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Amperometric biosensor based on denatured DNA for the study of heavy metals complexing with DNA and their determination in biological, water and food samples

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Abstract

Amperometric biosensor (BS) has been elaborated based on the stationary mercury-film electrode (SMFE) with silver support and cellulose nitrate (CN) membrane containing immobilized single-stranded DNA (ssIDNA). The sorption isotherms and ssDNA–heavy metal binding constants have been obtained with the BS. According to these data, the chosen heavy metals form the following series of binding strength with ssIDNA: Pb(II)>Fe(III)>Cd(II). It has been found that upon the competitive adsorption, there exists practically simultaneous sorption of different ions at ssIDNA containing membrane. The method of the determination of heavy metals based on preconcentration of metal ions on the BS followed by the destruction of DNA–metal complexes with ethylenediamine tetraacetate (EDTA) and voltammogram recording has been proposed. The lower limits of detectable contents are 1.0×10^{-10}, 1.0×10^{-9} and 1.0×10^{-7} mol l^{-1} for Pb(II), Cd(II) and Fe(III), respectively. Heavy metals have been assayed in natural and drinking water, milk and blood serum samples even under simultaneous presence with a selectivity factor of 1:10. The effect of matrix components has been estimated.

Keywords: Amperometric DNA biosensor; Heavy metals analysis; Adsorption

1. Introduction

Studying the process of complexing of DNA molecules and their parts with metals is an actual and interesting problem because it enables establishing features of DNA behaviour in the organism. Heavy metals are known to have a great affinity for DNA and cause muta- and cancerogenesis [1,2], the formation of malignant tumours being attended by an increase of heavy metals' concentration in the DNA of tumour cells [3]. Electrochemical biosensors (BS) based on immobilized DNA and its parts integrate sensitivity of detection with a high specificity of biomolecules, reduce the consumption of DNA and give rise to the development of modern methods of analysis of DNA effectors, including toxic ones, in environmental and biological objects [4–9].

The aim of this investigation is to perform voltammetric study of heavy metals (Fe(III), Pb(II), Cd(II)) complexing with denatured single-stranded DNA (ssDNA), and to use this complexing process for determining heavy metals in blood serum and in environmental objects with the developed amperometric BS based on ssDNA.

2. Experimental

2.1. Apparatus and reagents

Voltammetric measurements were performed with a SVA-1B-M-01 voltammetric system (Analytic, Bulgaria). The DNA-based BS constructed on the basis of a stationary mercury-film electrode (SMFE) with silver support ($d = 0.5$ mm) [10] or SMFE itself served as a working electrode. The reference electrode was a saturated calomel electrode (SCE). The solutions were deaerated with argon. An atomic-absorption spectrometer Z-6100 (Hitachi) was used. All measurements were performed at 298 ± 2 K. Chicken erythrocyte DNA (Reanal) at a concentration of 0.01 mg ml^{-1} in 0.9% NaCl was used. Medium-nitrogen cellulose nitrate (CN) films were employed. Highly purified organic solvents

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