Flow-Through Polarographic Cell for Flow-Injection Analysis. Determination of Nifedipine in Pharmaceutical Formulations

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ABSTRACT: A simple design of a polarographic flow-through cell is proposed in which a conventional dropping mercury electrode (DME) is used as working electrode, and a mercury pool and a platinum wire are used as the reference and counter electrodes, respectively. The mercury droplets falling from the DME coalesce with the pool, and a fairly constant hold-up cell volume is achieved by controlled removal of the mercury from the pool at an equivalent flow rate than that of the DME. The analytical features of the cell are illustrated by flow-injection (FI) determination of the nitro derivative nifedipine in pharmaceuticals. The flow-through detector can be used either amperometrically, under continuous flow operation, or voltammetrically in quiet solution, under continuous flow-stopped flow mode. Under continuous flow operation of the cell, the sampling rate obtained was 120 h^{-1}. The repeatability of the analytical signals, expressed as RSD, was always lower than 2.1%. The recovery of nifedipine in synthetic tablet formulations was 99.8 ± 0.6%. Lab Robotics and Automation **9:** 255–262, 1997

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INTRODUCTION

The most common instrumental techniques associated to detection in flow systems, as it is usual in the context of analytical methods, are photometry and colorimetry. In spite of this, reliable designs of electrochemical flow-through cells have shown that hydrodynamic electrochemistry is a good alternative for detection in high-performance liquid chromatography (HPLC) and flow-injection analysis (FIA) [1], taking into account that the cell design is of fundamental importance for proper functioning of the whole system. Thin layer and wall-jet cells are the two types most closely meeting the requirements of

good performance. In these cells, solid electrodes are usually used when oxidative electrochemistry is involved in the detection. On the other hand, reductive electrochemical determinations at negative potentials require normally the use of a mercury-based electrode and the removal of oxygen from the carrier and sample solutions.

In this context, a number of chromatographic, FIA, and gas-segmented flow systems have been proposed for determinations based on the use of mercury working electrodes [1–14]. One of the first designs of polarographic flow-through cell, including a dropping mercury electrode (DME), was reported by Lento [2] for the determination of cadmium, lead, and zinc using a gas-segmented flow system. In that assembly, a mercury pool served as the reference electrode and all fittings into the cell were sealed permanently with an epoxy resin. According to Lento, the convex shape of the mercury meniscus formed by the pool prevented excess buildup of mercury in the cell, since the droplets falling from the electrode did not coalesce with the pool and the droplets passed directly to the waste stream. This behavior could not be confirmed by Lund and Opheim [3], and a flow cell with a slightly different design was assembled in order to avoid the accumulation of mercury drops at the pool, which in this case was used as counter electrode. According to the authors, the mercury indeed passed through the exit tube without blocking the cell; however, this happened only at irregular intervals, and so much mercury then left the pool at once that it caused irregularities in the flow rate. In spite of the enhancements achieved with this design, the authors resolved to use a platinum electrode as counter electrode to evade the possibility of problems or irregularities associated with the mercury pool. Taking into account that the DME should be easy to remove from the cell for cleaning and exchange, the capillary was not permanently sealed to the cell, and a tight fitting of the capillary was obtained by means of a metal ring (sealed to the capillary), an O-ring, and a screw.

Forsman and Karlsson [4] intended an FI electroanalytical method for the determination of penicilloic acid. The penicilloate was detected polarographically at a dropping mercury flow-through cell. The flow stream meets the mercury drop in the opposite direction to the mercury flow and then passes out into the bulk solution in which the cell is embedded. The mercury drops fall out through the oval holes on the side of the Teflon body and are collected at the bottom of the flow cell. Counter and reference electrodes were placed in the bulk solution. This principle for a polarographic detector was first introduced by E. G. and G. Princeton Applied Research for use with the static mercury electrode, but these authors applied to conventional polarographic equipment.

Fogg et al. [5–7] reported the construction and applications of a simple wall-jet detector cell that can be used with a solid electrode or with a sessile mercury-drop electrode. Similarly to the previous cited work, the detector is used partly immersed in an electrolyte solution to give the contact with a counter and a reference electrode. This detector was applied to the determination of foods coloring matters, nitroprusside, nitrofurantoine, and acetazolamide.

Rabenstein and Saetre [8] developed a mercury-based two electrode system as flow-through electrochemical detector for liquid chromatography for the determination of glutathione and other biologically active sulfhydryl-containing molecules in eluates. The working electrode was a 0.8 mm diameter mercury pool that was constructed from a standard liquid chromatography tee connector. According to the authors, if adsorption occurs on the mercury pool of the detector, it does not limit its use.

