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Validation of a tubular bismuth film amperometric detector Determination of diclofenac sodium by multisyringe flow injection analysis

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Abstract

A tubular bismuth film electrode (BFE), installed as part of a multisyringe flow injection system, was used as an amperometric detector to determine the concentration of diclofenac sodium in pharmaceutical formulations. A tubular voltammetric detection cell was employed, in which the hydrodynamic flow conditions were not disturbed. This automated method allows the continuous regeneration of the BFE, preventing passivation of the detector and improving the sensitivity of detection. The influence of several variables on this sensitivity, such as the injection volume, deposition time and flow rate were evaluated; a two-level factorial experimental design was employed for this. In optimal conditions, the linear range of the calibration curve varied from 6.0–50.0 μmol L^{-1}, with a detection limit of 4.3 μmol L^{-1}. A sampling rate of 90 determinations/h was achieved; the relative standard deviation of analytical repeatability was <3.5%. After 30 injections the bismuth film on the electrode surface was automatically renewed. The method was validated by comparing the results obtained with those provided by RP-HPLC; no significant difference were seen ($p < 0.05$).

Keywords: Tubular bismuth film electrode; Multisyringe; Flow injection; Amperometry; Diclofenac sodium

1. Introduction

Over the last few decades, amperometric detection involving different electrodes has been used to determine the concentrations of a wide variety of reducible organic and inorganic compounds in different fluid media. Mercury electrodes, most notably the mercury drop electrode (MDE) [1,2], the hanging mercury drop electrode (HMDE) [3,4] and mercury film electrodes (MFE) [5,6], have been commonly employed for this purpose. The main disadvantage of these electrodes, which is becoming increasingly important in our environmentally conscious world, is the extreme toxicity of the mercury and mercury salts employed in their preparation. Electrode materials that might be able to replace mercury are therefore sought. In addition, the mechanical stability of mercury electrodes is low; they therefore require special handling and their use in flow analytical systems is limited. Other electrode materials that have been used in amperometric detection include glassy carbon (GC) [7,8], carbon paste (CP) [9,10] and gold [11,12]; recently, boron-doped diamond electrodes have become available [13,14]. These electrodes show good mechanical stability and allow surface or bulk modification, which increases their potential usefulness.

It is widely known, however, that solid electrodes suffer from memory effects due to the impossibility of surface

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renewal; in most cases surface regeneration (mechanical and/or electrochemical) is frequently needed. Recently, bismuth film electrodes (BFEs) were proposed as an alternative to mercury electrodes in stripping voltammetric trace analysis [15–17] and amperometric detection [18,19]. The characteristics of BFEs, such as their high hydrogen overpotential, low noise, their good mechanical stability, and their offering easy surface renewal by electrochemical deposition and stripping of the bismuth film, render them an attractive alternative for amperometric detection.

The coupling of automated flow analysis methods with electrochemical detectors allow accurate, reproducible determinations to be made. Multisyringe flow injection analysis (MSFIA) is a new flow analysis method [20,21] that is precise and robust. Based on the use of syringes it allows the simultaneous flow of several solutions. The advantages of this method include the small amounts of reagent consumed and the high sampling rate. In addition, it is easily automated, rendering it attractive for amperometric detection with BFEs.

This work reports the possible use of a multisyringe flow injection system with amperometric detection by tubular BFEs for the determination of diclofenac sodium (DS) in pharmaceutical preparations. Diclofenac sodium is a relatively safe and effective non-steroidal drug with pronounced anti-rheumatic, anti-inflammatory, analgesic and antipyretic properties. It is widely used in the treatment of degenerative joint diseases and other arthritic conditions [22]. Different methods for the quantitative determination of this drug in pharmaceutical preparations have been reported, including a number of spectrophotometric [23–25] and potentiometric [26,27] methods, sometimes coupled to flow [28,29] or chromatographic systems [30].

This work presents a construction procedure of a voltamperometric cell with tubular configuration to be used in flow assemblies. The constructed electrode is, to our knowledge, the first bismuth electrode with tubular configuration. The analytical methodology proposed for the DS determination aims to regenerate on-line the BFE, preventing the passivation of the detector and improving the analytical sensitivity. The influence on the detection sensitivity of variables, such as the injection volume, deposition time and flow rate were evaluated using a two-level factorial experimental design.

The method was validated by comparing the results obtained with those provided by reverse phase high performance liquid chromatography (RP-HPLC).

