 15 (2000): 353-362.

70. Vienneau, D. S. and Kindberg, C. G. "Development and validation of a sensitive method for tetracycline in gingival crevicular fluid by HPLC using fluorescence detection" J. Pharm. Biomed. Anal. 16 (1997): 111-117.

71. Croubels, S. M.; Vanoosthuyze, K. E. I. and Peteghem, C. H. V. "Use of metal

chelate affinity chromatography and membrane-based ion-exchange as clean-up procedure for trace residue analysis of tetracyclines in animal tissues and egg." J. Chromatogr. B 690 (1997): 173-179.

72. Agasøster, T. and Rasmussen, K. E. "On-line dialysis, liquid chromatography and post-column reaction detection of oxytetracycline in salmon muscle extracts." J. Pharm. Biomed. Anal. 10 (1992): 349-354.

73. Wan, G.-H.; Cui, H.; Zheng, H.-S.; Zhou, J.; Liu, L.-J. and Yu, X.-F. "Determination of tetracyclines residues in honey using high-performance liquid chromatography with potassium permanganate–sodium sulfite–β-cyclodextrin chemiluminescence detection." J. Chromatogr. B 824 (2005): 57-64.

74. Blanchflower, W. J.; McCracken, R. J.; Haggan, A. S. and Glenn, D. "Confirmatory assay for the determination of tetracyline, oxytetracycline, chlortetracycline and its isomers in muscle and kidney using liquid chromatography-mass spectrometry." J. Chromatogr. B 692 (1997): 351-360.

75. Kazemifard, A. G. and Moore, D. E. "Evaluation of amperometric detection for the liquid-chromatographic determination of tetracycline antibiotics and their common contaminants in pharmaceutical formulations." J. Pharm. Biomed. Anal. 16 (1997) 689-696.

76. Wangfuengkanagul, N. and Chailapakul, O. Electrochemcial analysis of acetaminophen using a boron-doped diamond thin film electrode applied to flow injection system." J. Pharm. Biomed. Anal. 28 (2002): 841-847.

77. Wangfuengkanagul, N. and Chailapakul, O. "Electrochemical analysis of D-penicillamine using a boron-doped diamond thin film electrode applied to flow injection system." Talanta 58 (2002): 1213-1219.

78. Siangproh, W.; Wangfuengkanagul, N. and Chailapakul, O. "Electrochemcial oxidation of tiopronin at diamond film electrodes and its determination by amperometric flow injection analysis." Anal. Chim. Acta 499 (2003): 183-189.

79. Siangproh, W.; Ngamukot, P. and Chailapakul, O. "Electrochemical determination of captopril at boron-doped diamond thin film electrode applied to a flow injection system." Sens. Actuat. B-Chem. 91 (2003): 60-66.

80. Boonsong, K.; Chuanuwatanakul, S.; Wangfuengkanagul, N. and Chailapakul, O. "Electroanalysis of lincomycin using boron-doped diamond thin film electrode applied to flow injection system." Sens. Actuat. B-Chem. 108 (2005): 627-632.

81. Bouvrette, P.; Hrapovic, S.; Male, K. B. and Luong, J. H. T. "Analysis of the 16 environmental protection agency priority polycyclic aromatic hydrocarbons by high performance liquid chromatography-oxidized diamond film electrodes." J. Chromatogr. A 1103 (2006): 248-256.

82. Terashima, C.; Rao, T. N.; Sarada, B. V.; Kubota, Y. and Fujishima, A. "Direct electrochemical oxidation of disulfides at anodically pretreated boron-doped diamond electrodes." Anal. Chem. 75 (2003): 1564-1572.

83. Kieber, R. J.; Helz, G. R. "Two method verification of hydrogen peroxide determinations in natural waters." Anal. Chem. 58 (1998): 2312-2315.

84. Holm, T. R.; Goore, G. K. and Barcelona, M. J. "Fluorimetry determination of hydrogen peroxide in groundwater." Anal. Chem. 59 (1987): 582-586.

85. Li, Y. and Townshend, A. "Evaluation of the adsorptive immobilization of horseradish peroxidase determination using fluorescene detection." Anal. Chim. Acta 359 (1998): 149-156.

86. Sakuragawa, A.; Taniai, T. and Okutani, T. "Flurometric determination of microamounts of hydrogen peroxide with an immobilized enzyme prepared by coupled horseradish peroxidase to chitosan beads." Anal. Chim. Acta 374 (1998): 191-200.

87. Navas, M. J.; Jiménez, A. M. and Galán, G. "Air analysis: determination of hydrogen peroxide by chemiluminescence." Atmospheric Environment 33 (1999): 2279-2283.

88. Kok, G. L. "Measurement of hydrogen peroxide in rain water." Atmospheric

Environment 14 (1980): 653-656.

89. Yoshizumi, K.; Aoki, K.; Nouchi, Y.; Okita, T.; Kobayashi, T.; Kamakura, S. and Taijima, M. "Measurements of the concentration in rainwater and of the Henry's law constant of hydrogen peroxide." Atmospheric Environment 18 (1984): 395-401.

90. Janasek, D.; Spohn, U. and Beckmann, D."Novel chemiluminometric H_2O_2 sensors for the sensitive flow injection analysis." Sen. Actuat. B-Chem. 51 (1998) 107-113.

91. Zhou, G.-J.; Wang, G.; Xu, J.-J. and Chen, H.-Y. "Reagentless chemiluminescence biosensor for determination of hydrogen peroxide based on the immobilization of horseradish peroxidase on biocompatible chitosan membrane." Sen. Actuat. B-Chem. 81 (2002): 334-339.

92. Ding, X.; Wang, P. and Liu, G. "New chemiluminescence analysis apparatus involving solid-state peroxyoxalate and immobilized fluorophore." J. Lumin. 40-41 (1988): 844-855.

93. Katayama, M.; Takeuchi, H. and Tanig , C. H. "Determination of hydrogen peroxide by flow-injection analysis with aryl oxalate-sulphorhodamine 101 chemiluminescence." Anal. Letts. 24 (1991): 1005-1015.

94. Quabβ, U. and Klockow, D. "Determination of Fe(II) and H_2O_2 in atmospheric liquid water by peroxyoxalate chemiluminescence." Int. J. of Environ. Anal.Chem. 60 (1995): 361-375.

95. Stigbrand, M.; Pontén, E. and Irgum, K. "1,1'-Oxalydiimidazole as chemiluminescence reagent in the determination of low hydrogen peroxide concentrations by flow injection analysis." Anal. Chem. 66 (1994): 1766-1770.

96. Stigbrand, M.; Karisson, A. and Irgum, K. "Direct and selective determination of atmospheric gaseous hydrogen peroxide by diffusion scrubber and 1,1'-Oxalydiimidazole chemiluminescence detection." Anal. Chem. 68 (1996): 3945-3950.

97. Yu, J. and Ju, H. "Amperometric of biosensor for hydrogen peroxide based on

hemoglobin entrapped in titania sol-gel film." Anal. Chim. Acta 486 (2003): 209-216. Drawback of HRP

98. Yang, W.; Li, Y.; Bai, Y. and Sun, C. Hydrogen peroxide biosensor based on myoglobin/colloidal gold nanoparticles immobilized on glassy carbon electrode by a nafion film." Sen. Actuat. B-Chem. in press.

99. Mascini, M.; Iannello, M. and Palleschi, G. "A liver tissue-based electrochemical sensor for hydrogen peroxide." Anal. Chim. Acta 138 (1982): 65-69.

100. Wijesuriya, D.; Lin, M. S. and Rechnitz, G. A. "Grape tissue-based electrochemical sensor for the determination of hydrogen peroxide." Anal. Chim. Acta 234 (1990): 453-457.