Baltensperger and Eggli [12] have examined the features of an amperometric flow-through detector using a renewable stationary mercury electrode. Its performance was assessed for 1,4-benzoquinone by FIA. The results from dependence of the signal on temperature, area of the mercury electrode, and flow rate were studied and compared with the appropriate theoretical models.

The use of coated solid electrodes is another common application of mercury-based electrodes, which has been largely applied in trace analysis batch electrochemistry. In this context, Luque de Castro and Izquierdo [13] reviewed the coupling of FIA with stripping analysis.

In this work, a new simple design of a polarographic flow-through cell for FIA is presented in which a conventional DME is used as working electrode, and a platinum wire and a mercury pool were used as the counter and reference electrodes, respectively. Controlled removal of mercury from below the pool allows one to work with a constant cell volume. However, when the stream was pointed directly onto the drop of the DME, the peak height was independent of the cell volume. Taking into consideration that the literature of the last few years shows that polarography as well as other voltammetric techniques are particularly applied in pharmaceutical and biological analysis [14–16], the nitro derivative nifedipine was used as the model analyte, in a media containing 0.1 M phosphate buffer (pH, 6) and ethanol in a volumetric ratio of 70:30. The determination proposed here can either be carried out voltammetrically in quiet solution, by recording the polarogram using the stopped flow approach or amperometrically, by recording the FIA-signal by application of a

constant potential at which the analyte is reduced transiently while passing through the cell.

EXPERIMENTAL

Reagents and Solutions

All chemicals used, except as noted, were of analytical reagent grade. Nifedipine was obtained from Laboratorio Chile (Santiago, Chile). Stock standard solutions (0.001 M) of nifedipine were prepared by dissolving the appropriate amount of the drug in a 70:30 v/v 0.1 M phosphate buffer (pH 6.0)–ethanol mixture. This same electrolyte solution was used to dissolve the tablets containing the drug, and also it was used as the carrier solution in the flow system. All solutions (samples and carrier) were deoxygenated with oxygen-free nitrogen before aspiration starts by using a similar assembly to that described previously [3]. Nitrogen was purged previously into ethanol, in order to avoid evaporation of ethanol from the carrier and sample solutions.

Apparatus and Instruments

A CV-27 Voltammograph (Bioanalytical Systems, Lafayette, IN, USA) was used as potentiostat/amperometric detector. A laboratory-made damping assembly was constructed for d.c. polarographic measurements. The current signals were recorded with a Graphtec WX 1200 XT recorder.

Figure 1 illustrates the design of the flow-through cell containing a three-electrode arrangement. The main body of the cell was made of Plexiglass, into which was fitted a conventional DME (Hg-capillary B405, Radiometer, Denmark). The correct location of the DME into the cell is when the flow stream, at the optimum flow rate (below 4.0 mL min^{-1}), directly impacts the mercury drops (Figure 1). The counter electrode was a platinum wire (0.2 mm diameter) that was inserted into the flow exit hole (0.7 mm). A mercury pool served as the reference electrode in which the mercury droplets falling from the DME coalesce. A fairly constant mercury volume in the pool (and consequently in the hold-up cell volume) was achieved by controlled removal of mercury, using a hammer of a Drop Life Timer (DLT1, Radiometer, Denmark) connected to the capillary where the mercury waste flows. Under the selected conditions, the mercury flow rate of the waste is made equivalent to that of the DME. The volume of the cell could be varied by modifying the volume of the mercury in the pool and, consequently, the distance between the DME surface and upper part of the pool, always keeping the location of the DME in the position shown in Figure 1, in which the stream is pointed directly onto the drop. The potential of the DME was controlled by the above-mentioned potentiostat.

The flow-injection manifold consisted of an Ismatec (MS-FIXO) four-channel peristaltic pump and a Rheodyne (model 5041) injection valve.

Manifold and Procedure

Figure 2 shows the one-channel manifold used. The sample (140 μL) is inserted into a buffer-supporting electrolyte stream (pH 6.0) at a flow rate of 2.0 mL min^{-1}. When the sample zone arrives at the cell, the flow is stopped in order to achieve voltammetric measurements. Contrarily, if normal FIA determinations are required, the amperometric transient signals are recorded by applying to the cell a potential of -0.8 V.

