

AN ISFET-BASED MICROLITRE TITRATOR INTEGRATION OF A CHEMICAL SENSOR-ACTUATOR SYSTEM*

BART VAN DER SCHOOT and PIET BERGVELD

Department of Electrical Engineering, Twente University of Technology, P O Box 217, 7500 AE Enschede (The Netherlands)

(Received June 6, 1985, accepted September 13, 1985)

Abstract

This paper describes the integration of pH-sensitive ISFETs with a coulometric pH-actuator system. The coulometric analyser is able to perform acid-base titrations in microlitre samples at high speed. Combination of chemical sensors with a corresponding actuator eliminates the need for frequent calibration and thus may increase the applicability of solid-state chemical transducers. A number of possible measuring methods are described.

Introduction

Potentiometric pH sensors such as the ISFET give information about one aspect of a solution, that is, its actual proton activity. In cases where the pH is determined by more than one protolyte, this knowledge is only of limited value. A possible method of obtaining more information about the buffering components in a solution is titration, which is of course a well-known technique in analytical chemistry. Furthermore, since titration is a dynamic method, calibration of the sensors is usually not required because one is only interested in relative changes in pH. With the application of ISFETs as solid-state chemical sensors, coulometry seems an appropriate technique for performing acid-base titrations. Therefore we integrated pH-sensitive ISFETs with noble-metal electrodes, evaporated onto the same substrate, into a chemical sensor-actuator system.

Another interesting aspect of this integrated system is its inherently small size. Integration of analytical chemical instrumentation into small units offers great advantages, not only with respect to sample size and reagent use, but also to the high speed at which analysis can be performed. In a recent paper Ruzicka and Hansen [1] describe what may be called a hybrid system for flow-injection analysis. They integrated the various components in a transparent PVC block a few cubic centimetres in size.

*Based on a paper presented at the Third International Conference on Solid-State Sensors and Actuators (Transducers '85), Philadelphia, PA, U S A, June 11 - 14, 1985

If miniaturization is to be taken further, other techniques are required as parts of the system become too fragile to be produced by normal fine-mechanical machining. Photolithographic etching techniques as used in the fabrication of microelectronics offer new possibilities. An excellent example of the use of this technology is the design of a gas chromatograph on a silicon wafer at Stanford University [2]. Integrated circuit technology not only enables the very accurate fabrication of small three-dimensional structures (micromachining) to be carried out but also allows the integration of solid-state sensors.

Design of the microlitre titrator

We describe here a coulometric analyser for acid–base titration realized on a silicon chip. Figure 1 shows the basic elements of the planar structure that forms the active part of the device. An n-channel ISFET is fabricated in a p-type silicon wafer using standard MOS technology. Thin-film metal electrodes are deposited on the wafer to operate as electrodes for coulometric generation of protons or hydroxyl ions and as the basis for a reference electrode. The generating electrode has a window that fits closely around the pH-sensitive gate area of the ISFET, so that the solution is in contact with the sensor. On top of this structure a silicon cover is placed at a distance of 30 - 100 μm in order to perform the titration in a very thin layer of the sample solution.

In our design the size of the generating electrodes is much larger than the dimensions perpendicular to the surface of the chip. Therefore, the generation of ions may be considered as practically homogeneous over a large area of the electrode in the middle of which these ions are measured by the ISFET. Diffusion perpendicular to the chip takes place in an order of