101. Navaratue, A. and Rechnitz, G. A. "Improve plant tissue-based biosensor using in vitro cultured tobacco callus tissue." Anal. Chim. Acta 257 (1992): 59-66.

102. Tai, G. Y.; Wen, M. L. and Wang, C. Y. "Bacteria-based biosensor for determination of hydrogen peroxide." Microchem. J. 53 (1996): 152-157.

103. Ricci, F. and Palleschi, G. "Sensor and biosensor preparation, optimisation and applications of Prussian Blue modified electrodes." Biosens.and Bioelectron. 21 (2005): 389-407.

104. Neff, V. D. "Electrochemical of oxidation and reduction of thin films of Prussian Blue." J. Electrochem. Soc. 125 (1998): 886-887.

105. Itaya, K.; Akahoshi, H. and Toshima, S. "Electrochemistry of Prussian Blue modified electrodes: an electrochemical preparation method." J. Electrochem. Soc. 129 (1982): 1498-1500.

106. Lin, M. S.; Tseng, T. F. and Shih, W. C. "Chromium (III) hexacyanoferrate (II)-based chemical sensor for the cathodic Analyst 123 (1998): 159-163.

107. Lin, M. S. and Shih, W. C. "Chromium hexacyanoferrate based glucose biosensor." Anal. Chim. Acta 381 (1999): 183-189.

108. Terashima, C.; Rao, T. N.; Sarada, B. V.; Spataru, N. and Fujishima, A. "Electrodeposition of hydrous iridium oxide on conductive diamond electrodes for catalytic sensor applications." J. Electroanal. Chem. 544 (2003) 65-74.

109. Furusawa, N. "Rapid high-performance liquid chromatographic determining technique of sulfamonomethoxine, sulfadimethoxine, and sulfaquinoxaline in eggs without use of organic solvents." Anal. Chim. Acta 481 (2003): 255-259.

110. Bard, A. J. and Faulkner, L. R. Electrochemical Methods. New York: John Wiley&Sons, 1980.

111. Stephen, C. R.; Murai, K.; Bruning, K. J. and Woodward, R. B.; "Acidity constants of the tetracycline antibiotics." J. Am. Chem. Soc. 78: (1956): 4155-4158.

112. Knox, J. H. and Jurand, J. "Mechanism of reversed-phase separation of tetracyclines by high-performance liquid chromatography." J. Chromatogr. 186 (1979): 763-782.

113. Tsuji, K.; Robertson, J. H. and Beyer, J. "High performance liquid chromatographic determination of tetracyclines." Anal. Chem. 46 (1974): 539-543.

114. Knox, J. H. and Jurand, J. "Separation of tetracycline by high-speed liquid chromatography" J. Chromatogr. 112 (1975): 103-115.

115. Ding, X.and Mou, S. Ion chromatographic analysis of tetracyclines using polymeric column and acidic eluent. J. Chromatogr. A 897 (2000): 205-214

116. Notsu, H.; Yagi, I.; Tatsuma, T.; Tryk, D. A. and Fujishima, A. "Surface carbonyl groups on oxidized diamond electrodes." J. Electroanal. Chem. 492 (2000): 31-37.

117. Khairutdinov, R. F. and Serpone, N. "Photoluminescence and transient spectroscopy free bass porphyrin aggregates." J. Phys. Chem. B 103 (1999): 761-769.

118. Kelbasuskas, L.; Bagdonas, S.; Dietel, W. and Rotamskis, R. "Excitation relaxation and structure of $TPPS_4$ J-gaggregate." J. Lumin. 101 (2003), 253-262.

119. Ogawa, T.; Tokunaga, E. and Kobayashi, T. "Giant electrooptic response of excitons in porphyin J-aggregate." Chem. Phys. Letts. 408 (2005): 186-191.

120. Ishida, A.; Majima, T. "Photocurrent generation of a porphyrin self-assembly monolayer on a gold film electrode by surface plasmon excitation using near-infrared light." Chem. Phys. Letts. 322 (2000): 242-246.

121. Ohno, O.; Kaizu, Y. and Kobayashi, H. "J-aggregate formation of a water-soluble porphyrin in acidic aqueous media." J. Chem. Phys. 99 (1993): 4128-4139.

122. Pasternack, R. F.; Fleming, C.; Herring, B.; Collings, P. J.; dePaula, J.; DeCastra, G. and Gibbs, E. J. "Aggregation kinetics of extended porphyrin and cyanine dye association." Biophys. J. 79 (2000): 550-560.

123. Pasternack, R. F.; Huber, P. R.; Boyd, P.; Engasser, G.; Francesconi, L. Gibbs, E.; Fasella, P.; Venturo, G. C. and Hind, L. deC. "On the aggregation of meso-substituted water-soluble porphyrin" J. Am. Chem. Soc. 94 (1972): 4511-4517.

124. Castriciano, M. A.; Romeo, A. Villari, V.; Micali, N. and Scolaro, L. M. "Structural rearrangement in 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrins J-aggregates under strongly acidic conditions" J. Phys. Chem. 107 (2003): 8765-8771.

125. Koti, A. S. R., Taneja, J. and Perisamy, N. "Control of coherence length and aggregate size in the *J*-aggregate of porphyrin." Chem. Phys. Letts. 375 (2003): 171-176.

126. Maiti, N. C.; Mazumder, S. and Periasamy, N.. "J- and H-aggregates of porphyrins with surfactants: fluorescence, stopped flow and electron microscopy." J. Porphyrins. Phthalocyanines 2 (1998): 369-376.

127. Maiti, N. C.; Mazumder, S. and Periasamy, N. "J- and H-aggregates of porphyrin-surfactant complexes: Time-resolved fluorescence and other spectroscopic studies." J. Phys. Chem. B 102 (1998): 1528-1538.

128. Koti, A. S. R. and Periasamy, N. "Cyanine induced aggregation in meso-tetrakis(4-sulfonatophenyl)porphyrin anions." J. Mater. Chem. 12 (2003): 2312-2317.

129. Koti, A. S. R. and Periasamy, N. "Self-assembly of template-directed J-aggregates of porphyrin." Chem. Mater. 15 (2003): 369-371.

130. Crusats, J.; Claret, J.; Díez-Pérez, Z.; El-Hachemi, H.; Gárcia-Ortega, H.; Rubires, R.; Sagues, F. and Ribó, J. M. "Chiral shape and enantioselective growth of colloidal particles of self-assembled meso-tetra(phenyl) and 4-sulfonatophenyl)porphyrins." Chem. Commun. (2003): 1588-1589.

131. Rubires, R.; Ferrera, J. –A. and Ribó, J. M. "Stirring effects on the spontaneous formation of chirality in the homoassociation of diprotonated meso-tetraphenylsulfonato porphyrin." Chem. Eur. 7 (2001): 436-446.

132. Zhang, L.; Yuan, J. and Liu, M. "Supramolecular chirality of achiral TPPS complexed with chiral molecular films." J. Phys. Chem. B 107 (2003): 12768-12773.

133. Jiang, S. and Liu, M. "Aggregation and induced chirality of an anionic meso-tetraphenylsulfonato porphyrin (TPPS) on a layer-by-layer assembled DNA/PAH matrix." J. Phys. Chem. B 108 (2004): 2880-2884.

134. Pasternack, R. F. and Collings, P. J. "Resonance light Scattering: A new technique for study chromophore aggregation." Sciences 269 (1995): 935-939.

135. Mati, N. C.; Ravikanth, M. Mazumdar, S. and Periasamy, N. "Fluorescence dynamic of noncovalently linked porphyrin dimers and aggregates." J. Phys. Chem. 99(1995): 17192-17197.