2. Experimental

2.1. Reagents and solutions

All solutions were prepared by dissolving the corresponding analytical grade reagent in filtered, deionised water with a specific conductivity <0.1 $\mu S\,cm^{-1}$; these were used without further purification. Acetate buffer solution was used as a supporting electrolyte and carrier solution. Stock solutions ($1000\,mg\,L^{-1}$) of DS (Aldrich, USA) were prepared weekly in acetate buffer solution (pH 4, $0.2\,M\,L^{-1}$). Working standard solutions were obtained by diluting the stock solution with the same buffer solution. A standard stock solution of bismuth ($1000\,mg\,L^{-1}$ atomic absorption standard solution (Aldrich, USA) was diluted as required.

Samples of four pharmaceutical products (all tablets) containing DS, all commercially available in Mexico, were also analysed. Ten tablets of each product were weighed and the mean calculated. These 10 tablets were pulverized and mixed; a stock solution of each sample (with $2.0 \times 10^{-3}\,mol\,L^{-1}$ in DS) was then prepared by weighing the corresponding quantity of powder and dissolving it in acetate buffer solution. Working solutions of $2.0 \times 10^{-5}\,mol\,L^{-1}$ were obtained by diluting the respective stock solution in acetate buffer solution.

2.2. Apparatus

An MSFIA system was used for volume-based DS determination (Fig. 1). This consisted of a programmable speed multisyringe burette (MicroBu 2030, Crison, Alella, Barcelona), which was used to aspirate and dispense the reagent solutions. The multisyringe burette had four syringes (10 mL) with a three-way isolation solenoid valve (N-Research, Cadwell, NJ, USA) on each head. Three additional, independent, three-way isolation solenoid valves (V1, V2, V3) were added. The instrumental devices were controlled using Autoanalysis 5.0 software. All tubing (i.d. 0.8 mm) connecting the different components of the flow system was made of Omnifit PTFE. Volume-based sampling was performed to avoid the sample contamination that occurs when time-based sampling is used [31]. Voltammetric measurements were made using a PGSTAT 10 Autolab electrochemical system (Eco Chemie, Switzerland), data was acquired using GPES software (v 4.6).

2.3. Construction of the electrochemical detector

The working and auxiliary electrodes were prepared from graphite–paraffin pellets by dissolving 0.25 g of paraffin wax in 10.0 mL of warm *n*-hexane (40 °C) in a beaker placed in a

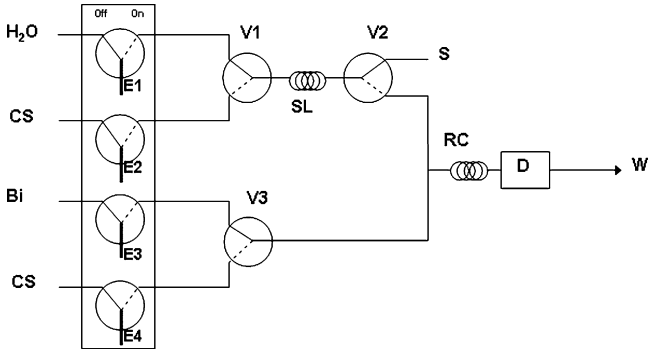


Fig. 1. Diagram of the MSFIA system used to determine diclofenac sodium. H_2O: water, CS: carrier solution (acetate buffer $0.2\,mol\,L^{-1}$, pH 4.0), Bi: bismuth solution ($5\,mg\,L^{-1}$), E(1–4): multisyringe solenoid valves, V(1–3): commutation valves, S: sample, SL: sample loop (200 μL), RC: reaction coil (10 cm length × 0.8 mm i.d.), D: tubular detector ($E = -0.8$ V, vs. Ag/AgCl) and W: waste.

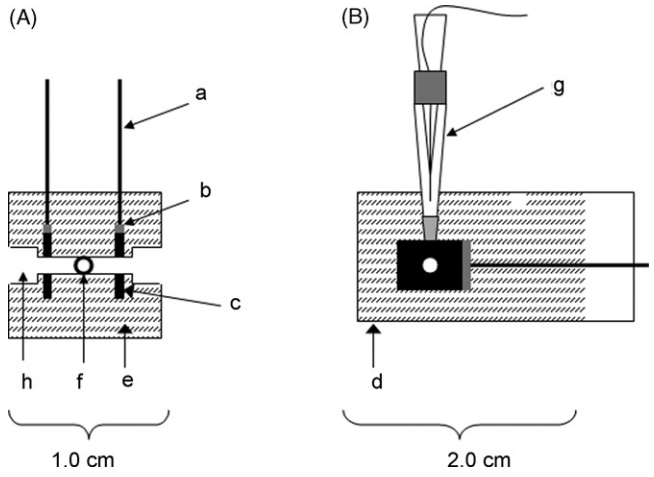


Fig. 2. Schematic representation of front (A) and side (B) view of the tubular voltammetric detector. (a) Electric shield cable, (b) rectangular silver plate, (c) carbon paste electrode (paraffin:graphite, 5:95), (d) perspex holder (1.0 cm × 1.0 cm × 3.0 cm), (e) non-conductive epoxy resin, (f) contact for the reference electrode, (g) reference electrode and (h) hole for Tygon tubing.

