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Evaluation of fibre optical chemical sensors for flow analysis systems

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Abstract

Optodes based on bromothymol blue (BB) and bromocresol purple (BP) acid–base indicators were constructed. The dyes were chemically immobilised on the surface of a bifurcated glass fibre optical bundle, which was previously silanised with 3-aminopropyl-triethoxysilane. A PTFE/acrylic cell was specially constructed, allowing the use of the optodes as detector in flow analysis systems. The optodes were evaluated for their pH responses in different flow approaches, such as continuous flow, flow injection and monosegmented flow systems. A steady state signal was achieved in the continuous flow system. The effect of the flow rate and the injected volume on the signal amplitude were studied. Signals up to 69 and 77% for BB optode and up to 82 and 92% for the BP optode of the steady state signal can be obtained by employing flow injection and monosegmented flow systems, respectively. The sampling frequency can be improved and sample consume can be lowered by employing the monosegment flow approach. © 1998 Elsevier Science S.A. All rights reserved.

Keywords: Optode; Optical fibre pH sensor; Flow analysis; FIA; MSFA

1. Introduction

Fibre optic chemical sensors (optodes) for the determination of metals and hydrogen ion in aqueous media are commonly constructed by supporting the sensitive reagent on the tip of a bifurcated optical fibre bundle [1–4]. Actually, the reagent is previously immobilised on a surface of a supporting material and then fixed on the tip of the bundle, with the aid of a polymeric grid. The immobilisation of the chemical reagent can be made by physical adsorption or electrostatic attraction on a polymeric support, such as Amberlite XAD-2, XAD-4 or XAD-7 [5–10], or by covalent chemical bonding on the surface of glass beads [11–13]. In the chemical immobilisation, the surface of the supporting material is previously activated by silanisation [11–13]. Sometimes, the reagent is physically entrapped into a polymeric film, which is fixed on the tip of the optical fibre bundle [14–18]. Frequently, these optode designs show long response times, typically 2–3 min. These response times depend basically on the thickness of the sensitive composite material, particle size and matrix quantity, indicator concentration, direction and magnitude of the pH changes, pH transition interval of the indicator and solution temperature [10].

Flow analysis systems, such as those based on flow injection analysis (FIA) [19] and monosegmented flow analysis (MSFA) [20,21] are widely used nowadays, since they are simple, reliable and allow a high sample throughput. In FIA systems, a sample aliquot is injected in a non-segmented flow, while in MSFA the sample is injected into the system between two air bubbles. These air bubbles avoid a direct contact between the sample and the carrier fluid, lowering significantly the sample dispersion, even for long residence times. Ordinarily, FIA and MSFA, despite their different flow patterns, provide similar analytical throughput, which is frequently high (ca. 120 sample h^{-1}) and therefore, the sample stays inside the flow cell for a short period (usually < 30 s). This characteristic of the flow systems requires a transducer with a relatively fast response time, in order to guarantee good sensitivity and provide an analytical signal whose intensity does not depend on transducer inertia.

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Considering the aspects pointed out above, an optode appears to have a response time incompatible with FIA or MSFA systems. In other words, when an optode is employed as a detector in these systems, the analytical signal does not reach the steady state, as a consequence of the response time of the sensor, causing loss of sensitivity. This problem does not affect the systems based on continuous flow, because the sample can be pumped continuously through the system, until the sensor reaches the steady state signal. However, this procedure increases sample consumption and also decreases sampling frequency.

The use of optodes as transducers in flow systems has been described in the literature. Sometimes the FIA approach has been employed [22,23], but most of the systems use the continuous flow approach [9,24–27]. Woods et al. [23] have compared the behaviour of three pH optodes based on different indicator strips in continuous flow and FIA conditions. Baker and Narayanaswamy [9] have employed a flow approach to investigate the influence of the support matrix, the immobilised indicator concentration and the solution ionic strength on the response of a pH optode based on 3,4,5,6-tetrabromophenolsulfonephthalein (TBPSP). Holobar et al. [24] studied the response time of a pH optode as a function of the flow rate, in order to evaluate the sensor for use in bioreactors. Despite these works, no systematic study has been carried out in order to verify the behaviour of the optode response under different flow conditions.

By considering these facts, this work is aimed at constructing pH optodes by immobilising a colorimetric reagent directly on the surface of a bifurcated optical fibre bundle, to study their behaviour in different flow conditions, such as FIA and MSFA, in order to improve sensitivity and/or sampling frequency.

2. Experimental

2.1. Reagents and solutions

All chemicals were of analytical reagent grade. Buffer solutions, in the pH range 3.0–9.0 were prepared according to standard procedures [28], by using boric acid, citric acid and trisodium orthophosphate. The ionic strength of the buffers was adjusted to 0.1 mol l^{-1} by the addition of suitable volume of 1.0 mol l^{-1} sodium chloride. The actual pH of the buffers were determined by employing a glass electrode and an Ag/AgCl reference electrode connected to a pH-meter (Micronal B374).

