

Full Research Paper

Flow-Injection Amperometric Determination of Tacrine based on Ion Transfer across a Water–Plasticized Polymeric Membrane Interface

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Abstract: A flow-injection pulse amperometric method for determining tacrine, based on ion transfer across a plasticized poly(vinyl chloride) (PVC) membrane, was developed. A four-electrode potentiostat with ohmic drop compensation was used, while a flow-through cell incorporated the four electrodes and the membrane, which contained tetrabutylammonium tetraphenylborate. The influence of the applied potential and of the flow-injection variables on the determination of tacrine was studied. In the selected conditions, a linear relationship between peak height and tacrine concentration was found up to 4×10^{-5} M tacrine. The detection limit was 1×10^{-7} M. Good repeatability was obtained. Some common ions and pharmaceutical excipients did not interfere.

Keywords: Interface between Two Immiscible Electrolyte Solutions; ion amperometry; plasticized polymeric membrane; tacrine determination; flow-injection analysis

1. Introduction

Ion transfer across the Interface between Two Immiscible Electrolyte Solutions (ITIES) is one of the main topics of the Electrochemistry of Liquid-Liquid Interfaces [1-4]. The terms “ion amperometry at ITIES” and “amperometric ion-selective electrodes” have been coined to denote the measurement of the electric current associated with the ion transfer across ITIES and the electrodes based on this principle, respectively.

The strategies used to overcome the mechanical instability of the liquid/liquid interface in order to develop flow amperometric detectors based on ion transfer imply the use of a porous membrane between both liquid phases [5-10] and the partial solidification of the organic phase with poly(vinyl chloride) (PVC) to become a polymer gel [11-12] which can be supported as an array of micro-interfaces [13-14], or even a plasticized PVC membrane [15-18] similar to those used in ion-selective electrodes (ISEs). These last ones have the advantage that, due to its typical composition containing a 2:1 (m/m) plasticizer to PVC ratio, are easy to handle, durable and can be easily accommodated in flow-through cells. Their physical and chemical properties are fairly well-known [19]. An advantage of the use of the amperometric transduction for these membranes over classical potentiometric transduction used in ion-selective electrodes is that the ion selectivity of the former can be tuned by altering the magnitude of the applied potential.

Tacrine is a parasympathomimetic and a centrally acting cholinesterase inhibitor. It was the first centrally-acting cholinesterase inhibitor approved for the treatment of Alzheimer's disease.



Several methods for the determination of tacrine using different techniques, including electrochemical oxidation and reduction [20-21], spectrofluorimetry on a luminiscence spectrometer [22] and liquid chromatography [23] have been proposed. In the present paper an amperometric method based on the transfer of protonated tacrine ion across a water-plasticized polymeric membrane interface is proposed.

2. Results and Discussion

Firstly, a hydrodynamic chronoamperometric study of the transfer of tacrine cation from the flowing solution to the membrane was made. LiCl solutions ($5 \times 10^{-2} \text{M}$) without and with $5 \times 10^{-5} \text{M}$ THCl were pumped. Fig. 1 shows the corresponding hydrodynamic chronoamperograms obtained for a single voltage pulse ($\Delta E = 200 \text{ mV}$, $\Delta t = 0.5 \text{ s}$) followed by the regenerating time (1s). The difference in current between both curves, also shown, corresponds to the transfer of tacrine cation TH^+ from the flowing solution to the membrane. The positive current observed during the application of the pulse corresponds to the ingress of TH^+ into the membrane. Because a portion of the current response at short times is apparently non-Faradaic, pulses shorter than 0.2 s are not recommended for a sensor containing a membrane with the same plasticizer to PVC ratio used [17-18]. A pulse duration of 0.5s was selected for the present work. The negative current observed when the potential is again lowered to the base potential corresponds to the egress of TH^+ from the membrane. As can be seen, the current returns to zero after 1 s and therefore this was the selected time between the end of a pulse and the beginning of the next one.