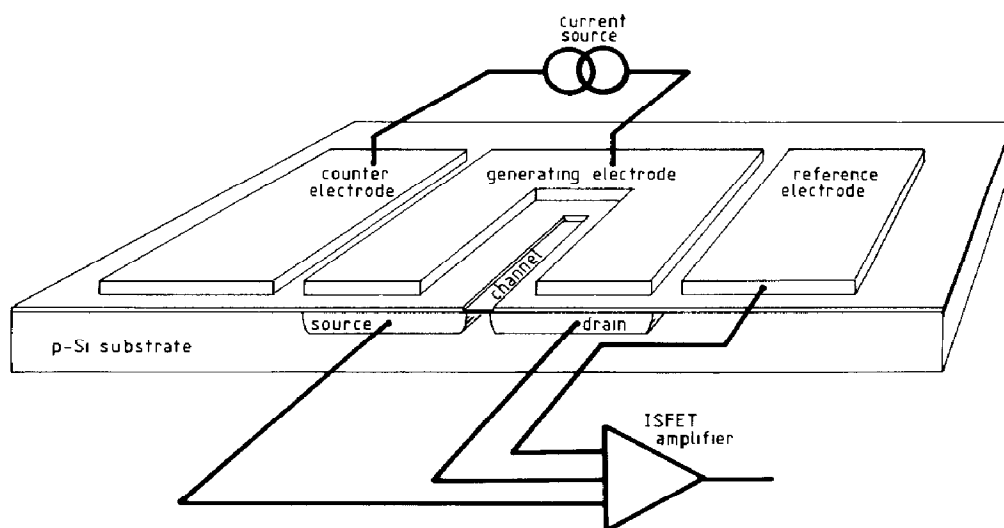


Fig 1 Basic elements of an integrated chemical sensor–actuator system for titration

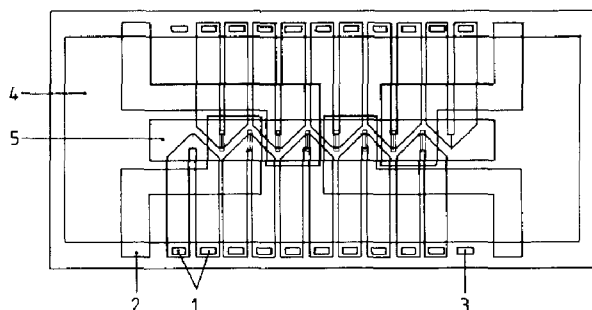


Fig 2 Coulometric analyser chip (1) Source and drain connections of the first ISFET, (2) one of four gold electrodes surrounding two ISFET gates, (3) substrate connection, (4) polyimide rim on which a silicon cover is placed and (5) sample channel

seconds, whereas lateral diffusion from cathode to anode will take up to minutes. Because of this, it is possible to perform acid–base titrations in the perpendicular direction very fast and there is no need to separate the anodic and cathodic reaction products by, for instance, a diaphragm. As the ISFET has a response time only of the order of milliseconds [3], this makes it an excellent detector for the registration of the titration curves.

In order to study the behaviour of a system as proposed above in practice, we designed the circuit shown in Fig 2. The silicon chip, which measures $9.5 \times 19 \text{ mm}^2$, contains 10 ISFETs arranged in a linear array. The pH-sensitive gate areas are spaced one millimetre apart. On the chip, four gold electrodes are deposited for coulometric generation. One of these electrodes can also be used as a pseudoreference electrode, as will be described later. The area of the sample channel is limited by a rim of polyimide. The volume of this channel is further determined by a cavity etched in a silicon cover that is sealed on top of the analyser chip. The sample solution is introduced through a hole in this cover and drained through a second hole by means of a peristaltic pump. A cross-section of the device can be seen in Fig 3. The sample channel is 1.5 mm wide and 12 mm long. The cover is

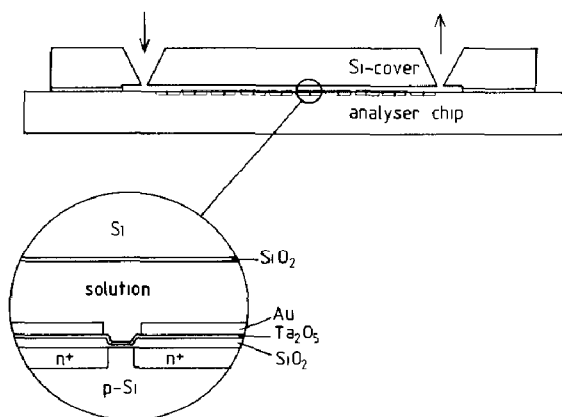
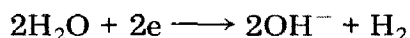


Fig 3 Cross-section of the coulometric analyser. The solution layer is between 30 and 100 μm thick.

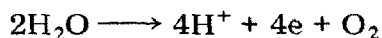
at a distance of 30 - 100 μm from the analyser chip and thus the total volume of the sample channel is 0.54 - 1.8 μl . The area of a generating electrode is 3 mm^2 and with a cover 30 μm away, the volume in which titration can be performed is only 90 nl. As can be seen in Fig. 2, this titration volume still contains two ISFETs and the sample channel is three times as wide as the ISFET's gate area. Therefore the titration volume could easily be further reduced to as little as 10 nl without difficulty.