Nitric acid, ethanol, *o*-xylene, 3-aminopropyl-triethoxysilane, bromocresol purple (BP) and bromothymol blue (BB) of analytical grades were used for the construction of the optode.

2.2. Construction of the pH optodes

A bifurcated fibre optic bundle with diameters of 6.0 mm (common end) and 4.0 mm (each branch) was assembled by employing borosilicate optical fibres (Reinchenbach®-SP-Brazil). The tip of the bundle was fixed in a polyester resin, in order to avoid the solution entering the interstices of the fibre bundle by capillary effect. The surface of the bundle was initially treated with ethanol (3 h), 5% nitric acid (1 h) and ethanol (1 h) and allowed to dry during 12 h at ambient temperature. The silanisation of the bundle surface was carried out by introducing the optical fibre tip into 5.0% (v/v) 3-aminopropyl-triethoxysilane in *o*-xylene for 1 h, with constant agitation. After this process, the tip was kept in 20 ml of *o*-xylene during 20 min, with agitation, in order to eliminate the excess of silane, washed exhaustively with ethanol to eliminate any excess of xylene and dried at ambient temperature during 1 h. The immobilisation of the acid–base indicators were done by introducing the optical fibre tip into a 0.04% (w/v) BB solution, containing 6.4×10^{-4} mol l^{-1} NaOH, or in 0.04% (w/v) BP, containing 1.93×10^{-3} mol l^{-1} NaOH, for 2 h at ambient temperature, with constant agitation. Finally, the optical fibres were washed with deionised water and allowed to dry at ambient temperature for 4 h.

2.3. Instrumentation

The instrumentation employed in this work consisted of a radiometric source (Oriel 6883) with a quartz-halogen lamp (12 V, 50 W), whose radiation was modulated at (190 ± 2) Hz, by an optical chopper (Oriel 75152) and focused onto one branch of an optical fibre bundle. The radiation light from the source was guided through the fibre optic to the pH sensing reagent in the end of a bundle. The reflected radiation was focused onto the entrance slit of a monochromator (McPhearson 275) and detected by a photomultiplier (Hamamatsu R106), connected to a lock-in amplifier (Stanford Research Systems SR 510), synchronised by the optical chopper frequency. Reflectance measurements were made at 562 ± 1 and 570 ± 1 nm for BB and BP, respectively. A PC-AT 486 microcomputer was employed to control the monochromator through a home-made parallel interface based on IC 8255 [29], to control the lock-in amplifier and for data acquisition through a RS232C serial interface. The software necessary to perform these tasks was written in Microsoft QuickBasic 4.5.

2.4. Flow cell and flow manifold

The flow cell was constructed by fixing the optical fibre bundle tip in an acrylic block, which was screwed to a black PTFE block. These two blocks (each one 34

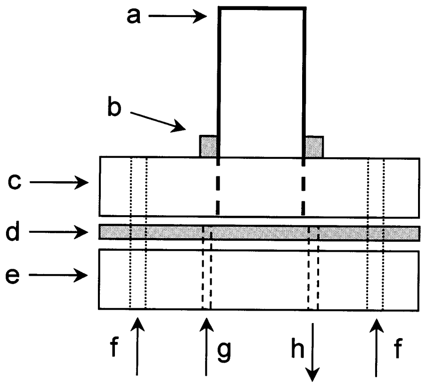

Fig. 1. Diagram of the flow cell: (a) cylindrical compartment to insert the fibre optical bundle (30 mm long and 9 mm id); (b) silicon glue; (c) acrylic block (34 × 34 × 10 mm); (d) rubber spacer (1 mm thick); (e) black PTFE block (34 × 34 × 10 mm); (f) screw holes; (g) flow inlet (1 mm id) and (h) flow outlet screw hole.

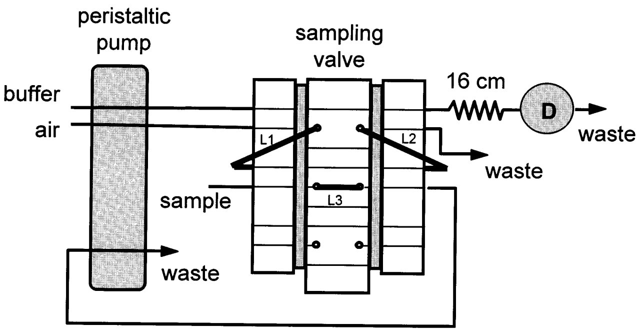

Fig. 2. Schematic diagram of the flow manifold. Sampling valve (in the sampling position) is arranged to perform the injection of a monosegment. D represents the flow cell.

mm long, 34 mm wide and 10 mm high) were separated by a 1 mm thickness rubber spacer in order to allow the solution to pass through the cell. Fig. 1 shows a schematic diagram of the cell.

