A PH OPTODE BASED ON THYMOL BLUE: APPLICATION TO DETERMINATION OF CO_2 USING FLOW INJECTION ANALYSIS SYSTEM

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Abstract: An optode based on thymol blue (TB), an acid-based indicator, has been constructed and evaluated as a detector in FIA system for CO_2 determination. The dye was chemically immobilised on the surface of a bifurcated glass optical fibre bundle, using silanisation in organic media. In FIA system, hydrogen carbonate or carbonate samples are injected in a buffer carrier solution, and then are mixed with phosphoric acid solution to generate CO_2, which diffuses through a PTFE membrane, in order to be collected in an acceptor carrier fluid, pumped towards to detection cell, in which the optode was adapted. The proposed system presents two linear response ranges, from 1.0×10^{-3} to 1.0×10^{-2} mol l^{-1}, and from 2.0×10^{-2} to 0.10 mol l^{-1}. The sampling frequency was 11 sample h^{-1}, with good repeatability (R.S.D < 4 %, n = 10). In flow conditions the optode lifetime was 170 h. The system was applied in the analysis of commercial mineral water and the results obtained in the hydrogen carbonate determination did not differ significantly from those obtained by potentiometry, at a confidence level of 95 %.

Keywords: CO_2, FIA, thymol blue, silanisation, pH-optode.

1 - INTRODUCTION

Quantification of carbon dioxide is an important parameter in areas such as medicine, environmental and industrial process [1,2], principally due to its important role in animal and plants metabolism and in the process of photosynthesis. Thus, measurement *in vivo* or in real time of blood P_{CO_2} in intensive care units is considering an essential parameter in monitoring, diagnosis, and treatment of critically ill patients subject to surgery [1,2]. In the atmosphere the CO_2 presence is the result of plant respiration (production) and photosynthesis (consumption). However, human activities, such as heading systems, transportation, industrial combustion, etc., and natural phenomenas, such as burning and degradation of biomass or volcanic eruptions, are responsible for much higher levels of this gas in some regions [3]. In this sense, CO_2 analysis is considered an essential element of environmental research [2]. CO_2 determination is also vital in many biochemical industries, where it serves for the process control. The continuous analysis of the fermentation process on line is critical for high product yields, minimal unwanted metabolic by products and an overall optimised control strategy [2].

With the aim of CO_2 determination, numerous analytical procedures are known in gases and fluids. Determination of CO_2 can be accomplished

by spectrophotometry based on pH-indicator systems [4], turbidimetric method [5] and infrared (IR) spectrometry [6,7]. Generally, these methodologies are fast and produces reliably quantitative results. However, they are expensive, bulky and not very much robust.

It is enough notorious than in all process for determination of CO_2, measurements in continuous form will be very convenient. In this aspect, flow systems become powerful tools; principally due to they are simple, reliable and allow a high sample throughput. For the other hand, chemical sensors are excellent devices for coupled in flow systems.

Between, the chemical sensors, that based on optical fibres, are usually sensitive, robust, cheap and easily miniaturised [8], also the optical sensors offer the possibility of remote, continuous, multianalyte analysis in low volume locations [2]. Thus, for the CO_2 determination numerous optodes have been developed. Since those reported by Zhujun and Seitz [9] and Optiz and Lübbers [10] in 1984, based on Severinghaus electrode [11]. In these optodes [12-19], the CO_2 molecules diffuse through a hydrophobic membrane, which separates the sodium bicarbonate buffer internal solution from the sample, the carbon dioxide is converted to HCO_3^- in the internal solution, changing its pH, which one is followed by using colorimetric or fluorescent pH indicators. However, the greater problems in these optodes are the internal solution use to detect the pH variations, and the type of membrane used to separate the internal and the analysed solutions. Consequently, these CO_2 optodes present limited lifetime and long response times [17] difficult their coupling in flow systems.

Due to the drawbacks presented by the optodes based on Severinghaus principle, gradual configurations have been proposed in order to obtain optodes with response times suitable for use in flow systems, and also to avoid the use of the internal bicarbonate solution.

In the ion-pairing method [6,7,20-24], ion-pair between a pH indicator and a phase transfer such as tetraoctylammonium hydroxide is entrapment in a layer of hydrophobic material such as silicone, permeable to CO_2 but not for H^+ [25]. These optodes show extremely fast response times,

when applied in gas phase measurements, but in liquid phase the sensor should be covered with a permeable membrane for the continuous and prolonged use [2].

