Acta Pharmaceutica Turcica
XLI (3) (141-148) 1999

AMPEROMETRIC MICROELECTRODE DESIGN BY RHODIUM DEPOSITION FOR IMPROVED NITRIC OXIDE MEASUREMENT

GELİŞTİRİLMİŞ NİTRİK ASİT ÖLÇÜMÜ İÇİN SODYUM DEPOZİSYONUYLA AMPEROMETRİK MİKROELEKTROD DİZAYNI

EMRAH KILINÇ[1], TAYFUN DALBASTI[2], GÜNAY YETİK[1], KAĞAN KERMAN[1], BURCU MERİÇ[1], MEHMET ÖZSÖZ[1]*

[1]Ege University, Faculty of Pharmacy,
[2]Ege University, Faculty of Medicine, 35100 Bornova - Izmir / TURKEY

Non-enzymatic, amperometric sensors are prepared by the deposition of rhodium (Rhodium Deposited-RhDp) to modify the surface of platinum microelectrodes (130 μm diameter) for nitric oxide (NO) monitoring at +0.55V (vs Ag/AgCl reference electrode). NO is an endogeneously synthesized biological mediator of great physiological and pathophysiological importance. In the detection of NO, amperometry has more spatial sensitivity than spectroscopic methods as micro- or nanoelectrodes of very small sizes (10 nm - 50 μm diameter) can be used. In literature amperometric NO detection is used to be done at around +0.90Volts, usually with additional membranes for the elimination of interferences. Selectivity could be improved with these additional membranes while compromising from sensitivity and response time. Besides, identical (electrode preparation by dip-coating with additional membranes is not a simple repeatable procedure and the sensors need to be calibrated individually. In this study we compared the performance of RhDp and plain (bare platinum) sensors at +0.55V with current-time recordings and calibration plots. Our results showed that at +0.55V, much lower than +0.90V, satisfactory current-time recordings and calibration plots for NO can be obtained with RhDp sensors while the plain sensors gave poor responses. It was also observed that interferring current due to the presence of ascorbate at physiological concentration (0,1 mM) has decreased considerably without additional membranes just by lowering the working potential to +0,55V. The remaining interference may be eliminated by employing thinner additional membranes without much deteriorating the response characteristics.

Keywords : *Nitric oxide; Rhodium deposition; Amperometry; Electrocatalysis*

+0.55 V 'da (Ag/AgCl referans elektroduna karşı) nitrik oksit (NO) tayini için platin mikroelektrotların (çap 130μm) yüzeyini rodyum depolanmasıyla geliştirerek (rodyum depolanmış = RhDP) enzimsiz, amperometrik sensörler hazırlandı. NO, büyük fizyolojik ve patofizyolojik önemi olan, endojen sentezli, biyolojik bir mediyatör maddedir. NO tayininde, çok küçük boyutda (çapı 10nm-50μm) nano- veya mikroelektrotlar kullanılabildiği için amperometri, spektroskopik metodlara kıyasla daha geniş bir duyarlılık aralığına sahiptir. Literatürde amperometrik NO tayini , genellikle girişimlerin yok edilmesi için ilave membranların eklenmesiyle , +0.90 V civarında yapılmıştır. Bu ilave membranlarla, duyarlılık ve cevap zamanındaki gerileme göz ardı edilerek , seçicilik geliştirilebilir. Bunun yanısıra , ilave membranlarla daldırarak kaplama yöntemiyle benzer elektrotların üretimi basit tekrarlanabilir bir işlem değildir ve sensörler tek tek kalibre edilmelidir. Çalışmamızda RhDP ve düz (çıplak platin) sensörlerin, +0.55 V 'da akım-zaman kayıtları ve kalibrasyon eğrilerini kullanarak performanslarını kıyasladık. RhDP sensörler +0.90 V 'da elde edilen cevapları +0.55 V 'da da vermiştir. Oysa düz sensörler aynı koşullarda zayıf cevaplar vermiştir. Aynı zamanda, fizyolojik konsantrasyonda (0,1 mM) askorbat enjeksiyonuyla oluşturulan girişim akımının, ilave membran olmadan sadece çalışma potansiyalinin +0.55 V 'a düşürülmesiyle gözle görülür biçimde azaldığı gözlemlenmiştir. Kalan girişim ise cevap akımı karakteristiklerini fazla etkilemeyecek şekilde daha ince ilave membranların kullanımıyla ortadan kaldırılabilir.