Basic theory

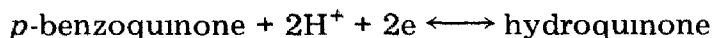
Titration of acids using electrogenerated hydroxyl ions can be carried out with high accuracy. Hydroxyl ions can be produced simply by the reduction of water at a noble-metal cathode:



At the same time the anodic reaction at the counter electrode yields protons according to:



The electrolysis of water occurs at relatively high electrode potentials and this places some restrictions on the composition of the solution in order to prevent undesired side reactions. Furthermore, at high current densities, hydrogen and oxygen gas bubbles may evolve. When an additional redox-couple that can be oxidized and reduced at lower electrode potentials is added to produce the desired pH changes, the undesired reactions can be suppressed. Quinhydrone, which is a 1:1 mixture of *p*-benzoquinone and hydroquinone, is such a redox-couple according to:



A disadvantage of the use of hydroquinone is that it is a weak acid ($\text{p}K_{\text{a}} = 10.47$) itself and thus affects the titration curves at high pH values.

The concentration of reagent (C) that is added to the solution by coulometric generation can be calculated according to:

$$C = \frac{It}{FA d} \quad (1)$$

Here I is the generating current, t the pulse length, F is Faraday's constant (96 487 mol/equiv), A the area of the generating electrode and d the thickness of the sample layer. As the volume to be titrated is relatively small with respect to the electrode area, the generating current density can be kept at a moderate value and still give a high titration speed. For example, with a generating current density of 1 mA/cm^2 and a sample layer thickness of 30 μm , the titration speed (C/t from eqn. (1)) is 3.3 milliequivalents/l/s.

Despite these moderate current values, the electrical resistance of the solution imposes an important restriction because of the small volume. This

resistance should be kept as low as possible to ensure a homogenous generation of ions across the electrode and therefore a high concentration of background electrolyte is required. However, the generating current between cathode and anode will still produce a voltage drop across the solution, which is measured by the ISFET with respect to the reference electrode. Thus an electrical artefact can be expected in the output registrations, but since it is only dependent in a linear way on the generating current, this artefact does not influence the final results.

Measurements

The analyser chip contains four gold electrodes. Coulometric titration in a stationary solution is performed by using one of these as the working electrode to produce hydroxyl ions. In order to obtain a homogeneously distributed generation of ions across this electrode, two counter electrodes are used, one on each side of the working electrode.

Because 10 ISFETs share a common reference electrode, source and drain follower circuits [4] are used to measure their output signal. The reference electrode may be an SCE connected to the coulometric cell by means of a tube. However, since several ISFETs are available on the chip, this enables another method of measurement to be used. While performing coulometric titrations in a stationary solution, only local pH changes are created. This means that in some places in the cell, the pH of the solution remains unaffected during the experiment. A normally pH-sensitive ISFET located at such a point can now be used as a reference. A differential measurement is performed using one ISFET at the site of the titration and one located at a neutral point of the cell. Both ISFETs share a common 'reference' electrode, which can now be a simple metal electrode and is, in our case, the fourth gold electrode not used for coulometric purposes. Any instabilities in the potential of this electrode are eliminated because they are measured by both ISFETs in the same way. Figure 4 shows a schematic drawing of the experimental set-up.

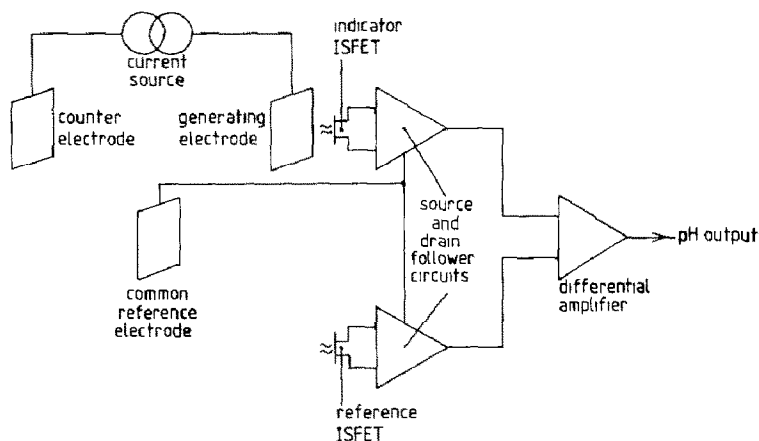


Fig 4 Schematic drawing of the set-up for static and dynamic measurements

The current source used for coulometric generation must be kept floating with respect to the pH-measuring circuit. It therefore operates on a battery supply and is controlled by means of optocouplers activated by an Apple IIe microcomputer. It actually consists of four parallel current sources of 2, 4, 8 and 16 μA respectively that can be switched on and off under digital control in connection with a timer circuit. Thus it is possible to generate current pulses between 0 and 30 μA in 2 μA steps and of any desired length.