Other configurations including the development of films sensors for CO_2 [26-29]; coupled the fibre optic in flow cell containing the indicator in solution [30] and copolymerised the dye in polymeric matrices [31]; or still immobilising polysiloxane directly on the glass optical fibre [32].

Previously, we report [33,34] optodes for pH measurements, constructed immobilised chemically acid–base indicators in a bifurcated borosilicate optical fibre bundle using the silanisation procedure in organic media. These optodes were evaluated in several flow systems showed response times and lifetime satisfactory for its use in these systems [33], and evaluated in the indirect determination of oxalate in food samples, which the CO_2 generated by the enzymatic reaction between oxalic acid and oxalate oxidase [34], was satisfactorily monitored.

Despite of the numerous optodes reported in literature, still exist the necessity to developed new experimental procedures for CO_2 determination. In this sense, this work describe the results obtained in the evaluation of the performance of a pH optode based on thymol blue (TB) acid-based dye, chemically immobilised on the glass optical fibre through silanisation, in the indirect determination of CO_2, as an alternative to the other existing methods.

2 - EXPERIMENTAL

2.1 - Reagents and Solutions

All chemical reagents were of analytical grade. Buffer solutions were prepared using distilled water and the actual pH of the solutions was determined by employing a pH electrode connected to a pH-meter (Micronal B374).

For the construction of the pH optode, nitric acid (Merk), ethanol (Nuclear), *o*-Xylene (Mallinckrodt), 3-aminopropyl-triethoxysilane (Aldrich) and thymol blue (Synth), were used. *o*-Xylene was previously dried overnight with anhydrous Na_2SO_4 before using in the silanisation.

2.2 - Construction of TB optode

The optode was constructed as previously described [33,34], by employing a bifurcated borosilicate optical fibre bundle (5 mm diameter), made with fibres of 70 µm i.d. The surface of the common end of the optical fibre bundle was initially treated with ethanol, nitric acid and ethanol, and allowed to dry at room temperature. Then, the silanisation was carried out by introducing the common end of the bundle into a 5.0 % (v/v) 3-aminopropyl-triethoxysilane in *o*-xylene. After this step, the silanised common end was rinsed with *o*-xylene, in order to eliminate any excess of silane, then with ethanol, to clean off the solvent, and allowed to dry at room temperature. The immobilisation of the dye was done by introducing the common end of the bundle into a 0.04 % (w/v) TB solution, containing 8.6×10^{-4} mol l^{-1} NaOH, and rinsed with deionised water. After all this steps the tip of the optical fibers showed an intense purple colour, indicating that the immobilisation was successful.

2.3 - Instrumentation

The instrumentation employed in this work consisted of a radiometric source (Oriel 6883), with a quartz-halogen lamp (12 V, 100 W), whose radiation was modulated at 200 ± 2 Hz by means of an optical chopper (Oriel 75152) and focused onto one arm of the optical fibre bundle. The modulated light source was guided to the common end of the optical fibre, where the TB was immobilised. The reflected radiation was focused onto the entrance slit of a monochromator (Oriel 77250) and detected by a photomultiplier tube (Oriel 77340), connected to a lock-in amplifier (Stanford Resear-

ch System SR 510), provided with a pre-amplifier (Stanford Reasearch System SR 552). The lock-in amplifier was synchronised to the frequency of the optical chopper. A PC-AT 486 microcomputer was employed to control the monochromator and the lock-in amplifier, through an electronic interface (ADLink ACL-811). Software was written in Microsoft VisualBasic 3.0 to perform these tasks.

