

Integrated Blood Gas Sensor for pO_2 , pCO_2 and pH based on Silicon Technology

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by

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This book is dedicated

to my wife Hélène,

to our son Linard,

and to my parents

Elisabeth and Caspar Arquint-Dubas,

for their continued support and encouragement.

IMPRIMATUR POUR LA THÈSE

Integrated Blood Gas Sensor for pO_2 , pCO_2
and pH based on Silicon Technology

de Monsieur Philippe Arquint

UNIVERSITÉ DE NEUCHÂTEL

FACULTÉ DES SCIENCES

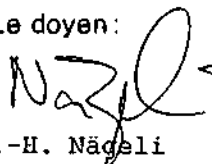
La Faculté des sciences de l'Université de Neuchâtel
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Le doyen:



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List of notations

Symbol	Description	Unit
a	activity	-
A	area	m ²
B	sensitivity parameter for ISFET	-
c	concentration	mol m ⁻³
C	capacitance	F
d	thickness of membrane or electrolyte layer	μm
D	diffusion coefficients	cm ² s ⁻¹
E	potential	V
f	activity coefficient	-
F	Faraday constant	9.6485·10 ⁴ C mol ⁻¹
H	Henry's law constant	mol mmHg ⁻¹ m ⁻³
I	current	A
j	summator	-
J	flux	mol m ⁻² s ⁻¹
k	Boltzmann constant	1.3807·10 ⁻²³ J K ⁻¹
k	reaction rate constant (first order)	s ⁻¹
K	equilibrium constant	-
K	distribution coefficient	-
l	distance between to points	m
L	ISFET / MOSFET channel length	μm
n	number of electrons involved in the O ₂ red.	-
p	partial pressure, i.e. pO ₂ : O ₂ partial pressure	mmHg
p	power, i.e. pK = - log ₁₀ K ⁺	-
q	unit charge	1.602·10 ⁻¹⁹ C
μ	mobility of ions / electron	cm s ⁻¹ V ⁻¹ mol ⁻¹
μ*	viscosity	cm ² s ⁻¹
r	radius	m
R	gas constant	8.3144 J K ⁻¹ mol ⁻¹
S	sensitivity	i.e. mV pH ⁻¹
t	time	s
t ₉₅ , t ₉₀	time for a 95 % , 90 % response, respectively	s, min
T	absolute temperature	K
τ	time constant	s
v	flow velocity	μl s ⁻¹

Symbol	Description	Unit
V	voltage	V
W	ISFET / MOSFET channel width	μm
x	1-dimensional coordinate	-
z	charge of an ion	C

Abbreviations:

AA	acrylamide
BHF	buffered hydrofluoric acid
BIS	N,N'-methylene-bisacrylamide
CE	counter electrode
CVD	chemical vapor deposition
DMAP	2,2-dimethoxy-2-phenyl-acetophenone
GPM	gas permeable membrane
HEMA	2-hydroxyethyl methacrylate
HG	hydrogel
ISFET	ion selective field effect transistor
KOH	potassium hydroxide (40 wt. %, 60 °C)
LPCVD	low pressure chemical vapor deposition
MOSFET	metal oxide semiconductor field effect transistor
NHE	normal hydrogen electrode
pAA	polyacrylamide
pHEMA	polymerized HEMA
PR+	positive photoresist
PR-	negative photoresist
RE	reference electrode
SCE	saturated calomel electrode
SE	shield electrode
TEGDMA	tetraethyleneglycol dimethacrylate
vs.	versus
WE	working electrode

1 Summary

The subject of the research described in this thesis is the design, the fabrication and the characterization of a miniaturized combined pO_2 , pCO_2 and pH sensor. This device is intended for the extracorporeal determination of the so-called "blood gas" values, which are the partial pressures of dissolved O_2 and CO_2 , and the pH value of arterial blood. Classical electrochemical sensing principles are used, realized in a planar and miniaturized form on a single silicon chip. The devices are fabricated by means of integrated circuit technology suitable for mass production. The pH and the Severinghaus pCO_2 sensor are based on the ion selective field effect transistor (ISFET), whereas the pO_2 is detected with a Clark-type sensor in a three electrodes configuration. Polymeric layers are employed for the inner electrolyte and the gas permeable membrane of the pO_2 and pCO_2 sensor. A special technique has been developed, which allows to pattern these polymeric membranes directly on wafer level by photopolymerization.

Two generations of the combined blood gas sensors have been realized and characterized in aqueous solutions and in transfusion blood. In particular the second generation of the sensor chip exhibits good operational performance, regarding linearity, response time, stability and lifetime. The results are promising for a future application of the device in a handheld blood gas analyzer or in a small bedside instrument. Tests in whole human blood are now being performed under clinical conditions.

2 Introduction

2.1 Practical relevance of blood gas analysis

2.1.1 Clinical importance

In modern health care, clinicians are increasingly dependent on the reliable and rapid measurement of chemical variables for accurate diagnostic and therapeutic decisions.

The measurement of the acidity (pH) and the partial pressure of dissolved oxygen (pO_2) and dissolved carbon dioxide (pCO_2) in arterial blood is important for the determination of the respiratory and metabolic condition of a patient, in order to ensure an adequate oxygenation and ventilation. It improves the ability to quantify and manage disruptions in the delivery of oxygen to the brain and the tissues. This can occur during surgery, in coronary care, in adult and neonatal intensive care, and in the fetus during labour and delivery. Blood gas analysis is established in the routine management of the critically ill, thus an intensive care unit without access to blood gas measurement seems impossible today. If patients are supported with mechanical ventilation, lack of this facility may prove to be disastrous. Furthermore, blood gas measurements are particularly important in preterm infants who are vulnerable to respiratory problems.

Blood gas analysis is one of the most frequent hospital diagnostic procedures. In 1987, in the USA at least 25 million arterial blood samples have been taken from patients for pO_2 , pCO_2 and pH determination [2.1].

2.1.2 Physiological role of O_2 , CO_2 and pH

The primary respiratory functions of blood are to transport oxygen from the lungs to the body tissues, and carbon dioxide from the tissues to the lungs. During the respiration process oxygen is absorbed and consumed, while CO_2 is produced and released by the body. The partial pressure of dissolved oxygen in arterial blood (pO_2) directly reflects the ability of the lungs to transport oxygen from inspired air into the bloodstream, in order to make oxygen available to the tissues. The partial pressure of dissolved carbon dioxide in arterial blood (pCO_2) reflects the ability of the lungs to eliminate this metabolic waste product. An change in the pCO_2 level not only indicates a possible respiratory problem, but also causes the pH of the blood to shift.

The pH value of blood is an indication for the status of acid production and elimination of the body. Hydrogen ions (H^+) are constantly released by acids through normal metabolism. An excess of H^+ is buffered by bicarbonate in the plasma. The kidneys excrete the appropriate amounts of acids and bases to keep the pH constant. The respiratory system also influences the pH value through the CO_2 content of blood by means of changing the rate of ventilation. Accordingly, disturbances of the acid-base balance, indicated by a change in the pH and/or pCO_2 level, can be caused by metabolic, respiratory, and/or renal problems. If both the pH and the pCO_2 are measured, the source for the acid-base disturbance can be identified.

There is a very fine balance between the pO_2 , pCO_2 and pH values of blood which is controlled by a complex biochemical and cardiopulmonary feedback system. It is a remarkable fact of nature that the pO_2 , pCO_2 and pH remain within a narrow, well defined bandwidth, which is shown in Tab. 2.1.

	pO_2 [mmHg]	pCO_2 [mmHg]	pH
normal	80 - 110	35 - 45	7.35 - 7.45
mild deviation	60 - 70	45 - 50	7.3 - 7.35
	130 - 140	27 - 35	7.45 - 7.5
moderate deviation	50 - 60	50 - 60	7.2 - 7.3
	140 - 150	20 - 27	7.5 - 7.6
severe deviation	< 50	> 60	< 7.2
	> 150	< 20	> 7.6

Tab. 2.1 Physiological ranges of arterial pO_2 , pCO_2 and pH values
(adapted from [2.2][2.3])

Oxygen is carried in blood in two forms. The major part (97 %) of the total content of oxygen is carried chemically combined with hemoglobin (Hb) in the red blood cells to form oxyhemoglobin (HbO_2). Only a small part (3 %) is oxygen physically dissolved in the plasma. It is essential that the blood has the capacity to carry a sufficient amount of bound oxygen and that the oxygen can dissociate from the hemoglobin in the tissues where the oxygen is needed. The amount of oxygen chemically bound to Hb depends directly on the pO_2 , but is also influenced by other parameters such as temperature, pCO_2 and pH. The degree to which oxygen is combined with Hb is expressed as blood oxygen saturation (sO_2), which is defined as the ratio of HbO_2 concentration to the total Hb concentration.

As contrasted with other necessities, such as food, water or vitamins, oxygen cannot be stored in the body over a long period. Therefore, it is essential to detect changes in the oxygen housekeeping of a patient. Lack of oxygen for only a few minutes causes unconsciousness, and brain damage or death may follow. If, on the other hand, the arterial pO_2 goes too high as a result of a

therapeutic intervention, damage to the eyes, which can result in blindness, can occur. This is in particular true for newborn infants. For adults, if an excess of oxygen is applied for over than 48 hours, oxygen can seriously damage the tissues of the lungs [24].

The total amount of CO₂ in the blood is partly transported in the form of bicarbonate ions (HCO₃⁻) (70 %), or bound to hemoglobin to form carbamino CO₂ (20 %). Thus there remains only a small amount in the form of physically dissolved carbon dioxide (10 %) [2.5]. The amount of carbonic acid (H₂CO₃) and carbonate (CO₃²⁻), which are also formed, can be neglected.

Using the Henderson-Hasselbalch equation, which has been become well known after works of Henderson in 1908 and Hasselbalch in 1916, it is possible to calculate the relationship between $p\text{CO}_2$, pH and the bicarbonate concentration $[\text{HCO}_3^-]$:

$$\text{pH} = \text{pK}' + \log_{10} \frac{[\text{HCO}_3^-]}{S \text{ pCO}_2}$$

with

K'	first apparent dissociation constant of dissolved CO ₂ in blood	
pK'	6.098 in whole blood	(at 37 °C)
S	solubility coefficient of CO ₂ in blood	
S =	0.0303 $\frac{\text{mmol/l}}{\text{mmHg}}$	(at 37 °C)

This equation is widely used to compute either the blood pH, the $p\text{CO}_2$ or the HCO_3^- level from the two other measured parameters. For the clinical use, special graphs (nomograms) have been developed to solve the Henderson-Hasselbalch equation in a graphical way (introduced by Ole Siggaard-Andersen) [2.5][2.6].

2.1.3 Accuracy requirements for blood gas analysis

Few measurements performed in clinical analysis have accuracy requirements as stringent as those for the determination of blood pH, pO_2 and pCO_2 . Critical decisions may change if there are differences as small as 10 % in these variables. The required accuracy is [2.7]:

pO_2 :	± 2.0 mmHg desirable	± 3.0 mmHg acceptable
pCO_2 :	± 2.0 mmHg	
pH:	± 0.03 pH	

Gambino [2.8] compared the performance of different blood gas instruments in order to determine whether they meet these requirements or not. The tests were performed on the same model of instrument, but in different laboratories, using standard blood gas control solutions. The maximum differences in readings, as compared to the "true" values, were:

pO_2 :	± 10.0 mmHg	(at 100 mmHg)
pCO_2 :	± 4.0 mmHg	(at 40 mmHg)
pH:	± 0.06 pH	(at pH 7.3)

This study demonstrates that the absolute readings, as obtained from different laboratories and/or from different instruments, vary more than is desired. For clinical routine applications, however, the precision of serial measurements on a given instrument is much more important than the absolute readings. The standard deviation of serial measurements is in the order of 0.005 pH and 1 mmHg for a typical benchtop blood gas instrument [2.9].

The critical requirements for biomedical instrumentation are reliability, ease, speed and efficiency of calibration and safety conditions. Furthermore, a clinical blood gas instrument should have a small sample size, a fast response time and should be ready for instant use at any time. It should feature an automatic calibration and consume as little gas and reagent as possible. Finally, it should be operable by non-laboratory personnel [2.8].

2.2 Measurement of blood gases

2.2.1 History of blood gas analysis

For an excellent historical survey on blood gas analysis, see [2.10][2.11]. The first accurate measurement of blood pH is credited to Hasselbalch and Lundsgaard using the hydrogen electrode with plasma in 1912. For nearly half a century, Donald D. van Slyke (1883-1971) was the dominant figure in both the theory and methods of acid-base balance and blood chemistry. In 1924 he reported the "manometric Van Slyke apparatus", which became a standard for the next 40 years in clinical laboratories. The dissolved gases in the blood were extracted by a vacuum pump and subsequently their partial pressure was measured with a mercury manometer. In 1952, a polio epidemic in Copenhagen stimulated Poul B. Astrup to develop a glass electrode by which pH could be measured in blood at 37 °C. He realized that he needed to determine $p\text{CO}_2$ and pH because several polio victims died in spite of ventilation in an "iron lung" or by means of manually operated respirators. He determined both the HCO_3^- and the $p\text{CO}_2$ of blood by measuring the actual pH value in the sample, then equilibrating the sample with two known CO_2 gas mixtures, and remeasuring the pH at each known $p\text{CO}_2$. The unknown HCO_3^- and $p\text{CO}_2$ could then be calculated. In 1956, Astrup developed a thermostated glass equilibration chamber containing a pH electrode, which was constructed by the Radiometer Company of Copenhagen and became known as the "Astrup method". Four years later, he presented together with Ole Siggaard-Andersen a capillary microelectrode, which was capable to measure pH in 25 μl samples of whole blood. The direct measurement of blood $p\text{CO}_2$ was first described by Richard W. Stow in 1957 and further developed by John W. Severinghaus in 1958. An electrode for blood $p\text{O}_2$ determination was first presented in 1956 by Leland C. Clark Jr. and is today well known as the "Clark electrode". In 1970 the appearance of integrated circuits led to the construction of the first fully automated microprocessor controlled blood gas analyzers. Today, the sensors in the benchtop blood gas instruments are still based on nearly the same technology as introduced more than thirty years ago by Astrup, Stow, Severinghaus and Clark.

2.2.2 *invasive and non-invasive methods*

For an excellent review on this subject, see [2.3].

Today, the major technique for measuring blood gases involves invasive withdrawal of a sample of blood from the artery and the subsequent analysis in an in-vitro diagnostic laboratory, usually located in some distance from the patient. Since serious changes in blood gas levels can occur in a few minutes, a timely therapeutic intervention becomes difficult as a consequence of the delay between sample acquisition and return of results (turn-around time) [2.12]. Therefore, many investigators are working on the development of continuous (or rapidly repeated) monitoring systems which can be brought to the bedside. These systems include invasive and non-invasive methods.

The invasive techniques can perhaps provide a more accurate reflection of the true biochemical and physiological status, however they have not yet been as successful as the non-invasive methods, due to the following disadvantages [2.1][2.13]-[2.17]:

- 1) they are potentially hazardous to the patient;
- 2) they bear problems associated with blood-incompatibility, resulting in a poor stability;
- 3) they need an extreme miniaturization;
- 4) servicing and operation are often very complex;
- 5) they are too expensive to be disposable;
- 6) they must be sterilizable;
- 7) in-vivo calibration is an unsolved problem;
- 8) a decrease in the signal stability is observed (wall-effect) when the sensor is placed close to the vascular wall [2.4].

Invasive gas analysis involves inserting the sensor into the patient's artery (in-vivo sensor) or inserting an extracorporeal circulation loop between the arterial and venous circulation of the patient (extracorporeal monitoring) [2.18].

Non-invasive methods include the classical blood gas analysis on benchtop instruments, the transcutaneous measurement of the pO_2 and pCO_2 on the skin, and the optical determination of the arterial hemoglobin-oxygen saturation sO_2 . Furthermore, capnography designates the continuous monitoring of the pCO_2 (and pO_2) in the airway. Combined with air flow measurement this allows calculation of the continuous O_2 consumption and CO_2 elimination [2.13].

2.2.3 In-vivo sensors

In-vivo monitoring is essential when the patient is in a labile condition, during which blood gas and pH levels are likely to change rapidly. It offers the possibility to evaluate physiological parameters in real-time. Many catheter-tip sensors for intravascular measurement or needle-type sensors for insertion into muscle and into cutaneous tissues have been designed since the 1950s to address this need. Normally, flexible polymer catheters are used to support the sensor, which carry electrical or fiber optic connections from it to the associated external instrumentation.

Oxygen sensors were the first used for in-vivo applications because they are relatively easy to miniaturize. Mostly amperometric devices have been fabricated, more recent sensors use also optical principles. The miniaturization of in-vivo pH electrodes was more difficult due to the fragile glass constructions. The advent of polymer ion-selective membranes, optical and semiconductor pH sensors have led to smaller intravascular electrodes. Optical sensors are in particular suited for in-vivo applications because they do not need a reference electrode. In-vivo sensors for $p\text{CO}_2$ monitoring generally use a pH sensor surrounded by a CO_2 permeable membrane. [2.14][2.16]

However, problems such as calibration and biocompatibility have prevented the introduction of such devices for routine monitoring so far [2.15].

2.2.4 Extracorporeal sensors

In order to reduce the risks of in-vivo measurements, sensor systems have been developed for continuous analysis in an extracorporeal blood circuit, thus combining the important advantages of both in-vivo and in-vitro monitoring. In these systems, the sensor is directly connected to the patient by means of an indwelling catheter, but remains ex-vivo. The data obtained may be considered to virtually represent in-vivo values. The advantage is that the entire analysis system can be controlled externally. Furthermore, intermittent calibration is possible and anti-coagulation fluids may be added to the system [2.15].

In the so-called "in-line systems", the blood sample is aspirated from the artery and is also returned to it, in order to avoid wasting of blood. The drawbacks are the potential risk for the patient of a blood returning system, and the difficult calibration. They are intended primarily for short-term heart bypass procedures. In our knowledge, one disposable optical sensor for continuous measurements of $p\text{O}_2$, $p\text{CO}_2$ and pH in blood is commercially available and has been successfully applied (Gas-STAT) [2.19]. A one-point calibration with in-line systems can be performed periodically by comparison of the sensor readings with the values obtained on conventional benchtop analyzers, using discrete blood samples.

A slightly different approach is the so-called "on-line system", where the blood is not returned to the patient, but drawn into waste. Such systems allow intermittent calibration cycles to be performed by diverting calibration solutions through the sensors. This increases the long-term stability of the system. Several systems of this type are under industrial development.

To avoid blood compatibility problems, one can separate the sensors from the extracorporeal blood stream by means of a semipermeable membrane, as commonly used for dialysis instruments. The sensors are in contact with an intermediate solution which is affected by the blood pH, pO_2 and pCO_2 through the membrane. By measuring these parameters in the intermediate solution, the blood values can be calculated [2.20].

2.2.5 Transcutaneous sensors

There are two different transcutaneous techniques involved with respiratory gas analysis: First, the measurement of local pO_2 and pCO_2 values on the skin, and second the optical determination of the oxygen saturation sO_2 through intact skin. These techniques do not represent real 'blood gas' measurements, although they do reflect the adequacy or inadequacy of gas exchange between the blood and the tissue.

pO_2 and pCO_2 values on the skin:

The pO_2 and pCO_2 values on the skin are measured either by means of classical electrochemical or optical sensors. The pO_2 on the surface of human skin at normal temperature is near zero. However, if the local blood flow in the skin is increased by increasing the temperature (to approximately 44 °C), the pO_2 measured on the surface of the skin can approach the arterial value quite closely. The measurement of the pCO_2 is similarly achieved by the use of a pCO_2 sensor and a heater attached to the skin of the patient.

This transcutaneous estimation of the arterial pO_2 and pCO_2 is a well established procedure in the clinic, in particular for newborn infants, because it is possible to non-invasively and continuously monitor the tissue oxygenation. However, there are some serious drawbacks. First, the relationship between the skin and the arterial pO_2 and pCO_2 values depends on the thickness, the temperature changes and the oxygenation of the skin [2.1][2.21][2.22]. In particular during shock, when the blood flow in the tissue is lowered, the correlation between arterial and skin pO_2 and pCO_2 may suddenly change [2.23], rendering the results useless. Nevertheless, it is especially in these critical moments that the clinician has to rely on correct blood gas values. Second, locally increasing the temperature of the skin constitutes a danger of burning, especially for newborn infants. Third, the determination of pH still requires a blood sample.

Determination of the oxygen saturation of hemoglobin (sO_2):

The spectrophotometric method of measuring the sO_2 , developed by Heilmeyer and others around 1943 and known as oximetry, relies on the observation that Hb and HbO_2 show differing absorption of light as a function of incident light wavelength. The tissue is rather transparent for radiation of wavelengths between 650 and 1100 nm, whereas hemoglobin has characteristic absorption spectra in this range [2.16]. The first oximetric devices were unable to differentiate between the absorption of light due to arterial blood and the absorption due to venous blood and tissue. A solution to this problem was to measure the changes in light transmitted through the skin that occur as a result of pulsation of the arteries. Since the other light absorbers in the optical path do not pulsate, only the arterial blood modulates the light transmitted through the tissue. This enables one to uniquely identify the arterial blood. This technique is called pulse oximetry and is the base for most of the present pulse oximeters. Mostly, two or more wavelengths of light are used, one sensitive to saturation, the other(s) not, in order to compensate for changes in tissue thickness, blood content, light intensity and other variables. The ratio of absorbances of light transmitted through thin sections of tissue, such as finger, ear-lobe or foot, is used to derive sO_2 .

This simple method has found a broad application, but one has to consider its limitation: A strong drawback of oximetry is the non-linear sO_2 - pO_2 relationship which hinders a reliable estimation of the arterial pO_2 . With a normal hemoglobin concentration, pO_2 levels of 80 to 100 mmHg provide a 97 % saturation. Hence, under normal physiological conditions, the oxygen saturation of hemoglobin is almost complete, and does vary very little with changing pO_2 levels. Other shortcomings of oximetry are related to the measuring mechanism. Since this technique relies on changes in light absorption produced by arterial pulsation, any event that significantly reduces vascular pulsations will result in an erroneous determination of the sO_2 value [2.1]. Also the occurrence of venous pulsations gives a wrong reading [2.16]. Furthermore, many tissues are too opaque for transmission oximetry, therefore it is impossible to determine the sO_2 of deep tissues. This leaves the fetus or important organs such as the brain beyond the range of current measurement [2.23].

2.2.6 In-vitro blood gas analysis

Some clinicians pretend, that there is still no reliable alternative to conventional blood gas analysis [2.24]. Accordingly, the major technique today is still an in-vitro analysis of arterial pO_2 , pCO_2 and pH, using instrumentation separated from the patient. The necessity to transport the sample to the analyzer poses significant drawbacks with regard to possible errors in sampling and sample handling, and with regard to the delay in obtaining the results (5 - 30 min) [2.25]. In emergency situations, results are needed within minutes after a sample is obtained, a fact that often leads to the creation of "satellite labs" in close proximity to critical care units [2.7]. Furthermore, the analysis of individual blood samples has the limitation that each analysis reflects a discrete measurement in time.

The blood sample is taken from the patient's artery in a glass or plastic syringe which has a light coating of heparin anticoagulant solution for preventing clot formation. Speed and gas-tight sampling apparatus are essential since O_2 and CO_2 must not be either gained by, or lost from, the sample. Also, since blood is a living tissue, metabolism will lower the blood pO_2 if the sample is not rapidly analyzed [2.26]. Arterial blood is the preferred type of sample because it reflects the true status of the pulmonary system. The withdrawal of the blood samples can be performed with minimal and controllable complications because often indwelling catheters for blood pressure measurements are used for critical care patients [2.24].

A state-of-the-art benchtop analyzer is self-calibrating, needs a sample volume of less than 100 μl , and the result is obtained after 45 s. Usually, many other blood gas parameters in addition to pO_2 , pCO_2 and pH are calculated. Some analyzers also perform electrolyte, hemoglobin or hematocrit measurements. For almost all these analyzers, electrochemical sensor principles are used. Generally, the gas permeable membrane of the sensors has to be exchanged monthly. The cost of an analyzer is between \$20'000 and \$40'000 [2.9].

2.3 Sensor technology

Invasive and non-invasive sensors, although having completely different applications, are often based on the same operating principles. Either classical electrochemical or optical sensors have found application.

2.3.1 *Electrochemical sensors*

The electrochemical pO_2 determination is based on the reduction of oxygen at a polarized electrode. It is utilized either as an extracorporeal sensor or as an in-vivo sensor.

The pCO_2 is determined by measuring the pH of an internal electrolyte which is separated from the sample by a gas permeable membrane. The miniaturization of this sensor has met with less success since it relies on a fragile glass pH electrode as the internal sensing element. However, by use of polymeric pH membranes, this problem can be circumvented [2.27][2.28][2.29]. Therefore, small tubular polymeric electrodes have been constructed which are flexible enough for the use as in-vivo sensors, thus preventing damage during intravascular catheter placement or removal. By impregnating the outer gas permeable tube with an appropriate ionophore, it is even possible to combine a pCO_2 sensor with an ion selective electrode (i.e. for K^+) [2.17].

The pH is usually determined by a pH glass electrode, also in a miniaturized version, or by using polymer membranes that are sensitive to pH [2.17]. For the pH determination, a reference electrode is necessary. For in-vivo sensors, most of the problems are encountered with the miniaturization of the reference electrode. In the last decade, semiconductor pH sensors have found an increasing interest, but not yet on a clinical level.

2.3.2 *Optical sensors*

Optical sensors for the determination of chemical species, named "optrodes" or "optodes" in analogy to the term electrodes, utilize an optical transduction of the analyte recognition process. The working principle is based on a selective interaction of a indicator reagent phase (located usually at the tip of the fiber optic) with the analyte, leading to a change in optical properties of the reagent phase. The optical property measured can be absorbance, reflectance or luminescence (indicators that are excited at one wavelength to emit light at another wavelength). There are three basic elements to each fiber optical sensor: an optoelectronic system, an optical fiber and an optode reagent on the tip of the fiber. Optodes became popular in the 1980's due to the advances in fiber optic technology [2.6].

For the determination of pO_2 , the quenching effect of O_2 on the luminescence of various indicator reagents is mostly used. In the presence of oxygen, the luminescence intensity of the reagent phase is decreased as a function of the pO_2 level in the sample. The advantage of O_2 optodes, as compared to amperometric electrodes, is that they do not consume oxygen because they need only a small amount of oxygen for equilibration [2.16] [2.30].

Optical pCO_2 sensors employ optical pH probes as inner transducers. The first pCO_2 optode for blood gas determinations was reported by Lübbers and Opitz in 1975. No direct CO_2 selective reagent has been found yet.

For pH determination, two separate approaches have been proposed. One group of investigators [2.22] uses luminescence indicators, other investigators use absorbance indicators [2.31].

Optical sensors offer several distinct advantages. These include insensitivity towards external (electrical) interferences, potential for low cost without sacrificing accuracy, and a unique suitability for miniaturization [2.22]. The long-term stability, however, can be difficult to achieve because of the low degree of quenching that is actually observed (for the pO_2 sensor), the instability of light source and detector, and the photochemical degradation of the immobilized indicator.

A combined intravascular fiber-optic sensor that measures arterial blood gases and pH has been put onto the market by Puritan-Bennett Corp., Carlsbad CA, in 1990 [2.25], and by Cardiovascular Devices Inc.

2.4 Silicon technology for blood gas analysis

For medical applications, silicon sensors offer a number of both technical and commercial advantages:

- 1) miniaturization allows for a smaller sample volume and a faster response;
- 2) the structural consistency of photolithography allows fabrication of identical, highly uniform and geometrically well-defined sensor elements;
- 3) it offers the possibility to combine several sensors on a single chip to get a multifunctional sensor;
- 4) preparation of ensembles of microelectrodes is easy, which is impossible by using conventional fabrication techniques [2.32];
- 5) large scale production leading to low-cost devices;
- 6) possible integration of interface electronics on the device for signal conditioning, multiplexing, and digitization. Sensors, electronics and actuators (micropumps, microvalves) can be integrated together to result in complete analysis systems [2.33].

However, the market for silicon-based electrochemical sensors such as ion-sensitive field effect transistor devices (ISFET) is growing only very slowly because of several fundamental problems. First, the direct physical contact between the sensor and the analyte needs an efficient encapsulation. Second, the miniaturization of the reference electrode has not yet been successful. Third, the promised cost benefits over existing technologies have never been proven. In the medical world, furthermore, it is hard to replace existing technologies that clinicians are used to work with, due to a certain reluctance. Fourth, the accuracy of the ISFET technology has not yet reached the standards of the classical techniques, nor the standards required by the clinical chemists.

Nevertheless, in the last 10 years, a lot of time and money has been invested in the field of solid-state sensors for blood gas analysis, both at the university and the industrial level, which is reflected by a distinct number of publications. In the following, some of these publications are summarized.

pH electrodes for continuous in-vivo measurements have been described such as metal/metal-oxide electrodes by de Rooij [2.34] and ISFETs by Bergveld [2.35] and Scheepel from Cordis Europa N.V. [2.36]. These measurements were always performed with a conventional reference electrode placed in a subcutaneous pocket close to the site of introduction of the catheter.

The first example of a multi-function chemical sensor based on silicon technology has been presented by Sibbald et al. [2.15][2.37] in 1985. They fabricated a quadruple function device for the on-line analysis of blood K^+ , Ca^{2+} , Na^+ and pH, based on ISFETs and ion-selective polymeric membranes. The sensor chip was mounted into a blood sampling cannula which simultaneously aspirated and heparinized the blood sample. A conventional reference electrode was used downstream of the sensor cell.

In 1984, Kohama and Nakamura [2.38] from Kuraray Co. Ltd. (Japan) published a clinical application of a needle-type in-vivo pCO_2 sensor. It was based on a pH ISFET and an Ag/AgCl reference electrode which were enclosed in a silicone tube. Clot formation around the sensor during implantation was prevented by slow infusion of heparin solution through a flushing device. Three years later, the same authors [2.39] described an in-line pH, pO_2 and pCO_2 monitoring system, composed of ISFET-based pH and pCO_2 electrodes and a platinum wire pO_2 electrode. The three sensors were bundled in an epoxy rod and held in place in a flow cell which was directly connected to an indwelling cannula.

Since 1987, Gumbrecht from SIEMENS (Germany) is working on an on-line monitoring system using a pO_2 , pCO_2 and pH sensor, based on ISFET (pCO_2 and pH) and thin film technology (pO_2). The three sensors are integrated on a 6.2 mm x 4.2 mm silicon chip, including on-chip signal processing. This "self-calibrating sample-taking sensor system" consists of a two-lumen catheter with a sensor flow-through cell (15 μ l) and a pump unit, which allows automatic calibration and flushing [2.40]-[2.42].

In order to reduce the size of an on-line system, Shoji [2.43] integrated a blood pH sensor together with a micro flow channel and a liquid flow control valve onto a single silicon chip. The sample volume for this "sampling-type micro-analyzing system" was 10 μ l, and the outer dimensions were 20 mm x 20 mm. The valve was used to switch between calibration solution and the blood sample, using an external peristaltic pump. An updated version of this system [2.44] integrated pH, pO_2 and pCO_2 sensors based on ISFET technology. For the reference electrodes, glass capillaries at the backside of the chip were used, which were filled with an electrolyte solution. Negative photoresist was used as gas permeable membrane.

Tsukada et al. [2.45][2.46] from Hitachi published in 1989/90 a combined pH, Na^+ , K^+ , Cl^- , pO_2 and pCO_2 sensor on a 4 mm x 4 mm chip, based on ISFET and polymeric membrane technology with integrated on-chip CMOS signal conversion and multiplexing. An external reference electrode was used.

In 1990, Nikolchev from MicroBionics Inc. [2.47] presented a silicon-based intravascular sensor for pH, pCO_2 and pO_2 , fitting into a standard 20 gauge catheter. The pH sensor was of the ion-selective membrane type, the pO_2 sensor was a Clark cell in 2 electrode configuration, and the Severinghaus

pCO₂ sensor consisted of a reference electrode and a pH sensitive metal oxide electrode. All three sensors were covered with the same "blood compatible" membrane.

2.5 Blood gas market

Physicians and nurses are relying more and more on the ability to evaluate patient parameters in real-time in emergency rooms, intensive care units, and during surgery. It has been estimated that every day in the USA, more than 100'000 blood gas analyses are performed. Worldwide sales of cardiac, respiratory, blood gas and other monitoring devices could reach \$1.2 billion by 1996, according to Market Intelligence Research Corp., Mountain View Ca. In 1989, these sales totaled approximately \$728 million [2.48].

The trend is undoubtedly proceeding in the direction of instruments that offer the possibility to obtain test results immediately at the patient's bedside, at the so-called point-of-care. Therefore, they need to be small, easy to operate and have a high reliability. A promising tool may be handheld blood analyzers, which are designed to bypass expensive and time-consuming in-house laboratories [2.49]. Furthermore, there is a strong need for reliable multiparameter sensors for blood components.

In the last two years, several instruments have been brought to the market, which offer both small size and multiparameter analysis, and which utilize new sensor technology.

In 1992, the portable clinical analyzer for whole blood from i-STAT Corporation (Princeton, NJ) was launched. This portable instrument represents the first multi-analyte system based on silicon technology to successfully enter the clinical chemistry market. It can be operated by a doctor or nurse and performs six of the most common blood tests (sodium, glucose, potassium, chloride, urea, hematocrit) giving results in about ninety seconds. This analyzer is made up of a handheld calculator and a disposable cartridge (\$10) attached to it, containing silicon based sensors and calibration solutions. I-STAT is planning to add silicon based sensors for pH, pO₂ and pCO₂ for the end of 1994.

PPG Sensors (PPG Industries, La Jolla, Ca) introduced in 1992 the StatPal® Blood Gas Analysis System, a bedside portable instrument. The sensor part of the instrument, contained in the syringe itself, is produced by thick film technology. PPG claims that the instrument "provides quick, accurate pH, pO₂ and pCO₂ measurements in 60 s at bedside" [2.48].

A portable blood gas, electrolyte and hematocrit (Hct) analyzer, called the GEM® Premier, was brought to the market in 1989 (Mallinckrodt Sensor Systems Inc., Ann Arbor, Michigan). The firm claims the self-calibrating system can be operated "by non-laboratory medical personnel", following a 20-minute training period. Miniaturized electrochemical sensors, calibrating solutions and a waste container are incorporated into one of three disposable cartridges designed for different reading combinations. One cartridge analyzes pO_2 , pCO_2 , pH, Na^+ , K^+ , iCa^{++} and Hct. A second analyzes pO_2 , pCO_2 , pH and Hct, and the third Na^+ , K^+ , iCa^{++} and Hct [2.48].

2.6 Conclusion

Blood gas analysis is an important tool in modern medicine. Today, the major technique is the in-vitro analysis of discrete blood samples on highly sophisticated instruments, separated from the patient. Invasive techniques have not found routine application because they are potentially hazardous to the patient. Therefore, non-invasive techniques, such as transcutaneous measurements, have a high popularity although the arterial pO_2 and pCO_2 can only be estimated.

For the future, in our opinion, the development will go in two different directions:

First, in direction of small handheld analyzers (Fig. 2.1) which offer a real-time analysis of withdrawn blood samples at the bedside of the patient. Such systems could be utilized also outside the clinical world.

Second, in direction of on-line sensor systems which are put into an extracorporeal blood circuit, offering continuous monitoring with intermittent calibration.

The sensor described in this thesis could be utilized for both these trends.

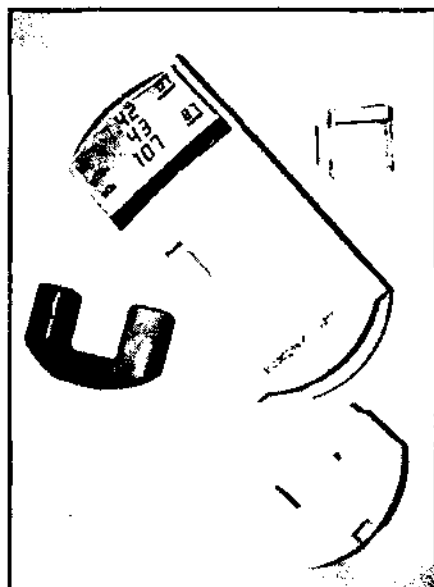


Fig. 2.1: Handheld blood gas analyzer (ATI Orion) [2.50]

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3 Design Considerations

3.1 Introduction

The aim of this chapter is to discuss the most critical parameters of the pO_2 , pCO_2 and pH sensors, in order to obtain quantitative and qualitative guidelines for the design of a planar, miniaturized blood gas sensor. These parameters include the sensitivity, the response time, the stability and many others. Theoretical models will be discussed and the literature is reviewed for this purpose.

The traditional blood gas sensors which are on the market today, are based on principles put forth by Clark for the pO_2 sensor, on the approach by Severinghaus for the pCO_2 sensor, and on glass electrode technology for the pH sensor. Because the clinical acceptance of these three detection principles is already well established, they are also chosen for the present combined sensor. Therefore, the discussion will be focussed on these three detection principles.

3.2 pO_2 sensor

3.2.1 *Measuring oxygen with amperometric methods*

There exists a large variety of techniques to measure the concentration of O_2 . Physical methods such as gas chromatography, magnetic susceptibility, mass spectrometry, and gasometric techniques are better suited to gas phase analysis than to liquid phase. For detection of dissolved oxygen in liquids, the amperometric method is widespread, which is also used to measure other electroactive gases. The amperometric gas sensor is an electrochemical cell where the desired compound is detected either by electrooxidation or electroreduction at a noble metal surface ("working electrode", WE) in contact with an electrolytic layer. The current generated by the electrochemical reaction of the analyte at the WE is directly proportional to the analyte partial pressure in the sample. The counter electrode (CE) completes the electrochemical cell by performing the other half cell reaction. The CE reaction is preferably in opposition to the WE reaction in order to minimize net chemical changes in the electrolyte, however this is often not the case. The CE has to be sufficiently large such that the current at the WE is not limited by the CE reaction. Often, a third

electrode, the reference electrode (RE) is used, which must provide a stable potential to polarize the WE. A potentiostat is used to provide a fixed potential for the WE relative to the RE, and to polarize the CE sufficiently to perform the opposite reaction.

The electrodes are covered with an electrolyte layer, and separated from the sample by a semipermeable membrane which protects the electrolyte from contamination. The current at the WE is in most cases controlled by the rate of mass transfer through the membrane. Ideally, the current is determined by the diffusion flux of the analyte through the membrane, and not by the electrocatalytic activity of the WE. The condition exists such that the catalytic activity is always high enough to guarantee reaction of all the analyte reaching the WE, or in other words, the reaction rate of the WE is considerably higher than the diffusion rate. Therefore, small changes in the catalytic activity, induced by depositions on the WE, by small variations of the applied potential, or by poisoning of the electrolyte, have no effect on the quality of the response. To maintain a high catalytic driving force at the WE, the applied voltage (overpotential) is kept sufficiently large, but not too large to prevent the decomposition of the electrolyte. If the sensor current is not diffusion limited, then the relationship between the current and the analyte partial pressure becomes more complicated. This now depends on the actual catalytic activity of the WE, on the adsorption of the analyte on the WE, on the possible (chains of) reactions, and on other parameters.

Selectivity of the amperometric sensor may be achieved by employing:

- 1) specific metals as the WE (catalyst). The WE selectivity is due to the differing ability of noble-metals to electrocatalyze reactions of specific analytes;
- 2) specific polarization potentials;
- 3) specific electrolytes;
- 4) specific permeable membranes which in the ideal case are permeable only for the desired compound.

There exist two arrangements of amperometric oxygen sensors: The first and most popular one is the "2 electrode configuration" with a working electrode ("cathode") and a combined reference / counter electrode ("reference anode"). The reference anode has to provide first a stable potential, and second serve as a counter electrode through which the current passes. In the following, the terms "cathode" and "reference anode" will be used only for the 2 electrode configuration.

The second type of amperometric sensors, the "3 electrode configuration", consists of 3 separated electrodes, namely working electrode (WE), counter electrode (CE) and reference electrode (RE). The current passes through the WE and the CE, while the RE is maintained electronically at a very high impedance to avoid a current uptake. Because there is no current flowing

through this electrode, the reference potential is more stable than in the 2 electrode configuration.

3.2.2 History of the Clark electrode

The Clark-type membrane covered oxygen electrode, so-called because of its introduction by Clark [3.1], is an amperometric oxygen sensor widely used in clinical diagnostics, environmental control and fermentation monitoring. Originally, this sensor was developed to measure blood oxygen tensions in the heart-lung machine.

In 1897, Danneel showed that dissolved oxygen in water can be electrolyzed to form hydroxyl ions at a wire connected to a negative potential. He observed that the current flowing was proportional to the concentration of the dissolved oxygen. This was the birth of the so called polarographic oxygen sensor which existed until 1956 simply as a platinum wire or disk immersed in a solution. The reference anode was a calomel bridge or a silver wire placed somewhere near the platinum wire. Several problems were encountered:

- 1) poisoning effects of materials absorbing onto the electrode surface;
- 2) dependence on the movement of the solution.

The rotation or vibration of the cathode was sometimes used to establish a fixed, known flow over the cathode thereby minimizing stirring effects. To prevent electrode poisoning, some authors employed an alternating pulsed polarization voltage, but with little success. Others found that poisoning effects could be reduced by coating the platinum wire with a thin layer of collodion, since such coatings were preferably permeable for oxygen and small ions. But collodion was fragile and tended to peel off. Attempts by Clark in 1953 to cover the cathode with cellophane and polyethylene succeeded only because there was a leakage path through the membrane or past the "seal" that held the membrane to the cathode. Otherwise, the cathode would be electrically isolated from the reference anode, which was located outside the membrane. The first description of the present version of the Clark sensor was given by Clark in 1956: The reference anode (silver) and cathode (glass insulated platinum) were put side by side. As internal electrolyte, a saturated potassium chloride solution was used. The electrodes and the electrolyte were separated from the sample by a 25 μm thick polyethylene oxygen permeable membrane. Therefore, the reduction current was independent of the composition of the sample, and no potential was applied to the sample solution because both electrodes were behind the membrane. This new sensor had a useful current output, was not poisoned, and was less sensitive to stirring effects than the bare cathode sensor.

3.2.3 Operation principle

Fig. 3.1 is a general schematic of a planar Clark electrode. The sensor consists of a noble metal WE (Au, Ag or Pt), a RE (usually Ag/AgCl) and a CE (Ag or Pt). The three electrodes are in contact with a film of electrolyte, typically 5 - 15 μm thick. The electrolyte is often a pH buffered solution with the addition of potassium chloride to stabilize the reference potential. For planar sensors, it is usually entrapped in a polymeric hydrogel layer (HG). A hydrophobic, gas permeable membrane (GPM) separates the electrolyte from the sample. This membrane is a good electrical insulator and is impermeable for liquids, ions and hydrophilic molecules. Suitable membrane materials are polypropylene, polyethylene, Teflon or Mylar, and their thickness is between 10 and 25 μm .

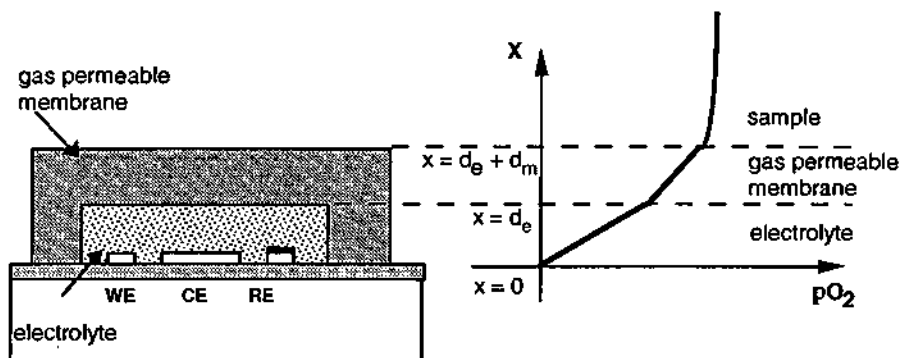


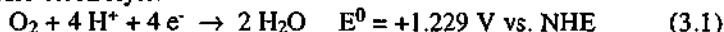
Fig. 3.1 Left side: cross section of a planar Clark type oxygen sensor
Right side: graph of the $p\text{O}_2$ distribution perpendicular to the WE surface
(during steady state polarization)

When a sufficiently large voltage is applied to the WE, oxygen in the electrolyte film is reduced and consumed:

in alkaline electrolyte:



in acidic electrolyte:



The resulting reduction current at the WE is taken as a measure of the O_2 partial pressure. Since O_2 near the WE is consumed, a deficiency of the analyte results. Hence, in order to maintain the reduction current, fresh O_2 has to be continuously resupplied from the sample solution through the membrane into the electrolyte.

