Triangle-Programmed Coulometric Nanotitrations Completed by Continuous Flow with Potentiometric Detection

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Coulometric nanotitrations were realized in a microchannel system using a continuous-flow titration technique with a triangle current−time profile. Redox and acid−base titrations were carried out on Fe(II) and nitric acid samples, respectively, with the same nanotitrator device. A linear relation between the concentration and the coulometric current transferred to the solution was found. The advantages of this universally applicable nanotitrator are fast response, low sample volume, high sensitivity, and high reproducibility as well as the convenience of handling an automated analyzer of the flow-through type.

Titrations are among the most widely used analytical techniques. For many years, considerable efforts were concentrated on the improvement of analysis times and detection limits, also in the field of titration methods. Two alternative approaches have been followed to reduce the titration times: first, the automation of classical titrations in a well-defined sample volume (batch procedure)[1,2] and, second, the introduction of titrations carried out in the flow-through mode.[1,3,4] The latter techniques are commonly known as flow injection analysis (FIA) titrations. It should be pointed out that these so-called titration techniques are basically kinetic methods, which do not fulfill the strict definition[5] of a standard titration. However, for the sake of simplicity, the terminology used for true titrations, including unconventional use of equivalence-point terminology, will be applied here as other authors[1−3,6,7] have done.

Automated titrators are familiar tools in routine analysis. They give an excellent reproducibility, but the analysis time required is relatively long. On the other hand, titrations carried out under continuous-flow conditions, either in a gradient chamber[6,8−10] or in a sequential system,[7,11,12] offer a higher rate and an intrinsic simplicity of analysis.

Pungor et al.[6,8] introduced in the early 1970s the so-called triangle-programmed titration technique for volumetric and coulometric titrations in flow injection analysis. The sample solution and the titrant were mixed in a gradient chamber located upstream from the sensor. The analysis time could be considerably decreased in comparison with that of conventional titrators, but still about 2 min was needed to carry out a titration. Progress in microtechnology opened new possibilities for a miniaturization of coulometric titration.[13,14] Preliminary results obtained with such microdevices were highly promising.

This paper reports the application of an inexpensive and universally applicable nanotitrator, which generally gives a very fast response and is suited for extremely small sample volumes. In contrast to those of a previous study,[14] measurements were carried out under continuous-flow conditions, using a triangle current−time profile. The very short analysis time is certainly one of the most valuable benefits of nanotitrations. Due to the large-scale miniaturization, the volume of the sample solution participating in the reaction is reduced to only a few microliters. The present microdevice also differs from the earlier macroscopic systems in that that no mixing unit is needed. In addition, the sensor is placed next to the chemical actuator.

THEORETICAL SECTION

Coulometry in Continuous-Flow Injection Analysis. If a continuously flowing analyte solution and a coulometrically generated reagent (titrant) are mixed in a certain section of a microchannel, the degree of titration in the outflow solution depends on the respective rates of molar input of the two educts. By changes in these rates, the degree of titration can be altered and monitored by an appropriate sensor placed in a flow-through detector cell. When a triangle current−time profile is applied at the coulometric electrodes, the flowing sample solution becomes

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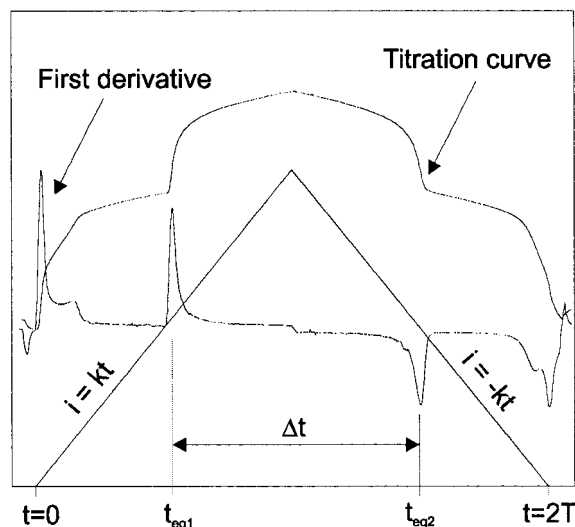
Figure 1. Triangle-programmed current−time profile and titration curve for continuous titrations of a flowing sample. The potentiometric titration curve and its first derivative were obtained for a 10 mM Fe(II) sample solution.