water-bath, and by adding 4.75 g of graphite powder (with stirring). After complete evaporation of the organic solvent, 0.20 g of the dry graphite powder, now enveloped in 5% paraffin wax (w/w), was pressed with a 10.0 mm diameter pellet press at 19,000 kg cm^2 for 5 min. Disks 10.0 mm in diameter and 1.2 mm thick were obtained. Electrical contact was made through a cable (Fig. 2A,a) attached by solder to a small rectangular silver plate (1.0 mm × 3.0 mm) (Fig. 2A,b). For this, a square-shaped fragment of the paraffin–graphite pellet (Fig. 2A,c) was glued using a conductive, silver-based epoxy resin. Two fragments of the pellet were then placed in a Perspex holder (1.0 cm × 1.0 cm × 3.0 cm) (Fig. 2A,d) filled with a non-conductive epoxy resin (Fig. 1A,e). The distance between the electrodes was about 3.0 mm. The final electrochemical cell was then left at 25 °C for 1 week. After hardening, a channel 0.8 mm in diameter was drilled perpendicular to the electrodes through the centre of the Perspex holder in order to assure perfect match geometry between the drilled channel and the flow tubing used assuring that the flow pattern is not disturbed.

A reference electrode (Fig. 2B,g) was made by sealing a silver wire in a polyethylene pipette tip. Approximately 4 cm of silver wire was attached to a shielded electrical wire with solder. Silver/silver chloride wires for the reference electrode were prepared by anodising silver wire in 3 mol L^{-1} KCl for 2 min at 0.10 V. A salt bridge was prepared by mixing 3 g of granular agar and 23.5 g of NaCl dissolved in 10 mL of water. The solution was boiled and a second pipette tip immersed into the boiling solution for 1 min while negative pressure was applied, thus drawing the agar solution into the tip of the reference electrode. This tip was immediately immersed in room temperature water to gel the agar. The reference electrode was completed by filling the tip with a 3 mol L^{-1} KCl solution and inserting the Ag/AgCl wire. Therefore, all the electrodes are separated by ca. 0.5 cm in order to reduce electrical noise.

Coupling of the voltamperometric cell was performed through two additional drills with 1.6 mm diameter and 3 mm depth (Fig. 2A,h), where small mouldable Tygon tubing pieces were poured. In this way, the Teflon tubing could be directly connected to the cell.

Once per day the surface of the tubular cell was moistened with double distilled water and polished using a cotton thread soaked in alumina. It was then rinsed with water.

2.4. Analytical cycle

Initially, a 1.0 mL aliquot of Bi(III) (5.0 mg L^{-1}) was injected (step 1) into the reaction coil (see Table 1). This was then directed towards the tubular cell by the carrier solution at a flow rate of 1.0 mL min^{-1} (step 2). A potential of −1.4 V was applied to generate a bismuth film on the internal surface of the working electrode. This was potentiostatically cleaned at +0.2 V (30 s) in flowing carrier solution after 30 injections. The electrochemical detector was then ready for a new analytical cycle. This prevented the passivation of the detector and improved the sensitivity of the detection [32].

Once the Bi film had formed, a 1.0 mL sample (step 3) was aspirated, filling the sample loop (200 μL) with DS solution. This was then injected into the carrier solution (acetate buffer 0.2 mol L^{-1}, pH 4.0) and detected at −0.8 V (versus Ag/AgCl) at a flow rate of 2.0 mL min^{-1}.

2.5. RP-HPLC comparisons

The concentration of DS in the analysed samples was also determined for comparative purposes using RP-HPLC, the standard technique used in pharmaceutical analyses. The apparatus

Table 1
Sequence of events in each analytical cycle

Event	Volume (mL)	Flow rate (mL min^{-1})	Position of solenoid valves						
			E1	E2	E3	E4	V1	V2	V3
1. Sample coil washing	2.0 d	6.0	Off	On	Off	Off	On	On	On
2. Bi film formation	1.0 p	1.0	Off	On	On	Off	On	On	Off
3. Washing of the electrochemical cell	1.0 d	6.0	Off	On	Off	On	On	On	On
4. Filling the sample coil	1.0 p	6.0	Off	Off	Off	Off	Off	Off	Off
5. Injection into carrier	1.0 d	2.0	Off	On	Off	On	On	On	On
6. Adjustment of the piston bar	1.0 p	6.0	Off	Off	Off	Off	Off	Off	Off
7. Repeat 2 times from step 4									