Several processes take part in the response mechanism of the sensor:

- 1) diffusion of O_2 through the membrane and the electrolyte layer;
- 2) dissolution of O_2 in the electrolyte;
- 3) adsorption onto the WE surface;
- 4) electrochemical reaction;
- 5) desorption of the reaction products;
- 6) diffusion of the products away from the WE.

Any of these steps can limit the current, thus determining the response characteristics. However, primarily the GPM is used to limit the current by controlling the rate of O_2 diffusion. In the "diffusion-limited current region", the magnitude of the current is practically independent of the electrode potential. The rate of diffusion through the membrane and, correspondingly, the current depends on several parameters: most important are the thickness and the gas permeability of the membrane.

It should be emphasized that the sensor actually responds to the partial pressure of O_2 in the sample which determines the rate of O_2 transport through the membrane, and not to the concentration of O_2 . Accordingly, if it is necessary to calculate the O_2 concentration of the sample, one has to know Henry's law constant at the given temperature and sample composition.

3.2.4 Sensitivity

The following assumptions are made for modelling the diffusion of oxygen to the WE:

- 1) When the WE is polarized, oxygen is fully and immediately reduced after reaching the WE surface. Thus the p_{O_2} is zero at the WE, as shown in Fig. 3.1.
- 2) At the outer surface of the GPM, no O_2 depletion layer is created. Thus the O_2 concentration outside the membrane is the same as in the bulk of the sample.
- 3) The oxygen diffuses only in the x-direction perpendicular to the electrode surface.
- 4) The diffusion coefficients within membrane and electrolyte are independent of the O_2 concentration, time and location.

The steady state oxygen flux J_m through the membrane is expressed by Fick's 1st law of diffusion:

$$J_m = \frac{D_m (c_{m;x=d_e+d_m} - c_{m;x=d_e})}{d_m} \quad (3.2)$$

with: D_m O_2 diffusion coefficient of the membrane
 $c_{m;x=d_e+d_m}$ O_2 concentration in the membrane at $x=d_e+d_m$
 $c_{m;x=d_e}$ O_2 concentration in the membrane at $x=d_e$
 d_m thickness of the membrane
 For the definition of the x-axis, see Fig. 3.1

The concentration and the partial pressure of oxygen within the membrane are related by Henry's law:

$$c_m = H_m p_m \quad \text{for } d_e \leq x \leq d_e + d_m \quad (3.3)$$

where H_m Henry's law constant for O_2 in the membrane
 p_m O_2 partial pressure for the membrane

The Henry's law constant is equivalent to the often used "solubility constant". Thus, eqn. (3.2) can be written as:

$$J_m = \frac{H_m D_m (p_{m;x=d_e+d_m} - p_{m;x=d_e})}{d_m} \quad (3.4)$$

with: $p_{m;x=d_e+d_m}$ O_2 partial pressure at $x=d_e+d_m$
 $p_{m;x=d_e}$ O_2 partial pressure at $x=d_e$

In analogy to eqn. (3.2) - (3.4), the flux J_e through the electrolyte layer is defined as follows, if the O_2 partial pressure at the WE surface is assumed to be zero:

$$J_e = \frac{H_e D_e p_{e;x=d_e}}{d_e} \quad (3.5)$$

with: H_e Henry's law constant for O_2 in the electrolyte
 D_e O_2 diffusion coefficient of the electrolyte
 $p_{e;x=d_e}$ O_2 partial pressure for the electrolyte at $x=d_e$
 d_e thickness of the electrolyte layer

At steady state, both fluxes are equal ($J_m = J_e$), and hence:

$$\frac{H_m D_m (p_{m;x=de+dm} - p_{m;x=de})}{d_m} = \frac{H_e D_e p_{e;x=de}}{d_e} \quad (3.6)$$

The partial pressures have the same values on both sides of the interfaces. It follows for the sample / membrane interface:

$$p_{m;x=de+dm} = p_s \quad (3.7)$$

with: p_s O_2 partial pressure for the sample solution

and for the membrane / electrolyte interface:

$$p_{e;x=de} = p_{m;x=de} \quad (3.8)$$

Thus, eqn. (3.6) can be rewritten as:

$$\frac{H_m D_m (p_s - p_{e;x=de})}{d_m} = \frac{H_e D_e p_{e;x=de}}{d_e} \quad (3.9)$$

and it follows for $p_{e;x=de}$:

$$p_{e;x=de} = \frac{\frac{H_m D_m}{d_m}}{\frac{H_e D_e}{d_e} + \frac{H_m D_m}{d_m}} p_s \quad (3.10)$$

According to Faraday's law, the steady state current is given as:

$$I_{t=\infty} = n F A J_e = n F A \frac{H_e D_e p_{e;x=de}}{d_e} \quad (3.11)$$

with: n number of electrons involved in the electrode reaction (eqn. (3.1))
 F Faraday constant
 A area of the WE

Finally, one obtains:

$$I_{t=\infty} = n F A \frac{H_e D_e}{d_e} \frac{\frac{H_m D_m}{d_m}}{\frac{H_e D_e}{d_e} + \frac{H_m D_m}{d_m}} p_s$$

$$I_{t=\infty} = n F A \frac{1}{\frac{d_m}{H_m D_m} + \frac{d_e}{H_e D_e}} p_s \quad (3.12)$$

Eqn. (3.12) can be compared with another formulation of the steady state current [3.2]:

$$I_{t=\infty} = n F A \frac{1}{\frac{d_m}{D_m K} + \frac{d_e}{D_e}} c_s \quad (3.13)$$

with: c_s O_2 concentration in the sample
 K distribution coefficient between
 solution / membrane

$$K = \frac{C_{m,x=d_e+d_m}}{C_s} = \frac{C_{m,x=d_e}}{C_{e,x=d_e}} \quad (3.14)$$

The last expressions use the oxygen concentration in the sample (c_s) rather than the oxygen partial pressure. In addition, the assumption is made that the distribution coefficient K is the same for both interfaces sample / membrane and membrane / electrolyte. This is only correct if the internal electrolyte solution and the sample solution have exactly the same oxygen solubility. To compare eqn. (3.12) and (3.13), we have to transform c_s into p_s and K into H_e and H_m :

Using eqn. (3.8), (3.14) and Henry's law, one obtains:

$$K = \frac{H_m}{H_e} \quad (3.15)$$

Under the condition that H_e is valid for both the electrolyte and the sample solution, it follows:

$$c_s = H_e p_s \quad (3.16)$$

Insertion of eqn. (3.15) and (3.16) into eqn. (3.13), leads to:

$$I_{t=\infty} = n F A \frac{H_e}{\frac{d_m}{D_m} \frac{H_e}{H_m} + \frac{d_e}{D_e}} p_s \quad (3.17)$$

which indeed corresponds to eqn. (3.12).

It may become rather problematic to use the oxygen concentration c_s according to eqn. (3.13) because the current is more generally related to the overall gradient of partial pressure. The quantity of the partial pressure is independent of the media involved. This is nicely shown for a test case where the sample fluid is changed from air-saturated water to water-saturated air. Since the pO_2 of the sample does not change, conservation of energy requires that the transport of oxygen also remains constant although the oxygen concentration in the sample (c_s) has changed dramatically from approximately 9 to 280 mg/l [3.3]. Therefore, one should always keep in mind that the concentration of oxygen in the sample can vary independently of the partial pressure.

The basic eqn. (3.12) is the formal description of a current that flows through two resistors in series, one arising from the membrane and the other from the electrolyte layer. The current is driven by the partial pressure of the sample. Depending on the materials and dimensions, either one of these resistors may be dominant.

Most of the theoretical models for the steady state current of a membrane covered oxygen electrode, published so far, are derived with the assumption that the transport of oxygen is determined by the membrane alone. Therefore, the electrolyte layer is usually neglected, which is valid only if:

$$\frac{d_e}{H_e D_e} \ll \frac{d_m}{H_m D_m} \quad \text{and} \quad \frac{d_e}{\sqrt{D_e}} \ll \frac{d_m}{\sqrt{D_m}}$$

Only a few authors [3.2], [3.4] and [3.5] included in their models diffusion through both the membrane and the electrolyte layer. Morf developed also an analytical solution for the time dependent response of the sensor, whereas Hale restricted his considerations to the steady state response.

3.2.5 Response time

One can differentiate between two non steady state situations: one is the response of the sensor to a step change in the oxygen partial pressure of the sample, and the other one is the switch-on response when the sensor is switched from open circuit to a defined polarization potential.

a) Response to a step change in oxygen partial pressure

To obtain an analytical solution for the sensor response to a step change in p_{O_2} , the following differential equations and boundary conditions have to be involved (with the same assumptions as for the calculation of the sensitivity):

Fick's first law for the diffusion in the electrolyte and in the membrane (the fluxes J_e and J_m are given in the direction towards the WE):

$$J_e = H_e D_e \frac{\partial p_e}{\partial x} \quad \text{for } 0 \leq x \leq d_e \quad (3.18)$$

$$J_m = H_m D_m \frac{\partial p_m}{\partial x} \quad \text{for } d_e \leq x \leq d_e + d_m \quad (3.19)$$

with the boundary condition at the interface:

$$J_e = J_m \quad \text{for } x = d_e \quad (3.20)$$

Fick's second law:

$$\frac{\partial p_e}{\partial t} = H_e D_e \frac{\partial^2 p_e}{\partial x^2} \quad \text{for } 0 \leq x \leq d_e \quad (3.21)$$

$$\frac{\partial p_m}{\partial t} = H_m D_m \frac{\partial^2 p_m}{\partial x^2} \quad \text{for } d_e \leq x \leq d_e + d_m \quad (3.22)$$

The partial pressures have the same values on both sides of the interfaces: at the sample / membrane interface:

$$p_m = p_s \quad \text{for } x = d_e + d_m \quad (3.23)$$

with: p_s O_2 partial pressure for the sample solution

at the membrane / electrolyte interface:

$$p_e = p_m \quad \text{for } x = d_e \quad (3.24)$$

In addition, it is assumed that, prior to the step change in partial pressure, the sensor is at steady state, i.e. the O_2 flux through the electrolyte and the membrane is independent of space and time.

Morf [3.2] presented an approximation for the time dependent current response to a step change in oxygen partial pressure, taking into account the diffusion through both the membrane and the electrolyte layer. The only requirement is that the partial pressure at the WE is zero. The corresponding solution reads:

$$I(t) \cong I_{\infty} + (I_{\infty} - I_{t<0}) 2 \sum_{j=1}^{\infty} (-1)^j \exp\left(-\frac{j^2 t}{\tau}\right) \quad (3.25)$$

with the time constant:

$$\tau = \left(\frac{d_m}{\pi \sqrt{D_m}} + \frac{d_e}{\pi \sqrt{D_e}} \right)^2 \quad (3.26)$$

In eqn. (3.25) the final steady state current for $t \rightarrow \infty$ is:

$$I_{\infty} = n F A \frac{1}{\frac{d_m}{H_m D_m} + \frac{d_e}{H_e D_e}} p_{s,t>0} \quad (3.27)$$

and the steady state current prior to the applied sample step change ($t<0$) is:

$$I_{t<0} = n F A \frac{1}{\frac{d_m}{H_m D_m} + \frac{d_e}{H_e D_e}} p_{s,t<0} \quad (3.28)$$

with: $p_{s,t<0}$ pO_2 of the sample prior to the step change ($t<0$)
 $p_{s,t>0}$ pO_2 of the sample after the step change ($t>0$)

As can be seen from eqn. (3.26), the time constant τ is proportional to the square of the thickness of the membrane and the electrolyte, respectively, and inversely proportional to the diffusion coefficient in the membrane and the electrolyte, respectively.

Fig. 3.2 shows the response of the oxygen sensor to step changes in the oxygen partial pressure, as calculated from eqn. (3.25). It can be seen that the response time is independent of the direction or the amount of the pO_2 change, which has been measured already in 1962 [3.6].

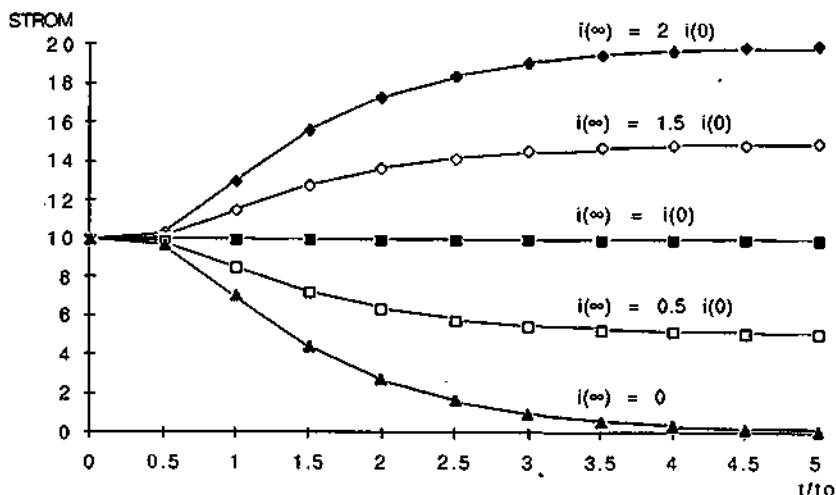


Fig. 3.2 Response of the pO_2 sensor to different step changes in partial pressure, calculated with eqn. (3.25). (Reproduced by permission of W.E. Morf [3.2])

Eqn. (3.25) can be compared with results given in the literature, i.e. from [3.7] or [3.8]. Hitchman and Mancy solved the problem with the assumption that the current is determined mainly by the membrane properties. Thus the electrolyte layer was regarded as non-existent, which is justified only if:

$$\frac{d_e}{H_e D_e} \ll \frac{d_m}{H_m D_m} \quad \text{and} \quad \frac{d_e}{\sqrt{D_e}} \ll \frac{d_m}{\sqrt{D_m}}$$

They obtained the following equation:

$$I(t) \cong I_{t=\infty} + (I_{t=\infty} - I_{t<0}) 2 \sum_{j=1}^{\infty} (-1)^j \exp \left(- \frac{j^2 \pi^2 D_m t}{d_m^2} \right) \quad (3.29)$$

with
$$I_{t=\infty} = n F A \frac{H_m D_m}{d_m} p_{s,t>0} \quad (3.30)$$

and
$$I_{t<0} = n F A \frac{H_m D_m}{d_m} p_{s,t<0} \quad (3.31)$$

If we simplify Morf's eqn. (3.25) by applying the same assumptions, we obtain:

$$\tau = \frac{d_m^2}{\pi^2 D_m} \quad I_{t \rightarrow \infty} = n F A \frac{H_m D_m}{d_m} p_{s; t > 0}$$

$$I_{t < 0} = n F A \frac{H_m D_m}{d_m} p_{s; t < 0}$$

which corresponds exactly to eqn. (3.29), (3.30) and (3.31).

b) The switch-on response

The second transient situation is observed when the sensor is switched from open circuit to a given potential. This transient situation is important for pulsed mode measurements, but of lower interest in the present case. Mancy et al. [3.6] have provided a solution for the response behavior, which can be summarized as follows.

Within a very short time interval after the start of polarization ($t < 0.1$ s), diffusion in the electrolyte layer alone is rate limiting, and the current is given as:

$$I(t) = n F A H_e \sqrt{\frac{D_e}{\pi t}} \left(1 + 2 \sum_{j=1}^{\infty} \exp \left(- \frac{j^2 d_e^2}{D_e t} \right) \right) p_s$$

which can be approximated by:

$$I(t) \cong n F A H_e \sqrt{\frac{D_e}{\pi t}} p_s \quad (3.32)$$

After this short initial time interval, the diffusion layer enters the membrane. If we assume that $\frac{d_e}{\sqrt{D_e}} \ll \frac{d_m}{\sqrt{D_m}}$, the rate of mass transfer within the membrane is now found to be predominant, as shown by eqn. (3.33), which is valid for $t < 10$ s:

$$I(t) = n F A H_m \sqrt{\frac{D_m}{\pi t}} \left(1 + 2 \sum_{j=1}^{\infty} \exp \left(- \frac{j^2 d_m^2}{D_m t} \right) \right) p_s$$

or by the approximation:

$$I(t) \cong n F A H_m \sqrt{\frac{D_m}{\pi t}} p_s \quad (3.33)$$

At longer time intervals, the diffusion layer will approach the outer face of the membrane. Then the current is given by:

$$I(t) = n F A H_m \frac{D_m}{d_m} \left(1 + 2 \sum_{j=1}^{\infty} \exp - \frac{j^2 \pi^2 D_m t}{d_m^2} \right) p_s$$

and approximates the steady state current:

$$I(t) \cong n F A H_m \frac{D_m}{d_m} p_s \quad (3.34)$$

As expected, the last expression corresponds exactly to the steady state current calculated for the response to a step change in partial pressure (eqn. (3.30)).

3.2.6 Flow dependence

A pO_2 sensor usually exhibits a flow dependence of the steady state current as well as a flow dependence of the response time.

a) Flow dependence of the steady state current

The reason behind the flow dependence of the steady state current is O_2 consumption from the sample. This results in a depletion layer near the membrane / sample interface, depending on the permeability of the membrane and on the flow velocity of the sample. Diffusion from the bulk of the liquid sample is not sufficient to replace the O_2 consumed by the WE, and therefore the oxygen is depleted near the interface. If a stirred or flowing liquid sample is used, then the current should increase with the rate at which fresh sample is supplied to the membrane surface. Correspondingly, higher current values result for stirred or flowing samples than for stagnant ones. This phenomenon is called stirring effect. For gaseous samples having the same pO_2 , the readings will be even higher because here the diffusion is limited only by the electrolyte / membrane, due to the extremely high diffusion rate in the gas phase. The difference in the readings between an aqueous and a gaseous sample of the same pO_2 is called the "liquid gas difference" or the "gas / water ratio". For blood gas sensors, the liquid gas difference should be as small as possible or at least very reproducible, which allows sensor calibration with humidified gases. In order to reduce the flow dependence, the O_2 consumption has to be reduced by using a smaller WE, a thicker membrane, or a membrane material with a lower diffusion coefficient. However, the requirements for flow

independence and for fast response / high sensitivity generally oppose each other, so that the best compromise has to be found for each individual application. For example, an IL (Instrumentation Laboratory) blood pO_2 sensor with a Pt wire of 12 μm diameter as WE (113 μm^2) and a 25 μm polypropylene GPM has a liquid gas difference smaller than 1 %.

To quantify the flow dependence, the following model can be used [3.9]. At the surface of the GPM, a thin layer of immobilized liquid is built up which adheres to the membrane and separates it from the moving stream. The thickness of this hydrodynamic layer varies with the flow velocity and the viscosity of the sample. The oxygen molecules have to diffuse through this layer to the surface of the membrane, which increases the total diffusion path. For the case of laminar flow, the thickness d_s of the boundary layer may be expressed by:

$$d_s \sim \sqrt{\frac{\mu^*}{v}} \quad (3.35)$$

with v flow velocity
 μ^* viscosity of the sample

The boundary layer is an additional diffusion layer, resulting in an additional "resistor" term in eqn. (3.12):

$$I_{\infty} = n F A \frac{1}{\frac{d_m}{H_m D_m} + \frac{d_e}{H_e D_e} + \frac{d_s}{H_s D_s}} p_s \quad (3.36)$$

or
$$\frac{1}{I_{\infty}} = f_1 + \frac{f_2}{H_s D_s} \sqrt{\frac{\mu^*}{v}} \quad (3.37)$$

with D_s O_2 diffusion coefficient in the sample
 H_s Henry's law constant for O_2 in the sample
 f_1, f_2 flow independent factors

b) Flow dependence of the response time

There are two reasons for the flow dependence of the response time: the O_2 depletion layer in the sample solution, and the carry-over effect of the measuring system. Both influences can be lowered by a higher flow rate. A higher flow rate rinses the system more efficiently, and therefore the "true" pO_2 value of the sample is faster established at the surface of the GPM.

3.2.7 CO_2 effect

Carbon dioxide is an acidic gas that permeates through the membrane and easily dissolves in the internal electrolyte. It finally shifts the pH of the electrolyte to lower values. The oxygen reduction at the WE is pH sensitive, however, requiring a less negative potential at lower pH values. Therefore, the voltammogram is shifted to more positive potentials at lower pH [3.10]. If the polarographic plateau is large, then the potential shift at the WE and the related change in reduction activity will have no influence on the resulting limiting current.

3.2.8 Temperature dependence

There are two main effects that cause a temperature dependence of the pO_2 sensor. The first one is the temperature dependence of the diffusion through the electrolyte and the membrane. Actually, the diffusion coefficients D can be related to the temperature as follows [3.6][3.7]:

$$D \sim \exp \frac{-f_1}{RT} \quad (3.38)$$

where R gas constant
 T absolute temperature

The second effect is the temperature dependence of the solubility of O_2 in the membrane and the electrolyte. As a rule, the Henry's law constant is given by [3.7]:

$$H \sim \exp \frac{+f_2}{RT} \quad (3.39)$$

The overall temperature dependence of the reduction current is therefore [3.9]:

$$I \sim \exp \frac{f_2 - f_1}{RT} \quad (3.40)$$

with f_1, f_2 temperature independent factors

3.2.9 Humidity effect

Yan and Liu reported in [3.11] that the electrocatalytic activity of the WE (platinum) deteriorates significantly under dry conditions. As a matter of fact, the electrolyte must guarantee at least a minimum conductivity for current flow. This conductivity is not maintained if the sensor dries out. The symptom manifested by the sensor in such cases is a gradual decrease of the signal [3.12].

3.2.10 Barometric pressure dependence

Since the Clark sensor measures the oxygen partial pressure, any change of the total pressure, which is the sum of the partial pressures of all components, will directly induce a change in the pO_2 [3.13]. For example, changes in hydrostatic pressure may be the cause for "anomalous signals" recorded during sample injection [3.14]. Staub [3.15] reported about a pressure effect on the current when using flow-through cuvettes.

Apparently, a hydrostatic pressure in the sample influences the signal, because the activity of dissolved oxygen at constant concentration is changed by this applied pressure [3.12].

3.2.11 Residual current

The current that flows in absence of oxygen is termed in the literature as background, dark, zero, offset or nitrogen current. This current determines the lower detection limit. Commercially available sensors have residual currents in the range of 0.01 to 1 % of the signal generated in air-saturated water. Different sources may be responsible for such residual currents:

- 1) Electrochemically active impurities under the membrane that are reduced at the same potential as used to reduce oxygen. Such impurities can be metal ions which are dissolved from the RE;
- 2) Electrical leakage through the insulating material separating RE, CE and WE;
- 3) Back diffusion of oxygen that is dissolved in the plastic components of a sensor [3.16];
- 4) Diffusion of reaction products from the counter electrode to the WE. O_2 is a major candidate [3.17];
- 5) Incorrect choice of the polarizing voltage [3.18][3.19].

There are some arguments that a large WE area produces relatively smaller residual currents than a small one [3.20]. Sensors used in the clinic are usually constructed with small WEs and have a significant residual current of 5 to 10 % of the current value measured for air [3.18].

3.2.12 Silver plating of the working electrode

For a two electrode system with a $Ag/AgCl$ reference anode, the Ag^+ ions generated at the reference anode are soluble in the internal electrolyte. The diffusion of the positively charged Ag^+ to the negatively polarized cathode and the subsequent reduction causes a plating of the cathode. It is therefore a common phenomenon to get a silver deposition on the cathode. The reduction of the dissolved ions at the cathode causes of course an undesired background current [3.12]. For miniature two electrode systems with a $Ag/AgCl$ reference anode, the continuous generation of Ag^+ and the

concomitant consumption of the AgCl reduces drastically the lifetime of the sensor, due to the limited amount of anode material [3.21].

For three electrode systems, the situation is less dramatic because there is no net current flowing at the RE, and hence no Ag^+ is produced electrolytically. However, due to the chemical dissolution of the AgCl layer in the electrolyte, a certain equilibrium concentration of Ag^+ is maintained in the electrolyte. This concentration is related to the Cl^- concentration by the solubility product: $[\text{Ag}^+][\text{Cl}^-] = 1.6 \cdot 10^{-10} \text{ M}^2$. The Ag^+ concentration reaches a minimum for $[\text{Cl}^-] \approx 0.1 \text{ M}$; at Cl^- concentrations above 0.1 M, the total concentration of dissolved silver ions increases due to formation of Ag^+ complexes of the type AgCl_2^- [3.22]. The same observation has been made by Severinghaus [3.23] who reported that the use of 0.1 M NaCl instead of saturated KCl solution results in a less pronounced dissolution of AgCl. Even when a three electrode system is used, the AgCl layer of the RE is slowly consumed and leads to an Ag plating of the WE. Sometimes the plating occurs over the whole area of the WE, an effect which is noticeable because of the change of coloration. This results in a variation of the WE activity and in changes of the way and the rate of oxygen reduction [3.17]. Silver plating occurs also at the periphery of the WE, as tree-like dendritic formations, thus extending the sensing area and leading to an increase in current. In extreme cases, these dendrites lead to a short circuit between the WE and the RE or CE [3.24][3.21].

There are several ways to reduce plating:

- 1) Choice of an electrolyte composition in which AgCl is less soluble (e.g. AgCl is much less soluble in glycerol than in H_2O);
- 2) Extension of the transport path between the RE and the WE or reduction of the diffusion rate (e.g. by incorporating the electrolyte into a hydrogel). In some pO_2 sensors, a porous ceramic plug separates the Ag/AgCl reference electrode from the main electrolyte compartment [3.25];
- 3) Use of a slightly negatively polarized shield or "guard" electrode between the RE and the WE, which inhibits the passage of metal ions to the WE [3.16]. However, the O_2 reduction occurring at this shield electrode should not interfere with the O_2 reduction at the WE.

3.2.13 Reaction at the WE

The term "n" in eqn. (3.1) is normally assumed to be equal to 4. However, there is some evidence that oxygen reduction to hydroxyl ions is not performed in one step [3.14][3.26]. The WE material also has an influence on the reduction activity. Several noble metals have been used as WE: platinum, gold and silver. Platinum is more generally used; however some

authors preferred Ag or Au, postulating that the oxygen reduction is less complex for these electrodes than for Pt [3.6][3.9].

3.2.14 Reaction at the CE

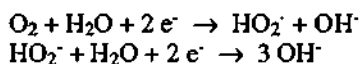
Any electrochemical reaction at the counter electrode can compensate for the current generated at the WE. The type of reaction depends on the electrolyte used and on the CE material. Probably, the main reactions are the oxidation of water to O₂ or the oxidation of Cl⁻ to Cl₂.

3.2.15 Electrolyte composition

The general requirements concerning the composition of the electrolyte layer are:

- 1) The electrolyte must carry the ionic current efficiently to reduce the ohmic voltage drop as much as possible [3.27]. Therefore, the electrolyte should maintain a certain humidity and salt concentration;
- 2) The solubility of the analyte should be high;
- 3) The electrolyte has to be compatible with both CE and WE reactions, and should contribute to a stable reference potential at the RE [3.19].

The pH of the electrolyte is of some importance because it influences the reaction at the WE. The pH determines which reaction path will be favoured, as shown in eqn. (3.1). Depending on the relative rates of these processes, four or less electrons will be required for the reaction of one O₂ molecule. This has been discussed in detail by Fletcher et al. [3.28] and Hahn et al. [3.26]. These authors suggest that the hydrogen peroxide ion HO₂⁻ is produced at the WE as an intermediate product of the O₂ reduction, therefore reaction (3.1) is in fact a two step process:



The second reaction takes place at more negative potentials than the first one. If the potential applied to the WE is not high enough, HO₂⁻ is not completely transformed according to the second reaction. Hence, the number of electrons transferred per O₂ molecule is less than 4, and HO₂⁻ is accumulated in the WE area. It was observed that, when using an electrolyte of pH 10 to 11, the voltammogram exhibits a larger diffusion-limited region. It seems that the production of HO₂⁻ is reduced by using a higher pH and by polarizing the WE to a highly negative potential, exceeding -900 mV, because then the second reaction proceeds faster. An accumulation of HO₂⁻ at the WE slows down the response of the sensor due to the slow decomposition of HO₂⁻. Suzuki believes that the accumulation of HO₂⁻ at the

WE causes electrochemical crosstalk between the WE and the CE [3.17]: If the accumulated HO_2^- flows into the CE area, it will cause rapid oxygen evolution by oxidation, resulting in overaccumulation of oxygen at the CE. This oxygen diffuses back to the WE, causing a residual current and a delay of the electrode response.

The pH of the electrolyte near the WE changes very rapidly when the potential is first switched on. Except for strong acids, the oxygen reduction is occurring essentially in an alkaline medium when the steady state current is finally achieved, no matter what the initial pH may have been. This change in pH arises from the generation of OH^- ions by the oxygen reduction. Hale and Hitchman [3.4] indeed found that the diffusion of the OH^- ions into the electrolyte is slower than the OH^- generation, resulting in a rapid accumulation of OH^- in the WE area. By modelling the diffusion of OH^- away from the WE, they were able to calculate the switch-on transients as a function of the initial pH of the electrolyte. They concluded that the attainment of the steady current is much faster for a strongly alkaline solution (0.5 M KCl + 0.5 M KOH), than for a 1 M KCl solution. This effect is understandable in terms of changing electrolyte conditions close to the WE.

3.2.16 Pulsed mode

A pulsed potential mode [3.29] is desirable for various (mainly theoretical) reasons:

- 1) To minimize the rate of consumption of oxygen (primary reason);
- 2) To be independent of a) temperature influences on the diffusion through the membrane and the electrolyte, and to be independent of b) the thickness variations of the electrolyte layer.

When working in the pulsed mode, only the transient switch-on current is taken as a measure of the pO_2 . Therefore, the sensor is not operated in the diffusion limited mode where the current is fully controlled by purely physical transport of O_2 through the membrane. In contrast, the current is now determined by the transient behavior of the catalytic activity, by the charging of the double layer capacitance, and by other transient effects, which are not always reproducible. Furthermore, memory effects and O_2 reservoirs in the bulk of the sensor can have a large influence on the kinetic current at the WE. Up to now, the pulsed mode operation has not been very successful.

3.2.17 *Microelectrodes*

The potential advantages of extremely small-sized electrodes for (amperometric) chemical sensors can be briefly stated as follows:

- 1) The limiting current density increases with decreasing electrode diameter, due to non-planar diffusion or even spherical diffusion and due to additional "edge effects" [3.19][3.25][3.15];
- 2) Reduced charging currents for the double layer capacitance;
- 3) Fast response because the steady state of mass transfer is established more rapidly [3.30];
- 4) Flow insensitivity because the O_2 consumption can be neglected [3.31][3.30];
- 5) Advantage over large electrodes for which the removal of the reaction products from the central part of the WE may be hindered, and the current density near the center of the electrode could be considerably lower [3.19].

A general disadvantage of small electrodes is the notoriously low current flow because the current is given as the product of the current density and the surface of the WE. In order to avoid this problem, many microelectrodes can be connected in a parallel array, thus providing a relatively large total current [3.32].

The following qualitative rules apply [3.2]:

- 1) The faradaic reduction current is approximately proportional to the perimeter of the electrode;
- 2) The capacitive background current is approximately proportional to the geometric area of the electrode [3.33][3.34];
- 3) From 1) and 2) follows that the signal to noise ratio is inversely proportional to the electrode diameter;

3.2.18 *Shortcomings of the Clark sensor*

The response depends on the rate of O_2 transport through the membrane, and anything which impedes this transport will reduce the output signal. Any change in membrane permeability caused by fouling, aging or thermally induced relaxation of the polymer will affect the rate of O_2 transport.

3.2.19 *Planar, miniaturized Clark electrodes*

Butler and Cobbold in 1976 [3.35] were the first to describe a Clark-type sensor that was fabricated by means of integrated circuit techniques. They were able to lay down 161 gold WEs each 7 μm in diameter and spaced 60 μm apart. Each WE was surrounded by a silver reference anode. With a

Teflon or polypropylene membrane, the sensor had a sensitivity of 0.5 nA/mmHg.

Liu et al. [3.32][3.36] used thick film metallization in the fabrication of a two electrode pO_2 sensors. With SiO_2 as a passivation layer, a multi-WE configuration was proposed. No gas permeable membrane or electrolyte layer was applied to this sensor structure.

Maseeh and Tierney [3.37][3.38] proposed the so-called "back-cell" configuration to decrease the response time of an amperometric (gas) sensor by eliminating the gas permeable membrane. The gas reaches the WE from the back side through a porous substrate rather than diffusing through a polymer electrolyte. The WE is also porous and is covered on top by a layer of polymer electrolyte (Nafion). The electrochemical reduction takes place at sites where the electrode, the electrolyte and the gas phase meet ("triple point"). As diffusion is at least five orders of magnitude faster in the gas phase than in an aqueous phase, the response is extremely fast. The drawbacks of this systems are: 1) the danger of poisoning the WE because there is no protection by a gas permeable membrane; 2) the transport of O_2 to the electrode seems to be limited by the reduction at the WE, and not diffusion limited, which causes a non-linear calibration curve; 3) a water reservoir is needed to provide sufficient humidity in the Nafion.

Suzuki et al. [3.17] proposed the use of silicon micromachining techniques to fabricate oxygen sensors. A glass substrate with a silver WE, gold CE and Ag/AgCl RE is bonded to a silicon substrate. The silicon substrate has etched grooves (9 μm deep) to provide small cavities to accommodate an electrolyte solution.

3.2.20 Conclusions

To conclude the description of the pO_2 sensor, the following qualitative design rules can be given:

	HG / GPM thickness d_e / d_m ↓	diffusion coefficients D_e / D_m ↑	Henry's law constants H_e / H_m ↑	WE size A ↓
sensitivity	↑	↑	↑	↓
response time	↓↓	↓	-	-
flow dependency	↑	↑	↑	↓

Tab. 3.1 Qualitative design rules for a planar pO_2 sensor (↓ means "decreasing", ↑ means "increasing")

3.3 pCO₂ sensor

3.3.1 *The Stow - Severinghaus pCO₂ sensor*

The CO₂ sensor initiated by Stow and Randall in 1957 [3.39] and improved by Severinghaus and Bradley in 1958 [3.23] is almost universally used for benchtop blood gas analyzers and invasive pCO₂ sensors. It is known as the Stow-Severinghaus pCO₂ sensor or Severinghaus pCO₂ sensor. The basic operating principle has remained unchanged since the introduction in 1957/58. The influence of dissolved CO₂ on the pH of an aqueous solution is used as the detecting principle. The essential idea is to separate the sample from a film of water by means of a membrane and to allow the CO₂ from the sample to permeate by diffusion through the membrane. Stow et al. used a commercial Beckman pH electrode in combination with a Ag/AgCl wire, over which a rubber membrane was stretched, trapping a very thin film of water. They observed a sensitivity of only -30.3 mV/decade because pure water was used as internal electrolyte. Severinghaus modified Stow's design by choosing a 25 µm teflon membrane, a layer of cellophane (50 µm when wet) to hold a better film of electrolyte between the glass and the teflon, and a 10 mM NaHCO₃ / 100 mM NaCl solution. They obtained a better stability due to the stabilizing effect of the NaCl on the Ag/AgCl reference electrode, a faster response and a doubled sensitivity due to the bicarbonate in the electrolyte.

3.3.2 *Sensitivity*

The CO₂ of the sample diffuses across the membrane into the film of internal electrolyte, driven by the difference in pCO₂ between the sample and the internal electrolyte. The diffusional partitioning of CO₂ reaches an equilibrium when the partial pressure of CO₂ is equal on both sides of the gas permeable membrane. After the hydration of the dissolved CO₂ and after the dissociation of the carbonic acid, the pH of the bicarbonate film is proportional to the pCO₂. The hydrogen ion activity is measured by a pH sensor vs. a reference electrode which are both in contact with the electrolyte. Since the potential of the pH electrode is related to the hydrogen ion activity by the Nernst equation, the theoretically expected response of the sensor to carbon dioxide is also Nernstian. Note that the Severinghaus sensor does not primarily respond to the concentration, but to the partial pressure of CO₂ of the sample [3.40].

The relationship between the $p\text{CO}_2$ and the concentration of CO_2 ($[\text{CO}_2]$) in the internal electrolyte is defined by Henry's law:

$$[\text{CO}_2] = H_e p\text{CO}_2 \quad (3.41)$$

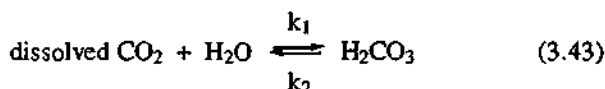
where H_e is the Henry's law constant for CO_2 in the electrolyte

The CO_2 dissolved in the internal electrolyte forms carbonic acid, which dissociates to hydrogen ion and bicarbonate ion. The overall reaction equilibrium is [3.41][3.42][3.43]:



The reaction of dissolved CO_2 in (3.42) consists of two sets of processes:

1) The first set of reactions is the formation of H_2CO_3 / regeneration of CO_2 :



The forward reaction (formation of H_2CO_3) is slow, determined by the rate equation:

$$-\frac{\partial[\text{CO}_2]}{\partial t} = k_1 [\text{CO}_2] \quad (3.44)$$

$$k_1 = 6.2 \cdot 10^{-2} \text{ s}^{-1} \quad (\text{zero ionic strength, } 25^\circ \text{C})$$

The reverse reaction (regeneration of CO_2) is faster, given by:

$$-\frac{\partial[\text{H}_2\text{CO}_3]}{\partial t} = k_2 [\text{H}_2\text{CO}_3] \quad (3.45)$$

$$k_2 = 23.7 \text{ s}^{-1} \quad (\text{zero ionic strength, } 25^\circ \text{C})$$

Equilibrium is reached, when forward (3.44) and reverse reaction (3.45) proceed at the same rate:

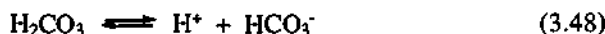
$$k_1 [\text{CO}_2] = k_2 [\text{H}_2\text{CO}_3] \quad (3.46)$$

with the equilibrium constant:

$$K_h = \frac{k_1}{k_2} = \frac{[\text{H}_2\text{CO}_3]}{[\text{CO}_2]} \quad (3.47)$$

At 25 °C, K_b is equal to $2.53 \cdot 10^{-3}$, therefore, less than 1 % of the dissolved CO_2 is present as H_2CO_3 .

2) The second set of reactions is the protolysis or dissociation of carbonic acid:



with the equilibrium constant for the dissociation of carbonic acid:

$$K_{i1} = \frac{[\text{H}^+][\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]} \quad (3.49)$$

$$\text{at } 25^\circ\text{C: } K_{i1} = 1.71 \cdot 10^{-4} \text{ M}$$

The rate of reaction (3.48) is very rapid.

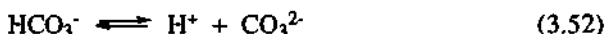
The acid formation and dissociation reactions may be combined and the respective constants summarized to the product, which results in the apparent dissociation constant pK' for the overall reaction (3.42): (H_2CO_3 is just an intermediate in the overall process)

$$K' = \frac{[\text{H}^+][\text{HCO}_3^-]}{[\text{CO}_2]} = K_b K_{i1} = 4.45 \cdot 10^{-7} \text{ M} \quad (3.50)$$

When only the reaction equilibrium (3.50) is taken into account, one could calculate the pH of the internal electrolyte as a function of the concentrations $[\text{HCO}_3^-]$ and $[\text{CO}_2]$, which is commonly done to define the sensitivity of the pCO_2 sensor:

$$\text{pH} = \text{pK}' + \log_{10} \left(\frac{[\text{CO}_2]}{[\text{HCO}_3^-]} \right) \quad (3.51)$$

But for completeness there are more reactions to consider. The bicarbonate ion can further dissociate to the carbonate ion:



$$\text{with } K_{i2} = \frac{[\text{H}^+][\text{CO}_3^{2-}]}{[\text{HCO}_3^-]} \quad (3.53)$$

$$\text{at } 25^\circ\text{C: } K_{i2} = 4.68 \cdot 10^{-11} \text{ M}$$

A further equilibrium, called "carbonate catalysis" may also be present in solutions containing CO_3^{2-} :



However, the contribution by (3.54) seems to be negligible in comparison with the previous equilibria.

At higher pH values, the dissolved CO_2 may react directly with OH^- :



$$\text{with: } K = \frac{K'}{K_w} = 4.45 \cdot 10^7 \text{ M}^{-1}$$

If a sodium bicarbonate (NaHCO_3) solution is used as the internal electrolyte, then the charge neutrality equation can be written as:

$$[\text{Na}^+] + [\text{H}^+] = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{OH}^-] \quad (3.56)$$

After insertion of the autoprotolysis constant for water,

$$K_w = [\text{H}^+][\text{OH}^-] = 1.0 \cdot 10^{-14} \text{ M}^2$$

it is finally possible to calculate the $[\text{H}^+]$ as a function of $[\text{CO}_2]$ and $[\text{Na}^+]$ and to construct the theoretical calibration curve of the pCO_2 sensor:

$$[\text{H}^+]^3 + [\text{H}^+]^2[\text{Na}^+] - [\text{H}^+](K_w + K_{a1}K_{a2}[\text{CO}_2]) - 2K_{a1}K_{a2}[\text{CO}_2] = 0 \quad (3.57)$$

In Fig. 3.3, the concentration of H^+ is calculated from the 3rd order eqn. (3.57) as a function of $[\text{CO}_2]$ and with $[\text{Na}^+]$ as parameter:

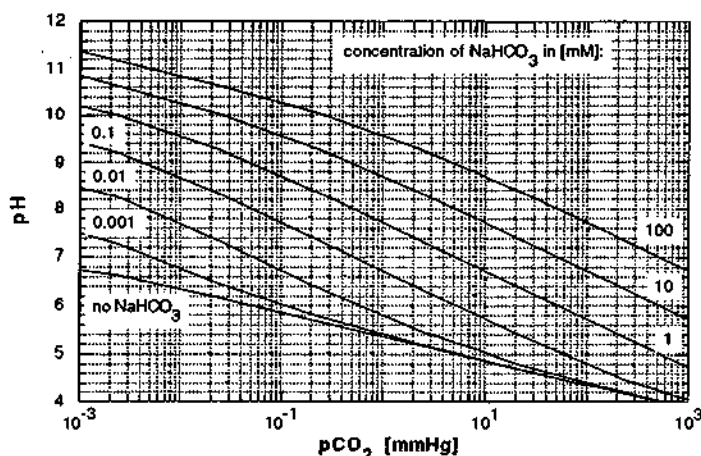


Fig. 3.3 Calculation of theoretical $p\text{CO}_2$ calibration curves from eqn. (3.57). The concentration of NaHCO_3 is taken as a parameter. It should be noted:

- 1) The sensitivity reduces to the half value, if no NaHCO_3 is added to the internal electrolyte. CO_2 in pure H_2O produces equal amounts of H^+ and HCO_3^- . However, if HCO_3^- (by the addition of NaHCO_3) is present in a large excess, the change in HCO_3^- due to CO_2 uptake is negligible but the change in H^+ is apparent;
- 2) If the NaHCO_3 concentration in the internal electrolyte is high, then the sensitivity decreases at low $p\text{CO}_2$ values.
- 3) If the NaHCO_3 concentration is low, then the sensitivity decreases at high $p\text{CO}_2$ values. Therefore, an appropriate value for the NaHCO_3 concentration in the internal electrolyte has to be chosen. If the working conditions conform to this optimal range, the pH can simply be calculated from the reduced eqn. (3.51).

3.3.3 Response time

The dynamic response of the $p\text{CO}_2$ electrode is governed by diffusion processes and by the kinetics of the above mentioned reactions. The following processes determine the dynamic response:

- 1) Diffusion of CO_2 through the membrane;
- 2) Diffusion of CO_2 in the internal electrolyte;
- 3) Formation of H_2CO_3 and regeneration of CO_2 in the electrolyte layer (simultaneously to process 2);
- 4) Diffusion of reaction products (carbonic acid, bicarbonate, carbonate) in the internal electrolyte layer.

