INFLUENCE OF THE BUFFER CAPACITY ON GLUCOSE POTENTIOMETRIC DETERMINATION IN SYNTHETIC SOLUTIONS AND IN REAL SAMPLES WITH DIFFERENT ACIDITIES

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Received July 30, 2007

Glucose oxidase-based enzyme electrodes were constructed by entrapping the enzyme in a semipermeable membrane (nylon) which was immobilized on the sensitive bulb of a glass electrode. The pH diminution in time, due to gluconic acid generation in the enzyme-catalysed reaction, was monitored at different glucose concentrations and different buffer capacities of the analysed solutions. The analyte (glucose) was dissolved in buffer solutions (pH = 6,9) with different phosphate concentrations: 10^{-4}M, 5×10^{-4}M, 10^{-3}M. Glucose concentrations ranged from 5×10^{-5}M to 10^{-3}M. Calibrations graphs were obtained at different buffer capacities, then real samples with different acidities (natural fruit juices and wine) were analysed, in order to assess the degree of appropriateness of the buffer capacity of the matrix to the acidity of the analysed sample. For the mentioned concentration range and for the acidic samples, it is better to choose a relatively great buffer capacity. The influence of the buffer capacity is less pronounced for nonacidic samples.

Key words: Glucose; Glucose oxidase; Potentiometry; Glass electrode; Buffer; Nylon.

INTRODUCTION

Biosensors are analytical tools applied in many fields of activity (food industry, biomedical analysis, environmental monitoring), due to the advantages that their use imply: high specificity, selectivity and sensitivity, fast response and low detection limit. They are able to detect a broad range of analytes (sugars, organic acids, aminoacids, lipids, vitamins, pesticides) in various media[1,2].

Biosensors are complex analytical instruments, incorporating a biocatalyst (*e.g.* enzyme) and a transducer, which senses the changes that take place in the system, as a result of the substrate (analyte)-biocatalyst (enzyme) interaction[3]. The transduced parameter can be electrical (intensity, potential/pH difference), optical (absorbance, reflectance, fluorescence, luminiscence), thermal, piezoelectrical [4].

Potentiometry, one of the most broadly used detection modality, makes use of different ion specific transducers: the pH glass electrode[5], field effect transistors[6] or other silicon-based transducers[7], metal oxides like SnO_2[8], Pt electrodes on which a polymer film with immobilized glucose oxidase was grown[9], glassy carbon electrodes on which a polyethyleneimine film was deposited[10], screen printed carbon electrodes[11], ammonium selective electrodes[12]. Wei and Ivaska[13] mention the use of a polyaniline film, as a transducer for the construction of a potentiometric glucose biosensor. Among these transducers, the pH-glass electrode is the most popular and widely used[5,14]. This method presents a series of advantages: good dynamic range, linearity, fast response, minimum of interferences, versatility.

One of the most widely employed immobilization technique used in potentiometric enzyme electrode construction is the use of semipermeable membranes. Cellophane or nylon membranes have already been used in the potentiometric detection of glucose[14-16], urea[17] and pesticides[18].

As the ability of nylon to act as a semipermeable membrane for potentiometric glucose detection has already been verified, and the influence of enzyme loading, membrane porosity and membrane surface groups has already been studied[15], we focused on the influence of the buffer capacity on glucose determination, by means of a Biodyne-based potentiometric biosensor. In this study, we also tried to adjust the buffer capacity of the matrix to the nature (acidity) of the sample analysed.

MATERIALS AND METHODS

Reagents and apparatus: glucose monohydrate analytical reagent (Peking Chemical Works), glucose oxidase Sigma Type X-S (210000 Units/g), monobasic potassium phosphate (Riedel de Haen), dibasic sodium phosphate (Riedel de Haen), sodium sulphate (Riedel de Haen), Biodyne membrane (nylon 6,6 positively charged, with surface $-NH_2$ groups, 0,45 μm porosity), digital pH-meter Radelkis OP-208 type, glass electrode EGA 31 type, Germany.