Flow directions: p (pick up) and d (dispense).

used was a PerkinElmer Series 200 liquid chromatograph (PerkinElmer MA, USA) equipped with a UV–vis detector at 286 nm and a manual injector connected to a 50 μL external loop. Chromatographic separation was achieved with a Microsorb 100-5C18 column (5 μm; 150 mm × 4.6 mm i.d.) (Varian, Palo Alto, CA, USA). The mobile phase was methanol-acetate buffer (50:50), pH 4.0, 0.1 mol L^{-1}. A flow rate of 1.2 mL min^{-1} was established at a constant temperature of between 23 and 25 °C.

3. Results and discussion

3.1. Electrochemical behaviour of diclofenac at the BFE

Cyclic voltammetry (in stop flow mode) was used to examine the electrochemical behaviour of the DS at the BFE. Fig. 3(A) shows a typical cyclic voltammogram (1.0×10^{-4} mol L^{-1}). The DS showed a single, well-defined reduction peak at −0.67 V.

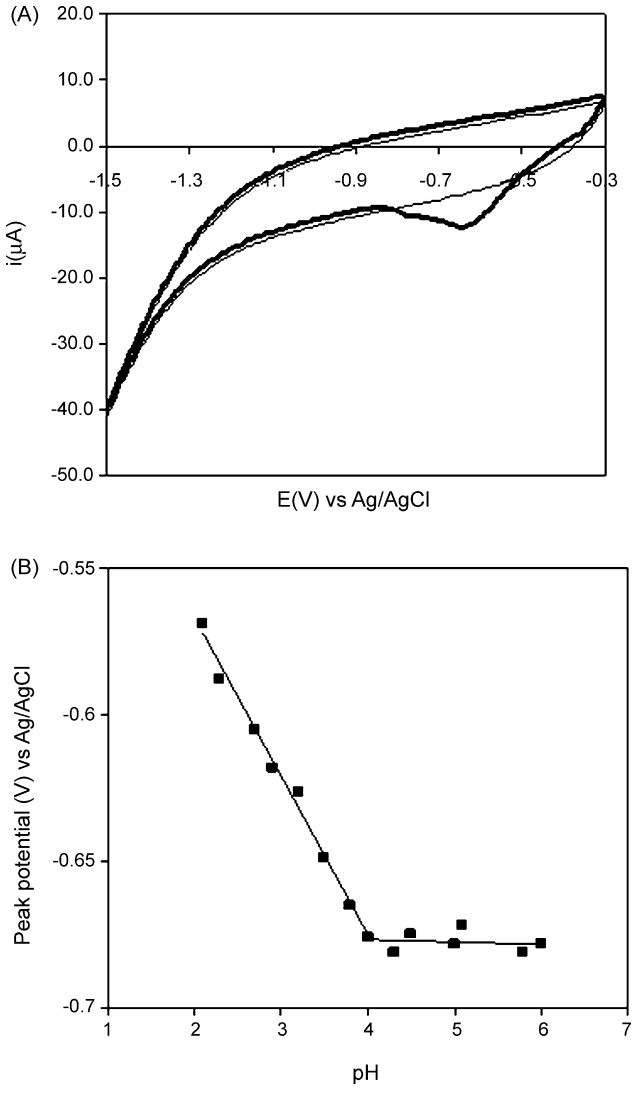
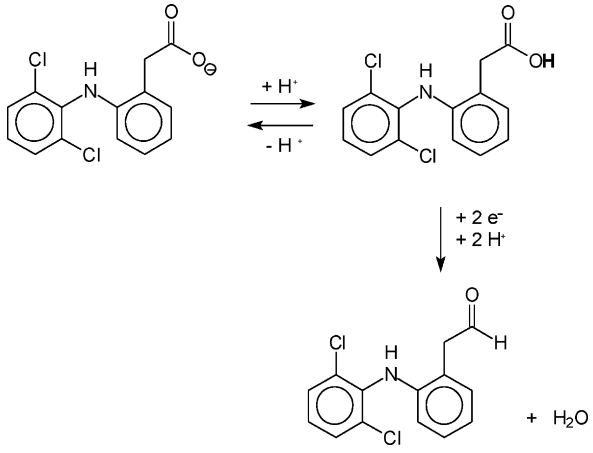


Fig. 3. (A) Cyclic voltammogram of 1.0×10^{-4} mol L^{-1} diclofenac sodium (DS) solution obtained at the BFE on-line deposited onto CPE. (—) Blank and (▬) DS solution. Supporting electrolyte: acetate buffer 0.2 mol L^{-1}, pH 4.0, scan rate: 250 mV s^{-1}; initial and final potential: −0.3 V; vertex potential. −1.5 V. (B) Dependence of peak potentials of DS with pH medium.