The response time of the pH sensor itself and the time for protolysis reactions are not taken into account because they are considered to be fast as compared to processes 1) to 4).

To obtain an analytical solution for the sensor response to a step change in carbon dioxide partial pressure, the following differential equations and boundary conditions have to be solved if, in a first step, only processes 1) and 2) are modelled.

The following assumptions are made to model the diffusion of carbon dioxide:

- 1) CO_2 diffuses only in a direction perpendicular to the membrane and the electrode surface;
- 2) No carbon dioxide penetrates into the surface of the pH sensitive membrane;
- 3) The diffusion coefficients in membrane and electrolyte are independent of the CO_2 concentration.

Fick's first law holds for the diffusion of CO_2 in the electrolyte and in the membrane, respectively. After replacing concentrations by partial pressures using Henry's law, one gets:

$$J_e = H_e D_e \frac{\partial p_e}{\partial x} \quad \text{for } 0 \leq x \leq d_e \quad (3.58)$$

$$J_m = H_m D_m \frac{\partial p_m}{\partial x} \quad \text{for } d_e \leq x \leq d_e + d_m \quad (3.59)$$

(the fluxes J_e and J_m are given in the direction towards the electrode, see Fig. 3.1 for the definition of the x-axis)

The following boundary condition is valid at the membrane / electrolyte interface:

$$J_e = J_m \quad \text{for } x = d_e \quad (3.60)$$

The second boundary condition holds at the pH sensing interface where the CO_2 flow is zero:

$$J_e = 0 \quad \text{for } x = 0 \quad (3.61)$$

Fick's second law leads to the relations:

$$\frac{\partial p_e}{\partial t} = H_e D_e \frac{\partial^2 p_e}{\partial x^2} \quad \text{for } 0 \leq x \leq d_e \quad (3.62)$$

$$\frac{\partial p_m}{\partial t} = H_m D_m \frac{\partial^2 p_m}{\partial x^2} \quad \text{for } d_e \leq x \leq d_e + d_m \quad (3.63)$$

The partial pressures have the same values on both sides of the interfaces: at the sample / membrane interface:

$$p_m = p_s \quad \text{for } x = d_e + d_m \quad (3.64)$$

with: p_s : CO_2 partial pressure for the sample solution

at the membrane / electrolyte interface:

$$p_e = p_m \quad \text{for } x = d_e \quad (3.65)$$

The last boundary condition follows from the assumption that, prior to the step change in partial pressure applied at $t=0$, the sensor is in an equilibrium state, i.e. the initial partial pressures in the electrolyte, the membrane and the sample solution are the same.

Morf [3.2] presented an approximation for the time dependent response to a step change in carbon dioxide partial pressure, including parameters for both the membrane and the electrolyte. The final result for the potential response of the pH electrode reads:

$$E(t) \cong E_{t=\infty} + S \log_{10} \left(1 - \left(1 - \frac{p_{s;t < 0}}{p_{s;t > 0}} \right) \exp -\frac{t}{\tau} \right) \quad (3.66)$$

where S sensitivity (in theory Nernstian)

In eqn. (3.66), the time constant τ can have different limiting values, depending on the dimensions and on the material constants of membrane and electrolyte:

if $\frac{H_m}{H_e} \sqrt{\frac{D_m}{D_e}} \geq 1$: (both membrane and electrolyte are rate limiting)

$$\tau = \left(\frac{2 d_m}{\pi \sqrt{D_m}} + \frac{2 d_e}{\pi \sqrt{D_e}} \right)^2 \quad (3.67)$$

if $\frac{H_m}{H_e} \sqrt{\frac{D_m}{D_e}} \ll 1$: (the low permeability of the membrane is limiting the diffusion)

$$\tau = \frac{d_m d_e H_e}{H_m D_m} \quad (3.68)$$

In eqn. (3.66), the final equilibrium potential $E_{t=\infty}$ for $t \rightarrow \infty$ is given by:

$$E_{t=\infty} = \text{const} + S \log_{10} p_{s;t > 0} \quad (3.69)$$

whereas the initial potential $E_{t < 0}$ prior to the applied partial pressure step ($t < 0$) is :

$$E_{t < 0} = \text{const} + S \log_{10} p_{s;t < 0} \quad (3.70)$$

As already mentioned, the time constant τ can have different values, depending on the geometrical parameters and the diffusion coefficients of the electrolyte and the gas permeable membrane.

Eqn. (3.67) is valid if the membrane and the electrolyte layer have similar diffusion coefficients and comparable Henry's constants, respectively. In this case, the response of the sensor is determined by the diffusion of CO_2

through both layers. As can be seen in eqn. (3.67), τ is then proportional to the square of the thickness of the membrane and the electrolyte, respectively, and inversely proportional to the diffusion coefficient in the membrane and the electrolyte, respectively.

Eqn. (3.68) is valid, if the membrane has a low permeability compared to the electrolyte layer. In this case, the membrane can transport CO_2 only at a highly reduced rate, due to the low concentration and mobility of dissolved CO_2 in the membrane. The membrane is therefore limiting the CO_2 flux and, hence, the final equilibrium is only slowly established. The response time is here determined by the time needed to fill up the electrolyte layer with CO_2 "siphoning" in through the membrane.

Fig. 3.4 shows the time response of the carbon dioxide sensor to step changes in partial pressure, as calculated from eqn. (3.66). It is also evident from Tab. 3.2 that the response vs. time behavior depends on the direction and on the extent of the step change.

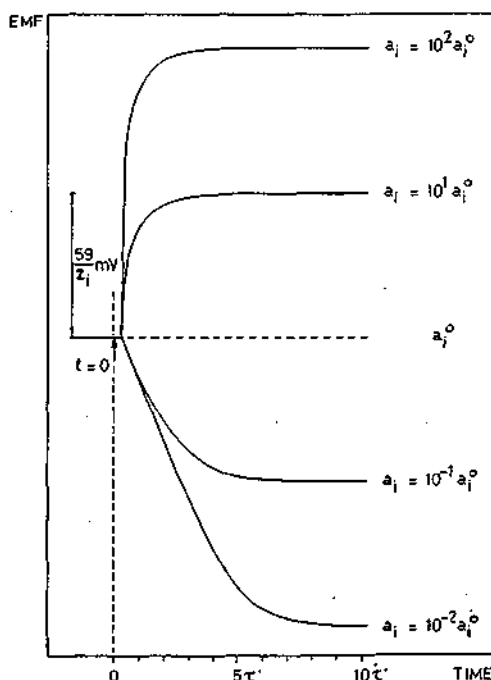


Fig. 3.4 Response of the pCO_2 sensor to different step changes in partial pressure, as calculated from eqn.(3.66). a_i is the activity of CO_2 after the step change, and a_i^0 prior to the step change. (Reproduced by permission of W.E. Morf [3.44])

The time t_{95} for a 95 % response can be calculated from eqn. (3.66) as a function of the time constant τ :

$$t_{95} = \tau \ln \frac{1 - \frac{p_{s,t \leq 0}}{p_{s,t > 0}}}{1 - \left(\frac{p_{s,t \leq 0}}{p_{s,t > 0}} \right)^{0.05}} \quad (3.71)$$

In Tab. 3.2, t_{95} is calculated for different step changes of $p\text{CO}_2$.

	$\frac{p_{s,t \leq 0}}{p_{s,t > 0}} = 100$	$\frac{p_{s,t \leq 0}}{p_{s,t > 0}} = 10$	$\frac{p_{s,t \leq 0}}{p_{s,t > 0}} = 0.1$	$\frac{p_{s,t \leq 0}}{p_{s,t > 0}} = 0.01$
t_{95}	5.9τ	4.3τ	2.1τ	1.6τ

Tab. 3.2 t_{95} calculated for different step changes of $p\text{CO}_2$

So far, only the physical diffusion of CO_2 through the gas permeable membrane and the electrolyte has been modelled, neglecting the (chemical) processes 3) and 4) mentioned at the beginning of this chapter. Van der Schoot [3.45] has predicted the complete dynamic behavior of the Severinghaus sensor on the basis of computer simulations. In his simulation model, he included all four processes. Fig. 3.5 shows that there is only a minor difference in the simulated responses, obtained when processes 3) and 4) are taken into account or not. His conclusion is that the delay in response arising from chemical reactions of CO_2 is rather small and can be neglected, especially for CO_2 sensors with thicker membranes and electrolyte layers. The qualitative shape of the sensor response is identical to Morf's analytical solution (eqn. (3.66)).

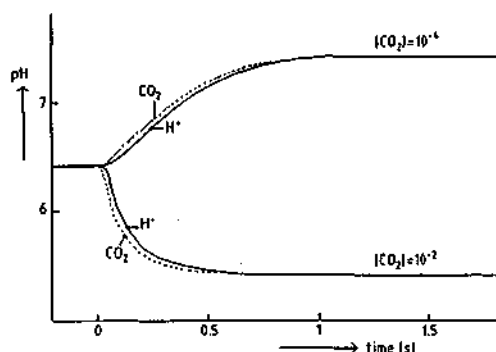


Fig. 3.5

Response curves of a $p\text{CO}_2$ sensor obtained by computer simulations, showing the difference between the change in CO_2 concentration (dotted line) and pH at the surface of the glass electrode (straight line). (Reproduced by permission of B.H. van der Schoot [3.45])

In the past, several authors [3.23][3.38] believed that their sensor response was limited by the hydration / dehydration of CO_2 . They tried to speed up the response by adding carbonic anhydrase to the electrolyte. Carbonic anhydrase catalyzes the formation / dehydration of H_2CO_3 , which enables rapid exchange of CO_2 in red blood cells and in the alveoli of the lungs. The corresponding reaction of CO_2 in the presence of the enzyme is approximately 5000 fold faster! However, the authors observed that the pCO_2 sensors did not exhibit a faster response after addition of the enzyme to the electrolyte.

3.3.4 Flow dependence

A pCO_2 sensor should not exhibit a flow dependence of the steady state signal because at equilibrium there is no net consumption of CO_2 and therefore no CO_2 depletion layer is built up in the sample. On the other hand, the pCO_2 sensor shows a flow dependence of the response time, which can be attributed to a depletion layer in the sample during the transient CO_2 exchange between sample and internal electrolyte, as well as to the carry-over effect of the measuring system (see flow dependence of the pO_2 sensor). Such phenomena have been reported by several authors, e.g. [3.46].

3.3.5 Temperature dependence

In contrast with the earlier findings for the pO_2 sensor, the temperature dependence of the diffusion coefficient and the solubility of CO_2 in the HG and the GPM have no effect on the sensitivity of the pCO_2 sensor. This is due to the fact that, in equilibrium, no CO_2 is consumed and hence, the transport properties of the HG and GPM have no influence. On the other hand, the temperature dependence of the pH sensing element of the Severinghaus sensor cannot be neglected. Thus, the pCO_2 sensor should exhibit a temperature dependence close to the one of the pH sensor.

3.3.6 Humidity dependence

According to theory, the loss of water from the internal electrolyte should have no effect on the pCO_2 response, when the pH electrode and the RE are located at the same place. This is demonstrated by the following [3.47]. The overall sensor signal E corresponds to the difference of potential contributions by the pH electrode (eqn. (3.51)) and the RE:

$$E \approx S \log_{10} \left(\frac{[\text{CO}_2]}{[\text{HCO}_3^-]} \right) - (-S) \log_{10} [\text{Cl}^-] + \text{const}$$

$$E = S \log_{10} \left([\text{CO}_2] \frac{[\text{Cl}^-]}{[\text{HCO}_3^-]} \right) + \text{const}$$

where S sensitivity (in theory Nernstian)

If the internal electrolyte is loosing humidity, both the concentrations of $[\text{Cl}^-]$ and of $[\text{HCO}_3^-]$ will increase with the same rate, and the term $\frac{[\text{Cl}^-]}{[\text{HCO}_3^-]}$ will remain constant. Therefore, at least theoretically, E should not change with a change in humidity. In practice, however, the humidity in the gel has distinct effects on the sensitivity S and on the equilibrium constant K' . Sensors with nonhydrated or partially hydrated hydrogel electrolytes exhibit lower slopes. This reduction in slope for drier electrolytes may be the result of buffering side groups on the hydrogel polymer chain, of an increase in the HCO_3^- concentration, or of a decrease in the water available for CO_2 hydration [3.38].

3.3.7 Shortcomings

The Severinghaus sensor has several shortcomings:

- 1) Slow response and long recovery time at low gas concentrations (due to diffusion and the sluggish kinetics of the CO_2 hydration reaction);
- 2) Poor selectivity (cross-sensitivity) relative to other acidic or basic gases which can diffuse through the membrane and change the pH in the electrolyte;
- 3) Precision limitations associated with the logarithmic response mode;
- 4) Detection limits which are not low enough [3.48].

3.3.8 Miniaturized Severinghaus sensors

Severinghaus sensors using Ion Selective Field Effect Transistors (ISFET) as pH sensing elements have been presented by [3.49] (encapsulation in a polymeric tube with an Ag/AgCl wire as reference electrode). Lai et al. [3.46] constructed a pCO_2 sensor using a miniaturized pH glass electrode.

3.3.9 Alternative CO_2 sensing principles

An amperometric CO_2 sensor in which CO_2 is reduced in a non-aqueous solvent (dimethylsulphoxide), was presented by Alberly et al. [3.50] [3.51] and lately by Küter et al. [3.52]. A problem is that O_2 is more easily reduced than CO_2 and hence interferes with the CO_2 determination. To exclude the O_2 interference, the authors propose to use a metallized membrane as an electrochemical filter for the removal of O_2 , allowing the

CO₂ to pass. By measuring the O₂ reduction current on that filter, it is possible to determine the O₂ partial pressure simultaneously.

Swette et al. [3.53] presented in 1990 an catheter-mounted electrochemical CO₂ sensor using the so-called "anodic stripping" method at a platinum electrode. The CO₂ measurement involves an adsorption step ('reduced CO₂') at a low potential, followed by anodic stripping (anodic ramp between 0.7-1.1 V during which the reduced CO₂ is oxidized). The integral amount of oxidation charge is used as a quantitative measure of the CO₂ partial pressure.

In 1988, van der Schoot [3.54] proposed a so-called "coulometric pCO₂ sensor". In this sensor, CO₂ is not determined from the pH value of the inner electrolyte, but by an acid-base titration performed in the internal electrolyte solution. Therefore, the internal pH sensing element of the Severinghaus pCO₂ sensor is replaced by an integrated sensor-actuator system that is able to perform a coulometric titration. An ISFET is used as the sensor to monitor the pH changes induced by the actuator, which is a gold electrode that fits closely around the gate area.

Another, more exotic way to design an amperometric CO₂ sensor is to convert a Clark-type oxygen electrode into a CO₂ sensor by combining it with a biological receptor. Suzuki [3.55][3.56] described a disposable sensor, on which autotrophic bacteria are immobilized in a gel, covered with a gas permeable membrane.

Opdycke and Meyerhoff et al. [3.57][3.58] replaced the glass pH electrode of the Severinghaus sensor by an internal polymeric membrane electrode sensitive to H⁺, thus achieving a less expensive and less fragile design.

3.3.10 Conclusions

To conclude the detailed description of the pCO₂ sensor, the following qualitative design rules can be given:

	HG / GPM thickness d_c / d_m ↓	diffusion coefficients D_e / D_m ↑	Henry's law constants H_e / H_m	addition of NaHCO ₃
sensitivity	—	—	—	↑
response time	↓↓	↓	1)	—

1) has an effect only if the permeability of the GPM is low

Tab. 3.3 Qualitative design rules for a planar pCO₂ sensor (↓ means "decreasing",
↑ means "increasing")

3.4 pH sensor

3.4.1 Definition of pH

The pH is defined in terms of the hydrogen ion activity a_{H^+} :

$$pH = -\log_{10} a_{H^+} \quad (3.72)$$

The activity of the hydrogen ion can be defined by its relation to the hydrogen ion concentration $[H^+]$ and its activity coefficient f_{H^+} :

$$a_{H^+} = f_{H^+} [H^+] \quad (3.73)$$

If the activity coefficient is unity, then activity is equal to concentration, which is nearly the case in dilute solutions with low ionic strength. The factors that affect the activity coefficient are the ionic strength, the temperature, the dielectric constant, the ion charge, the size of the ion and the density of the solvent. When defining pH, aqueous samples are usually implied. The activity coefficient for these solutions can be determined by using empirical standardization procedures. Calibrations for pH analysis should therefore be performed in standards which must have a composition as close as possible to the sample solution [3.59].

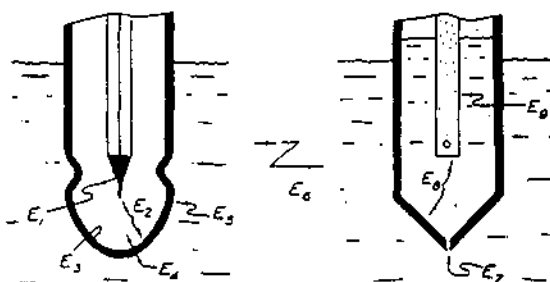
3.4.2 History of the pH glass electrode

In 1906, Cremer found that, when a thin membrane of glass was interposed between two aqueous solutions, an electric membrane potential difference was generated which was very sensitive to changes in acidity. This observation was further characterized by Haber who developed the first pH glass electrode in 1909 together with Klemensiewicz. Stadie developed in 1929 a pH meter using the first vacuum tube amplifier capable of measuring the small current available from high resistance glass electrodes. It took more than 20 years to introduce the commercial pH electrodes in 1932. Intense commercial activity has led to great improvements in the performance and applicability of glass electrodes to various conditions of measurement.

3.4.3 pH glass electrode

The most popular way of measuring the pH is by using a glass electrode. When a thin membrane of pH sensitive glass is interposed between two solutions, an electrical potential difference is built up across the glass, which is related to the pH of the two solutions. The measuring system consists of an indicating glass electrode and a reference electrode, both in contact with the solution under investigation. The overall potential difference between these

two electrodes is measured at extremely low currents. There are actually many potentials involved, as shown in Fig. 3.6, but ideally all of the potential contributions, except for the pH sensitive one, are stable, reproducible and pH independent.



Sources of Potentials

Potential	Between
E_1	Internal and solution
E_2	Internal and inside glass membrane
E_3	Solution and inside glass membrane
E_4	Insider and outer glass membrane
E_5	Sample and reference electrodes
E_6	Glass and reference electrodes
E_7	Liquid junction and sample
E_8	Internal and filling solution
E_9	Internal elements

Fig. 3.6 Diagram of a pH electrode (left) and a reference electrode (right), showing all potentials involved. (Reproduced from [3.60])

When the pH sensitive glass bulb is immersed in a solution, the surface of the glass swells slightly and a hydrated, leached layer is formed as the water penetrates into the silicate network. An ion exchange equilibrium is established between the hydrogen ions in the solution and the ions in the glass surface. The potential of this equilibrium (E_5) depends on the hydrogen ion activity. E_5 is defined by the Nernst equation:

$$E_5 = E_5^0 + \frac{RT}{F} \ln a_{H^+} = E_5^0 + 2.303 \frac{RT}{F} \log_{10} a_{H^+}$$

By inserting eqn. (3.72), one obtains:

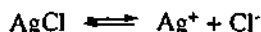
$$E_5 = E_5^0 - 2.303 \frac{RT}{F} \text{pH} \quad (3.74)$$

where

R	gas constant
F	Faraday constant
T	absolute temperature
E_5^0	standard potential, corresponding to the electrode potential for $a_{H^+} = 1$

$$\text{at } 25^\circ\text{C: } 2.303 \frac{RT}{F} = 59.16 \text{ mV.}$$

The glass electrode is constructed in a tubular form with a pH sensitive glass bulb. In the interior are an inner reference electrode element and a filling solution with a constant hydrogen ion activity and a constant chloride activity. The hydrogen ion activity is involved in the exchange equilibrium with the inner glass surface and defines potential E_3 (Fig. 3.6). E_3 is given in analogy to E_5 , but due to the constant a_{H^+} in the filling solution, E_3 is constant. The constant chloride ion activity of the filling solution establishes a constant potential E_1 with the Ag/AgCl internal reference element. The reaction equilibrium at the surface is:



$$K = [\text{Ag}^+][\text{Cl}^-] = 10^{-10.2} \text{ M}^2 \text{ at } 25^\circ\text{C}$$

E_1 is given by:

$$E_1 = E_1^0 - \frac{RT}{F} \ln a_{\text{Cl}^-} \quad (3.75)$$

where a_{Cl^-} chloride activity, supposed to be constant

3.4.4 Reference electrode

The glass electrode alone is not sufficient to measure the overall potential; an external reference electrode (RE) is needed that contacts the sample solution and completes the measuring circuit. The RE has to provide a stable and reproducible reference potential. It incorporates an internal reference element, normally calomel or Ag/AgCl, and an internal reference electrolyte contained in a glass or plastic body. The electrical contact between the reference electrolyte and the sample solution is maintained through a liquid junction.

The internal reference element:

The internal element is basically an ion selective electrode which uses a reversible redox couple to convert between ionic and electronic conduction environments. Both the reference electrode and the pH glass electrode need such an internal reference element. The most commonly used couple is solid AgCl on Ag in contact with an electrolyte solution having a given chloride activity. The potential of the reference electrode is fixed by the constant level of electroactive ions in the reference electrolyte. Thus, the potential E_9 is given by in analogy to E_1 , but with a negative sign due to the opposite orientation of the electrode / solution interface:

$$E_9 = - \left(E_9^0 - \frac{RT}{F} \ln a_{Cl^-} \right) \quad (3.76)$$

The internal reference electrolyte:

The internal reference electrolyte is normally nearly saturated potassium chloride (3.5 M KCl) saturated with silver chloride. The electrolyte should not react with the sample nor contaminate the sample, and the diffusion mobilities of the anion and the cation should be nearly equal to eliminate the diffusion potential across the liquid junction. In the design of macro RE, large reservoirs of reference electrolyte are used to minimize the effect of electrolyte loss through the liquid junction [3.61].

The liquid junction:

The reference electrolyte is separated from the test solution by means of a physical diffusion barrier. The barrier, also referred to as the liquid junction, provides the electrical contact to the sample. Within the liquid junction there is a concentration gradient of all the species which are found on both sides. The liquid junction should allow contact and moderate leakage of the filling solution into the sample, but at the same time it should restrict diffusional losses/gains from/to the reference electrolyte, which would alter the RE potential due to changes in the ionic composition or concentration concentration. These two requirements, low diffusion and low impedance, are mutually incompatible, and trade-offs must be made to optimize the reference electrode stability and lifetime. The liquid junctions are made of various materials: porous ceramic plugs, fabric wicks, or ground-glass sleeves. Some reference electrodes have a free-flowing tube as liquid junction. The main difference between the various kinds of constructions and materials used are the junction potential and the rate at which the filling solution leaks through the junction. Normally a junction with a moderate flow rate (up to 1 ml/day) will provide low resistance and a low junction potential. The junctions with slower flow guarantee much less contamination

of the sample, but are more easily clogged. A contaminated or clogged junction is a major source of error in pH measurement because the junction potential and the response time are seriously increased. The potential at the junction between the reference electrode solution and the sample (E_7) is generally built up when these two solutions have differing compositions and/or concentrations. It is caused by both diffusion and migration. Since different ions diffuse with different mobilities, the electrical charge tends to be carried unequally across the junction. This results in a potential difference whose magnitude and stability depends on the composition of the solutions and the type of junction. The liquid junction potential can be calculated by using the combined Nernst-Planck equation for each individual ion. With a few simplifying assumptions, the Henderson eqn. (3.77) is derived [3.62]:

$$E_7 = \frac{- \sum_i z_i u_i (c_{i,s} - c_{i,e})}{\sum_i z_i^2 u_i (c_{i,s} + c_{i,e})} \frac{RT}{F} \ln \frac{\sum_i z_i^2 u_i c_{i,s}}{\sum_i z_i^2 u_i c_{i,e}} \quad (3.77)$$

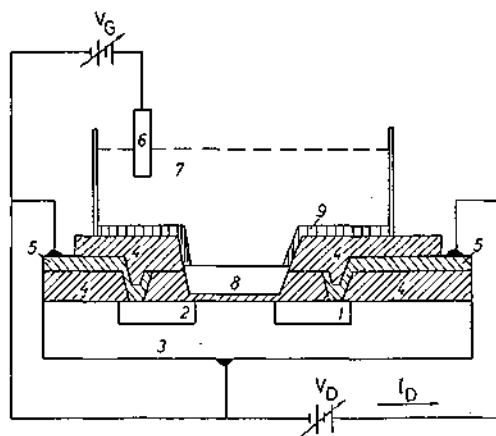
where	$\sum_i$	sum taken over all charged species
	z_i	ionic charge of the ion i
	c_i	concentration of the ion i ,
		subscript s: sample, subscript e: electrolyte
	u_i	mobility of the ion i

The practical significance of this complex equation is that the liquid junction potential E_7 depends on the ionic mobility, concentration and charge of each individual ion in the solutions on both sides of the liquid junction. Tables for the ionic mobility can be found in [3.20]. The variations of the liquid junction potential can be reduced to a minimum, in particular for relatively dilute sample solutions, by the use of concentrated solutions of equitransferent electrolytes on the RE side (such as KCl). In such solutions both the cation and the anion have nearly the same mobility. On the other hand, the liquid junction potential values for blood as sample are probably larger and less stable because blood is not simply a dilute solution of singly charged particles. What is called "blood pH value" thus turns out, as the result of unknown and unpredictable liquid junction potentials, to be no value of absolute precision. The remaining uncertainty of such determinations is about ± 0.03 to ± 0.05 pH units [3.63].

3.4.5 ISFET for pH measurements

In 1970, it was reported by Bergveld that microelectronic impedance converting elements could be used as a transducing element for the potential of a pH sensitive membrane. The Ion Selective Field Effect Transistor (ISFET) can be considered as an Metal Oxide Semiconductor Field Effect Transistor (MOSFET), where the metal gate is replaced by a reference electrode, an electrolyte solution, and an ion selective material deposited over the gate insulator. The use of an ISFET as the in situ impedance converting element is attractive because it has high input and low output impedance, small dimensions, robust solid state nature, and a fast response. However, there are some disadvantages in comparison with traditional pH glass electrodes, namely a lower signal stability and the need for encapsulation.

In Fig. 3.7 is shown a schematic diagram of an ISFET.



Schematic diagram of an ISFET

- | | |
|--------------|---------------------------|
| 1) drain | 6) reference electrode |
| 2) source | 7) solution |
| 3) bulk | 8) ion-selective material |
| 4) insulator | 9) encapsulant |
| 5) metal | |

Fig. 3.7 Schematic diagram of an ISFET. (Reproduced from [3.64])

Different ion selective materials are available. Inorganic membranes such as SiO_2 , Si_3N_4 , Al_2O_3 and Ta_2O_5 serve as pH sensitive materials, and polymeric membranes with immobilized ionophores can be used as K^+ , Ca^{++} , Na^+ selective membranes [3.65].

The theory of the ISFET characteristics has been treated elsewhere [3.64][3.66][3.67][3.68]. An ISFET consists of a silicon substrate with source and drain diffusion regions separated by a channel that is covered with a pH sensitive gate oxide. Changes in the electrical field perpendicular to the gate oxide surface result in a variation of the conductance in the source-drain-channel. This is analogous to the MOSFET, where the electrical field is induced by a metal gate instead of the pH sensitive surface potential. Therefore, the device operation of an ISFET can be deduced from the MOSFET behavior, where the drain current in the unsaturated linear region is given by [3.69]:

$$I_D = \mu C_{ox} \frac{W}{L} \left((V_{GS} - V_T) V_{DS} - \frac{V_{DS}^2}{2} \right) \quad (3.78)$$

with	I_D	drain current
	V_{GS}	gate - source voltage
	V_{DS}	drain - source voltage
	V_T	threshold voltage
	μ	electron mobility
	C_{ox}	insulator capacitance per unit area
	W	channel width
	L	channel length

In the case of pH ISFETs using inorganic oxides as pH sensitive materials, the threshold voltage V_T is the only pH dependent term, which is given by [3.68]:

$$\frac{\partial V_T}{\partial pH} = 2.303 \frac{kT}{q} \frac{\beta}{\beta+1} = 2.303 \frac{RT}{F} \frac{\beta}{\beta+1} \quad (3.79)$$

with	β	sensitivity parameter
	q	unit charge

The observed pH dependence of the above mentioned materials can be related to the state of dissociation of hydroxyl groups at the surface. These X-OH or X-O⁻ groups (X = Si, Al, Ta, and other elements) act as discrete and H⁺ specific sites for chemical reactions and modify the surface potential of the material when brought in contact with an electrolyte solution. The pH sensitivity of thin membranes is usually lower than the one predicted by the Nernst equation. This may be explained by the so called site-binding theory [3.67] according to which the Nernst equation does not apply to such ionogenic surfaces or microlayers. In eqn. (3.79), the term $\beta/(1+\beta)$ is

shifting the sensitivity to lower values. β is proportional to the charge density and the reactivity of the surface groups. A higher site density and reactivity results in a larger β and therefore in a larger pH sensitivity. The ISFET would behave in an ideal Nernstian way if β is very high. This theoretical explanation is confirmed by experimental determination of β , as shown in Tab. 3.4.

	surface density cm^{-2}	β	sensitivity mV/pH
SiO_2	$5 \cdot 10^{14}$	0.14 [3.67]	30 - 40
Al_2O_3	$8 \cdot 10^{14}$	4.8 [3.67] 14 [3.64]	48 - 56 55
Ta_2O_5	$1 \cdot 10^{15}$	62 [3.70]	58
Si_3N_4	$5 \cdot 10^{14}$		54 [3.71]
hydrated glass	$>10^{16}$ [3.22]		Nernstian

Tab. 3.4 Surface density, sensitivity parameter β , and sensitivity to pH for different pH sensitive materials

There is some evidence that the X-O⁻ surface density of thin film pH sensitive layers is low compared to the site density in thick hydrated layers of conventional glass electrodes. Because glass is much more porous than the above mentioned materials, the hydrated layers at the glass surface are up to 1000 Å thick and their Si-OH respectively Si-O⁻ density is higher than 10^{16} cm^{-2} . However, for very thin glass membranes, a reduced sensitivity is also observed [3.46]. The limited density of sites in thin film materials has also an effect on the drift behavior. A higher drift is observed, caused by variations in the number of active sites. For macroscopic glass electrodes having an excess of active sites, changes in the population of sites have no observable effect on the sensitivity.

3.4.6 Operation mode of the ISFET

As can be seen from eqn. (3.78) and (3.79), the characteristics of an ISFET are analogous to those of a MOSFET, but with a pH dependent threshold voltage V_T . The ISFET dipped in a solution, in combination with a reference electrode which contacts the solution, shows exactly the same qualitative behavior as a MOSFET. In Fig. 3.8, the drain current I_D of an ISFET is plotted as a function of the potential which is applied to the reference electrode with respect to the source contact.

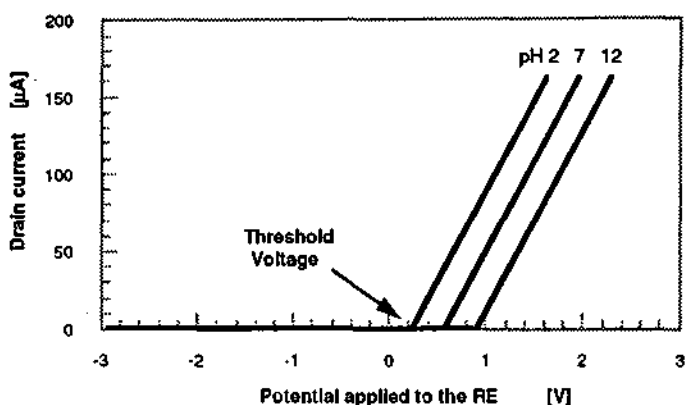


Fig. 3.8 Plot of the drain current I_D of an ISFET as a function of the potential, which is applied to the reference electrode with respect to the source contact. Note that the threshold voltage V_T is shifted to higher potentials with increasing pH. V_T is defined as the potential which has to be applied to the reference electrode before the drain current begins to flow.

The potential which is plotted on the x-axis of Fig. 3.8, is actually the sum of several potentials involved in the measuring circuit, as shown in Fig. 3.9.

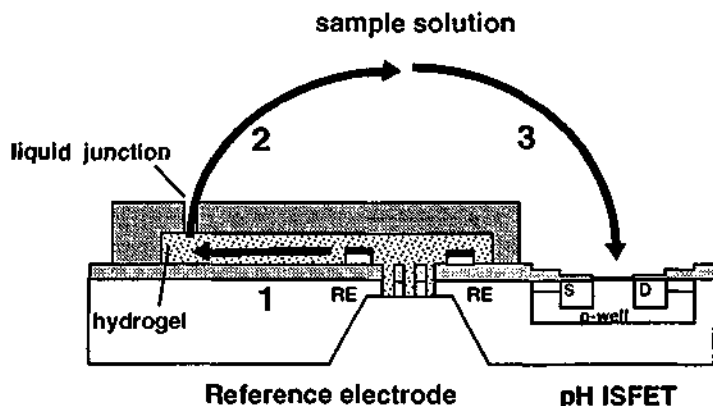


Fig. 3.9 Diagram of the involved potentials. The drain current I_D is determined by the overall potential between the Ag/AgCl electrode and the source contact of the ISFET, which is the sum of three individual potentials: 1: the potential between the Ag/AgCl electrode and the hydrogel; 2: the liquid junction potential between the hydrogel and the bulk of the sample solution; 3: the (pH sensitive) potential between the bulk of the solution and the source contact of the ISFET.

The potential 1 of Fig. 3.9 depends on the chloride activity in the hydrogel and is defined by eqn. (3.75). The potential 2 is the liquid junction potential, defined by eqn. (3.77). Eventual streaming potentials could also be added to this term. To measure the pH of a sample correctly, the potentials 1 and 2 should be held as constant as possible. The potential 3 is the pH sensitive part of the overall potential, which is equivalent to the term V_{GS} in eqn. (3.78). It is the potential between the bulk of the solution and the source contact of the ISFET. The drain current is determined by the sum of all these three potentials. Instead of taking the drain current directly as a measure of pH, it is advantageous to operate the ISFET in such a way that the output signal is presented in the form of a voltage analogous to that of a conventional pH electrode. This can be achieved by using the device in the so-called constant drain current mode by means of an external feedback loop that controls the Ag/AgCl electrode - source potential. The output signal in this operation mode is the potential between the Ag/AgCl electrode and the source contact of the ISFET, as shown in Fig. 3.9.

The overall output signal is therefore given by:

$$V_{tot} = V_1 + V_2 + V_3 \quad (3.80)$$

V_1 and V_2 correspond to $-E_9$ and $-E_7$, respectively, and are given as defined in (3.76) and (3.77). V_3 is basically equivalent to V_{GS} in eqn (3.78). Thus, after transformation, one obtains:

$$V_3 = V_{GS} = V_T + \frac{L}{\mu C_{ox}} \frac{I_D}{W V_{DS}} + \frac{V_{DS}}{2} \quad (3.81)$$

If I_D and V_{DS} are held constant by means of a feedback loop, one gets the result:

$$V_3 = V_T + \text{const} \quad (3.82)$$

The pH sensitivity of the overall potential V_{tot} can finally be defined as:

$$\frac{\partial V_{tot}}{\partial \text{pH}} = \frac{\partial V_3}{\partial \text{pH}} = \frac{\partial V_T}{\partial \text{pH}} = 2.303 \frac{RT}{F} \frac{\beta}{\beta+1} \quad (3.83)$$

The sensitivity of the overall potential V_{tot} to a variation of the chloride activity (a_{Cl^-}) in the hydrogel can be written as:

$$\frac{\partial V_{tot}}{\partial (\log_{10} a_{Cl^-})} = \frac{\partial V_1}{\partial (\log_{10} a_{Cl^-})} = -2.303 \frac{RT}{F} \quad (3.84)$$

Therefore, the overall potential V_{tot} gets more positive with increasing pH, and more negative with increasing chloride activity. In the following, the overall potential V_{tot} will be named simply as the "gate potential".

3.4.7 Drift and hysteresis of the ISFET pH response

The drift behavior of ISFETs has been studied in detail in [3.72] and can be explained by a combined effect of chemical drift and solid state drift. The ISFETs exhibit the highest drift during the first exposure to a solution. This can be explained by a gradual hydration of the sensing surface or by a diffusion / migration of mobile species into the surface [3.73]. After some hours of immersion (10 to 20 hours), the observed drift for Al_2O_3 ISFETs typically reduces to 1 - 2 mV/day, which is generally attributed to a persistent solid state drift.

Studies on the hysteresis of ISFETs have been presented in [3.74], where a hysteresis in the order of 3 to 6 mV is observed, when the pH is cycled between 3 and 11. Bousse explains the hysteresis by the presence of two different effects. First, a fraction of the Al-OH sites responds more slowly to pH changes, caused by some degree of porosity of the Al_2O_3 layer. Second, solutions with strong OH^- and F^- ion concentrations cause an irreversible drift towards a more negative threshold voltage, which also affects the measured hysteresis. This is probably due to a slow dissolution of the Al_2O_3 layer in these solutions.

3.4.8 Temperature dependence

There are two temperature dependent effects. The first results from the Nernst equation (3.74) where the pH sensitivity is proportional to the temperature. The second effect is related to the solid-state characteristics of the ISFET. The overall temperature dependence of Al_2O_3 ISFETs, determined at constant pH, is approximately $\frac{\partial V_{\text{tot}}}{\partial T} = -1 \frac{\text{mV}}{^\circ\text{C}}$.

The temperature dependence of the pH sensitivity is $\frac{\partial V_{\text{tot}}}{\partial \text{pH} \partial T} = +0.23 \frac{\text{mV}}{\text{pH } ^\circ\text{C}}$ [3.64].

3.4.9 Miniaturized reference electrodes

An essential and mandatory component of miniaturized electrochemical sensors, the reference electrode, is often neglected. In most reports, traditional reference electrodes are used, preventing the development of a completely miniaturized system based on solid-state technology. The advantages of the ISFETs are virtually eliminated without a reference electrode with similar characteristics. Although miniaturization of the sensitive part of such systems has been considerably successful,

implementation of a stable micro-reference electrode has been more difficult. An appropriate reference electrode should be at least as small as the associated ISFET and should be completely realizable on wafer scale with IC compatible methods. Nevertheless, this miniaturized reference element should provide the required stability over time.

A number of attempts have been made to fabricate various types of miniature reference electrodes, based on silicon technology. Some have simply deposited Ag/AgCl electrodes, which are in direct contact with a test solution of constant Cl⁻ concentration, others coated the Ag/AgCl layers with a hydrogel layer. Another approach is to scale down conventional reference electrodes by using micro-machined cavities in the silicon substrate. These cavities serve as the housing for the internal electrolyte and have an opening to the sample solution. The opening (or liquid junction) is either a porous glass glued on top of the chip [3.75] or a microfabricated porous silicon membrane [3.76][3.77]. In the gel-type internal electrolyte with a volume of approximately 1 μ l, a Ag/AgCl wire is immersed. However, as the size and therefore the volume of the internal electrolyte decreases, it is much more difficult to maintain a constant internal electrolyte concentration. The impedance of the liquid junction can be increased, but then the noise susceptibility will also increase [3.61]. According to Bühler's rule [3.22], the lifetime of the RE (in months) is given by the product of the reference electrolyte volume (in milliliters) and the liquid junction impedance (in kilohms). Evidently, this relationship imposes very serious limitations to the miniaturization of conventional junction-type reference electrodes.

3.4.10 Alternatives to conventional RE

Janata proposed in 1978 [3.78][3.79] a method with a differential arrangement, integrating an ion-sensitive structure and an ion-insensitive structure (REFET). A pair of ISFETs is differentially operated, having one ISFET with a bare pH sensitive surface, and the other ISFET (REFET) coated with a polymeric layer. This polymeric coating decreases as much as possible the pH sensitivity of the REFET, to obtain a differential pH sensitivity between the ISFET and the REFET. The features of the insensitive structure should be similar to those of the ISFET, requiring the same fabrication technology, the same stability, and an adequate lifetime. However, a sufficiently high differential sensitivity and a satisfying stability could not be achieved, although much effort has been put in this approach [3.80][3.81][3.82]. The main problem is to stabilize (as a function of time) the partial insensitivity of the REFET which, by definition, is not in a thermodynamically defined equilibrium state.

In dynamic systems (FIA, Analyte Chopping), a pseudo-REFET in a differential measurement setup can be used [3.79][3.83][3.84][3.85]. The pseudo-REFET is an ISFET with a retarded response time, as induced by covering the gate area with a hydrogel layer which acts as a diffusion delaying barrier for ions. The reference potential has to be stable only for the duration of the analyte detection, and the delayed and temporary deviation from a defined baseline level is not of interest. If both ISFETs are on the same chip, their solid-state properties, for example drift, temperature and light sensitivity should be the same, so that these effects are more or less canceled. On the other hand, the creation and definition of a thermodynamically well defined baseline level remains a serious problem of such REFETs. This problem is even more acute since the hydrogel layer behaves not only as a diffusion retarding barrier, but also as the source of an additional liquid junction potential.

3.4.11 Conclusions

For the reference electrode, the following qualitative design rules can be given:

	volume of the inner electrolyte ↓	liquid junction resistance ↑
lifetime / stability	↓	↑
Cl ⁻ sensitivity	↑	↓
noise	-	↑

Tab. 3.5 Qualitative design rules for a reference electrode (↓ means "decreasing", ↑ means "increasing")

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4**1st Design****4.1 Introduction**

At the beginning of this project, numerous questions were open concerning the feasibility of a triple function sensor based upon silicon technology. Questions regarding the choice of the measuring principles, technological uncertainties concerning the fabrication, other questions about the demands of clinical applications, and many more. Obviously, it was not possible to immediately find an answer to all these questions, and proceeding with the project, more questions and problems would appear. Thus, a step by step approach has been chosen to study all these topics, one after the other. To start, a simple, first generation of the chip was designed in order to have an efficient experimental tool and testing playground. The idea was to obtain experience and gain know-how concerning the fabrication and the operation of such a sensor.

4.2 Layout**4.2.1 Introduction**

A relatively large device surface of 10 mm x 10 mm was chosen, with sufficient spacing between the three sensing elements. The dimensions are kept large because many fabrication parameters are not known, i.e. how precisely the polymeric layers can be patterned, how well they adhere to the substrate and how well they adhere to each other.

The chip layout of the 1st Design is shown in Fig. 4.1.

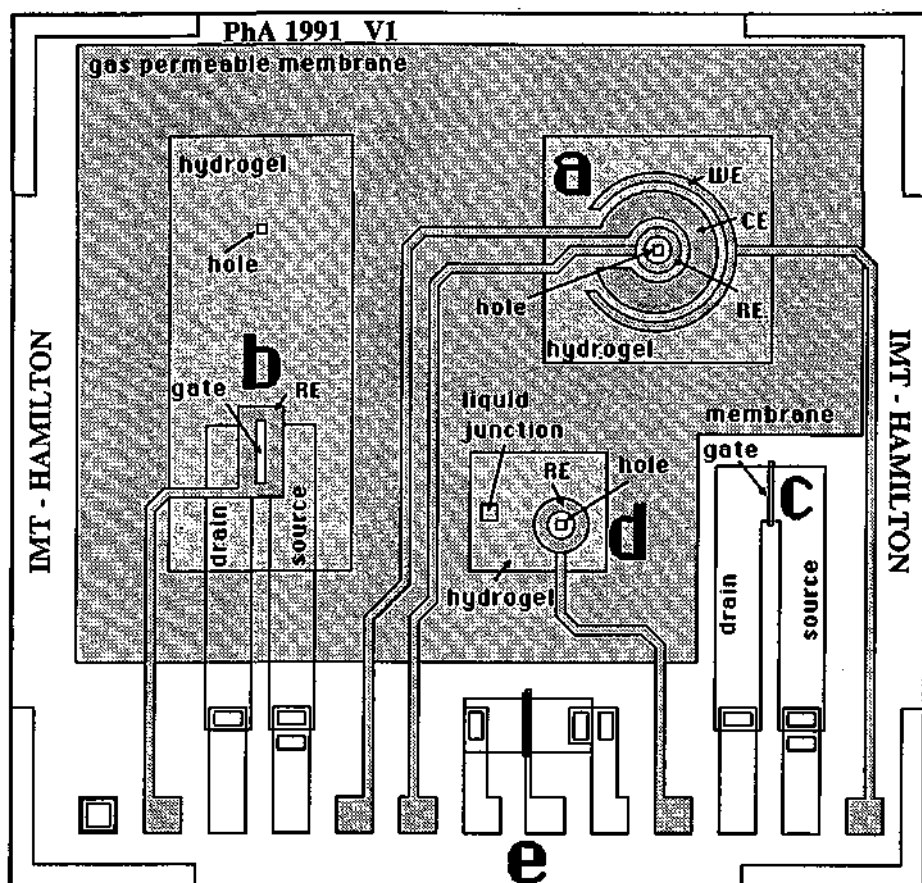


Fig. 4.1 Layout of the 1st Design: a combined pO_2 , pCO_2 and pH chip, integrated on a planar silicon chip of 10 mm x 10 mm

- a) pO_2 sensor with the working (WE), counter (CE) and reference electrode (RE), covered by the hydrogel (HG) and the gas permeable membrane (GPM). In the center is the opening to the backside of the wafer;
- b) pCO_2 sensor consisting of an ISFET and an Ag/AgCl RE, both covered by the HG and the GPM. At the upper end of the HG is located the opening to the backside;
- c) pH sensor (ISFET);
- d) RE for the pH sensor, consisting of a Ag/AgCl layer covered by the HG and the GPM. In the center of the Ag/AgCl layer is located the opening to the backside of the chip. A small opening in the GPM serves as the liquid junction;
- e) MOSFET for testing.

