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Chloride determination in serum by a flow-injection analysis precipitation pseudo-titration technique using a flow-through all-solid-state silver electrode*

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Abstract: A simply constructed, tubular, all-solid-state, flow-through silver electrode for flow-injection analysis (F.I.A.) is described. Use of a single line manifold accommodating the silver electrode, with a low level of silver ion (5×10^{-4}M) in the carrier stream, is a useful method to determine chloride in serum, by means of a precipitation pseudo-titration F.I.A. technique. The sampling frequency is about 60/h.

Keywords: *Silver(I)-selective electrode; flow-through electrode; flow-injection analysis (F.I.A.); chloride determination; pseudo-titration; serum.*

Introduction

The growing demand for analytical methods in industrial, environmental and clinical analyses has stimulated the development of flow-through techniques such as segmented flow analysis and flow-injection analysis (F.I.A.). The technique of F.I.A. is based on the controlled and reproducible dispersion of a sample zone when introduced into an unsegmented continuously flowing carrier stream and provides an attractive alternative in clinical determinations [1–3]. The running costs are low and the systems are relatively easy to operate and maintain. Recent reviews on clinically relevant species determined by F.I.A. have been published [4, 5].

The use of potentiometric detection in F.I.A. is more attractive than any other detection technique because of its simple instrumentation requirements, low cost and freedom from sample colour and turbidity interference [6]. Moreover, these latter characteristics make potentiometric F.I.A. methods especially suitable for biological samples.

Among the wide range of detectors used, there are different types of flow-through

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electrodes and potentiometric cells. An important feature of these cells is that their geometry can cause much variation in the hydrodynamic conditions of flow, and so can alter the behaviour of the electrode (stability, reproducibility, response time, etc.). In F.I.A., the use of a tubular configuration seems ideal, as the flow characteristics can be kept constant throughout the system. Recently, some novel flow-through tubular arrangements for ion-selective electrodes have been reported [7–10].

The desire to miniaturise, simplify and produce cheaper ion-selective electrodes (ISE) has motivated attempts to interface tubular, all-solid-state, ion-selective electrodes based on inorganic salts with F.I.A. [11]. Muller [12] developed a silver sulphide-based silver chloride detector for use particularly in the measurement of chloride in blood by a differential technique.

Recently, the present authors have proposed a general procedure for the construction of flow-through tubular electrodes without inner reference solution accommodating PVC membranes as well as heterogeneous crystalline membranes [13, 14].

The determination of chloride in blood is a frequently performed test in the clinical laboratory. Direct potentiometry presents two drawbacks. The usual concentration of chloride in serum is *ca* 0.1 M and only a difference of about 5 mV occurs between the two limiting values in optimum conditions and Nernstian response. On the other hand, the influence of albumin sulphide groups can cause serious interference [12].

"Titrimetric" F.I.A. procedures for the determination of several species, which rely on the controlled dispersion aspect, have been introduced [15]. Pardue and Fields [16, 17] have critically examined if the term "titrimetry" is suitable for these procedures; however, the question is not settled [18]. Meanwhile, Stewart [19] has proposed the abbreviation T.B.F.I.A. for time-based flow-injection analysis that employs pulse-width measurement and the term "pseudotitrations" to classify the technique. Leaving aside the questions of appropriate nomenclature, such methods are clearly superior to other types of continuous flow titration systems in speed and precision. A variety of detection modes such as spectrophotometry, potentiometry or conductimetry have been used [20] but hitherto no precipitation titration has been proposed.

The main aim of the present work was first to construct a simple and very compact, all-solid-state, flow-through silver electrode, and then to test the suitability and behaviour of the electrode in F.I.A. precipitation titrations in the determination of chloride in serum over the very narrow range ($9.6–10.4 \times 10^{-2}$M) usually found in serum.

Experimental

Apparatus and reagents

The flow-injection system is shown in Fig. 1a.

Solutions were propelled by a Gilson Minipuls 2 peristaltic pump. A Rheodyne 5020 injection valve was used. The connections were made by Teflon tubing (0.7 mm i.d.). The manifold accommodates a pulse suppressor, ground electrode, a device for a flow-through reference electrode (as previously described) and a dilution chamber [9, 10, 14]. The electrode signal was monitored with a Crison 501 pH meter connected to a Knauer recorder. An Orion 90-02-00 double-junction reference electrode was used.