The behaviour observed at the BFE was consistent with that seen at mercury electrodes [2].

Cyclic voltammograms were recorded over the pH range of 2.0–6.0 to study the dependence of the peak potential on this variable. Consistent with results obtained for DS and similar non-steroidal drugs when using mercury electrodes [33], a linear shift of the peak potential towards more negative values was observed as the pH increased from 2.0 to 4.0 (Fig. 3(B)). At higher pH values the peak potential remained constant. This behaviour may be associated with the acid-base properties of DS (its reported pK_a value is 4.0) [34]. The slope value obtained was −54 mV pH^{-1}; the corresponding r^2 was 0.992. This result indicates that the reduction of DS involves a 1:1 ratio of protons to electrons, as predicted; this is the same as that seen with mercury electrodes [35]. According to the results obtained, the reduction of DS proceeds by the mechanism shown below:



3.2. Amperometric studies

To evaluate the amperometric detection of DS at the BFE under flow conditions, the carrier/electrolyte solution composition was optimised.

Initially the Bi film was prepared by injecting 1.0 mL of 5.0 mg L^{-1} Bi(III) standard solution into the tubular flow cell at a flow rate of 1.0 mL min^{-1} while a potential of −1.4 V was applied. Once the BFE on the electrode surface was prepared, the optimum pH of the carrier/electrolyte solution was determined using a potential of −1.4 V, a flow rate of 0.5 mL min^{-1}, and a sample injection volume of 50 μL ([DS] = 1.0×10^{-5} mol L^{-1}). The effect of pH (from 2 to 8) on the analytical signal was evaluated using hydrochloric acid, acetate and phosphate buffer solutions. According to the results obtained, lowering the pH of the carrier/electrolyte imparts a compromise situation between an increase of current signal of diclofenac reduction and hydrogen gas formation. A pH of 4.0 (acetate buffer solution) was therefore selected for subsequent analyses.

The influence of the potential applied to the working electrode on the amperometric signal was evaluated in hydrodynamic voltammogram experiments using the tubular BFE and a carbon paste electrode (CPE). Hydrodynamic voltammograms were obtained by plotting the applied potential (V versus Ag/AgCl) against the detector response (analytical signal, μA) after the

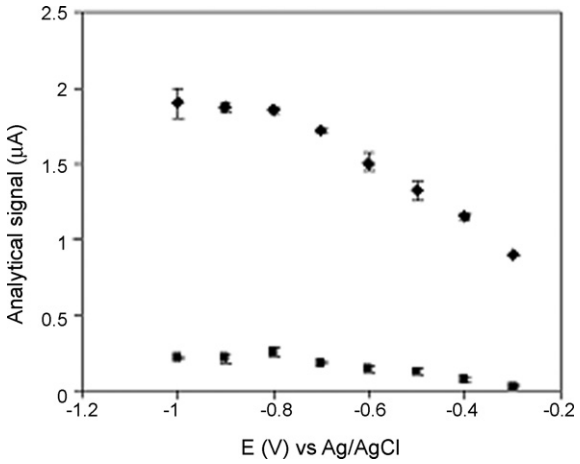


Fig. 4. Analytical signal (μA) obtained for a 1.0×10^{-5} mol L^{-1} DS solution as function of potential (E) of the working electrode: (■) CPE and (♦) BFE. Supporting electrolyte: acetate buffer 0.2 mol L^{-1}, pH 4.0.

injection of a DS standard solution of 1.0×10^{-5} mol L^{-1} (Fig. 4). The analytical signal increased from −0.3 to −0.8 V and then remained constant for both electrodes. The latter value was therefore used in the following analyses.

Fig. 4 shows that the BFE allowed the attainment of a higher analytical signal value (and with good repeatability [R.S.D. of 1.6%; $n = 10$]), than that obtained with the CPE. The BFE therefore enhanced the detection signal.

3.3. Optimisation of flow parameters

The proposal of a new analytical method requires the optimisation of all variables, including the chemical and instrumental factors that may influence the analytical signal. For this, chemometric experiments were performed [36].

Factorial experimental designs can be used to screen for the important variables affecting a selected response, and as a tool for exploring and modelling the latter response. Optimisation using factorial designs is a rigorous yet simple method for finding the experimental conditions that allow the best responses of a chemical system to be obtained [37].