Anahtar Kelimeler : *Nitrik asit; Rodium depozisyonu; Amperometri; Elektrokataliz*

*Correspondence

Introduction

NO is involved in a wide range of physiological processes in living tissue. NO, the oxidant radical, as a mediator may play a role in the pathophysiology seen in persistent pulmonary hypertension of the newborn(1). In another study it shown that NO takes part in the activation effect on tumor necrosis factor-alpha which automatically inhibited the activation of transcription factor NF-kappa B(2). Among several other functions NO is responsible for hypoxia and reoxygenation injury due to its free radical nature and high reactivity with the superoxide radical to yield peroxynitrite, an oxidant molecule(3). NO was also found to be involved in the gastroprotection (against ethanol-induced gastric mucosal lesions) induced by cholecystokinin-8 and pentagastrin(4). On the other hand, NO is known to be the mediator of potential importance in numerous physiologic and inflammatory processes in the lung(5). In the past few years numerous studies were published about the importance of NO(6-41). There were some techniques recommended for the determination of NO(42). Electrochemistry has been employed often in NO detection(43-46). An amperometric sensor using reduction current was also employed in NO detection(19). The tip of the sensor was covered with a hydrophobic membrane and contained an internal electrolyte. Platinum was used for working and counter (auxiliary) electrodes and Ag/AgCl as the reference electrode. The NO that diffused to the working electrode was first oxidized to NO^+, which was then reduced to NO, and the reduction current was determined. Interferences were considerably less as observed in this method but the sensor construction was not simple as a hydrophobic membrane and an internal electrolyte of potassium bromide and sulfuric acid were employed. A spectroscopic method was proposed for NO detection(18). A mammalian enzyme, ferrochelatase, has [2FE-2S] cluster which is destructed by NO. UV-visible absorption spectroscopy of the enzyme incubated with NO indicates a rapid loss of the visible absorption spectrum. Another electrochemical determination of NO was proceeded by employing a Clark-type NO-sensitive electrode where an essential factor of NO synthases, tetrahydrobiopterin, was found to induce rapid oxidation of NO(13). A fiberoptic sensor was constructed for the determination of NO and response characteristics were examined(47). O-phenylenediamine(OPD)-modified carbon fiber electrodes for amperometric detection of NO by direct oxidation were also fabricated(48). Working electrodes used were 30 μm diameter carbon fibers modified with o-phenylenediamine and Nafion. Amperometric measurements were performed at +0.90V(vs. Ag/AgCl reference). Nitrite, ascorbate, dopamine interferences at + 0.90V were decreased by the use of o-phenylenediamine and Nafion coatings. But these electropolymerization and coating procedures of the two membranes brought additional steps in the fabrication of the amperometric electrodes. Our study demonstrates a very simple amperometric electrode construction when compared with other initial amperometric electrode configurations(19,48). With the use of electrocatalytic rhodium(Rh) deposition on platinum microelectrodes, it was possible to obtain satisfactory results at a working potential of +0.55V. Electrocatalytic effect of Rh and some other

VIII B group metals have been discussed previously in detection of some other compounds(49-80). According to our literature survey electrodeposition of Rh or the other VIII B group metals have not been employed in amperometric NO determination. Response currents of our Rh-modified electrodes compared with that of bare platinum microelectrodes. Our findings showed that Rh particles play an excellent electrocatalytic role on the oxidation of NO as poor responses are obtained with bare Pt microelectrodes at +0.55V. Interferring effect of a very common interference, ascorbic acid, was also studied at physiological concentration. The ascorbate interference was decreased by lowering the working potential from +0.90V to +0.55V, but could not be eliminated. With the use of additional membranes more satisfactory results may be obtained.