4.2.2 General design parameters

A critical part of a planar miniature chemical sensor is the hydrophilic polymer layer (hydrogel, HG), which incorporates the internal electrolyte for the Clark and Severinghaus cell and for the reference electrode. The humidity and the electrolyte concentration in this layer should be kept as stable as possible during operation. Due to the miniaturization, the volume of the internal electrolyte is very small, which hinders a reasonable buffering of the electrolyte. In order to circumvent this problem, an electrolyte reservoir on the backside of the wafer is provided for each individual sensor. The electrolyte reservoir is connected to the hydrogel layer, which is deposited on the topside of the wafer, through a hole. Thus, the HG is supplied with fresh electrolyte from the backside of the chip. The hole through the silicon substrate is pierced by micromachining techniques. The electrolyte reservoir is integrated in a holder, which serves as a chip support, and which contains the necessary volume of the electrolyte solution. The layout of the hydrogels is chosen with the following design rules:

- 1) The sensitive areas of the individual sensors are at a distance of at least 1 mm from the border of the respective hydrogel layers, in order to avoid side or border effects;
 - 2) the surface of the hydrogel layers are chosen large enough to ensure a mechanically stable attachment to the transducer surface;
 - 3) the hydrogels for the 3 individual sensors are not connected together to avoid any chemical crosstalk or poisoning (from the liquid junction);
- For the gas permeable membrane (GPM), the following design rules are followed:
- 1) One single and entire piece of GPM is used, which covers all 3 independent hydrogel layers;
 - 2) the GPM extends 1 mm beyond the hydrogel on all sides to ensure proper attachment of the GPM to the silicon oxide surface;
 - 3) The Pt and Ag electrodes are covered in the non-active regions by the GPM. Therefore, the GPM should act as a perfect insulation layer in order to avoid that those parts of the electrodes, which are covered only by the GPM, become electrochemically active.

4.2.3 pO_2 sensor

The pO_2 sensor is a miniaturized, amperometric Clark-type oxygen sensor with three thin film electrodes (Fig. 4.2). The working (WE) and counter electrode (CE) material is platinum and the reference electrode (RE) is an Ag layer covered with AgCl. The electrodes are arranged in a concentric configuration in order to obtain a symmetrical electrical field without corner effects. The RE is placed in the center (0.125 mm^2), the CE around it (1 mm^2), and the WE as the outer ring (0.5 mm^2). The CE surface is twice the WE surface in order to avoid a current limitation by the CE. RE and WE are $100 \text{ }\mu\text{m}$ wide, the CE is $300 \text{ }\mu\text{m}$ wide, and the spacing between the electrodes is $100 \text{ }\mu\text{m}$.

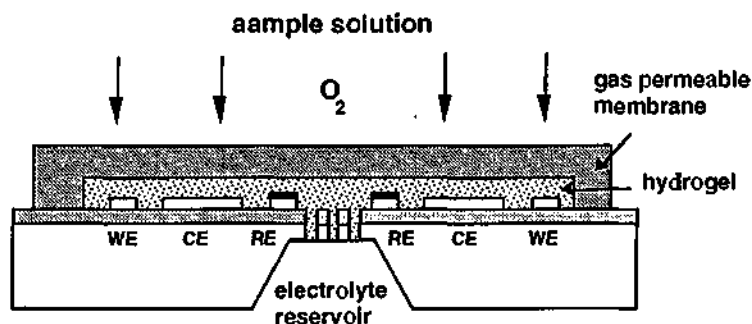


Fig. 4.2 Cross section through the planar pO_2 sensor, illustrating the concentric electrode configuration and the opening to the electrolyte reservoir on the backside of the chip

The WE and RE must be separated as much as possible to prevent or minimize the silver deposition on the WE. The RE could either be placed within the reservoir at the backside of the chip, or the on-chip RE around the opening to the backside can be used. The opening is put in the center to supply all 3 electrodes homogeneously with fresh electrolyte.

The 3 electrodes are covered by a polyacrylamide hydrogel containing an electrolyte solution (100 mM KCl, 50 mM NaHCO_3). The hydrogel is separated from the sample by an outer, gas permeable polysiloxane membrane.

4.2.4 $p\text{CO}_2$ sensor

The $p\text{CO}_2$ sensor is of the Severinghaus type, using an Al_2O_3 ISFET as pH sensing element and a thin film Ag/AgCl reference electrode (RE). Both the ISFET and the RE (0.43 mm^2) are covered by a polyacrylamide hydrogel layer. The surface of the RE is chosen to be small in order to prevent adhesion problems since the hydrogel cannot anchor to AgCl . The hydrogel is separated from the sample by a polysiloxane membrane (Fig. 4.3).

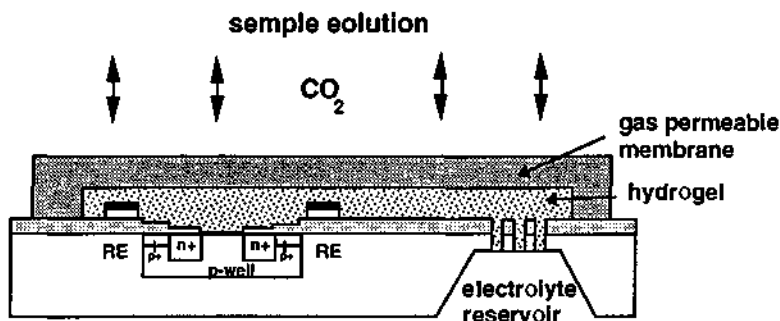


Fig. 4.3 Cross section through the $p\text{CO}_2$ sensor, showing the pH ISFET and the Ag/AgCl RE in close distance, and the opening to the electrolyte reservoir at the other end of the hydrogel

For the gas permeable membrane and the hydrogel, the same polymers are used as for the $p\text{O}_2$ sensor and the same electrolyte is impregnated in the HG. The gate of the ISFET and the Ag/AgCl reference electrode are arranged as close as possible, to minimize the dependence on humidity, and are located at some distance from the border of the HG to avoid interference effects. The distance between the gate/RE and the opening to the reservoir is sufficiently large. This ensures that the electrolyte solution of the reservoir does not buffer the pH at the sensing area. The electrolyte flow from the reservoir to the sensing area has to pass a long distance underneath the membrane. Therefore a CO_2 distribution equilibrium can be easily established in the electrolyte before it reaches the gate area.

4.2.5 pH sensor

The pH of the sample is measured by an Al_2O_3 ISFET with a thin film Ag/AgCl reference electrode (RE)(Fig. 4.4).

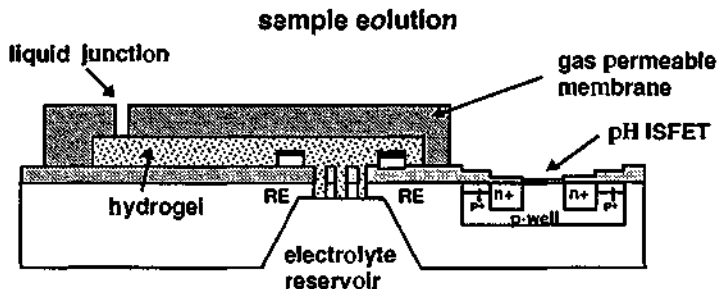


Fig. 4.4 Cross section through the pH sensor and the reference electrode. The ISFET is in direct contact with the sample solution. The Ag/AgCl layer of the reference electrode surrounds the opening to the electrolyte reservoir on the backside of the chip, and is in some distance from the liquid junction to the sample solution.

The gate of the ISFET is in direct contact with the sample and is therefore not covered with the GPM. n-channel ISFETs are used which are placed in a p-well to electrically isolate them from the other ISFET and MOSFET on the same chip. The length of the channel is $16\text{ }\mu\text{m}$ and the width is $600\text{ }\mu\text{m}$. In operation, the n-channel between drain and source is formed by depletion of the p-dopants due to the applied positive electrical field on the gate (for the pCO_2 sensor, exactly the same type of ISFET is used).

4.2.6 Reference electrode for the pH sensor

The reference electrode consists of a Ag/AgCl electrode layer which surrounds the opening to the reservoir (Fig. 4.4). The Ag/AgCl layer (0.21 mm^2) is covered by the HG and by the GPM. A simple opening ($0.5\text{ mm} \times 0.5\text{ mm}$) in the GPM serves as the liquid junction. In order to minimize poisoning of the internal electrolyte by the sample solution, and in order to maintain the Cl^- concentration near the Ag/AgCl electrode as constant as possible, the following rules have been followed:

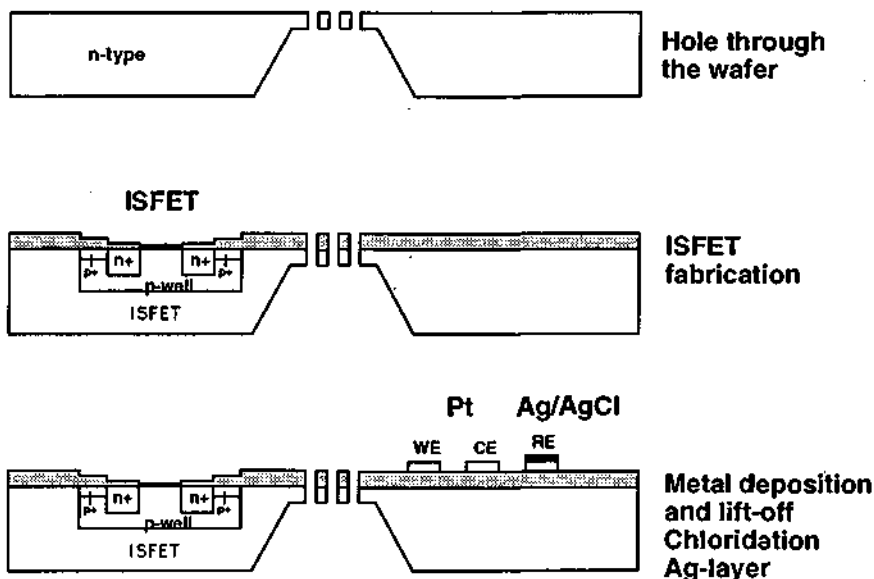
- 1) An extended distance between the liquid junction and the Ag/AgCl layer is chosen to increase the diffusional resistance. However, this distance should not be too large in view of a moderate electrical resistance;

- 2) The volume of the HG, where the internal electrolyte is entrapped, should be as large as possible. However, a compromise has to be found in order to minimize memory effects of potentially entrapped analyte;
- 3) The Ag/AgCl layer surrounds the opening to the backside of the chip and is therefore in direct contact with the electrolyte reservoir. A Ag/AgCl wire in the reservoir can be used alternatively to the on-chip Ag/AgCl layer, which would further increase the stability of the RE.

4.3 Fabrication

The fabrication of the chip involves five main processing sequences which are shown in Fig. 4.5:

- A) In the first sequence, holes are etched through the wafer.
- B) In the second step the ISFETs are fabricated.
- C) The metal electrodes are deposited by electron beam evaporation and patterned by the lift-off method. The partial chloridation of the reference electrodes is performed in FeCl_3 solution.
- D) Then the hydrogels are deposited as prepolymers on wafer level and patterned by photopolymerization.
- E) In the 5th sequence, the gas permeable membranes are patterned using the same method as for D), but starting from a different polymeric material.



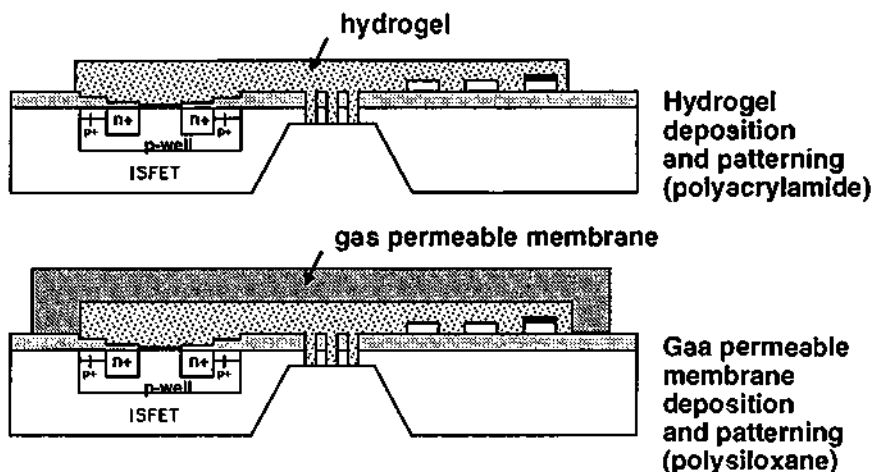


Fig. 4.5 The 5 main processing sequences of the chip fabrication

4.3.1 Holes through the wafer

The sensors are fabricated on 380 μm thick, n-type silicon wafers with a resistivity of 15-35 Ωcm and a $\langle 100 \rangle$ crystal orientation. A 3000 $\text{\AA}$ thick thermal SiO_2 is grown and then 1500 $\text{\AA}$ of Si_3N_4 is deposited by low pressure chemical vapor deposition (LPCVD). With mask 1, positive photoresist (PR+) is patterned on the backside of the wafer and then windows are etched in the Si_3N_4 by plasma etching. The SiO_2 in the windows is removed in buffered hydrofluoric acid (BHF) solution (1). In KOH, the silicon is etched anisotropically from the backside, in order to achieve a silicon membrane of 20 μm thickness (2). The surface of the membrane at the topside of the wafer measures 120 μm x 120 μm .

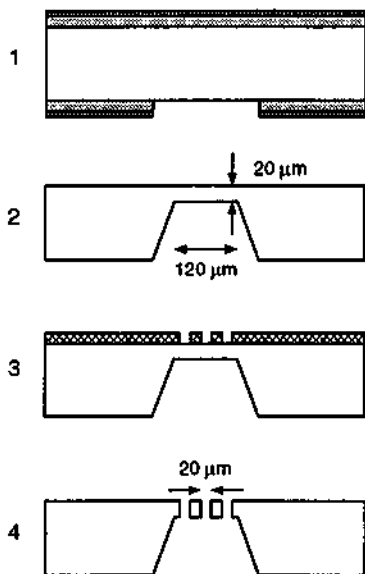


Fig. 4.6 Fabrication of the holes through the wafer

The remaining Si_3N_4 and SiO_2 layers are removed by plasma etching and BHF etching, respectively. PR+ is spun on the topside of the wafer and patterned with mask 2 (3). By a plasma etching process using a $\text{C}_2\text{ClF}_5 / \text{SF}_6$ gas mixture and a PR+ mask [4.1], the $20\text{ }\mu\text{m}$ thick silicon membrane is pierced (4) (for each membrane 9 holes of $20\text{ }\mu\text{m} \times 20\text{ }\mu\text{m}$). Almost vertical sidewalls are obtained due to redeposition of PR+ on the sidewalls. In Fig. 4.7, a photograph of the membrane is shown, after piercing the small holes.

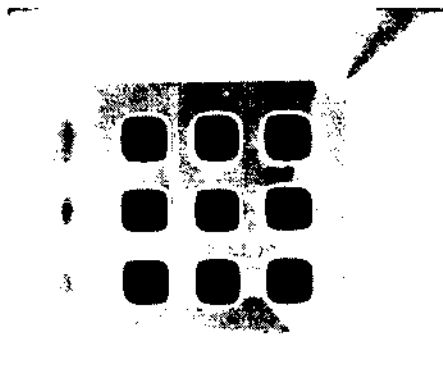
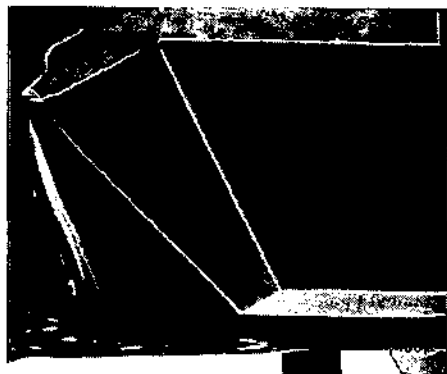


Fig. 4.7 On the left: photograph of a cross section of the anisotropically etched wafer with the $20\text{ }\mu\text{m}$ thick membrane at the bottom.
On the right: photograph of the $120\text{ }\mu\text{m} \times 120\text{ }\mu\text{m}$ silicon membrane, seen from the backside of the wafer, with the 9 holes having $20\text{ }\mu\text{m} \times 20\text{ }\mu\text{m}$.

Comments:

The structuring of the holes through the wafers is chosen as the first step of the sensor fabrication because it would be extremely difficult to perform the same processes in a later stage. The expected problems are the influence of the plasma etching on the characteristics of the ISFETs, and the difficulty to protect the topside of the wafer during the KOH etching. Furthermore, if the holes are etched at the end of the fabrication, it is impossible to deposit an isolating layer at the sidewalls in order to electrically separate the bulk of the silicon from the electrolyte solution.

The holes in the silicon membrane have a size of $20\text{ }\mu\text{m} \times 20\text{ }\mu\text{m}$, in order to reduce problems during all the subsequent photoresist spinings in the sensor fabrication. In fact, one can imagine, that spinning of a liquid photoresist on a surface with holes would result in very irregular photoresist layers. However, if these holes have very small size, then they are almost "invisible" for viscous photoresists. These holes are opened in two steps: first by anisotropic etching from the backside and then from the topside using dry

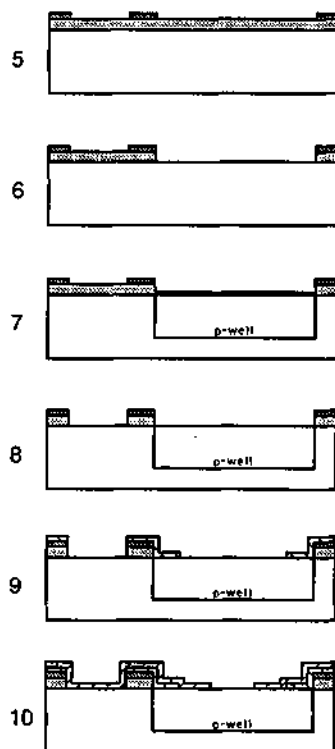
plasma etching, which allows the definition of very small openings at the top. If the holes are opened directly in one single anisotropic etching process, then the size of the opening at the top of the wafer cannot be defined precisely.

4.3.2 ISFET fabrication

(for more information see [4.2])

By wet oxidation, a 7800 Å thick SiO_2 layer is grown at 1100 °C, which also covers the sidewalls of the holes through the wafer and therefore acts as an isolating layer. Subsequently, 2000 Å Si_3N_4 is deposited by LPCVD. With mask 4, which defines the layout of the p-wells, PR+ is patterned. By plasma etching, the Si_3N_4 is selectively opened (5). With negative photoresist (PR-) and mask 5, the substrate contacts are covered.

The areas for the p-wells are opened in BHF (6). A 950 Å thick thermal SiO_2 is grown, which is used as protection layer for the implantation. The p-well is implanted with a dose of $1.0 \cdot 10^{12}$ boron-ions/ cm^2 and an energy of 80 keV. The annealing of the crystal structure and the diffusion of the implanted ions is performed during 24 h at 1100 °C (7). This results in a junction depth of 4 µm and a sheet resistance of 21.5 $\text{k}\Omega/\square$, which corresponds well to the values obtained by numerical simulation. After the implantation, the protection layer of SiO_2 is removed and in parallel the substrate contact is opened in BHF (8). A 3500 Å boron doped SiO_2 layer (borosilicate glass) is deposited by chemical vapor deposition (CVD), which later serves as a boron source for the diffusion of the channel stopper. PR+ is patterned on top of it using mask 6, which defines the zones of the channel stopper. The boron doped SiO_2 layer is selectively



etched in BHF (9) and on top, a 3500 Å phosphorous doped SiO_2 layer is deposited by CVD, which later acts as a phosphorous source for the drain and source diffusion. With mask 7, PR+ is patterned on top of the phosphorous doped SiO_2 to define the drain and source zones. In BHF, the phosphorous doped SiO_2 layer is removed at the gate area (10). At 1100 °C, the gate oxide is grown (950 Å), and in parallel, the dopants are driven from the doped SiO_2 layers into the silicon substrate (30 min with O_2 stream and 50 min without) (11).

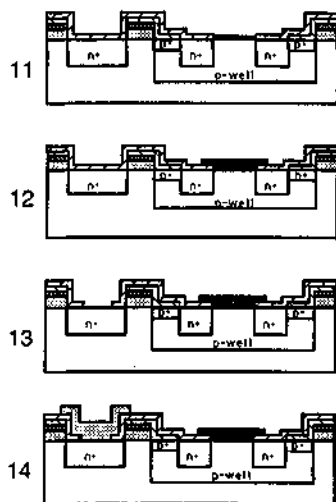


Fig. 4.8 ISFET fabrication

For the channel stopper, a junction depth of 1 μm and a sheet resistance of 1.4 $\text{k}\Omega/\square$ results, and for the drain and source areas 1.5 μm and 54.6 $\Omega/\square$ respectively. The pH sensitive Al_2O_3 layer (600 Å) is deposited by means of CVD at 900 °C, followed by a CVD deposition of an undoped SiO_2 layer (4000 Å), which later acts as the mask material for the wet etching of the Al_2O_3 . A PR+ layer is structured on top of this SiO_2 layer using mask 8. The masking SiO_2 is etched in BHF and the photoresist is removed. In 85 % H_3PO_4 at 185 °C, the Al_2O_3 is etched, using the SiO_2 layer on top as mask. Then the SiO_2 mask material is removed in BHF (12). With mask 9, a PR- layer is patterned, which defines the openings for the drain, source and substrate contacts. Then these contacts are opened by BHF etching, followed by the removal of the PR- (13). 1.5 μm of Al is evaporated and a PR+ layer is patterned on top using mask 10, which defines the Al metallization paths. The Al layer is etched in a mixture of phosphoric acid, nitric acid and acetic acid at 42 °C, followed by the PR+ stripping. The Al is finally annealed during 10 min at 450 °C (14).

Observed problems:

During the deposition of the Al_2O_3 layer (12), some of the membranes with the 20 $\mu\text{m} \times 20 \mu\text{m}$ holes exploded literally, as shown in Fig. 4.9. The reason for this high mechanical stress, which is acting apparently on the silicon membrane, is probably the different thermal expansion coefficients of all the layers covering the membrane (thermal SiO_2 , CVD SiO_2 , Si_3N_4). The Al_2O_3 deposition is performed at 900 °C and the wafers are introduced into the

furnace without any ramping of the temperature. Thus, during loading and deloading, the thermal shock is high.



Fig. 4.9 Photograph of exploded silicon membranes during the Al_2O_3 deposition

No damage of the membranes was observed during the processes (5), (7) and (11), where the silicon membranes were exposed to 1100°C during over 24 h. For these processes, the final temperature in the furnace is achieved by ramping from a low start temperature, thus minimizing thermal shock.

4.3.3 Deposition and patterning of the noble metal electrodes

The Pt electrodes for the WE and the CE are patterned by the lift-off method [4.3][4.4]. First, a PR+ layer is patterned (mask 11) and then hardened at the surface by immersion in a chlorobenzene soak. Onto this PR+ layer, $1500\text{ \AA}$ of Pt is deposited by e-gun evaporation, with $500\text{ \AA}$ Ti as adhesion layer. The PR+ is then dissolved in an acetone bath, thus all the Pt which is laying on the PR+ is lifted off (15).

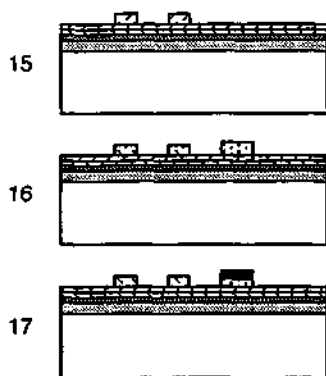


Fig. 4.10 Deposition and patterning of noble metal electrodes

With the same technique, the Ag for the RE is structured: First, the PR+ layer is patterned (mask 12) and treated with the chlorobenzene. Then 1.2 μm of Ag is evaporated, with a 300 Å Ti adhesion layer. The lift-off is again performed in acetone (16). Onto the structured Ag, a PR- is patterned using the mask 13, which defines the areas, where the Ag has to be chloridized. The partial conversion of the Ag layers (10-30 %) to AgCl is performed in 25 mM FeCl_3 solution during 2 s (17).

Observed problems:

- 1) The lift-off for the Ag layer is more problematic than the one for Pt because a 1.2 μm thick Ag layer is used and because Ag is more elastic than Pt. The prebake, the exposure time, the development time and the time in the chlorobenzene have to be optimized to achieve a good "overhang" structure of the photoresist prior to the Ag evaporation. Fig. 4.11 shows a typical profile of a silver layer after the lift-off.

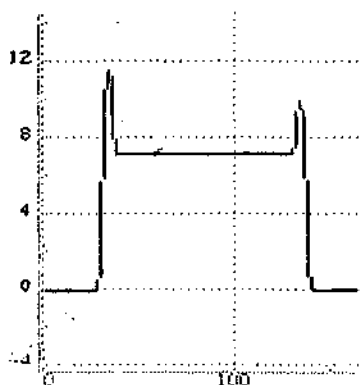


Fig. 4.11

Typical profile of a silver layer after the lift-off: The peaks at the border of the silver layer are due to Ag evaporation on the sidewall of the photoresist

It is important to strictly control the chloridation time in the 25 mM FeCl_3 solution. If too much of the Ag layer is transformed into AgCl, then the adhesion of the Ag on the Ti adhesion layers becomes very bad.

- 2) After processes 15 and 16, the threshold voltage of the Al_2O_3 ISFETs is shifted in a random distribution to negative potentials. The reason for this phenomenon is not clear; perhaps it is the γ -radiation emitted from the e-gun during the evaporation of Ag and Pt. This radiation could induce charges in the gate oxide of the ISFETs. With a temperature annealing at 250 °C and 18 h, this shift of the threshold voltage is partially removed. However, this annealing causes a reaction between the Al and the AgCl to aluminium chloride, causing a deposition on the Al contacts.

4.3.4 Deposition and patterning of the polyacrylamide hydrogel layer

The processing for the on-wafer deposition and photolithographic patterning of polymeric layers, used for the HG and the GPM, is described in detail in chapter 6. After a silanization of the transducer surface, a given volume of the acrylamide monomer solution is cast on the wafer. The viscous, but liquid monomer layer (50 - 100 μm) is leveled using a glass plate. The wafer is then exposed for 3 minutes to UV light having an intensity of 10 mW/cm^2 . Mask 13 is used, on a standard mask aligner. After the polymerization, the unexposed parts are removed in water.

4.3.5 Deposition and patterning of the polysiloxane gas permeable membrane

The gas permeable membrane is deposited following the same procedure as that described for the polyacrylamide hydrogel. The silanization is not repeated prior to the deposition. For the patterning of typical membrane thicknesses of 200 μm , the exposure time through mask 14 is between 3 and 4 min and xylene is used for the development.

The complete device after all fabrication steps is shown in Fig. 4.12:

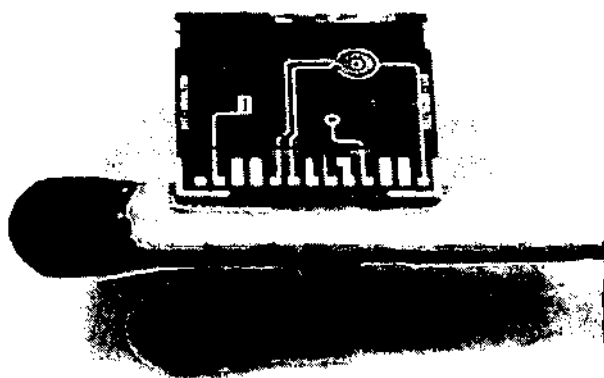


Fig. 4.12 Photograph of a complete device

4.4 Characterization

4.4.1 Introduction

After dicing the wafer, the chip is glued onto an epoxy printed circuit board (PCB), and then the wire bonding is performed. The borders of the chip and the wire bonds are covered with epoxy. An epoxy glue is used which cures at room temperature to avoid exposing the HG and the GPM to elevated temperatures. The chip on the PCB is mounted in a polycarbonate flow-through cell. This cell consists of an upper and a lower block, which take up the chip in a sandwich assembly. The upper block provides an inlet and an outlet for the analyte and a flow chamber, where the sample is exposed to the sensitive parts of the chip. This block is pressed onto the topside of the chip, in particular onto the border regions of the GPM. Thus the GPM is used to seal the analyte chamber. The lower block incorporates three chambers, which are filled with an electrolyte solution. By pressing the lower block to the backside of the wafer, these electrolyte reservoirs are connected to the openings in the chip, using commercial O-rings for sealing. A Ag/AgCl wire can optionally be put into the electrolyte reservoir.

The lower block with the electrolyte reservoirs is not very helpful because the electrolyte does not easily flow through the openings in the chip into the hydrogel layers. Usually, these openings are blocked with air bubbles which prevent any passage of liquid. Furthermore, the handling of the lower block is unpractical (three electrolyte solutions to handle). Thus, all the following characterizations have been performed without using the electrolyte reservoir on the backside.

However, these holes through the wafer have been very useful for the hydration of the whole sensor before it is placed in operation. For this preconditioning, the sensor is immersed in the desired electrolyte solution, which results in an efficient hydration of the HG due to the direct contact with the electrolyte solution. If, on the other hand, these holes through the wafer are closed with tape, then the HG is humidified only by water vapor diffusing through the GPM. This is very slow, and results in an ill-defined concentration of KCl and NaHCO₃ in the HG. If the sensor is well hydrated at the beginning of the operation and then constantly held in contact with a liquid sample, the water content in the HG remains fairly constant due to water vapor equilibration. Therefore, an electrolyte reservoir at the backside of the wafer is not longer necessary during operation.

Surprisingly, it has been observed that the adhesion of the hydrogel to the SiO₂ transducer surface decreases with increasing duration of hydration. This may be explained either by a degradation of the chemical links obtained from silanization of the SiO₂, or by the higher mechanical stress in the hydrogel due to the increased swelling.

4.4.2 Methods

Preparing O₂/CO₂/N₂ gas mixtures:

For the preparing of the gas mixtures a static gas blender is used (Digital Static Gas Blender DSGB-20, Bi-M Instrument Company). To obtain aqueous solutions equilibrated with defined gas mixtures, the gases are bubbled through the solution during at least 30 min in a closed glass beaker of 100 ml.

Preparing solutions with defined pCO₂:

An easy way of preparing solutions with defined CO₂ concentrations has been described by Mostert in [4.5]. A phosphate pH buffer of pH 7.4 is used, consisting of a mixture of 50 ml of 100 mM KH₂PO₄ and 39.1 ml of 100 mM NaOH with an ionic strength of 0.156. By adding NaHCO₃ to this buffer, the concentration of CO₂ can be controlled according to the following relationship:

$$[\text{CO}_2] = 0.0586 [\text{HCO}_3^-] \quad \text{both in concentrations mM}$$

The Henry's law constant of CO₂ in aqueous solution at 25 °C is $0.0433 \frac{\text{mM}}{\text{mmHg}}$ CO₂ under atmospheric pressure and by neglecting the water vapor pressure. Accordingly, the pCO₂ is obtained as:

$$\text{pCO}_2 = \frac{0.0586}{0.0433} [\text{HCO}_3^-] = 1.353 [\text{HCO}_3^-]$$

with pCO₂ in mmHg and [HCO₃⁻] in mM

If the concentration of NaHCO₃ in g/l is used (1 M is equivalent to 84 g/l), the final result reads:

$$\text{pCO}_2 = 16.11 [\text{NaHCO}_3] \quad (4.1)$$

with pCO₂ in mmHg and [NaHCO₃] in g/l

This relationship is valid only for limited concentrations of NaHCO₃. The pH of the phosphate buffer solution does not change up to a concentration of 1 g/l. At 2 g/l, the pH increases by 0.06 units, at 4 g/l by 0.16 units, and at 8 g/l by 0.26 units. Therefore, to calibrate correctly, the concentration of NaHCO₃ should not exceed 2 g/l.

The advantage of this method is, beside the simple preparation, that an eventual leak in the GPM can be immediately recognized: the constant pH of the sample also determines the inner electrolyte in the case of a leak, therefore the sensor does not respond to $p\text{CO}_2$ changes (only a transient peak is visible, but the signal returns to its pH determined baseline).

Measurements in transfusion blood:

Transfusion blood is obtained from the Hôpital des Cadolles in Neuchâtel. For blood transfusion, a concentrate of erythrocytes in a buffer solution is used instead of whole blood. This transfusion solution can be stored for several months at 4 °C. It is prepared in the hospital starting from 450 ml of fresh human blood, from which the plasma and the blood platelets are removed in a centrifuge. A 200 ml volume of a concentrate of erythrocytes results, which is added to 100 ml of citrate phosphate dextrose solution (CPD). CPD is necessary to prevent clotting of the erythrocytes during storage; when introduced into the human body, however, it is quickly absorbed minimizing danger of bleeding. Since CPD contains some citric acid, the pH of transfusion blood is around 6.4, lower than the value for whole blood.

For equilibrating the blood samples with $\text{O}_2/\text{CO}_2/\text{N}_2$ gas mixtures, a tonometer (Instrumentation Laboratory 1L 237) is used. Eight ml of blood is introduced into a thermostated (37 °C) sample vessel, which rotates so that a thin film of blood deposits on the walls of the vessel. This thin blood film is in contact with the humidified gas mixture, thus a good equilibration is achieved. The gas mixture is fed into the tonometer directly from the gas blender. The tonometry is performed during at least 30 min, at a constant gas stream.

The sensor is mounted in the flow-through cell, and the peristaltic pump is connected downstream of the sensor cell. The blood samples are aspirated through the cell, directly out of the tonometer, after completion of the tonometry. A PVC inlet tubing is used, being as short as possible to minimize memory effects of the tubing walls and influences of the ambient air, and in order to keep the temperature of the tonometered blood as constant as possible. The sensor cell itself is not thermostated, thus the temperature of the blood sample is somewhat decreased when it arrives in the cell. The sensor signal is not temperature corrected.

Between the blood samples, the flow-through cell is rinsed with a solution of 50 mM NaHCO_3 and 100 mM KCl equilibrated with N_2 .

4.4.3 pO_2 sensor

Sensitivity in aqueous solution:

Fig. 4.13 shows a calibration curve of the pO_2 sensor, as obtained when the chip is immersed in a unstirred solution equilibrated with different O_2/N_2 gas mixtures. The WE is polarized only in a pulsed mode and the reading is taken after 1 min of polarization. A higher sensitivity (5 nA/mmHg) is observed at pO_2 lower than the air saturated level, and a lower sensitivity (3.5 nA/mmHg) at higher pO_2 . This is probably due to the fact that it is difficult to establish precise pO_2 levels in the solution due to gain / loss from the ambient air.

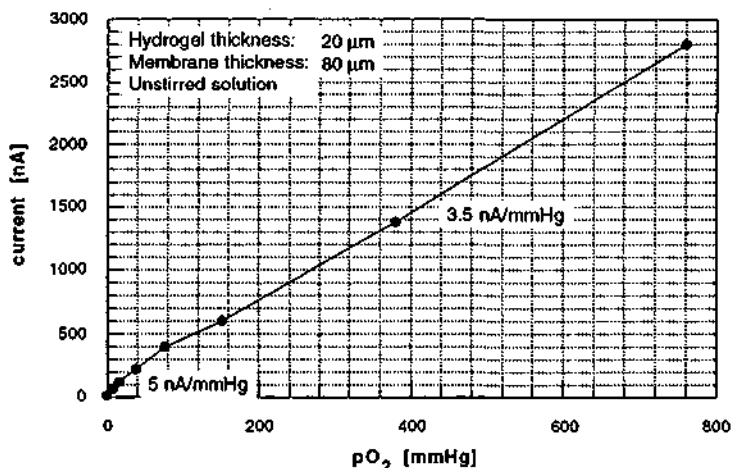


Fig. 4.13 Calibration curve of the pO_2 sensor immersed in an unstirred 50 mM $NaHCO_3$ / 50 mM Na_2CO_3 / 1 mM KCl solution equilibrated with different O_2/N_2 gas mixtures. The reading is taken always 1 min after switching on the potential (-650 mV).

Fig. 4.14 displays the calibration curve for the pO_2 sensor mounted in the flow-through cell; a constant polarization potential is applied. A perfectly linear calibration plot is obtained, resulting in a correlation coefficient of the linear regression of 0.9997.

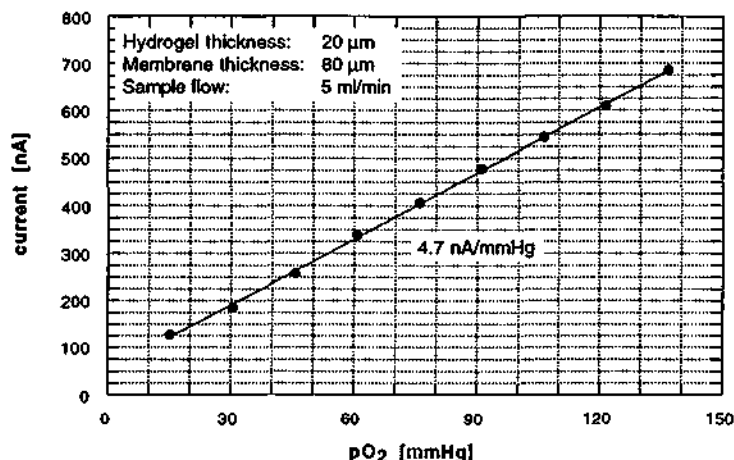


Fig. 4.14 Calibration curve of the pO₂ sensor mounted in the flow-through cell and constantly polarized (-650 mV). The solution (50 mM NaHCO₃ / 50 mM Na₂CO₃ / 1 mM KCl) is first equilibrated with different O₂/N₂ gas mixtures and then pumped through the cell with a flow of 5 ml/min. The steady state current is taken as reading.

The theoretical sensitivity, as calculated from eqn. (3.27), is in the range of 4.4 to 11.6 nA/mmHg for a sensor with 20 μm HG / 80 μm GPM. These values are obtained using the following assumptions. The diffusion coefficient of O₂ is $D_m = 1.6 \cdot 10^{-5} \text{ cm}^2/\text{s}$ for polysiloxane and $D_e = 3.0 \cdot 10^{-6}$ to $9.0 \cdot 10^{-6} \text{ cm}^2/\text{s}$ for polyacrylamide. For O₂ in polyacrylamide, the diffusion coefficient has been determined by the Cottrell method, as described in chapter 6. It is found that the hydrogel used in this work has a D_e which is 2 to 6 times lower than the diffusion coefficient reported for pure water ($1.8 \cdot 10^{-5} \text{ cm}^2/\text{s}$). The solubility for O₂ in the hydrogel is set equal to that in water, and the O₂ solubility for GPM is set 10 times higher than for water. Of course, these values are only approximately known, and the theoretical model neglects the presence of a O₂ depletion layer in the sample solution. The typical sensitivity of around 5 nA/mmHg O₂, or about 1 μA ($\pm 200 \text{ nA}$) in air saturated solution, which has been observed for all characterized sensors, is therefore well in the expected range.

Sensitivity in transfusion blood:

In Fig. 4.15, the calibration curve of the pO_2 sensor for transfusion blood is plotted. The measurements are performed as described in ch. 4.4.2.

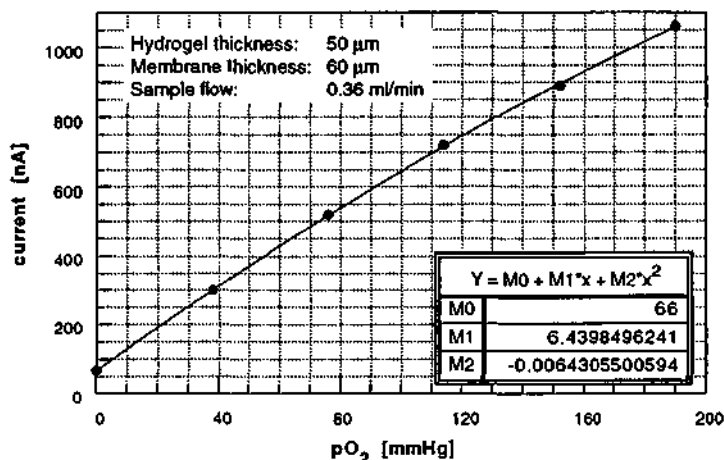


Fig. 4.15 Calibration curve of the pO_2 sensor in tonometered transfusion blood at 34 °C. The sensor is mounted in the flow-through cell and constantly polarized (-700 mV). The sample flow is 0.36 ml/min. Between the blood samples, the cell is rinsed with an aqueous solution, equilibrated with N_2 .

Evidently, this calibration curve is not as linear as the calibration curve obtained before in aqueous solution. Due to the low sample flow used for the blood measurements, the influence of the gas permeability of the flow-through system is believed to be higher in this case. The cell is rinsed between two blood sample measurements with an aqueous solution equilibrated with pure N_2 . The carry over of the oxygen free rinsing solution to the blood samples appears to be more important when the oxygen content of the sample is high. This is the reason for the observed decreasing sensitivity for increasing pO_2 . The slow response of the sensor can also be attributed to the low sample speed and to the memory effect of the flow-through system. It is therefore necessary to use calibration / rinsing solutions with pO_2 levels in the physiological range.

Voltammogram:

For choosing the operation potential for the reduction of O_2 , it is important to know the current / potential relationship (voltammogram). The operating potential has to be in the limiting current plateau in order that small variations in the polarization will have no significant effect on the measured current. A voltammogram of the pO_2 sensor is shown in Fig. 4.16. A narrow current plateau appears at -700 to -900 mV.

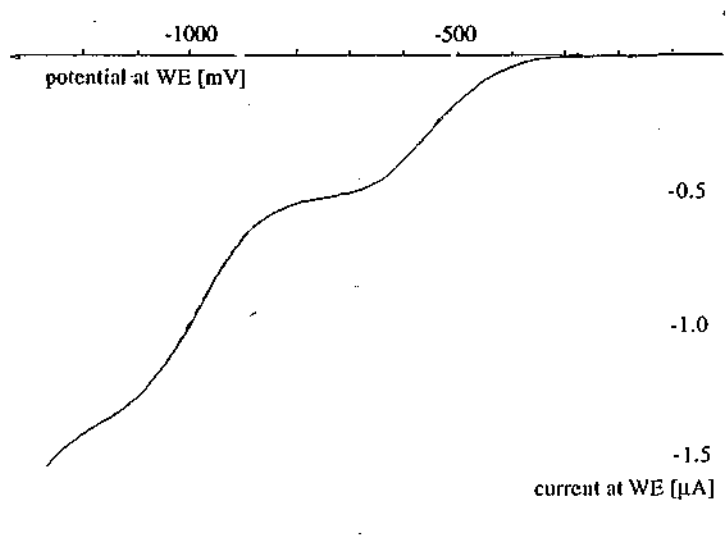


Fig. 4.16 Voltammogram recorded at a scan rate of 5 mV/s in air saturated 50 mM $NaHCO_3$ / 50 mM Na_2CO_3 / 1 mM KCl solution. The sensor is immersed in a beaker and is not mounted in the flow-through cell. The solution is not stirred.