Experimental results and discussion

Static measurements

Figure 5 shows the output measured with an ISFET as a result of the applied current pulses. The electrical artefact caused by the resistance of the solution is obvious. It shows as a stepwise change in the output voltage at the beginning and end of the input current pulse. After a coulometric pulse it takes some seconds before the induced pH change is homogeneously distributed across the volume above the generation electrode. The pH value that is reached after an input pulse of 56 μC is approximately pH 8, which is close to the equivalence point of the titrated acid. In the Figure it can be seen that the pH change reaches a maximum value and then drops back. This effect is believed to be caused by lateral diffusion of protons generated at the counter electrodes. The solution is, of course, most sensitive to this effect at pH values around the equivalence point when the buffer capacity is at its minimum. At pH values below and above this point, the buffer capacity is higher and the induced pH change is more stable.

When the pH change is measured some seconds after the application of the generation pulse, it is not influenced by transport effects, because at the time of measurement it is homogeneously distributed. After the experiment, fresh solution is introduced into the cell and the measurement can be repeated. Thus, registration of each discrete point of the titration curve will take a number of seconds. We shall call this method, which must be performed in a stationary solution, static measurement.

When the experiment is repeated for a number of points, the titration curve can be constructed as shown in Fig. 6. The input charge needed to

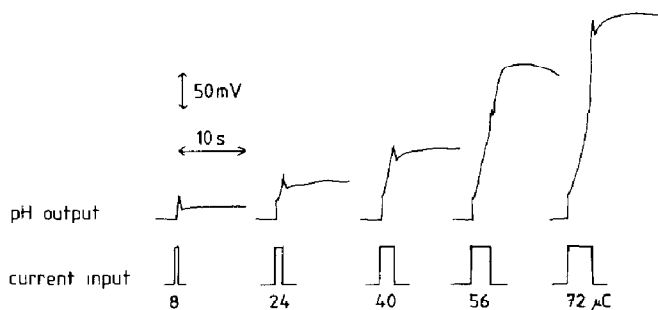


Fig. 5 Response of the system after current pulses of increasing magnitude in a 0.005 M acetic acid solution