Determination in Pharmaceutical Formulations

Tablet formulations containing a nominal 10 mg of nifedipine in a total mass of approximately 130 mg were analyzed. Twenty tablets were thoroughly ground and mixed. Samples equivalent to about 3.5 mg of nifedipine were accurately weighed and dissolved in the supporting electrolyte in a 25 mL calibrated flask. The contents of the flasks were shaken for 10 minutes and then allowed to settle. The samples were injected into the manifold. The contents of the drugs in the tablets were determined by reference to the calibration plot. In order to establish the reliability of the method, nifedipine was determined in synthetic samples containing the drug in common tablet excipients (magnesium stearate, gelatin, lactose, and starch).

RESULTS AND DISCUSSION

The electrochemical behavior of nifedipine has been studied previously [17, 18]. Nifedipine exhibits only one irreversible polarographic wave throughout the whole pH range. This wave is due to the four-electron reduction of the nitro group to a hydroxylamine derivative. According to Ellaithy and Zuman [18], at pH < 6, the hydroxylamine derivative undergoes an acid-catalyzed dehydration yielding a quinonemethide that is further reduced to the amine. Consequently, the reduction of the nitro group occurs in a single six-electron step at these pH values. Similar processes have been reported for some substituted 5-nitrofurans [19, 20], nitrobenzophenones [21], and for some nitropyrazoles and nitroimidazoles [18].

The continuous flow method reported here was

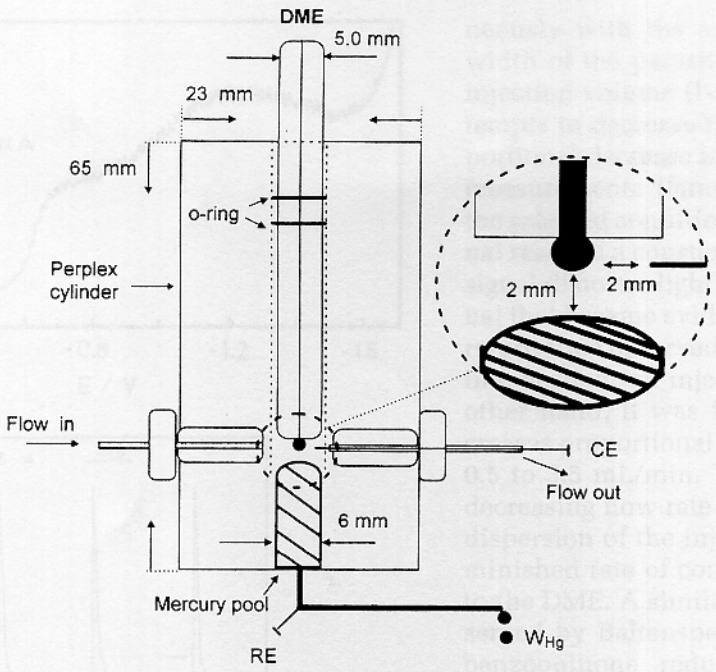

Figure 1. The detector unit. DME, dropping mercury electrode; RE, reference electrode; CE, counter electrode; W_{Hg}, mercury waste.

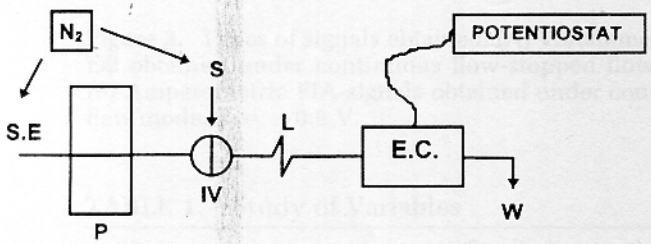

Figure 2. Schematic diagram of the flow-injection system. N_2, nitrogen; S.E., supporting electrolyte; S, sample; IV, injection valve; L, coil; E.C., electrochemical cell.

based on the facility of reduction of the nitro group in mercury electrodes. By using the manifold depicted in Figure 2, the analyte can be determined either voltammetrically or amperometrically. Figure 3 shows the two different signals obtained. In the first case (Figure 3A), the d.c. polarogram was recorded after the pump was halted when the entire volume of the cell was filled with the sample injected. Under these conditions, the reduction is diffusion-controlled as shown by the linear dependence of the wave height with $h^{1/2}$. As can be seen at potential more negative than the main polarographic wave, an ill-defined current increase was observed, which according to Ellaithy and Zuman [18] corresponds to two overlapping waves. These typical current–voltage curves in quiet solution cannot be obtained with previous cell designs based on a flow-through polarographic detector immersed in an electrolyte bulk solution in which are placed the other two electrodes [4–7]. The second type of signal (Figure 3B) was obtained under continuous flow conditions, by applying a constant potential for transient reduction of the analyte. Contrarily, in this case, convection dictates the mass transport to the electrode. The former situation must be the selected if mechanistic information about the electrode process is required, while the later, which is considerably faster, is proposed for analytical purposes.