All reagents were of analytical grade and double-distilled water was used. The aqueous chloride standards were prepared by successive dilutions of a sodium chloride stock solution. The baseline supporting electrolyte solution was 0.1 M potassium nitrate with 5.0×10^{-4}M silver nitrate as precipitant.

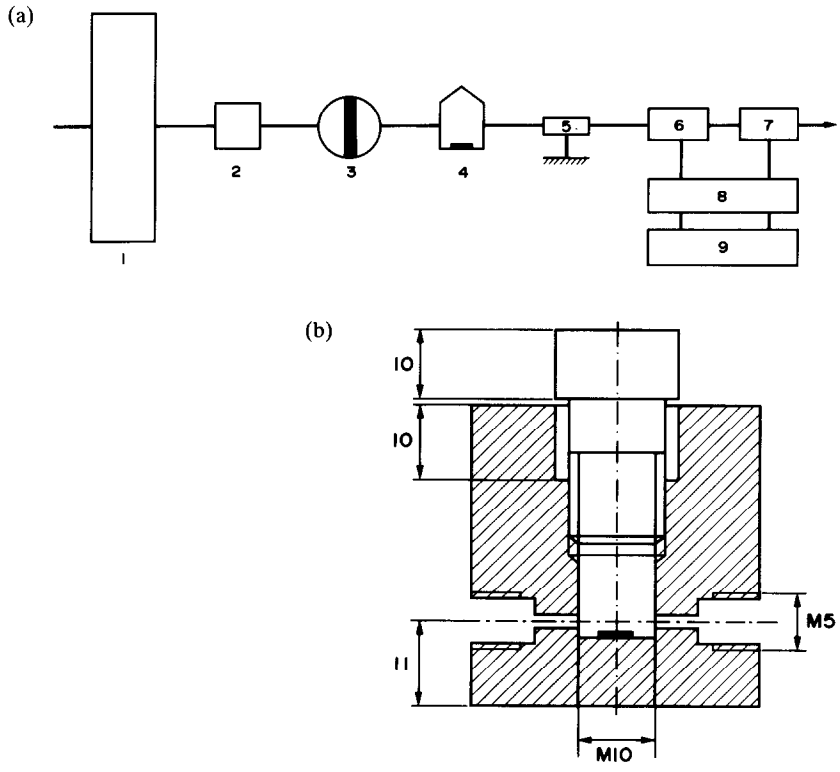

Figure 1
(a) Flow-Injection system: 1, peristaltic pump; 2, pulse suppressor; 3, injection valve; 4, gradient chamber; 5, ground electrode; 6, silver electrode; 7, reference electrode; 8, potentiometer; 9, recorder. (b) Variable volume gradient chamber.

Construction of the tubular electrode

In previous work [13], a new procedure for the construction of all-solid-state heterogeneous membrane electrodes sensitive to silver(I) ion was proposed. The procedure is especially suitable for preparation of potentiometric sensors for F.I.A.

The first step in the present work was to find a more convenient internal reference than the one used previously [13], because the silver foil contact used is not appropriate for a tubular electrode configuration. A commercially available plastic conductive support, with silver powder dispersed over epoxy resin (Epotek 410), has been used to replace the rigid silver foil internal contact.

The basic design and the construction process of the tubular electrode is shown in Fig. 2. It is composed of three separated blocks, a potentiometric flow cell and two adaptors.

The electrode body of the potentiometric flow cell was constructed by filling a silicon tube (i.d. 11 mm) with commercial epoxy resin (Araldite CW 2215 and hardener H.R.) and curing it at 40°C for 12 h. Then, the cylindrical epoxy was cut transversely (8 mm wide). Each of those pieces was drilled longitudinally with a 5 mm diameter drill; the silver epoxy resin was placed inside and cured at 105°C for 2 h. Later, the conductive silver epoxy support was drilled longitudinally with a 3 mm diameter drill. The sensor membrane mixture was placed into the cavity and cured at 40°C for 24 h. Finally a 0.7 mm i.d. hole was drilled.