Operation mode: the enzyme-pH electrode for glucose was obtained by entrapping the enzyme in a liquid layer, around the sensitive bulb of the glass electrode: 0,3 ml of the enzyme solution (3000 U/ml in phosphate buffer 0,1M, pH=7,0 prepared by mixing monobasic potassium phosphate and dibasic sodium phosphate in a volumetric ratio 3,88/6,12, as described before [19]) was poured in the center of the nylon (Biodyne B) membrane; the membrane was left overnight at 4^0C; the membrane containing the enzyme solution was placed tightly over the sensitive bulb of the glass electrode and held in place with a rubber ring. The enzyme electrode was kept for about an hour prior to use in the buffer solution 0,1 M, pH=7,0. Measurements were carried out in phosphate buffer solutions, pH=6,90, with different buffer capacities (phosphate concentrations), which were prepared by mixing monobasic potassium phosphate and dibasic sodium phosphate in a volumetric ratio 4,48/5,52, as described[19]); the concentrations of phosphate used in the buffer solution in which the analyte (glucose) was dissolved were: 10^{-3}M, 5×10^{-4}M, 10^{-4}M. The phosphate buffer solutions also contain sodium sulphate (0,1 M) in order to keep constant ionic strength.

The analyte concentrations ranged from 5×10^{-5} to 10^{-3}M. Real samples (juices and wine) were diluted before analysis in the respective buffer solutions, at the appropriate degree of dilution (see Table 1).

RESULTS AND DISCUSSIONS

For the obtained enzyme electrode, we followed the pH decrease, at different glucose concentrations and at different buffer capacities of the analysed glucose solutions (Figs. 1–3). The pH diminution in time increases with the analyte concentration, because the amount of gluconic acid generated during the glucose oxidase-catalysed reaction is greater.

To underline the effect of buffer capacity, we reformulated the results presented in graphs 1–3, by presenting the pH variation in time, at a given glucose concentration, at different buffer capacities of the matrix (Figs. 4–6). By analysing the obtained results (Figs. 4–6), we notice that for the same analyte concentration, the pH variation decreases with the increase of phosphate concentration in the buffer solution. With the increase of buffer capacity, more protons will be neutralized by the phosphate present in the buffer and the transducer (the glass electrode) will sense a smaller pH variation. The influence of the buffer capacity becomes more accentuated, when glucose concentration increases. Calibration graphs (Fig. 7) were obtained at different buffer capacities, for the glucose oxidase-based enzyme electrode.

Glucose analysis in natural juices and wine (see Table 1) required only sample dilution in the respective buffer solutions. The obtained results were confirmed by the standard addition method and by a volumetric technique-Schoorle method for determination of reducing sugars.

By analysing the obtained results, presented in table 1, we notice that it is advisable to work at a relatively strong buffer capacity (in 10^{-3}M buffer solution) especially for acidic samples (citric juices and wine). The greater results obtained for these acidic products in the most diluted buffer solution (10^{-4}M) can be explained by the presence of organic acids (citric, ascorbic, tartaric etc., whose influence could not be thoroughly minimized at that buffer capacity.

The increase of the obtained (measured) glucose concentration with the decrease of the buffer capacity, especially for wine, must also be correlated to the degree of sample dilution (maximum 1/200 for wine), compared to orange juice (1/2000). The influence of the buffer capacity becomes almost negligible for less acidic samples (carrot juice).