2.4 - Flow Manifold and Procedure

The flow manifold was assembled as shown in Figure 1. Was employing a peristaltic pump (Ismatec® IPC8), furnished with tygon tubes to propel the solutions. In the FIA system, hydrogen carbonate or carbonate samples were injected into a 0.1 mol l^{-1} phosphate buffer at pH 7.6 stream (PBS), using a sliding central bar sampling valve [35], and then were mixed with a phosphoric acid solution, in order to generate CO_2. The CO_2 diffused through a PTFE (Black Swam MFG. Co.) membrane placed in the gas diffusion cell "2" [36], and was collected by an adequate acceptor fluid, which was continuously pumped towards to detection system "D", that include the TB optode. The change of the pH in the acceptor fluid, causes a colour change of the TB immobilised on the optical fibre bundle, generating a variation in the reflectance intensity, which was proportional to sample concentration.
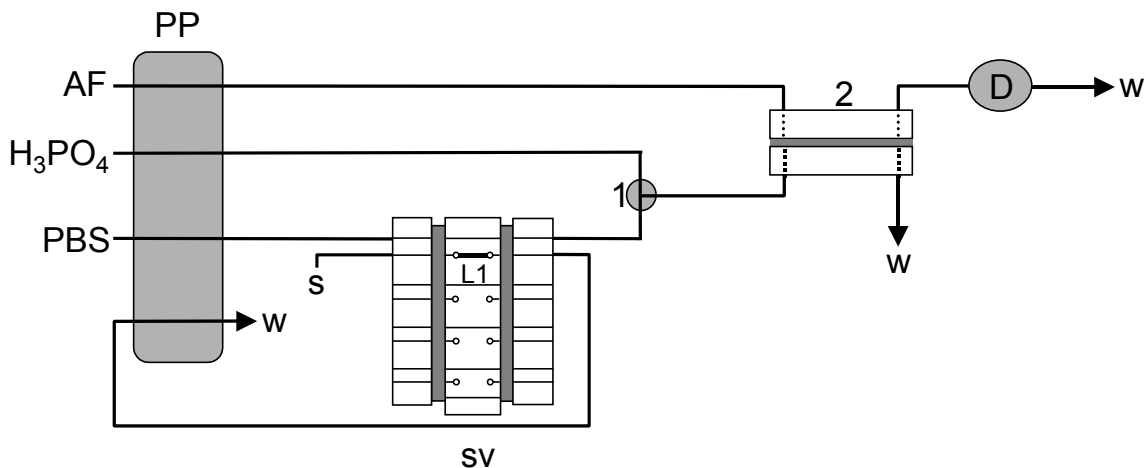
Figure 1. Schematic diagram of the flow manifold. PP, peristaltic pump; AF, acceptor fluid; PBS, phosphate buffer solution; s, sample; L1, loop of sampling; sv, sampling valve; 1, mixture point; 2, gas diffusion cell; D, detection system and w, waste.

2.5 - Determination of hydrogen carbonate in mineral water samples

Six commercial mineral water samples collected in the Campinas city (São Paulo – Brazil) were analysed, in which the total concentration of hydrogen carbonate was determined. Each sample was injected directly into the FIA system, and the reflectance intensity variation was monitored. The concentration of hydrogen carbonate in the sample was determined by interpolation, employing the analytical curve obtained with $NaHCO_3$ standards solutions. These results were compared with those obtained by potentiometric titration [37], using a pH-meter (Micronal B374) equipped with a glass electrode. The hydrogen carbonate titration in the samples was carried out used a 0.0100 mol l^{-1} HCl standard solution.

3 - RESULTS AND DISCUSSION

3.1 - Characteristics of the thymol blue optode

Thymol blue (TB) was chosen due to its aqueous solution presents two pH regions of colour change (from 1.2 to 2.8 and from 8.0 to 9.6). On the other hand, studies carried out by Gonçalves *et al.* [4] have demonstrated the efficiency of the TB dye in aqueous solution for CO_2 determination, using FIA system and spectrophotometric detection. When TB was immobilised in a optical fibre bundle, the pH optode presented two linear response ranges, allowing to detect pH changes from 4.0 to 11.0 as showed in Figure 2. It is a good result, because generally, optodes based on acid-base dyes presented a response range of two pH units or $pK_a \pm 1$. This peculiar profile of the TB optode can be probably explained considering the TB dye characteristics, which presents two-pH range of colour change in aqueous solutions. The extended ranges can be attributed to the chemical immobilisation of the colorimetric reagent on different sites of the optical fibre bundle surface, as explained previously [33], where the indicators molecules can be either strongly or weakly bon-