In the present work, the flow rate, the injection volume and the deposition time for the formation of the bismuth film were tested. Optimal working values were established following a two-level factorial design [38] and using a 1.0×10^{-5} mol L^{-1} DS standard solution. The two levels tested (shown in Table 2) were selected based on the results of preliminary work.

Table 2
Factors optimised and levels tested

Factors	Levels	
	Low (−)	High (+)
Flow rate (F, mL min^{-1})	0.80	2.00
Injection volume (V, μL)	20.0	200.0
Deposition time (t, s)	60.0	300.0

Table 3
Level combinations and results obtained

F	V	t	Signal height (μA)		Mean
−	−	−	0.53	0.65	0.59
+	−	−	0.60	0.64	0.62
−	+	−	2.93	2.96	2.95
−	−	+	0.60	0.72	0.67
−	+	+	2.57	2.80	2.69
+	−	+	0.96	0.98	0.97
+	+	−	5.72	5.95	5.84
+	+	+	5.54	5.51	5.53

F = flow rate, V = injection volume and t = deposition time.

Table 3 shows the design matrix and values obtained for the peak heights. The treatment of these data showed the mean effects exerted by each factor on the detection signal (Table 4, column 2). In turn, these values were used to calculate the variance of each factor employing the Yates algorithm (column 3) [39]. After comparing the variance shown by each with the variance of the residuals (0.009), a Fischer's F-test was performed to validate each source of variation. These tests indicated that, at a significance level of $p = 0.05$, the critical factors were the flow rate (F), the injection volume (V), and the interaction flow rate–injection volume (FV) and injection volume × deposition time (Vt).

Together, these results show that the flow rate and (especially) the injection volume have significant effects on the peak intensity. The deposition time did not significantly affect the results obtained. Quantitative evidence of the strong influence exerted by the injection volume in the MSFIA system is provided by the variance obtained for the interactions between this factor and the flow rate and deposition time. This influence is commonly observed in electrochemical and even spectrophotometric detection methods [39–41].

Based on the results shown in Table 4, the following optimal conditions are proposed: a flow rate of 2.0 mL min^{-1}, an injection volume of 200.0 μL and a deposition time for the bismuth film of 60.0 s. These levels were selected with the intention of acquiring analytical signals with adequate stability and sensitivity without affecting the sampling rate.

Table 4
F-factors obtained by the two-level factorial experimental design used to evaluate the effect of flow variables on the amperometric signal

Factor	Effect	Variance	$F_{calculated}$
F[a]	1.517	9.206	**1019.29**
V[a]	3.537	50.041	**5540.53**
t	−0.038	0.006	0.63
FV[a]	1.347	7.259	**803.68**
Ft	0.056	0.012	1.38
Vt[a]	−0.248	0.246	**27.25**
FVt	−0.080	0.026	2.83

[a] Factors in bold differ significantly with respect to the ANOVA results ($F_{calculated} > 5.32$ at the 95% confidence level).

3.4. Interference studies

The effect of the constituent pharmaceutical excipients (sucrose, sorbitol, sodium benzoate, glycerol and citric acid) present in the tablets was then studied. Solutions containing 1.0×10^{-5} mol L^{-1} of DS and the foreign compound at higher concentrations (maximum 100:1) were analysed. The interfering concentration of each compound was considered that which caused a variation in the response greater than or equal to ±5% compared to the response obtained in its absence. The results showed that, at the concentrations in which they were present in the samples tested, none of these excipients interfered in the determination of DS.

3.5. Analytical properties of the procedure

A standard curve for DS was constructed under optimal experimental conditions. Each standard solution was analysed in duplicate and the mean values plotted. Table 5 shows the regression variables taken from this standard curve. The limits of detection were calculated according to IUPAC criteria [42], i.e., three times the value of s_e/b_1, where s_e is the square root of the residual variance of the standard curve and b_1 is the slope. The intermediate precisions of the procedure, expressed as the relative standard deviation (R.S.D.) for six determinations (made on different days) made on a synthetic sample containing analyte concentrations of 1.0×10^{-5} or 5.0×10^{-5} mol L^{-1}, were 3.71 and 1.93% respectively.

To investigate the effect of successive injections on the life of the bismuth film, a 1.0×10^{-5} mol L^{-1} DS standard was continuously injected under optimal conditions. After 30 determinations, the analytical signal value showed good repeatability; the R.S.D. was 3.27%. After 30 injections a new bismuth film was automatically renewed on the electrode surface.

3.6. Determination of diclofenac sodium in pharmaceutical formulations

The proposed amperometric method was used to determine DS in four commercially available pharmaceutical products (all tablets). Table 6 shows the results obtained. For comparative purposes, the DS in the samples was also determined by RP-HPLC [43].

