

Chapter 10

Amperometric biosensor for the enantioanalysis of L-lysine in serum samples

10.1 Introduction

Advanced single-crystal diamond has enabled the development of a wide range of monocrystalline diamond products to meet the exacting requirements of for an amperometric transducer [1]. The reliability obtained for the electrical properties of single-crystal diamond is encouraging for research in the electrochemical sensors based on monocrystalline diamond, as well, it proves that the doping of monocrystalline diamond is not necessary which minimizes the time affected for electrode's construction and also simplified the steps adapted for the design of such electrochemical sensors [2]. Amperometric biosensors are a good alternative for chromatographic methods due to the fact that they can be used for the direct measurement of the enantiomers in solutions without any prior separation of the substance that has to be determined [3]. The biochemical reaction is very selective and sensitive [4]. The sensitivity of the enzymatic reaction must be correlated to the sensitivity of the transducer; the best electrochemical transducer for this biosensor is the amperometric one [5]. Design of amperometric biosensors based on monocrystalline diamond paste proved higher sensitivity and selectivity [5,6].

Lysine is one of the indispensable amino acids that can not be manufactured by the human body, but must be acquired from food sources [7]. L-lysine (Figure 10.1) is widely available to the public as a non-prescription of oral supplement. L-lysine can be transported into brain [8]. In the metabolism of L-lysine, lysine is the precursor of

acetoacetyl-CoA, which is a very important enzyme in the biosynthesis of acetylcholine in the central nervous system [7].

Lysine is also the precursor in the biosynthesis of L-carnitine, which is involved in the transport of acyl groups into mitochondria for beta-oxidation [9]. L-carnitine is an essential cofactor in the transfer of long-chain fatty acids across the inner mitochondrial membrane [10] and significantly decrease the infarct volume compared with saline [11].

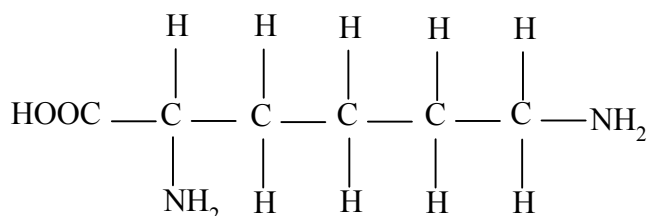


Figure 10.1 L-lysine

The determination of lysine was carried out by liquid chromatography [12,13], ion exchange chromatography [14] and capillary electrophoresis [15]. Unfortunately, these methods may be unsuitable for the analysis of large sets of samples since the separation step is often tedious and time consuming and need expensive equipments. For this reason, there is a great interest in developing faster, less expensive and simpler methods for L-lysine analysis. Biosensors offer the possibility of fast and inexpensive analysis. Four different enzymes have been used as biological components in the construction of these biosensors [16-19]. L-lysine oxidase is the widely used in enzymatic reaction of L-lysine. This enzyme was isolated from *Trichoderma Viride* Y244-2 (EC 1.4.3.14) [20]. The reaction of the enzymatic conversion of L-lysine is shown in Figure 10.2. The enzyme has been reported to immobilize by cross-linking with glutaraldehyde onto a polymer film directly on the

working electrode [26], or covalently on silanized silica gel [23], on nylon [27], or on immobilization membranes [24].

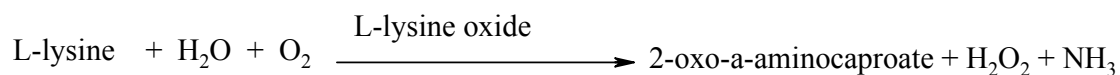


Figure 10.2 Enzymatic conversion of L-lysine

In this chapter, a diamond paste based amperometric biosensor is proposed for the analysis of L-lysine. The proposed amperometric biosensor based on physical immobilization of L-lysine oxidase in a monocrystalline natural diamond paste is designed for the enantioselective analysis of L-lysine in serum samples.

10.2 Reagents and Materials

Natural diamond powder with particle size 1 μm (99.9%), β -alanine, creatine and creatinine were purchased from Aldrich (Milwaukee, USA); paraffin oil and L-lysine were purchased from Fluka (Buchs, Switzerland); L-glutamate, D-lysine and L-lysine oxidase (L-lysine oxygen oxidoreductase [deaminating]; (EC 1.4.3.14)) were purchased from Sigma (USA); asparagine and phosphate buffer (pH=7.0) were purchased from Merck (Darmstadt, Germany). D-lysine was purchased from Sigma (Germany). L-(+)-serine was purchased from Reidel-Haen (Germany) L-cystine from Hopkin & Williams LTD (England) L-leucine from Aldrich (Germany); histamine dihydrochloride from BDH (Poole, England); glycine from Analar, BDH (Poole, England).

De-ionized water from a Modulab System (Continental Water Systems, San Antonio, TX, USA) was used for all solution preparations. Stock solution of L-lysine was used (10^{-4} mol/L) for standard solutions preparation (10^{-16} - 10^{-4} mol/L) by serial dilution.

10.3 Diamond Paste Based Amperometric Biosensor

Plain diamond was prepared by mixing 0.1 g of diamond powder with 20 μ L paraffin oil, and filled in a plastic pipette peak leaving an empty space of 3-4 mm in the top part to be filled with a modified diamond paste.

The diameter of the biosensor was 3 mm. Electrical contact was obtained by inserting a silver wire into the diamond paste. The biosensor tip was gently rubbed on fine abrasive paper to produce a flat surface. The surface of the biosensor was wetted with de-ionized water and then polished with alumina paper (polished strips 30144-011, Orion) before use. The biosensor was stored at 4°C, when not in use. The enzyme solution used for the biosensor design was prepared in 0.1 mol/L phosphate buffer of pH = 8.0.