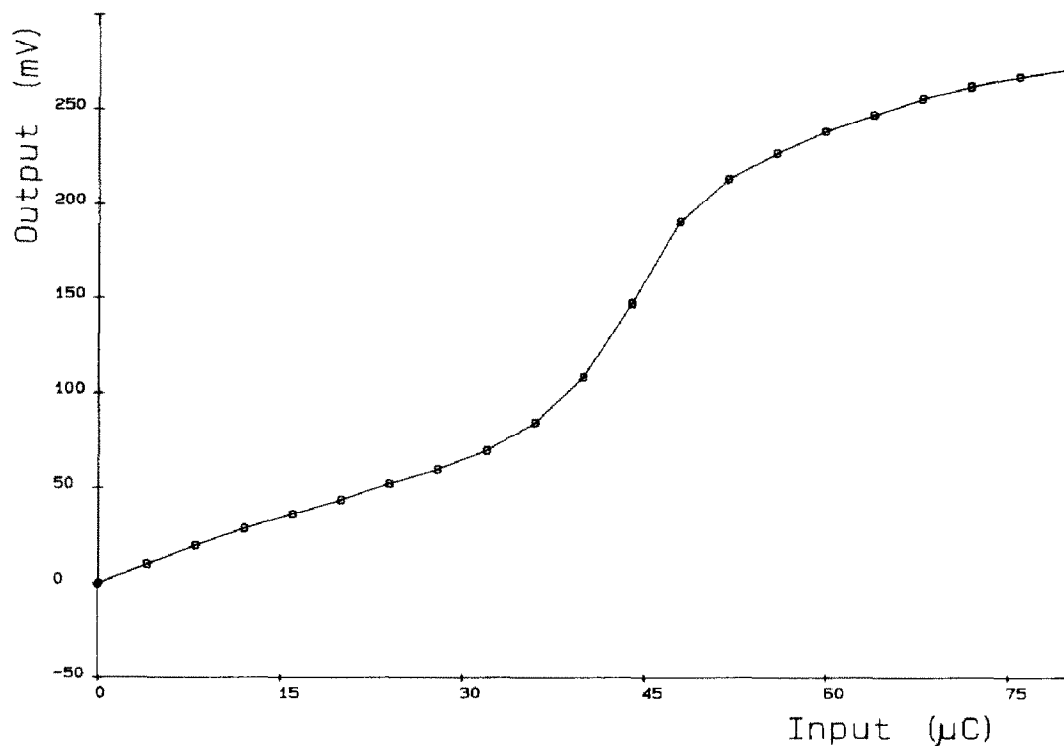


Fig 6 Construction of a titration curve from static measurements in 0.005 M acetic acid

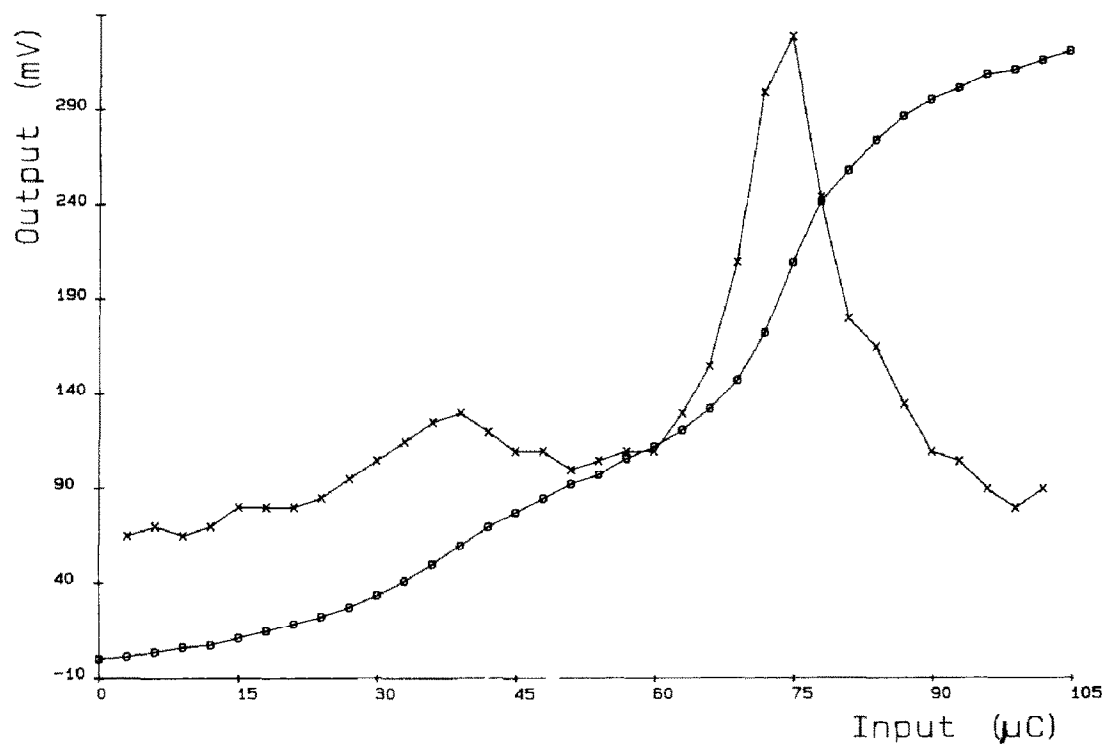


Fig 7 Titration curve (o) and first derivative (x) of a mixture of 0.005 M HCl and 0.005 M acetic acid

reach the equivalence point is in good agreement with the theoretical value according to eqn (1) The usefulness of the method is also shown in Fig 7, where a mixture of two acids is titrated Both hydrochloric and acetic acid are present in a concentration of 5 millimoles/l and the first derivative of the titration curve shows two maxima at the appropriate places

Dynamic measurements

Although the accuracy of the static measurements as described above is good, the method is rather time consuming Therefore it is interesting to