Materials and Methods

Reagents

Sodium nitrite ($NaNO_2$) and 95-98 % (w/w) H_2SO_4 were obtained from Merck and were used without further purification. Phosphate buffer (pH=7.4) containing 1.36 g/L KH_2PO_4 and 6.96 g/L K_2PO_4 was prepared daily. Rh atomic absorption solution (1000 ppm) was obtained from Aldrich and used after dilution to 100 ppm with distilled water. Teflon-insulated, multistranded Pt wire (130 μm id) (10% Ir-90% Pt) was purchased from Medwire®. Potassium iodide (KI) was purchased from a local source (Analiz Kimya) and was used as received.

Working Electrode Preparation

All the working electrodes (microelectrodes) were prepared from teflon-insulated Pt wire (130 μm diameter) by removing 3 mm of the teflon coating (from the tip of the wire) using ordinary flame(Fig.1). Prepared electrodes were preconditioned in 0.5 M H_2SO_4 solution by applying a constant potential of +1.9V for 30 secs followed by a cyclic voltammetry between the ranges -0.25 to +1.1V with a scan rate of 100 mV/sec for 10 min. Deposition of rhodium was performed at -0.80V for 10 mins in 100 ppm rhodium atomic absorption solution without stirring.

Apparatus

The potentiostats used were : EG&G PAR Model (Princeton Applied Research) 264 A Voltammetric Analyzer and 626 Polarecord Metrohm Voltammetric Analyzer/Recorder. An Orion Model 290 A pH-meter was used in phosphate buffer preperations. Batch experiments were performed in a BAS (Bioanalytical Systems) Model VC-2 electrochemical cell. Working electrode, reference electrode (Ag/AgCl, Model RE-1 BAS) and ordinary platinum wire auxiliary electrode (1 mm i.d.) were placed in the electrochemical cell through the holes in its teflon cover. A magnetic stirrer provided convective transport.

Procedure

0.1 M KI, 0.1 M KNO_2 and 0.1 M H_2SO_4 solutions were prepared daily with distilled water. Working potential of +0.55V was applied and transient currents were allowed to decay to steady-state values before the injection of the NO solution. 300 μL of KI, KNO_2 and H_2SO_4 solutions were mixed in a glass tube which resulted in the formation of NO :

$$2KNO_2+2KI+2H_2SO_4 \rightarrow 2NO+I_2+2H_2O+2K_2SO_4$$

Then immediately 50 μL of NO solution was injected into the electrochemical cell where NO was oxidized to NO_3^- :

$$NO + 4OH^- \xrightarrow{+0.55\ V} NO_3^- + 2H_2O + 3e^-$$

At the same time current was monitored under batch conditions at room temperature, with 400 rpm stirring in the electrochemical cell containing 10 mL phosphate buffer.

Results and Discussion

Fig.1 shows the side view and the cross section of the working electrode prepared using teflon coated multistranded platinum wires (b) (10%Ir+90%Pt). A section of 300μm(a) was treated with flame to remove the teflon coating. After

