Thin Layer Samples Controlled by Dynamic Electrochemistry

Maria Cuartero§, Gastón A. Crespo, and Eric Bakker*

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Abstract: We summarize here recent advancements on thin layer samples controlled by dynamic electrochemistry techniques for determining different ions that include potassium, calcium, protamine, nitrate, nitrite and halides in diverse environmental and clinical samples. Generally, a thin layer liquid (less than 100 µm in thickness) is confined between a working electrode and an ion-selective membrane. Once an external electrical perturbation (*i.e.* constant potential) is applied to the system, an exhaustive ion transfer process is imposed in the entire thin layer solution. As a result, the observed charge (integration of the current decay) is proportional to the depleted ion concentration. Other electrochemical protocols such as linear sweep voltammetry were also explored aiming at the discrimination of several ions presented in the thin layer. One of the most attractive examples involves the detection of a mixture of three halides (iodide, bromide and chloride), which can be well resolved at moderated scan rates (10 mV.s^{-1}). Paper-based coulometric sensing on thin layers defined by cellulose papers are being developed in view of translating these new concepts into a reliable and low cost sensing platform.

Keywords: Coulometry · Desalinator unit · Ionophore-based membranes · Paper-based microfluidic devices · Thin layer sample

1. Motivation

The undeniable necessity of gaining more knowledge on human activity and its relation with the ecosystem has catalyzed the development of new fundamental sensing concepts. As a result, there is an urgent need for reliable chemical sensors that are able to extract information about a desired target in real and complex matrices.[1] Additional required characteristics of such sensors include the ability for '*in situ*' determinations, and they should be disposable, portable, and involve low consumption of sample and resources. Clinical diagnostics,[2] environmental monitoring,[3] and quality control[4] (mainly food and drinkable water) are attractive areas for these types of sensors and innovative scientific efforts have recently been put forward in this direction. In this context, we summarize here the establishment of thin layer sample modulation by dynamic electrochemistry as a new and absolute counting platform for the determination of ions in diverse samples that range from human blood to seawater as well as food supplements.

2. Thin Layer Concept

The establishment of thin layer samples (less than 100 µm in thickness) allows one to impose the total conversion/depletion of the ion of interest once an appropriate driving force (*e.g.* constant or linear sweep potential) is applied to the system. As a result of the electrical perturbation, an anion (X$^-$) is deposited on the working electrode and coupled to the transport of the counterion (*e.g.* K$^+$) across an ion-selective membrane. Owing to the fact that the current is mainly limited by processes occurring in the aqueous sample phase, the thickness of the aqueous layer defines the time of the experiment. Mass transport is primarily limited to ion diffusion (convection and migration are typically insignificant at the selected conditions). Fig. 1a shows a simplified scheme of this concept for potassium determination by applying a constant potential for an appropriate time.[5] As observed, the thin layer sample is confined between a working electrode and an ion-selective membrane for potassium. First reports about this concept were performed with tubular materials for both electrode and ion-selective membrane elements (see below and Fig. 2).[5,6]

As extensively demonstrated with ion-transfer experiments between two immiscible liquids, there is an ion-transfer potential that is a function of the partitioning of the ion of interest between the two phases.[7] If the applied potential is smaller than a threshold value, ion-transfer is not observed (giving only capacitive currents as readout). When the applied potential becomes higher than the threshold value (constant potential), a current decay is observed as a result of the establishment of the ion-transfer process (Fig. 1b).[8] Because ion-selective membranes are considered as initially non-polarized interfaces (the same ion is shared in both phases)[8b] the phase boundary potential for each interface is well defined and is used as a reference value for the membrane regeneration process, which is imposed after the interrogation pulse. In the example used here, the organic membrane is doped with a potassium ionophore, rendering the ion transfer process selective for the potassium ion. During the interrogation step, therefore, potassium ion is preferentially transferred to the membrane phase compared to the cation of the background electrolyte and chloride ions are simultaneously electrochemically deposited on the Ag/AgCl element (working electrode) to fulfill the electroneutrality condition in the thin layer sample (see Fig. 1b).