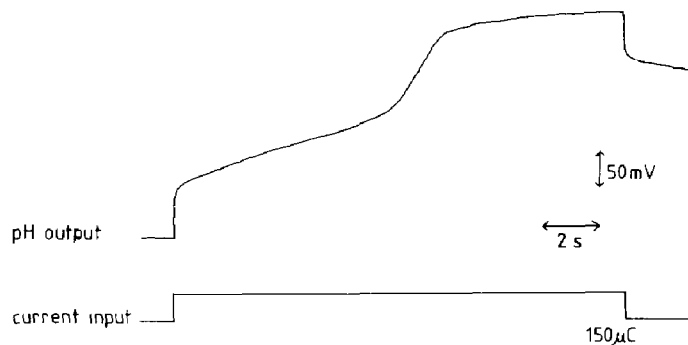


Fig 8 Response of the system during a current pulse in a 0.005 M acetic acid solution

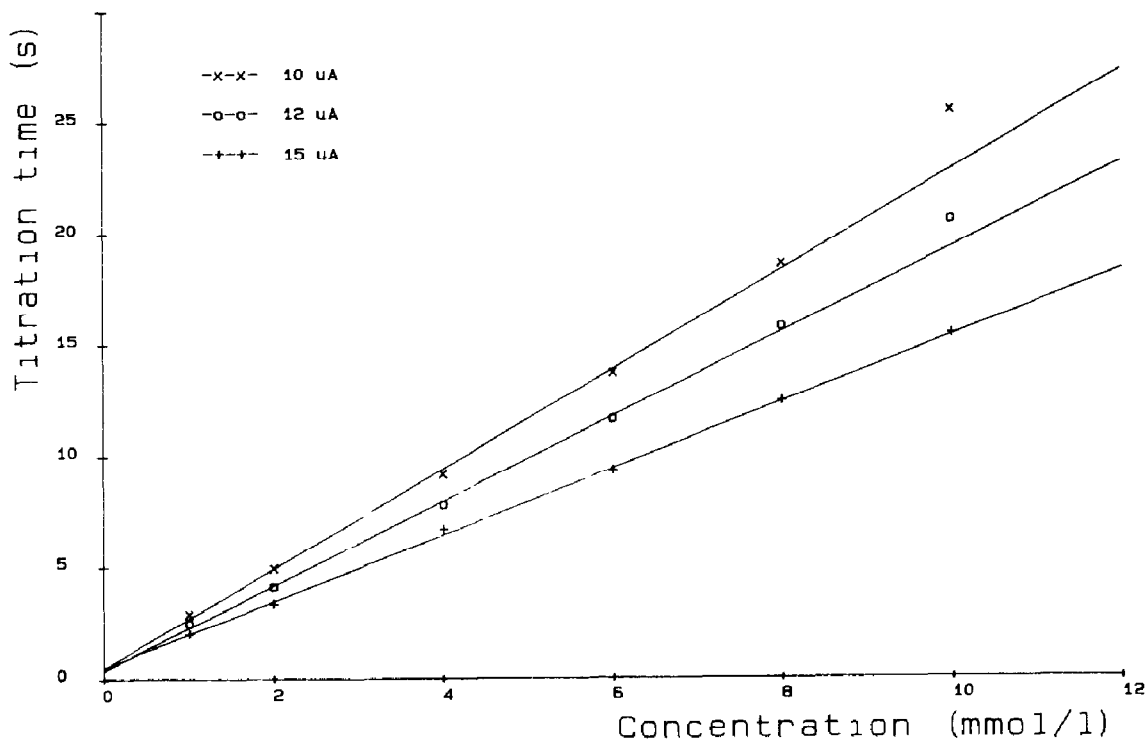


Fig 9 Titration times from dynamic measurements as a function of acid concentration at various current values

examine the behaviour of the system during a generation pulse that is long enough to perform a complete titration. We shall call the measurements performed in this way, dynamic measurements. The resulting pH response will, of course, be influenced by diffusion of ions from the generating electrode to the ISFET and across the volume above the electrode. Despite these diffusion effects, the recorded pH output shows a fair approximation to the conventionally measured titration curve, as can be seen in Fig. 8.

The time needed to reach the equivalence point will, of course, be proportional to the concentration of acid. Figure 9 shows the titration time as a function of acid concentration for three current values. The relationship is linear over a wide range, but at times exceeding approximately 20 seconds a deviation occurs. We assume that lengthening of titration times is caused by lateral diffusion, resulting in the mixing of cathodic and anodic products. Of course this behaviour is a function of the geometry of the device, such as the electrode size and the distance between cathode and anode.

Titration time is inversely proportional to the generating current, as is shown in Fig. 10 for two different acid concentrations.

