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Spectrophotometric Biosensor for Flow Injection Analysis of Glucose on Real Samples.

LOPES, Flavio Marques[1]; **FERNANDES**, Kátia Flávia [2]

Keywords: biosensor spectrophotometric; Glucose-oxidase; Peroxidase, Polyaniline

1.INTRODUCTION:

The detection of glucose in biological fluid is one of the most important analysis for diagnosing several diseases. These analysis must be made combining precision, specificity, reproducibility and high speed of processing. Similarly, the determination of glucose concentration in foods, beverages and pharmaceutical products constitute on of the routine measurement in the quality control area. On this context, the biosensors appears as very effective tool because they assemble these properties in one single device.

2.MATERIALS AND METHODS:

The sensor was composed by glucose oxidase (GOD) and peroxidase (HRP) immobilized onto polyaniline activated with glutaraldehyde (PANIG). The immobilized enzymes were assembled into a reaction camera and the products formed in the enzymatic reaction were conducted to the spectrophotometer (Fig. 01).

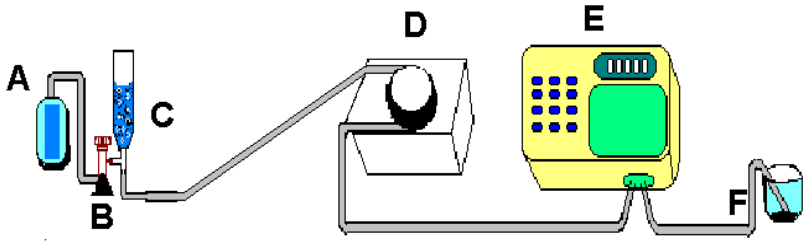

Fig. 01 – Air pump; B- Commuter valve; C- Reaction camera; D- Peristaltic pump; E- Spectrophotometer; F- Waste.

3.RESULTS AND DISCUSSIONS:

The immobilization parameters were optimized for GOD, with a maximum immobilization yield of 16% when 5.0 mg of PANIG and 8.9 EU prepared in 0.1 mol L-1 phosphate buffer, pH 7.0 were left to react for 60 min, at 4oC, under gently stirring. HRP maximum immobilization yield of 24.5% was obtained when immobilization conditions were 162 EU prepared in 0.1 mol L-1phosphate buffer, pH 6.0 were offered to 5.0 mg of PANIG for 120 min, at 4oC, under gently stirring. Three reactor designs were tested, being the fluidized ascendant bed reactor that showed best performance. On this reactor, the operational parameters were optimized and under these conditions, the linear operational range for glucose determination was between 0.5 and 6.0 mg mL-1(Fig. 02). The biosensor showed high response reproducibility, with maximum relative standard deviation of 0.003 (Fig 03). This biosensor was utilized for glucose determination in real samples, such as blood serum (Table 01), medicine syrups (Table 02), glucose serum (Table 02), fruit juices (Table 03), energetic drinks and honey (Table 03). The results obtained in the biosensor were compared to those with free enzymes (commercial kits),

and then submitted to validation tests, in confidence interval rage of 95%, using the paired t test.

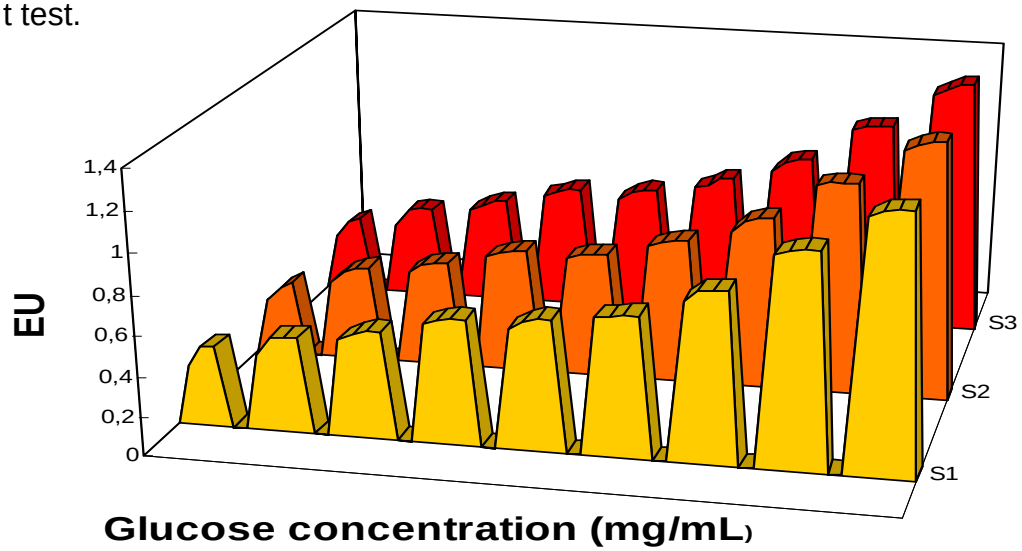

Fig 2- Biosensor response for increasing glucose concentration. (Concentrations: 0; 0.5; 1.0 1.5; 2.0; 2.5; 3.0; 4.5 and 6.0 mg/mL)