Fig. 4.17 shows voltammograms of test Pt microelectrodes differing in their design from the pO_2 sensor presented here. A bare Pt electrode, the same electrode covered with a hydrogel layer, and an electrode covered with both hydrogel and gas permeable membrane are used to study the voltammogram under different conditions.

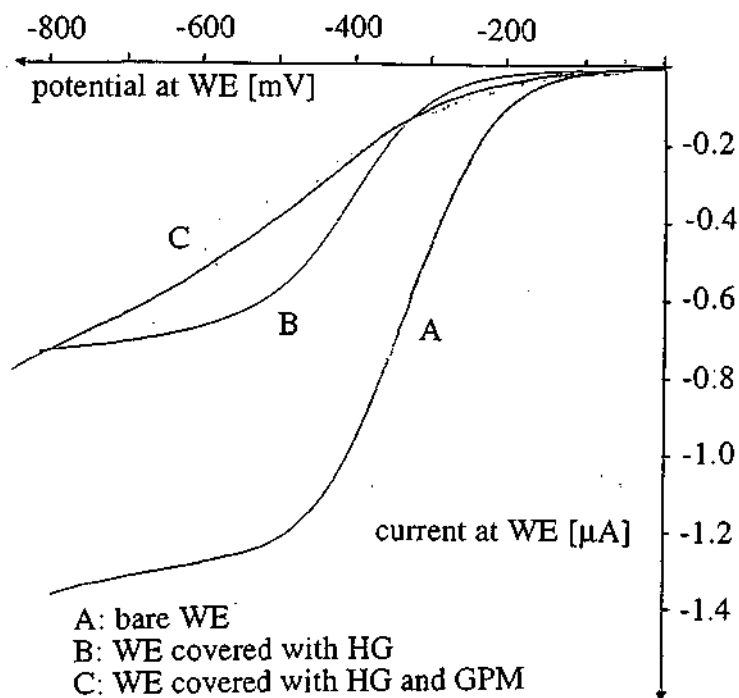


Fig. 4.17 Voltammogram of a Pt electrode (WE: 0.085 mm^2), with a RE and CE on the same chip: A) bare WE; B) same WE covered with a hydrogel layer; C) same WE covered with a hydrogel and a gas permeable membrane. Measurements performed in $50 \text{ mM NaHCO}_3 / 50 \text{ mM Na}_2\text{CO}_3$ solution saturated with $100 \% \text{ O}_2$. The scan speed is 25 mV/s .

One can clearly recognize, the more the electrode is covered, the less the limiting current plateau is developed. There are several reasons for such behavior:

- 1) Perhaps, the scan speed is too fast for establishing a steady state at each point of the voltammogram. As a matter of fact, the voltammograms of traditional pO_2 electrodes are usually recorded during 12 hours of scan time between 0 and -1000 mV [4.6]. The HG and the GPM have entrapped a certain volume of oxygen which has to be consumed before the WE is really sensing the true oxygen level of the sample. It can be observed that the rate, at which the potential is scanned, influences the shape of the voltammogram. The observation that the plateau is more

pronounced in unstirred solution than in stirred one, is indicative for the high O_2 consumption by this sensor.

- 2) A second reason could be that the activity of the Pt electrode is reduced due to the lower humidity of the HG. If the water content is too low, an extremely negative potential would be necessary to cause a diffusion-limited current.
- 3) Also the pH of the electrolyte is of considerable importance for the activity of the WE. If the initial pH is too low, the accumulation of OH^- ions in the vicinity of the WE will change its activity.
- 4) Furthermore, the surface of the Pt WE is well known to have a certain memory: previously applied potentials have an influence, due to adsorption effects, oxide layers at the surface, and other effects. Even passivation phenomena may appear in extreme cases.

This poorly defined limiting current plateau is at variance with voltammograms observed for traditional Clark electrodes, where the (published) plateaus are always very wide and flat [4.7]. However, for microelectrodes, several authors have reported similarly poor defined plateaus [4.8], [4.9], [4.10].

Response time:

The response time of a pO_2 sensor with a $10\text{ }\mu\text{m}$ HG and a $160\text{ }\mu\text{m}$ GPM, mounted in the flow-through cell and exposed to O_2/N_2 gas mixtures, is 12 s for t_{90} and 18 s for t_{95} . These values correspond well to theoretical values which are 5.4 to 6.3 s for t_{90} and 7 to 7.7 s for t_{95} when eqn. (3.26) is used ($D_m = 1.6 \cdot 10^{-5}\text{ cm}^2/\text{s}$ and $D_e = 3.0 \cdot 10^{-6}$ to $9.0 \cdot 10^{-6}\text{ cm}^2/\text{s}$). Fig. 4.18 shows repeated responses to N_2 and air.

However, the same sensor mounted in the flow-through cell, but exposed to solution equilibrated with O_2/N_2 gas mixtures, is 10 times slower: 120 s for t_{90} and 180 s for t_{95} . This liquid / gas difference of the response cannot be explained with eqn. (3.26) because the theoretical model neglects all influences arising outside the GPM. In Fig. 4.18, at the right side, the sensor is exposed to N_2 saturated solution, which clearly results in a slower response.

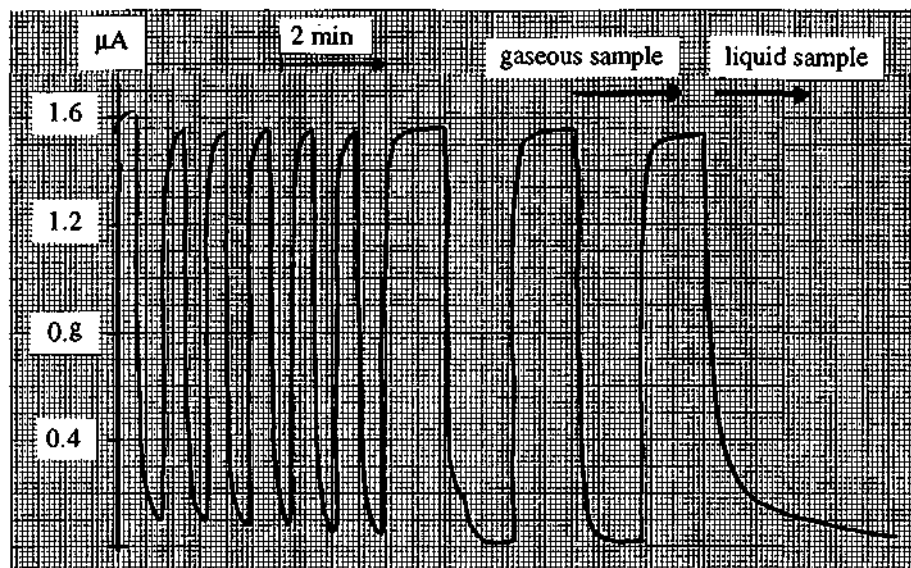


Fig. 4.18 Response to N_2 and air as gaseous samples. At the right side: the response to N_2 saturated solution.

This sluggish behavior in solution has probably two reasons:

- 1) The O_2 diffusion layer, which extends into the sample, is slowly established. In gaseous samples, due to the high diffusion coefficient, this depletion layer at the outer side of the GPM does not exist. For the above mentioned sensor, the current in air is actually 50 % higher than in air saturated solution, which indicates the presence of a fairly thick aqueous diffusion layer.
- 2) The response measured in the flow-through cell is not a priori the response time of the sensor, but more likely the response time of the whole measuring setup. The flow-through cell has its own response time which is the time needed to establish at the surface of the GPM the true pO_2 value of the sample. It is observed that the response of the sensor, when directly immersed into an unstirred or stirred solution, is faster than the response of the same sensor mounted in the flow-through cell. The reason for this is a memory effect existing for the polymeric tubings of the inlet system which are transparent for O_2 . Thus it takes considerable time to equilibrate correctly all the tubings and the housing of the flow-through cell.

If the sample flow is increased, the response of the system gets faster because the flow rate influences both above mentioned effects.

When measuring in solution, it can be observed that the response is faster for a rising step in oxygen partial pressure than when going down. In the literature, this phenomenon is often attributed to accumulation and slow decomposition of hydrogen peroxide in the vicinity of the WE, as well as to the slow rate at which it is consumed at the WE [4.11], [4.12]. According to the present work, a slow response is often dictated by the measuring setup. As a consequence, equilibrium is faster achieved when the end point is located close to ambient pO_2 , and slower when the end point is very different from ambient pO_2 .

In the literature, Friesen and McIlroy [4.13] described the fastest pO_2 electrode having a response time of 0.08 s (measured in gas) when using a 3 μm thick Teflon membrane stretched to just below the breaking point. Standard values for the 90 % response time of blood oxygen sensors are about 10 s for 6 μm thick Teflon membranes and 30 s for 25 μm thick Teflon membranes.

The term "response time" is often not properly defined. For example, values are given for 90, 95 and 99 % step response, respectively, and they refer to measurements in gases or in liquids. Very often, however, no details on the mode of determination are specified.

Stability and lifetime:

After complete conditioning, the pO_2 sensor can be continuously operated during two weeks in air saturated solution with a stability of ± 2 %. The steady state current fluctuates less if the sensor is mounted in the flow-through cell, than if it is directly immersed in an unstirred solution (± 5 %) or in a stirred solution (± 10 %). In the case of the unstirred solution, this is due to small fluctuations in the thickness of the oxygen depletion layer at the surface of the GPM, which depends on the natural convection in the unstirred sample. If the solution is held in motion with a magnetic stirrer, the flow velocity at the surface of the GPM is still partly defined by random, and the current fluctuates again. In the flow-through cell, on the other hand, the flow at the GPM is precisely and reproducibly defined.

A slow drift of the steady state current is usually observed in both directions, depending on the extent of hydration in the HG, and the deposition rate of interfering silver on the WE.

If the sensor is held in an air saturated solution, but not in operation, and if it is polarized only every third day during a 15 min measurement, then the steady state current is reproduced with a precision of ± 1 %. However, if the

sensor is taken out of the solution, dried during 6 days and rehydrated during 1 day, then the steady state current can differ by $\pm 20\%$.

The lifetime of the sensor in continuous operation is in the order of 2 weeks. In most cases, the loss of adhesion of the HG and the GPM limits the lifetime. Sometimes, the silver deposition on the WE leads to a short circuit between WE and CE (see also below).

Flow dependence:

Fig. 4.19 illustrates the flow dependence of the pO_2 sensor mounted in the flow-through cell.

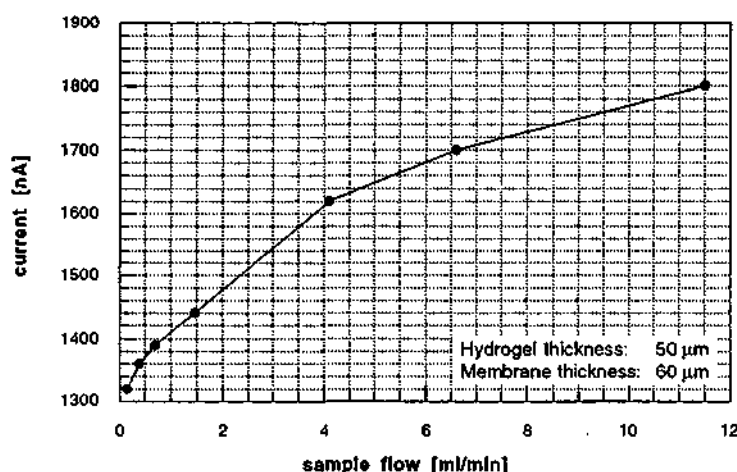


Fig. 4.19 Flow dependence measured in the flow-through cell, using air saturated phosphate buffer solution of pH 7.4 at different flow rates. The polarization voltage is -700 mV.

According to eqn. (3.37), the relationship between the steady state current (I_{∞}) and the flow velocity (v) is given by:

$$\frac{1}{I_{\infty}} = f_1 + f_2 \sqrt{\frac{1}{v}} \quad \text{with } f_1, f_2: \text{factors}$$

Therefore, the reciprocals of the steady state current values ($\frac{1}{I_{\infty}}$) in Fig. 4.19 are plotted in a new figure (Fig. 4.20) as a function of the flow velocity and are fitted on the basis of the above equation:

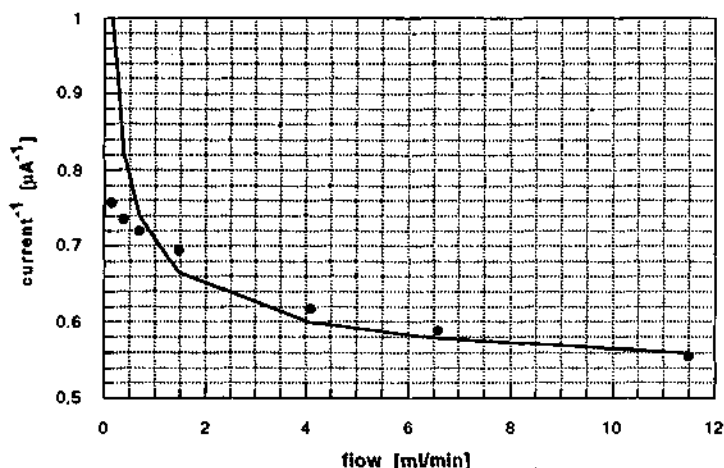


Fig. 4.20 $\frac{1}{\bar{I}_{\infty}}$ is plotted as a function of the flow velocity (data from Fig. 4.19). The dots are the measured values. The line is a curve fit with the regression function $0.5 + 0.2 \sqrt{\frac{1}{v}}$ (using the indicated dimensions).

The curve fit agrees well in the velocity range higher than 0.7 ml/min. In the lower range, the fit predicts a much higher flow dependence than actually measured. From eqn. (3.36) it follows that:

$$\frac{f_1}{f_2 \sqrt{\frac{1}{v}}} = \frac{\frac{d_m}{H_m D_m} + \frac{d_e}{H_e D_e}}{\frac{d_s}{H_s D_s}}$$

With the simplifications $D_s \equiv D_e \equiv D_m$, $H_s \equiv H_e$, and $H_m \approx 10 H_e$, one can estimate the thickness d_s of the stagnant layer in the sample at the surface of the GPM:

$$d_s \approx \frac{f_2 \sqrt{\frac{1}{v}}}{f_1} \left(\frac{d_m}{10} + d_e \right)$$

For this particular sensor, the thickness of the HG and the GPM is $d_e = 50 \mu\text{m}$ and $d_m = 60 \mu\text{m}$, respectively. Hence, the thickness d_s of the stagnant layer is approximately $30 \mu\text{m}$ at 0.7 ml/min, $16 \mu\text{m}$ at 2 ml/min, $8 \mu\text{m}$ at 8 ml/min and $6.5 \mu\text{m}$ at 12 ml/min.

CO₂ effect:

As discussed in chapter 3, the CO₂ diffusing into the HG decreases the pH of the internal electrolyte, and leads to a shift of the voltammogram to more positive potential values by an amount of 59 mV/pH. This follows from the Nernst equation for the equilibrium potential of the oxygen reduction:



$$E_{\text{eq}} = E^0 - \frac{RT}{4F} \ln \left(\frac{[\text{OH}^-]^4}{[\text{O}_2] [\text{H}_2\text{O}]^2} \right) \quad (4.2)$$

$$E_{\text{eq}} = E^0 - \frac{RT}{F} \ln [\text{OH}^-] + \frac{RT}{4F} \ln [\text{O}_2] \quad (4.3)$$

and with $K_w = [\text{H}^+][\text{OH}^-] = 1.0 \cdot 10^{-14}$

one obtains:
$$E_{\text{eq}} = E^0 + \frac{RT}{F} \ln [\text{H}^+] - \frac{RT}{F} \ln K_w + \frac{RT}{4F} \ln [\text{O}_2] \quad (4.4)$$

$$E_{\text{eq}} = E^0 - 2.303 \frac{RT}{F} \text{pH} - \frac{RT}{F} \ln K_w + \frac{RT}{4F} \ln [\text{O}_2] \quad (4.5)$$

If the limiting current plateau is wide and flat, then this pH shift does not change the WE current. However, if the limiting current plateau is not well developed, the shift of the voltammogram leads to a different current depending on the CO₂ partial pressure, as explained in Fig. 4.21:

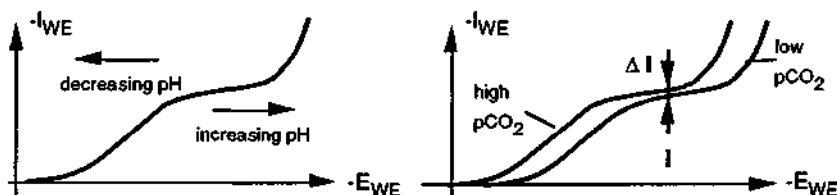


Fig. 4.21 On the left side: a decreasing pH shifts the voltammogram to more positive potentials; an increasing pH to more negative potentials, according to eqn. (4.5).

On the right side: differences in the pCO₂ shift the voltammogram, resulting in a different current reading (at a fixed potential). The current results to be more negative with increasing pCO₂.

Furthermore, the pH of the hydrogel also influences the shape of the voltammogram, as discussed in chapter 3.

As can be seen in Fig. 4.22, the measured current at the WE is in fact increasing with increasing $p\text{CO}_2$. In a concentration range up to 0.8 g/l of NaHCO_3 (corresponding to 13 mmHg of CO_2), the relative increase of the current is in the order of 6 % if the NaHCO_3 concentration is doubled. At higher concentrations, however, the CO_2 effect becomes considerably smaller.

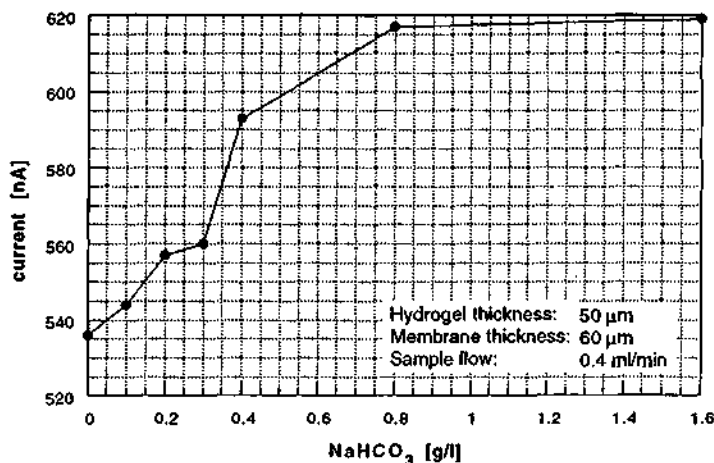


Fig. 4.22 Dependence of the reduction current on the $p\text{CO}_2$ of the sample. Current values measured in the flow-through cell using phosphate buffer solution of pH 7.4 with different concentrations of NaHCO_3 . Before adding the NaHCO_3 , the buffer solution was equilibrated with air. The sample flow is 0.4 ml/min and the polarization voltage is -700 mV.

The determination of the CO_2 effect by using phosphate buffer solutions with different concentrations of NaHCO_3 is rather problematic. If the buffer solution is air saturated prior to the addition of NaHCO_3 , the $p\text{O}_2$ is well defined. However, if NaHCO_3 is added, the $p\text{CO}_2$ of the solution is set to a new level, and therefore the total pressure of the solution and/or the individual partial pressures of the dissolved gases should change. Thus, the $p\text{O}_2$ after the addition of NaHCO_3 is not exactly known. The CO_2 effect could be investigated more precisely by using solutions that are equilibrated with $\text{O}_2 / \text{CO}_2 / \text{N}_2$ gas mixtures of constant $p\text{O}_2$.

Temperature dependence:

The temperature dependence of the pO_2 sensor is plotted in Fig. 4.23. The measured values at 25, 30, 35 and 40 °C are fitted according to eqn. (3.40), where an exponential temperature dependence is predicted. However, in the measured range, this exponential behavior is almost not visible. The linearized dependence in the range of 25 to 40 °C is 10 nA/°C. The relative dependence is therefore 1 %/°C, which is fairly low in comparison with the literature (usual range: 2 - 3 %/°C).

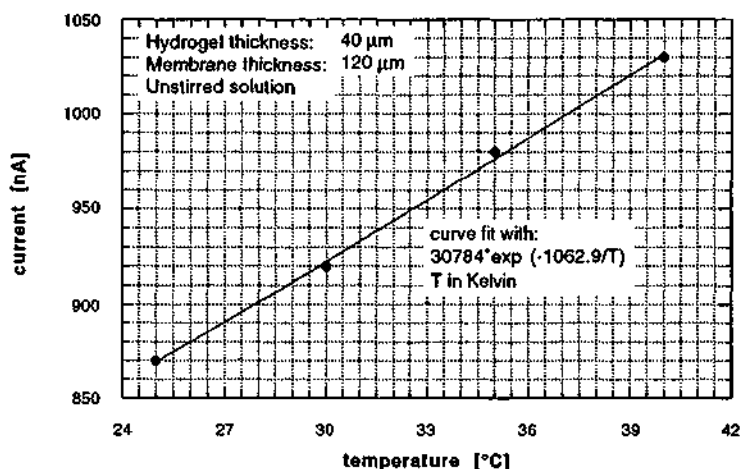


Fig. 4.23 Temperature dependence measured at 25, 30, 35 and 40 °C (dots). The chip is immersed in an unstirred air saturated solution (50 mM NaHCO_3 / 50 mM Na_2CO_3 / 1 mM KCl) which is held at a constant temperature (± 0.1 °C). The applied potential is -700 mV. The curve fit (line) is done according to eqn. (3.40).

Humidity dependence:

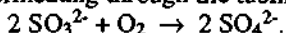
When the chip is immersed in solution, the hydrogel can be hydrated only by water vapor which permeates from the sample solution through the gas permeable membrane into the hydrogel. Full hydration needs more than 24 hours, and the swelling of the hydrogel due to the water uptake is clearly visible. During hydration, the current at the WE is continuously increasing, and also the shape of the voltammogram is changing. The steady state current obtained with the fully hydrated gel can be more than 20 times higher than for the nearly dry gel. One could imagine that, due to the swelling of the hydrogel, the current would decrease because the thickness of the hydrogel is growing, and thus the sensitivity would decrease, according

to eqn. (3.12). However, eqn. (3.12) is only valid, if the standard reaction rate at the WE is much higher than the diffusion rate of O_2 towards the WE. This condition is obviously not fulfilled if the gel is not completely hydrated. The reason for this could be a loss of the catalytic activity of the Pt surface, or, more likely, a high resistance between WE and CE, leading to a near loss of electrolytic contact under dry conditions. Furthermore, diffusion coefficients in the dry gel are considerably lower than in the hydrated gel, which could be another reason for the observed change in sensitivity. In addition, as H_2O is taking part in the O_2 reduction reaction, a reduced water content perhaps also slows down this reaction.

The reverse process, the dehydration of the hydrogel, can be studied by measurements in dry gas stream. The sensitivity is rapidly decreasing during the exposure to the dry gas. After 1 hour, the oxygen reduction current is almost zero. When the sensor is again exposed to a liquid sample, then the hydration takes much more time than the dehydration. The current is continuously increasing and reaches the previous level only after over 12 h of contact with the air saturated solution.

Residual current:

The residual current measured with N_2 saturated solution is typically between 2 to 5 % of the current found for air saturated solution. This value is limited more by the preparation of the solution than by the sensor itself. If a O_2 free solution is prepared by adding 2-5 % of Na_2SO_3 to the solution, the measured residual current is below 1 %. This is evidently due to the fact that Na_2SO_3 consumes all O_2 stored in the flow-through cell as well as the O_2 permeating through the tubings. The basic reaction is the following:



Ag^+ deposition on the WE:

Due to the negative potential applied on the WE, dissolved Ag^+ originating from the RE is continuously deposited on the WE. The deposition starts at the inner border of the WE, which is oriented towards the RE (Fig. 4.24). The silver plating continues to grow and finally covers the whole WE. Tree-like dendritic structures grow at the edges of the WE, where the electrical field is most inhomogeneous. These dendritic structures in the end lead to a short circuit between WE and CE.

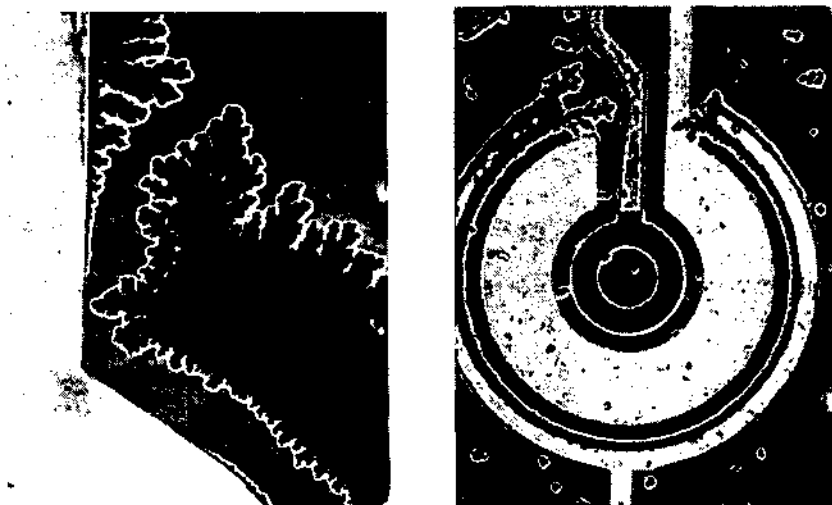


Fig. 4.24 Ag^+ deposition on the WE: situation after 2 weeks of continuous operation. On the right side: The inner borders of the WE are already covered, and dendritic structures are grown at the edges of the WE. The uncovered Pt surface appears white, the covered surface appears black. On the left side: detailed view of the edge.

It has been noted that in fully hydrated hydrogels the deposition rate is much slower, since apparently less Ag^+ is dissolved. By inverting the polarization of the WE for some hours, the deposited Ag^+ is removed from the surface of the WE, and re-dissolved in the gel, and finally it plates the CE.

On one pO_2 sensor, the GPM is removed, leaving only the HG, and an external RE is used instead of the on-chip RE. When this chip is operated continuously during 1 month, absolutely no plating on the WE is observed. This observation confirms that Ag^+ dissolved in the gel is clearly originating from the on-chip RE. Furthermore, the above sensor exhibits a much smaller current drift than the usual ones, which are covered with the GPM. Apparently, the current drift is partly caused by Ag^+ interference at the WE.

Reaction at the CE:

As mentioned in ch. 3.2.14, it is not clear, what kind of reaction is happening at the CE. Usually, the exact CE reaction is of minor importance, as long as it does not interfere with the WE reaction or deteriorate the electrolyte. However, for chips of the 1st Design where an external CE is combined with a bare WE structure, a different current at the WE is observed as compared with the same chip combination when using the on-chip CE. Two reasons may explain this observation:

- The on-chip CE is one of the limiting elements of the electrochemical cell, and hence the CE is limiting the current at the WE.
- The CE reaction produces electroactive species which diffuse to the WE where they are reduced. Thus, an additional current is generated at the WE, which is not due to reduction of the sample gas.

The possibility a) is checked using a bare structure of the 1st Design either with an external CE (Pt wire) or with the on-chip CE. When the on-chip CE limits the current, then the sensitivity should be higher in the experiment with the external CE. But exactly the opposite situation is observed, as shown in Fig. 4.25. Thus, the CE does not limit the current at the WE, and possibility a) does not apply.

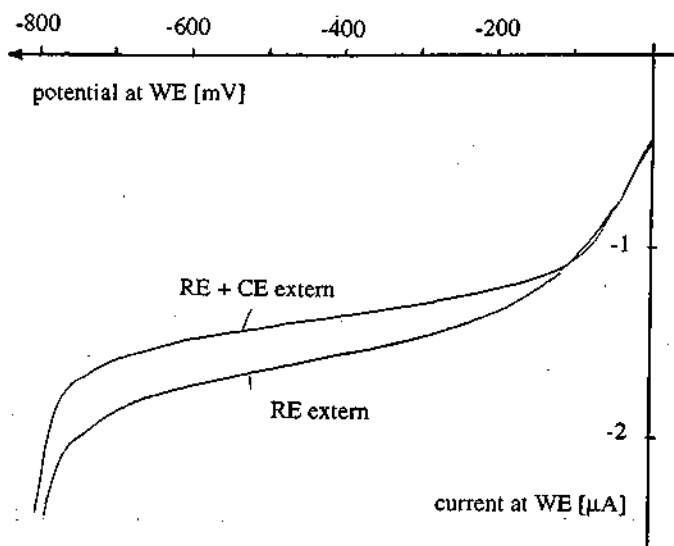


Fig. 4.25 Voltammogram of a bare pO_2 sensor of the 1st Design (HG and GPM are not deposited), recorded in air-saturated solution which is not stirred. The scan speed is 5 mV/s; an external Metrohm RE (SCE) is used. In the upper plot, an external CE is used; in the lower plot, the on-chip CE is used. A higher current is observed with the on-chip CE.

According to b), it is postulated that the products of the CE reaction interfere with the WE reaction, an effect which is known as electrochemical crosstalk. Fig. 4.25 shows that the current is higher with the on-chip CE; this additional current must be caused by products of the CE reaction that diffuse to the WE. Possible CE reactions are the oxidation of Cl^- to Cl_2 , or the oxidation of H_2O to O_2 . Since the first reaction proceeds only in the presence of Cl^- , a different voltammogram should result for measurements with chloride-free solution. However, in practice, such a voltammogram looks exactly like Fig. 4.25. Therefore, the main reaction at the CE is the oxidation of H_2O which probably produces O_2 ; the product diffuses laterally to the WE and causes the observed reduction current increase. This reaction can obviously not be stopped by extracting the water from the electrolyte. However, if the stirring rate near the electrodes is so high that the lateral diffusion CE and WE cannot build up, there should finally be no difference in the currents for on-chip and external CE. In fact, this result can be observed in Fig. 4.26, where the two voltammograms perfectly agree.

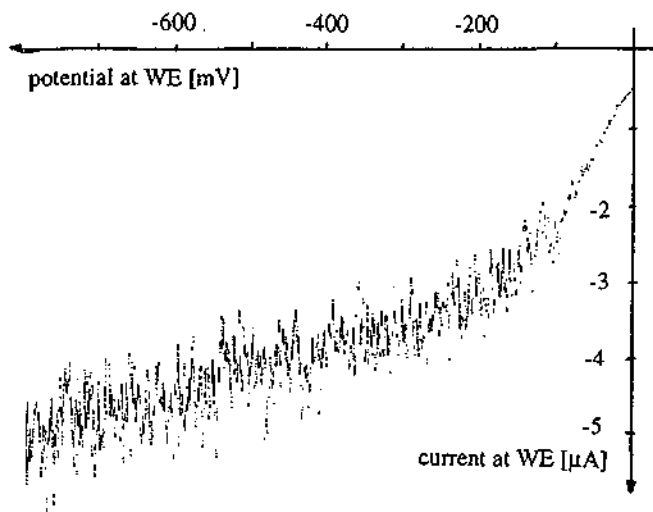


Fig. 4.26 Voltammogram of a bare structure of the design 1 (HG and GPM are not deposited), recorded in air-saturated solution with a high stirring rate. The scan speed is 5 mV/s; an external Metrohm RE (SCE) is used. The two voltammograms, one with an external CE and the other with the on-chip CE, are virtually identical. The fluctuation of the signal is due to the stirring.

According to the hypothesis b) the amount of O_2 diffusing from the CE to the WE is proportional to the total amount produced at the CE, and thus to the total current flowing between WE and CE. Therefore, if we increase the total current, the current difference between the experiments with external and on-chip CE should increase by the same factor. This can be verified by operating the sensor in samples with different pO_2 . Tab. 4.1 shows the ratios (given in %) between the current obtained with external CE and the current with on-chip CE in samples of different pO_2 . In fact, these current ratios are independent on the pO_2 of the sample and on the polarization voltage.

	Polarization voltage -500 mV	Polarization voltage -600 mV	Polarization voltage -700 mV
pO_2 : 38 mmHg	79.1	80.0	78.9
pO_2 : 152 mmHg	78.0	80.3	79.3
pO_2 : 760 mmHg	81.3	80.0	80.8

Tab. 4.1 Ratio of current values obtained with external CE and with on-chip CE ($100 \frac{i_{\text{external}}}{i_{\text{on-chip}}}$) (in %) in samples of different pO_2 and at different polarization potentials. Chips with a bare structure of the design 1 (HG and GPM are not deposited) are used in aqueous, unstirred solution saturated with O_2/N_2 gas mixtures. An external Metrohm RE (SCE) is applied.

4.4.4 $p\text{CO}_2$ sensor

If not mentioned differently, the ISFET of the $p\text{CO}_2$ sensor is operated with $I_D = 100 \mu\text{A}$ and $V_{DS} = 0.5 \text{ V}$. The measurements are performed at room temperature, and the chip is shielded against light.

Sensitivity in aqueous solution:

The sensitivity of the $p\text{CO}_2$ sensor can be measured in different ways: in solutions that are equilibrated with CO_2/N_2 gas mixtures, in phosphate buffer solutions of constant pH where the $p\text{CO}_2$ is determined by the bicarbonate concentration, or directly in CO_2/N_2 gas mixtures. Fig. 4.27 shows a calibration curve for solutions equilibrated with different CO_2/N_2 gas mixtures.

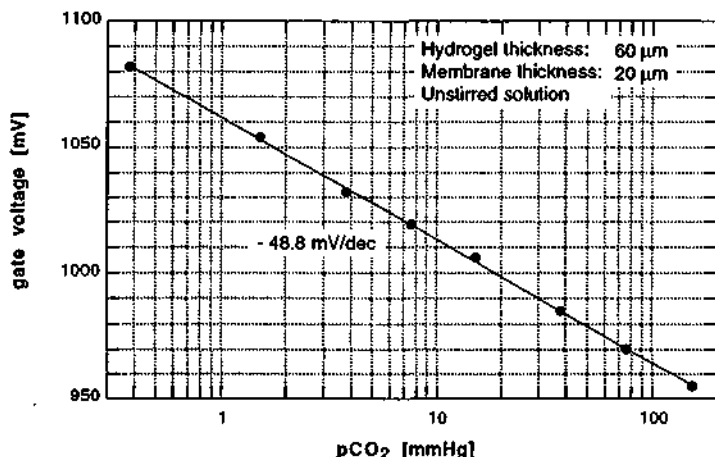


Fig. 4.27 Calibration curve of the $p\text{CO}_2$ sensor immersed in a 100 mM KCl solution which is equilibrated with CO_2/N_2 gas mixtures. The solution is not stirred. The correlation coefficient of the linear regression is 0.9997.

Results on the sensitivity of the pCO_2 sensor in buffer solutions of constant pH, to which NaHCO_3 is added to adjust the CO_2 partial pressure, are given in Fig. 4.28.

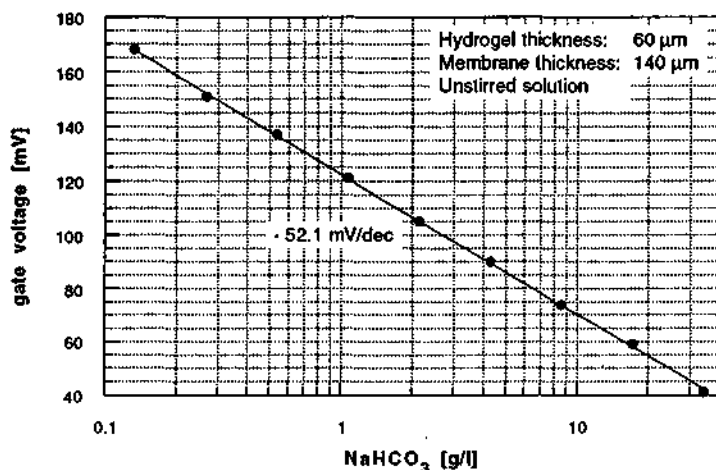


Fig. 4.28 Calibration curve of the pCO_2 sensor immersed in phosphate buffer solution of pH 7.4 with different concentrations of NaHCO_3 . The solution is not stirred. The correlation coefficient of the linear regression is 0.9998. The pCO_2 of the solution in mmHg can be calculated by multiplying the concentration of NaHCO_3 in g/l by the factor 16.11 (eqn. (4.1)).

In Fig. 4.29, two calibration curves of the same sensor are compared, one obtained with phosphate buffers, and the other measured directly on CO_2/N_2 gas mixtures. The two measurements agree fairly well in the absolute level of the sensor signals, and confirm the validity of eqn. (4.1). However, a slightly higher sensitivity is observed when using gas mixtures. The measurements on the buffer solutions are performed at 0.4 ml/min and 4 ml/min without any difference in the signal; thus the sensitivity of the pCO_2 sensor is independent on the sample flow velocity, as predicted by theory.

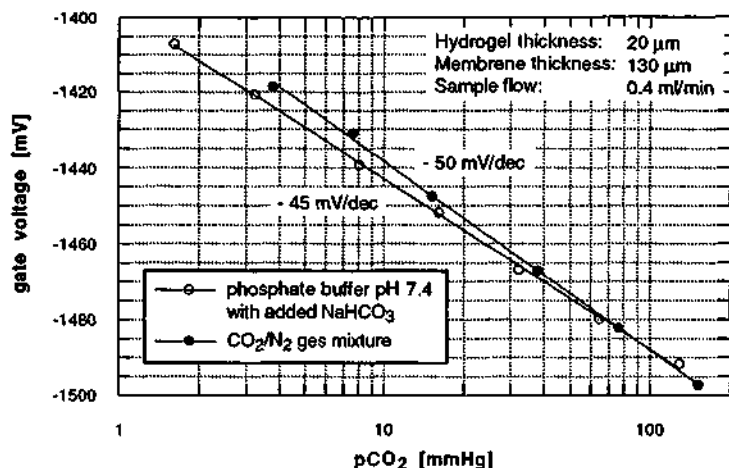


Fig. 4.29 Calibration curve of the $p\text{CO}_2$ sensor mounted in the flow-through cell. One set of measurements uses phosphate buffer of pH 7.4 with different concentrations of NaHCO_3 . The flow rate is 0.4 ml/min and 4 ml/min, respectively, without any difference in the signals. The $p\text{CO}_2$ of the solution in mmHg is calculated from eqn. (4.1). The correlation coefficient of the linear regression is 0.9997. The second set of measurements is performed directly in CO_2/N_2 gas mixtures. The correlation coefficient is 0.9996.

The linearity for all sensors studied is almost perfect in a wide range of $p\text{CO}_2$ levels. However, the sensitivity differs significantly from one sensor to another, ranging from -40 to -55 mV/dec. Reasons for such differences will be discussed later.

Sensitivity in transfusion blood:

In Fig. 4.30 is plotted the calibration curve of the $p\text{CO}_2$ sensor for measurements in transfusion blood. The measurements are performed as described in ch. 4.4.2.

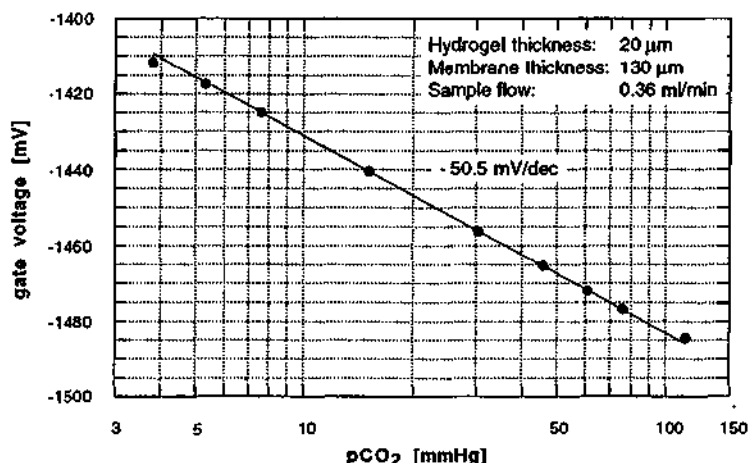


Fig. 4.30 Calibration curve of the $p\text{CO}_2$ sensor in tonometered transfusion blood at 34 °C. The sensor is mounted in the flow-through cell. The sample flow is 0.36 ml/min. Between the blood samples, the cell is rinsed with an aqueous solution, equilibrated with N_2 .

The calibration curve is very linear in the range of 5 to 80 mmHg, but it indicates a reduced sensitivity at $p\text{CO}_2$ values above 80 mmHg and below 5 mmHg. Exactly the same behavior is observed if the $p\text{CO}_2$ values are measured on aqueous solution equilibrated with the same gas mixtures, or if the $p\text{CO}_2$ values are sensed directly in the gas stream. All these measurements are compared in Fig. 4.31. The calibration curves for the blood and the aqueous samples correspond very well, the signals obtained for aqueous samples being shifted to slightly higher $p\text{CO}_2$ values. The measurements on the gas mixtures exhibit exactly the same sensitivity as for the blood and the aqueous samples, and they show the same decrease of sensitivity at low $p\text{CO}_2$ values. However, the calibration curve is uniformly shifted to more negative voltages, which corresponds to higher $p\text{CO}_2$ readings. This can be explained by the earlier statements that, for gaseous samples, the memory effect and the permeability of the flow-through system have no influence. Furthermore it has to be taken into account that, during the studies on liquid samples, the flow-through cell is always rinsed with a

N₂ equilibrated solution. Due to the memory effect, the readings of the samples will therefore tend towards lower pCO₂ levels.

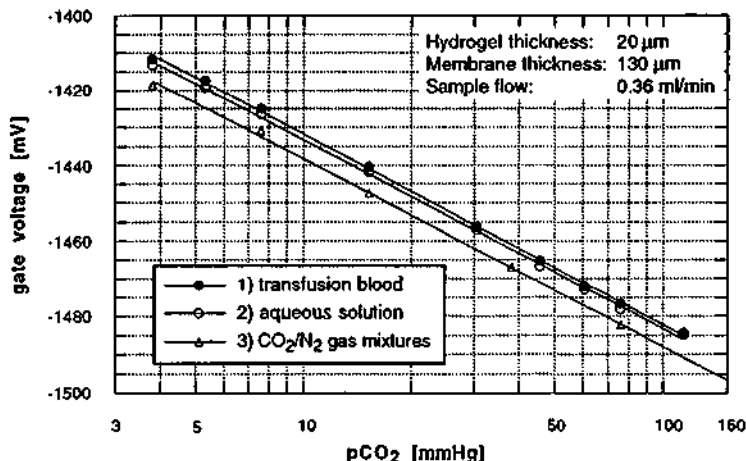


Fig. 4.31 Comparison of three different calibration curves of the same pCO₂ sensor:

- 1) In tonometered transfusion blood at 34 °C (as shown in Fig. 4.30). The sensitivity is -50.5 mV/dec, and the correlation coefficient of the linear regression is 0.9997.
- 2) In tonometered aqueous solution at 34 °C. The sensitivity is -49.9 mV/dec and the correlation coefficient of the linear regression is 0.9996.
- 3) In humidified CO₂/N₂ gas mixtures at room temperature. The sensitivity is -49.6 mV/dec and the correlation coefficient of the linear regression is 0.9996.

For these measurements, the sensor is mounted in the flow-through cell. The sample flow for the liquid samples is 0.36 ml/min. For the gaseous samples the flow is not precisely determined.

Humidity dependence:

The $p\text{CO}_2$ sensitivity seems to be strongly dependent on the humidity of the hydrogel, as can be seen in Fig. 4.32 and 4.33. When a sensor with dry hydrogels is taken into operation, the sensitivity is very low, but starts to increase upon exposure to a liquid. A second observation is that the pH of the internal electrolyte is generally shifted to higher values during hydration, and to lower values during drying. Hydration through the GPM is not very efficient because the humidity has to pass as water vapor through the GPM. This takes several days, depending on the thickness of the GPM. By contacting the hole through the wafer with an external electrolyte solution, water uptake by the hydrogel becomes much faster. But often, trapped air bubbles in the hole prevent such a hydration from the backside.