gradually titrated by coulometrically generated ions. The evolution of the reaction is monitored by the indicator electrode. During the first half-period (the leading edge) of the current−time profile, the usual titration curve is recorded, whereas, during the second half-period (the trailing edge), the reverse titration curve is monitored. Equivalence points of the titration appear in both directions. The first equivalence point is situated on the leading edge, and the second, on the trailing edge of the titration curve. The effective peak width of the titration curve, denoted below as time span (Δt), is determined by the time elapsed between the minimum and the maximum of the first derivative (Figure 1).

Simple Model. A simple description of the titration end point can be obtained from a balance of the flows of the species participating in the chemical reaction. For a redox titration, the reaction is the following:

$$\nu_A A_{red} + \nu_B B_{ox} \Leftrightarrow \nu_A A_{ox} + \nu_B B_{red} \qquad (1)$$
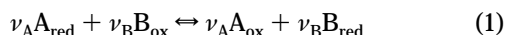
where ν_A and ν_B are the stoichiometric coefficients. The mass flow rate $R_{A,red}$ of the coulometrically generated educt is determined by the current−time profile (i vs t) and depends on the respective proportionality factor k [A/s]:

$$R_{A,red} = \frac{i}{nF} = \frac{\nu_A}{\nu_B} \frac{kt}{F} \qquad [mol/s] \qquad (2)$$

where n is the number of electrons transferred per ion and F is the Faraday constant. The rate of mass flow $R_{B,ox}$ of the analyte species to the titration cell is given by the product of the initial concentration c_0 [M] of the sample solution and the volumetric flow velocity Q [L/s]:

$$R_{B,ox} = Qc_0 \qquad [mol/s] \qquad (3)$$

Equation 2 holds for the leading edge of the titration curve. At the corresponding equivalence point after the time $t_{eq,1}$, the
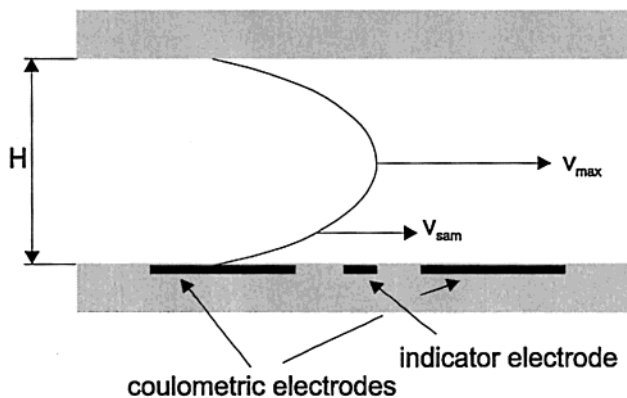
Figure 2. Parabolic flow profile in the microchannel. The effective volume of the sample solution titrated moves with an average velocity of $v_{sam} = \alpha v_{max}$.

two reagent mass flows are balanced:

$$\nu_B R_{A,red} = \nu_A R_{B,ox} \qquad (4)$$

Accordingly, the first equivalence time $t_{eq,1}$ becomes

$$t_{eq,1} = \frac{Q}{k} Fc_0 \qquad [s] \qquad (5)$$

Equations 4 and 5 hold for the idealized case, where the mixing between the two reagent flows is complete (see, however the extended model described below). In analogy, the second equivalence time $t_{eq,2}$, as found for the trailing edge of the titration process, can be described as

$$t_{eq,2} = 2T - \frac{Q}{k} Fc_0 \qquad [s] \qquad (6)$$

where $2T$ is the period of the titration cycle. Finally, the time span Δt is given by

$$\Delta t = t_{eq,2} - t_{eq,1} = 2T - 2\frac{Q}{k} Fc_0 \qquad [s] \qquad (7)$$

Extended Model. The simple model leading to eq 7 does not account for physicochemical effects arising in the microchannel, namely, the actual hydrodynamics of flow and the diffusion of ions.

