ETHANOL CONTENT DETERMINATION IN BEERS, WINES, AND HARD LIQUOR DRINKS USING A CATALYTIC FUEL CELL. COMPARISON WITH OTHER TWO CONVENTIONAL ENZYMATIC BIOSENSORS: CORRELATION AND STATISTICAL DATA.

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Abstract – A catalytic fuel cell has been employed to check ethanol content in several samples of commercial wines, beers and hard liquor drinks. Two other conventional biosensors, based on catalase or alcohol oxidase enzyme, were also used, to the same purpose, on the same samples. Data obtained by three sensor methods have been compared and correlated. Lastly t-test and F-test were applied.

Keywords: analysis , statistical data, ethanol beverages, three sensors

1. INTRODUCTION

Our team recently investigated the feasibility of using a small catalytic 'fuel cell' originally constructed for the purpose of obtaining energy from methanol or ethanol, but now adapted for analytical purposes [1,2], based on the following reaction: $C_2H_5OH + 3H_2O \longrightarrow 12H^+ + 12\ e^- + 2CO_2$. The intention was to see whether this kind of device can also be used for ethanol determination in real samples.

2. METHODS

To this end water-alcohol solutions containing increasing percentages of ethanol are added to the cell, recording the current increase that occurs between two electrodes of the cell, working in potentiostatic format. Lastly the current was achieved at the steady state, after each hydroalcoholic addition. it was so possible to obtain calibration curves for ethanol.

Owing good analytical results obtained operating on standard ethanol solutions, the fuel cell was then used to check the ethanol content in several wine, beer and hard liquor commercial drink samples.

Results obtained analyzing red and white wines or beers are also compared with that obtained using two conventional different enzyme electrodes that have been fabricated by immobilizing either alcohol oxidase, or catalase, in kappa-Carrageenan gel layer overlapped to an amperometric gaseous diffusion Clark type oxygen electrode. The variation of the oxygen concentration in the aqueous solution, due to the enzymatic reactions was measured at a constant applied potential of – 650 mV. In the case of the catalase electrode [3,4], the measurement was performed by adding hydrogen peroxide to a buffer solution which was diffused through the dialysis membrane towards the enzymatic layer where the reaction catalysed by the catalase enzyme took place: $H_2O_2 \xrightarrow{\text{catalase}} 1/2\ O_2 + H_2O$. Since this reaction led to the production of oxygen, the concentration of the latter in the measurement solution increased. This increase triggered an increase in the cathodic current, which increased from the original value to a new value corresponding to a new stationary state. At this stage further additions were made (equal to 20 µL) of a standard solution of ethanol; after each addition a reaction of the following type occurred which was catalyzed by catalase: $CH_3CH_2OH + H_2O_2 \xrightarrow{\text{catalase}} CH_3CHO + 2\ H_2O$. The second reaction removed part of the H_2O_2 substrate from the first reaction, which was slowed down; this slowdown was accompanied by a decrease in the level of oxygen produced in the solution during the first reaction; that was evidenced by the decrease in the measured cathodic current which attained a new stationary state after each addition of alcohol solution. The current variation was read off after each alcohol addition [3, 4]. When the biosensor used alcohol oxidase, the operating procedure was

much simpler [3] as it consisted of directly making successive additions of the standard ethanol solution to a buffer solution in which the measurement was being performed. After each addition a reaction catalyzed by the alcohol oxidase enzyme of the following type took place: $CH_3CH_2OH + O_2 \xrightarrow{\text{alcohol oxidase}} CH_3CHO + H_2O_2$. The reaction led to the oxygen present in the solution being consumed with a consequent decrease in the cathodic current, which was measured until a new stationary state was reached. Also in this case, after each addition, the current variation was read off. All the experiments were carried out in a reaction cell thermostated at 23°C containing 15 mL of 0.05 mol L^{-1} phosphate buffer solution.

3. RESULTS

The effect of pH on the response of the conventional biosensors, investigated in detail, showed as the best pH was found to be 7.5 for the catalase electrode and 8.0 for the alcohol oxidase electrode, respectively. The catalase biosensor displayed a much greater sensitivity to ethanol than, for instance, to methanol, unlike the alcohol oxidase biosensor. The catalase biosensor also displayed a stability and a life-time at least triple than that of the alcohol oxidase biosensor, as well as an LOD at least one decade lower. It also displayed a better repeatability and reproducibilty for ethanol standard solutions. The alcohol oxidase biosensor was instead found to be more sensitive to methanol than to ethanol, but allowed the test to be carried out slightly faster than with the catalase device. The only drawback of the catalytic fuel cell was its long response time, but its lifetime was very long in comparison with those of the two conventional enzyme electrode.