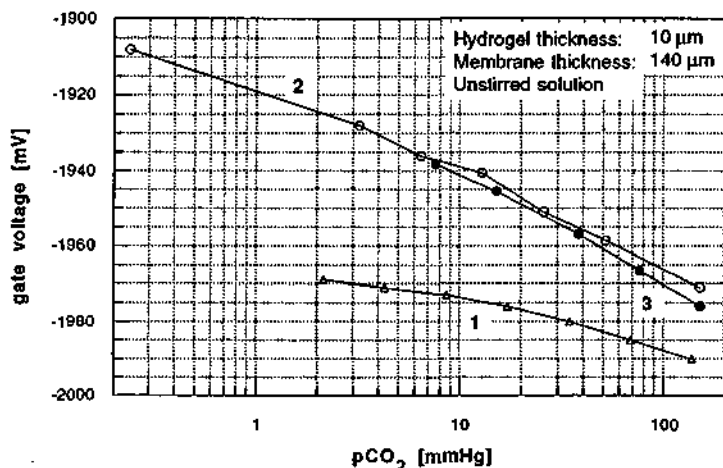


Fig. 4.32 Calibration curves of the $p\text{CO}_2$ sensor with partly humidified hydrogel.

- 1) Dry hydrogel without hydration; measurements in phosphate buffer of pH 7.4, with NaHCO_3 added to adjust the $p\text{CO}_2$; sensitivity is between -7 and -17 mV/dec
- 2) After 6 days of hydration through the GPM; measurements under the same conditions as for 1); sensitivity is -23 mV/dec
- 3) After 9 days of hydration through the GPM; measurements on CO_2/N_2 gas mixtures; sensitivity is -29 mV/dec

In Fig. 4.33, the hydrogel is hydrated during one night directly through a hole in the GPM, which is closed prior to the measurements. This direct hydration is more efficient than the hydration through the connection to the backside of the wafer.

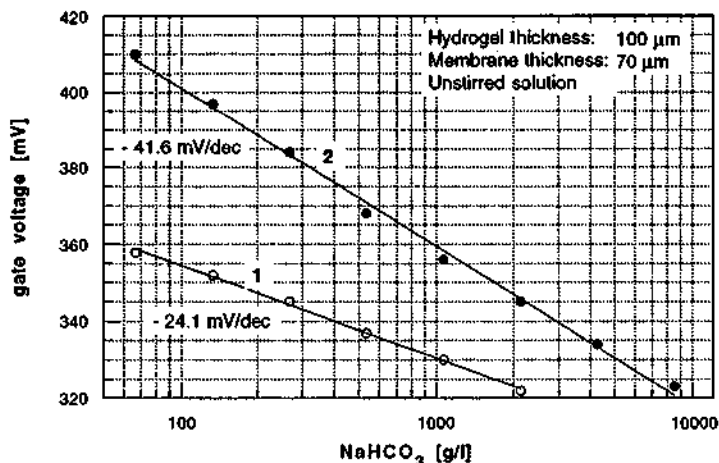


Fig. 4.33 Sensitivity of the pCO₂ sensor with partly humidified hydrogel.

- 1) The hydrogel has been hydrated over night through the GPM; measurements in phosphate buffer of pH 7.4 with NaHCO₃ added to adjust the pCO₂: sensitivity is -24.1 mV/dec and the correlation coefficient of the linear regression is 0.9991.
- 2) The hydrogel has been opened with a needle and hydrated over night in 50 mM NaHCO₃ and 100 mM KCl. The opening in the GPM is finally closed with silicone rubber. Measurements under the same conditions as for 1): sensitivity is -41.6 mV/dec and the correlation coefficient of the linear regression is 0.9982.

Reasons for the reduced sensitivity observed with dry hydrogel could be:

- a) a reduced pH sensitivity of the ISFET due to the low hydration of the sensitive layer, or
- b) a predominant dissociation of the CO₂ in the dry hydrogel, due to a low concentration of HCO₃⁻.

Point a) cannot be completely excluded. If the HG and the GPM of a pCO₂ sensor with low sensitivity is removed, and the pH calibration curve is recorded in aqueous solution, then a normal pH sensitivity is observed. The ISFET therefore operates correctly when immersed in a solution. However,

under dry conditions, a reduced pH sensitivity may result, as it is often mentioned for typical ISFETs (see below).

Evidence for point b) is seen in Fig. 4.32, where the sensitivity is reduced at low $p\text{CO}_2$ levels. The shape of these curves is quite similar to the theoretical calibration plots calculated in ch. 3 (Fig. 3.3). Throughout, a reduced sensitivity is predicted at moderately low $p\text{CO}_2$ levels if the NaHCO_3 concentration of the electrolyte is too low. It is possible, when using an unconditioned hydrogel, that the concentration of dissolved NaHCO_3 near the ISFET is much lower than after conditioning. Thus, the resulting pH change may be considerably lower than 1 pH/dec.

Response time:

As discussed in ch. 3, the response of the $p\text{CO}_2$ sensor depends on several parameters: on the direction and the amount of the $p\text{CO}_2$ change, on the thickness of the GPM and of the HG, and on the flow rate. These theoretical predictions are corroborated by experimental evidence.

a) Response time for increasing and decreasing $p\text{CO}_2$ steps:

Fig. 4.34 shows a typical response vs. time curve, recorded in gases. The different response times for increasing or decreasing $p\text{CO}_2$ are clearly manifest.

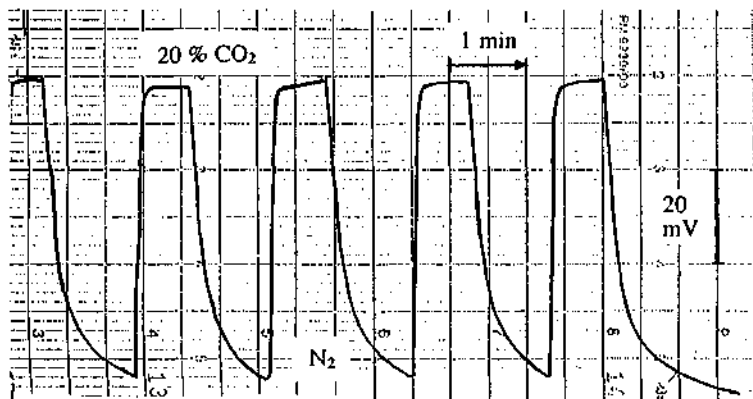


Fig. 4.34 Time response of the $p\text{CO}_2$ sensor to N_2 and to a mixture of 20 % CO_2 / 80 % N_2 (gaseous samples).

b) Response time for different amounts of the $p\text{CO}_2$ change:

The dependence of the response time on the amount of the step change is shown in Fig. 4.35. The line indicates the theoretical value of t_{90} in τ units, and the dots are measured values for t_{90} (in solution). It is clearly demonstrated that the actual response follows the predicted behavior.

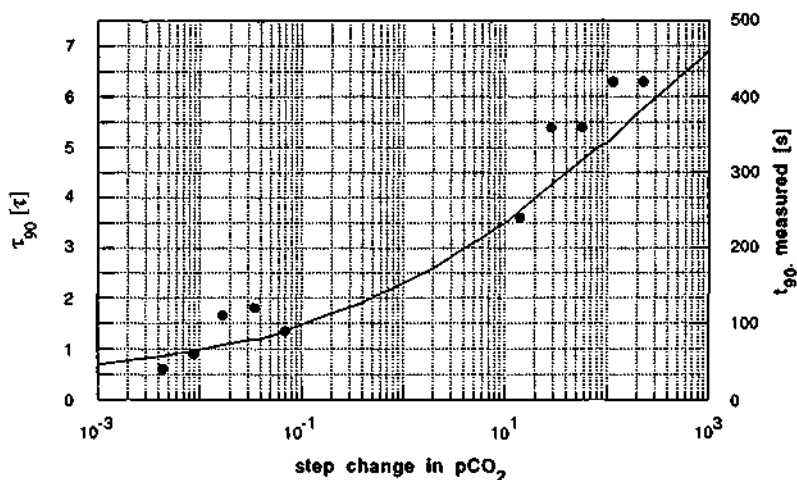


Fig. 4.35 Response time values for different $p\text{CO}_2$ step changes. The x-axis represents the value $\frac{p\text{CO}_2(t<0)}{p\text{CO}_2(t>0)}$. The line is the theoretical curve calculated from eqn. (3.67) and expressed in τ units for a $10 \mu\text{m HG} / 140 \mu\text{m GPM}$. The dots are measured values for t_{90} (in solution), in seconds.

c) Response time for different thicknesses of HG and GPM:

Tab. 4.2 shows the response times of four $p\text{CO}_2$ sensors, having different thicknesses of HG and GPM. The measured response times are compared with the time constants τ from eqn. (3.67):

d_e [μm] (hydrogel)	d_m [μm] (membrane)	t_{95} [min] $p\text{CO}_2 \uparrow$	t_{95} [min] $p\text{CO}_2 \downarrow$	time constant τ
20	60	2	7	2.5 - 3.6
40	160	2.5	21	15 - 21
30	130	2.5	50	10 - 13
100	70	5	85	12 - 25

Tab. 4.2 Response times t_{95} for four $p\text{CO}_2$ sensors with different thicknesses of HG and GPM, for increasing $\uparrow$ and decreasing $\downarrow$ $p\text{CO}_2$ steps, measured by immersion in phosphate buffers of pH 7.4 with different concentrations of NaHCO_3 . The measured values are compared with the calculated time constants τ resulting from eqn. (3.67), for $D_m = 1.1 \cdot 10^{-5} \text{ cm}^2/\text{s}$ and $D_e = 3.0 \cdot 10^{-6}$ to $9.0 \cdot 10^{-6} \text{ cm}^2/\text{s}$.

d) Response time for different flow rates:

Beside the response differences found for increasing / decreasing $p\text{CO}_2$ steps, the response vs. time behavior depends also on the flow rate of the sample, as it has been discussed for the $p\text{O}_2$ sensor. In Tab. 4.3, the response time values t_{90} and t_{95} are given for measurements in gases and solutions at different flow rates.

flow [ml/min]	t_{90} [s] $p\text{CO}_2 \uparrow$	t_{95} [s] $p\text{CO}_2 \uparrow$	t_{90} [s] $p\text{CO}_2 \downarrow$	t_{95} [s] $p\text{CO}_2 \downarrow$
gas	9	12	78	120
0.5	150	300	-	-
1.7	150	270	390	960
3.8	60	120	390	720
10.6	70	110	510	720
24.6	-	-	330	480

Tab. 4.3 Response times t_{90} and t_{95} for a $p\text{CO}_2$ sensor with 10 μm HG / 140 μm GPM in gas and solution, respectively, for increasing $\uparrow$ and decreasing $\downarrow$ $p\text{CO}_2$ steps at different sample flow rates. The response in gas is measured by using air (0.03 % CO_2) and 20 % CO_2 in N_2 . The response in solution is measured by using an air-saturated phosphate buffer of pH 7.4 and the same phosphate buffer with 3.2 g/l NaHCO_3 (53 mmHg CO_2).

The theoretical response times calculated from eqn. (3.67) for a sensor with 10 μm HG / 140 μm GPM and for a change 0.03 % CO_2 / 20 % CO_2 are 7 - 7.5 s (t_{90}) and 11 - 12 s (t_{95}) for increasing $p\text{CO}_2$, and 55 - 60 s (t_{90}) and 63 - 70 s (t_{95}) for decreasing $p\text{CO}_2$. These values are quite close to the response times measured in gas. The CO_2 diffusion coefficients used for the calculations are $D_m = 1.1 \cdot 10^{-5} \text{ cm}^2/\text{s}$ and $D_e = 3.0 \cdot 10^{-6} - 9.0 \cdot 10^{-6} \text{ cm}^2/\text{s}$. The CO_2 solubility of the hydrogel is set equal to that of water, and the CO_2 solubility of the GPM is assumed to be 2.6 times higher than for water.

Temperature dependence:

Fig. 4.36 displays the change of the steady state signal induced by a temperature variation. In the range of 20 to 40 $^{\circ}\text{C}$, the temperature effect is very linear with $\frac{\delta V_{\text{GS}}}{\delta T} = -0.8 \text{ mV}/^{\circ}\text{C}$. A temperature change of 1 $^{\circ}\text{C}$ therefore causes a relative error of about 3 - 3.5 % in the $p\text{CO}_2$ determination (without recalibration).

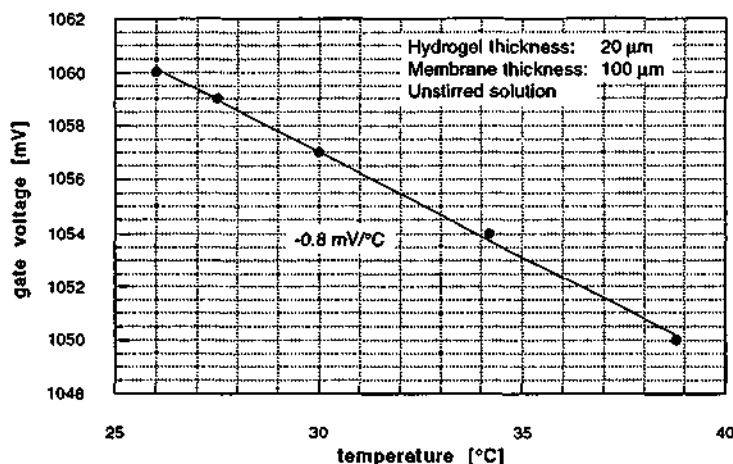


Fig. 4.36 Temperature dependence, measured at 26, 27.5, 30, 34.2 and 38.8 $^{\circ}\text{C}$ (dots). The chip is immersed in an unstirred air saturated Merck Titrisol pH buffer held at a constant temperature (± 0.1 $^{\circ}\text{C}$). The readings are taken 1 hour after the temperature has reached a stable position.

As mentioned in ch. 3, the temperature dependence of the steady state signal should be similar to the one of the pH sensing element. In our case, the pH ISFETs exhibit a temperature dependence of $\frac{\delta V_{\text{GS}}}{\delta T} = -0.7$ to $-0.8 \text{ mV}/^{\circ}\text{C}$ [4.2], which corresponds very well with the findings for the $p\text{CO}_2$ sensor.

Stability and lifetime:

Exactly the same drift behavior is observed for the pCO_2 sensor as for the pH sensor described below. If the pCO_2 sensor is well hydrated, the drift of the baseline is in the order of 1 to 2 mV/day.

The lifetime of the pCO_2 sensor is mainly determined by the adhesion of the hydrogel to the transducer surface, and is in the range of 2 weeks. The hydrogel is anchored fairly well to the SiO_2 of the transducer, but has no adhesion to the Ag/AgCl layer of the RE arranged around the gate of the ISFET. Since the Ag/AgCl surface is rather large, the HG always starts to peel in this region. This does not affect the sensitivity of the sensor, but the response time becomes longer.

4.4.5 pH sensor

If not mentioned differently, the ISFET of the pH sensor is operated with $I_D = 100 \mu\text{A}$ and $V_{DS} = 0.5 \text{ V}$. The measurements are performed at room temperature, and the chip is shielded against light.

Sensitivity of the pH sensor combined with a commercial reference electrode:

Fig. 4.37 shows the drain current (I_D) as a function of the applied voltage at the reference electrode (V_{GS}), as obtained when the ISFET is immersed in different pH buffers. One can recognize the characteristic behavior of a MOSFET, given by eqn. (3.78). With increasing pH, the threshold voltage (V_T) is shifted towards more positive potentials, as theoretically demonstrated in eqn. (3.79), and illustrated in Fig. 3.8.

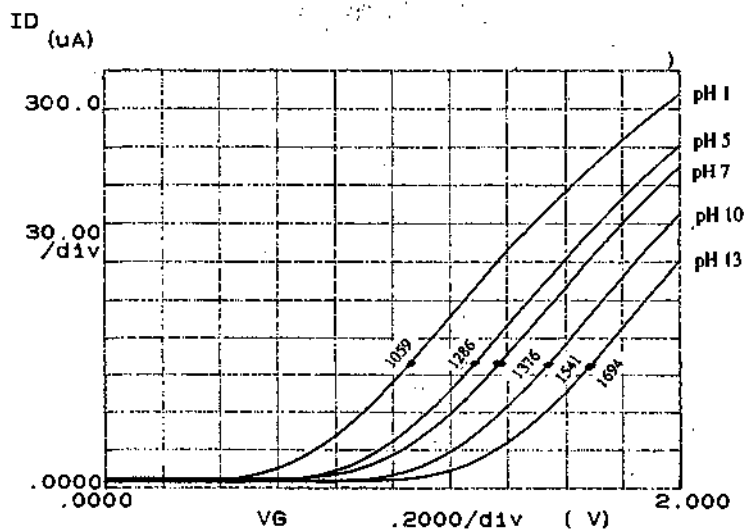


Fig. 4.37 I_D characteristics as a function of applied potential at the RE, recorded for the pH sensor in Merck Titrisol pH buffers of pH 1, 5, 7, 10 and 13, respectively. A Metrohm reference electrode (SCE) is used.

In normal operation, I_D is held constant using the external closed loop electronic circuit. This circuit therefore automatically changes V_{GS} to keep I_D constant. Thus, when changing from one pH buffer to another, the change in V_{GS} corresponds directly to the change in surface potential at the pH sensitive membrane. When V_{GS} at constant I_D and V_{DS} is plotted as a function of the pH, one obtains the pH calibration curve.

This is shown in Fig. 4.38, where the calibration curves of seven individual pH sensors are plotted. Merck Titrisol pH buffers of pH 1, 5, 7, 10 and 13, respectively, and a Metrohm reference electrode are used. The mean sensitivity of the sensors reported in Fig. 4.38 is 53.2 mV/pH, with a standard deviation of only 0.22 mV/pH. The correlation coefficients of the linear regression are within 0.9996 and 0.9998.

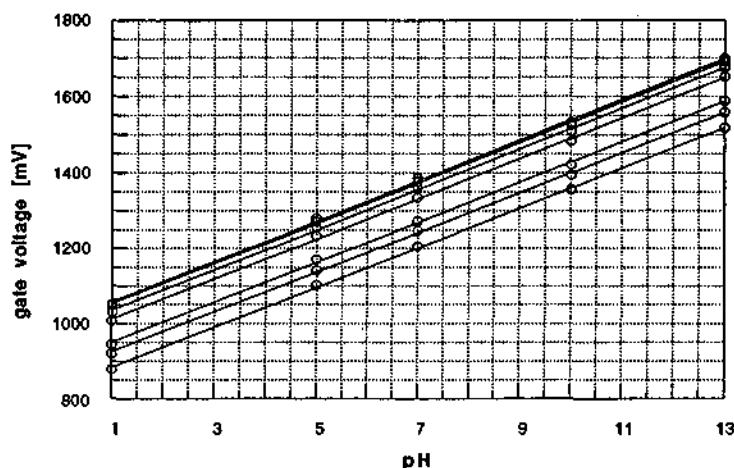


Fig. 4.38 Calibration plots of seven individual pH sensors, determined in Merck Titrisol pH buffers of pH 1, 5, 7, 10 and 13, respectively. A Metrohm reference electrode (SCE) is used.

The sensitivity of the ISFET depends on the amount of hydration of the pH sensitive layer, as discussed in chapter 3. Fig. 4.39 and 4.40 illustrate the dependences of the sensitivity and of the absolute gate potential at pH 7 on the storage conditions. The sensitivity apparently increases with time, more slowly during storage in air, and more rapidly during storage in aqueous solution. The maximum sensitivity of about 56 mV/pH (Al_2O_3 ISFET) is reached after 8 days of wet storage and is not further increased. The gate potential continuously drifts to more positive values, and reaches a nearly stable value more rapidly during wet storage than during dry storage. An appropriate conditioning of the ISFET prior to the first use is therefore crucial for high precision measurements.

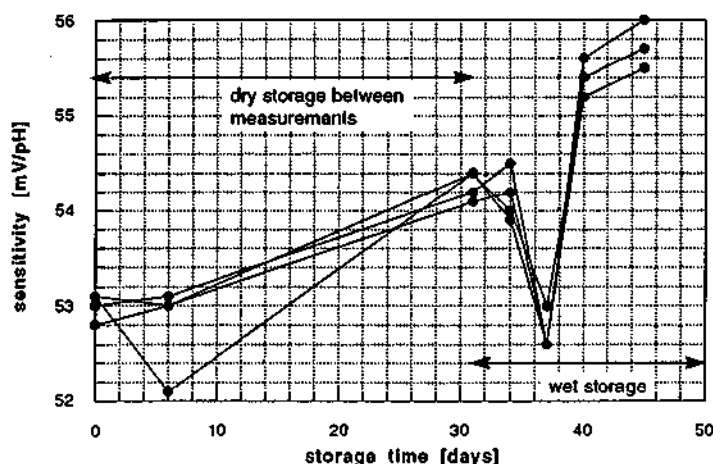


Fig. 4.39 Dependence of the sensitivity on time and conditions of storage. The sensitivity is evaluated throughout in Merck Titrisol pH buffers of pH 1, 5, 7, 10 and 13, respectively. A Metrohm reference electrode (SCE) is used. During "dry storage", the ISFET is stored in air between measurements; during "wet storage", it is stored in deionized water.

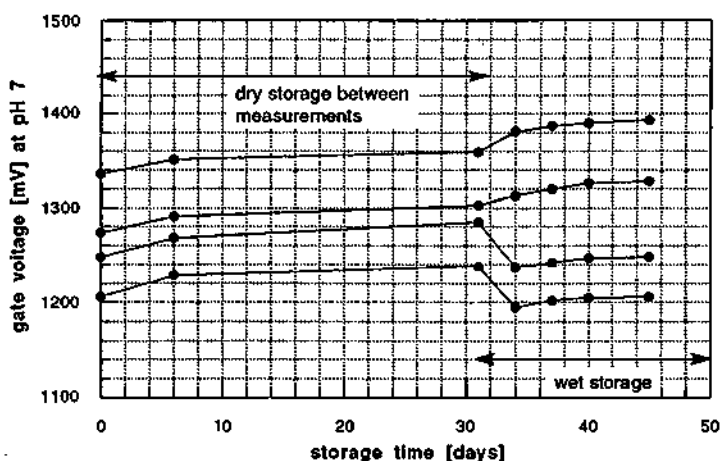


Fig. 4.40 Dependence of the gate potential of pH 7 on time and conditions of storage. The same conditions as in Fig. 4.39 are valid.

Sensitivity with the on-chip reference electrode:

In Fig. 4.41 is plotted the calibration curve of the pH sensor using the on-chip RE. As sample solutions, phosphate pH buffer solutions with a constant (50 mM) KCl concentration are used to keep the potential of the RE constant. The sensor signal as shown in Fig. 4.41 is drift compensated by means of an intermittent calibration measurement at pH 6.98 between the sample solutions. The maximum sensor deviation from the curve fit is $-0.012 / +0.007$ pH values, calculated over all measurements. The correlation coefficient of the linear regression is 0.9998.

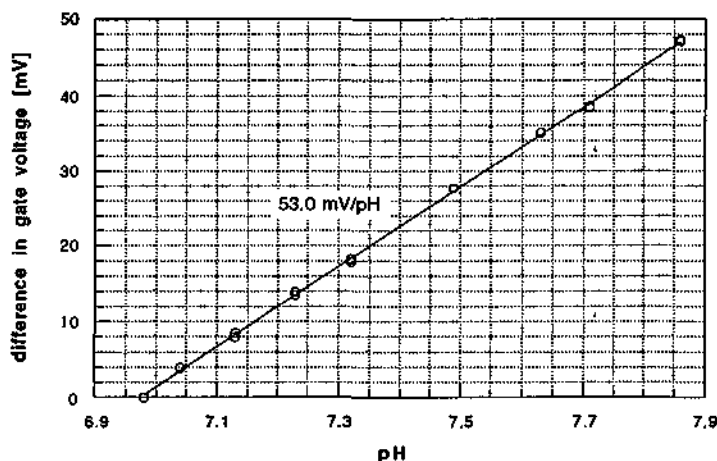


Fig. 4.41 pH calibration curve measured in the flow-through cell using the on-chip RE. The sample solutions are phosphate buffers, which are a mixture of (100 mM KH_2PO_4 / 50 mM KCl) and (100 mM NaOH / 50 mM KCl). The sample is controlled prior to injection with a Metrohm pH electrode in order to obtain a reference pH value. The sample flow is 1 ml/min. Each sample is measured twice. The sensor signal is drift corrected by means of an intermittent calibration at pH 6.98.

It is also useful to specify the changes in sensitivity obtained when increasing or decreasing pH steps are performed. The ISFET and the on-chip RE are immersed in different Merck Titrisol pH buffer with 100 mM KCl in the sequence pH $7 \rightarrow 4 \rightarrow 7 \rightarrow 10 \rightarrow 7 \rightarrow \dots$, allowing for a short rinsing with H_2O before each step. The reading is performed 3 and 5 min after immersion. Tab. 4.4 shows the resulting sensitivities.

The pH sensitivities found in measurements with the Hamilton RE (Ag/AgCl) are within 53.3 to 55.3 mV/pH, and with the on-chip RE within 52.4 to 56.1 mV/pH. Both sets of data exhibit a higher sensitivity in the pH range 4 to 7

than in the range 7 to 10. The direction of the step change has only a minor influence on the resulting sensitivity.

	on-chip RE 3 min [mV/pH]	on-chip RE 5 min [mV/pH]	Hamilton 3 min [mV/pH]	Hamilton 5 min [mV/pH]
pH 4 to 7	55.8	56.1	54.7	54.9
pH 7 to 4	56.3	56.5	55.1	55.3
pH 7 to 10	53.0	53.0	53.8	53.9
pH 10 to 7	52.2	52.4	53.1	53.3

Tab. 4.4 Sensitivity in mV/pH for pH steps from pH 4 to 7, 7 to 4, 7 to 10, and 10 to 7. The readings are taken 3 and 5 min after immersion in the pH buffer (Merck Titrisol pH buffer with 100 mM KCl). The on-chip RE is used in comparison with a Hamilton "single pore" (Ag/AgCl) reference electrode. In both measurements, the same ISFET is used.

Sensitivity in transfusion blood:

In Fig. 4.42 are plotted intermittent measurements on transfusion blood and on phosphate buffers of pH 7.0 and 7.4, respectively, using the on-chip RE. If the gate potential value for pH 7.0 is taken as a reference value for drift correction, then the pH reading in transfusion blood is repeated with a standard deviation of 0.6 mV (over the shown 8 measurements). Note the low pH level of transfusion blood, as explained in chapter 4.4.2.

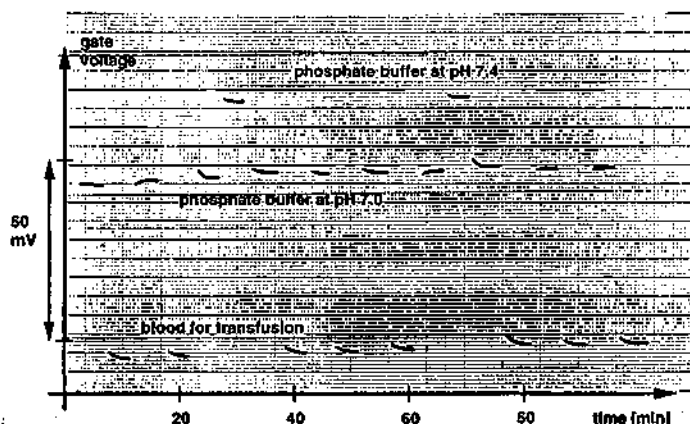


Fig. 4.42 Intermittent pH measurements on transfusion blood, and on phosphate buffers of pH 7.0 and 7.4 (50 mM KCl). The on-chip RE is used, and the sensor is immersed in the unstirred solution. The measurements are performed at room temperature.

Drift of the ISFETs:

As already mentioned for the sensitivity, the degree of hydration of the Al_2O_3 layer determines the drift rate of the sensor. Fig. 4.43 shows a typical drift behavior when an ISFET is continuously immersed in solution. A high drift is observed in the first 20 hours of immersion, and later a roughly constant drift of 100 to 150 $\mu\text{V/h}$ is attained.

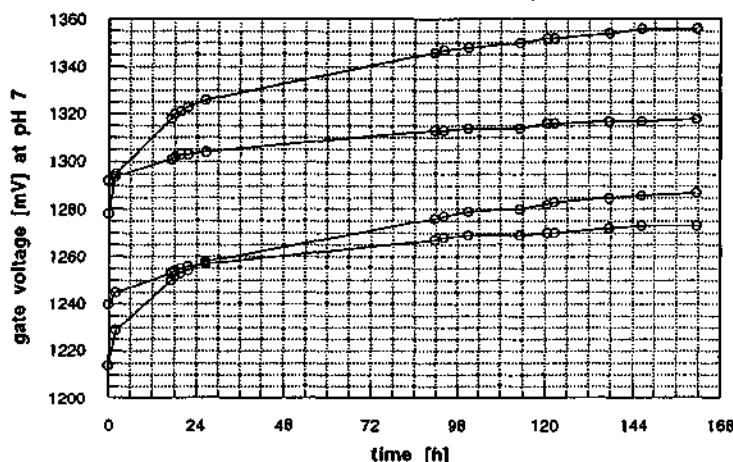


Fig. 4.43 Drift of the gate potential in Merck Titrisol pH buffer of pH 7. The ISFET remains in the solution and is continuously operated. A Hamilton "single pore" reference electrode (Ag/AgCl) is used.

Response time:

The response time of the pH sensor combined with the on-chip RE or an external commercial reference electrode is shown in Tab. 4.5. Merck Titrisol pH buffer solutions, to which 100 mM KCl is added, are used as samples. The chip is immersed in the respective solution (together with the external RE) after a short rinsing with H_2O . The times between the immersion of the sensor and the point where the sensor signal changes by less than 0.5 mV/min are given as the response time values in Tab. 4.5.

The on-chip RE turns out to be significantly slower for decreasing pH steps than for increasing steps. Partly the same behavior is exhibited by the Hamilton RE (Ag/AgCl), whereas the Metrohm RE (SCE) tends to an inverse behavior.

The asymmetry in the response time behavior may be a consequence of the slightly different sensitivities between pH 4 to 7 and 7 to 10, as was discussed in a previous section.

	ISFET vs on-chip RE	ISFET vs Hamilton "single pore"	ISFET vs Metrohm 6.0702.100
pH 4 to 7	0.5	0.5	0.5
pH 7 to 4	3.5	2	1
pH 7 to 10	0.5	0.5	3
pH 10 to 7	2	0.5	0.5

Tab. 4.5 Response time in min, i.e. time needed for the sensor signal to decay below a signal change of 0.5 mV/min. The time starts with the immersion into the pH solution (Merck Titrisol pH buffer with 100 mM KCl). For all measurements, the same ISFET is used.

The response time of the pH sensor in combination with the on-chip RE and mounted in the flow-through cell depends on the magnitude of the pH step change. For pH changes below 0.5 pH unities, the sensor needs less than 30 s to achieve a signal that is within 0.5 mV of the final steady state signal. For pH changes by 1 pH unity or more, the signal ± 0.5 mV from the steady state is reached after 2 min. In this case, no difference in the response time for decreasing or increasing pH steps is observed. These measurements are performed at a flow of 1 ml/min, using phosphate pH buffers with constant KCl concentration.

Hysteresis:

The hysteresis of a measurement at pH 7 is determined in the following way. The ISFET and the Metrohm reference electrode are introduced into Merck Titrisol pH buffer solutions in the sequence pH 7 $\rightarrow$ 5 $\rightarrow$ 1 $\rightarrow$ 7 $\rightarrow$ 10 $\rightarrow$ 13 $\rightarrow$ 7, with intermittent short rinsing with water. The readings are performed 2 min after immersion of ISFET and reference electrode. The three values taken for pH 7 are within ± 1 mV.

The same measurements are performed for the same ISFET, but this time in combination with the on-chip RE. Merck Titrisol pH buffers are used with the addition of 100 mM KCl. If the readings are taken 3 min after immersion, the three values at pH 7 are within ± 2 mV.

Stability and lifetime:

The signal stability of the pH ISFET alone is discussed in a previous section. The lifetime of the sensors is in the order of several months. In no case, the lifetime of the sensor was limited by the ISFET.

The on-chip RE alone exhibits a drift of about 0.1 to 0.3 mV/h when immersed in a solution of constant KCl concentration. This value is measured by comparison with a conventional RE which may also contribute a certain drift. When the KCl concentration in the sample is changed, then the (transient) drift of the on-chip RE increases to about 1 mV/h. This is caused by the equilibration of the KCl levels in the hydrogel and in the sample. The time needed for such an equilibration depends on the extent of the chloride step change. If KCl is completely removed from the sample, then the sensor drifts over 10 h before it reaches a nearly stable signal.

The operating lifetime of the on-chip RE is rather short (around 40 h). Generally, after this time, the Ag/AgCl layer peels off from the underlaying Ti adhesion layer. There are several possible reasons for such failure.

- 1) During fabrication, too much Ag has been converted into AgCl, which may seriously decrease the adhesion of the Ag on the Ti
- 2) During operation, AgCl is continuously dissolved in the hydrogel and diffuses through the liquid junction into the sample. This effect is very dramatic, when the sensor is frequently used in solutions with very low chloride concentration. In such cases, the AgCl layer may partly or completely peel off.

4.4.6 Parallel operation of all three sensors

The parallel operation of all three sensors is problematic due to the following reason. The Pt and Ag electrodes, from the bonding pads to the active zones, are only covered by the GPM. No additional insulation layer is used. As already mentioned, water vapor penetrates through the GPM and leads to hydration of the HG. The uptake of water vapor through the GPM causes a sufficiently conducting ohmic contact between the different Pt and Ag layers. Hence, electronic crosstalk is observed between different sensors or electrodes, which increases with continuing exposure to aqueous samples. When a potential is applied to the WE of the pO_2 sensor, for example, the potential at the RE of the pCO_2 sensor may also change. Such interferences could be excluded only by further improvements of the chip design.

4.5 Conclusion

The 1st Design of the blood gas sensor proves that it is possible to fabricate a combined pO_2 , pCO_2 and pH sensor using silicon technology. The individual sensors perform well in aqueous solutions and in transfusion blood as well, exhibiting an excellent linearity and stability. However, the characterization of the chip has revealed some problems. The most serious drawback of the 1st Design is the electronic crosstalk between the individual sensors which renders parallel operation difficult. The operational lifetime of the sensor chip being 2 weeks is limited mainly by the adhesion of the hydrogel and of the Ag/AgCl layers to the transducer surface. Furthermore, the pO_2 sensor exhibits a fairly large flow dependence, and also problems caused by the Ag^+ deposition on the WE. An important feature of a blood gas sensor is its response time, which is too high for the present chip. This problem, however, is partly sensor inherent, partly related to the flow-through system used. This slow response time is primarily due to the deadspace associated with the input tubing and the flow cell and due to the gas permeability of the input tubing. Furthermore, the mounting of the sensor chip into the system is complicated and it is difficult to achieve a reproducible flow behavior in the flow cell.

Because the observed problems are not of fundamental nature, there is a strong indication that by an appropriate modification of the sensor design, the performance can be enhanced, in order to achieve the desired characteristics. A 2nd Design of the sensor chip will probably show more clearly the advantages and the limits of this sensor technology.

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5 2nd Design

5.1 Introduction

Based on the experience of the 1st Design, a new sensor chip ("2nd Design") is developed, fabricated and characterized. The aim is to optimize the performance of the device, in particular the response time, the lifetime and the simultaneous operation of the combined pO_2 , pCO_2 and pH blood gas sensor. Furthermore, the 2nd Design focuses on the simple integration of the chip into a flow-through system by providing a flow-through channel on-chip, which greatly simplifies the mounting of the sensor.

5.2 Layout

5.2.1 Introduction

Tab. 5.1 specifies the performance limitations which were encountered with the 1st Design, and the corresponding modifications for the 2nd Design.

Limitations of the 1st Design	Modifications for the 2nd Design
slow response (pO_2 and pCO_2) due to: - thick layer of hydrogel (HG) and gas permeable membrane (GPM) - high gas permeability of the flow-through system	→ thinner polymeric layers → smaller dimensions of the flow-through system
operational lifetime < 2 weeks due to: - poor adhesion of HG & GPM - dissolution of Ag layers	→ Si_3N_4 layer as anchoring material → thicker Ag layers (galvanization)
simultaneous operation of all three sensors difficult (electrical crosstalk)	→ Si_3N_4 passivation layer covering the platinum electrodes
hydration of HG very slow	→ hydration channels
flow dependence of the pO_2 sensor	→ smaller working electrode (WE)
Ag ⁺ deposition on the WE	→ shield electrodes between reference electrode (RE) and WE

Tab. 5.1 Performance limitations encountered with the 1st Design and the corresponding modifications for the 2nd Design.

The operating principle of the sensor cells remains identical. However, to eliminate the problems observed for the 1st Design, the sensor geometry is adapted. The main difference is that the holes through the wafer, used in the 1st Design to provide a connection to an external reservoir, are not integrated anymore.

5.2.2 Polymeric flow-through channel

In the 2nd Design, a flow-through channel is directly structured on the chip (Fig. 5.1). The aim is to fully utilize the possibilities of miniaturization, not only for the sensor part, but also for the flow-through cell. With the precisely defined flow-through channel a reproducible and homogeneous flow behavior, a small sample volume and a fast response can be achieved. Furthermore, this channel provides an identical treatment of all samples and a strict reproduction of the measurement conditions. The flow-through system offers the possibility to compensate the sensor drift by using intermittent calibration. The flow channel should avoid bubble trapping or deposition of blood components by using an adequate flushing. The sample solution is brought into contact with the channel for a minimum time period. For the most part of time, the channel is exposed to a simple electrolyte solution with an ion content similar to the one expected in the analyte.

The flow-through channel is 18 mm long and 2 mm wide. With these dimensions, the channel fits into the miniaturized chemical analysis system developed in our laboratory, which combines chemical sensors and micropumps fabricated in silicon [5.1][5.2]. The height of the polymeric sealing ring, which is approximately 500 μm , determines the internal volume of the channel. If the gas permeable membrane is 100 μm thick, then the volume of the channel is in the order of 15 μl . The channel is closed by means of a glass plate which has an inlet and an outlet opening (diameter 1 mm) at the left and the right end of the channel, respectively. The polymeric ring itself is 1 mm wide, which ensures a sufficient sealing to the glass plate. Polysiloxane is an excellent sealing material, and therefore almost no force has to be applied to the glass plate in order to hermetically close the channel (Fig. 5.2). Accordingly, the mounting/dismounting of the chip into the flow-through system is easily accomplished.

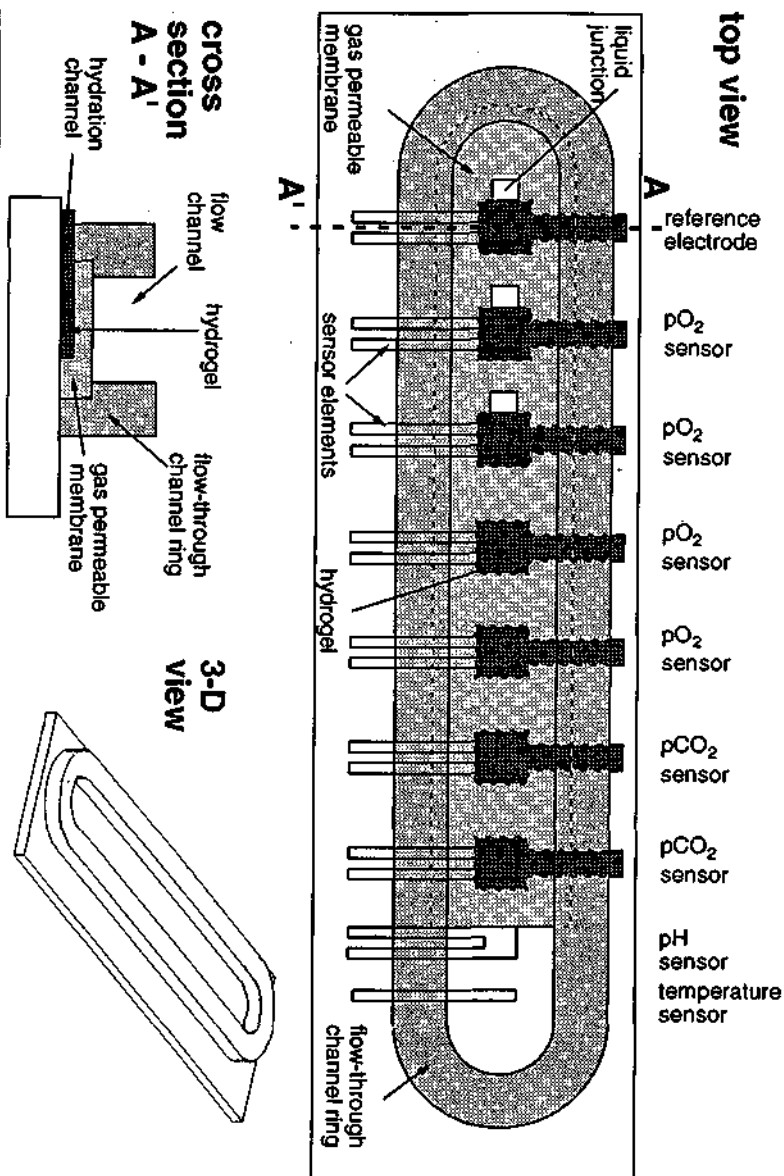


Fig. 5.1

Layout of the 2nd Design (22 mm x 6 mm): The 18 mm long and 2 mm wide flow-through channel is formed by the polymeric sealing ring, in which 8 sensing elements (shown schematically) are aligned in a row, having a spacing of 2 mm. The 7 sensing elements on the left are covered with a HG layer of 1 mm x 1 mm. These HG layers have an extension underneath the sealing ring ("hydration channel"). The first 3 elements on the left have a 0.5 x 0.5 mm opening in the GPM, which serves as a liquid junction. The 8th element is the pH ISFET, which is not covered by the GPM.

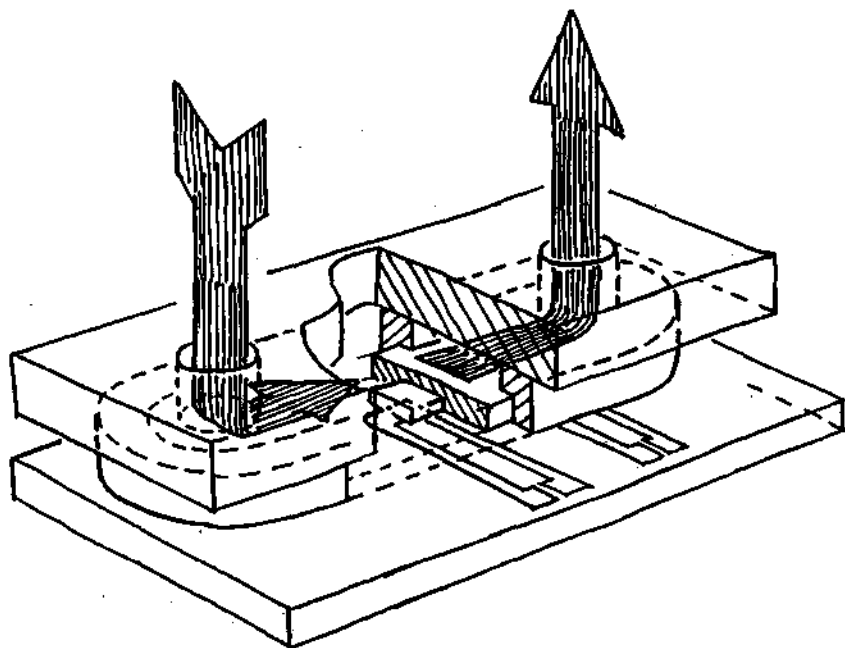


Fig. 5.2 Sensor chip (bottom), having the polymeric flow-through ring which is closed with the glass plate (top). The glass plate provides an inlet and an outlet opening for the sample stream.

5.2.3 Sensor cells

In the flow-through channel, eight individual sensing elements are aligned in a row, having a spacing of 2 mm (Fig. 5.1). Four potentiometric and four amperometric cells are available.