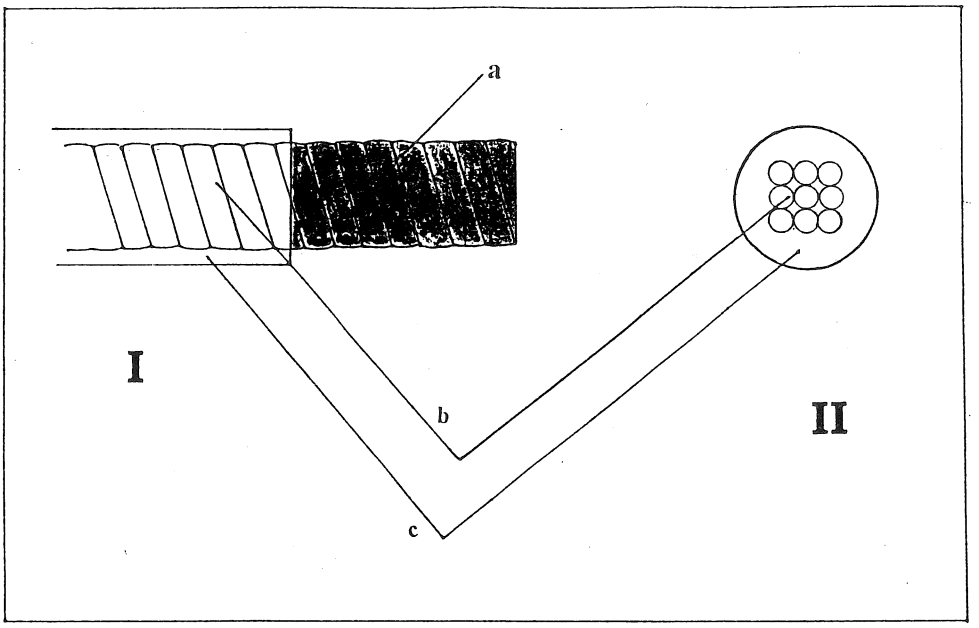

Fig.1. Side view(**I**) and cross section(**II**) of microelectrode prepared using multistranded (9 strands) platinum wires(**b**)(130 μm total diameter) with 25 μm teflon coating(**c**). Rhodium is deposited on the section(**a**)(300 μm in length) where teflon coating is removed using ordinary fire.

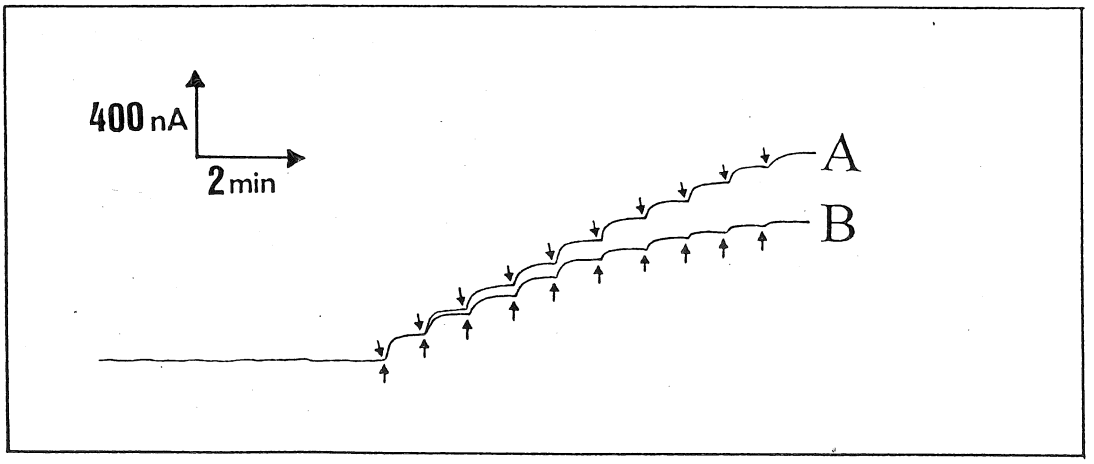

Fig.2. Current-time diagrams obtained at RhDp(A) and plain(B) sensors by increasing the concentration of NO in $5x10^{-4}$ M steps. Batch experiment, stirring the solution at 400 rpm and +0.55 V (vs Ag/AgCl) operating potential. Solution 0.05 M phoshphate buffer (pH = 7,4).

preconditioning, Rh was immobilized on this section to provide electrocatalysis. Except this section teflon coating remained on the rest of the electrode body(c).

Our results showed that the response currents of the RhDp sensor to the injection of NO at +0.55V were observed higher than the response of the plain sensor. As shown in Fig.2, after 10 injections of 50 μL of NO solution the current-time diagrams of RhDp(A) and plain(B) sensors obviously differ from each other.. After the first injection of 50 μL of NO solution, both sensors gave the same starting current values of 100 nA.