The manifold was assembled by employing a peristaltic pump (Ismatec MP13GJ-4), furnished with tygon tubes to propel the solutions. A sliding central bar sampling valve [30] was employed to inject buffer solution into both the FIA and the MSFA systems. Fig. 2 shows the flow manifold, with the sampling valve arranged to inject a sample monosegment. In the sampling position, as shown in this figure, loops L1 and L2 are filled with air, while loop L3 is filled with sample. When the sampling valve is comutated to the injection position, the buffer fluid carries the monosegment (constituted by air loop L2, sample loop L3 and air loop L1) towards the detector. In the FIA approach, the air loops L1 and L2 are removed and the sample is injected directly into the carrier fluid. A PTFE tube with 0.8 mm internal diameter and 160 mm length was employed as transmission line between the injector and the flow cell. In the MSFA system, the buffer solutions were injected between two air bubbles of 35 µl.

3. Results and discussion

The chemical immobilisation, by covalent bonds, is used in the construction of an optode to improve durability of the sensor, because it reduces the leaching of reagent. This procedure has been described frequently in the literature. However, so far, the immobilisation has been carried out on the surface of glass beads [11–13], which are fixed on the tip of a bifurcated optical fibre bundle. The thickness of the glass beads layer directly affects the response time of the sensor, since the mass transport is slow [31,32]. This behaviour is also found when the reagent is physically adsorbed on a polymeric support [10]. In addition, the quantity of the immobilised indicator also affects the response time of the optode [10]. On the other hand, the present work shows that a thin layer of reagent can be obtained by its direct immobilisation on the surface of the optical fibre bundle, which can diminish the response time of the sensor, as previously described elsewhere [33].

Initially, the responses of the optodes were evaluated with continuous flow conditions, that is, by continuously pumping the buffer solution through the cell. In

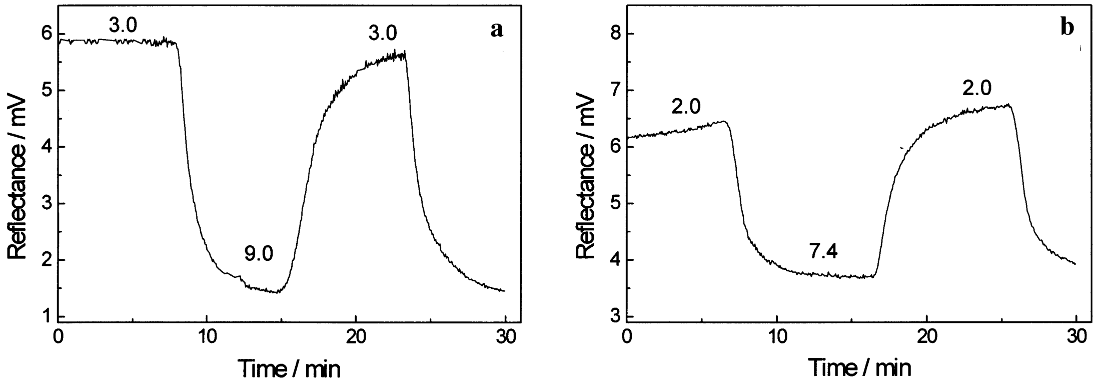

Fig. 3. Signals recorded in continuous flow conditions for reversibility studies. pH changes were from 3.0 to 9.0 and 2.0 to 7.4 for the optodes based on BB (a) and BP (b) acid–base indicators, respectively.

Table 1
Performance of the optodes based on BB and BP acid–base indicators

Parameter	BB optode	BP optode
Analytical pH range	3–8	2–6.8
Correlation coefficient	0.997	0.995
Operational life time (s)	180	210
Drift (mV day^{-1})	+0.2 mV (after 1 day) −0.4 mV (others days)[a]	+0.6 mV (after 2 days) −0.1 mV (others days)[b]

[a] Working 11 h day^{-1}.
[b] Working 14 h day^{-1}.

Table 2
Response times of the optodes in continuous flow conditions

Flow rate (ml min^{-1})	Response time (s)	
	BB optode[a]	BP optode[b]
1.0	228	190
1.8	212	146
2.7	166	126
3.6	148	112

[a] pH change from 4.0 to 7.4.
[b] pH change from 2.0 to 8.2.

these experiments, a steady state response is obtained. Reversibility studies were carried out by employing buffer solutions in the pH ranges of 3.0–9.0 and 2.0–7.4 for BB and BP optodes, respectively, with the ionic strength adjusted in 0.10 mol l^{-1} with sodium chloride. Fig. 3 shows the analytical signals recorded in these studies and Table 1 summarises the main characteristics of the optodes. Maximum signal differences of (4.29 ± 0.16) and (3.18 ± 0.08) mV were obtained for BB and BP optodes, respectively, when the pH was changed from 3.0 to 9.0. These signal differences decrease with the use of the optodes, due to leaching of the immobilised indicators. However, periodic sensor calibration can be used to overcome this problem. Useful life times of at least 180 and 210 h for BB and BP optodes, respectively, were obtained in flow conditions, which are better than others described in the literature [24].