The amperometric cells can be used as oxygen sensors, or they serve as transducers for a glucose sensor to be realized in the future. Two of these amperometric cells have a liquid junction; therefore, the corresponding reference electrode is not located within the cell, but on the other side of the liquid junction (these cells will be termed in the following as "Open Clark" cells). The other two cells are Clark type sensors ("Closed Clark" cells), with a 3 electrode configuration of WE, CE and RE. A fourth electrode, the shield electrode (SE), can be operated to interrupt the Ag^+ migration between the RE and the WE.

The four potentiometric cells consist of an ISFET and an Ag/AgCl layer, and exist in three different versions:

- Two cells are completely covered with HG and GPM, and are used for the $p\text{CO}_2$ sensors. In the following, they will be called "Closed ISFET".
- One cell is uncovered and used as the pH sensor ("Open ISFET"). In this cell, no Ag/AgCl layer is provided.
- One cell is covered by the HG and the GPM, but has a liquid junction ("Semi-open ISFET"). The Ag/AgCl layer of this cell can be used as a conventional reference electrode for the pH sensor. Furthermore, the cell can be operated in a differential measurement setup as a pseudo-REFET against the Open ISFET for pH measurements.

The chip offers the possibility to determine redundantly the $p\text{O}_2$, $p\text{CO}_2$ and the pH of the sample.

The sensor cells, apart from the Open ISFET, consist of a 1 x 1 mm hydrogel layer which is centered on top of the amperometric or potentiometric transducing element. The hydrogel is extended under the sealing ring to the upper end of the chip. This extension, called "hydration channel", is 0.5 mm wide and 1.8 mm long and allows a direct and quick hydration during the preconditioning of the sensor. The liquid junctions of the first three sensor cells (Fig. 5.1, left) are openings in the GPM of 0.5 mm x 0.5 mm, which allow a contact of the sample solution to the hydrogel in an area of 100 x 500 μm .

To obtain an optimum adhesion of the hydrogel to the transducer, large areas of Pt or Ag/AgCl are avoided because a chemical anchoring of the polymers onto metallic layers is difficult. If, however, a certain surface of the metallic layer is necessary, the electrode is cut into small bands that are interconnected underneath the insulating Si_3N_4 layer.

5.2.4 Amperometric sensor cells

In the following, only the Closed Clark cells will be discussed.

Four different layouts of the Closed Clark cell are provided on each wafer. On a single chip, only one layout is used.

1) The first layout, shown in Fig. 5.3, is an array of 72 platinum working microelectrodes connected electrically in parallel. Each microelectrode has an active area of $5\ \mu\text{m} \times 5\ \mu\text{m}$ (total WE area: $1.8 \cdot 10^{-3}\ \text{mm}^2$). The distance from one WE to the other is $100\ \mu\text{m}$. The CE and RE are placed into the hydration channel, separated from the WE by the SE. The Ag^+ migration between the RE and the WE is interrupted by a slightly negative polarization of the SE. Reaction products of the CE can escape in direction of the channel, or are also eliminated by the SE.

This layout is called "M05" in the following, where "M" stands for microelectrodes, and "05" for the size of the WE.

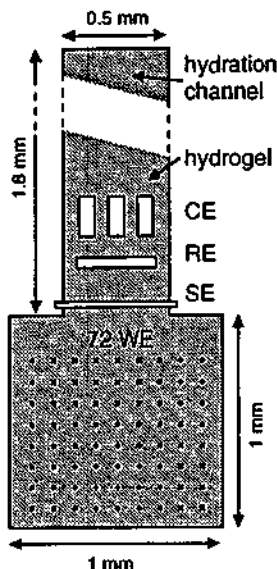


Fig. 5.3 Closed Clark "M05" with an array of 72 working electrodes of $5\ \mu\text{m} \times 5\ \mu\text{m}$

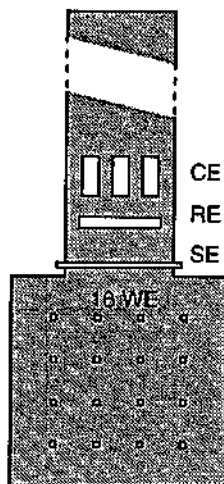


Fig. 5.4 Closed Clark "M20" with an array of 16 working electrodes of $20\ \mu\text{m} \times 20\ \mu\text{m}$

2) The second layout, called "M10", corresponds exactly to "M05", but the size of the 72 working microelectrodes is $10\ \mu\text{m} \times 10\ \mu\text{m}$, giving a total WE area of $7.2 \cdot 10^{-3}\ \text{mm}^2$.

3) The third layout, shown in Fig. 5.4, is called "M20" and follows the same idea as "M05" and "M10". However, the size of the 16 working microelectrodes is increased to $20\ \mu\text{m} \times 20\ \mu\text{m}$ (total WE area: $7.2 \cdot 10^{-3}\ \text{mm}^2$), and the spacing between the electrodes is $200\ \mu\text{m}$.

- 4) The fourth layout (Fig. 5.5) is a concentric electrode arrangement similar to the 1st Design. The WE (radius: $40\text{ }\mu\text{m}$, area: $5.0 \cdot 10^{-3}\text{ mm}^2$) is arranged in the center, separated from the RE and CE by the SE. The RE is placed between the CE and the WE to obtain an optimum distribution of the electrical field. The CE area is higher than twice the area of the WE and the SE to prevent any current limitation by the CE. The SE is separated from the WE by a distance of $200\text{ }\mu\text{m}$ to avoid any influence on the O_2 concentration gradient near the WE. This layout will be called Closed Clark "C", where the "C" stands for concentric.

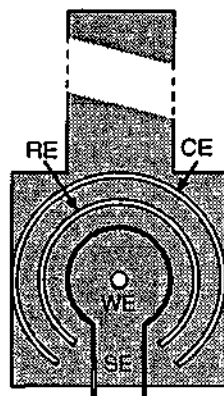


Fig. 5.5 Closed Clark "C" with a concentric electrode configuration.

5.2.5 Potentiometric sensor cells

Three different types of potentiometric cells are provided on each chip: two Closed ISFET, one Open ISFET and one Semi-open ISFET.

- 1) The layout of the Closed ISFET is shown in Fig. 5.6 (on the left). The gate of the ISFET is arranged in the center. Two bands of Ag/AgCl are placed on both sides, being interconnected and used as the RE. The hydrogel is completely covered by the GPM.
- 2) The Semi-open ISFET (Fig. 5.6, on the right) has exactly the same layout as the Closed ISFET, apart from the liquid junction which consists of an opening in the GPM of $0.5\text{ mm} \times 0.5\text{ mm}$.
- 3) The Open ISFET is an uncovered ISFET without Ag/AgCl layer.

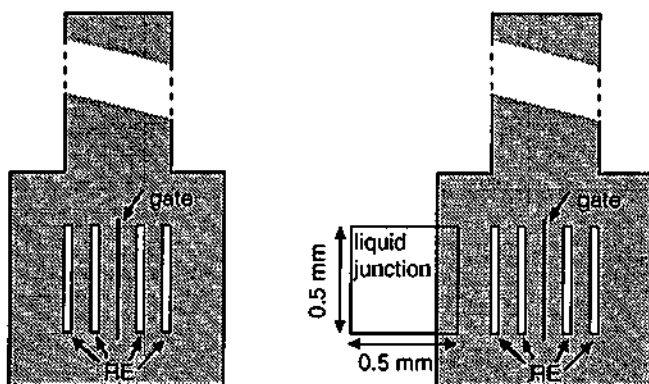


Fig. 5.6 On the left: layout of the Closed ISFET with the gate in the center and four bands of Ag/AgCl
On the right: layout of the Semi-open ISFET which corresponds to the Closed ISFET, except for the liquid junction.

5.3 Fabrication

5.3.1 Introduction

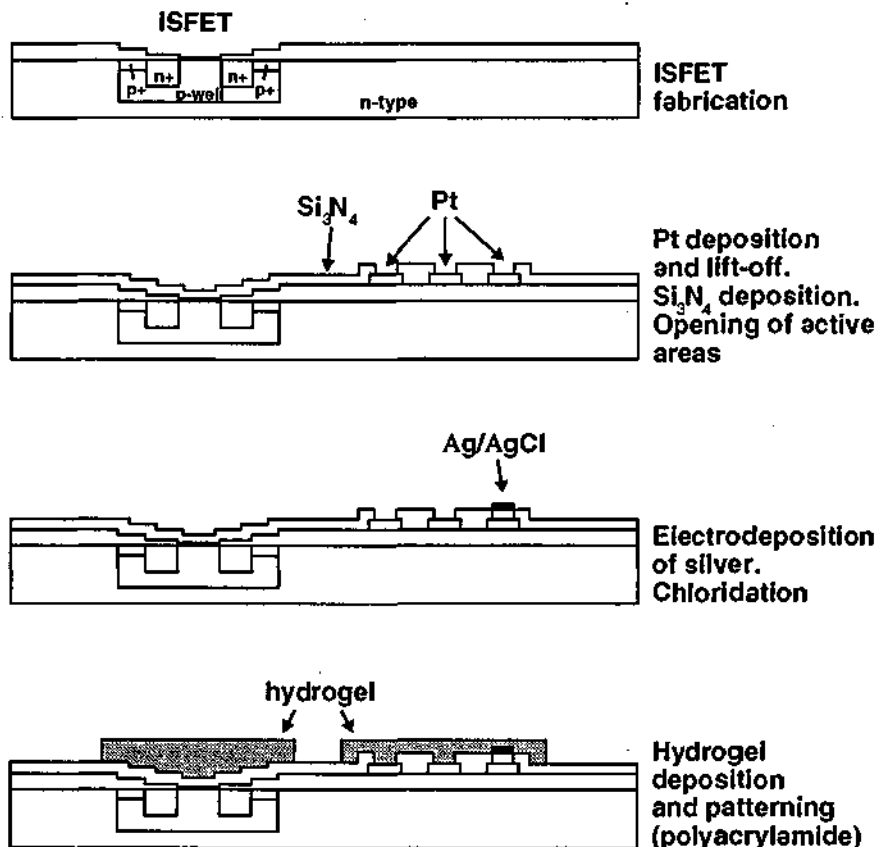
Several fabrication steps are changed in comparison to the 1st Design:

- the Pt layers are covered with an insulating Si_3N_4 layer to avoid any crosstalk between the individual Pt electrodes. The Si_3N_4 layer is opened only in the regions where the Pt has to be chemically active.
- the Si_3N_4 insulating layer is simultaneously used as pH sensitive membrane
- instead of e-gun evaporation of Ag layers, the Ag is electrodeposited by galvanization on an underlaying Pt structure. This method allows the deposition of very thick Ag layers up to $10\text{ }\mu\text{m}$, which adhere very well to Pt.
- the chloridation of the Ag layers is performed electrochemically, resulting in a very uniform and thick AgCl layer up to $5\text{ }\mu\text{m}$. With this thick AgCl layer, a more stable reference potential is obtained.
- as additional and last step, the $500\text{ }\mu\text{m}$ thick flow channel ring is deposited by photopolymerization of polysiloxane.

Although several processing steps have been added, the fabrication is less complex than for the 1st Design because the holes through the wafer are omitted. The complete fabrication sequence needs 12 masks.

In Fig. 5.7, the six main fabrication steps are shown:

- A) In the first sequence, the ISFETs are processed
- B) In the second step, Pt is deposited by evaporation and structured by the lift-off method. Then the Pt is covered with a passivation layer (Si_3N_4). This layer is opened in the active zones of the Pt electrodes.
- C) 10 μm of Ag is electrodeposited on a given part of the Pt structures, and a 25 % of this Ag layer is transformed into AgCl by electrochemical chloridation
- D) The hydrogel layers are patterned by photopolymerization
- E) The gas permeable membrane is patterned by photopolymerization
- F) The flow-through channel ring is patterned by photopolymerization



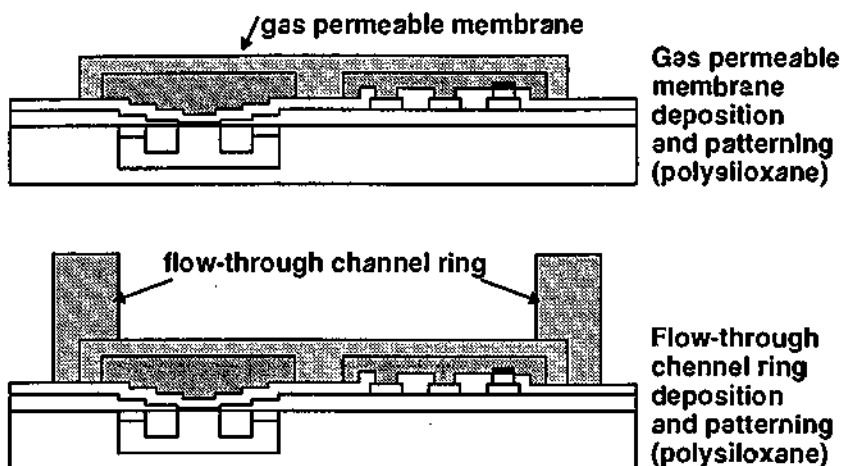


Fig. 5.7 The 6 main processing steps of the chip fabrication

5.3.2 ISFET fabrication

For the ISFET fabrication, the same process sequence is used as for the 1st Design, except for the deposition of the pH sensitive membrane. Instead of Al_2O_3 , a layer of Si_3N_4 is used, which has two purposes: first, it serves as the insulation material for the coverage of the Pt electrodes, and secondly, it is used as pH sensitive material on the gate of the ISFETs. If we compare the fabrication with that of the 1st Design, as shown in Fig. 4.8 of ch. 4, the 2nd Design starts from step 5, and the following processes up to number 11 remain unchanged. From 5 to 11, the p-well is implanted, the drain and source zones are defined, and the gate silicon dioxide is grown.

5.3.3 Pt patterning and coverage with Si_3N_4

After step 11, the process continues with the opening of the drain, source and substrate contacts. Then, 1500 Å of platinum are deposited by e-gun evaporation, using a 100 Å thick Ta adhesion layer. The Pt is patterned using the lift-off method, and is then covered with 1800 Å of Si_3N_4 , deposited at 800 °C by low pressure chemical vapor deposition (LPCVD). The Si_3N_4 is etched in H_3PO_4 at 185 °C, using a 4000 Å thick SiO_2 layer as masking material. The gate oxide of the ISFETs remains covered with the Si_3N_4 layer. Wet etching of Si_3N_4 is preferred to plasma etching, to avoid any deterioration of the gate oxide quality. Finally, 1.5 μm of Al is deposited and patterned to create the contact pads.

5.3.4 Electrodeposition of Ag and electrochemical chloridation

In the next step, Ag is electrochemically deposited on those Pt electrodes which later will serve as Ag/AgCl reference electrodes. All these reference electrodes are connected in parallel and can be contacted at one single point on the wafer. This allows galvanization and electrochemical chloridation to be performed directly on wafer level. By cutting the wafer into individual chips at the end of the fabrication, the connection between all the reference electrodes is automatically interrupted.

The wafer is immersed into an AgCN solution, except for the electrical contact point. A negative potential is applied to this contact versus a platinum wire which serves as a counter electrode, to achieve a constant current of $100 \mu\text{A}/\text{mm}^2$. This results in an Ag deposition rate of about $1 \mu\text{m}/\text{min}$. Positive photoresist is used to cover those areas of the platinum where no Ag has to be deposited. The resulting Ag layer is $10 \mu\text{m}$ thick and has a density close to the one of bulk silver ($10.5 \text{ g}/\text{cm}^3$). This can be calculated by relating the deposited volume to the total charge transferred during the deposition. The adhesion of Ag to the underlaying Pt is excellent. The electrochemical partial chloridation of the Ag layer is performed in 50 mM KCl solution by applying a positive potential to the Ag versus a platinum counter electrode. In order to achieve a homogeneous AgCl layer, a low current density of $5 \mu\text{A}/\text{mm}^2$ is used. 25 % of the initial Ag is converted into AgCl by transferring the corresponding amount of charge. The coloration of the AgCl is dark brown, indicating a thick layer. The electrochemical chloridation is preferred over the chemical chloridation in FeCl_3 solution because the latter method seems to chloridize the Ag only within a thin surface layer, which limits the long term stability of the electrode. It was observed for the 1st Design that the chloridation in FeCl_3 solution can cause a complete loss of adhesion of the Ag/AgCl to the transducer surface. With the electrochemically deposited Ag, even if the Ag is completely converted to AgCl, the AgCl layer does not peel off from the underlaying platinum.

5.3.5 Deposition and patterning of the hydrogels

This step is performed by using the same process as for the 1st Design. However, the acrylamide monomer composition is adapted, as will be discussed in ch. 6.

5.3.6 Deposition and patterning of the gas permeable membrane

The same process and monomer mixture is used as for the 1st Design. A critical point is the opening of the 0.5 x 0.5 mm liquid junctions during the development of the siloxane in xylene. These liquid junctions are zones of unpolymerized monomer, surrounded by a large area of polymerized siloxane. Due to the high viscosity of the monomer, and due to the small openings, the unpolymerized monomer solution is somehow trapped in these windows. It can be removed only by applying a jet of xylene in the direction of the openings. Possibly also the light scattering during the UV exposure contributes to this problem because the liquid junctions correspond to dark points on the photomask, surrounded by a large transparent area.

5.3.7 Deposition and patterning of the polymeric sealing ring of the flow-through channel

The deposition and patterning of the polymeric sealing ring is performed by using the same monomer solution as for the gas permeable membrane. However, a much greater thickness of 500 μm is needed. In order to obtain this thickness, a sufficient volume of the monomer solution is deposited on the wafer by means of a pipette. Three spacers with a thickness of 500 μm are placed at the outer edge of the wafer. The contact force, which is applied by the mask aligner between the photomask and the wafer, diminishes the monomer layer down to a homogeneous thickness, defined by the 3 spacers. This is done prior to exposing the monomer to UV light.

Channels of more than 700 μm thickness (with other spacers) and 1 mm of channel width have been achieved, with a pattern definition better than $\pm 100 \mu\text{m}$.

The completed device is shown in Fig. 5.8:

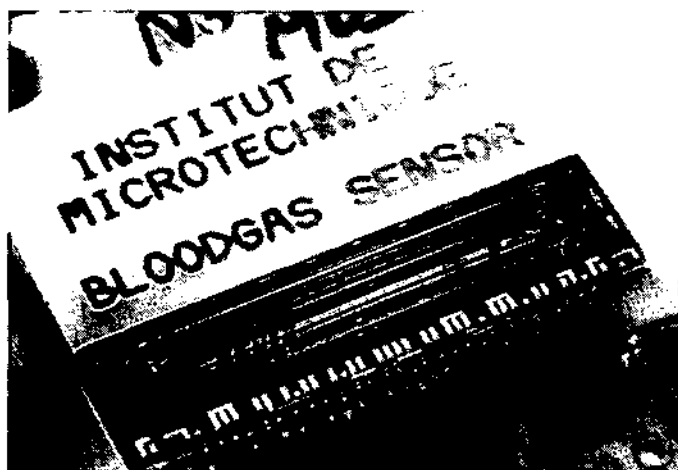


Fig. 5.8 Photograph of the completed device (after dicing the wafer), mounted onto the printed circuit board, after wire bonding

5.4 Characterization

5.4.1 Introduction

As already shown in Fig. 5.2, the flow-through channel integrated on the chip is closed on top with a glass plate which has an inlet and an outlet opening. The glass plate and the chip, which is glued onto a printed circuit board, are held in position by a holder made from polycarbonate. This holder also provides the connections for the inlet and outlet tubing. The outlet tubing is connected to a peristaltic pump which sucks the sample solutions through the flow-through system (Fig. 5.9). The inlet tubing is connected to the output of a 2-way valve. With the two input lines of the valve, two different sample solutions can be intermittently transferred to the measuring cell. The timing of the magnetic valve is controlled electronically by a programmable timer. The tubings between the sample and the valve, and between the valve and the sensor chip, are either glass capillaries or stainless steel tubings with an internal diameter of 0.5 mm. They are as short as possible to minimize memory effects.

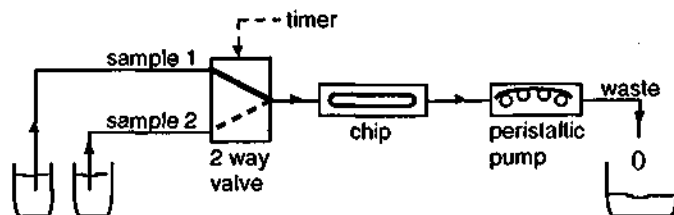


Fig. 5.9 Schematic diagram of the flow-through system

5.4.2 pO_2 sensor

Sensitivity in aqueous solution:

Fig. 5.10 shows the calibration plot of a "M10" type oxygen sensor. A high linearity is exhibited in a very broad range of pO_2 from 70 to 600 mmHg. The correlation coefficient of the linear regression is 0.9998. The extrapolated residual current is 7 nA, which corresponds to about 20 mmHg of pO_2 .

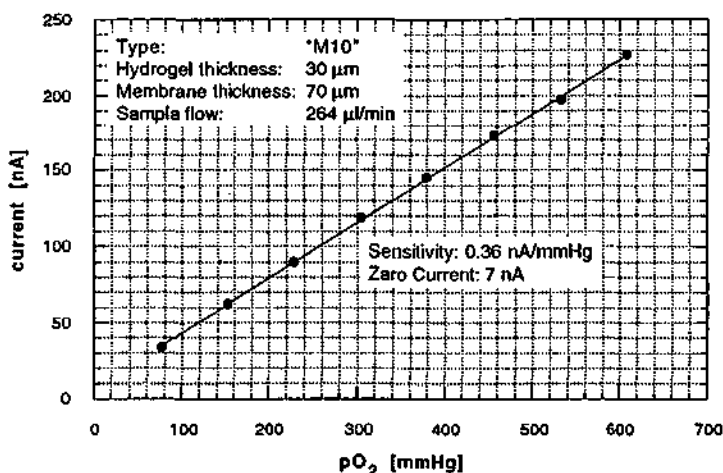


Fig. 5.10 Calibration plot recorded in 100 mM KCl solution equilibrated with O_2/N_2 gas mixtures at room temperature. The current is corrected by using an intermittent baseline measurement (in air saturated solution). The polarization of the WE is -800 mV. The measurements are performed at the 5th day after the hydration.

The sensitivity of the different pO_2 sensor types depends on the WE area: "M10" has a sensitivity of 0.62 nA/mmHg, "M05" has 0.19 nA/mmHg and "C" has 0.25 nA/mmHg (measured immediately after hydration). Furthermore, the sensitivity / area ratio for the array types ("M05", "M10") is slightly higher as for the single WE ("C") oxygen sensors: the current density in air saturated solution is 16 $\mu A/mm^2$ for "M05", 14 $\mu A/mm^2$ for "M10" and 8 $\mu A/mm^2$ for "C", which agrees with the microelectrode theory predicting a higher current density with decreasing WE area.

The sensitivity of the pO_2 sensor is strongly influenced by the humidity of the hydrogel. When the sensor is hydrated prior to its operation using the hydration channel, and mounted into the flow-through cell, then the sensitivity is the highest. During continued operation, the sensitivity decreases slowly, simultaneously with a visible decrease in the thickness of the hydrogel. The hydrogel slowly loses its humidity, probably through the hydration channel, which is exposed to air during the measurement. The amount of humidity, which passes from the sample through the GPM into the HG, seems to be smaller than the loss through the hydration channel. For a "M05" / "M10" / "C" oxygen sensor, the current in air saturated solution is in the order of 30 / 100 / 40 nA, when measured immediately after the hydration. When the sensor is in continuous use for 10 days, the current decreases to 5 / 20 / 10 nA (8 %/day), at which level it remains constant. If the upper end of the hydration channel is covered with silicone rubber after the hydration, the loss of humidity is much slower, and the current decrease is in the order of 1 to 2 %/day, also reaching a stable value after some days of operation. This steady state is probably reached in the moment when the loss/gain of humidity is in equilibrium.

Further investigations would be required, of course, for controlling the influence of the humidity on the sensitivity. On the other hand, if the sensor is calibrated frequently, the humidity dependence of the response is not really a problem because linearity and reproducibility are excellent for both the humid and dry condition.

Fig. 5.11 illustrates the good reproducibility of the pO_2 sensor, for a repeated measurement of two phosphate pH buffer solutions, equilibrated with air and 10 % O_2 / 90 % N_2 , respectively.

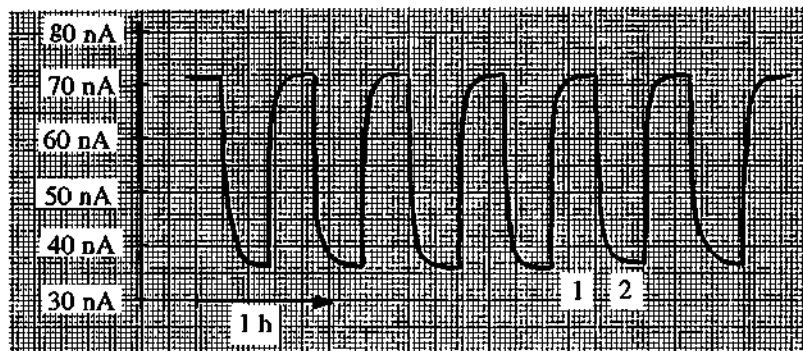


Fig. 5.11 "M10" type oxygen sensor: Repeated measurements of two phosphate buffer solutions of pH 7.4: (1) is air saturated ($pO_2 \approx 160$ mmHg), (2) is equilibrated with a 10 % O_2 / 90 % N_2 gas mixture ($pO_2 \approx 76$ mmHg). The polarization voltage is -800 mV and the flow is 650 μ l/min.

Voltammogram:

As discussed in ch. 4.4.3, the shape of the voltammogram depends strongly on the recording parameters, on the pH of the internal electrolyte, and on the pretreatment of the platinum WE. In order to study the influence of all these parameters in more detail, the GPM of a "M10" oxygen sensor is removed, thus only the HG remains on top of the WE. With this setup, the solution which contacts the WE can be easily exchanged. Changing the pH of the solution in contact with the WE does not have a significant effect on the shape of the voltammogram. However, by changing the cycling sequence of the potentials, distinct difference in the shape are observed. In Fig. 5.12, two voltammograms are compared, one recorded from +1000 to -1000 mV, and the other only from 0 to -1000 mV, but both at the same scan rate. The voltammogram obtained after prepolarization at positive potentials exhibits a pronounced current limiting plateau, the other one exhibits no plateau.

The pretreatment, i.e. a prior application of positive potentials, seems to change the state of oxidation of the Pt surface, with the result that the oxygen reduction proceeds more easily and at less negative potentials. This effect has also been described in an earlier study by [5.3]. They found that, by changing the potential limits of the voltammetric cycle, the oxygen reduction potential and the shape of the voltammogram changed significantly. They observed furthermore that the diffusion limited plateau is more pronounced by using 0.1 M NaOH as internal electrolyte, instead of using a 0.1 M NaCl solution.

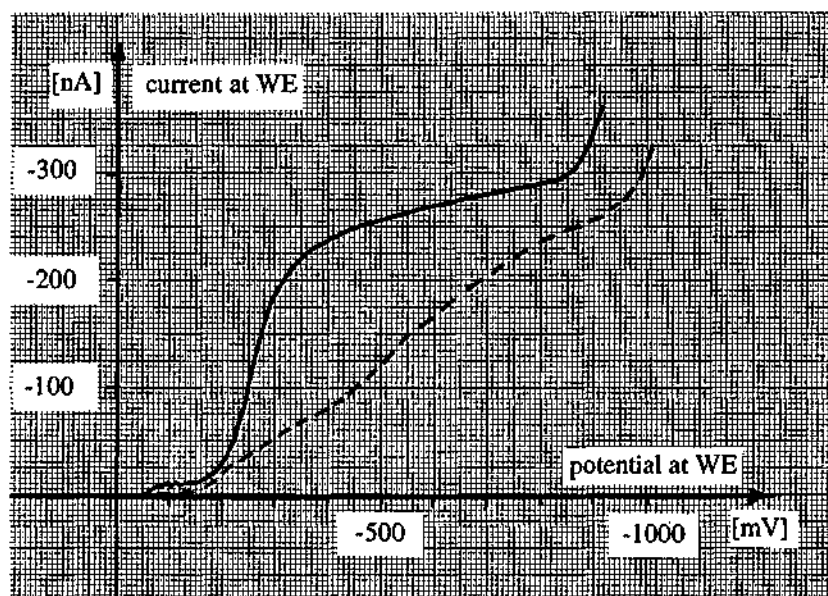


Fig. 5.12 Voltammogram of a "M10" type oxygen sensor, where the gas permeable membrane has been removed. One voltammogram is recorded with a scan from +1000 mV to -1000 mV (plain line), the other from 0 to +1000 mV (dashed line), both at a scan rate of 10 mV/s. The sample solution is air saturated phosphate buffer solution of pH 11, and the flow speed is 240 $\mu\text{L}/\text{min}$.

In Fig. 5.13, the potential scan from +1000 mV to -1000 mV is repeated for an intact "C" type sensor, having both the HG and the GPM. In order to have the WE in contact with the same electrolyte as for the voltammogram shown in Fig. 5.12, the sensor is hydrated through the hydration channel with phosphate buffer solution of pH 11. Again, the current limiting plateau appears only nicely in the case where the voltammogram is recorded with a scan from +1000 mV to -1000 mV.

In practical applications, the pO_2 electrode is constantly polarized. Thus the pretreatment may have an influence at the beginning of polarization, but effect is probably minimized with continued operation.

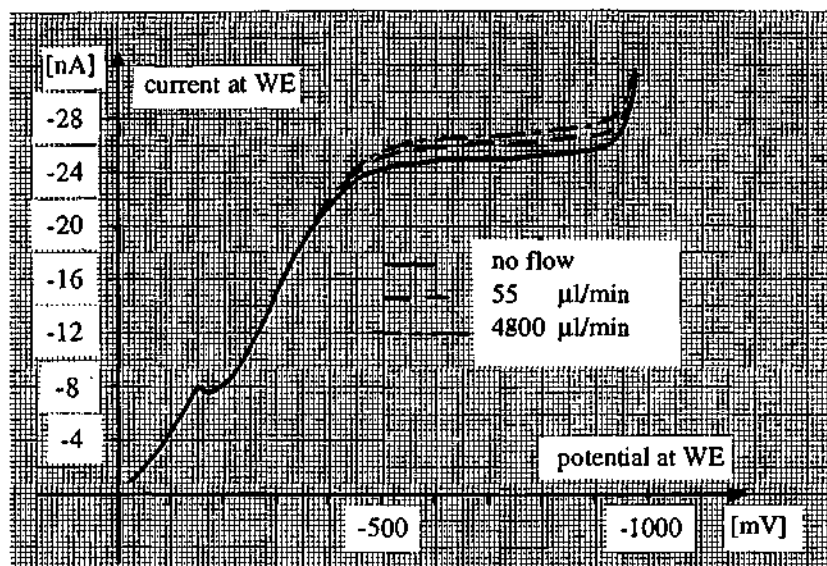


Fig. 5.13 Voltammogram of an intact "C" type oxygen sensor having both the HG and the GPM. The HG is hydrated prior to the measurement with phosphate buffer solution of pH 11. The potential is scanned from +1000 mV to -1000 mV with a rate of 10 mV/s. The sample flow is zero (plain line), 55 $\mu\text{l/min}$ (dashed line) and 4800 $\mu\text{l/min}$ (dashed/pointed line). The sample solution is air saturated phosphate buffer at pH 11.

Response time:

The response time of the sensor, mounted in the flow-through cell and exposed directly to a gas stream of O_2/N_2 gas mixtures, is 6 s for t_{90} and 8 s for t_{95} . When a liquid sample is used, t_{95} is in the range of 120 s for a sample flow of 250 $\mu\text{l/min}$. Again, a striking difference of the response time between the measurement in gas and in liquid is observed, in analogy to the findings for the 1st Design. Due to the fact that the observed response time characterizes the response of the complete flow-through system, and not of the sensing element alone, the response behavior strongly depends on the rinsing efficiency of the sample stream, and particularly on the flow velocity (Tab. 5.2). In addition to the flow velocity, the shape of the flow-through channel is mainly decisive for the dynamic characteristics of the system.

flow [$\mu\text{l}/\text{min}$]	t_{90} [s] $\text{pO}_2 \uparrow$	t_{95} [s] $\text{pO}_2 \uparrow$	t_{90} [s] $\text{pO}_2 \downarrow$	t_{95} [s] $\text{pO}_2 \downarrow$
250	100	126	-	-
2500	28	38	34	62

Tab. 5.2 Response times t_{90} and t_{95} of the pO_2 sensor for increasing ($\uparrow$) and decreasing ($\downarrow$) pO_2 steps and for different sample velocities. The measurements are performed on air saturated and N_2 saturated phosphate buffer solutions of pH 7.4.

We observed that swelling of the hydrogels causes an undesired hydrodynamic behavior in the flow-through channel, as shown in Fig. 5.14. Due to increased hydrogel volume, the channel is narrowed on top of the sensing elements, which causes the fluid to pass mainly at the outer side of the channel. On top of the sensing elements the channel is rinsed very poorly, thus slowing down the response.

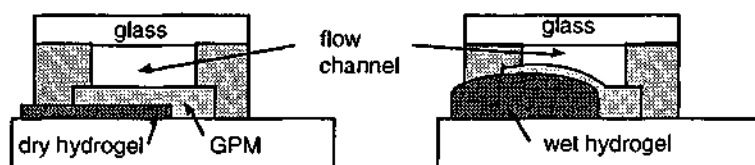


Fig. 5.14: Cross section of the flow-through channel for the cases where the hydrogel is dry (left) and wet (right). The flow-through channel is narrowed due to the swelling of the hydrogel, causing a reduced rinsing efficiency on top of the sensing elements.

An improvement can be achieved by using a channel, which is not wider than the hydrogels of the sensing elements (1 mm). First tests using a 1 mm wide channel, instead of 2 mm, show a considerably faster response. Furthermore, it can be found that, by increasing the height of the channel, the sensor becomes faster. The rinsing efficiency seems to be poor, if the height of the channel is small compared to its width. An optimum is achieved, if width and height have the same dimensions.

It is also observed that the response becomes faster, when solutions are used in which the oxygen content is chemically held constant (oxygen buffers). The response from air saturated to oxygen free solution is considerably faster when using a solution in which the oxygen level is held nearly at zero by an excess of Na_2SO_3 , as compared to a solution simply equilibrated with N_2 . Na_2SO_3 is reacting with and consuming oxygen that is stored in the sidewalls of the flow-through system, or is leaking into the measuring

chamber. Accordingly, the true pO_2 value of the sample is established faster in the measuring chamber.

The choice of material for the flow-through channel ring has a serious impact on the memory effect of the system. As the solubility of oxygen in silicone rubber is 9.9 times higher than in aqueous solution (for carbon dioxide: 2.6 times higher), the polymeric ring is a huge sink/source for O_2 and CO_2 , respectively. This seriously decreases the speed of response as a result of the necessary charging/decharging of the polymeric sealing ring. In standard blood gas analysis, the blood sample (between 70 and 150 μl) is stopped as soon it has entered the measuring chamber, and the measurement is performed on the resting sample. This procedure would be impossible with our setup because oxygen and carbon dioxide would immediately equilibrate with the polymeric sealing ring, thus falsifying the results of the measurement.

To circumvent this problem, the O_2 and CO_2 solubility of the sealing ring has to be seriously reduced by choosing a different polymeric material. Another approach would be to etch the flow-through channel directly in the covering glass which is now pressed onto the gas permeable membrane (Fig. 5.15).

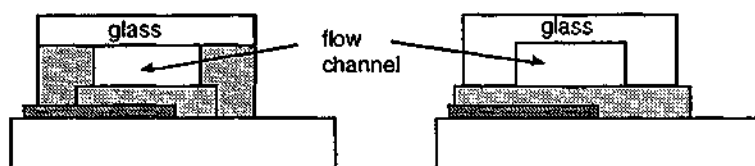


Fig. 5.15: Left side: actual setup using the polymeric sealing ring which is closed on top with a flat glass plate
Right side: the channel is etched into the glass plate which is directly pressed onto the gas permeable membrane. The polymeric sealing ring is no longer necessary.

For the actual setup, the response is still too slow for clinical demands. However, with a better choice of the materials and an optimum shape of the flow-through channel, the response time of the system can be shortened to an acceptable value. It should be mentioned that in conventional blood gas analysis, where 40 s "response time" are a standard, the steady state readings are always mathematically extrapolated, but never really awaited. Therefore, the actual response times t_{95} of commercially available sensors are higher than 40 s. This indicates that the response of the present sensor is not far away from an acceptable value.

CO₂ effect:

To correctly determine the CO₂ effect on the current of the pO₂ sensor, sample solutions are used that have a defined pO₂ and pCO₂ level. These solutions are prepared by equilibration with distinct gas mixtures having a controlled and constant pO₂ level. Fig. 5.16 shows a typical repeated measurement on two solutions. It is observed that the current of the pO₂ sensor is influenced only at the beginning of a step change in pCO₂, and then falls back to the steady state value. 2 min after the pCO₂ step change, the current is again on the previous level. Therefore, the static current of the pO₂ sensor seems to be completely independent of the pCO₂ level. This can be explained by the following reasoning. The oxygen reduction at the WE produces OH⁻ which defines the pH value in the surrounding of the WE. When the pCO₂ of the sample is changed, the equilibrium pH near the WE will also be disturbed for a short moment. However, due to the continued OH⁻ production, the pH falls back to its original value. This equilibrium pH at the WE is defined by the diffusion of OH⁻ away from the WE, and the diffusion of CO₂ into the WE area. Apparently, the OH⁻ production by the O₂ reduction is more important than the influence of CO₂.

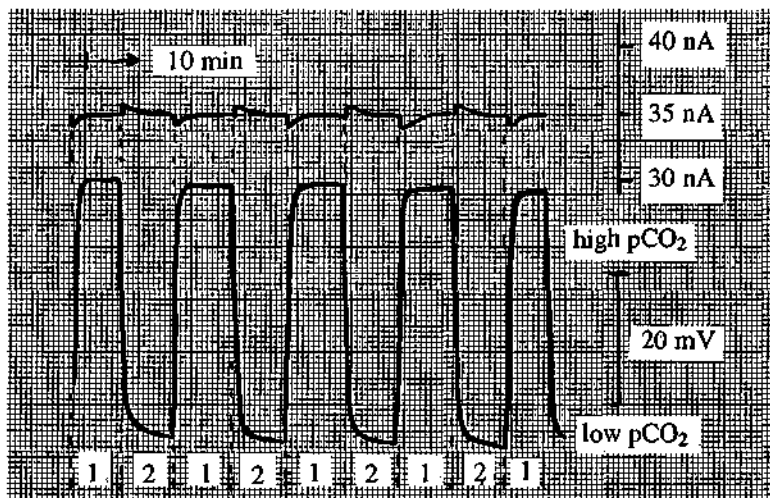


Fig. 5.16: Parallel operation of a "M10" pO₂ sensor (upper curve) and a pCO₂ sensor (lower curve) on the same chip. Repeated measurement on two 100 mM KCl solutions having a constant pO₂ of 160 mmHg, but a different pCO₂ (15 mmHg and 91 mmHg). Solution 1 is equilibrated with a gas mixture of 21 % O₂ / 12 % CO₂ / 67 % N₂; solution 2 with 21 % O₂ / 2 % CO₂ / 77 % N₂. The flow is 240 µl/min. The pO₂ sensor is polarized at -800 mV.

When the $p\text{CO}_2$ decreases, the current increases for a short time, and vice versa. The dynamic deflection of the reduction current is in the order of 4 % for a step change in $p\text{CO}_2$ between 15 and 91 mmHg. The observed influence of the $p\text{CO}_2$ on the oxygen determination is very small, especially when it is considered that the change in $p\text{CO}_2$ covers more than the physiological range.

The present measurements on the 2nd Design are at variance with those on the 1st Design where it was observed that the current increases with increasing $p\text{CO}_2$, not only for a short moment, but also in the steady state. However, the measurements on the 1st Design were performed by using phosphate buffer solutions, in which the $p\text{CO}_2$ level was defined by adding NaHCO_3 . As already discussed, the $p\text{O}_2$ of such a solution is not properly defined. The measurements with the 2nd Design are therefore much more reliable since here the samples are equilibrated with a defined oxygen partial pressure.

Flow dependence:

In Fig. 5.17 is plotted the flow dependence of the two Closed Clark cells of a "C" type oxygen sensor, operated in parallel on the same chip.

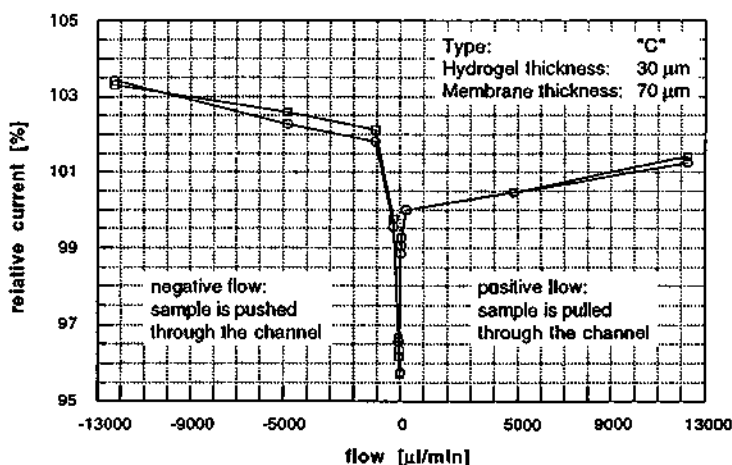


Fig. 5.17 Flow dependence studied in the flow-through cell using air saturated phosphate buffer solution of pH 7.4. The sample is either pulled through the cell, thus having the pump downstream (positive flow velocity), or pushed through the cell, having the pump upstream (negative flow velocity). The relative current [%] is plotted, using the current at a flow of $+260 \mu\text{l}$ as a reference value. The polarization voltage is -800 mV .

The flow dependence follows the predicted behavior, as discussed in ch. 3.2.6, with a current minimum at flow 0, and increasing currents at higher flow rates. The relative current changes are within $\pm 3\%$ over a broad flow range of -13 to $+13$ ml/min. Thus the flow effect is small as compared to the 1st Design. In the range of 200 to 13000 $\mu\text{l/min}$, the relative current change is even smaller than 1 %. The flow effect is not the same when the sample is pulled or pushed through the flow-through channel: the curve is shifted to higher $p\text{O}_2$ values when the flow is negative. We believe that this phenomenon is due to the hydrostatic pressure of the sample in the flow-through channel, which is decreased if the sample is sucked through the channel, or increased if the sample is pushed. As the total pressure is the sum of the partial pressures of all components, the partial pressure of oxygen follows the fluctuations of the total pressure, as shown in Fig. 5.17. It is observed that this effect is increased, due to the larger flow resistance, if the height of the channel is small.

A parameter related to the flow dependence is the so called liquid gas difference. For the measuring system studied in Fig. 5.17, the current value found for humidified air corresponds to the current measured for air saturated solution at a flow of $+260$ $\mu\text{l/min}$. Thus the liquid gas difference of the $p\text{O}_2$ sensor is almost zero. As for liquid samples, the same fluctuations of the reduction current are observed for gaseous samples when the total pressure of the gas stream is changed. The current increases with increasing total pressure in the flow-through channel, and vice versa. It is not clear whether this pressure dependence is exclusively caused by a change in O_2 partial pressure, or not. A second reason could be that a pressure induced change in the thickness of the hydrogel influences the current. This explanation would agree with findings for the $p\text{CO}_2$ sensor where a much smaller dependence on the total pressure of the sample is observed. As a matter of fact, the signal of the $p\text{CO}_2$ sensor is independent of the thickness of the hydrogel.

The flow dependence and the related liquid gas difference of the 2nd Design have been reduced almost to zero by using small WE and WE arrays. The influence of the hydrostatic pressure may bear some problems in view of practical applications. Therefore, the flow resistance of the flow-through system has to be kept as low as possible. Such precautions lead to a reduction of pressure changes in the flow-through channel, as induced by differing pump velocities.

5.4.3 $p\text{CO}_2$ sensor

If it is not mentioned otherwise, the ISFET of the $p\text{CO}_2$ sensor is operated at $V_{\text{DS}}=0.5\text{ V}$ and $I_{\text{D}}=100\text{ }\mu\text{A}$. The measurements are performed at room temperature, without temperature compensation. The ISFET is shielded against light.