136. Akins, D. L.; Zhu, H. –R. and Guo, C. Absorption and raman scattering by

aggregated meso-tetrakis(p-sulfonatophenyl)porphine." J. Phys. Chem. 98 (1994) : 3612-3618.

137. Kubat, P.; Lang, K.; Janda, P. and Anzenbacher, P., Jr. "Interaction of porphyrins with a dendrimer templates: Self-aggregation controlled by pH." Langmuir 21 (2005): 9714-9720.

138. Tabata, M. and Tanaka, M. "A new method for the determination of the stability constant of metalloporphyrins: Use of the catalytic effect on mercury(II) on metalloporphyrin formation." J. Chem. Soc., Chem. Commun. (1985): 42-43.

139. Skoog, D. A.; West D. M.; Holler, F. J. and Crouch, S. R. Fundamental of Analytical Chemistry. 8th Ed. California: Thompson, 2004.

140. Dean, J. A. Analytical Chemistry Handbook New York: McGraw-Hill, 1995.

APPENDICES

APPENDIX A

PUBLICATIONS

Available online at www.sciencedirect.com
SCIENCE DIRECT
Talanta 64 (2004) 1183–1188
Talanta
www.elsevier.com/locate/talanta

A flow injection method for the analysis of tetracycline antibiotics in pharmaceutical formulations using electrochemical detection at anodized boron-doped diamond thin film electrode

N. Wangfuengkanagul, W. Siangproh, O. Chailapakul*

Department of Chemistry, Faculty of Science, Chulalongkorn University, 254 Phyathai Road, Patumwan, Bangkok 10330, Thailand

Received 1 March 2004; received in revised form 26 April 2004; accepted 26 April 2004
Available online 23 August 2004

Abstract

A method using flow injection (FI) with amperometric detection at anodized boron-doped diamond (BDD) thin films has been developed and applied for the determination of tetracycline antibiotics (tetracycline, chlortetracycline, oxytetracycline and doxycycline). The electrochemical oxidation of the tetracycline antibiotics was studied at various carbon electrodes including glassy carbon (GC), as-deposited BDD and anodized BDD electrodes using cyclic voltammetry. The anodized BDD electrode exhibited well-defined irreversible cyclic voltammograms for the oxidation of tetracycline antibiotics with the highest current signals compared to the as-deposited BDD and glassy carbon electrodes. Low detection limit of 10 nM (signal-to-noise ratio = 3) was achieved for each drug when using flow injection analysis with amperometric detection at anodized BDD electrodes. Linear calibrations were obtained from 0.1 to 50 mM for tetracycline and 0.5–50 mM for chlortetracycline, oxytetracycline and doxycycline. The proposed method has been successfully applied to determine the tetracycline antibiotics in some drug formulations. The results obtained in percent found (99.50–103.01%) were comparable to dose labeled.

Keywords: Tetracycline; Chlortetracycline; Oxytetracycline; Doxycylcine; Flow injection system; Anodized boron-doped diamond thin film electrode

1. Introduction

Tetracyclines are broad spectrum antibiotics for their high activity against nearly all gram-positive and gram-negative bacteria. Tetracycline, chlortetracycline, oxytetracycline and doxycycline are commonly used in food protection animals (including honeybee), because of their board spectrum activity and low production cost. However, tetracycline residues in milk or meat are also toxic and can cause allergic reactions in some hypersensitive individuals in human. Owing to their extensive use in infectious diseases therapy, there have been several analytical methods available for these compounds in pharmaceutical preparations, biological samples and milk samples. Official methods such as microbiological-based techniques can also provide both qualitative and quantitative analysis for tetracyclines [1–3]. These methods have characteristic disadvantages: microbiological tests are not only time consuming, laborious and expensive but also poor in term of sensitivity and selectivity. There are also some batch procedures which employ spectrophotometry [4,5], chemiluminescence [6–9], spectrofluorimetry [10], and electrochemical method [11].

Flow injection (FI) is a well-known tool that offers improvement in most batch methods, especially the high sample throughput. For the tetracyclines, there are some FI methods available with all types of detection such as spectrophotometry [12,13], chemiluminescence [14,15] and electrochemical method [16–18]. The spectrophotometric FI method detections [12,13] for tetracyclines are based on the formation of a color product by their reaction with 4-aminophenazone and hexacyanoferrate(III). However, this method has a limited concentration range. Under the optimized conditions, tetracyclines were determined in the ranges of 1–20 and 20–250 mg L^{-1}. The lowest limit of detection is 0.2 mg L^{-1} for doxycycline [12]. For the chemiluminescence method [14], a highly toxic and expensive agent,

* Corresponding author. Tel.: +66 2 218 7615; fax: +66 2 254 1309.
E-mail address: corawon@chula.ac.th (O. Chailapakul).

0039-9140/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2004.04.032

N-bromosuccinimide is used. The reported working range are 0.05–3.00 mg L^{-1} for tetracycline, 0.50–5.00 mg L^{-1} for oxytetracycline and 0.50–7.00 mg L^{-1} for doxycycline and chlortetracycline.

Electrochemical techniques such as amperometric and potentiometric detection are good alternative methods that are widely used in pharmaceutical applications. The techniques are usually easy to operate, fast and inexpensive. The sensitivity of electrochemical methods is often greater than the spectrophotometric method. For example, the detection limits using HPLC amperometric detection varied from 0.1 to 1 mg L^{-1} for tetracycline family [19].

Procedures in electroanalysis strongly depend on working electrode materials, so the searching and development of new electrode material is necessary. Boron-doped diamond (BDD) thin film electrodes have been emerged as new electrode material in the many fields in electrochemistry, especially in electroanalysis applications [20–29]. The most importance electrochemical properties are: (i) the wide working potential window in aqueous and non-aqueous media; (ii) a stable and low background current, leading to improved signal-to-background and signal-to-noise ratios; (iii) slight adsorption of polar molecules and long term stability of the response, leading to improved resistance to electrode deactivation and fouling. Recently, a diamond electrode has been chemically pretreated by electrochemical oxidation [30–32]. The anodized diamond electrodes retain the excellent properties of the as-deposited diamond electrode even though, the electrode surface of anodized diamond electrode is applied by a high anodic potential. Anodized diamond electrodes are suitable for determination of various analytes such as chlorophenol [30], dopamine and uric acid in the presence of ascorbic acid [31] and homocysteine [32]. These anodized electrodes have exhibited some attractive properties such as excellent stability and high reproducibility. In this present work, the anodized diamond electrode was used for the electrochemical determination of tetracyclines using cyclic voltammetry. Glassy carbon (GC) and as-deposited diamond electrodes were also used for comparison. The anodized diamond electrode was exploited as working electrode for amperometric determination of antibiotic drugs, using flow injection system.

2. Experimental

2.1. Chemicals and reagents

All reagents were analytical reagent grade. All solutions were prepared in ultrapure water. Standards (hydrochloric forms) of tetracycline, chlortetracycline, oxytetracycline and doxycycline were supplied by Sigma Chemical (USA). Potassium dihydrogen phosphate (BDH), disodium hydrogen phosphate, sodium hydroxide (Merck) and phosphoric acid 85% (J.T. Baker) were used to prepare the buffer solution. Phosphate buffer (0.1 M) solutions with pH ranges from 5.0 to 8.0, were prepared by dissolving the appropriate amounts of potassium dihydrogen phosphate and disodium hydrogen phosphate. Phosphoric acid and sodium hydroxide were used to adjust the desired pH that is out of the above range.

The standard tetracycline antibiotics were prepared by dissolving an appropriate amount of the tetracycline antibiotics in the buffer solution. The solutions were prepared daily.