For the present system, a Reynolds number of about 4 is calculated for the highest flow rate used in this study (120 μL/ min). This indicates that the flow is strictly laminar; i.e., a parabolic flow profile is built up in the microchannel. The near proximity of the chemical actuator and of the sensor implies that the titration occurs mainly in the boundary zone of the channel (see Figure 2). Therefore, the effective volume of the titrated sample solution moves with an average velocity v_{sam}, which is related to the maximum rate v_{max} by a reduction factor α. The value of α must be experimentally determined from a calibration curve, since the effective volume titrated is not known.

In addition, the response time asymmetry t_r of the potentiometric sensor,[15] revealed by the asymmetry of the titration curve, can also be accounted for in eq 7, which finally yields the relationship

$$c_0 = \left(T - \frac{\Delta t}{2} - t_r\right)\frac{k}{\alpha FHWv_{max}} \qquad (8)$$

where H is the channel height, W is the channel width, and v_{max} is the maximum velocity at the center of the parabolic flow profile.

Potentiometric Detection. A direct potentiometric method is used to detect the titration end points. The potential is measured between the indicator electrode, located where the titration takes place, and the reference electrode, placed 7 mm upstream in the microchannel. The reference electrode was located far from the titration cell to prevent interference from the redox reactions. Both electrodes were made from platinum. Platinum is an inert metal and does usually not participate in the redox chemistry, except as a conductor of electrons and as an indicator of the activity ratio of a redox couple. The platinum reference electrode can be considered as a pseudoreference electrode, since its potential depends on the composition of the native sample solution. As a relative signal arising between the potentiometric electrodes is sufficient for the determination of the end-point titration, this simple reference electrode is adequate for the present measurement. The emf E of the electrode cell is given by the Nernst equation.[16]

EXPERIMENTAL SECTION

Design of the Nanotitrator. An array of platinum planar electrodes of different sizes are deposited on a silicon substrate, within a microchannel etched in a glass cover plate. This cover plate is anodically bonded on the silicon substrate. The whole process of the microfabrication of the coulometric nanotitrator was reported earlier.[14,17]

Figure 3 shows a schematic view of the nanotitrator. The coulometric titration method applied in this study makes use of two coulometric electrodes (a generator and a counter electrode) and of two potentiometric electrodes (an indicator and a 250 μm wide reference electrode). The widths of the generator and the counter electrode are both 250 μm. Both coulometric electrodes are separated from the 5 μm wide indicator electrode by a distance of 5 μm.

The length and width of the microchannel are 20 mm and 1 mm, respectively. The height of the channel used in this study is either 30 or 50 μm. Thus, the total volume of the whole channel is 0.6 or 1 μL, respectively.

Equipment and Setup. All the experiments were automated, so that the triangle-programmed coulometric titrations could be well reproduced. Either a piston pump (700 Dosino-2 mL, Metrohm) or a peristaltic pump (MV CA-4, Ismatec) was used to continuously and precisely inject the sample solution into the microchannel. Pulses of the Dosino have a resolution of 0.2 μL.
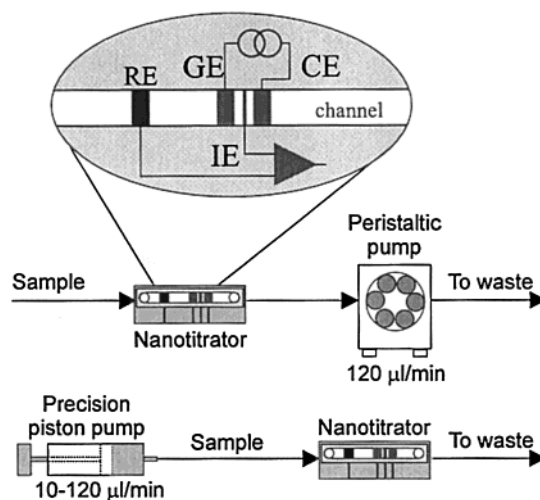

Figure 3. Experimental setup and schematic view of the μTAS for coulometric nanotitrations. IE, RE, GE, and CE denote indicator, reference, generator, and counter electrodes, respectively.