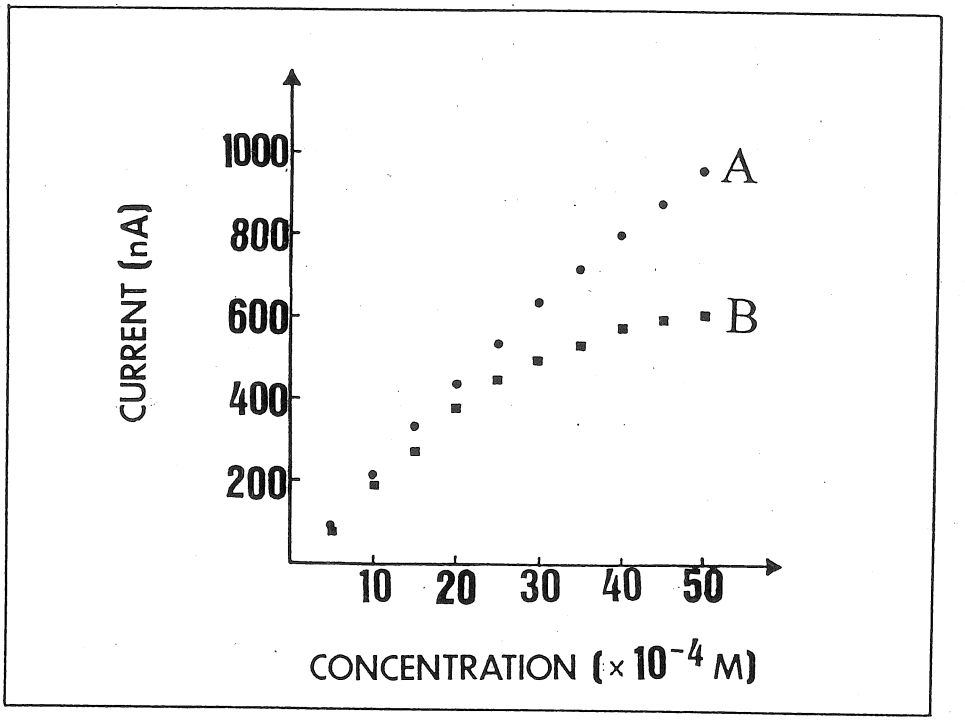

Fig.3 : Resulting calibration plots of RhDp(A) and plain(B) sensors. Conditions as in Fig.2.

As more alinquots of NO were injected, the responses of the RhDp sensor climbed up to 961nA while the response of the plain sensor reached to a plateau at 610 nA, i.e. electrocatalytic effect of deposited rhodium boosted the response current with an increase of approximately 50% in the sensor response.

In Fig.3 the calibration responses of both sensors were plotted. For the plain sensor (B), response curve was linear between the ranges 0-20x10^{-4} M NO concentration. After 20x10^{-4} M concentration level, current readings for the plain sensor began to drift away from linearity. On the other hand, response characteristic of the RhDp sensor (A) was linear until 30 x 10^{-4} M NO concentration, which means 50% extended range. Increasing the concentration of the NO beyond this level resulted in the limitation of linearity in response. A very common interference; ascorbic acid (at phys. conc. 0.1mM) was also studied (Fig.4) to determine the effect of lower working potential; +0.55V. Three injections of 50 µL of NO solution followed by a single injection of 50 µL of ascorbic acid solution were done at +0.90V(A) and +0.55V (B) respec-tively. At +0.90 V good sensor responses were obtained with NO injections, but also the ascorbate interference was too high (700 nA). Working at +0.55V the ascorbate interference decreased nearly to the half of the first value (440 nA) as comparably slight decrease was observed in NO responses. Therefore it can be concluded that ascorbate interference can be decreased considerably by detecting NO at +0.55V, which is enabled by electrocatalytic effect of deposited rhodium, without any other additional membranes or other