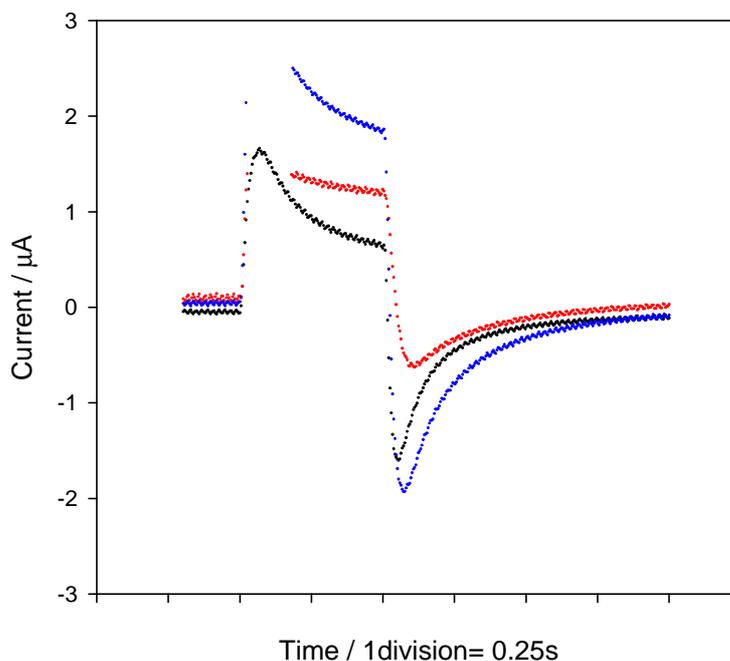


Figure 1. Hydrodynamic chronoamperograms corresponding to a 5×10^{-2} M LiCl flowing solution containing none (black) and 5×10^{-5} M THCl (blue), and the difference between both curves (red).

Fig. 2 shows the pulse amperometric detector response obtained for the injection of a 2×10^{-5} M THCl working solution following the procedure described in 3.4. As can be seen, a well-defined flow-injection peak corresponding to the transfer of TH^+ from the flowing solution to the plasticized PVC membrane was obtained.

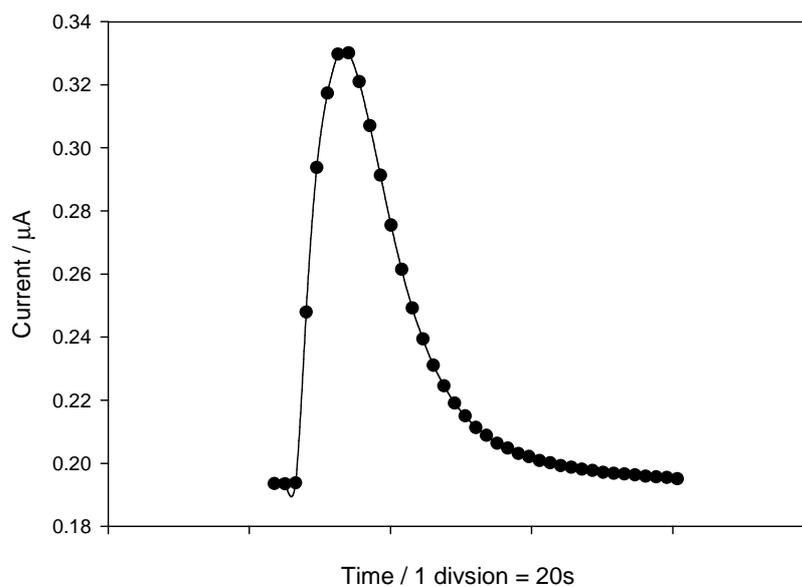


Figure 2. Flow injection peak corresponding to the injection of a $250 \mu\text{l}$ of 2×10^{-5} M THCl working solution. Flow rate 2.0 ml min^{-1} ; $E_f = 500 \text{ mV}$; $\tau = 0.5 \text{ s}$; $\Delta t = 1.5 \text{ s}$.

2.1. Influence of the applied potential

The influence of the applied potential E_f on the peak height corresponding to tacrine transfer was studied in the range 200–600 mV. The results obtained are shown in Fig. 3. As can be seen, the peak height increased as the potential was increased up to 500 mV, above which the peak height remained constant. The background current increased as the potential was increased over the whole potential range studied and reached high values above 500 mV. Taking all this into account, an E_f value of 500 mV was selected.