The triangle current–time profiles were provided by a model 273A galvanostat (EG&G Princeton Applied Research). The data acquisition was accomplished using a homemade acquisition circuit, driven by a National Instruments (Lab-PC+) acquisition board.

Each measuring cycle consisted of a 20 s titration period, during which the triangle current–time profile was applied and the potential response was recorded. The next cycle started 5 s later, to replace the sample solution in the microchannel. Cross-contamination between the sample solutions was inhibited by pumping the new sample solution in the nanotitrator for about 5 s before the beginning of the titration cycle. The titrations were repeated seven times for each concentration. The electrodes were preconditioned during the first two titrations. Results obtained during the following five titrations were averaged, and the standard deviation was calculated.

Materials. All reagents were purchased from Aldrich except when specified otherwise. $FeSO_4$ was used as the analyte for redox titrations. An excess of 20 mM cerium(III) chloride was added to the iron(II) solution. The salts were dissolved in deionized water. A background electrolyte of 1 M H_2SO_4 was used for redox titrations. A 97% quinhydrone solution was used as a redox couple to carry out acid–base titrations with nitric acid as a sample solution (HNO_3, Titrisol, 0.1 M, Merck). A background electrolyte of 0.1 M KNO_3 (p.a.) was used for acid–base titrations.

RESULTS AND DISCUSSION

Redox Titrations. The redox couple $Fe(II)/Fe(III)$ was chosen as a typical example suited for redox titrations. To prevent the occurrence of hydrolysis by water, an excess of $Ce(III)$ was added to the solution as an electron-transferring reagent.[16,18] This ion is oxidized at a lower potential than that for water.

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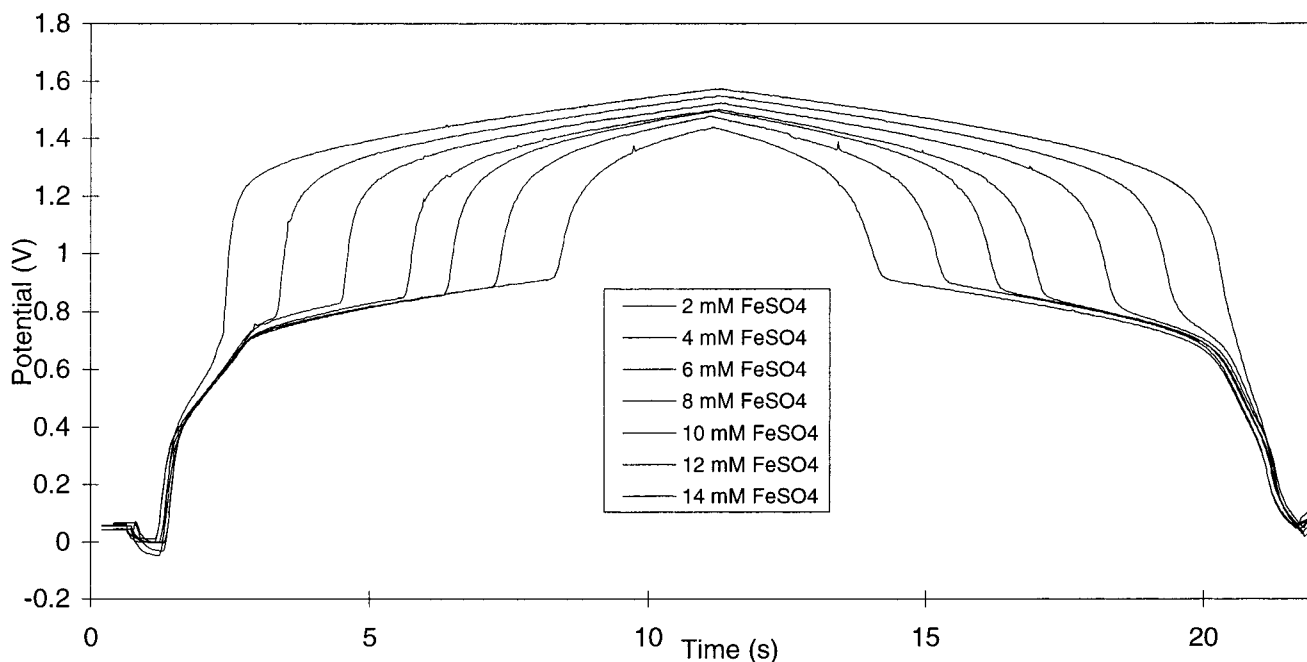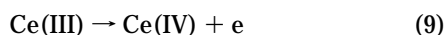(18) Lingane, J. J. *Electroanalytical Chemistry*; Interscience: New York, 1958; pp 139, 559–560.

