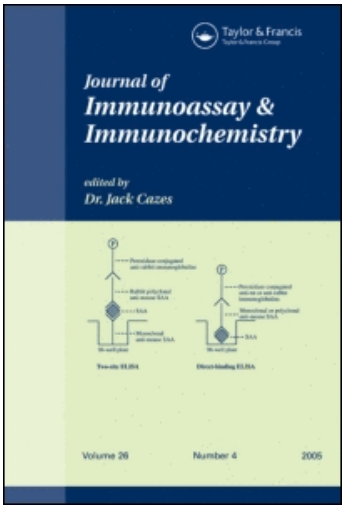

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Diamond Paste Based Immunosensor for the Determination of Azidothymidine

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Diamond Paste Based Immunosensor for the Determination of Azidothymidine

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ABSTRACT

An amperometric immunosensor, based on diamond paste (diamond powder and paraffin oil), has been constructed for the assay of azidothymidine (AZT). The diamond paste is impregnated with anti-AZT. The immunosensor can be used reliably for the assay of azidothymidine in its pharmaceutical formulations. The potential used for azidothymidine assay was + 240 mV vs. Ag/AgCl electrode. The surface of the immunosensor can be regenerated by simply polishing, thereby obtaining fresh immunocomposite ready to be used in a new assay. The new amperometric immunosensor, based on diamond paste, gives reliable results for the assay of AZT as raw material and from its pharmaceutical formulation.

Key Words: Diamond; Amperometric Immunosensor; Azidothymidine; Zidovudine.

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INTRODUCTION

Azidothymidine (zidovudine, AZT) (Figure 1) is a thymidine analogue antiretroviral drug which is active against the human immunodeficiency virus (HIV). AZT proved effective in reducing the incidence of opportunistic infections and neoplasms in acquired immunodeficiency syndrome (AIDS) and AIDS related complex patients.[1,2] Up to now, AZT remains the mainstay in the treatment of patients infected with HIV.[3]

Among electrochemical immunosensors, amperometric immunosensors represent the best combination of sensitivity and selectivity; hence, amperometric transducers ensure the highest sensitivity and the immunoreaction ensures the best selectivity.[4–6] The reliability of immunosensor construction is influencing the reliability of the analytical information and it will also contribute to the validation of the immunosensors for pharmaceutical analysis.[7] Accordingly, a physical immobilization of the antibody into diamond paste is preferred for the design of the amperometric immunosensor.[4]

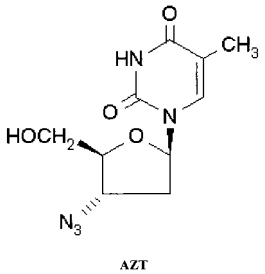
CZE,[8,9] HPLC,[10–12] RIA,[13] polarographic[14,15] techniques, as well as UV/Vis spectrometry,[16] and mass spectrometry,[16] have been described for the assay of AZT.

In this paper, an amperometric immunosensor based on the physical immobilization of anti-AZT in a diamond paste is proposed for the assay of AZT.

EXPERIMENTAL

Chemicals

The immunological system composed from azidothymidine was obtained from Sigma (St. Louis, MO, USA). APO-Zidovudine capsules



AZT

Figure 1. Structure of AZT.

were obtained from APOTEX, Inc, Ville St. Laurent, Que, Canada. Diamond powder with a particle size ca. 50μ was obtained from Aldrich. Paraffin oil was obtained from Fluka (Buchs, Switzerland). All other reagents were of the highest available analytical grade. All solutions were prepared using deionized water.

Apparatus

A 663 VA Stand (Metrohm, Herisau, Switzerland) connected to a μAutolab and software version 4.8 were used for all amperometric measurements. A platinum electrode and a Ag/AgCl (0.1 mol/L KCl) electrode served as counter and reference electrodes in the cell.

Amperometric Immunosensor Design

The antiserum was diluted to a working dilution of 1:30 in 0.01 mol/L phosphate buffer saline, pH = 7.4, containing 0.1% sodium azide. The paraffin oil and diamond powder were mixed in a ratio of 1:4 (w/w) and then it was added to the diluted anti-AZT to obtain a final composition of 0.9% (w/w) in anti-AZT. The diamond paste (diamond powder and paraffin oil) was filled into a plastic tip, leaving about 3 to 4 mm empty in the top to be filled with the chemically modified diamond paste that contains anti-AZT. The diameter of the immunosensor was 3 mm. The electric contact was done by inserting a silver wire in the diamond paste.

Before each use, the surface of the electrode was wetted with double distilled water and then polished with an alumina paper (polishing strips 30144–001, Orion). When not in use, the amperometric immunosensor was stored in a dry state at 4°C.

Recommended Procedure

Direct Amperometric Assay

The technique used for the direct amperometric assay was chronoamperometry; the potential applied was +240 mV vs. Ag/AgCl. The working temperature was 25°C. The sensor was dipped into a thermostatic cell (25°C) containing 10 mL of phosphate buffered saline, pH = 7.4 containing 0.1% sodium azide. Different aliquots of stock

AZT solution ($c = 10^{-4}$ mol/L) were added to generate a series of concentration steps.

Content Uniformity Test of Apo-Zidovudine Capsules

Ten Zidovudine capsules (100 mg AZT/capsule) are individually placed in ten 100 mL calibrated flasks, and dissolved in the phosphate buffer. The apparatus cell was filled with the prepared solution and the current developed was measured. The unknown concentration was determined from the calibration graph.

RESULTS AND DISCUSSION

Electrode Response

The electrode response was determined using the chronoamperometric technique ($E = +240$ mV vs. Ag/AgCl). The calibration equation obtained for the amperometric immunosensor is as follows:

$$I = 3.05 + 42.7c; \qquad r = 0.9999$$

where I ($\langle \mathrm{I} \rangle = \mu\mathrm{A}$) is the intensity of the current and c ($\langle c \rangle =$ fmol/L) is the concentration of AZT.

The limit of detection for the amperometric immunosensor is 2×10^{-4} fmol/L with a working concentration range between 4×10^{-4} and 6×10^{-2} fmol/L. The response time of the amperometric immunosensor is 60 s. The response obtained for the immunosensor revealed a good stability and reproducibility for one week over the tests performed.

Analytical Application

The immunosensor proved to be useful for the purity tests of AZT using the chronoamperometric ($E = +240$ mV vs. Ag/AgCl electrode) technique. An average recovery of $99.96 \pm 0.03\%$ ($n = 10$) was recorded for the assay of AZT raw material.

AZT can be reliably assayed from the APO-Zidovudine capsules with an average recovery of $98.71 \pm 0.21\%$ ($n = 30$). The results are in good

agreement, and within the range given in The United State Pharmacopoeia XXIV[17]: 90 to 110% AZT per capsule.

CONCLUSIONS

The construction of the immunosensor is simple and reproducible. The reliability of the analytical information is assured by the RSD value obtained in the recovery tests. The proposed amperometric immunosensor is suitable for the assay of AZT raw material as well as from its pharmaceutical formulations. The main advantage of the proposed method over the other methods described for AZT assay is the possibility of its determination directly, without any prior separation, with a high precision, rapidity, and low consumption of sample and buffer.

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