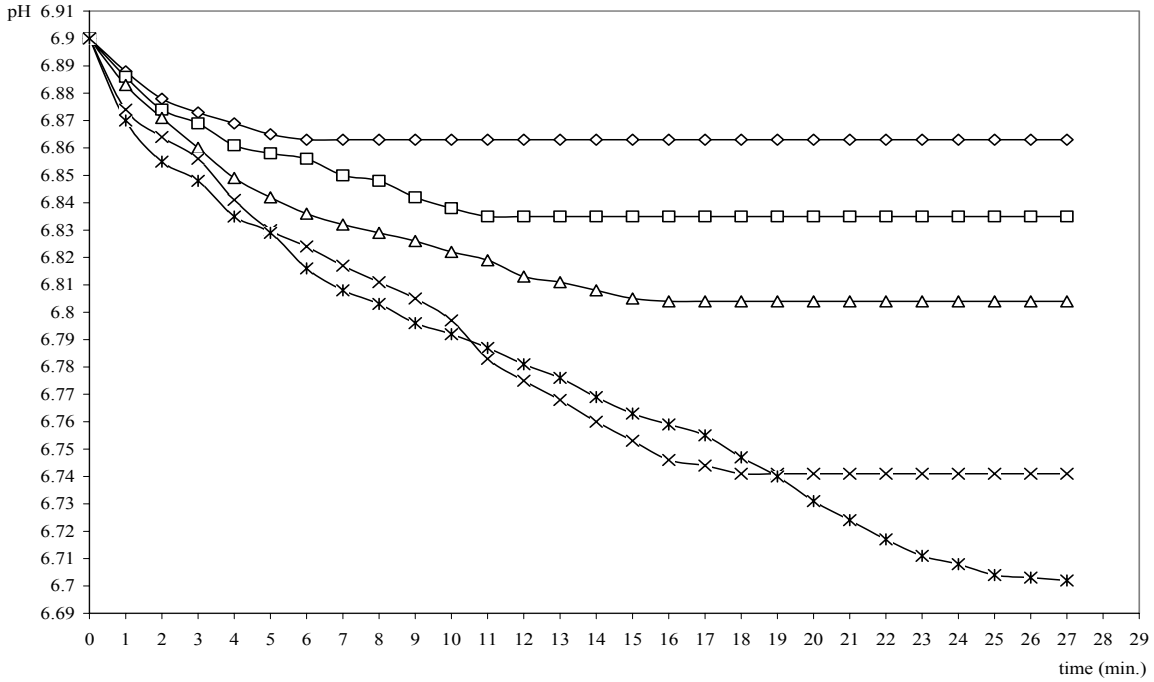

Fig. 1. pH diminution in time, in the buffer solution with 10^{-3}M phosphate, at different glucose concentrations: ◊–10^{-4}M; □–2×10^{-4}M; Δ–5×10^{-4}M; x–$7{,}5\times10^{-4}$M; *–10^{-3}M.

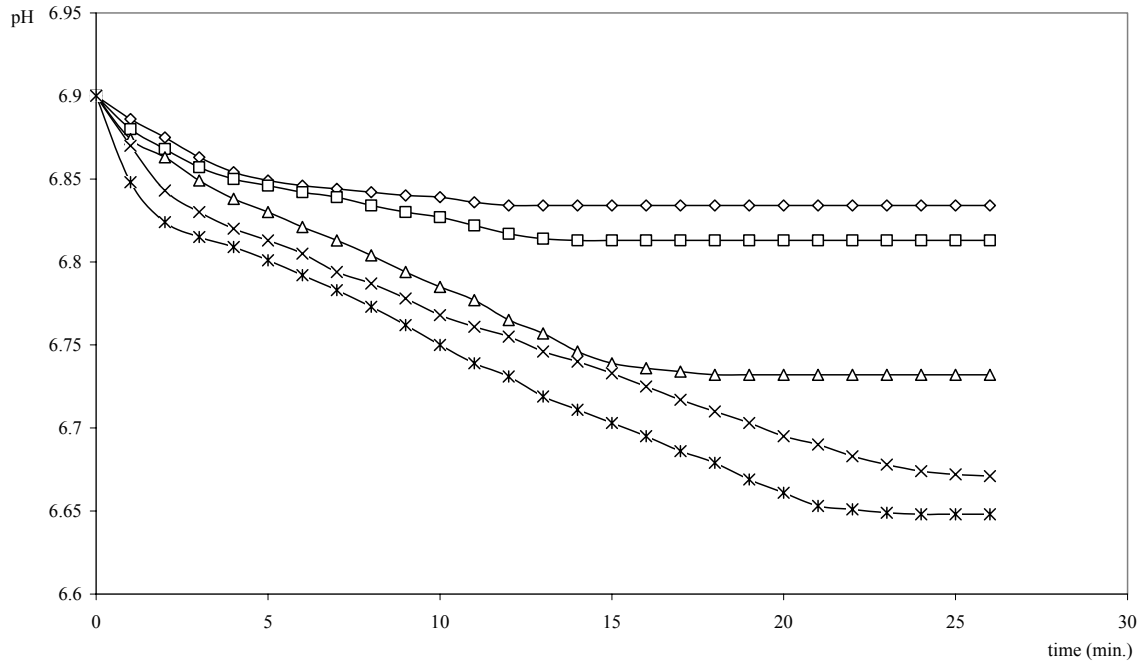

Fig. 2. pH diminution in time, in the buffer solution with 5×10^{-4}M phosphate, at different glucose concentrations: ◊–10^{-4}M; □–2×10^{-4}M; Δ–5×10^{-4}M; x–$7{,}5\times10^{-4}$M; *–10^{-3}M.