Table 5
Regression parameters of the calibration plots of peak height (in μA) vs. DS concentration (in μmol L^{-1})

Parameter	Value
Square root of residual variance, s_e	0.184
Number of standards	7
Determination coefficient, r^2	0.990
Intercept confidence interval, $b_0 \pm t\, s(b_0)$	-0.395 ± 0.400
Slope confidence interval, $b_1 \pm t\, s(b_1)$	0.128 ± 0.013
Linear range	6.0–50.0
Detection limit	4.3
Sampling rate (samples h^{-1})	90

Table 6
Concentrations (mean and %R.S.D.; $n = 5$) of DS in the pharmaceutical products as determined by the proposed method and RP-HPLC

Sample	MSFIA	RP-HPLC
1	24.6 (2.6)	23.3 (3.1)
2	49.3 (2.71)	50.5 (1.24)
3	74.4 (2.32)	77.6 (2.83)
4	100.6 (2.06)	101.1 (3.15)

Concentration = mg $tablet^{-1}$.

The mean DS concentrations ($n = 5$) for each sample obtained using the two methods were compared using the Student t-test, assuming comparable variances (confirmed by an F-test). The values of $t_{calculated}$ were then compared to a $t_{tabulated}$ with 4 degrees of freedom at the 95% confidence level ($t = 2.78$). No significant differences were seen between the results obtained with each method.

4. Conclusions

This work describes a miniaturized tubular amperometric BFE detector, prepared on-line on CPE that provides a novel alternative for flow analytical determination of DS. The constructed voltamperometric cell can be easily constructed with ordinary laboratory material. The coupling procedure and the tubular configuration adopted provided robust attach into any point of the flow system without significant flow disturbance. These characteristics makes the detector appropriate to flow systems operating in positive or negative pressure [44], allows coupling of more detectors for multi-parameter determinations and additionally, it can be used for multi-site detection [45]. The BFE can be easily renewed on-line electrochemically, which improves both the repeatability and intermediate precision of the detection signal. This feature represents a significant advantage over traditional electrodes since no polishing procedures are needed. BFE electrode showed to be a good substitute of the traditional mercury electrodes as it's less toxic and provides similar analytical performance for DS.

The proposed methodology based on MSFIA and amperometric detection, is fully automated, economic, enables high determinations rate and provided DS determinations comparable to those obtained by the RP-HPLC reference method.

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References

[1] A.G. Kazemifard, D.E. Moore, A. Mohammadi, J. Pharm. Biomed. Anal. 30 (2002) 257–262.
[2] M. Xu, L. Chen, J. Song, Anal. Biochem. 329 (2004) 21–27.