Variables Affecting the Performance of the FIA Detection

The main purpose of this work is to study the characteristics of the detector cell when it is used reductively under continuous flow conditions. A 70:30 v/v 0.1 M phosphate buffer (pH 6.0)–ethanol mixture was used as carrier electrolyte solution to evaluate the other variables. The peak height and return time of the signal were the parameters considered to select the optimum conditions (Table 1).

Effect of the FI Variables

Increased sample volumes resulted in proportionally increased analytical signals and return times. A vol-

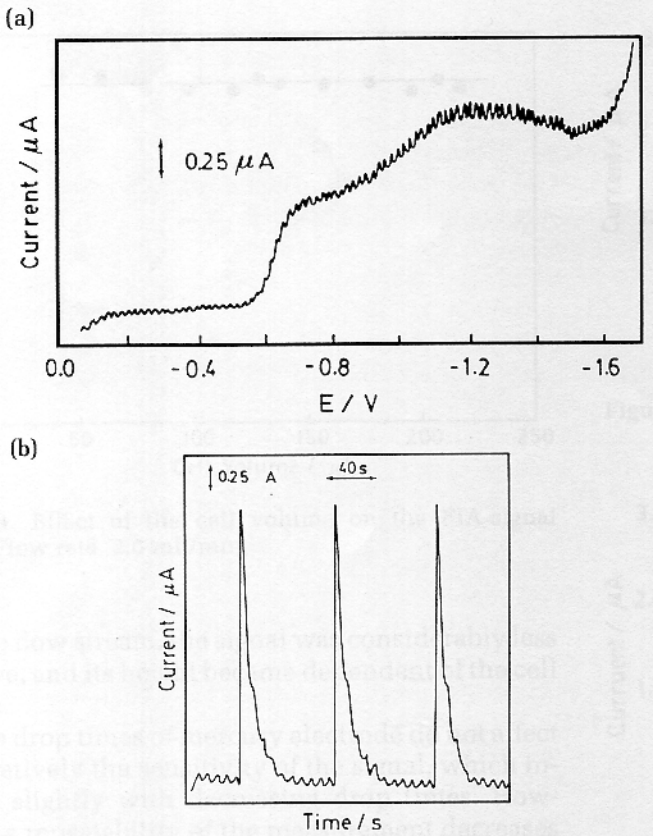

Figure 3. Types of signals obtained. (A) Voltammetric signal obtained under continuous flow-stopped flow mode. (B) Amperometric FIA-signals obtained under continuous flow mode, $E = -0.8$ V.

TABLE 1. Study of Variables

Variable	Studied Range	Selected Value
FIA		
Injected Volume (IV), μL	30–235	140
Delay Coil (L), cm	10–200	30
Flow Rate (q), mL min^{-1}	0.5–3.5	2.0
Delay Time, s	0–22	0
Flow Cell		
Cell Volume, μL	30–220	47
Potential ($-E$), mV	400–1200	800
Drop Time (t), s	2.4–10	7.5

ume of 140 μL was chosen as a compromise between sensitivity and sample throughput. The effect of the delay coil length was investigated by varying the coil length from 10 to 200 cm. A short delay coil of 30 cm was used to minimize the dispersion effect on the drug samples. It was observed that injection of the samples (and blanks) gave rise to a physical perturbation in the flow profile. This perturbation was recorded as a parasite signal that appears simultaneously with the analytical peak. The height and width of the parasite signal depend directly on the injection volume (IV)/delay coil length (L) ratio. Attempts to decrease this interference resulted in proportional decrease in the sensitivity of the analytical measurements (faradaic current). Consequently, in the selected conditions (Table 1), the interference signal reached a constant value of 0.34 μA. This parasite signal distorts slightly the return time of the FIA-signal that became evident in the abnormally prolonged return time observed for the signal (Figure 3B) taking into account an injection volume of 140 μL. On the other hand, it was found that the peak current increases proportionally with increasing flow rate from 0.5 to 3.5 mL/min. The decrease in the signal with decreasing flow rate is due not only to the increased dispersion of the injected sample but also to the diminished rate of convective transport of the analyte to the DME. A similar flow-rate dependence was observed by Baltensperger and Eggli [12] for the 1,4-benzoquinone reduction using an amperometric flow-through detector with a renewable stationary mercury electrode. Because the flow stream impacts directly the drop flowing from the DME, flow rates over 4.0 mL/min interfered with the drop stability giving rise, consequently, to less reproducible signals.