Examining Figs. 9 and 10, it can be seen that the curves cross the time axis at approximately 0.5 s. This delay time, represented by t_d , may be caused by diffusion from the generating electrode to the ISFET's gate area because there is inevitably a small distance (approximately 20 μm) between

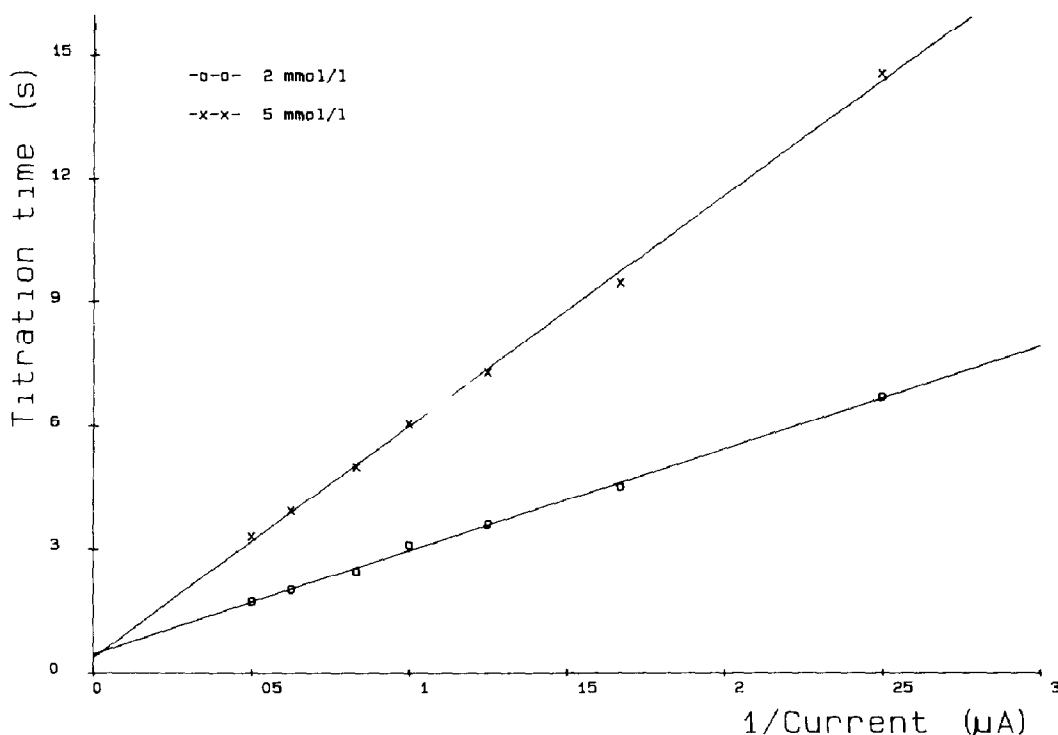


Fig. 10 Titration times from dynamic measurements for two acid concentrations plotted against the inverse generating current

the two. At this point it should be noted that the total amount of charge needed to reach the equivalence point in this case is larger than the theoretical value that can be found for a homogeneous titration according to eqn (1). Based on the linear relationships in Figs 9 and 10, we therefore introduce a factor α . If t_e is the titration time needed to reach the equivalence point, the effective titration time is now given by $(t_e - t_d)/\alpha$. The relationship between concentration and titration time for this type of measurement can now be found from a slight modification of eqn (1)

$$C = \frac{I}{FA d} \times \frac{(t_e - t_d)}{\alpha} \quad (2)$$

Both parameters t_d and α depend on the geometry of the cell and thus need to be determined only once. For a sample layer thickness of $30 \mu\text{m}$, α was found to be 1.25 and t_d was 0.5 s. The maximum concentration of acid that can be titrated accurately can now be estimated using eqn (2). When titration is limited to 15 seconds, the thickness of the sample layer is $30 \mu\text{m}$ and the maximum generating current is 1 mA/cm^2 , the largest possible concentration is about $4 \times 10^{-2} \text{ mole/l}$.

Continuous measurements

Flow injection titrations [5 - 6] usually require the formation of a concentration gradient of the sample in a reagent solution so that the reaction can be monitored as this gradient passes a detector. Coulometric generation enables the concentration of reagent to be varied continuously. This means that either smaller samples can be measured when using the methods described above or that a continuous titration can be performed.