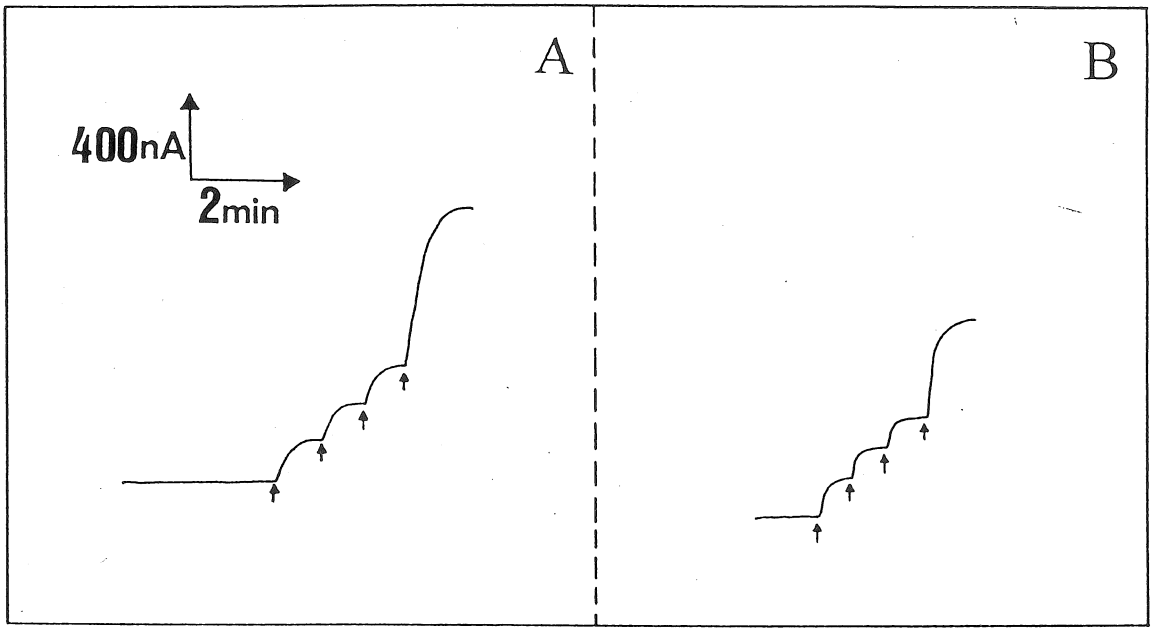

Fig.4. The RhDp sensor response to ascorbate inteference. Three successive injections of NO in $5x10^{-4}$ M steps followed by one ascorbate injection of 0.1 mM at +0.90 V(A) and +0.55 V(B). Other conditions as in Fig.2.

barriers. The mechanism of the electrocatalysis by rhodium or other VIII B group metals hasn't been explained briefly although it was used commonly in literature(49-80).

CONCLUSION

Response characteristics of the amperometric sensors for the detection of NO can be improved with the electrocatalytic effect of deposited rhodium. Almost identical sensors may be produced with this method which saves individual calibration steps. Working at +0.55V decreased interferences, extended linearity and response currents without compromising from the response time. Additional membranes like polyphenylenediamine, Nafion or cellulose acetate can be used for further elimination of the interferences.

Acknowledgement

This research was supported by a grant from TUBITAK - SBAG-U-15/6.

The authors wish to thank Metrohm AG - Switzerland for technical support.

References

1. R.H. Steinhorn, S.L. Millard, C.F. Morin: *3rd. Clin. Perinatol.* 22, 405 (1995)
2. H.B. Peng, P. Libby, J.K. Liao: *J. Biol. Chem.*, 270, 14214 1995
3. L. Yu, P.E. Gngaro, M. Niederberger, T.J. Burke, R.W. Schrier: *Proc. Natl. Acad. Sci. U. S. A.* 91, 1691 (1994)
4. T. Stroff, N. Lambrecht, B.M. Peskar: *Eur. J. Pharmacol.*, 260, R1-2 (1994)
5. L. Kobzik, D.S. Bredt, C.J. Lowestein, J. Drazen, B. Gaston, D. Sugarbaker, J. S. Stamler: *Am. J. Respir. Cell. Mol. Biol.* 9, 371(1993)
6. V. Schreiber: *Cas. Lek. Cesk.* 133, 137 (1994)
7. E. Mienl, F. Aloisi, B. Ertl, F. Weber, R. de-Waal-Malefyt, H. Wekerle, R. Hohlfeld *Brain* 117, 1323 (1994)
8. S. S. Smith, J. Li: *Mol. Pharmacol.* 43, 1 (1993)