2.2. Electrodes

The as-deposited BDD electrodes used in this work are from Professor Akira Fujishima' s Laboratory. The electrodes were prepared by deposition of the BDD thin films on highly conductive *n*-Si(1 1 1) substrate using the technique of microwave plasma-assisted chemical vapor deposition. Deposition was usually carried out for 10 h to achieve a film thickness of approximately 30 μm. The nominal B/C atomic ratio in the gas phase was 1:100, and the typical boron-doped level in the film was ca. 10^{21} cm^{-3}. The experimental conditions and the apparatus used for the diamond film growth have been described in detail elsewhere [23]. A piece of diamond was rinsed with ultrapure water prior to use for electroanalysis.

Anodized BDD electrode was prepared by treating an as-deposited BDD electrode in 0.1 M KOH solution. The potential was applied between 0 and 2.2 V versus Ag/AgCl using cyclic voltammetry for 30 min. The anodized BDD electrode was also rinsed with ultrapure water before use.

The GC electrode was purchased from Bioanalytical System, Inc. (area 0.07 cm^2). It was pretreated by sequential polishing with 1 and 0.05 μm of alumina/water slurries on felt pads, followed by rinsing with ultrapure water prior to use.

2.3. Cyclic voltammetric investigation

Electrochemical experiments were conducted out in a single compartment three-electrode glass cell. The BDD electrode was pressed against a smooth ground joint at the bottom of the cell, isolated by an *O*-ring vitron (area 0.07 cm^2). Ohmic contact was made by placing the backside of the Si substrate. The GC carbon was used as working electrode for the comparison. The Ag/AgCl with salt bridge and Pt wire were used as reference electrode and counter electrode respectively. The voltammetric measurement was performed with the three types of electrodes using Autolab Potentiostat 100 (Eco-Chemie, The Netherlands).

2.4. Flow injection with amperometric detection

The flow injection system consisted of a thin layer flow cell (Bioanalytical System, Inc), a 20 mL stainless steel loop of injection port (Rheodyne 7725), a peristaltic pump, and

electrochemical detection (Autolab potentiastat 100). The carrier solution (0.1 M potassium dihydrogen phosphate pH 2) was regulated at the flow rate of 1 mL min^{-1} by measuring the volume of carrier solution in 1 min. The thin layer flow cell consisted of a silicone rubber gasket as a spacer, a Ag/AgCl electrode as the reference electrode and a stainless steel tube as an auxiliary electrode and an outlet of the flow cell. The experiments were performed in a copper faradaic cage to reduce the electronic noise. All experiments were done at room temperature.

2.5. Sample preparation

The contents within the pharmaceutical capsule (as described on the label) were: 250 mg tetracycline hydrochloride™ (Tetracycline hydrochloride); 250 mg Aureomycin™ (chlortetracycline); 250 mg Oxycline™ (oxytetracycline); 100 mg Medomycin™ (doxycycline). The powder of twenty capsules of each drug was accurately weighed and homogenized so as to obtain the mean capsule weight. A portion of the homogenized powder corresponding to the mean capsule weight was then transferred into volumetric flask and dissolved in 0.1 M phosphate buffer (pH 2), and then mixed thoroughly. A portion of the sample solutions was filtrated through 0.45 mm nylon membrane. The filtrated solutions were further diluted using 0.1 M phosphate buffer (pH 2) to make the final concentration in the range of 0.96–5.15 mg mL^{-1} within the linear dynamic range. All tetracycline antibiotic solutions were protected from light by covering with aluminum foil and stored at 4 °C.

3. Results and discussion

3.1. Cyclic voltammetry

3.1.1. Voltammetric study

According to Fig. 1, the anodized BDD electrode (Fig. 1c) provided well-resolved irreversible oxidation cyclic voltammogram with the highest current responses in comparison to the as-deposited BDD and the glassy carbon electrodes, Fig. 1a and b respectively. These electrodes produced oxidative waves at +1.0 V versus Ag/AgCl, however the as-deposited BDD electrode produced no voltammetric peak shape in contrast to the results at the anodized BDD electrode. The results obtained from the other antibiotics, i.e., chlortetracycline, oxytetracycline and doxycycline were analogous to the results shown for tetracycline in Fig. 1. These results can be rationalized as follows. The surface of the BDD, after the anodizing treatment, is partly covered with oxygen atoms [30–32]. In acidic condition, all the tetracycline are positively charged. This therefore encourage electrostatic interaction between the negatively charged BDD anodized surface and the analytes. In contrast, the as-deposited BDD, the surface is non-polar (covered with hydrogen atoms), and therefore the electrostatic interaction

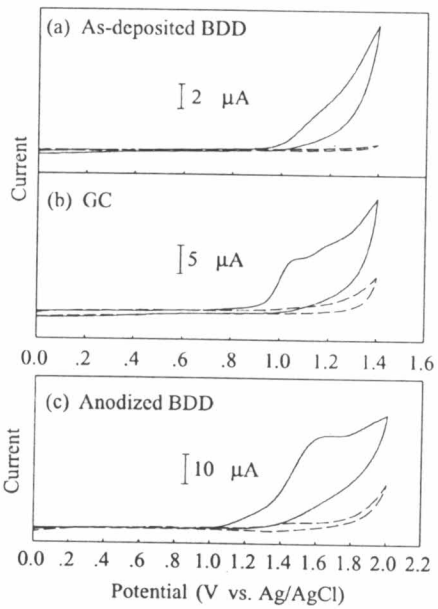

Fig. 1. Cyclic voltammetric results of 500 μM tetracycline (solid line) in 0.1 M potassium dihydrogen phosphate (pH 2) with the corresponding background current (dashed line) at: (a) as-deposited BDD; (b) glassy carbon; and (c) anodized BDD electrodes.

is less permitted. This may be the reason for the ill-defined peak current, in Fig. 1a, for example.

3.1.2. pH dependence

The effect of pH was investigated from pH 2–9 for all four compounds using anodized BDD electrode. A plot of pH versus Ep has been investigated. It was found that results were ambiguous, and the expected pK was inconsistant with the pK value of the dissociation in the literature.

For quantitative purposes, the information concerning about the current response was also investigated. It was found that in acidic buffer, tetracyclines provided the well-defined oxidative responses cyclic voltammograms. However, in neutral and alkaline buffer, ill-defined voltammetric waves were obtained. The current response decreased with increasing the pH. Results for all, the antibiotics demonstrated that pH 2 gave the highest current signal (Fig. 2). Therefore, pH 2 was chosen as the optimal pH for the rest of experiments.

It can be explained that tetracyclines are derived from a system of four membered rings arranged linearly with characteristic double bonds. They are amphoteric compounds with high polarity, and an isoelectric point between 4 and 6. From the literature [33,34], three dissociations have been observed for tetracyclines, with $pK_1 = 3.3$, $pK_2 = 7.5$ and $pK_3 = 9.4$. In strongly acidic pH, the tetracycline molecule exists in its fully protonated form as a singly charged cation. As proposed [30–32], the surface of anodized BDD electrode covered with the negative charge, electrostatically attracts the positive tetracycline. Hence, at pH 2 the positively charged tetracycline provided the highest response.