Figure 4. Triangle-programmed coulometric titrations of Fe(II). The sample solutions with concentrations ranging from 2 to 14 mM (curves from top to bottom) were titrated using a current−time slope of 8 μA/s and a flow rate of 120 μL/min (peristaltic pump).

$$Ce(III) \rightarrow Ce(IV) + e \qquad (9)$$

The cerium(IV) produced diffuses rapidly from the electrode surface and oxidizes an equivalent amount of iron (II):

$$Fe(II) + Ce(IV) \rightarrow Fe(III) + Ce(III) \qquad (10)$$
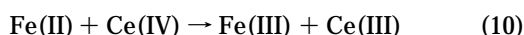
The overall reaction is finally given by

$$Fe(II) \rightarrow Fe(III) + e \qquad (11)$$
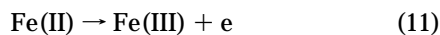
Figure 4 shows redox titrations for different sample concentrations, carried out in a 30 μm high channel. A cycle $2T$ of 20 s was chosen for the triangle current−time profile; this cycle could of course be shorter. The titration rate was found to be sufficiently high for sample concentrations ranging between 2 and 14 mM and for a current scan of 8 μA/s. The excellent symmetry of the curves in Figure 4 indicates that the titration conditions are extremely stable in the microchannel. Accordingly, a high reproducibility was achieved for all measurements, the standard deviation being always below 1%, except for equivalence times larger than 17 s (up to 4%). In comparison with earlier redox titrations carried out at a constant current in static sample solutions,[14] the present measurements showed a slightly better reproducibility. This may be due to the establishment of a well-defined stagnant layer at the indicator electrode.

Figure 5 exhibits the linear relationship between the equivalence time and the sample concentration for different current−time slopes. The total equivalence time is defined as the difference between the period of a cycle and the time span. Equation 8 was used to fit the data obtained for 8 μA/s. The reduction factor α was found to be 0.022, and the response time asymmetry t_r of the sensor was 0.09 s. All the other data were fitted on the basis of the same parameters, using the respective current−time slopes.
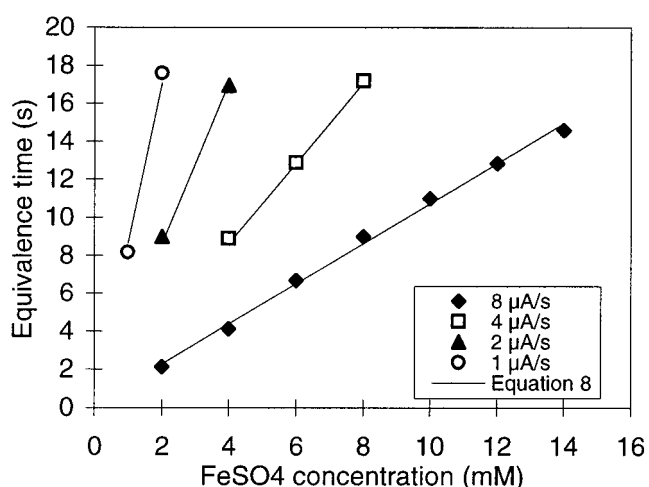

Figure 5. Linear relation between the Fe(II) concentration and the total equivalence time, $2T - \Delta t$, for different current−time slopes. Equation 8 was used to fit the experimental data ($\alpha = 0.022$ and $t_r = 0.09$ s).

The observed reduction factor α indicates that only 2% (about 400 nL for a half-period of 10 s) of the sample solution is titrated above the sensor at a flow rate of 120 μL/min. The average speed of the titrated sample, v_{sam}, is calculated from these results as 0.04 mm/s.