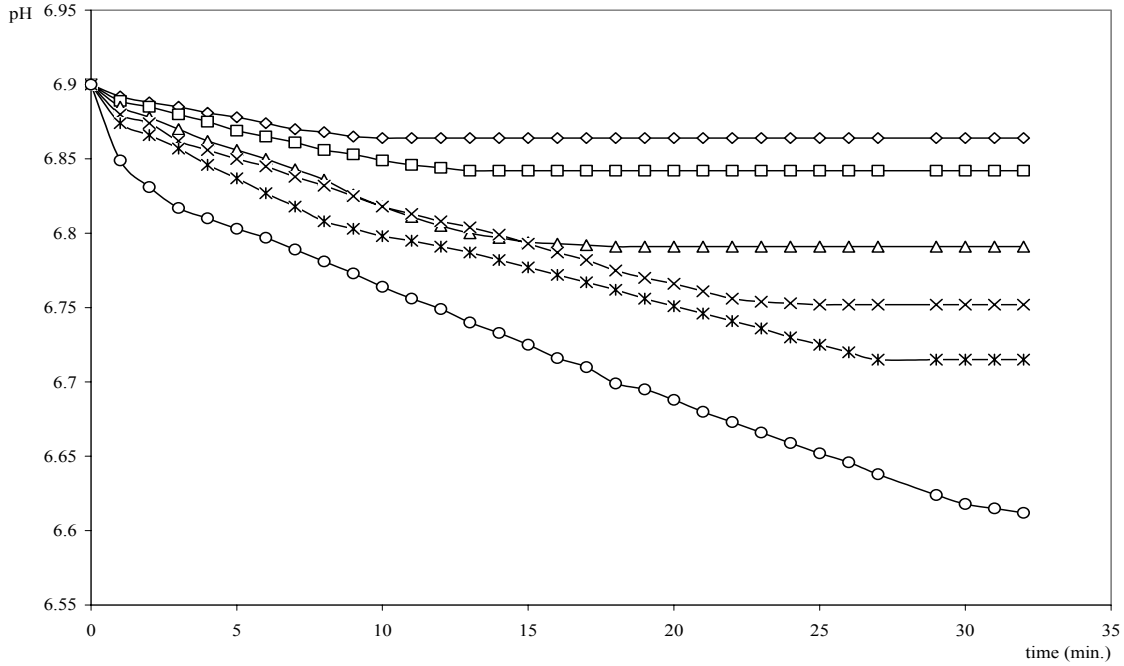

Fig. 3. pH diminution in time, in the buffer solution with 10^{-4}M phosphate, at different glucose concentrations: ◊–5×10^{-5}M; □–$7{,}5\times10^{-5}$M; Δ–10^{-4}M; x–$1{,}25\times10^{-4}$M; *–2×10^{-4}M; o–5×10^{-4}M.