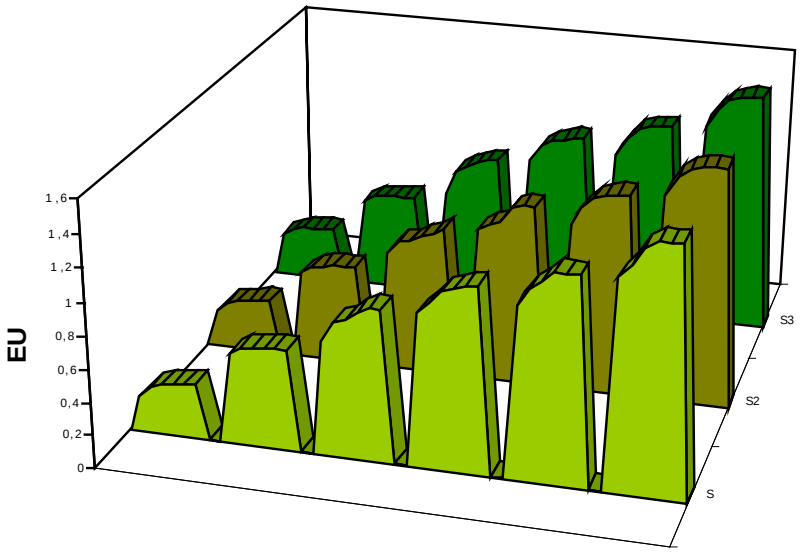

Fig 3- Biosensor response as function of residence time. (Times: 5; 10 ; 15; 20; 25 and 30 min

Table 01 – Glucose Concentration In Human Plasma

Laboratory Glucose (mg/mL)	Biosensor		Laboratory Glucose (mg/mL)	Biosensor	
	Glucose (mg/dL)	RSD		Glucose (mg/dL)	RSD
82	82	0,02	118	94	0,06
96	128	0,03	90	89	0,04
73	73	0,03	90	103	0,05
86	94	0,02	99	92	0,01
74	73	0,05	103	92	0,02

84	69	0,06	119	88	0,04
96	93	0,03	70	84	0,05
79	78	0,06	75	80	0,06
98	91	0,06	100	104	0,05
100	132	0,06	74	81	0,02
96	93	0,07	74	86	0,06
88	87	0,03	88	82	0,06
252	223	0,04	86	86	0,02
226	203	0,04	82	83	0,03

t Test in 95% Confidence. (t Table = 2,05; t Calculated = 0,48)

Table 02 – Glucose concentration in Nourishing products

Nourishing Products	Laboratory		Biosensor	
	Glucose (mg/mL)	RSD	GLUCOSE (mg/mL))	RSD
Gatorate (Isotonic)	41,8	0,03	53,7	0,06
Marathon (Isotonic)	36,3	0,005	48,5	0,07
Energil C (Isotonic)	46,15	0,011	50,4	0,2
On Line (Energetic)	61,7	0,02	87,7	0,12
Red Bull (Energetic)	67,1	0,03	64	0,23
Flyng Hourse (Energetic)	49	0,02	47	0,14
Tampico (Citrus Juice)	48,9	0,02	53,6	0,06
Honey	37,4	0,04	30,2	0,02

t Test in 95% Confidence. (t Table = 2,36; t Calculated = 1,77)

Table 3 – Glucose concentration in Pharmaceutical products

Pharmaceutical Products	Laboratory		Biosensor	
	Glucose (mg/mL)	RSD	GLUCOSE (mg/mL))	RSD
Syrup Claritin	240,5	0,08	215,5	0,02
Syrup Bisolvan	0,003	0,03	0,002	0,05
Syrup Melagrião	111,5	0,09	92,1	0,03
Serum Glucose 5% (Equiplex)	59,1	0,02	64,7	0,02
Glucose Ampoule 25% (Equiplex)	245,7	0,01	219,6	0,02

t Test in 95% Confidence. (t Table = 2,77; t Calculated = 2,03)

4. CONCLUSIONS:

All the results were validated, being possible to use this biosensor for glucose determination in substitution of those classic enzymatic tests commercially available on the market.

SUPPORT – FUNAPE

[1] Bolsista de pós-graduação (Doutorado). - flaviomarques@ueg.br
[2]Orientadora/ Instituto de Ciências Biológicas/UFG/ katia@icb.ufg.br