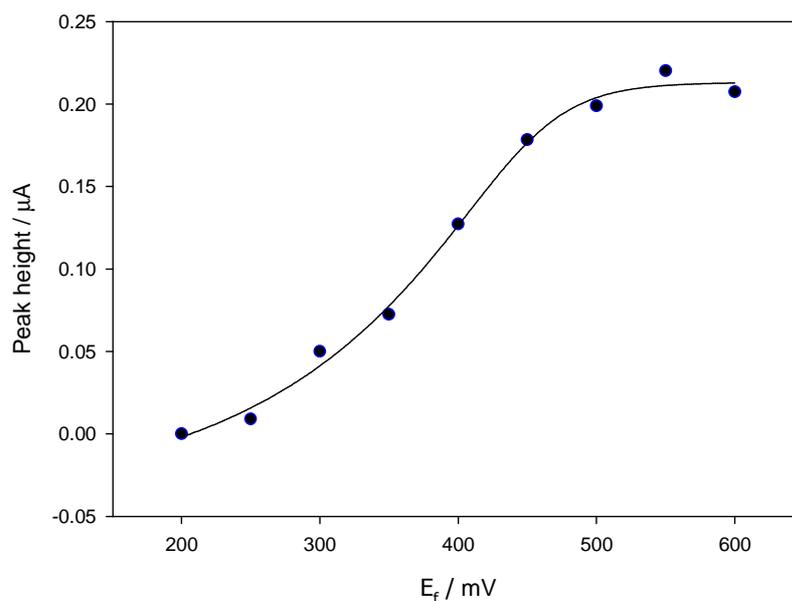


Figure 3. Influence of the applied potential on the peak height corresponding to the injection of 250 μl of 3×10^{-5} M THCl working solution. Flow rate 2.0 ml min^{-1} ; $\tau = 0.5\text{s}$; $\Delta t = 1.5\text{s}$.

2.2. Influence of the flow-injection variables

The effect of the flow rate on the determination of tacrine was studied at a concentration of THCl 4×10^{-5} M, by injecting aliquots of 250 μl of the working sample solution into a LiCl 5×10^{-2} M carrier solution and by varying the flow rate between 0.25 and 3.0 ml min^{-1} . Maximum peak height values were obtained between 2.0 and 3.0 ml min^{-1} but the reproducibility worsened above 2.0 ml min^{-1} . Therefore a flow rate of 2.0 ml min^{-1} was selected for further studies.

The influence of the sample volume was studied over the range 160 – 290 μl in the selected conditions. The peak height increased as the sample volume was increased up to 250 μl , above which the height remained almost constant. A sample volume of 250 μl was used for further studies.

2.3. Features of the method

In the selected condition, a linear relationship between the peak height and tacrine concentration was obtained up to 4×10^{-5} M THCl. The regression equation was $H(\mu\text{A}) = 6.8 \times 10^3 [\text{THCl}](\text{M}) + 5.0 \times 10^{-4}$ with a regression coefficient of 0.9958. The detection limit obtained from three times the standard

deviation of the baseline noise was 1.0×10^{-7} M. A comparison of the sensitivity of the proposed method with those corresponding to the other methods referred for determination of tacrine [20-23] shows that the proposed method is more sensitive than the electrochemical method based on the oxidation of tacrine (detection limit 2.6×10^{-7} M), and less sensitive than the spectrofluorimetric and the high performance liquid chromatography methods (detection limits 1×10^{-9} and 4×10^{-9} M, respectively). An advantage of the proposed method over the formers could be its higher sampling rate and lower cost.

The repeatability of the proposed method was evaluated by performing ten consecutive injections of 4×10^{-5} M THCl working solution. The relative standard deviation obtained for the peak heights was $\pm 3.6\%$. The maximum sampling rate of the method was 70 samples per hour.

2.4. Effect of other species

The effect of other species on the flow-injection amperometric method for the determination of THCl was studied by injecting solutions of 2×10^{-5} M THCl working solution containing different concentrations of several inorganic and organic species. Cation solutions were used in the form of chlorides. The tolerance limit was taken as the concentration causing an error of not more than $\pm 5\%$ in the determination of tacrine. K^+ , Na^+ , Ca^{2+} , glucose, sucrose and lactose did not interfere at least up to a species/tacrine molar ratio of 100. Organic cations may interfere, the higher cation lipophilicity the higher interference.

In order to check the viability of using the amperometric method here proposed to the determination of tacrine in human serum, a calibration graph was carried out in artificial serum. The detection limit found was 4.7×10^{-7} M. The therapeutic range for tacrine is 5-80 ng ml⁻¹ which lies under the detection limit and therefore a preconcentration step should be necessary to apply the proposed method to human serum.