A comparison of the main analytical results for three used devices is done in the table 1.

Table1. Main analytical results.

Method	Linearity range(M)	LOD(M)	Life time (days)	Analysis time (min)
Catalase biosensor	2.0×10^{-6} –2.0×10^{-5}	0.4×10^{-6}	≈30	≈34
Alcohol oxidase biosensor	9.2×10^{-6} –3.4×10^{-4}	3.6×10^{-6}	≈7	≈25
Fuel Cell (SC) potentiostatic format at OAP	1.0×10^{-3} –4.0×10^{-2}	8.0×10^{-4}	>90	≈55

A comparison among the results obtained applying the catalytic fuel cell and two conventional amperometric enzymatic sensors, was also performed by studying the correlation of results obtained by applying three methods for ethanol content determination in several commercial wines and beers (see Fig. 1-3).

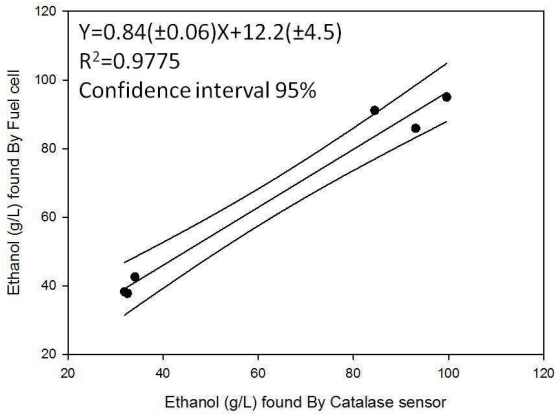

Fig. 1. Correlation between results found by Fuel cell and Catalase biosensor.

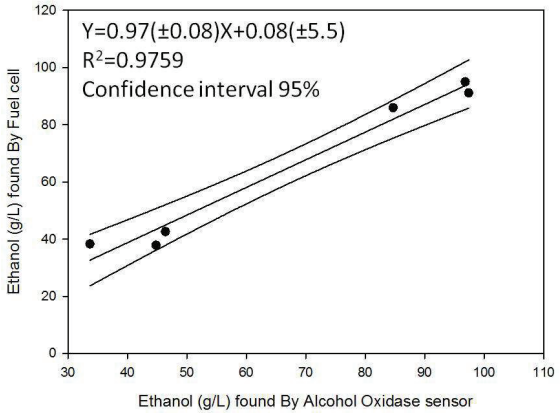

Fig. 2. Correlation between results found by Fuel cell and Alcohol oxidase biosensor.

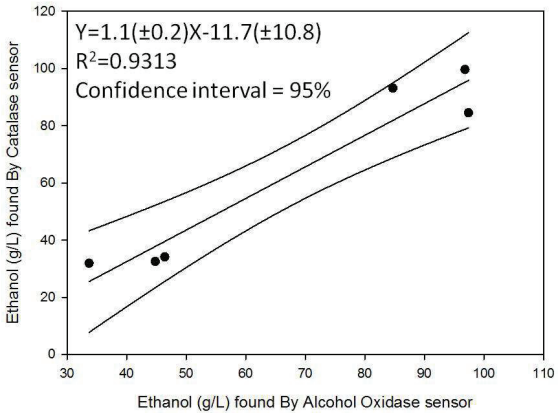

Fig. 3. Correlation between results found by Catalase biosensor and Alcohol oxidase biosensor.

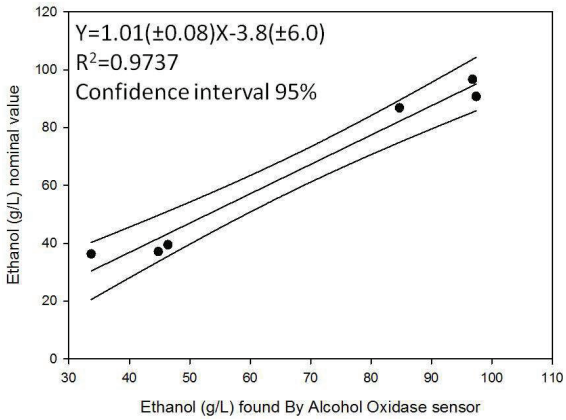

Fig. 6. Correlation between nominal values and results found by Alcohol oxidase biosensor.