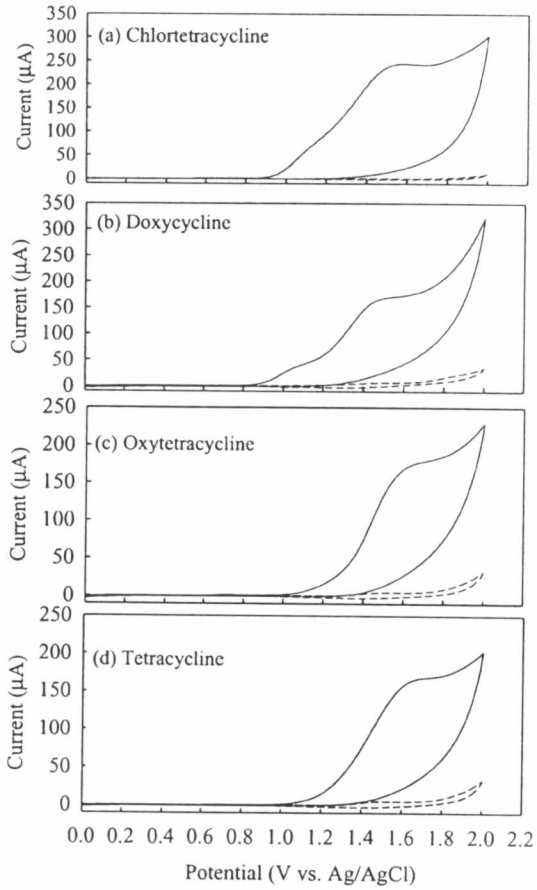

Fig. 2. Cyclic voltammograms at anodized BDD electrode vs. Ag/AgCl in 500 μM chlortetracycline, doxycycline, oxytetracycline, and tetracycline in 0.1 M phosphate buffer pH 2 (solid lines) and 0.1 M phosphate buffer pH 2 (dashed lines). The scan rate was 50 mV s^{-1}; area of electrode, 0.07 cm^2.

Moreover, the anodized diamond surface can be recovered by in situ reactivation (applying a high positive potential 2.6 V versus Ag/AgCl) during the flow injection measurement [30].

3.1.3. Scan rate dependence

The scan rate dependence study was carried out by varying the scan rate ranging from 0.01 to 0.3 V s^{-1}. From the result, we found for all four compounds, that the relationship between current and square root of scan rate was linear with the correlation coefficient of approximately 0.99 (data not shown). The results indicated that the oxidation currents are controlled by the diffusion of tetracyclines in the interfacial reaction zone.

3.2. Flow injection with amperometric detection

3.2.1. Hydrodynamic voltammetry

Hydrodynamic voltammogram was obtained from the average of three injections at aliquot of 20 μL of 100 mM tetracycline antibiotic solutions in the flow injection system. In this case the applied potential was gradually increased from 1.1 to 2.0 V versus Ag/AgCl. The carrier solution was 0.1 M potassium dihydrogen phosphates (pH 2). Fig. 3a shows the hydrodynamic voltammogram of tetracycline antibiotics with the corresponding background currents. The hydrodynamic voltammogram of tetracycline antibiotics did not exhibit the sigmoidal shape of the signal versus potential probably due to the high oxidation potential of tetracycline. To obtain the optimum potential, we calculated the S/B ratios from the results in Fig. 3a at each potential and plotted the ratios versus potentials (Fig. 3b). The maximum S/B ratio was found at 1.5 V (versus Ag/AgCl) for chlortetracycline and at 1.6 V (versus Ag/AgCl) for tetracycline, oxytetracycline and doxycycline. Hence, these optimum potentials were used for the quantification experiments in amperometric flow injection.

3.2.2. Analytical performance of the FI system at anodized BDD electrode

A series of analytical features were performed on the proposed method for the quantification of tetracycline, chlortetracycline, oxytetracycline and doxycycline. Calibration parameters and the linear ranges for all the drugs are summarized in Table 1. The limit of detection (3S/N) was 10 nM for all compounds. To obtain the repeatability, 10 replicates of injections were carried out at three concentrations (5, 10, 50 mM). The peak variability defined as the relative standard deviation (R.S.D. %), were found to be 1.3–1.7 (for tetracycline hydrochloride), 2.4–3.0 for chlortetracycline, 1.6–3.0

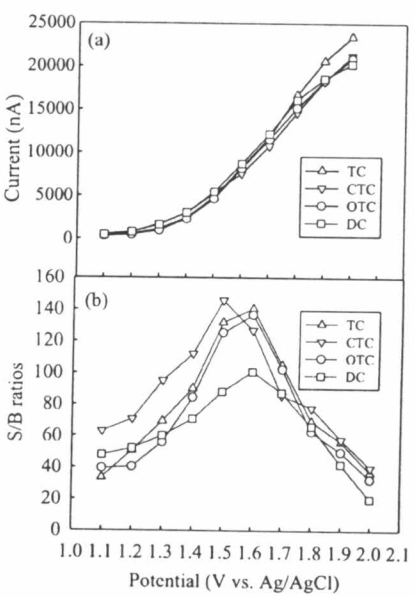

Fig. 3. Hydrodynamic voltammetric results for 100 μM tetracycline antibiotics. (a) (△): tetracycline, TC; (▽): chlortetracycline, CTC; (○): oxytetracycline, OTC; and (□): doxycycline, DC in 0.1 M potassium dihydrogen phosphate (pH 2), using 0.1 M potassium dihydrogen phosphate (pH 2) as carrier solution. The flow rate was 1 mL min^{-1}. (b) Hydrodynamic voltammogram of signal-to-background ratios.

Table 1
Regression parameters and linear range for the determination of tetracycline antibiotics by flow injection with amperometric detection using anodized boron-doped diamond electrode ($n = 2$)

Analytes	Linear range (μM)	a	b	r^2
Tetracycline	0.1–50	110.0 ± 5.9	192.1 ± 5.0	0.997
Chlotetracycine	0.5–50	76.9 ± 3.9	199.7 ± 29.7	0.997
Oxytetracycline	0.5–50	90.2 ± 3.0	165.2 ± 28.9	0.999
Doxytetracycline	0.5–50	96.2 ± 3.8	213.7 ± 48.9	0.997

$ay = ax + b$, where y: current (nA); x: concentration (μM); a: slope (nA/μM); b: intercept (nA).

Table 2
Determination of tetracycline antibiotics in drug formulations ($n = 2$)

Drug formulations	Label amount (mg)	Found (mg)	Found (%)
Tetracycline	250	249.4 ± 1.7	99.8 ± 0.7
Aureomycine	250	249.7 ± 1.3	99.87 ± 0.5
Oxycline	250	254.6 ± 2.5	101.85 ± 1.0
Medeomycin	100	98.4 ± 1.0	98.4 ± 1.0

for oxytetracycline and 1.7–2.7 for doxycycline. The stability of the amperometric response using flow injection at the anodized BDD electrode was obtained by conducting 100 repetitive injections of 50 mM chlortetracycline. It was observed that the peak variation was about 3.4%. The throughput of sample is approximately seventy injections per hour.

3.2.3. Application

Our method was applied for determination of each compound in four drug formulations using the standard addition method. The results are presented in Table 2. The percentages of recovery indicate that the amounts are comparable between the labeled quantity and the value obtained from the proposed method.

4. Conclusion

Anodized treatment has significantly improved the analytical performance of the BDD thin film for the analysis of tetracycline drugs. Current signal of the anodized BDD electrode is greater than the current signal of the glassy carbon electrode. It was observed from the cyclic voltammetric study that pH strongly influenced the current response of the voltammograms with pH 2 being selected for the method development, since the highest current was obtained with a well-defined voltammetric signal.

Amperometric detections of four compounds, i.e., tetracycline, chlortetracycline, oxytetracycline and doxycycline was employed in the method development, using flow injection. The developed method has been successfully applied to determine four types of tetracyclines in various pharmaceutical formulations. The tetracycline antibiotics content of the drug was found to be in excellent agreement with the declared amount on the label.