ded to the optical fibre, resulting in groups with different p*Ka*´s and, therefore, extending the linear pH range.
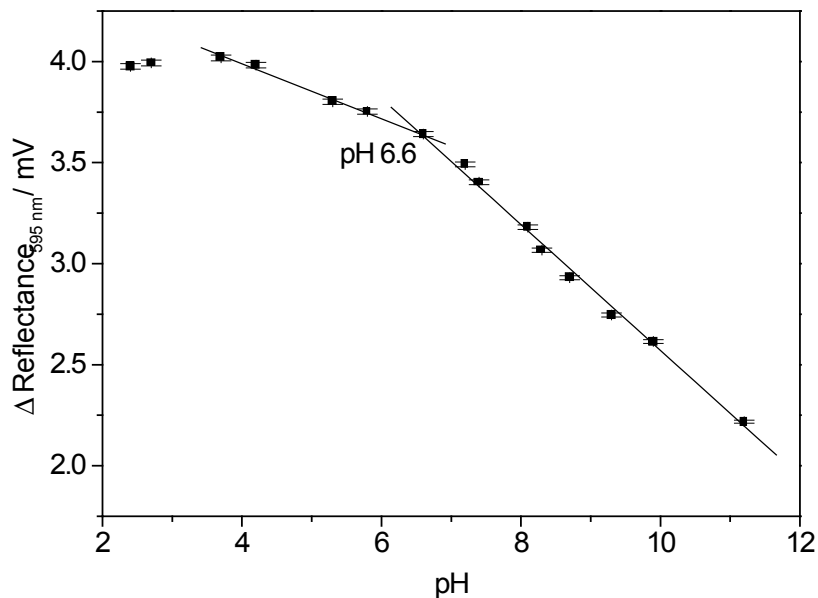

Figure 2. TB optode response profile as pH function.

3.2 - Optimisation of the FIA system

In the determination of the CO_2 with the proposed FIA system, parameters such as composition, pH and concentration of the acceptor fluid (AF) have important effects on the sensitivity and linear response range of the method. Thus, was carry out a study on different acceptor fluids. Solutions of different compositions were employed with pH values in the range from 4.0 to 11.0, considering the color changes of the TB optode. The results obtained with acceptor solutions with pH lower than 5.0 were not reproducible, due to the formation of small bubbles of CO_2. For 1.0×10^{-3} mol l^{-1} phosphate buffer solution (pH 7.5) provided the best results for CO_2 determination in the FIA system, were obtained (Table 1). On the other hand, in basic solutions (pH > 9) a little leaching of the TB immobilised was observed.

Table 1. Characteristics of the flow system as a function of pH and acceptor fluid composition (ionic strength adjusted at 0.05 mol l^{-1} with NaCl).

Receiving carrier fluid	Linear range/mol l^{-1}	Sensitivity/mV l mol^{-1}
NaOH (pH 11.0)	$2.5 \times 10^{-3} - 1.0 \times 10^{-2}$	18.0 ± 0.6
	$1.0 \times 10^{-2} - 0.10$	7.6 ± 0.2
NaOH (pH 10.5)	$2.5 \times 10^{-3} - 1.0 \times 10^{-2}$	25.7 ± 1.3
	$1.0 \times 10^{-2} - 0.10$	8.1 ± 0.2
Phosphate buffer	$2.5 \times 10^{-3} - 1.0 \times 10^{-2}$	32.2 ± 0.9
1.0×10^{-3} mol l^{-1} (pH 7.5)	$2.5 \times 10^{-2} - 0.10$	13.2 ± 0.6

V_i = 1.0 ml; flow rate = 2.9 ml min^{-1} ; acid solution = 0.1 mol l^{-1} H$_3$PO$_4$.

The effect of the acceptor fluid ionic strength on the FIA system performance was evaluated. This study was carried out because is known that optodes constructed using acid-base dyes are sensitive to variation of ionic strength leading to incorrect results [38]. In this study, the ionic strength of the acceptor fluid was adjusted in 0.01, 0.05 and 0.10 mol l^{-1} with NaCl. The analytical curves showed in Figure 3 indicate that the ionic strength adjusted to 0.01 mol l^{-1} provides the best results, mainly for the more diluted range.
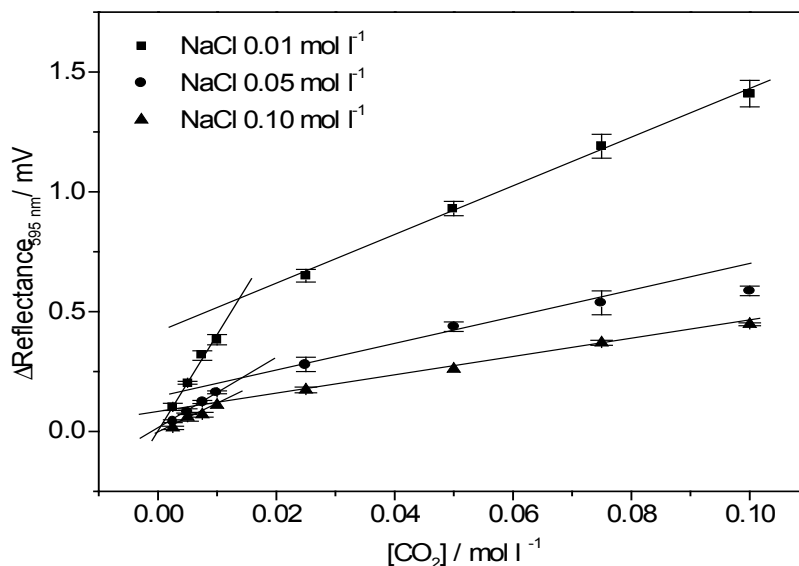

Figure 3. Analytical curves obtained in the study carried out to evaluate the influence of the ionic strength of receiving carrier fluid in the FIA system response.