The time necessary for the optode to reach the steady state signal depended on the flow rate. Table 2 shows that the response times of the optodes constructed in this work, decrease when the flow rate is increased. This result is expected because as far as the flow rate is increased, the solution in contact with the surface of the optode is renewed faster, allowing shorter response times. These results were obtained on the same day and it is important to emphasise that as long as the optodes are used, their response times are decreased. However, this fact does not mean a performance improvement of the optodes, but only reflects the leaching of the immobilised indicators. This result agrees with that described by Alabbas et al. [10], who showed that the response time of an optode is shortened if the quantity of the immobilised indicator is decreased. This fact can also explain the difference of response times between the BB and BP optodes. Although the BB was immobilised from a little more concentrated solution than the BP, it was probably more efficiently bonded to the surface of the optical fibre bundle than BP. As a consequence, BB optode shows response times longer than BP optode, as shown in Table 2.

Considering these results, experiments were carried out in FIA and MSFA systems, by varying the injected volume and flow rate in order to determine conditions of good sensitivity and high sampling frequency. In the MSFA system, the buffer solution was injected between two air bubbles, which were allowed to pass through the flow cell. The air bubble signals were easily identified and did not affect the precision of the measurements. The carriers were buffer solutions of pH 3.0 and 2.0 and signals were obtained by the injection of buffer solutions of pH 9.0 and 7.4 for BB and BP optodes, respectively, as shown in Figs. 4 and 5. Tables 3 and 4 show the results obtained in these experiments expressed as a fraction, in percentage, of the steady state signal. As can be seen, the signal increases if the injected volume increases, but decreases if the flow rate increases. This is in accordance with optode kinetic responses because, in flow conditions, the analytical signal is directly proportional to the time interval in which the sensor is in contact with the sample, before the steady signal is reached. In the MSFA system the sample is injected between two air bubbles, significantly diminishing its dispersion, allowing, as a consequence, an increasing in the analytical signal intensities. Furthermore, the change in the pH of the sample due to mixture with the carrier is minimised (often eliminated), whatever its buffer capacity, because the sample is separated from the carrier by the air bubbles. This problem can occur in FIA systems, since the mixture of sample and carrier is promoted by dispersion. Tables 5 and 6 show the sampling frequencies obtained with BB and BP optodes, respectively. As can be seen, by considering the same injected volume, FIA usually provides a higher sampling frequency than MSFA system. Since the sample dispersion is very low in MSFA, one could expected the highest analytical throughput with this system because the cleaning time is also expected be short. However, due to the low sample dispersion, MSFA provides high analytical signals and the sampling frequency is decreased because the response times of the optodes are relatively long. On the other hand, by considering analytical signals of the same magnitude, the MSFA spends less sample and provides higher sampling frequency than the FIA approach, as can be noted by looking at the some values in Tables 3–6.

