GLUCOSE QUANTIFICATION IN COMMERCIAL SERUMS BY MEANS OF AMPEROMETRIC BIENZIMATIC BIOSENSOR BASED ON A BIOCOMPOSITE RIGID MATRIX.

Rivera B[1], Ramírez-Silva M.T[2], Morales-Pérez A[1] *
1 Facultad de Estudios Superiores Cuautitlán, UNAM, Av. Primero de mayo S/N, Cuautitlán Izcalli, Edo de México C. P 54750 México. 2 Universidad Autónoma Metropolitana-Izt. 2Michoacán y Purísima S/N México D.F C.P 09340 México.
Corresponding author e-mail mpa@servidor.unam.mx.

INTRODUCTION. In the last years the study of amperometrics bienzimatics biosensors has grown, due to the possibility of increasing the sensitivity when more than one enzyme is used **[1-5]**. In these investigations the biological material is immobilized superficially. The main with this kind biosensors is their useful time, which, generally is not more than few days. The objective of the present work consists on the construction and optimization of an amperometric bienzimatic biosensor of type for the quantification of glucose in serums. The biosensor is based on the combined immobilization of the enzymes glucose oxidase (GOD) and horseradish peroxidase (HRP) in a biocomposite of rigid matrix. When this immobilization technique is used, the advantages in terms of biosensor lifetime and the possibility to reduce the protoheme group (HRP) to the active form when exist an intimate contact of the enzymes with the graphite powder, applying a potential of -300 mV *vs* Ag/AgCl the active place is regenerated **[6]**.

EXPERIMENTAL. The optimization consists on determining the experimental conditions in which the maximum signal of the bienzimatic biosensor is obtained studying some factors such as the content of biological material in the biocomposite temperature, concentration of the electrolyte supports and pH. The optimal proportion of biological material in the biocomposite was prepared as follow: 13.5 mg of GOD, 8.5 mg of HRP, 151.9 mg of graphite powder and 650.3 mg of epoxy resin. The effect of the temperature in the biosensor signal was studied. It was determined experimentally that from 318^{o} to 322^{o}K the response of the biosensor was independent of the temperature. Therefore it was decide to work at 320^{o}K. For the concentration of the supports electrolyte and pH, it was established experimentally that the maximum response was achieved at pH 6.5 using 0.1 M phosphate buffer. When the optimal operation conditions of the bienzimatic biosensor were established, it was determined a good linearity in an interval of 5.6 to 16 mM of glucose. For the studies of the lifetime, it was determined experimentally that in a period of 3 months, the signal of the bienzimatic biosensor is under the confidence limits of 99%. Finally, the bienzimatic biosensor (method 1) was tested to quantify the content of glucose in serums commercial Mexicans. In order to have a comparison parameter the commercial kit was provided by Sigma-Aldrich Co. (method 2). The obtained results are shown in the table 1.

Table 1. Glucose quantification of in glucoside serums. The reported values are in grams of glucose for 100 mL of solution.

Serum	Label	Method 1	Method 2
1	2	1.986±0.0051	1.994±0.0291
2	5	4.917±0.0651	4.976±0.0341
3	2	1.999±0.0069	2.067±0.0581
4	5	4.947±0.0427	4.987±0.0789

CONCLUSIONS. It is possible to incorporate two enzymes in oneself rigid biocomposite and they are well immobilized. It was demonstrated how the temperature, pH and concentration of the supports electrolyte affect the response of the biosensor. Finally, it was possible to quantify glucose in a series of commercial serums using this approach and a good correlation was observed when our method was compared with a reference one. The must important advantage of the developed biosensors resides in the time of analysis, which is not more of 5 minutes.

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