In Table 2 are shows the FIA system characteristics as a function of injected volume (V_i) of sample. V_i equal to 500 µl was used, since it provided good sensitivity and good sampling frequency. However, if the sensitivity must be improved, 1000 µl can be used.

Table 2. Sensitivities and sampling frequencies obtained in the linear range from 2.5×10^{-2} to 0.10 mol l^{-1}, as function of the injected volume (V_i).

V_i / µl	Sensitivity / mV l mol^{-1}	Sampling frequency / sample h^{-1}
125	0.8 ± 0.2	13
250	2.5 ± 0.1	12
500	3.6 ± 0.1	11
750	3.7 ± 0.1	9
1000	6.9 ± 0.4	8

AF = 1.0×10^{-3} mol l^{-1} phosphate buffer (pH 7.5), with ionic strength adjusted at 1.0×10^{-2} mol l^{-1} with NaCl; 0.10 mol l^{-1} H$_3$PO$_4$ acid solution and flow rate = 2.9 ml min^{-1}.

Table 3 shown the results obtained in the study carried out to evaluate the effect of the flow rate on the FIA system performance. Even though the best results were obtained using a flow rate of 1.3 ml min^{-1}, it was employed a flow rate of 1.6 ml min^{-1}, as a mean of improving the sampling frequency.

Table 3. Effect of the flow rate of the carrier fluid on the response and sampling frequency. ΔReflectance at flow rate 1.3 ml min^{-1} = 0.64 mV; [Na$_2$CO$_3$] = 0.010 mol l^{-1}.

Flow rate / ml min^{-1}	Sampling frequency/sample h^{-1}	Percentage of reflectance variation
1.3	9	100
1.6	10	72
2.6	11	57
3.5	12	30

AF (acceptor fluid) 1.0×10^{-3} mol l^{-1} phosphate buffer (pH 7.5), with ionic strength adjusted at 1.0×10^{-2} mol l^{-1} with NaCl; 0.10 mol l^{-1} H$_3$PO$_4$ acid solution and V_i = 500 µl.

The composition of the acidic solution used to generate CO$_2$ was also studied, employing succinate buffer (pH 3.0), H$_3$PO$_4$ and H$_2$SO$_4$ solutions. Results summarised in Table 4 show that 0.5 mol l^{-1} phosphoric or sulphuric acid solutions provide the best results. Phosphoric acid was chosen in the present work because the signal was greater than that one obtained with sulphuric acid solution.

Table 4. Reflectance variation for various acid solutions. [Na$_2$CO$_3$] = 0.010 mol l^{-1}.

Confluent fluid	H$_3$PO$_4$ / mol l^{-1}		H$_2$SO$_4$ / mol l^{-1}		Buffer
	0.5	0.1	0.5	0.1	(pH 3.0)
Δ Reflectance/mV	0.72 ± 0.02	0.64 ± 0.01	0.69 ± 0.01	0.52 ± 0.02	0.35 ± 0.02

*standard deviation for three replicates.

AF (acceptor fluid) 1.0×10^{-3} mol l^{-1} phosphate buffer (pH 7.5), with ionic strength adjusted at 1.0×10^{-2} mol l^{-1} with NaCl; flow rate = 1.6 ml min^{-1} and V_i = 500 µl.