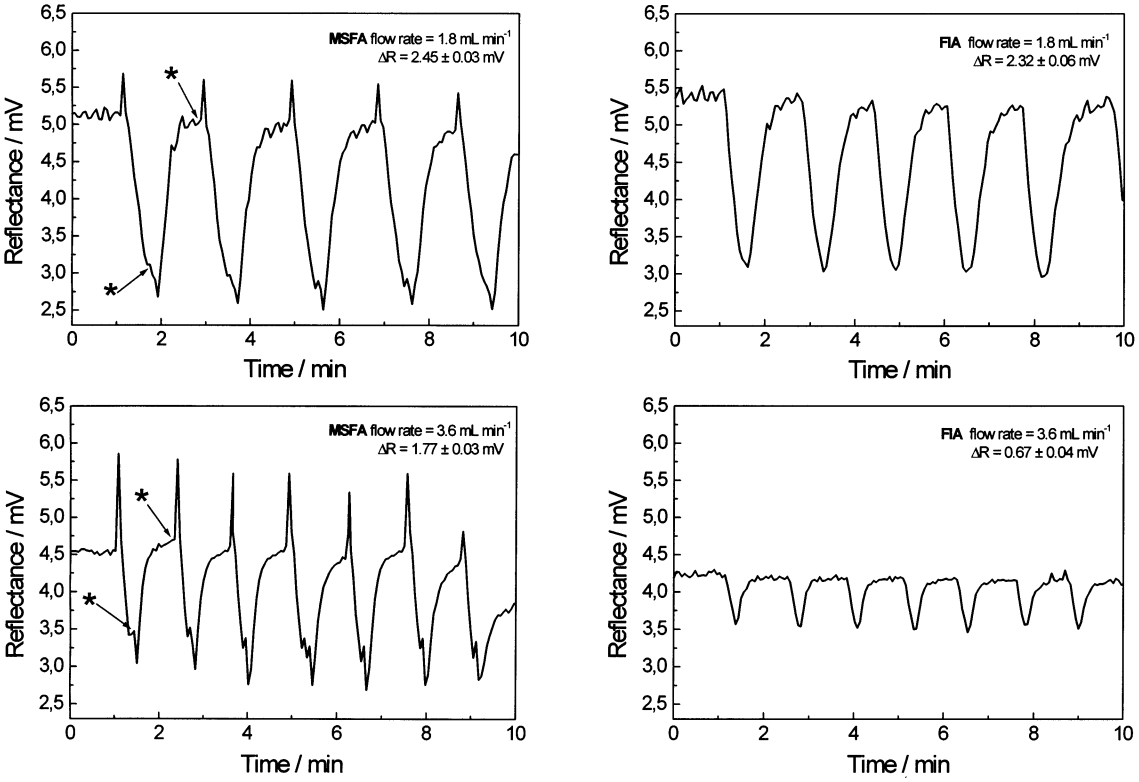

Fig. 4. Signals obtained with the injection of 750 µl of a buffer solution of pH 9.0 in a buffered carrier stream of pH 3.0, employing the optode based on BB acid–base indicator. (*) indicates air bubbles.

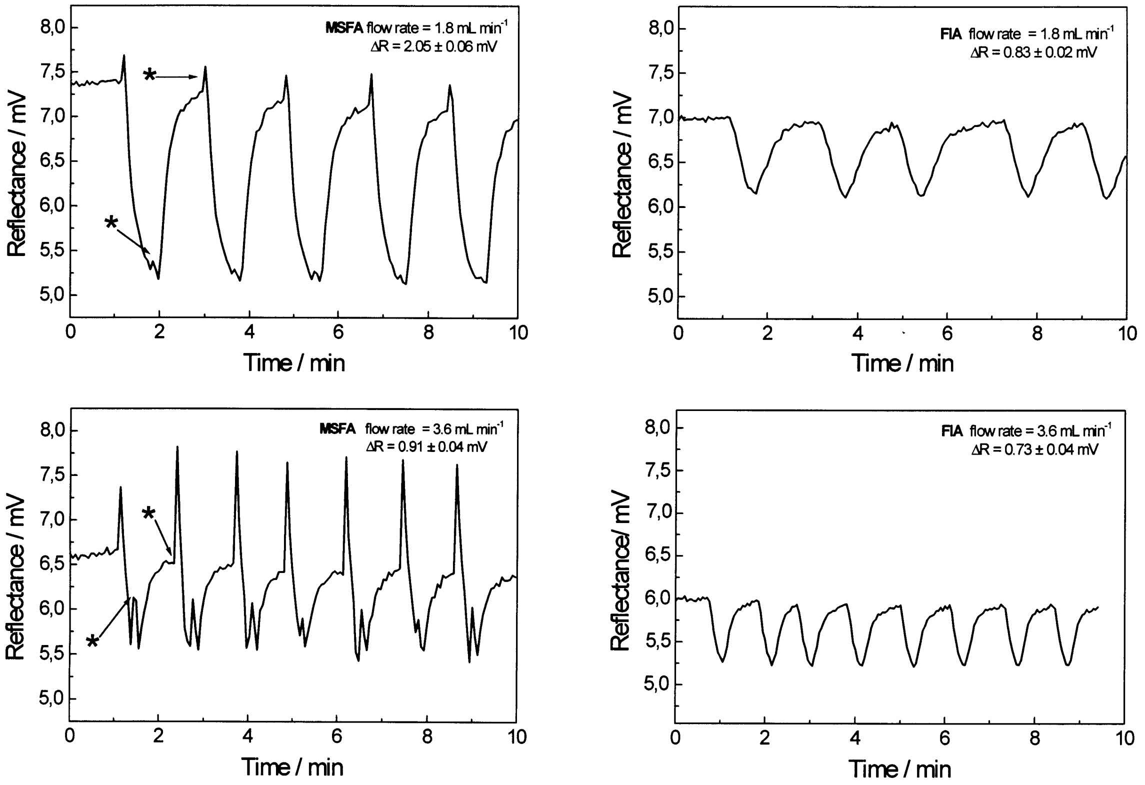

Fig. 5. Signals obtained with the injection of 750 µl of a buffer solution of pH 7.4 in a buffered carrier stream of pH 2.0, employing the optode based on BP acid–base indicator. (*) indicates air bubbles.