Influences of the Current−Time Slope and the Flow Rate. As expected, the current−time slope and the flow rate are decisive for the titration efficiency. A higher current−time slope is expected to increase the speed of the titration. Figure 6a shows the response curves obtained for the titration of an 8 mM Fe(II) sample at different current−time slopes. The variation of the current intensities allows the coverage of a wide concentration range. According to eq 8, the time span Δt is related to the reciprocal of the current−time parameter k. These predictions are confirmed by experimental results in Figure 6b. The same coefficients, α and t_r, were used as in Figure 5.

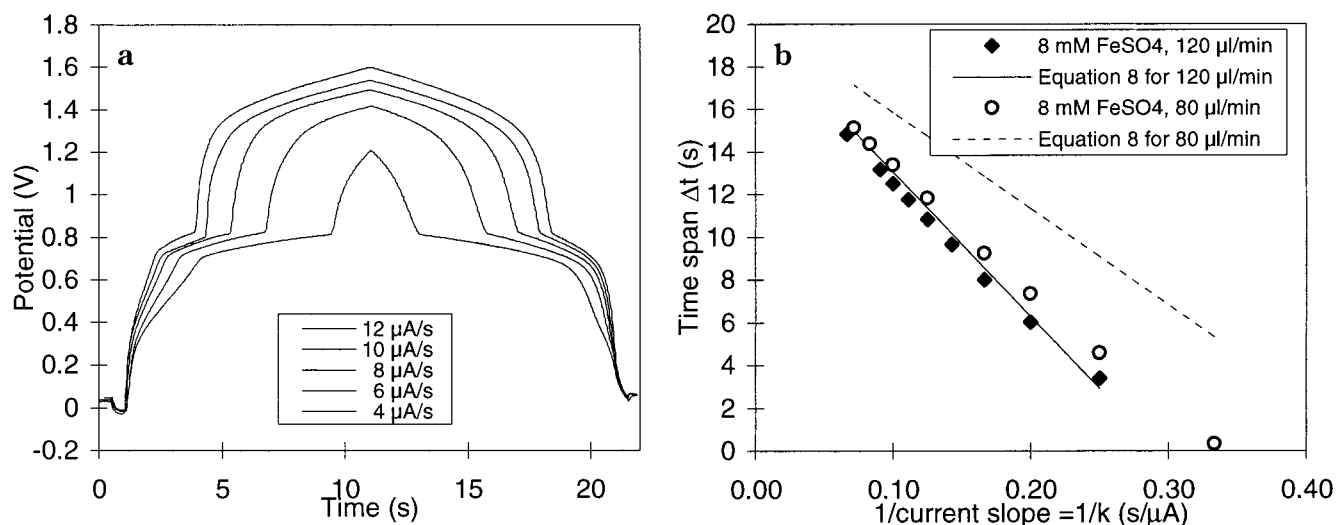**Figure 6.** (a) Triangle-programmed coulometric titrations of an 8 mM Fe(II) sample, using current−time slopes of 12−4 μA/s (curves from top to bottom). A flow rate of 100 μL/min was chosen (piston pump). (b) Relationship between the time spans Δt and the reciprocals of the applied current−time slopes. Theoretical curves were fit according to eq 8 using the same parameters α and t_r as in Figure 5.
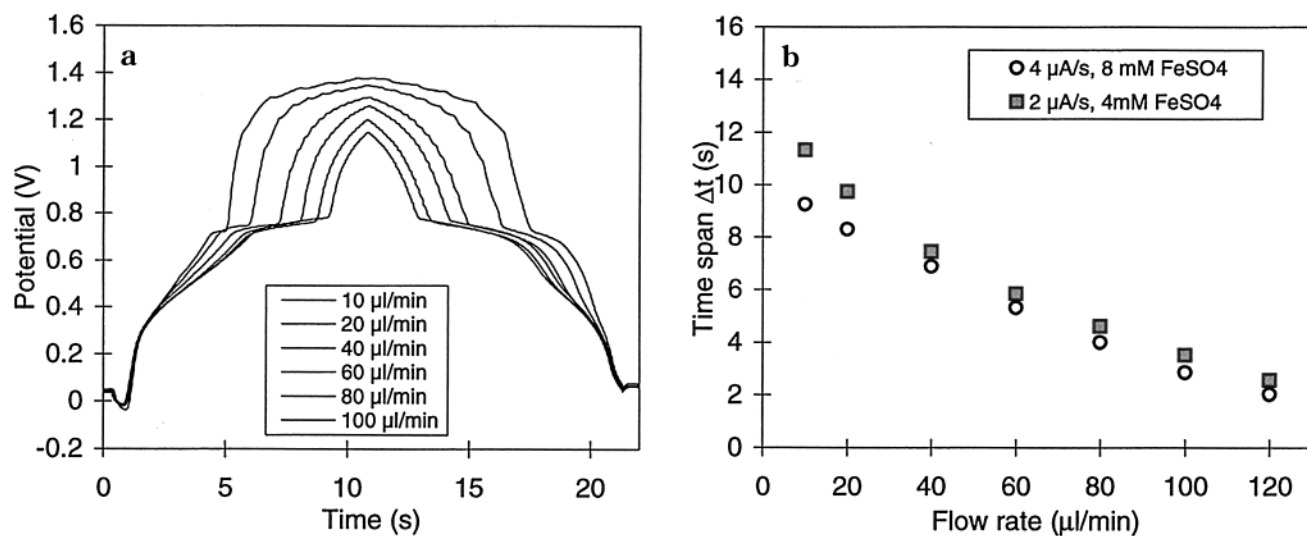