Acknowledgements

This research was supported by the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0209/2544) and Methee Vijai Grant, the Ratchadaphisek Somphot Endowment Grant and TJTTP-OECF. Special thanks are extended to Professor A. Fujishima (The University of Tokyo) for the BDD electrodes used in this research. The authors also would like to thank Dr. Nathan Lawrence (New Mexico State University) for editing the manuscript.

References

[1] The United Pharmacopoeia, 21st revision, Easton PA, Mack Printing Company, USP XXI, 1985
[2] The British Pharmacopeia, HMSO, London, 1988
[3] J. Kurittu, S. Lonnberg, M. Virta, M. Karp, J. Agric. Food. Chem. 48 (2000) 3372.
[4] K.M. Emara, H.F. Askal, G.A. Saleh, Talanta 38 (1991) 1219.
[5] U. Saha, A.K. Sen, T.K. Das, S.K. Bhowal, Talanta 37 (1990) 1193.
[6] X.R. Zhang, W.R.G. Baeyens, A. Vandenborre, G. Vanderweken, A.C. Calokerinos, S.G. Schulman, Analyst 120 (1995) 463.
[7] H. Han, Z. He, Y. Zeng, Anal. Sci. 15 (1999) 467.
[8] S. Marczynski, Biopolymers 57 (2000) 365.
[9] A. Pena, L.P. Palilis, C.M. Lino, M.I. Silveira, A.C. Calokerinos, Anal. Chim. Acta 405 (2000) 51.
[10] S. Croubels, C.V. Peteghem, W. Baeyens, Analyst 119 (1994) 2713.
[11] S. Sabhawal, K. Kishore, P.N. Moorthy, J. Pharm. Sci. 77 (1988) 78.
[12] R. Karlicek, P. Solich, Anal. Chim. Acta 285 (1994) 9.
[13] S.M. Sultan, F.E.O. Suliman, S.O. Duffuaa, I.I. Abu-Abdoun, Analyst 117 (1992) 1179.
[14] S.A. Halvatzis, M.M. Timotheou-Potamia, A.C. Calokerinos, Analyst 118 (1993) 633.
[15] A.B. Syropoulos, A.C. Calokernos, Anal. Chim. Acta 255 (1991) 403.
[16] C.M.C.M. Couta, J.L.F.C. Lima, M. Conceicao, B.S.M. Montenegro, S. Reis, J. Phram. Biomed. Anal. 18 (1998) 527.
[17] H. Ji, E. Wang, Analyst 113 (1988) 1541.
[18] W. Oungpipat, P. Southwellkeely, P.W. Alexander, Analyst 120 (1995) 1559.
[19] A.G. Kazemifard, D.E. Moore, J. Pharm. Biomed. Anal. 16 (1997) 689.
[20] N. Spataru, B.V. Sarada, E. Popa, D.A. Tryk, A. Fujishima, Anal. Chem. 73 (2001) 514.
[21] J. Xu, G.M. Swain, Anal. Chem. 70 (1998) 1502.
[22] M.D. Koppang, M. Witek, J. Blau, G.M. Swain, Anal. Chem. 71 (1999) 1188.
[23] B.V. Sarada, T.N. Rao, D.A. Tryk, A. Fujishima, Anal. Chem. 72 (2000) 1632.
[24] N. Wangfuengkanagul, O. Chailapakul, J. Pharm. Biomed. Anal. 28 (2002) 841.
[25] N. Wangfuengkanagul, O. Chailapakul, Talanta 58 (2002) 1213.
[26] T.N. Rao, B.V. Sarada, D.A. Tryk, A. Fujishima, J. Electroanal. Chem. 491 (2000) 175.
[27] M.C. Granger, J. Xu, J.W. Strojek, G.M. Swain, Anal. Chim. Acta 397 (1999) 145.
[28] T.A. Ivandini, B.V. Sarada, C. Terashima, T.N. Rao, D.A. Tryk, H. Ishiguro, Y. Kubota, A. Fujishima, J. Electroanal. Chem. 521 (2002) 117.

[29] T.N. Rao, B.H. Loo, B.V. Sarada, C. Terashima, A. Fujishima, Anal.Chem. 74 (2002) 1578.
[30] C. Terashima, T.N. Rao, B.V. Sarada, D.A. Tryk, A. Fujishima, Anal. Chem. 74 (2002) 895.
[31] E. Popa, Y. Kubota, D.A. Ttyk, A. Fujishima, Anal. Chem. 72 (2000) 1724.
[32] O. Chailapakul, W. Siangproh, B.V. Sarada, C. Terashima, T.N. Rao, D.A. Tyrk, A. Fujishima, Analyst 127 (2002) 1164.
[33] X. Ding, S. Mou, J. Chromtogr. A 897 (2000) 205.
[34] J.H. Knox, J. Jurand, J. Chromatogr. 186 (1979) 763.

Chemistry Letters Vol.34, No.1 (2005)

Change in Conformation of J-aggregate 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin (H_2TPPS) by Addition of Nonionic Surfactant (Triton X-100)

Nattakarn Wangfuengkanagul and Masaaki Tabata*†

Department of Chemistry, Faculty of Science, Chulalongkorn University, 254 Payathai Rd. Patumwan, Bangkok 10330, Thailand

†*Department of Chemistry, Faculty of Science and Engineering, Saga University, 1 Honjo-machi, Saga 840-8502*

(Received September 21, 2004; CL-041099)

Drastic conformational change of J-aggregate of 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin (H_2TPPS) was observed by addition of nonionic surfactant (Triton X-100) in acidic medium. The CD spectra of the aggregate $(H_4TPPS)_n$ changed to opposite signed CD spectra in the presence of Triton X-100. The interaction of $(H_2TPPS)_n$ with Triton X-100 was studied by the measurements of UV–vis and fluorescence spectra as well as CD spectra at different concentrations of Triton X-100.

Molecular aggregates in which monomers are arranged in a regular form are of particular interest because of their unique electronic and spectroscopic properties.[1–3] There are two important kinds of molecular aggregation: J- and H-aggregates arranged in different way. J-aggregates exhibit a red-shift in absorption spectra and are one-dimensional molecular arrangement in which the transition moments of individual monomers are aligned parallel to the line joining their centers (end-to-end arrangement or side-by-side arrangement). H-aggregates exhibit a blue shifted absorption band and monomers are aligned parallel to each other but perpendicular to the line joining their centers (face-to-face arrangement). 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin (H_2TPPS) that is one of water-soluble porphyrins can form aggregates in acidic solution[4–6] or in high ion strength containing inorganic cations.[7] For the recent years, there are many papers studied on aggregation by addition of cationic molecules, e.g. cationic surfactants as cetyltrimethyl ammonium bromide (CTAB),[8] cationic dyes cyanide (i.e. 3,3′-diethyloxacarbocyanine iodide {2-[3-(3-ethyl-2,3-dihydrobenzoxazolylidene)propenyl]-3-ethylbenzoxazolium iodide} (DiOC2(3) and 3,3′-dihexyloxycarbocyanine iodide {2-[3-(3-hexyl-2,3-dihydrobenzoxazolylidene)propenyl]-3-hexyl-benzoxazolium iodide} (DiOC6(3)).[9] The J-aggregate of H_2TPPS was also induced by polymers, e.g. polylysine used as a template for aggregate.[10] It is well known that H_2TPPS is an achiral compound in the monomer form while J-aggregate, $(H_4TPPS)_n$, is a chiral compound that gives induced CD spectra. It has been suggested that the sign of the CD spectra of the J-aggregates is changed by stirring-direction of solution[11,12] or by addition of enantiomer compounds, e.g. D- or L-tryptophan.[13,14] We have found that nonionic surfactant (Triton X-100) alters CD spectra of J-aggregate to opposite sign. In this paper we will describe the interaction between the J-aggregate and Triton X-100 and change in conformation of the J-aggregate with different concentration of Triton X-100.