3.3 - FIA system performance

Figure 4 shows a typical response profile for this FIA system and in Figure 5 is showed the corresponding analytical curves.
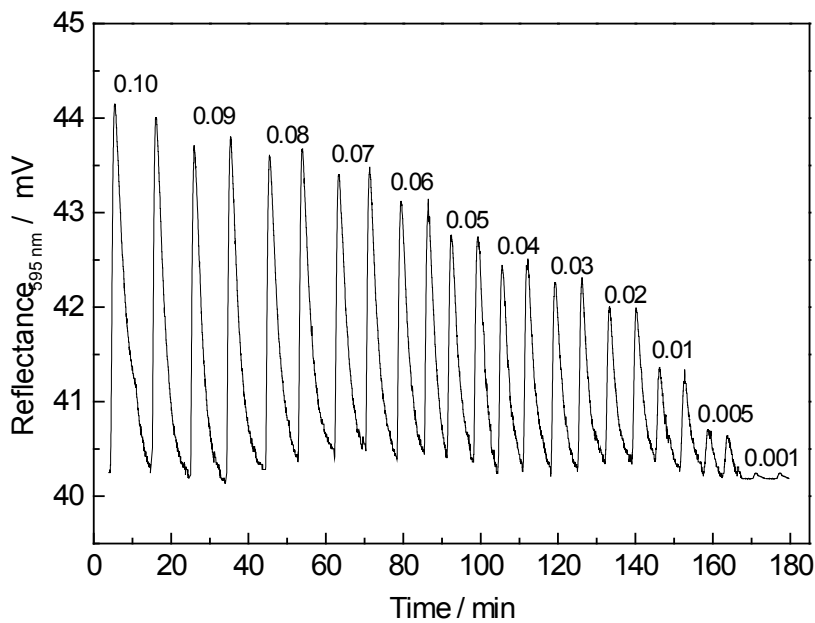

Figure 4. FIA signals obtained in the CO_2 determination (values above the peaks indicate the concentration of the solution, in mol l^{-1}).
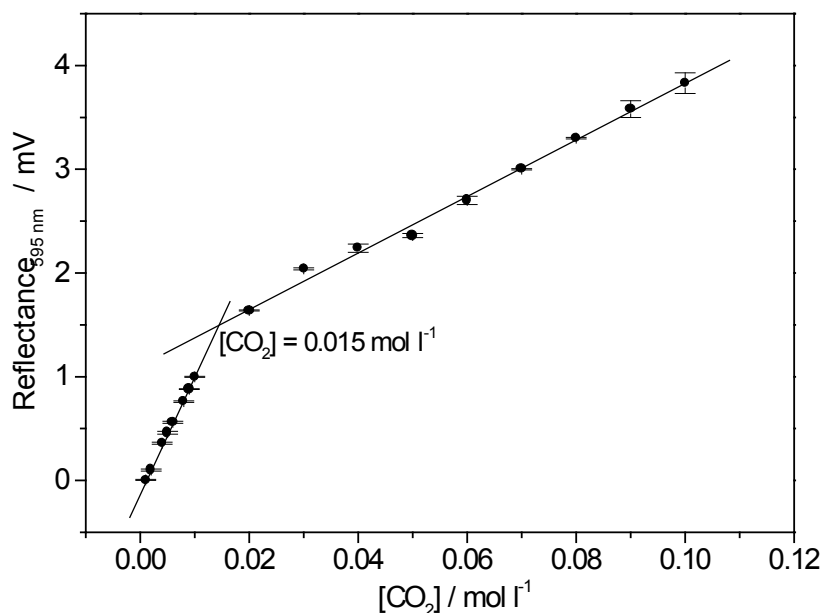
Figure 5. Analytical curves obtained in FIA system proposed. Measurements carried out injected 500 μl of samples in a carrier of 0.1 mol l^{-1} phosphate buffer (pH 7.6) with 0.1 mol l^{-1} NaCl and 1.0 x 10^{-3} mol l^{-1} EDTA. The acid solution was 0.50 mol l^{-1} H$_3$PO$_4$ and accepter fluid 1.0 x 10^{-3} mol l^{-1} buffer solution (pH 7.5) with 1.0 x 10^{-2} mol l^{-1} NaCl in a rate of 1.60 ml min^{-1}.

At Figure 5 it can be observed two linear response ranges, the first from 0.001 to 0.010 mol l^{-1} and the second response range from 0.020 to 0.10 mol l^{-1}, which presented the following linear regressions, respectively;

$$\Delta Reflectance = -0.103(\pm 0.017) + 109.597(\pm 2.007) \ [CO_2] \quad (r=0.9991, n=8) \tag{1}$$

$$\Delta Reflectance = 1.115(\pm 0.033) + 27.164(\pm 0.621) \ [CO_2] \quad (r=0.9982, n=9) \tag{2}$$

This peculiar profile of this FIA system can be probably explained considering the response profile of TB optode as a function of pH (Figure 2). When hydrogen carbonate or carbonate samples, are injected the pH of the accepter fluid changes. These pH changes are detected by TB optode, which responds linearly until the concentration of the sample causes a pH change in the acceptor fluid of 6.6 (see Figure 2). In the proposed FIA system, the injection of samples with concentrations higher than 0.015 mol l^{-1}, causes a pH shift in the acceptor fluid for values smaller than 6.6 (Figure 2), corresponding to the straight line with minor inclination of Figure 5. For concentrations higher than 0.015 mol l^{-1}, a straight line from 0.020 to 0.10 mol l^{-1} is obtained.