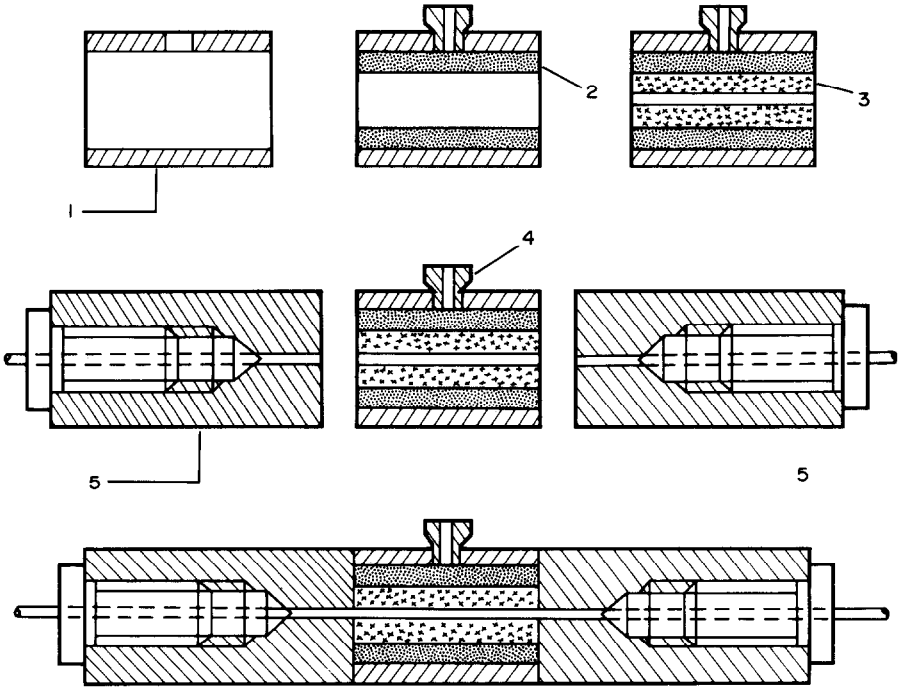

Figure 2
Construction process of the tubular electrode: 1, electrode body; 2, conductive epoxy resin; 3, sensor membrane; 4, electric plug; 5, adaptors.

The composition of this mixture was Ag_2 S-epoxy resin (3:2, m/m).

The adaptors (15 × 0.7 mm i.d.) were made as shown in Fig. 2. The three parts were assembled with fresh epoxy resin.

Results and Discussion

Behaviour of the tubular electrode

Initial results showed baseline noise that originated from the peristaltic pump. A pulse suppressor, ground electrode, and a device for flow-through reference electrode as previously described [14], largely eliminated noise. The carrier solution was 0.1 M potassium nitrate containing 10^{-6}M silver nitrate to stabilise the baseline. The behaviour of the potentiometric flow cell was studied as before [9]. It was found that the use of an injection volume of 50 µl and a tube length of 30 cm allowed the flow rate to be varied over a wide range (1–6 ml min^{-1}) without affecting the quality of the electrode response. This is very convenient for versatility in the subsequent measurement in precipitation–titration procedures. Under these conditions, the electrode gave a Nernstian response for 10^{-5}–10^{-1}M silver ion with a slope of 62 ± 0.5 mV.

Chloride titrations using the silver tubular electrode

The flow system leads to a concentration gradient by dispersion of the sample in an adequate device. Peak width is in this case the analytical signal. The silver electrode was

inserted into the flow-injection system (Fig. 1a). The carrier solutions comprised 0.1 M potassium nitrate with several amounts of silver nitrate added. The concentration of silver nitrate in the carrier clearly determines the linear range and sensitivity of the determination. Under the best conditions, using a 5×10^{-4}M silver nitrate (background) carrier, good sensitivity was obtained to *ca* 10×10^{-2}M chloride concentration. All attempts to reach the usual range of concentrations of chloride in serum failed because too much precipitate was formed and the tubes became blocked frequently. However, a simple 1:10 dilution of the sample serum injected allowed use of the carrier mentioned above. Additionally, this dilution decreased protein interference.