Correspondence: Prof. Dr. E. Bakker
Department of Inorganic and Analytical Chemistry
University of Geneva
Quai E.-Ansermet 30
CH-1211 Genève
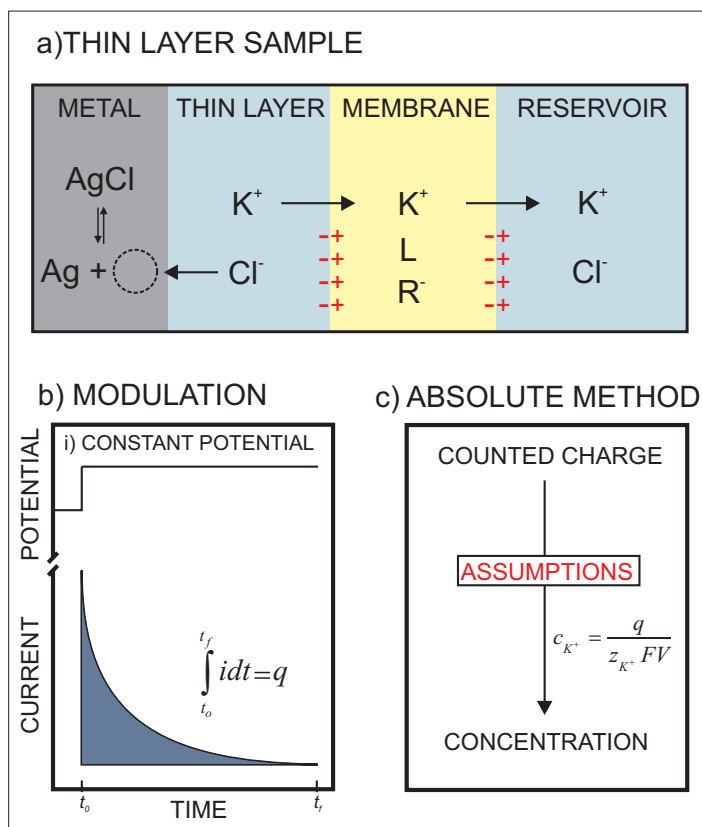E-mail: eric.bakker@unige.ch

a) THIN LAYER SAMPLE

b) MODULATION   c) ABSOLUTE METHOD

Fig. 1. a) Scheme of the electrochemical mechanism upon application of a cathodic potential to a thin layer of KCl. (L = potassium ionophore and R⁻ = cation exchanger). b) Schematic illustration of the current decay obtained during the application of a constant potential pulse. Integration of this curve corresponds to the charge of the ion. c) Scheme of the relationship between the charge and the ion concentration. (C_{K+} is the potassium concentration, q is the charge, Z_{K+} is potassium ion charge, F is the Faraday constant and V is the volume)

brane[18] and data correction by taking into account the capacitance of the cell.[14]

2.1 Experimental Set-up

Thin layer coulometry was performed using an electrochemical cell based on tubular materials.[6] Fig. 2 shows the current design of the custom-made microfluidic electrochemical cell.[14,16] This cell is based on a tubular working electrode, typically a silver or chloridized (Ag/AgCl) silver wire introduced inside a tubular ion-selective membrane that has a somewhat larger inner diameter (600 μm for polypropylene and 360 μm for Nafion). The gap formed between the wire and the membrane delimits a thin space of limited volume that is filled by the corresponding sample. Volumes between 0.6[16] and 26 μL[13] are obtained depending on the membrane used and the length of the tubing. The membrane is placed in a reservoir that contains an outer solution in which reference and counter electrodes are immersed. The sample is introduced through the inlet by means of a pump working at low flow rates (3–5 μL/min).

The integration of the current decay profile gives the total number of transferred potassium charge (coulometric readout) in the entire thin layer if some key assumptions are satisfied: i) the transfer process is chemically selective; ii) the current must be limited by the thin layer sample; iii) the volume of thin layer sample must be known and constant with time, iv) the ion-transfer process must be exhaustive for the target of interest (Fig. 1c). Accordingly, in order to accomplish these assumptions, significant efforts have been put forward from our group and others.

The ion transfer selectivity has been modulated using ion-selective membranes doped with different ionophores for cations[9] and anions.[10] We demonstrated the use of highly selective membranes in thin layer coulometry to determine potassium,[5] calcium,[6,11] protamine,[12] as well as nitrate,[13] and nitrite.[14] On the other hand, ion-exchange Donnan exclusion membranes such as Nafion and FKL,[15] which show a selectivity pattern of the Hofmeister type, can also be used. In this case the selectivity can be introduced by the electrochemical reaction at the electrode in contact with the thin layer sample[16] (see below). In addition, the use of fast diffusive materials for membrane preparation is convenient to limit the current by mass transport in the aqueous phase only. It was therefore necessary to replace poly(vinyl chloride) used in conventional plasticized polymeric membranes by other materials with higher diffusion coefficients

such as porous polypropylene doped with organic liquids.[6] The thin layer volume of appropriate thickness and constant sample volume is achieved by an optimization of the experimental setup (Fig. 2). Finally, a homogeneous and complete electrochemical transformation of the target ion in the sample is required. Ideally, the analyte in the thin layer sample has to be exhaustively depleted allowing for an absolute measurement. However, other cations present in the sample can also be transferred even when the membrane is highly selective to the analyte. In order to avoid this as well as undesired non-Faradaic processes, a range of procedures were developed, including the introduction of an additional excitation pulse for background correction,[17] the incorporation of a physical separator between the working electrode and the mem-