9. G. E. Boeckxstaens, P. A. Pelckmans, H. Bult, J.G. De-Man, Y. M. van-Maercke: *Br. J. Pharmacol.* 102, 434 (1991)
10. J. Murray, C. Du, A. Ledlow, J. N. Bates, J. L. Conklin: *Am. J. Physilo.* 261, G401 (1991)
11. S. L. Archer, N. J. Cowan: *Circ. Res.* 68, 1569 (1991)
12. H. Mitsuhata, R. Shimizu, M. M. Yokoyama: *J. Clin. Immunol.* 15, 277 (1995)
13. B. Mayer, P. Klatt, E. R. Werner, K. Schmidt: *J. Biol.Chem.* 270, 655 (1995)
14. P. Vallence, S. Moncada: *J. R. Coll. Physicians. Lond.* 28, 209 (1994)
15. M. W. Radomski: *Ann. Med.* 27, 321 (1995)
16. V. Cattell: *Nephrol. Dial. Transplant.* 10, 759 (1995)
17. Y. Watanabe, K. Terashima, H. Hidaka: *Biochem. Biophys. Res. Commun.* 219, 638 (1996)
18. V. M. Sellers, M. K. Johnson, H. A. Dailey: *Biochemistry* 35, 2699 (1996)
19. W. Xu, S. J. Yao, S. K. Jr. Wolfson: *ASAIO. J.* 41, M416 (1995)
20. S. Moncada: *J. Lab. Clin. Med.* 120, 187(1992)
21. C. Du, J. Murray, J. N. Bates, J. L. Conklin: *Am. J.Physiol.* 261, G1012(1991)
22. W. S. Shin, Y. H. Hong, H. B. Peng, R. De-Caterina, P. Libby, J. K. Liao: *J. Biol. Chem.* 11317 (1996)
23. E. Anggard: *Lancet* 343, 119 (1994)
24. K. A. Rockett, M. M. Awburn, E. J. Rockett, I. A. Calack: *Am J. Trop. Med. Hyg.* 50, 735 (1994)
25. M. Kihara, P. A. Low: *Exp. Neurol.* 132, 180 (1995)
26. M. Jimenez, P. Vergara, F. Christinck, E. E. Daniel: *Am. J. Physiol.* 269, G22 (1995)
27. N. E. Cameron, M. A. Cotter: *Eur. J. Clin. Invest.* 25, 19 (1995)
28. M. Wahl, L. Schilling, A. A. Parsons, A. Kaumann: *Brain Research* 637, 204 (1994)
29. A. Meulemans: *Neurosci. Lett.* 157, 7 (1993)
30. A. V. Nowicky, L. J. Bindman: *J. Neurophysiol.* 70,1255 (1993)
31. C. W. Shuttleworth, K. M. Sanders, K. D. Keef: *Br. J. Pharmacol.* 109, 739 (1993)
32. C. Iadecola, F. Zhang, X. Xu: *Am. J. Physiol.* 264, R738 (1993)
33. T. J. A. Fox, E. E. Daniel, F. Christinck, Z. Woskowska, S. Cipris, T. J. McDonald: *Peptides* 14, 1171 (1993)
34. M. C. Oz, D. J. Pinsky, S. Koga, H. Liao, C. C. Marboe, D. Han, R. Kline, V. Jeevanandam, M. Williams, A. Morales: *Circulation* 88, II291 (1993)
35. M. P. Kavanaugh: *Biochemistry* 32, 5781 (1993)
36. S. Archer: *FASEB-J.* 7, 349 (1993)
37. G. F. Chen, D. W. Cheung: *Circ. Res.* 70, 270 (1992)
38. T. Nagao, P. M. Vanhoutte: *Am. J. Physiol.* 261, H1034 (1991)
39. T. Yamamura: *Nippon. Yakurigaku. Zasshi.* 107, 173 (1996)
40. E. Noiri, T. Peresleni, F. Miller, M. S. Goligorsky: *J. Clin. Invest.* 97, 2377 (1996)
41. T. Nagafuji, M. Sugiyama, T. Matsui, A. Muto, S. Naito: *Mol. Chem. Neuropathol.* 26, 107 (1995)
42. F. Bedioui, S. Trevin, J. Devynck, *Electroanalysis* 8, 1085 (1996)
43. F. Bedioui, S. Trevin: *Biosens. Bioelectronics* 13, 227 (1998)
44. S. Trevin, F. Bedioui, J. Devynck: *Talanta* 43, 303 (1996)
45. C. Privat, F. Lantoine, F. Bedioui, E. Millanvoye van Brussel, J. Devynck, M.-A. Devynck: *Life Sciences* 61, 1193 (1997)
46. S. Trevin, S. Andre, J. Devynck, J. L. Boucher, F. Bedioui: *Anal. Comm.* 34, 69 (1997)
47. X. Zhou, M. A. Arnold: *Anal. Chem.* 68, 1748 (1996)
48. M. N. Friedemann, S. W. Robinson, G. A. Gerhardt: *Ibid.* 68, 2621 (1996)
49. P. Atanasov, S. Gamburzev, E. Wilkins: *Electroanalysis* 8, 158 (1996)
50. Y-K. Lai, K-Y. Wang: *Ibid.* 6, 919 (1994)
51. J. T. Ohara, R. Rajagopalan, A. Heller: *Anal. Chem.* 65, 3512 (1993)
52. J. T. Ohara, R. Rajagopalan, A. Heller: *Anal. Chem.* 66, 2451 (1994)
53. J. Wang, G. Rivas, M. Chicharro: *Electroanalysis* 8, 434 (1996)
54. I. E. Iwuuoha, R. M. Smyth, G. J. Vos: *Ibid.* 6, 982 (1994)
55. M. Ozsoz, A. Erdem, E. Kilinc, L. Gokgunnec: *Ibid.* 8, 147 (1996)
56. J. Wang, N. Naser, L. Angnes, H. Wu, L. Chen: *Anal. Chem.* 64, 1285 (1992)