Table 3
Signals obtained in FIA and MSFA with the optode based on BB acid–base indicator, expressed as percentage of the steady signal observed in continuous flow

Flow rate (ml min^{-1})	V_i							
	100 µl		250 µl		500 µl		750 µl	
	FIA	MSFA	FIA	MSFA	FIA	MSFA	FIA	MSFA
1.0	*	19.6 ± 1.3	27.3 ± 1.6	38.6 ± 1.8	52.2 ± 3.4	71.2 ± 3.3	68.8 ± 4.6	76.8 ± 3.7
1.8	*	10.3 ± 0.9	15.2 ± 1.2	28.6 ± 1.3	44.0 ± 2.0	47.2 ± 1.9	59.8 ± 2.6	63.1 ± 2.4
2.7	*	7.2 ± 1.5	12.2 ± 3.8	27.2 ± 3.0	30.1 ± 2.0	53.3 ± 5.7	55.6 ± 3.1	65.2 ± 4.1
3.6	*	*	*	20.7 ± 2.1	11.1 ± 1.5	33.3 ± 4.6	20.7 ± 1.9	54.6 ± 4.0

Standard deviation for six replicates.
* No signal was observed.
pH changes from 3.0 to 9.0.
V_i, injected volume.

Table 4
Signals obtained in FIA and MSFA with the optode based on BP acid–base indicator, expressed as percentage of the steady signal observed in continuous flow

Flow rate (ml min^{-1})	V_i							
	100 µl		250 µl		500 µl		750 µl	
	FIA	MSFA	FIA	MSFA	FIA	MSFA	FIA	MSFA
1.0	14.6 ± 0.7	61.3 ± 1.1	32.5 ± 0.4	78.8 ± 0.4	50.7 ± 1.1	81.8 ± 1.5	39.8 ± 1.1	91.6 ± 5.1
1.8	16.9 ± 1.4	35.9 ± 3.0	27.7 ± 2.1	59.1 ± 4.3	43.0 ± 3.4	76.4 ± 6.1	34.3 ± 2.5	84.7 ± 6.4
2.7	*	*	18.5 ± 1.9	36.3 ± 2.4	30.0 ± 0.8	55.9 ± 2.9	38.5 ± 0.9	64.1 ± 2.6
3.6	*	*	17.7 ± 0.9	26.1 ± 1.8	24.8 ± 1.8	36.6 ± 3.1	32.3 ± 1.8	40.3 ± 1.8

Standard deviation for six replicates.
* No signal was observed.
pH changes from 2.0 to 7.4.
V_i, injected volume.

Fig. 6 shows the analytical curves obtained in continuous flow, MSFA and FIA systems with the BB and BP optodes, demonstrating the lower sensitivity for the FIA system. These results are expected, because FIA provides higher sample dispersion than the other two systems. As shown, linear response ranges higher than the pH ranges of the indicators in aqueous solution (6.0–7.6 for the BB and 5.2–6.8 for the BP) were obtained. The extended ranges can be attributed to the chemical immobilisation of the colorimetric reagents on different sites of the optical fibre bundle surface [34]. The indicators molecules can be either strongly or weakly bonded to the optical fibre, resulting in groups with different pK_a's and, therefore, extending the linear pH range [9]. A shift in the response ranges to more acid values for both immobilised indicators was also observed, which means that the pK_a of the indicator decreased, concerning the values presented in solution (7.0 and 6.3 for the BB and BP, respectively). By considering that the surface of the optical fibre bundle was silanised with an amino-silane reagent, this result agree with those described by Baker and Narayanaswamy [9], who found that anion-exchange resins which contained amino-groups (such as IR-400, IR-45 and Dowex 4), due to their basicities, produce a decrease in pK_a of 3,4,5,6-tetrabromophenolsulfonephtalein physically immobilised on their surfaces.

4. Conclusions

Immobilisation of acid-base indicators directly on the surface of the optical fibre bundle is simple and easy to perform, providing optodes with characteristics which allow their use as a detector in flow analysis. Due to the results obtained in this work, it can also be concluded that the MSFA system can improve optode performance, increasing both sensitivity and analytical throughput, which can be modified according to the volume of injected sample and the flow rate employed.

Table 5
Sampling frequency in FIA and MSFA with the optode based on BB acid–base indicator, expressed in sample h^{-1}

Flow rate (ml min^{-1})	V_i							
	100 µl		250 µl		500 µl		750 µl	
	FIA	MSFA	FIA	MSFA	FIA	MSFA	FIA	MSFA
1.0	*	28	36	27	24	19	18	18
1.8	*	42	54	41	36	36	32	29
2.7	*	60	60	48	54	42	42	36
3.6	*	*	*	60	56	48	49	40

* No signal was observed.
pH changes from 3.0 to 9.0.
V_i, injected volume.