3. Thin Layer Cyclic Voltammetry – Multianalyte Detection

As noted above, constant potential thin layer coulometry is an adequate readout protocol for the detection of a single analyte. Samples containing multiple analytes may be detectable as well if the electrochemical protocol is adapted. The application of linear sweep potential to thin layer samples has been recently explored in our group using the cell shown in Fig. 2 based on a Nafion membrane and a silver electrode.[16] The thin layer voltammograms have the benefit of providing peak-shaped responses, giving a different peak for each ion. These peaks can be better analyzed than the monotonous current decays observed with the application of a constant potential. The selectivity of the method originates in the formation of insoluble
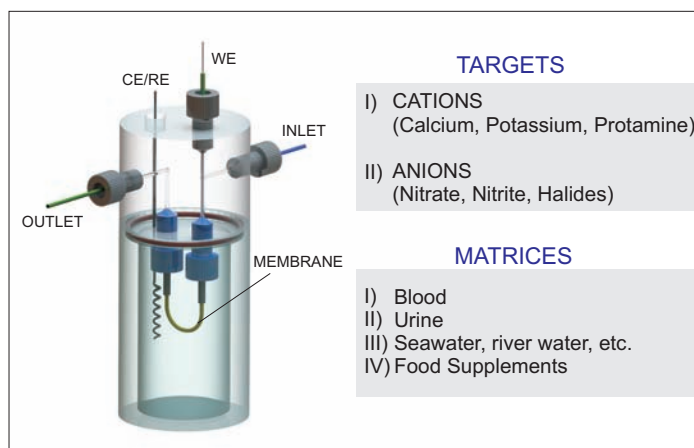

Fig. 2. Design of the tubular cell used for thin layer coulometry. The coulometric cell consists of a counter/reference electrode (CE/RE) and working electrode (WE) made of Ag/AgCl. The working electrode is a wire (300 μm diameter and 15 mm length) which is inserted into a Nafion membrane (360 μm internal diameter and 40 mm length).

TARGETS

I) CATIONS
    (Calcium, Potassium, Protamine)

II) ANIONS
    (Nitrate, Nitrite, Halides)

MATRICES

I)   Blood
II) Urine
III) Seawater, river water, etc.
IV) Food Supplements

halide salts on the silver element, as explained below.

The approach was applied to mixed samples of chloride, bromide and iodide.[16] By increasing the applied potential, halide ions present in the thin-layer sample are electrodeposited on the working electrode as silver halides, while their respective counterions (sodium) are transported across the Nafion to the outer solution (see scheme in Fig. 1a). Owing to the disparate solubility products for AgCl, AgBr and AgI, the electrodeposition of each halide occurs at different potentials leading to three different peaks in the cyclic voltammogram. Fig. 3a shows a scheme of peaks obtained for chloride, bromide and iodide at different scan rates. As observed, better differentiated peaks are obtained using lower scan rates (optimum scan rate was 5 mV s^{-1}) because this allows for an exhaustive electrochemical process at each stage of the voltammogram.[16]

Fig. 3b exhibits experimental voltammograms for different halide concentrations in mixed samples, showing three well-defined peaks. The integrated charge from each peak was found to be linear with the corresponding halide concentration present in the sample.[16] Excellent reproducibility of the calibration parameters (2.5% RSD), even from cell to cell, indicates that the method is potentially calibration-free. Moreover, analysis of chloride, bromide and iodide in tap, mineral and river water as well as in seawater was successfully achieved. Samples only need to be filtered before being analyzed without any other pretreatment such as dilution. This fact constitutes a valuable advantage over other methods such as ion chromatography[19] since the high amount of chloride in seawater normally masks the determination of other ions. The electrochemical reduction of the chloride concentration without influencing the concentration of the remainder of the ions is especially attractive. Therefore, a simple electrochemical treatment utilizing the cell showed in Fig. 2 was proposed for a rapid removal of NaCl in seawater in a matter of minutes.[20] The method is capable of reducing chloride concentration from 0.6 M to 3 mM in artificial seawater, constituting an important progress in view of nutrient analysis since an electrochemical nutrient detection may be coupled online to this type of thin layer desalination unit. For this purpose, new cell configurations are currently being explored to increase the desalinated sample volume from a few (~20 μL) to hundred μL.

3.1 *Paper-based Thin Layer*

A synergy between previous fundamental investigations with a low cost paper platform results in a suitable configuration to obtain disposable and portable ion sensors. Paper has been widely used as sampling element in microfluidic paper-based analytical devices.[21] However, its ability to create a well-defined thin aqueous layer has not yet explored in electrochemical sensor design.