Other experiments were performed in order to optimise the flow-injection system parameters, the volume of the gradient chamber, flow rate and sample volume. In precipitation pseudotitrimetric F.I.A. procedures the use of a mixing chamber has shown to be an appropriate device to create an exponential concentration gradient. The stirring assures a well-dispersed precipitate, and a moderate volume of the chamber with an appropriate flow rate avoids loss of sensitivity and permits an acceptable sample throughput. The use of a variable volume mixing chamber (Fig. 1b) optimises this parameter simultaneously with flow rate. A volume of 0.55 ml and a flow rate of *ca* 1 ml min^{-1} was finally chosen because it is a good compromise between sample throughput rate and reagent consumption.

The sample volume has little influence on the slope of the graph of $log[Cl^-]$ versus time (sensitivity) but large sample volumes cause a low sample throughput rate. A volume of 50 μl was finally chosen.

The procedure does not qualify as a titration but better as a peak-width measurement-based F.I.A. determination. Under these conditions, it has been shown that the choice of "set points" is arbitrarily made [21]. The width of the pulse (t) at a fixed signal intensity is called the "set point". Obviously, it is not necessary to reach the baseline after each injection. A signal of 330 mV has been chosen as potential at the "set points". This potential is *ca* 20 mV over the baseline potential of the tubular silver electrode at $[Ag^+] = 5 \times 10^{-4}$M. With these assumptions a new injection is made when this potential is reached and so the sample throughput can be largely increased.

The system was calibrated injecting (in quadruplicate) five aqueous chloride standards at the same ionic strength of the carrier in the range 8.0×10^{-3}M–1.2×10^{-2}M. The quantitative evaluation was based on peak-width measurement in mm (speed recorder 1 mm s^{-1}). In this way the "set points" were represented in s.

The calibration plot yields a straight line ($r = 0.9966$; $N = 24$) at least in the mentioned range, covering the normal fasting level and pathological values of chloride in blood, with sensitivity higher than that of the direct potentiometric determination. The calibration plot and corresponding values are shown in Fig. 3.

The good reproducibility of the system is in general ±0.5 mm, equivalent to ±0.5 s. This compares favourably with that of direct potentiometric F.I.A. determination.

Determination of chloride in serum

The test conditions were optimised in order to facilitate distinction between 9.6 and 10.4×10^{-3}M Cl^-. Figure 3 shows that a difference of about 8 mm occurs between the two limiting values. The samples were conducted on control sera (Precinorm S, Boehringer, Mannheim) which, prior to measurements, were diluted (1:10). The relative standard deviation ($N = 12$) is about 1.75%. The deviation of the results related to standard serum (0.1 M) [12] was 0.43%. Matrix effects are considerably diminished

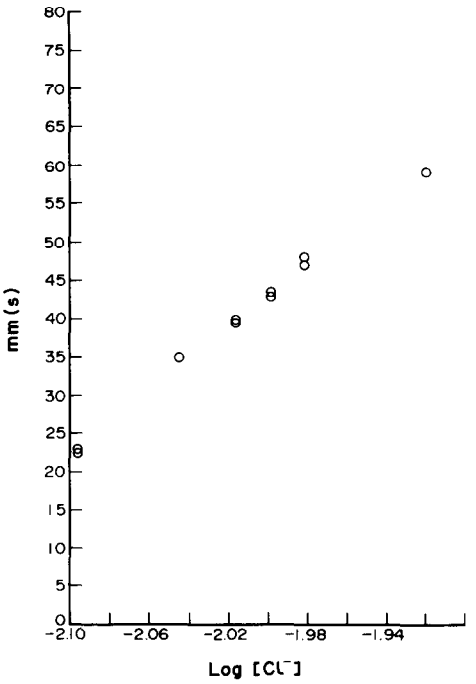

Figure 3
Calibration plot. Peak-width (mm) (equivalent to time in s) against log [Cl^-].

versus direct potentiometry by dilution of the sample (before the injection and into the gradient chamber). The sampling frequency is about 60/h.

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