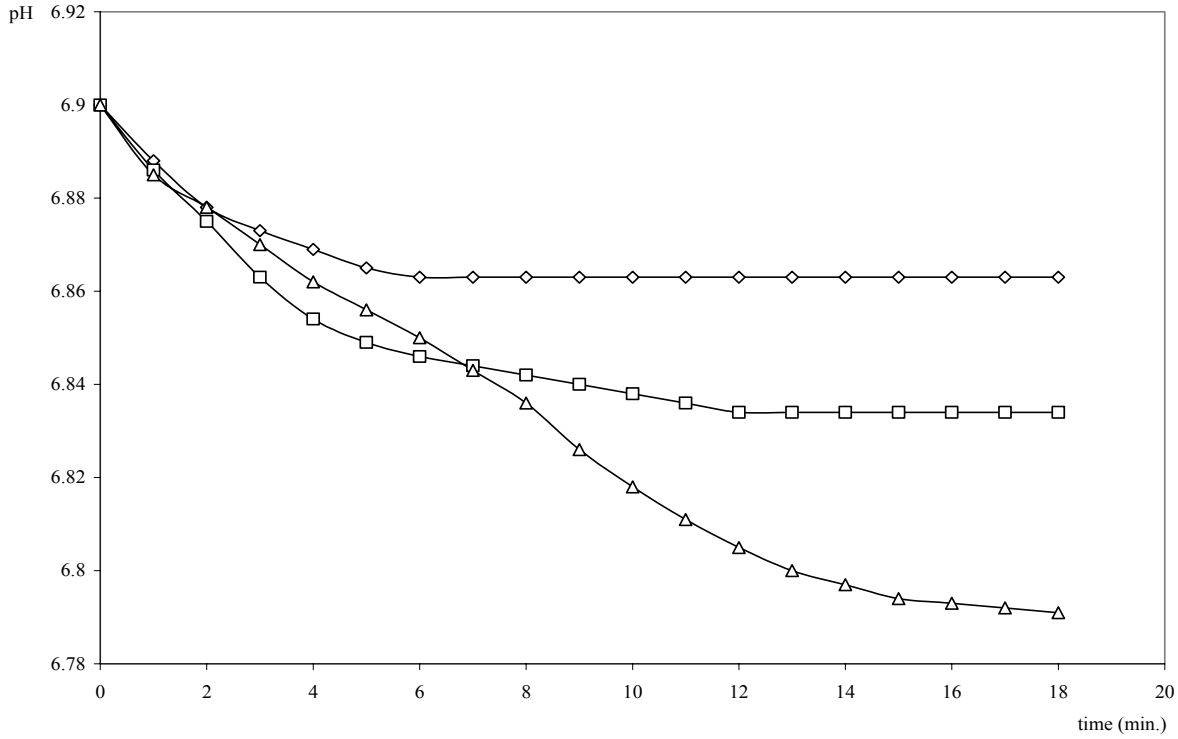

Fig. 4. pH diminution in time at 10^{-4} M glucose, at different phosphate concentrations in the buffer solution: ◊–10^{-3}M; □–5×10^{-4}M; Δ–10^{-4}M.