57. J. Mtonaka, R. L. Faulkner: *Ibid.* 65, 3258 (1993)
58. J. Wang, L. Angnes, *Anal. Chem.* **1992**, 64, 456
59. J. Wang, Q. Chen: *Ibid.* 66, 1007 (1994)
60. J. Furbee, T. Jr. Kuwana, S. R. Kelly: *Ibid.* 66, 1575 (1994)
61. H. Gunasingham, B. C. Tan: *Analyst* 114, 695 (1989)
62. F. S. White, P. F. A. Turner, D. R. Schmid, U. Bilitweski, J. Bradley: *Electroanalysis* 6, 625 (1994)
63. K. H. Lubert, M. Guttmann, L. Beyer: *Ibid.* 8, 320 (1996)
64. I. G. Casella, E. Desimoni, *Ibid.* 8, 447 (1996)
65. J. Wang, L. Chen, J. Liu, F. Lu: *Ibid.* 8, 1127 (1996)
66. Z. Zhang, C. Lei, J. Deng: *Analyst* 121, 971 (1996)
67. Q. Deng, S. Dong: 121, 1123 (1996)
68. M. Hedenmo, A. Narvaez, E. Domingues, I. Katakis: *Ibid.* 121, 1891 (1996)
69. O. Adeyoju, E. I. Iwoha, M. R. Smyth, D. Leech: 121, 1885 (1996)
70. E. S. M. Lutz, E. Dominguez: 8, 117 (1996)
71. I. G. Casella, *Ibid.* 8, 128 (1996)
72. A. Ciszewski, E. Kubaszewski, M. Lozinski: *Ibid.* 8, 293 (1996)
73. A. Napier, J. P. Hart: *Ibid.* 8, 1006 (1996)
74. X. Qi, R. P. Baldwin, H. Lin, T. F. Guarr: *Ibid.* 3, 119 (1991)
75. T-M. Park, E. I. Iwuoha, M. R. Smyth, B. D. Mac Craith: *Anal. Comm.* 33, 271 (1996)
76. K. Mac Ateer, R. D. O'Neill: *Analyst* 121, 773 (1996)
77. S. Gamburzev, I. Iliev, P. Atanasov, A. Kaisheva, F. Scheller, D. Dfeifer: *Electroanalysis* 3, 861 (1991)
78. E. Wang, H. Ji, W. Hov: *Ibid.* 3, 1 (1991)
79. D. Leech, J. Wang, M. R. Smyth: *Ibid.* 3, 37 (1991)
80. P. J. O'Connell, C. K. O'Sullivan, G. G. Guilbault: Proco of the Fifth World Congress on Biosensors, Berlin, Germany, June 3-5, Oral Presnt., pp.201 **1998**

Accepted:16.11.1998