The repeatability of the FIA system was evaluated by ten successive injections of 5.0 x 10^{-2} mol l^{-1} sodium carbonate solutions, obtained a relative standard deviation value (R.S.D.) of 3.5%. This result indicates the good performance of the proposed FIA system.

The TB optode lifetime in flow conditions was c.a. of 17 days, which is in accordance with others optodes, previously reported [33,34].

3.4 - Determination of CO_2 in mineral water samples

The system was evaluated by hydrogen carbonate determination in mineral water. No significant differences were found at a confidence level of 95 %, when the results were compared with those obtained with potentiometric method, as showed in Table 5.

Table 5. Results obtained in the determination of hydrogen carbonate concentration in mineral waters by proposed and potentiometric methods.

Sample	[HCO_3^-] / mol l^{-1}	
	Potentiometric titration	Proposed method*
01	1.99×10^{-3}	$(1.97 \pm 0.10) \times 10^{-3}$
02	2.01×10^{-3}	$(2.04 \pm 0.12) \times 10^{-3}$
03	2.67×10^{-3}	$(2.68 \pm 0.01) \times 10^{-3}$
04	2.72×10^{-3}	$(2.63 \pm 0.05) \times 10^{-3}$
05	2.90×10^{-3}	$(2.92 \pm 0.01) \times 10^{-3}$
06	2.02×10^{-3}	$(1.99 \pm 0.01) \times 10^{-3}$

* standard deviation for six replicates

4 - CONCLUSIONS

The chemical immobilisation of TB dye on a borosilicate optical fibre bundle allowed the preparation of a pH optode, which presented response times sufficiently short and long lifetime to be used as a detector in a FIA system. The results show that this system is able to determine satisfactorily carbonate, with good repeatability and accuracy.

Acknowledgements

Resumo: Este trabalho mostra a construção de um optodo baseado no indicador ácido – base azul de timol (TB) e sua aplicação como detector de um sistema para análise por injeção em fluxo (FIA) para determinação de CO_2. O indicador foi quimicamente imobilizado na superfície de um feixe bifurcado de fibras ópticas de vidro, usando silanização em meio orgânico. No sistema FIA, amostras de carbonato ou bicarbonato são injetadas em uma solução tampão carregadora, e a seguir são misturadas com solução de ácido fosfórico para gerar CO_2, o qual se difunde através de uma membrana de PTFE, para ser coletado em um fluido carregador aceptor, o qual é bombeado em direção à cela de detecção, na qual encontra-se o optodo. O sistema proposto apresenta duas faixas lineares de resposta, entre $1,0 \times 10^{-3}$ e $1,0 \times 10^{-2}$ mol l^{-1}, e entre $2,0 \times 10^{-2}$ e $0,10$ mol l^{-1}. A freqüência de amostragem foi de 11 amostras h^{-1}, com boa repetibilidade (R.S.D < 4 %, n = 10). Sob condições em fluxo o tempo de vida do optodo foi de 170 h. O sistema proposto foi aplicado na análise de águas minerais comerciais, e os resultados obtidos

na determinação de bicarbonato foram similares daqueles obtidos pelo método potenciométrico, a um nível de confiança de 95%.

Palavras-chave: CO_2, FIA, azul de timol, silanização,optodo para medidas de pH.

5 - References

[1] M.P. Xavier, G. Orellana, M.C. Moreno-Bondi, J. Díaz-Puente, Quim. Anal. 19 (2000) 118.

[2] A. Mills, K. Eaton, Quim. Anal. 19 (2000) 75.

[3] T.D. Rhines, M.A. Arnold, Anal. Chim. Acta 231 (1990) 231.

[4] R.I. Gonçalves, Determinação da pressão parcial do CO_2, na atmosfera e em água do mar por injeção em fluxo contínuo, com detector espectrofotométrico, Ph.D. Thesis, USP, São Paulo, 1996.