Figure 7. (a) Triangle-programmed coulometric titrations of a 4 mM Fe(II) sample at a current scan of 2 μA/s for different flow rates (10−100 μL/min, from top to bottom). (b) Relationship between the time spans Δt and the sample flow rates. Results from two different series of experiments are given.

Figure 6b also reveals that eq 8 fits well the experimental data obtained for a flow of 120 μL/min, but only qualitatively for 80 μL/min. This discrepancy can be explained by diffusion effects, which become more important at smaller flows. Apparently, the effective volume of titrated sample is larger at 80 μL/min than at 120 μL/min. Nevertheless, the similarity between the experimental results obtained at the two flow rates indicates that diffusion phenomena play a minor role. A more systematic study of the influence of the sample flow rate on the titration behavior is presented in Figure 7. Evidently, a 10-fold increase of the flow rate leads to a reduction of the time span between the titration end points by a factor of about 3−4.

The pulsation of the piston pump used can clearly be seen in Figure 7a, especially for low flow rates. At a flow rate of 10 μL/min, 200 nL is pumped every 1.2 s.

Acid−Base Titrations. Acid−base titrations were carried out using an indirect detection method. The pH determination was based on the measurement of the redox potential of quinhydrone.[19]

By saturation of the sample with quinhydrone, an equimolar mixture of quinone and hydroquinone was established. The basic equilibrium for the hydroquinone couple is

$$C_6H_4O_2 + 2H^+ + 2e \rightarrow C_6H_4(OH)_2 \tag{13}$$
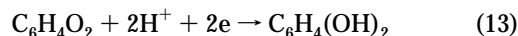
This is a reversible redox reaction in which hydrogen ions participate. Nitric acid solutions were titrated by this procedure in a 50 μm high microchannel. Figure 8 illustrates typical titration curves obtained for different sample concentrations. For this titration type, a linear relationship between the equivalence time and the sample concentration was found for almost 2 orders of magnitude (0.2−8 mM) when the appropriate current scan was used. In all measurements, a high reproducibility was achieved, the standard deviation being always below 1%, except for equivalence times larger than 14 s (standard deviation up to 10%).