H_2TPPS (TCI), Triton X-100 (ICN Biochemicals, Inc.), acetic acid (Wako Chemical), and sodium perchlorate (Wako Chemical) were used without further purification. The solutions were prepared with double distilled water (Milli-Q, Millipore). UV–vis spectra were measured by a UV–vis spectrophotometer (JASCO V-550) at 25 °C. CD spectra of J-aggregate, $(H_4TPPS)_n$, were measured by a CD spectrophotometer (JASCO Spectrophotometer, Power Supply 91N, Japan) three times and averaged. Fluorescence spectra were measured by a fluorescence spectrophotometer (Hitachi F-4500) under excitation at 490 nm. J-aggregate, $(H_4TPPS)_n$, was prepared in 0.01 mol dm^{-3} (=M) CH_3COOH and 0.1 M $NaClO_4$, and kept in an incubator at 25 °C for 1 day before measurements.

In acidic medium, H_2TPPS turns to diacid or protonated form (H_4TPPS) ($pK_{a3} = 4.76$; $pK_{a4} = 4.99$).[15] The diacidic form of H_4TPPS has a positive charge at four protonated pyrroles that interacts with the peripherally sulfonatophenyl anionic groups and induces the aggregation of H_4TPPS by ion-pair formation or electrostatic interaction that causes charge-neutralization. The UV–vis absorption spectra of $(H_4TPPS)_n$ exhibited the characteristic peaks of J-aggregation at 490 and 706.5 nm in acetic acid solution. Moreover, it has peaks at around 434 and 645 nm that are attributed to monomer H_4TPPS. The J-aggregate species were also measured by fluorescence and polarized fluorescence spectra. Fluorescence spectrum of $(H_4TPPS)_n$ showed the maximum emission spectrum at around 716 nm in acidic medium. The polarized fluorescence spectra of $(H_4TPPS)_n$ were measured as a function of excitation wavelength. A positive polarization peak was observed at around 490 nm for $(H_4TPPS)_n$. UV–vis spectra of $(H_4TPPS)_n$ were also investigated at different concentrations of Triton X-100. The UV–vis spectra depended on the concentrations of Triton X-100, especially, concentration below, near or above critical micelle concentration (cmc.) of Triton X-100 (Figure 1).

Effects of the concentrations of Triton X-100: (a) *(Triton X-100 below cmc. (0.001% v/v).* The UV–vis spectrum of H_4TPPS solution exhibited absorption maximum peaks at 434 and 490 nm. The peak intensity at the 490 nm decreased in the

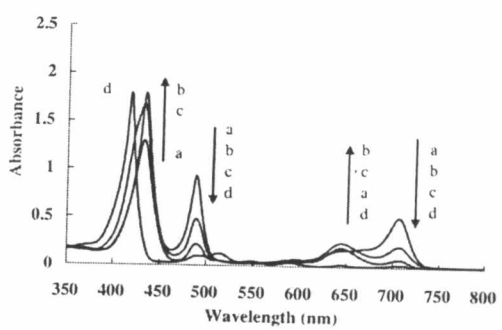

Figure 1. UV–vis spectra of H_4TPPS (1×10^{-5} M) in the presence of Triton X-100 of (a), 0% (pH 3.3); (b), 0.001% (pH 3.3); (c), 0.1% (pH 3.3) and (d), 5% in v/v (pH 3.6) in 0.01 M acetic acid and 0.1 M sodium perchlorate.

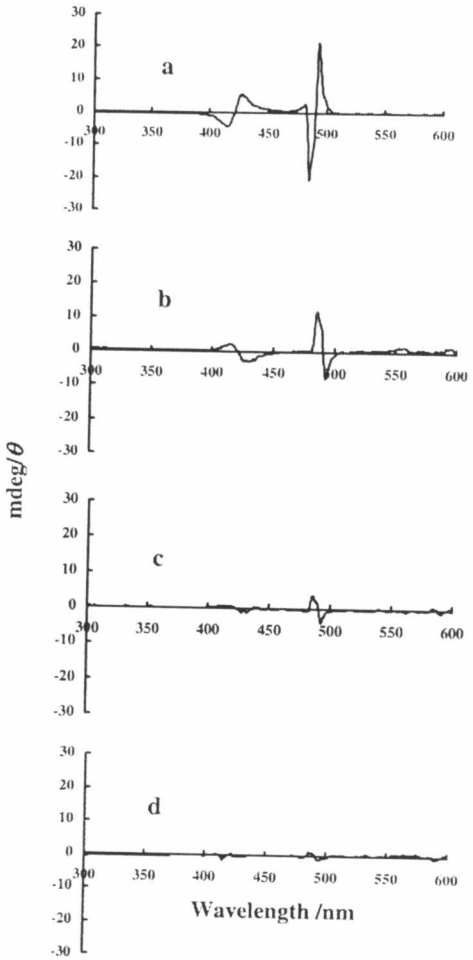

Figure 2. CD spectra of H_4TPPS (1×10^{-5} M) in the presence of Triton X-100 of (a), 0% (pH 3.3); (b), 0.001% (pH 3.3); (c), 0.1% (pH 3.3) and (d), 5% in v/v (pH 3.6) in 0.01 M acetic acid and 0.1 M sodium perchlorate.

presence of Triton X-100 that is attributed to the interaction of Triton X-100 with $(H_4TPPS)_n$ and to the dissociation of $(H_4TPPS)_n$ into monomer H_4TPPS. (b) *Triton X-100 near cmc. (0.1% v/v).* Triton X-100 forms premicelle under the conditions. The UV–vis spectrum gives a board peak that has an absorption maximum wavelength at around 430 nm. The peak is a mixture of two species, $(H_4TPPS)_n$ and H_2TPPS incorporated into the micelle, that exhibited two maximum fluorescence spectra at 650 and 716 nm. (c) *Triton X-100 above cmc. (5% v/v).* Triton X-100 forms micelle under the conditions. UV–vis spectra showed a maximum wavelength at 418.5 nm, while the peak of 434 nm disappeared and the peak at 490 nm decreased. The results suggest that $(H_4TPPS)_n$ dissociates to monomer and is solubilized into the micelle as the form of deprotonated free base porphyrin (H_2TPPS).

Figure 2 shows change in CD spectra of J-aggregate, $(H_4TPPS)_n$ in the presence of Triton X-100 at different concentrations under the same conditions as UV–vis spectral measurements. The CD spectrum of the J-aggregate changed to opposite sign by addition of Triton X-100 at the concentration below cmc. (Figure 2b) and the intensity decreased with increase of Triton X-100 (Figure 2c) and finally disappeared at the concentration above cmc. The turnover of CD spectra by addition of Triton X-100 implies a specific interaction of Triton X-100 with $(H_4TPPS)_n$.

From the changes in UV–vis and CD spectra of the J-aggregate in the presence of different concentrations of Triton X-100, it is expected that the following two reactions occur. One is the interaction of J-aggregate with Triton X-100, leading to the change in chirality of $(H_4TPPS)_n$, and the other is the incorporation of the J-aggregate into the micelle of Triton X-100 after releasing proton to form monomer of H_2TPPS. The change in chirality of the J-aggregate could be explained by the following possible mechanism. H_4TPPS molecules assemble each other through their induced dipole moments to form linear or helical J-aggregate by side-by side arrangement of H_4TPPS.[7] Triton X-100 binds to the J-aggregate, $(H_4TPPS)_n$, on the outside of the J-aggregate, and the bound Trion X-100 molecules assemble each other, that may cause the change in chirality of the J-aggregate, $(H_4TPPS)_n$ to opposite direction. The detailed reaction mechanism will be reported elsewhere.