The analyser chip provides the possibility of generating hydroxyl ions and measuring pH at practically the same place. As a number of ISFETs are integrated along the sample channel, this enables the continuous measurement of concentration gradients as a function of position in the solution, whereas with one sensor the gradient can only be measured as a function of time. A continuous measurement can be performed by using all four gold electrodes as cathodes to produce hydroxyl ions, the counter electrode in this case being a platinum wire placed in the tube outside the cell.

When a solution is flowing through the sample channel, it becomes gradually titrated by the generated ions. Consecutive points of the titration curve can be read with the ISFET array. Of course the titration speed is dependent on the flow rate of the solution through the analyser, as can be seen in Fig 11. The effective charge input into the sample solution increases as it passes the generating electrodes. Generation starts 0.5 mm before ISFET no 2 and stops 0.5 mm after ISFET no 9. From the drop in pH at ISFET no 10, it can be seen that only part of the solution is titrated. Calculations show that at all flow rates only about 25% of the solution takes part in the reaction. The continuous measurements shown here are only preliminary results, a more thorough investigation will soon be performed.

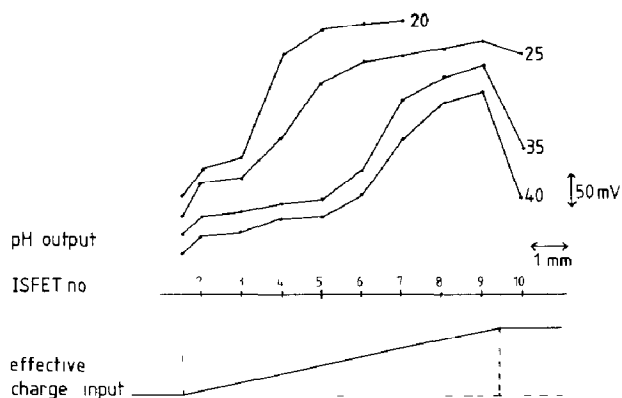


Fig 11 Continuous titrations in a flowing solution. Titration depends on the flow rate, which is indicated on the curves in microlitres per minute. The average fluid speed is 2 - 4 mm/s.

Titration depends on the flow rate of the solution, which must therefore be known accurately. Our device, however, can also be used successfully as a flow meter. When a stepwise change in pH is injected at the first generation electrode, it will pass the consecutive ISFETs as the solution flows through the cell. The flow rate can now be easily calculated. It should be noted that the flow is laminar in all cases, the Reynolds number for the highest flow rate in Fig 11 being about 0.8.

Conclusions

In this paper we have presented a number of applications of an integrated chemical sensor-actuator system. The small dimensions allow the system to perform chemical analysis within a few seconds and its operation is quite straightforward. Of course the potential uses of such a system are by no means limited to the examples given here.

Coulometry is an absolute method of measurement, therefore calibration of the chemical sensitivity of the sensors is not necessary. The parameters of importance are the dimensions of the cell, which are constant and determined by the fabrication process, and, in the case of continuous measurements, the flow rate of the solution, which can easily be measured with the same device.

Because the need for frequent calibration is eliminated by the integration of a chemical sensor and a corresponding actuator, such a system can be used unattended for longer periods of time. This fact is, of course, of great importance when measurements are performed in automated systems. The application of solid-state chemical transducers may thus become closer to reality in fields such as implanted biomedical devices, environmental control and process industry.

Acknowledgements

The authors thank Dr M Bos and Professor Dr W E van der Linden from the Department of Chemical Engineering for their interest and useful suggestions

References

- 1 J Ruzicka and E H Hansen, Integrated microconduits for flow injection analysis, *Anal Chim Acta*, 161 (1984) 1 - 25
- 2 S C Terry, J H Jerman and J B Angel, A gas chromatographic air analyzer fabricated on a silicon wafer, *IEEE Trans Electron Devices*, ED-26 (1979) 1880 - 1886
- 3 B H van der Schoot, P Bergveld, M Bos and L J Bousse, The ISFET in analytical chemistry, *Sensors and Actuators*, 4 (1983) 267 - 273
- 4 P Bergveld, The operation of an ISFET as an electronic device, *Sensors and Actuators*, 1 (1981) 17 - 29
- 5 J Ruzicka, E H Hansen and H Mosbaek, Flow injection analysis, Part IX, A new approach to continuous flow titrations, *Anal Chim Acta*, 92 (1977) 235
- 6 A U Ramsing, J Ruzicka and E H Hansen, The principles and theory of high speed titration by flow injection analysis, *Anal Chim Acta*, 129 (1981) 1