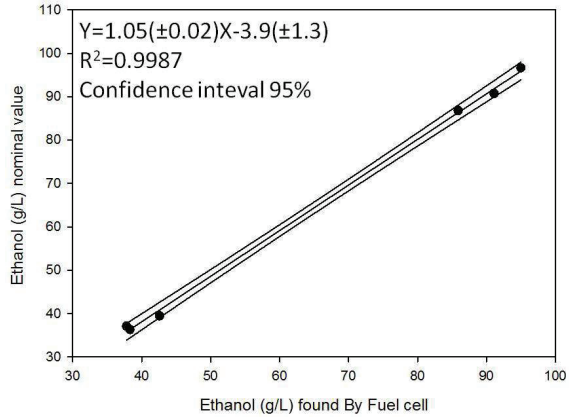

Fig. 4.Correlation between nominal values and results found by Fuel cell.

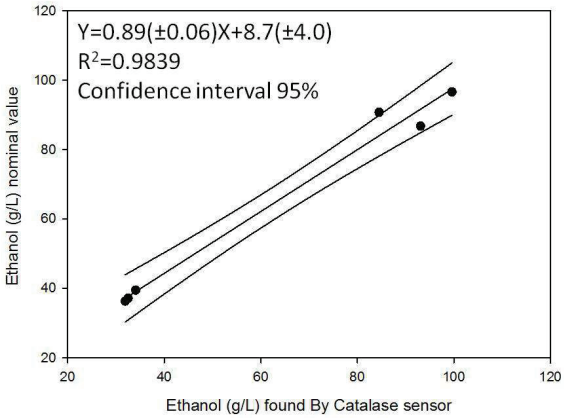

Fig. 5. Correlation between nominal values and results found by Catalase biosensor.

In addition also the correlation of data from each method and nominal values given by beverages producer firms (Fig. 4,5 and 6) was also evaluated. Lastly also t-test was applied(table 2).

Table 2. Paired t-test, two sided, ν=5, (p=95%) applied to values obtained by three methods for beers and wines samples vs nominal values .

By Catalase sensor	By Alcohol oxidase sensor	By Fuel cell
t-experimental		
-0.8881	1.421	0.8476
t-critical		
2.571	2.571	2.571
|t-exp.| < t-cr.	|t-exp.| < t-cr.	|t-exp.| < t-cr.
Results of t-test		
Not significant	Not significant	Not significant

Finally a statistical evaluation of variance by F-test was performed. See table 3.

Table3. F-test:comparison among precisions , two sided (p=95%).

	Result of the F-test		
Samples	Alcohol ox. *vs.* Catal.	Fuel cell *vs.* Catal.	Fuel cell *vs.* Alcohol ox.
B.n.1	N.S.	S.	S.
B.n.2	N.S.	N.S.	N.S.
B.n.3	N.S.	S.	S.
W.n.1	N.S.	N.S.	N.S.
W.n.2	N.S.	N.S.	N.S.

W.n.3	N.S.	N.S.	N.S.
W.n.4	N.S.	N.S.	N.S.

N.S.= Not Significant ; S.= Significant
B.= beer; W.= wine

Lastly in Fig. 7 a comparison of first results of research still in progress, concerning the ethanol content determination in conventional hard liquor drinks, using catalytic fuel cell and nominal values given by producers has been shown.

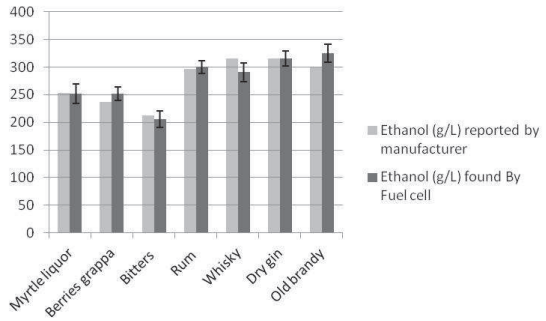

Fig. 7.Agreement between nominal values and results found by Fuel cell for the super alcoholic samples.

4.CONCLUSIONS

Results demonstrated a good correlation among three different methods as well as by the nominal values. The F-test evidenced as the repeatability of three methods is usually of the same order. An evaluation of obtained data evidence as the catalytic fuel cell may be considered a suitable analytical devices to check ethanol content in commercial beverages at slow cost and in a simple way. The comparison with two other conventional enzymatic biosensor methods confirm this assertion. It is also interesting for instance to observe as the best correlation between experimentally determined and nominal values (see R^2) was found just in the case of the catalytic fuel cell device.

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