Effect of the Detection Unit Variables

The effect of the cell volume on the analytical signal was investigated by changing the distance between the DME surface and mercury pool from 2 up to 5 mm, by moving the mercury pool, but always keeping the location of the DME in the optimum position (Figure 1). As should be expected, the peak current would decrease and the return time would extend with the increment of the cell volume because the dilution effect is favored; however, because the flow stream always impacts the drop of the DME, the height of the signal was constant between this volume range (Figure 4) in a wide range of flow rates. On the other hand, the peak width or return time of the signals apparently was also independent of the cell volume, because all signals showed the same return time (ca. 30 s) in the volume range from 30 to 220 μL. However, the physical perturbation in the flow profile during sample injection produces the parasite peak that is responsible of this atypical return time observed for the analytical signals, as indicated above.

The correct location of the DME into the cell (Figure 1) is when the flow stream directly impacts the mercury drops. Contrarily, if the mercury drop was

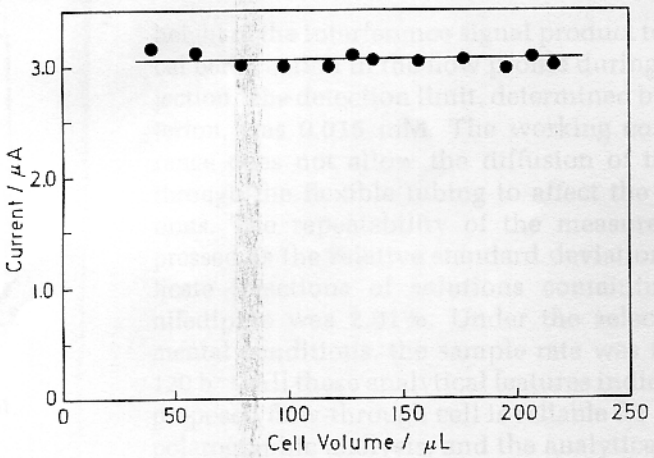

Figure 4. Effect of the cell volume on the FIA-signal height. Flow rate, 2.0 mL/min.

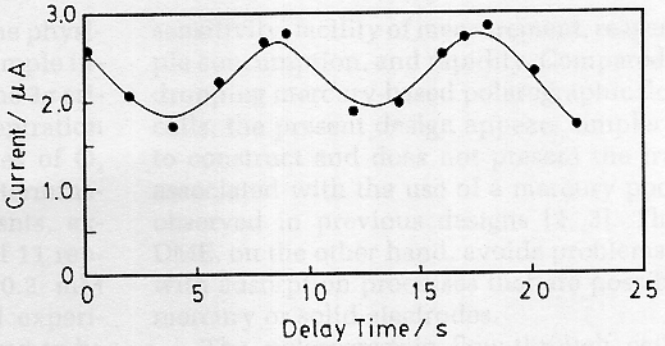

Figure 5. Effect of the delay time on the FIA-signal height.

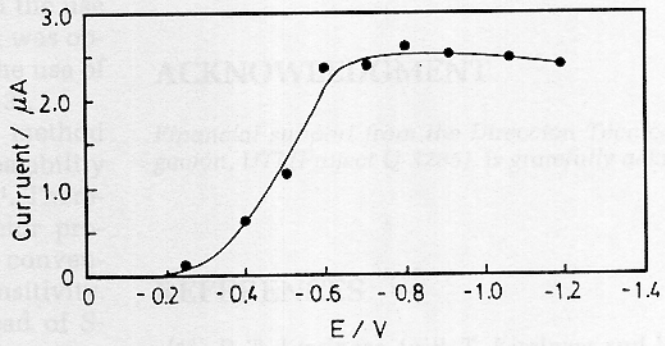

Figure 6. Effect of the applied potential on the FIA-signal height.

over the flow stream, the signal was considerably less sensitive, and its height became dependent of the cell volume.