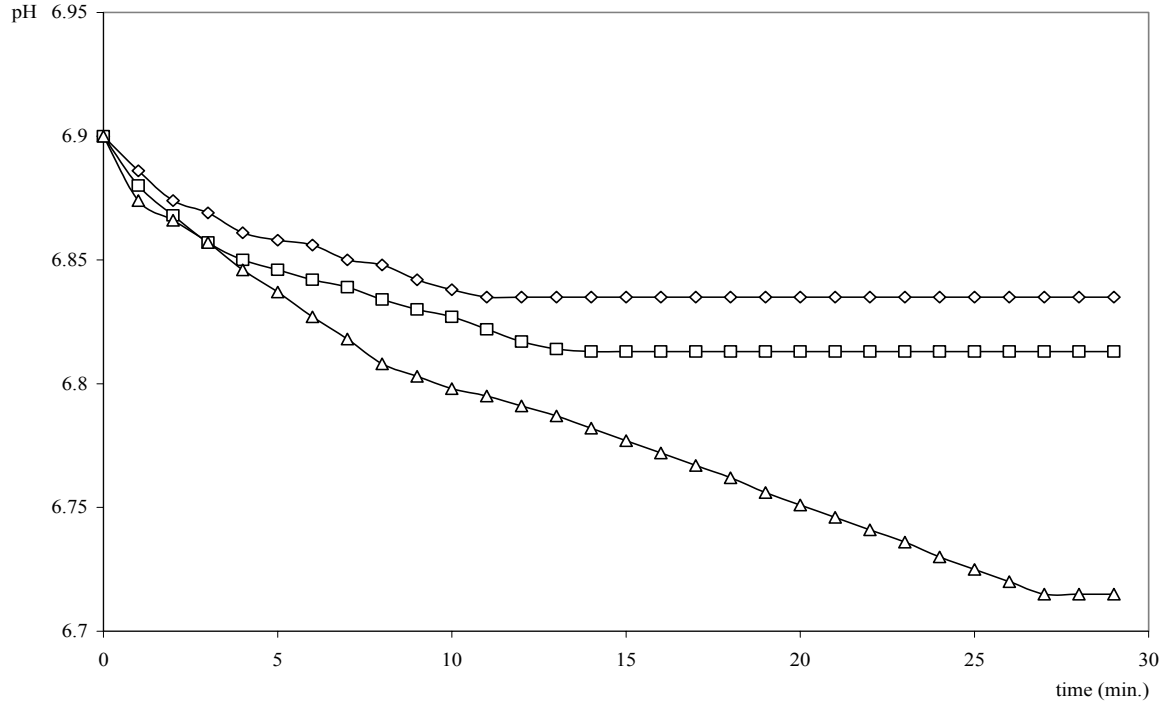

Fig. 5. pH diminution in time at 2×10^{-4} M glucose, at different phosphate concentrations in the buffer solution: ◊–10^{-3}M; □–5×10^{-4}M; Δ–10^{-4}M.

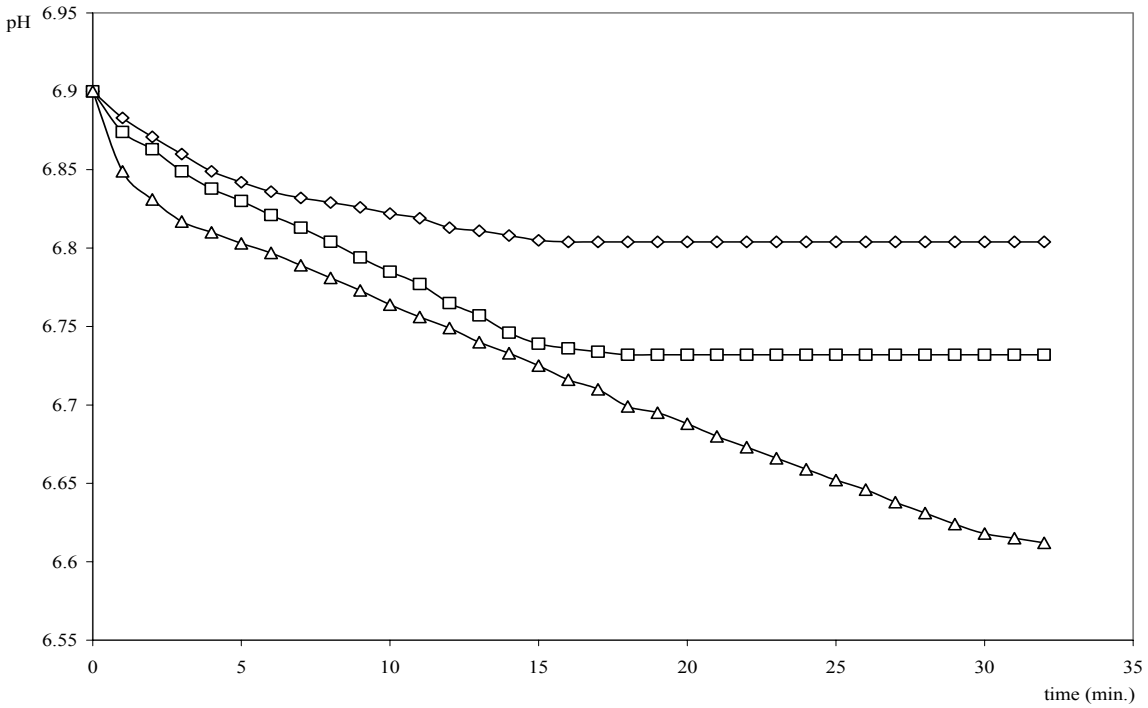

Fig. 6. pH diminution in time at 5×10^{-4} glucose, at different phosphate concentrations in the buffer solution: ◊–10^{-3}M; □–5×10^{-4}M; Δ–10^{-4}M.