Table 6
Sampling frequency in FIA and MSFA with the optode based on BP acid–base indicator, expressed in sample h^{-1}

Low rate (ml min^{-1})	V_i							
	100 µl		250 µl		500 µl		750 µl	
	FIA	MSFA	FIA	MSFA	FIA	MSFA	FIA	MSFA
1.0	36	30	25	24	22	20	19	17
1.8	48	48	40	40	34	35	28	29
2.7	*	*	54	54	45	41	36	36
3.6	*	*	66	57	60	48	48	42

* No signal was observed.
pH changes from 2.0 to 7.4.
V_i, injected volume.

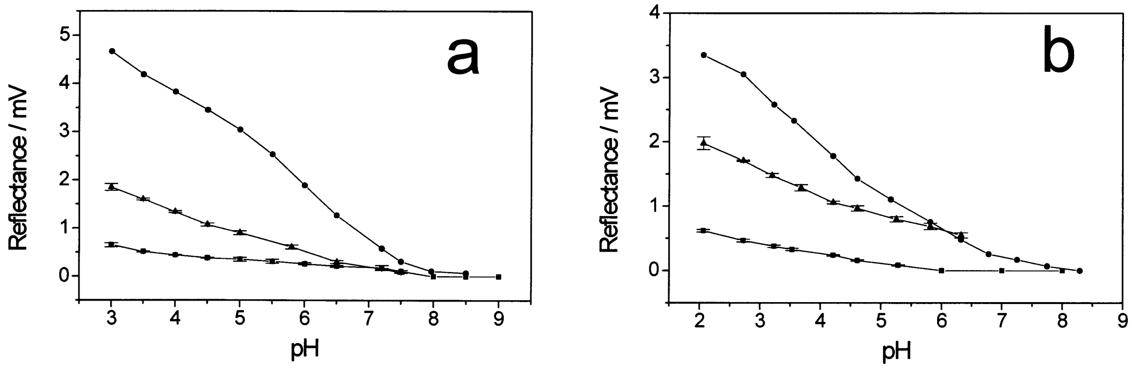

Fig. 6. Analytical curves obtained in continuous flow (●), MSFA (▲) and FIA (■) systems, by employing optodes based on BB (a) and BP (b) acid–base indicators. (a) Flow rate, 4.1 ml min^{-1}; sample volume, 750 µl and (b) flow rate, 1.0 ml min^{-1}; sample volume, 250 µl (bars indicate the standard deviation for three replicates).

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References

[1] D.A. Blair, T. Cynkowski, L.G. Bachas, Fluorocarbon-based immobilization of a fluoroionophore for preparation of fiber optic sensors, Anal. Chem. 65 (1993) 945–947.
[2] W.A. de Oliveira, R. Narayanaswamy, A flow-cell optosensor for lead based on immobilized dithizone, Talanta 39 (1992) 1494–1503.

[3] M.K. Carroll, F.V. Bright, G.M. Hieftje, Fiber-optic time-resolved fluorescence sensor for the simultaneous determination of Al^{3+} and Ga^{3+} or In^{3+}, Anal. Chem. 61 (1989) 1768–1772.

[4] K. Suzuki, K. Tohda, Y. Tanda, H. Ohzara, S. Nishihama, H. Inoue, T. Shirai, Fiber-optic magnesium and calcium ion sensor based on a natural carboxylic polyether antibiotic, Anal. Chem. 61 (1989) 382–384.

[5] G. Serra, A. Schirone, R. Boniforte, Fibre-optic pH sensor for sea-water monitoring using a single dye, Anal. Chim. Acta 232 (1990) 337–344.

[6] G.F. Kirkbright, R. Narayanaswamy, N.A. Welti, Fibre-optic probe based on the use of an immobilised colorimetric indicator, Analyst 109 (1984) 1025–1028.

[7] S.H. Alabbas, D.C. Ashworth, R. Narayanaswamy, Design and performance features of an optical-fibre reflectance pH sensor, Anal. Proc. 26 (1989) 373–375.

[8] S. Motellier, P. Toulhoat, Modified acid–base behaviour of resin-bound pH indicator, Anal. Chim. Acta 271 (1993) 323–329.

[9] M.E.J. Baker, R. Narayanaswamy, The modeling and control of the response of an immobilised indicator, Sensors Actuators B 29 (1995) 368–373.

[10] S.H. Alabbas, D.C. Ashworth, B Bezzaa, S.A. Momin, R. Narayanaswamy, Factors affecting the response time of an optical-fibre reflectance pH sensor, Sensors Actuators A 51 (1996) 129–134.

[11] J. McGlip, Glass cages make sense, Phys. World 11 (1991) 23–25.