[5] W. Lang, H.U. Wolf, R. Zander, Anal. Biochem. 92 (1979) 255.

[6] G. Neurauter, I. Klimant, O.S. Wolfbeis, Anal. Chim. Acta 382 (1999) 67.

[7] A. Mills, A. Lepre, L. Wild, Sens. Actuators B 38–39 (1997) 419.

[8] K.T.V. Grattan, D. T. Sun, Sens. Actuators B 82 (2000) 40.

[9] Z. Zhujun, R. Seitz, Anal. Chim. Acta 160 (1984) 305.

[10] N. Optiz, D.W. Lübbers, Adv. Exp. Med. Biol. 180 (1984) 757.

[11] J.W. Severinghaus, A.F. Bradley, J. Appl. Phys**.** 13 (1958) 1574.

[12] M.D. DeGrandpre, M.M. Baehr, T.R. Hammar, Anal. Chem. 71 (1999) 1152.

[13] J.W. Parker, O. Laksin, C.Yu, M.L. Lau, S. Klima, R. Fisher, I. Scott, B.W. Atwater, Anal. Chem. 65 (1993) 2329.

[14] M.B. Tabacco, M. Uttamial, M. McAllister, D.R. Walt, Anal. Chem. 71 (1999) 154.

[15] C. Goyet, R. David, R. Walt, P.G. Brewer, Deep-Sea Res. 39 (1992) 1015.

[16] M.F. Choi, P. Hawkins, Anal. Chim. Acta 309 (1995) 27.

[17] M.D. DeGrandpre, Anal. Chem. 65 (1993) 331.

[18] C. Munkholm, D.R. Walt, F.P. Milanovich, S.M. Klainer, Talanta 35 (1988) 109.

[19] O. S. Wolfbeis, Fibre Optic Chemical Sensors and Biosensors, CRC Press, Boca Raton, 1991.

[20] B.H. Weigl, A. Holobar, W. Trettnak, I. Lklimant, H. Kraus, P. O'Leary, O.S. Wolfbeis, J. Biotechnol. 32 (1994) 127.

[21] B. Müller, P.C. Hauser, Analyst 121 (1996) 339.

[22] G.J. Mohr, T. Werner, I. Oehme, C. Preininger, I. Klimant, B. Kovacs, O.S. Wolfbeis, Adv. Mater. 9 (1997) 1108.

[23] C. Mallins, B.D. McCraith, Analyst 123 (1998) 2373.

[24] H.N. McMurray, J. Mater. Chem. 2 (1992) 401.

[25] J. Lin, TrAC, Trends Anal. Chem. 19 (2000) 541.

[26] Y. Kawabata, T. Kamichika, T. Imasaka, and N. Ishibashi, Anal. Chim. Acta 219 (1989) 223.

[27] A. Mill, L. Monaf, Analyst 121 (1996) 535.

[28] B.H. Weigl, A. Holobar, N.V. Rodriguez, O.S. Wolfbeis, Anal. Chim. Acta 282 (1993) 335.

[29] C. Malins, H.G. Glever, T.E. Keyes, J.G. Vos, W.J. Dressick, B.D. MacCraith, Sens. Actuators B 67 (2000) 89.

[30] G. Orellana, M.C. Moreno-Bondi, E. Segovia, M.D. Marazuela, Anal. Chem. 64 (1992) 2210.

[31] C. Munkholm, D.R. Walt, F. Milanovich, Talanta 35 (1988) 109.

[32] I. Kasik, M. Pospísilová, V. Matejec, M. Chomát, K. Rose, L. Sasek, Book of Abstracts of the Europt(r)ode IV, 1998, sII11a.

[33] P.T. Sotomayor, I.M. Raimundo Jr., G. Oliveira-Neto, W.A. Oliveira, Sens. Actuators B 51 (1998) 382.

[34] M.D.P.T. Sotomayor, I.M. Raimundo Jr., G. Oliveira-Neto, L.T. Kubota, Anal. Chim. Acta 447 (2001) 33.

[35] H. Bergamin Fº, J.X. Medeiros, B.F. Reis, E.A.G. Zagatto, Anal. Chim. Acta 101 (1978) 9.

[36] O.J. Hall, R.C. Aller, Limnol. Oceanogr. 37 (1992) 1113.

[37] A. Vogel, Quantitative Chemical Analysis, Longman Group, England, 5nd edn., 1989, pp. 278-279.

[38] M.J.P Leiner, P. Hartman, Sens. Actuators B 11 (1993) 281.