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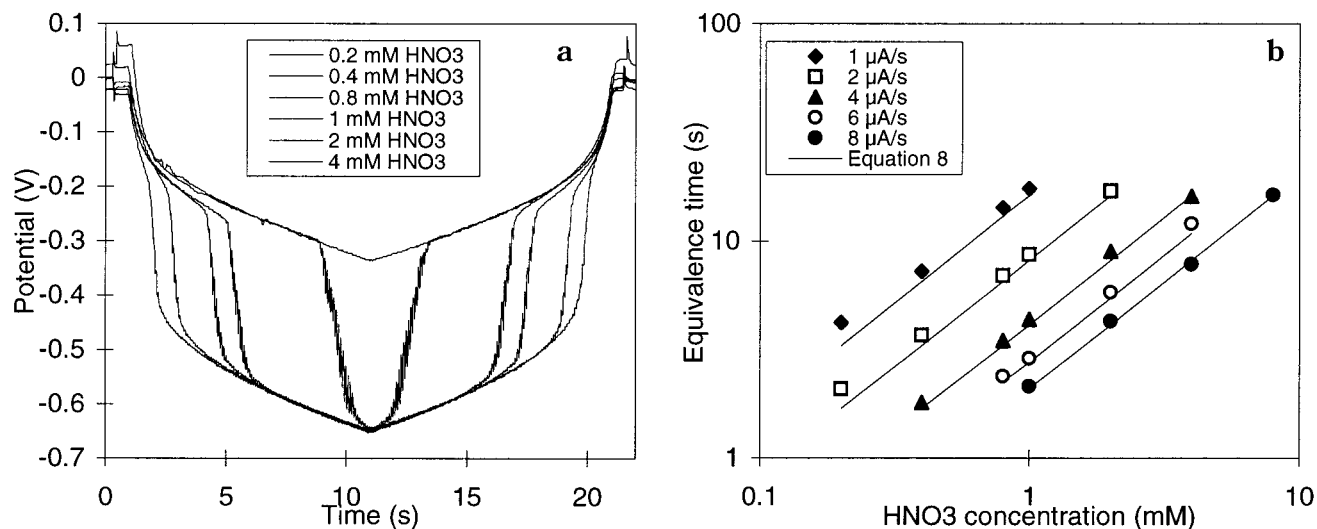
Figure 8. (a) Triangle-programmed coulometric titrations of nitric acid. The sample solutions with concentrations ranging from 0.2 to 4 mM (curves from bottom to top) were titrated using a current−time slope of 2 μA/s and a flow rate of 120 μL/min. (b) Linear relation between the Fe(II) concentration and the total equivalence time, $2T - \Delta t$, for different current−time slopes. Equation 8 was used to fit the experimental data ($\alpha = 0.041$ and $t_r = -0.045$ s). Slopes of the fits were 16.07, 8.03, 4.01, 2.67, and 2.00 1/mM for 1, 2, 4, 6, and 8 μA/s, respectively.

Equation 8 was used to fit the data obtained for 8 μA/s. In this case, the reduction factor was found to be 0.041 and the response time asymmetry t_r of the indicator electrode was −0.045 s. All other data were fitted on the basis of the same parameters, using the respective current−time slopes.

The difference observed between the reduction factors α for the titrations of Fe(II) ($\alpha = 0.022$) and the titrations of nitric acid ($\alpha = 0.041$) may be explained by the height differences of the microchannels used for the experiments, 30 μm for Fe(II) titrations and 50 μm for HNO$_3$ titrations, respectively. In both cases, the response time asymmetry is very small, which is the due to the proximity of the indicator electrode to the coulometric electrodes.

CONCLUSION

The present results show that the reported nanotitrator was successfully applied for triangle-programmed coulometric titrations. As compared with earlier studies by Pungor et al.[6,8] on a macroscopic device, the titration time could significantly be reduced in the present case. An excellent symmetry and a good reproducibility of the titration curves were achieved due to the small dimensions of the microfabricated device and the close

arrangement of the electrodes. It must be pointed out here that, since the symmetry of the response is excellent, a reduction of the measurement time by a factor of at least 2 can be envisaged, because only a half-cycle of the titration is necessary for the determination of the sample concentration. This modification would eliminate the need for the bidirectional current ramp.

Because the flowing solution sample is titrated only in a small zone near the electrodes, a calibration of the nanotitrator is required for quantitative analyses. The high precision found in the present studies documents that the titration volumes were well reproducible in all experiments.

It should also be noted that more than 1000 measurements were performed using the same device in the same configuration.