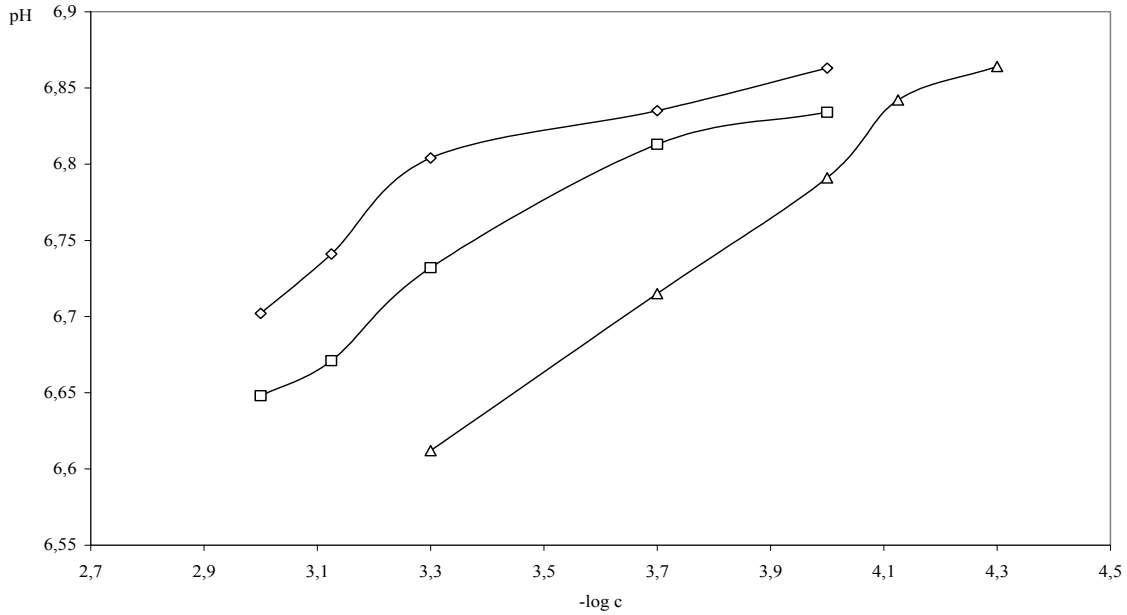

Fig. 7. Calibration graphs obtained at different phosphate concentrations in the buffer solution : ◊–10^{-3}M; □–5×10^{-4}M; Δ–10^{-4}M.

Table 1

Results (%) obtained at glucose determination in natural juices and wine

Sample	Schoorle	C_{buffer} M	Dilution	Biosensor	Add 1 theoretical	Recovery 1 (%)	Add 2 theoretical	Recovery 2 (%)
Carrot	2.75	10^{-3}	1/1000	2.92	4.92	97.15	6.92	96.53
Carrot	–	5×10^{-4}	1/1000	2.88	4.88	104.10	6.88	105.81
Carrot	–	10^{-4}	1/1000	3.03	5.03	102.78	7.03	96.59
Orange	3.81	10^{-3}	1/500	3.93	4.93	101.93	5.93	95.95
Orange	–	5×10^{-4}	1/500	4.12	5.12	96.87	6.12	104.90
Orange	–	10^{-4}	1/2000	4.24	5.24	94.08	6.24	104.49
Wine	0.40	10^{-3}	1/100	0.42	0.62	104.84	0.82	104.88
Wine	–	5×10^{-4}	1/100	0.44	0.64	104.69	0.84	95.24
Wine	–	10^{-4}	1/200	0.49	0.69	94.93	0.89	102.25

Add.1 theoretical and Add.2 theoretical represent the theoretical values after the first and the second addition, respectively.
Recovery(%)1 and Recovery (%)2 are the percents of the obtained (measured) values (after the first and the second addition, respectively), calculated from the theoretical values (which are considered 100).

CONCLUSIONS

For the same enzyme loading and for the same buffer capacity, the analytical signal increases with the analyte concentration, because the amount of gluconic acid generated during the enzymic reaction is greater.

At the same enzyme loading and for the same analyte concentration, the pH diminution decreases with the increase of buffer capacity, because the glass electrode senses a smaller amount of protons when the phosphate concentration in the buffer is greater. The influence of buffer capacity is more accentuated as glucose concentration increases.

The method has been applied with good results to glucose determination in real samples (orange juice, carrot juice and wine). The obtained results have been confirmed by a conventional volumetric technique (Schoorle) and by the standard addition method.

It is advisable to work at a relatively strong buffer capacity for acidic samples, in order to minimize the influence of organic acids found in citric juices and wines. Of course, this influence

must be correlated to the degree of sample dilution. The influence of buffer capacity is much less pronounced for nonacidic samples (ex. carrot juice).

In order to reach a good degree of accuracy, it is compulsory to adjust the buffer capacity of the matrix to the nature of the analysed sample.

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