Recently, we reported on the creation of thin layer samples with very low sample volume together with a coulometric readout using a paper-based cell for multianalyte halides detection.[22] The use of 75 μm thick papers combines two main operational functions: the physical transport of the sample into the cell and the achievement of a thin layer sample, resulting in an exhaustive and potentially calibration-free methodology. Fig. 4a shows a scheme of the cell design entirely composed by flat elements. The sample is introduced into the cell using the filter paper which brings the solution in direct contact with the working electrode (silver foil element) and the membrane. The membrane used here is FKL, a cation-exchange Donnan exclusion membrane. The opposite side of the membrane is in contact with a second filter paper that contains another solution of sodium halide in contact with a silver/silver halide element part, which operates as the reference/counter electrode. All elements (two papers, two electrodes and the membrane) are squeezed together by magnetic elements embedded into two acrylic blocks.

Following the same electrochemical procedure used with the tubular cell concept for halide determination, we established an inexpensive, robust and reliable paper-based device for the determination of chloride, bromide and iodide in complex matrices (different types of waters and a food supplement).[22] Fig. 4b shows the voltammogram obtained for the food supplement as an example. The advantages of this paper-based configuration compared to the tubular cell include a simpler device fabrication, the use of low-cost disposable materials and a more portable system since it is not necessary to use a pump.

4. Conclusions

We present here an overview on recent advances in thin layer samples controlled by dynamic electrochemistry for sensing ions. Thin layer samples are generally developed by confining the sample between the working electrode and the ion-selective membrane. The application of a constant potential imposes a total conversion/depletion of the ion of interest with a monotonous current decay. Multianalyte detection
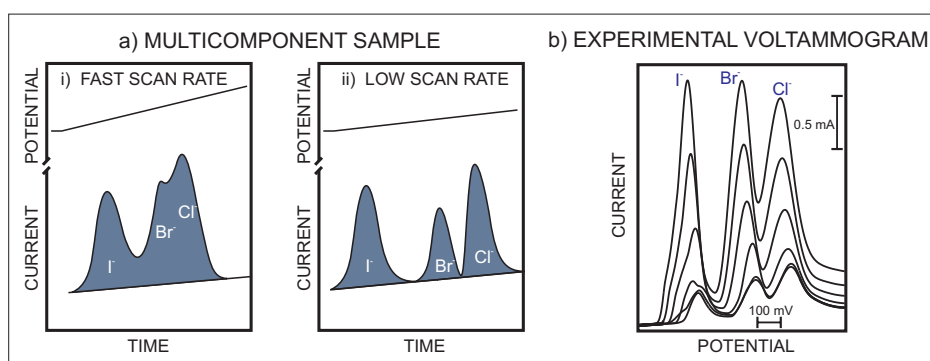
Fig. 3. a) Comparison of the three peaks obtained in thin layer voltammograms at a silver electrode at low and fast scan rates for chloride, bromide and iodide. b) Thin layer cyclic voltammograms at a silver electrode obtained for different concentrations of chloride, bromide and iodide in mixed samples. (From the top to the bottom: 0.1, 0.06, 0.03, 0.01, 10^{-3} and 10^{-4} M).
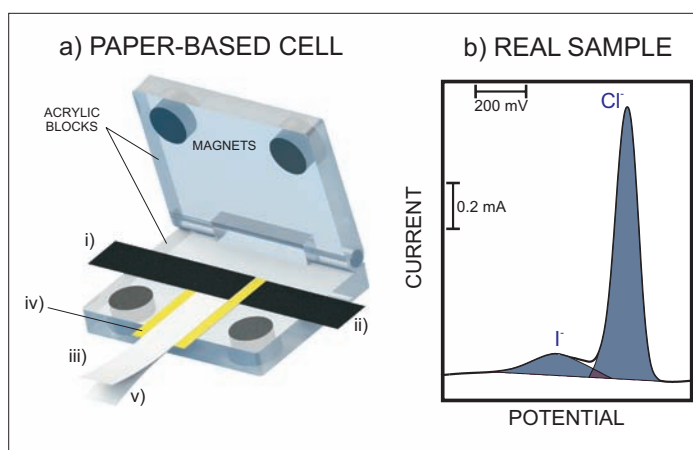
Fig. 4. a) Scheme of the paper-based electrochemical cell for halide detection. i) Working electrode, ii) counter/reference electrode, iii) paper for sampling, iv) FKL membrane, v) paper for the outer solution. b) Experimental thin layer voltammogram of halides obtained for a food supplement.

in complex samples is not possible with this type of protocol. Instead, thin layer cyclic voltammetry may resolve a mixture of different ions while maintaining the exhaustive nature of the analysis. As a case in point, the multianalyte determination of chloride, bromide and iodide in mixed ion samples is demonstrated. Additionally, the use of paper to confine thin layer samples provides a disposable and portable device, which is extremely simple in terms of manipulation, cost and contamination risk. This methodology forms the conceptual basis to develop new systems based on solid-contact ion-selective electrodes for ion sensing in the future.

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