This research was supported by the Thailand Research Fund through the Royal Golden Jubilee Ph. D. Program (Grant No. PHD/0209/2544) and Association of International Education, Japan (AIEJ) scholarship and Grand-in-Aid (No. 15350046) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

1 R. F. Khairutdinov and N. Serpone, *J. Phys. Chem. B*, **103**, 761 (1999).
2 H. Kano and T. Kobayashi, *Bull. Chem. Soc. Jpn.*, **75**, 1071 (2002).
3 L. Kelbauskas, S. Bagdonas, W. Dietel, and R. Rotomskis, *J. Lumin.*, **101**, 253 (2003).
4 O. Ohno, Y. Kaizu, and H. Kobayashi, *J. Chem. Phys.*, **99**, 4128 (1993).
5 R. F. Pasternack, C. Fleming, S. Herring, P. J. Collings, J. dePaula, G. DeCastro, and E. J. Gibbs, *Biophys. J.*, **79**, 550 (2000).
6 R. F. Pasternack, P. R. Huber, P. Boyd, G. Engasser, L. Francesconi, E. Gibbs, P. Fasella, G. C. Venturo, and L. deC. Hinds, *J. Am. Chem. Soc.*, **94**, 4511 (1972).
7 C. Maiti, S. Mazumdar, and N. Periasamy, *J. Porphyrins Phthalocyanines*, **2**, 369 (1998).
8 A. S. R. Koti, J. Taneja, and N. Periasamy, *Chem. Phys. Lett.*, **375**, 171 (2003).
9 A. S. R. Koti and N. Periasamy, *J. Mater. Chem.*, **12**, 2312 (2002).
10 J. Crusats, J. Claret, I. Díez-Pérez, Z. El-Hachemi, H. García-Ortega, R. Rubires, F. Sagués, and J. M. Ribó, *Chem. Commun.*, **2003**, 158.
11 A. S. R. Koti and N. Periasamy, *Chem. Mater.*, **15**, 369 (2003).
12 R. Rubires, J.-A. Farrera, and J. M. Ribó, *Chem.—Eur. J.*, **7**, 436 (2001).
13 N. C. Maiti, S. Mazumdar, and N. Periasamy, *J. Phys. Chem. B*, **102**, 1528 (1998).
14 L. Zhang, J. Yuan, and M. Liu, *J. Phys. Chem. B*, **107**, 12768 (2003).
15 M. Tabata and M. Tanaka, *J. Chem. Soc., Chem. Commun.*, **1985**, 42.

Published on the web (Advance View) December 4, 2004; DOI 10.1246/cl.2005.38

APPENDIX B

DEFINITION AND CALCULATION [139, 140]

Accuracy

Accuracy refers to the closeness of agreement between the true value of the analyte concentration and the mean result that is obtained by applying the experiment procedure. There are various ways and units in which the accuracy can be expressed. Recovery is a term often used to describe accuracy, the equation for recovery is:

$$\%\text{Recovery} = \frac{\text{Measured value}}{\text{True value}} \text{x} 100$$

If the recovery were poor, it would be a good indication of a problem with the method. Standard addition is one technique that can be used to determine the recovery of spiked analyte. Relative error is the other term that can express the accuracy. The equation is shown below:

$$\%\text{error} = \frac{(\text{Measured value} - \text{True value})}{\text{True value}} \text{ x } 100$$

Precision

The precision of an analytical procedure expresses the clossness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducible.

Repeatability is one method that is used to describe precision. It is done by multiple injections of a homogeneous sample and calculation of the relative standard deviation (%RSD). The equation for %RSD is shown below:

$$\%\text{RSD} = \frac{\text{standard deviation}}{\text{Mean}} \text{ x } 100$$

Repeatability also expresses the precision under the same conditions over a short interval time. Repeatability is also termed intra-assay precision.

Intermediate precision expresses within-laboratories variations: different day (inter-assay), different analytes, different equipment, etc.

Reproducibility expresses the precision between the laboratories variations: (collaborative studies, usually applied to standardization of methodology)

Linearity (Linear range)

A linear range is the concentration range that can be determined with a linear calibration curve. The lower limit of the dynamic range is generally considered the limit of detection. The upper end of the range is usually taken as the concentration at which the analytical signal or the slope of the calibration curve deviates by a specified amount from the linear relationship. The working sample concentration and samples tested for accuracy should be in the linear range.

Sensitivity

Sensitivity is the change in the analytical response divided by the corresponding change in the concentration of a standard (calibration) curve, i.e. the slope of the analytical calibration.

Limit of detection (LOD)

The detection limit of a method is the lowest analyte concentration that can be determined to be different from an analyte blank. There are numerous way that detection limit have been defined. An example, is the lowest analyte concentration that is above the noise level of the system, typically, three time the noise level (S/N = 3) or the lower limit concentration of the linear dynamic range. This term is used to describe low analyte concentrations (< 10 μM).

For high analyte concentrations, the detection limit is defined as the lowest concentration that provides a signal to background ratio S/B of three. The equation of S/B ratio is shown below:

$$\text{S/B ratio} = \frac{(\text{total signal} - \text{blank signal})}{\text{blank signal}}$$

Limit of quantitation (LOQ)

The limit of quantitation (LOQ) is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The term limit of quantitation is preferred to limit of determination to differentiate it from LOD. LOQ has been defined as 3 times higher than the LOD.

Formal dissociate constant of monobasic weak acid (K_a)

$K_a = [H^+][A^-]/[HA]$; $pK_a = -\log[H^+][A^-]/[HA]$

K_1, K_2, K_3, K_i, K_n = Successive formal dissociate constants of basic weak acid H_nA

$K_1 = [H^+][H_{n-1}\ A^{-1}]/[H_nA]$; $pK_1 = -\log [H^+][H_{n-1}\ A^{-1}]/[H_nA]$

$K_2 = [H^+][H_{n-2}\ A^{-2}]/[H_nA]$; $pK_1 = -\log [H^+][H_{n-2}\ A^{-2}]/[H_nA]$

$K_3 = [H^+][H_{n-3}\ A^{-3}]/[H_nA]$; $pK_1 = -\log [H^+][H_{n-3}\ A^{-3}]/[H_nA]$

$K_i = [H^+][H_{n-i}\ A^{-i}]/[H_nA]$; $pK_1 = -\log [H^+][H_{n-i}\ A^{-i}]/[H_nA]$

VITA

Name : Miss Nattakarn Wangfuengkanagul

Date of Birth : 13 May 1977

Place of Birth : Bangkok, Thailand

Gender : Female

Educations :

1995-1999	Bachelor Degree of Science (Industrial Chemistry, 2nd honor), King Mongkut's Institute of Technology Ladkrabang
1999-2002	Master Degree of Science (Analytical Chemistry), Chulalongkorn University
2002-2006	Post graduate study leading to a Doctoral of Philosophy (Analytical Chemistry), Chulalongkorn University

Research scholarships

- The Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (Grant No. 0209/2544, 2002-2006)
- The Association of International Education, Japan (AIEJ) scholarship (2003-2004)

Publications

1. Wangfuengkanagul, N.; Siangproh, W. and Chailapakul, O. "A flow injection method for the analysis of tetracycline antibiotics in pharmaceutical formulations using electrochemical detection at anodized boron-doped diamond thin film electrode." Talanta, 64(2004): 1183-1188.
2. Wangfuengkanagul, N. and Tabata, M. "Change in conformation of J-aggregate 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin (H_2TPPS) by addition of nonionic surfactant (Triton X-100)." Chem. Letts., 34 (2005): 38-39.