[12] F. Baldini, S. Bracci, F. Cossi, P. Bechi, F. Pucciani, Controlled-pore glasses embedded in plastic optical fibers for gastric pH sensing purposes, Appl. Spectrosc. 48 (1994) 549–552.

[13] O.S. Wolfbeis, H. Offenbacher, Fluorescence sensor for monitoring ionic strength and physiological pH values, Sensors Actuators B 9 (1986) 85–91.

[14] S.G. Shulman, S Chen, F. Bai, M.J.P. Leiner, L. Weis, O.S. Wolfbeis, Dependence of the fluorescence of immobilized 1-hydroxypyrene-3,6,8-trisulfonate on solution pH: extension of the range of applicability of a pH fluorosensor, Anal. Chim. Acta 304 (1995) 165–170.

[15] J.P. Jones, M.D. Porter, Optical pH sensor based on the chemical modification of a porous polymer film, Anal. Chem. 60 (1988) 404–406.

[16] T. Werner, C. Huber, S. Heinl, M. Kollmannsberger, J. Daub, O.S. Wolfbeis, Novel optical pH-sensor based on a boradiaza-indacene derivative, Fresenius J. Anal. Chem. 359 (1997) 150–154.

[17] T.J. Cardwell, R.W. Cattrall, L.W. Deady, M. Dorkos, G.R. O'Conell, A fast response membrane-based pH indicator optode, Talanta 40 (1993) 765–768.

[18] Y. Kostov, S. Tzonkov, L. Krysteva, Membranes for optical pH sensors, Anal. Chim. Acta 280 (1993) 15–19.

[19] J. Ruzicka, E.H. Hansen, Flow injection analysis. Part I. A new concept of fast continuous flow analysis, Anal. Chim. Acta 78 (1975) 145–157.

[20] C. Pasquini, W.A. Oliveira, Monosegmented system for continuous flow analysis. Spectrophotometric determination of chromium (VI), ammonia, and phosphorus, Anal. Chem. 57 (1985) 2575–2579.

[21] W.E. Van der Linden, Classification and definition of analytical methods based on flowing media, Pure Appl. Chem. 66 (1994) 2493–2500.

[22] B.A. Woods, J. Ruzicka, G.D. Christian, N.J. Rose, R.J. Charlson, Measurements of rainwater pH by optosensing flow injection analysis, Analyst 113 (1988) 301–306.

[23] B.A. Woods, J. Ruzicka, G.D. Christian, R.J. Charlson, Measurement of pH in solutions of low buffering capacity and low ionic strength by optosensing flow injection analysis, Anal. Chem. 58 (1986) 2496–2502.

[24] A. Holobar, B.H. Weigl, W. Trettnak, R. Benes, H. Lehmann, N.V. Rodriguez, A. Wollschlager, P. O'leary, P. Raspor, O.S. Wolfbeis, Experimental results on an optical pH measurement system for bioreactors, Sensors Actuators B 11 (1993) 425–430.

[25] G.F. Kirkbright, R. Narayanaswamy, N.A. Welti, Studies with immobilized chemical reagents using a flow-cell for the development of chemically sensitive fibre-optic devices, Analyst 109 (1984) 15–17.

[26] H. Posch, M.J.P. Leiner, O.S. Wolfbeis, Towards a gastric pH-sensor: an optrode for the pH 0–7 range, Fresenius J. Anal. Chem. 334 (1989) 162–165.

[27] T. Werner, O.S. Wolfbeis, Optical sensor for the pH 10–13 range using a new support material, Fresenius J. Anal. Chem. 346 (1993) 564–568.

[28] D.D. Perrin, B. Dempsey, Buffers for pH and Metal Ion Control, Chapman and Hall, London, 1974, p. 156.

[29] D.J. Malcome-Lawes, Microcomputers and Laboratory Instrumentation, 2nd edn, Plenum Press, New York, 1988, pp. 147–178.

[30] H. Bergamin Fo, J.X. Medeiros, B.F. Reis, E.A.G. Zagatto, Solvent extraction in continuous flow injection analysis. Determination of molybdenum in plant material, Anal. Chim. Acta 101 (1978) 9–16.

[31] J.O.W. Norris, Current status and prospects for the use of optical fibres in chemical analysis, Analyst 114 (1989) 1359–1372.

[32] R. Narayanaswamy, Optical chemical sensors: Transduction and signal processing, Analyst 118 (1993) 317–322.

[33] C. Munkholm, D.R. Walt, Polymer modification of fibre optic chemical sensors as a method of enhancing fluorescence signal for pH measurement, Anal. Chem. 58 (1986) 1427–1430.

[34] M. Bacci, F. Baldini, A.M. Scheggi, Spectrophotometric investigations on immobilized acid-base indicators, Anal. Chim. Acta 207 (1988) 343–348.

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