The drop times of mercury electrode do not affect significatively the sensitivity of the signal, which increases slightly with decreasing drop times. However, the repeatability of the measurement decreases considerably with lower drop times, probably due to the difficulty to coordinate, in this instance, that the detection occurs always (in each injection) on the same area of the mercury drop. The relative standard deviation ($n = 10$) of the signal using drop times of 7.5 and 2.4 seconds were 0.5 and 4.2%, respectively. A drop time of 7.5 seconds was selected; consequently, one drop is sufficient to delimit the maximum of the analytical signal.

Because the area of the DME is time dependent, the arrival of the analyte to the cell must be synchronized in order for the sample zone to meet, in each injection, the equal electrode area. In this context, the time elapsed (delay time, Table 1) between injection of the sample and the fall of the mercury drop was studied. As can be seen in Figure 5, the peak height varies with this time according to the electrode area that the sample zone meets when it passes through the cell. Consequently, the instant of injection must be synchronized with the electrode area in order to obtain repeatability in the analytical measurements.

The peak height of the flow-injection signal was recorded with different potentials applied to the DME. A typical hydrodynamic voltammogram showing only one wave was obtained (Figure 6) making injections at different potentials. The ill-defined current increases that appear in the polarogram obtained under static conditions (Figure 3B) was not observed under hydrodynamic conditions. Differences between both voltammograms are normally observed because the conditions in both instances are completely different, taking into account both the time implicit in the measurements and the mass transport mechanism involved in each instance [5]. A potential of -0.8 V was selected for FIA determination of nifedipine, which is in the plateau of the plot.

Features of the Method

Under the optimum conditions stated in Table 1, a linear relation was observed between the peak height and nifedipine concentration in the range 0.05 and 0.5 mM. The equation ($n = 8$) of the regression line obtained was:

$$I(\mu A) = 6.056\ [\text{nifedipine}]_{mM} + 0.34$$

The correlation coefficient for this plot was equal to 0.9998. Despite the fact that a sensitivity of 6.056 μA/mM is not sufficient in trace analysis, this value is quite satisfactory for analysis of pharmaceutical formulations.

The intercept of 0.34 μA is consequent with the

height of the interference signal product to the physical perturbation in the flow profile during sample injection. The detection limit, determined by the 3σ criterion, was 0.015 mM. The working concentration range does not allow the diffusion of traces of O_2 through the flexible tubing to affect the determinations. The repeatability of the measurements, expressed as the relative standard deviation of 11 replicate injections of solutions containing 0.2 mM nifedipine was 2.01%. Under the selected experimental conditions, the sample rate was found to be 120 h^{-1}. All these analytical features indicate that the proposed flow-through cell is reliable for continuous polarographic analysis, and the analytical responses do not present irregularities associated with the use of a DME together with a mercury pool, as it was observed previously in cell designs based on the use of both mercury electrodes simultaneously [2, 3].

The conventional d.c. polarographic method shows a sensitivity of 5.079 $\mu A\ mM^{-1}$, a repeatability (RSD) of 2.19%, and a sample rate of 12 h^{-1}. Therefore, the amperometric flow-through detector proposed here offers analytical advantages over conventional polarographic methods, in terms of sensitivity, facility of measurement (peak height instead of S-shaped wave height), sample and reagent consumption, and rapidity.

Applications

Ten determinations were carried out on a synthetic mixture containing nifedipine in common tablet excipients. The recovery was typically equal to 99.8 ± 0.6%, which indicates that the method is free from these interferences. Ten assays on pharmaceutical formulations containing a nominal 10 mg of the drug per tablet gave a mean value of 9.8 mg per tablet with an RSD of 1.9%.

CONCLUSIONS

A design of a polarographic cell for use in FIA has been proposed, in which a conventional DME is used as working electrode together with a mercury pool as the reference electrode and a platinum wire as counter electrode. The cell can be used not only under continuous flow mode but also under continuous flow-stopped flow mode in order to obtain information about the electrode process, by recording the current-potential signals in static solution, which is not feasible when the flow cell is immersed in an electrolyte solution [4–7]. The approach described here showed analytical advantages respective to the conventional polarographic methods as regards to sensitivity, facility of measurement, reagent and sample consumption, and rapidity. Compared with other dropping mercury-based polarographic flow-through cells, the present design appears simpler and easier to construct and does not present the irregularities associated with the use of a mercury pool as it was observed in previous designs [2, 3]. The use of a DME, on the other hand, avoids problems associated with adsorption processes that are possible in static mercury or solid electrodes.

The polarographic flow-through cell was successfully applied to the determination of nifedipine in pharmaceutical formulations.

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