[3] A.H. Alghamdi, F.F. Belal, M.A. Al-Omar, J. Pharm. Biomed. Anal. 41 (2006) 989–993.
[4] M.M. Ghoneim, M.A. El Ries, E. Hammam, A.M. Beltagi, Talanta 64 (2004) 703–710.
[5] A. Economou, P.R. Fielden, Trends Anal. Chem. 16 (1997) 286–292.
[6] D.A. El-Hady, M.I. Abdel-Hamid, M.M. Seliem, V. Andrisano, N.A. El-Maali, J. Pharm. Biomed. Anal. 34 (2004) 879–890.
[7] R.I.L. Catarino, A.C.L. Conceicao, M.B.Q. Garcia, M.L.S. Goncalves, J.L.F.C. Lima, M.M. Correia dos Santos, J. Pharm. Biomed. Anal. 33 (2003) 571–580.
[8] V. Pfaffen, P.I. Ortiz, Anal. Sci. 22 (2006) 91–94.
[9] M.F.S. Teixeira, L.A. Ramos, O. Fatibello-Filho, E.T.G. Cavalheiro, Anal. Bioanal. Chem. 376 (2003) 214–219.
[10] M. Pedrero, P. Salas, R. Gálvez, F.J.M. de Villena, J.M. Pingarron, Fresen. J. Anal. Chem. 371 (2001) 507–513.
[11] T. Charoenraks, S. Palaharn, K. Grudpan, W. Siangproh, O. Chailapakul, Talanta 64 (2004) 1247–1252.
[12] V.A. Pedrosa, D. Lowinsohn, M. Bertotti, Electroanalysis 18 (2006) 931–934.
[13] T.A. Ivandini, B.V. Sarada, C. Terashima, T.N. Rao, D.A. Tryk, H. Ishiguro, Y. Kubota, A. Fujishima, J. Electroanal. Chem. 521 (2002) 117–126.
[14] W. Siangproh, N. Wangfuengkanagul, O. Chailapakul, Anal. Chim. Acta 499 (2003) 183–189.
[15] J. Wang, J.M. Lu, S.B. Hocevar, P.A.M. Farias, B. Ogorevc, Anal. Chem. 72 (2000) 3218–3222.
[16] A. Economou, Trends Anal. Chem. 24 (2005) 334–340.
[17] J. Wang, Electroanalysis 17 (2005) 1341–1346.
[18] E.A. Hutton, B. Ogorevc, S.B. Hocevar, F. Weldon, M.R. Smyth, J. Wang, Electrochem. Commun. 3 (2001) 707–711.
[19] E.A. Hutton, B. Ogorevc, M.R. Smyth, Electroanalysis 16 (2004) 1616–1621.
[20] M. Miro, V. Cerda, J.M. Estela, Trends. Anal. Chem. 21 (2002) 199–210.
[21] M.A. Segundo, L.M. Magalhaes, Anal. Sci. 22 (2006) 3–8.
[22] S.C. Sweetman (Ed.), Martindale:The Complete Drug Reference, 35th ed., Pharmaceutical Press, London & Chicago, 2006.
[23] A.A. Matin, M.A. Farajzadeh, A. Jouyban, II Farmaco 60 (2005) 855–858.
[24] J.C. Botello, G. Perez-Caballero, Talanta 42 (1995) 105–108.
[25] Y.C. de Micalizzi, N.B. Pappano, N.B. Debattista, Talanta 47 (1998) 525–530.
[26] A.M. Pimenta, A.N. Araujo, M.C.B.S.M. Montenegro, Anal. Chim. Acta 470 (2002) 185–194.
[27] S.S.M. Hassan, R.M. Abdel-Aziz, M.S. Abdel-Samad, Analyst 119 (1994) 1993.
[28] S. Garcia, C. Sanchez-Pedreno, I. Albero, C. Garcia, Mikrochim. Acta 136 (2001) 67–71.
[29] P. Ortega-Barrales, A. Ruiz-Medina, M.L. Fernandez-de Cordova, A. Molina-Diaz, Anal. Sci. 15 (1999) 985–989.
[30] O. Kuhlmann, G. Stoldt, H. Struck, G. Krauss, J. Pharm. Biomed. Anal. 17 (1998) 1351–1356.
[31] M.A. Segundo, H.M. Oliveira, J.L.F.C. Lima, M.I.G.S. Almeida, A.O.S.S. Rangel, Anal. Chim. Acta 537 (2005) 207–214.
[32] G. Kefala, A. Economou, Anal. Chim. Acta 576 (2006) 283–289.
[33] J.A. Acuna, M.D. Vazquez, M.L. Tascon, P. Sanchez-Batanero, J. Pharm. Biomed. Anal. 36 (2004) 157–162.
[34] B. Cantabrana, J.R. Perez Vallina, L. Menéndez, A. Hidalgo, Life Sci. 57 (1995) 1333–1341.
[35] A.M. Bond, Modern Polarographic Methods in Analytical Chemistry, Marcel Dekker, New York, 1980, p. 185.
[36] D.C. Montgomery, Design and Analysis of Experiments, Wiley & Sons, New York, 2000.
[37] J.M. Bosque-Sendra, L. Gamiz-Gracia, A.M. Garcia-Campana, Anal. Bioanal. Chem. 377 (2003) 863–874.
[38] D.L. Massart, B.M.G. Vandegiste, L.M.C. Buydens, S. de Jong, P.J. Lewi, J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics: Part A, Elsevier, Amsterdam, 1997.
[39] E. Barrado, J.A. Rodríguez, M.B. Quinaz, J.L.F.C. Lima, Int. J. Environ. Anal. Chem. 86 (2006) 563–572.
[40] I. Nemcova, P. Rychlovsky, M. Havelcova, M. Brabcova, Anal. Chim. Acta 401 (1999) 223–228.
[41] C. Vannecke, E. Van Gyseghem, M.S. Bloomfield, T. Coomber, Y.V. Heyden, D.L. Massart, Anal. Chim. Acta 446 (2001) 411–426.
[42] International Union of Pure and Applied Chemistry, Anal. Chem. 48 (1976) 2294–2296.
[43] L. Zecca, P. Ferrario, P. Costi, J. Chromatogr. 567 (1991) 425–432.
[44] M.L.S. Silva, M.B.Q. Garcia, J.L.F.C. Lima, E. Barrado, Talanta 72 (2007) 282.
[45] R.I.L. Catarino, A.C.L. Conceição, M.B.Q. Garcia, M.L.S. Gonçalves, J.L.F.C. Lima, M.M.C. Santos, J. Pharm. Biomed. Anal. 33 (2003) 571.