3. Experimental

3.1. Apparatus

The four-electrode potentiostat and the four-electrode flow cell used were described in [15]. Ag/AgCl electrodes were used as reference electrodes and Pt electrodes were used as counter electrodes. A one-channel flow-injection assembly was used (Fig. 4). The distance between the injection valve and the cell was 30 cm. A Merck Hitachi L-7110 isocratic pump, Omnifit injection valve, connecting tubing of 0.5mm bore, PTFE tubing and various end fittings and connectors were used to construct the flow injection system. A glass ring of 28mm inner diameter and 30mm height, glass plate, vial and punch were purchased from Fluka for the construction of the membranes.

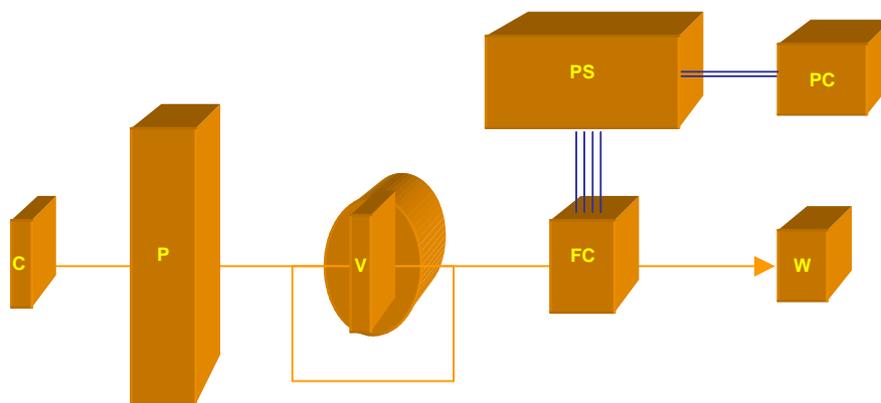


Figure 4. Diagram of the flow-injection system: (C) carrier solution; (P) pump; (V) injection valve; (FC) flow-cell; (W) waste; PS: Four-electrode potentiostat; (PC) personal computer.

3.2. Reagents and solutions

Poly(vinyl chloride) (PVC) high molecular mass, 2-nitrophenyl octyl ether (NPOE), sodium tetraphenylborate (NaTPB) and tetrahydrofuran (THF) were Selectophore products from Fluka. Tetrabutylammonium tetraphenylborate (TBATPB), tetrabutylammonium chloride (TBACl) and tacrine hydrochloride (THCl) were purchased from Sigma. A 1×10^{-2} M solution of THCl was prepared by dissolving in water. Working solutions were prepared by diluting with 5×10^{-2} M LiCl. Glucose, lactose, sucrose and starch were purchased from Probus. All the other reagents used were of analytical reagent grade. Nanopure water ($18\text{-M}\Omega$) prepared with a Milli-Q (Millipore) system was used throughout.

The composition of the artificial serum was Na_2HPO_4 1.5 mM, NaH_2PO_4 8 mM, CaCl_2 2.0 mM, MgCl_2 0.8 mM, KCl 4.5 mM, NH_4Cl 0.05 mM, NaCl 140 mM, Glucose 4.7 mM and Urea 140 mM.

3.3. Membrane preparation

The membranes were prepared by dissolving 200 mg NPOE, 100 mg PVC and 8.4mg TBATPB in 3 ml THF. This solution was poured into the glass ring resting on the glass plate and was left overnight to allow the solvent to evaporate slowly. A 7-mm diameter piece was cut out with the punch and incorporated into the flow-through cell. The electrochemical cell can be expressed as



The applied potential E is defined as the potential difference between the right and left hand terminals. E is controlled by means of the four-electrode potentiostat that applies the necessary potential between the right and left counter electrodes. A positive current corresponds to the transfer of cations from the sample phase into the membrane phase.

3.4. Flow-injection pulse amperometric procedure for the determination of tacrine

A 5×10^{-2} M LiCl carrier solution was pumped through the FI system at a flow rate of 2 ml min^{-1} . The potential applied to the detector cell was held at a base potential E_b of 300 mV, at which no current

flows; voltage pulses of the same duration ($\tau = 0.5\text{s}$) and amplitude ($\Delta E = E_f - E_b = 200\text{mV}$) were applied at a constant interval $\Delta t = 1.5\text{s}$. The current was sampled and averaged during the last 50 ms of each pulse. For the determination of tacrine, 250 μl aliquots of working sample solutions ($1 \times 10^{-6} - 4 \times 10^{-4}$ M THCl) were injected into the carrier solution. Calibration graphs were prepared by plotting the peak height versus THCl concentration.

For the calibration graph in artificial serum, the serum was used as carrier solution and tacrine working solutions prepared in serum were injected.

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