A modified diamond paste was prepared as follows: 400 mg of natural monocrystalline diamond powder was mixed with 30 μ L paraffin oil to form a diamond paste. 50 μ L from the enzyme solution (1 mg enzyme/mL phosphate pH = 8.0) was added to the diamond paste.

10.4 Apparatus

A663 VA Stand (Metrohm, Herisau, Switzerland) in combination with a PGSTAT 100 and software Ecochemie (version 4.9) were used for all chronoamperometric

measurements. A Pt electrode and an Ag/AgCl electrode served as the counter and reference electrodes in the cell.

10.5 Recommended Procedures

The chronoamperometric technique was used for the measurement of the intensity of current at 650 mV. The electrode were dipped into a cell containing 10 mL of phosphate buffer, pH = 8.0 and different volumes of L-lysine solution were added. The intensity of current measured was plotted versus the concentration of L-lysine. The unknown concentrations of L-lysine in serum and tablet solutions were determined from the calibration graph.

10.6 Determination of L-lysine in serum samples

Five serum samples were collected from healthy volunteers. The serum samples were buffered with (pH = 8.0) and spiked with different volume of L-lysine. Direct amperometry was involved to determine the concentration of L-lysine in serum samples.

10.7 Results and discussion

10.7.1 Response characteristics of the amperometric biosensors

The response characteristics of the biosensors were measured at 650mV. The reason of the selection of the potential is to obtain the maximum sensitivity and selectivity for the assay of hydrogen peroxide produced by the enzymatic reaction (Figure 10.2) [25]. The biosensor exhibits a linear concentration range from 1 to 100 nmol/L with limit of detection of 4 pmol/L magnitude order. The biosensor response was highly stable and reproducible over two months, when the biosensor was intensively used everyday for measurement and was kept into the fridge at 4 °C when not in use. The

low limits of detection and high reliability of the biosensor is due to the diamond paste matrix used for its design.

The response of the biosensor to different L-lysine concentrations was linear over wide concentration ranges and can be described by the following calibration equation:

$$I = 0.23 C + 2.28; \quad r = 0.9866$$

where I is the intensity of the current in μA , C is the concentration of L-lysine in nmol/L , and (r) is the regression coefficient.

The limits of detection were calculated using the equation proposed by Otto [26]

$$DL = \frac{I_B + 3\sigma_S - a}{S}$$

where I_B is the background current, σ_S is the standard deviation for the measurement of the background current, a is the intercept of the calibration equation and S is the slope of the calibration equation.

10.7.2 Enantioselectivity of the amperometric biosensor

The selectivity of the biosensor was checked using both separate and mixed solution methods over D-lysine, L-(+)-serin, L-cystine, L-leucine, L-glutamic acid, asparagine, histamine dihydrochloride, glycine, β -alanine, creatine and creatinine. Amperometric selectivity coefficients, K_{sel}^{amp} , were determined following the method proposed by Wang [27] for the same potential (650 mV) that was used for the determination of response characteristics of the proposed amperometric biosensor. The ratio between the concentrations of L-lysine and the interfering species was 1:10. All the values of

the amperometric selectivity coefficients (pK_{sel}^{amp}) obtained by using the mixed solution method, for the biosensor designed for L-lysine are higher than 2 (Table 10.1) demonstrating the enantioselectivity and selectivity of the proposed biosensor.

Table 10.1 Amperometric selectivity coefficients of the biosensor^a

Interfering Species (j)	pK_{sel}^{amp}
D-lysine	2.55
L-serine	3.36
L-leucine	2.15
L-aspartic acid	2.01
L-glutamic acid	3.15
Histamine	2.09
Glycine	2.07
β -alanine	3.15
Creatine	2.37
Creatinine	2.91

^aAll measurements were made at room temperature; all values are the average of ten determinations

10.7.3 Analytical applications

The recovery test of L-lysine was conducted in the presence of its antipode, D-lysine. The results obtained (Table 10.2) demonstrated the suitability of the proposed biosensor for the enantioanalysis of L-lysine due to the high recovery values obtained for the assay of L-lysine in the presence of D-lysine. No significant differences in the recovery values were recorded for the ratio between L:D varying from 1:9 to 1:99.9.

Table 10.2 Recovery of L-lysine in the presence of D-lysine^a

L:D	% L-lysine, Recovery
2:1	99.19 \pm 0.07
1:1	99.89 \pm 0.06
1:2	99.93 \pm 0.06
1:4	99.82 \pm 0.07
1:9	99.84 \pm 0.06

^aAll measurements were made at room temperature; all values are the average of ten determinations.

The results obtained for the analysis of L-lysine in serum samples are shown in Table 10.3. Blood samples were collected from healthy volunteers and spiked with different aliquots of L-lysine solutions. The results obtained using the proposed biosensor at 650 mV working potential are in good concordance with the values added to the serum samples.

Table 10.3 Recovery of L-lysine in serum samples

Sample No.	% L-lysine, Recovery
1	99.01±0.05
2	98.99±0.06
3	99.12±0.05
4	99.07±0.05
5	99.18±0.06

^aAll measurements were made at room temperature; all values are the average of ten determinations.

10.8 Conclusion

The proposed design of the amperometric biosensor is simple and reproducible. The analytical information obtained using the proposed biosensor is highly reliable. One of the features of the biosensor is its utilization for *in vivo* determination of L-lysine.

10.9 References

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