Sensitivity in aqueous solution:

Fig. 5.18 shows the calibration curve of the $p\text{CO}_2$ sensor in phosphate pH buffer solution with different concentrations of NaHCO_3 . A high linearity is observed over a range of 2 decades of $p\text{CO}_2$. The correlation coefficient of the linear regression is 0.9990. However, the sensitivity of 39 mV/dec is fairly low.

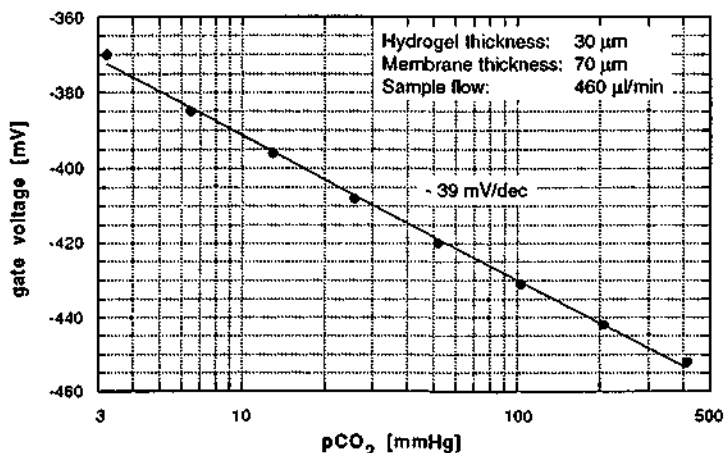


Fig. 5.18 Calibration plot of the $p\text{CO}_2$ sensor recorded in phosphate buffer solution of pH 7.4. The $p\text{CO}_2$ is adjusted with different concentrations of NaHCO_3 (see eqn. (4.1)).

The sensitivity strongly depends on the humidity of the hydrogel and varies between 30 and 45 mV/dec. Maximum sensitivity is achieved immediately after hydration of the HG through the hydration channel. As soon as the sensor is operated in the flow-through system, the sensitivity decreases slowly, as already mentioned for the $p\text{O}_2$ sensor. After an equilibrium humidity has been established in the HG (after some days of operation), the sensitivity again remains constant and reproducible for both $p\text{CO}_2$ sensors on the chip, as shown in Fig. 5.19. To achieve high sensitivity, one should find a

way to keep the humidity in the HG as high as possible, for example by adding glycerol to the monomer solution. We tried to equilibrate the HG with a mixture of water/glycerol by immersing the encapsulated chip into this solution. However, glycerol does not pass through the hydration channel, probably due to its high viscosity and molecular size. Therefore, glycerol has to be added to the acrylamide monomer solution prior to the HG deposition.

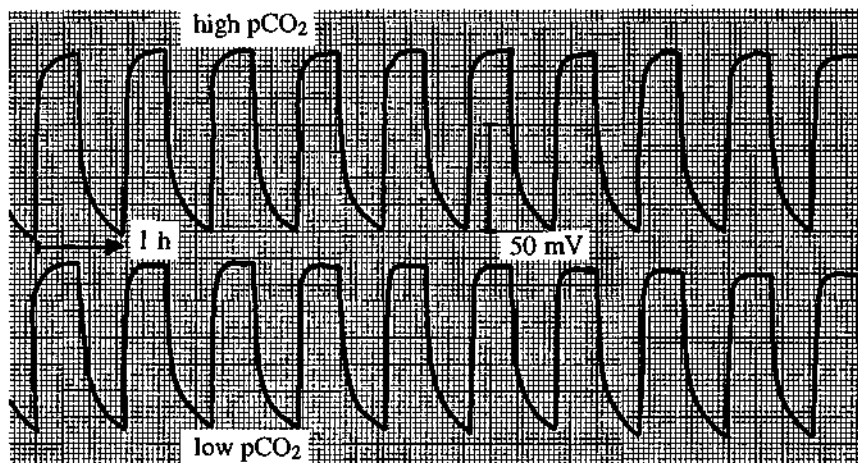


Fig. 5.19: Parallel response of two on-chip $p\text{CO}_2$ sensors to two phosphate buffer solutions of pH 7.4, one without NaHCO_3 (air saturated: 0.25 mmHg; "low $p\text{CO}_2$ "), the other with 5 g/l NaHCO_3 (80 mmHg; "high $p\text{CO}_2$ "). The flow is 83 $\mu\text{l}/\text{min}$.

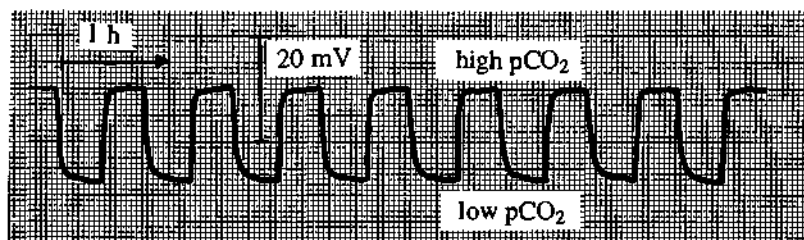


Fig. 5.20: Response of a $p\text{CO}_2$ sensor to two phosphate buffer solutions of pH 7.4, one with 0.26 g/l NaHCO_3 (4.3 mmHg; "low $p\text{CO}_2$ "), the other with 1.33 g/l NaHCO_3 (21.5 mmHg; "high $p\text{CO}_2$ "). The flow is 83 $\mu\text{l}/\text{min}$.

As can be seen from Fig. 5.19 and 5.20, the $p\text{CO}_2$ sensor exhibits a high reproducibility and stability. Furthermore, it can be operated for more than 2 months. The offset drift of the signal is in the order of 1-2 mV/day. If the sensor is frequently calibrated, the observed humidity effect does not cause any problems in practical applications. By choosing a hygroscopic hydrogel, the sensitivity can be increased and stabilized.

Response time:

The response time t_{95} for increasing $p\text{CO}_2$ steps, measured in liquid samples, is in the order of 1 to 2 min, depending on the flow rate of the sample, as shown in Tab. 5.3:

flow [$\mu\text{l}/\text{min}$]	t_{90} [s] $p\text{CO}_2 \uparrow$	t_{95} [s] $p\text{CO}_2 \uparrow$	t_{90} [s] $p\text{CO}_2 \downarrow$	t_{95} [s] $p\text{CO}_2 \downarrow$
13	300	780	-	-
83	90	120	-	-
126	75	105	450	720
470	60	90	300	420

Tab. 5.3 Response times t_{90} and t_{95} of the $p\text{CO}_2$ sensor, for increasing ($\uparrow$) and decreasing ($\downarrow$) $p\text{CO}_2$ steps and for different sample velocities. Measurements are performed on air saturated phosphate buffer solution of pH 7.4, and on the same phosphate buffer with 25 g/l NaHCO_3 .

The way how the response time for decreasing $p\text{CO}_2$ steps is measured, is not realistic because the $p\text{CO}_2$ level of air saturated solution is very low. The same remarks can be made here as for the response time of the $p\text{O}_2$ sensor.

Dependence on the pH of the sample:

Because the GPM is impermeable to ions, the response of the $p\text{CO}_2$ sensor should be independent of the pH of the sample. This fact can be used to check the membrane integrity. As soon as the GPM is pierced or leaking, the $p\text{CO}_2$ sensor starts to follow the pH changes of the sample solution, indicating a faulty sensor.

Dependence on the $p\text{O}_2$ of the sample:

The influence of the oxygen partial pressure on the $p\text{CO}_2$ measurement in a sample with constant $p\text{CO}_2$ has been determined using a 100 mM KCl solution equilibrated with $\text{O}_2/\text{CO}_2/\text{N}_2$ gas mixtures. The $p\text{CO}_2$ level is held constant at 38 mmHg, the $p\text{O}_2$ is changed from 76 to 608 mmHg. The resulting signal of the $p\text{CO}_2$ sensor is not influenced by the $p\text{O}_2$.

5.4.4 pH sensor

If it is not mentioned otherwise, the ISFET of the pH sensor is operated at $V_{DS}=0.5$ V and $I_D=100$ μ A. The measurements are performed at room temperature without temperature compensation. The ISFET is shielded against light. As reference electrode, the Ag/AgCl layer of the "Semi-open ISFET" cell is used.

Sensitivity in aqueous solution:

The pH sensitivity of the ISFET vs. the on-chip RE (Semi-open ISFET) is 51 mV/pH, measured in samples with constant chloride concentration (Fig. 5.21). For the 2nd Design, Si_3N_4 has been used as pH sensitive material, which explains the slightly lower sensitivity as compared to Al_2O_3 (1st Design).

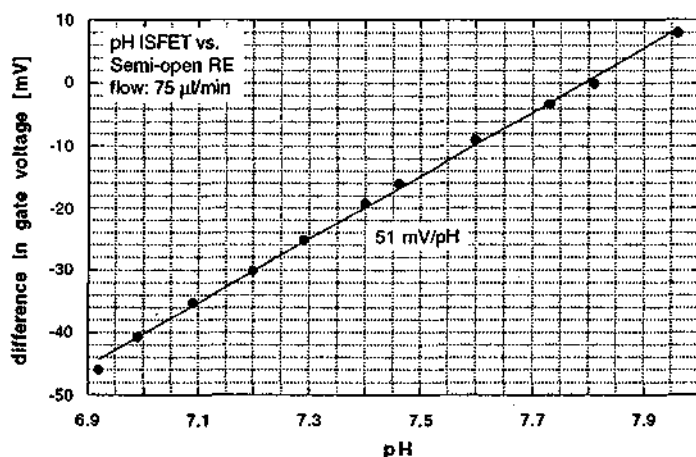


Fig. 5.21: Calibration plot of the pH sensor. The chip is mounted in the flow-through cell and the on-chip RE (Semi-open) is used. The sample solutions are phosphate buffers, which are mixtures of (100 mM KH_2PO_4 / 50 mM KCl) and (100 mM NaOH / 50 mM KCl). Thus, the chloride concentration of the sample is constant. Prior to injection, the samples are measured with an Orion pHure electrode in order to obtain a reference pH value. The sample flow is 75 μ l/min. The sensor signal is drift corrected, by means of an intermittent calibration at pH 7.8. The correlation coefficient of the linear regression is 0.9984.

Fig. 5.22 and 5.23 show the results of repeated measurement on two phosphate buffer solutions having a pH of 7.06 and 7.32 pH, and pH 6.95 and 7.9, respectively. The difference in the sensor signal between the two pH buffers is reproduced with a standard deviation of 0.14 mV, which corresponds to about 0.003 pH. During this measurement, the baseline drifts continuously with a value of 0.25 mV/h, which is believed to be mainly due to a drift of the reference electrode potential.

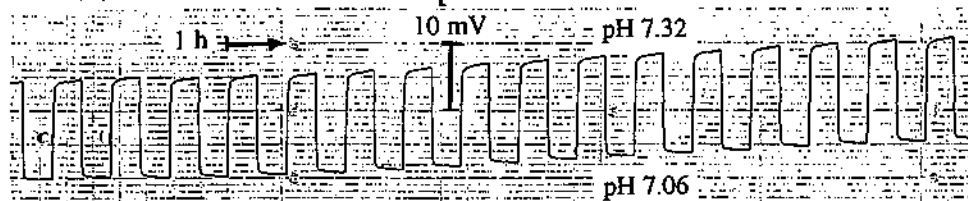


Fig. 5.22: Repeated measurement on two phosphate buffer solutions at pH 7.06 and 7.32, which are mixtures of (100 mM KH_2PO_4 / 50 mM KCl) and (100 mM NaOH / 50 mM KCl). The on-chip RE (Semi-open) is used. The flow is 36 $\mu\text{l}/\text{min}$.

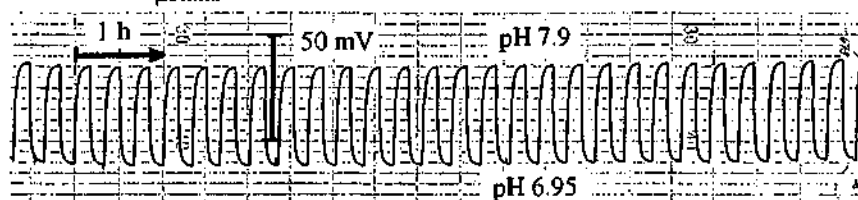


Fig. 5.23: Repeated measurement on two phosphate buffer solutions of pH 6.95 and 7.9, which are mixtures of (100 mM KH_2PO_4 / 50 mM KCl) and (100 mM NaOH / 50 mM KCl). The on-chip RE is used. The flow is 36 $\mu\text{l}/\text{min}$.

Response time:

The response time of the pH sensor, mounted in the flow-through cell, is given in Tab. 5.4. The response time of the pH sensor is in the same range as for the pO_2 and pCO_2 sensors (1 to 2 min for t_{95}), which indicates once again that the rinsing efficiency of the flow-through system is mainly responsible for the sluggish response, and not the sensor itself which in the case of the pH sensor is very fast.

flow [$\mu\text{l}/\text{min}$]	t_{90} [min]	t_{95} [min]
83	3	4
130	2	2.5
240	1	1.5

Tab. 5.4 Response times t_{90} and t_{95} of the pH sensor, measured for a step change from pH 6.95 to 7.7 in a phosphate buffer solution with 50 mM KCl. The response times are equal for increasing or decreasing pH steps.

Cl⁻ sensitivity of the reference electrode:

If the chloride concentration is not identical from one sample to the other, then the overall response of the pH sensor will consist of the response of the ISFET to the pH change, and of the retarded response of the reference electrode to the Cl⁻ activity change. This behavior is shown in the upper curve of Fig. 5.24, where a repeated response to two phosphate buffer solutions with different pH values and different Cl⁻ concentrations is recorded. The potential response of the Open ISFET vs. the reference electrode of the Semi-open cell is illustrated. In the first 2 min after changing the solution, the variation in the overall signal is only determined by the pH response, and later it is governed by the Cl⁻ response. Thus, the correct pH reading has to be taken in the first 2 min after changing the sample. In the lower curve of Fig. 5.24, the response of the Semi-open ISFET to its reference electrode is recorded. In this case, both the ISFET and the Ag/AgCl layer are covered with the hydrogel and are exposed to the analyte only through the liquid junction. Thus, both the pH and the Cl⁻ response are retarded in the same manner, and the overall signal is determined by a mixed pH/Cl⁻ response. Hence, a separate determination of the pH response is no longer possible.

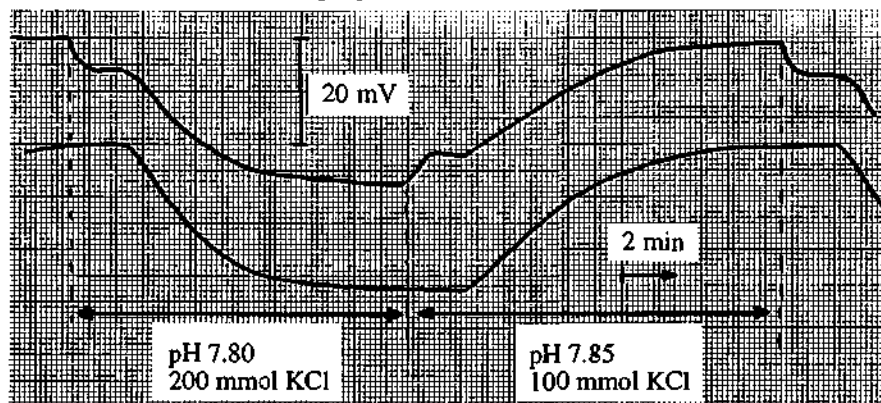


Fig. 5.24: Repeated response of the pH sensor to two phosphate buffer solutions with pH 7.8 / 200 mM KCl and pH 7.85 / 100 mM KCl, respectively. The flow rate is 37 μ l/min.

Upper curve: Response of the Open ISFET vs. the Ag/AgCl layer of the Semi-open cell. First, the uncovered ISFET responds to the pH change, while after a delay of 2 min, the RE responds to the change in KCl concentration. Thus, the pH response can be clearly identified.

Lower curve: Response of the Semi-open ISFET vs. the Ag/AgCl layer of the Semi-open cell. Because both the ISFET and the Ag/AgCl layer are covered with the hydrogel, a mixed response is observed, and a separate pH reading is not possible.

Because both curves, the upper and lower one, measure the pH vs. the Cl^- response, their total signal change must be identical, which is confirmed in Fig. 5.24. The response of the reference electrode to the change in Cl^- concentration has a t_{95} of 10 min. The determination of the Cl^- sensitivity in phosphate pH buffer solutions with constant pH and with KCl concentrations of 50, 75 and 100 mM KCl, respectively, confirms the almost ideal Nernstian behavior of the RE.

For practical use, where samples with unknown Cl^- concentration have to be measured, the Cl^- influence has to be circumvented by taking only differential readings of the sensor signal. This requires of course a reproducible retarding behavior of the reference electrode, as demonstrated in Fig. 5.25 and 5.26. In these two figures, the response of the pH sensor vs. the Semi-open RE to samples with different Cl^- concentrations is plotted. The sensor system responds very reproducibly to the pH and Cl^- changes, and exhibits a good separation in time between the pH and Cl^- response.

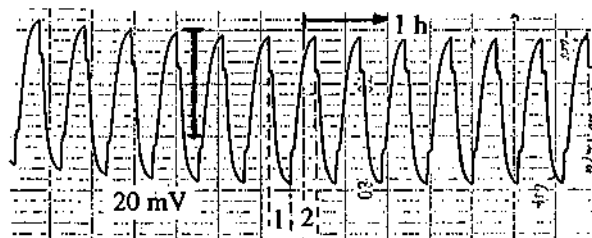


Fig. 5.25: Repeated response of the pH sensor to two phosphate buffers with pH 7.8 / 200 mM KCl (solution 1) and at pH 7.85 / 100 mM KCl (solution 2), respectively, at a flow rate of 37 $\mu\text{l}/\text{min}$. The Open ISFET vs. the Ag/AgCl layer of the Semi-open cell is used.

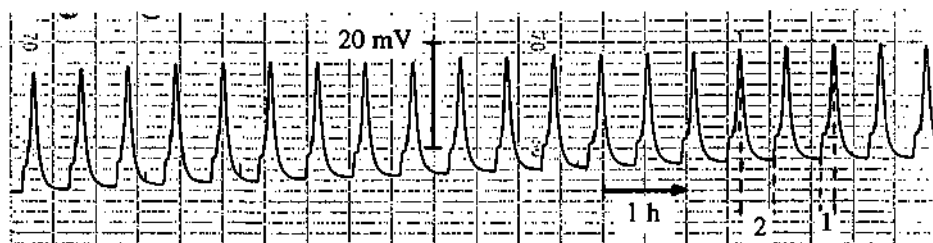


Fig. 5.26: Repeated response of the pH sensor to Merck Titrisol pH buffer of pH 7.0. Solution 1 is the pure buffer without any KCl, having a pH of 6.98. Solution 2 is the same buffer, but with 50 mM KCl added, resulting in a pH of 6.92. The Open ISFET vs. the Ag/AgCl layer of the Semi-open cell is used at a flow rate of 37 $\mu\text{l}/\text{min}$.

As predicted in eqn. (3.83) and (3.84), the response of the pH sensor is shifted to more positive values either by increasing the pH, or by decreasing the Cl^- concentration of the sample, and vice versa.

The observed Cl^- sensitivity is an inherent drawback of the miniaturization of the reference electrode. However, the response of the pH sensor is very reproducible, which means that by using correct timing of the measuring cycles, the pH can be properly determined, independently of the Cl^- concentration of the sample. On the other hand, for highly precise determinations of the true pH values, a correction for diffusion potentials arising at the liquid junction between the sample and the Semi-open RE cell, appears to be necessary (see also below).

Influence of the ionic strength:

In order to evaluate the effect of the ionic strength on the overall response of the pH sensor, different concentrations of KNO_3 are added to phosphate pH buffer (0, 20 mM, 100 mM and 200 mM KNO_3). To check the exact pH values of these solutions, they are measured with a Metrohm pH electrode. The addition of KNO_3 to phosphate buffer shifts the pH reading of the glass electrode to lower values: the pure phosphate buffer without KNO_3 has a measured pH of 7.22, whereas after the addition of 200 mM KNO_3 , a pH of 7.10 is measured with the glass electrode. These shifts are believed to be caused by a change in the liquid junction potential (ch. 3.4.4). It is likely that they are not caused by a change in the activity of H^+ . The ISFET vs. the Semi-open RE follows exactly the same shifts of the pH readings as observed with the glass electrode. In Fig. 5.27 is shown a repeated response of the pH sensor to a phosphate buffer solution without KNO_3 and with 200 mM KNO_3 , respectively. The effect of the ionic strength is relatively small and corresponds to the effect observed for conventional glass electrodes.

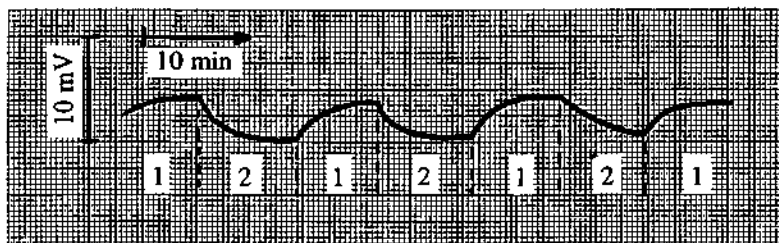


Fig. 5.27: Repeated response of the pH sensor vs. the Semi-open RE to phosphate pH buffer solutions without KNO_3 (solution 1) and with 200 mM KNO_3 (solution 2). The nominal pH values determined with a Metrohm pH glass electrode are pH 7.22 (1) and 7.10 (2), respectively. The flow rate is 240 $\mu\text{l}/\text{min}$.

Influence of the flow rate:

pH measurements in the flow-through cell show a dependence on the flow velocity. The flow dependence is different when the on-chip RE is used, or when a conventional RE is placed into the input line.

When using the on-chip RE (Fig. 5.28), it is observed that the overall potential of the pH sensor is shifted to higher pH values with increasing flow rate. After changing the flow velocity, the potential shifts slowly to a new equilibrium value which is reached only after 1 or 2 hours. The sensitivity of the pH sensor, however, remains unchanged.

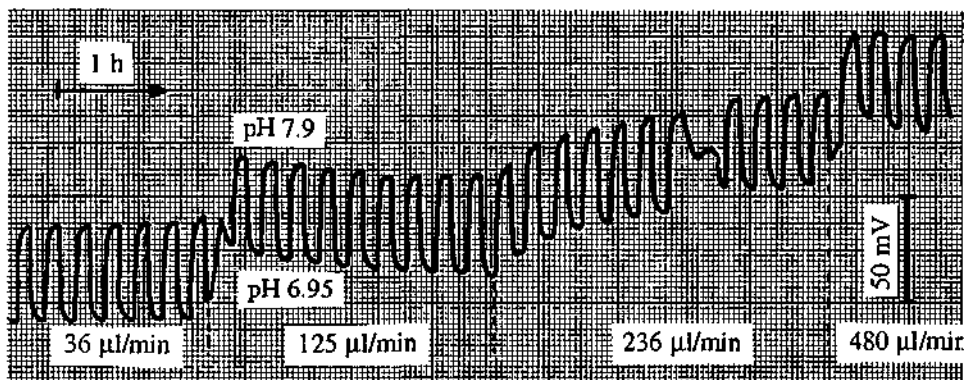


Fig. 5.28: Repeated measurement on two phosphate buffer solutions of pH 6.95 and 7.9, which are mixtures of (100 mM KH_2PO_4 / 50 mM KCl) and (100 mM NaOH / 50 mM KCl). The Semi-open RE is used. The flow is changed from 36 $\mu\text{l}/\text{min}$ to 480 $\mu\text{l}/\text{min}$.

When a conventional RE is used, which is placed into the input line upstream of the flow-through cell, similar (but smaller) potential shifts are observed, as shown in Fig. 5.29. In analogy to the measurements with the on-chip RE, an increasing flow rate shifts the signal to higher pH values. However, the potential shifts are established rapidly, in contrast to the on-chip RE. Furthermore, the sensor signal oscillates with the movements of the peristaltic pump, exhibiting an amplitude in the range of 10 mV. The amplitude increases with the pump frequency, and as soon as the pump is stopped, the signal stops oscillating. The oscillation and the offset of the signal are not influenced by the flow direction. The oscillation is also present using the on-chip RE, but with an amplitude below 0.5 mV and therefore not visible in Fig. 5.28.

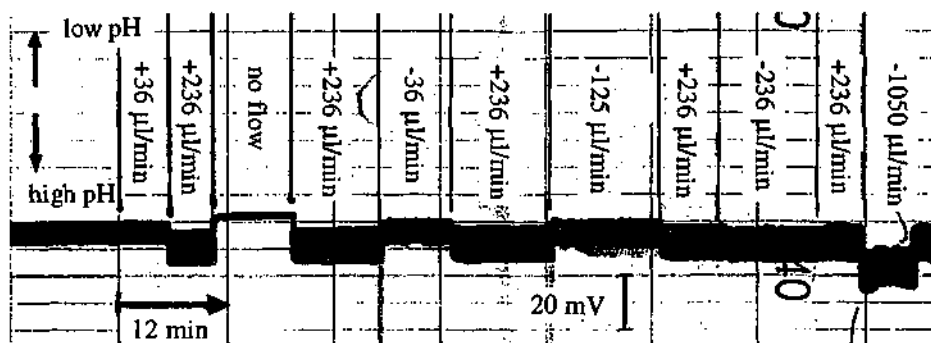


Fig. 5.29: Flow dependence of the Open ISFET vs. an external Ag/AgCl RE (Hamilton Single Pore) located upstream in the input line. The flow velocity is varied in positive direction (sample is pulled through the flow-through cell, RE is upstream) or negative direction (sample is pushed through the flow-through cell, RE is downstream). Phosphate buffer of pH 7.4 is used.

In the literature, potential variations of potentiometric sensors as a function of the flow velocity have been reported repeatedly and ascribed to various causes. Fleet et al. [5.4] observed a sensor signal that fluctuated with the movements of the peristaltic pump. They attributed this flow dependence to liquid junction potential changes at the reference electrode. They suppressed these signal changes by using a capillary free flow liquid junction downstream of the sensing electrode and by using a pressure pulse suppressor in the inlet tubing. It is not clear, which one of the two modifications eliminated the flow effect. Ruzicka et al. [5.5] reported two different interactions between the pump and the electrode signal: one due to static electricity effects, and the other due to changes in pressure caused by the peristaltic pump. They eliminated the static electricity effects by proper earthing of the inlet and outlet of the flow cell. The pressure dependence was reduced by use of a bubble chamber in the inlet tubing, which served as a pressure pulse suppressor. Van den Winkel et al. [5.6] attributed the observed flow dependence to streaming potentials in the liquid caused by pressure differences. A streaming potential occurs, when a liquid is forced through a narrow capillary by a pressure gradient.

The streaming potential can be described by the Helmholtz-Smoluchowski equation (for laminar flow):

$$E_{str} \sim \frac{v l \rho}{r^4}$$

with	v	flow velocity
	l	distance between the reference electrode and the sensing electrode
	r	radius of the (tubular) flow cell
	ρ	electrical resistivity of the sample solution

As a consequence, a streaming potential is set up over the distance between the reference electrode and the sensing electrode, and is added to the overall potential measured between the two electrodes. Van den Winkel et al. believe that the observed potential shift is not due to a change in the liquid junction potential because they observed the same potential variations in measurements between the metallic input and output connections of the flow-through system. On the other hand, large values of the streaming potential are expected only for highly diluted solutions with a high electrical resistivity.

Our observation that the sensor signal oscillates with the pressure pulses of the peristaltic pump agrees basically and qualitatively well with the mentioned publications. The oscillations are much higher when the external RE is used because, in this case, the distance between the RE and the ISFET is increased at least by a factor of 10. If the flow-through channel is narrowed by the swollen hydrogels, as shown in Fig. 5.14, then the oscillation is even higher. Grounding of the solution at the input and the output of the channel does not remove the observed phenomena, which excludes static electricity effects. Therefore it seems evident that pressure is the main cause for these fluctuations, and Van den Winkel's explanation fits well with our observation when using the external RE. However, when the on-chip RE is used, the observed shift of the signal offset is slow, whereas Van den Winkel suggests that the streaming potential is established instantaneously. Conventional blood gas analyzers are normally pulling the sample into the measuring chamber, and then stop the flow for the measuring cycle. Therefore, in view of practical applications of the present sensor system, the observed problem has to be studied in detail in order to eliminate fluctuations of the signal due to the flow/stop cycle.

5.4.5 Parallel operation of all three sensors

An easy way to test the parallel performance of all on-chip sensors is by using blood gas standard solutions which are available from several manufacturers. They are normally used in the clinical pO_2 , pCO_2 and pH analysis to provide a reliable quality control of the calibration and performance characteristics of a commercial analyzer. For our tests, we work with BIO-RAD standards; three different solutions are available, having pO_2 , pCO_2 and pH levels in the clinical range:

	pO_2 [mmHg]	pCO_2 [mmHg]	pH
level 1	63.9 ± 5.8	58.1 ± 1.4	7.21
level 2	102.5 ± 2.8	35.6 ± 1.0	7.41
level 3	141.8 ± 4.8	19.5 ± 1.1	7.62

The indicated pO_2 , pCO_2 and pH values are the mean values of the standard solutions, as obtained from 21 commercial blood gas analyzers and specified by the manufacturer. The indicated ranges are the standard deviations of the readings on these 21 analyzers.

In Fig. 5.30 is shown a measurement cycle using BIO-RAD blood gas control solutions and an aqueous calibration solution, which is phosphate buffer solution of pH 7.2 with 50 mM KCl. The response time t_{95} of all three sensors is below 2 min. The response gets even faster when air bubbles are put in between the sample and calibration solutions. These air bubbles seem to rinse the flow-through cell very efficiently [5.7]. The repeated readings for the calibration solution are reproduced very precisely. For the calibration, air saturated phosphate buffer is used, which is a good choice for the pO_2 and the pH sensor. However, the pCO_2 of an air saturated solution is far below the clinical range. Therefore, the pCO_2 sensor does not reach a steady state signal during the calibration phase. Thus, it is recommended for further applications to use a different pCO_2 calibration level within the clinical range.

If the signals of the three sensors are plotted as a function of the pO_2 , pCO_2 and pH levels in the sample solutions, linear calibration curves are obtained (Fig. 5.31). The true values for the samples are reproduced with a precision of $-1.3/+2.5$ mmHg for the pO_2 sensor, $-1.3/+1.2$ mmHg for the pCO_2 sensor and $-0.005/+0.006$ for the pH sensor (maximum deviation of the actual sensor signal from the linear regression).

The results obtained by parallel operation of all sensors are very promising in view of the tests on whole blood. No interdependence of the three sensors is observed.

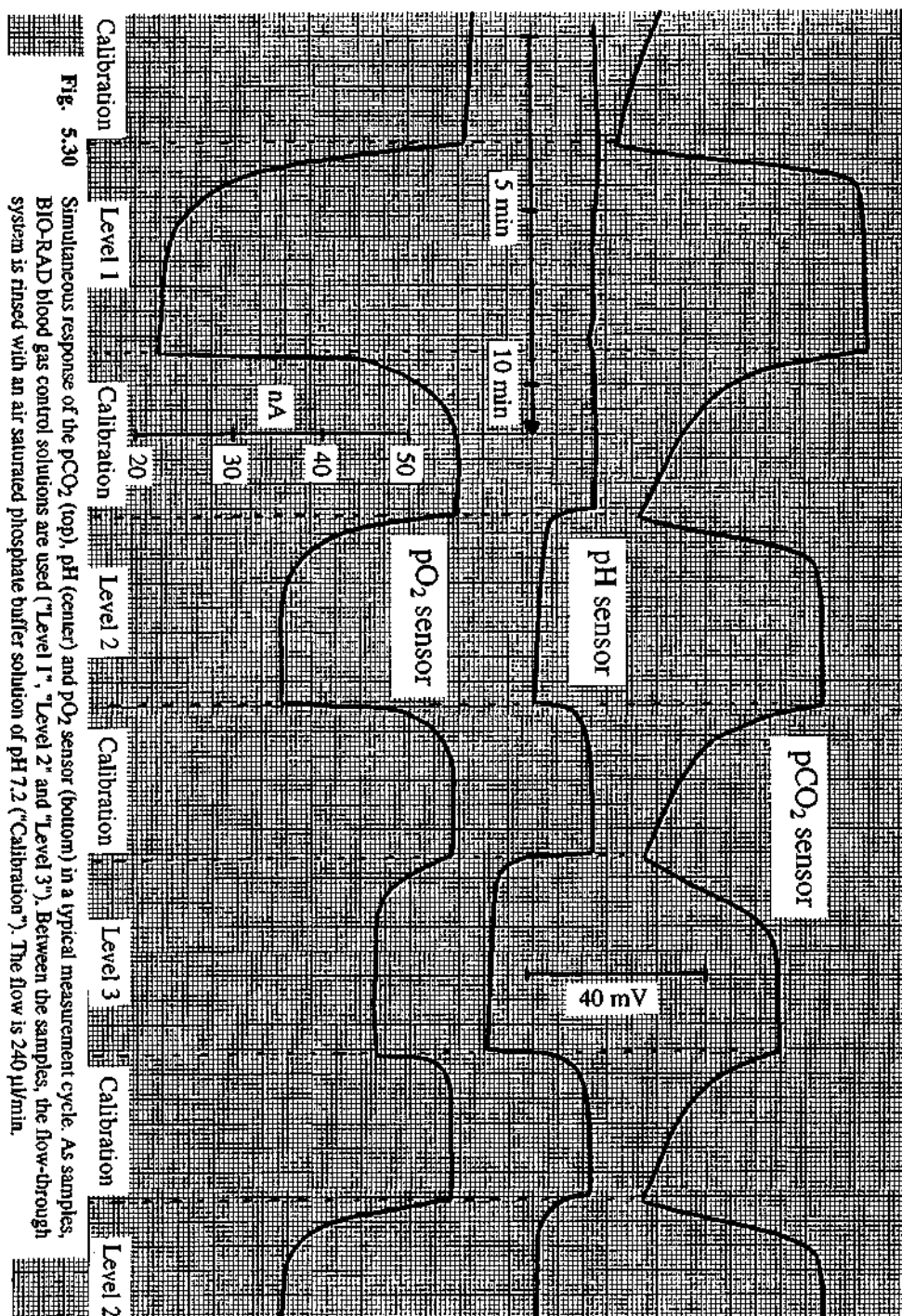


Fig. 5.30

Simultaneous response of the pCO₂ (top), pH (center) and pO₂ sensor (bottom) in a typical measurement cycle. As samples, BIO-RAD blood gas control solutions are used ("Level 1", "Level 2" and "Level 3"). Between the samples, the flow-through system is rinsed with an air saturated phosphate buffer solution of pH 7.2 ("Calibration"). The flow is 240 μ l/min.

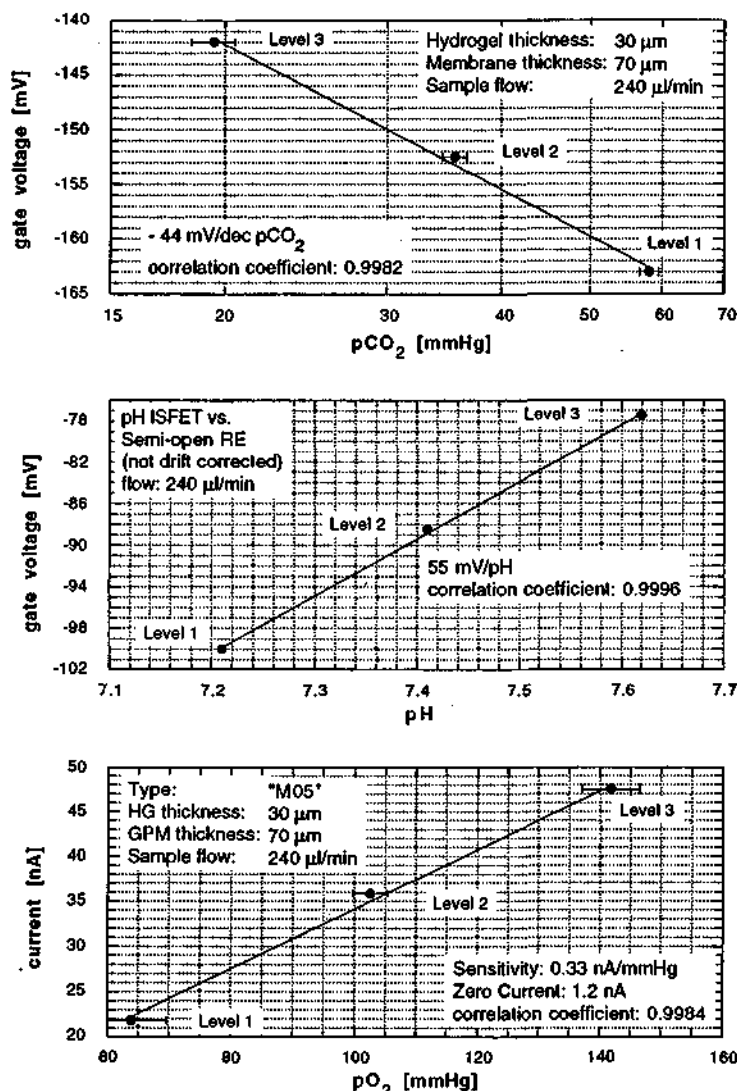


Fig. 5.31: Plot of the sensor responses to the BIO-RAD control solutions as a function of the $p\text{CO}_2$, pH and $p\text{O}_2$ level, respectively. The sensor readings correspond to the plots in Fig. 5.30. At each level, an error bar indicates the variation of the solutions, as it is specified by BIO-RAD.

5.5 Conclusion

Layout

The integration of the flow-through channel on-chip has significantly simplified the mounting of the device into the measuring setup. The precisely defined channel guarantees a highly reproducible and homogeneous flow, and eliminates problems such as bubble entrapment or blood clotting. Polysiloxane as a channel material has proven to have excellent sealing capability. On the other hand, due to its high solubility for O_2 and CO_2 , this polymeric material is not appropriate for blood gas measurements, in particular when the sample size has to be minimized.

The hydration channel, which is used for a quick hydration during the preconditioning, decreases the time needed to put the chip in operation. However, a way has to be found to keep the humidity in the hydrogel layer as constant as possible during operation.

By covering the platinum electrodes with a Si_3N_4 passivation layer, the electrical crosstalk between the individual sensor elements has been completely eliminated. This is indispensable for the simultaneous operation of all sensors. Furthermore, the structuring of the Si_3N_4 layer allows the fabrication of extremely small WE with a size of $5 \times 5 \mu m$, arranged in an array. It is highly difficult to fabricate WE of this size by using direct patterning of platinum, for example with the lift-off technique. The use of arrays of small WE reduces the flow dependence and the liquid gas difference of the pO_2 sensor almost to zero.

By adding a shielding electrode to the amperometric pO_2 sensor, the silver deposition on the WE has been stopped, and the lifetime and the long-term stability of the device has been increased.

The deposition of a $10 \mu m$ Ag layer for the RE by galvanization has two important advantages: the adhesion of the Ag to the underlaying Pt is excellent, and the increased amount of Ag contributes to a longer lifetime of the RE. Furthermore, by growing a thick AgCl layer by means of electrochemical chloridation, the stability of the reference potential has been increased significantly.

Fabrication

The fabrication of the sensor chip has been considerably simplified in comparison with the 1st Design because the holes to the backside of the wafer have been omitted. By the use of one single Si_3N_4 layer as passivation layer and as pH sensitive membrane, two important fabrication steps are combined. Furthermore, the threshold voltage of the ISFETs is more precisely controlled by using LPCVD deposition of Si_3N_4 , instead of CVD

Al_2O_3 . The slightly reduced sensitivity of Si_3N_4 is no problem for the performance of the sensors.

By means of all the modifications of the fabrication, the reproducibility from one device to the other is very good, and the yield on a wafer with more than 90 % is excellent.

Performance of the sensors

A profit of the careful design and fabrication of the device is the highly reproducible performance of the sensor elements, in particular for long-term measurements. The lifetime of the device in operation is more than 2 months, and the storage lifetime under dry conditions is unlimited. By having two identical sensors for the same parameter on one device, high reliability can be guaranteed. The reproducibility of sensitivity and offset of two identical sensor elements on the same chip is within $\pm 3\%$.

All three sensors exhibit an excellent linearity and, after a certain time of preconditioning, a stable sensitivity. However, the final sensitivity of the pO_2 and the pCO_2 sensor is dependent on the humidity of the hydrogel layer. Thus, for future devices, the humidity should be kept as constant as possible by changing the composition of the hydrogel.

The response time t_{95} of the system is in the order of 1 to 2 min at a flow rate above 200 $\mu\text{l}/\text{min}$. This is not yet acceptable for clinical applications. On the other hand, by choosing a more adequate material for the flow-through channel, t_{95} can be reduced at least by a factor of two. This is confirmed by a t_{95} value of less than 10 s for gaseous samples, in which case the gas permeability of the system has no influence.

The cross-sensitivity between the three sensors is very low. The largest effect observed is an influence of the CO_2 content of the sample on the current response of the pO_2 sensor, which however is below 4 % of the current signal for a pCO_2 shift over more than the physiological range. All other effects are negligible.

The flow dependence is very low for the pO_2 and the pCO_2 sensor. However, for the pH sensor, the pressure changes in the flow channel, as induced by different flow velocities, cause streaming potentials. This effect is more important if a flow-through channel is used with a high flow resistance.

A particular problem of the pH sensor is the Cl^- sensitivity of the RE. Due to the high reproducibility of the sensor signals, this effect can be eliminated by means of a differential readout mode.

The characteristics of the 2nd Design have been greatly improved as compared to the 1st Design. Due to the promising results, the combined pO_2 , pCO_2 and pH sensor has found a considerable interest in the clinical instrumentation industry.

The performance of the 2nd Design using whole blood samples will be tested in April '94, in collaboration with one of the leading companies in the blood gas analysis market. For this test, the sensor will be mounted into a commercial benchtop blood gas analyzer, thereby replacing the existing conventional sensor elements. Only with such more realistic tests, the device can prove its applicability for clinical use.

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6 Polymeric Membrane Deposition

6.1 Introduction

Common to many electrochemical sensors of both the potentiometric and the amperometric type is the need for a polymeric membrane which has to be deposited over the underlying transducer. These membranes have many functions such as modifying surface properties of the electrodes, controlling the transport of the species of interest, or as an immobilization matrix. Most of the miniaturized sensors that have been reported so far consist only of microfabricated electrodes on a suitable planar substrate, without integrated electrolytes and membranes. In order to benefit from the ease of microfabrication for both parts of the sensor, transducer and membrane, the problem of the structurization of the polymeric layers has to be addressed. A deposition technique has to be developed, which is compatible with microelectronic processing, and which is capable to deposit these membranes on wafer level with a high reproducibility. Such technique allows to process the complete sensor entirely on wafer, thus excluding costly manipulations on the individual chip. This significantly simplifies the device fabrication, which is of course necessary in order to reduce the costs in view of mass production.

The deposition techniques that are commonly used in association with planar transducer technology range from dip-coating and casting, through lift-off, screen printing, and plasma etching, electrochemical deposition to photolithography. Dip-coating is usually performed manually on-chip level and is widely used because of its simplicity, in spite of the costly processing and the difficulties in achieving reproducible membranes. The casting technique is the deposition of a well-controlled volume of dissolved membrane material onto the transducer surface or into small cavities patterned on the surface in photoresist or polyimide layers. There are some approaches to fill such cavities with automatic dispensing robots directly on wafer level. The lift-off technique, adapted from microelectronic processing, can be used only for membranes with a thickness up to 1.5 μm . Screen printing of the membrane material through openings in a metallic screen is also applicable on wafer level, however, the lateral resolution is poor.

Selective etching of organic polymeric membranes can be performed by dry plasma etching using oxygen containing plasmas or by wet etching. The critical steps are the application of the masking material on top of the membrane and the final stripping procedure. Electrochemical deposition is mostly used to entrap enzymes in a polymeric matrix, on top of a conducting metallic electrode, and yields a good spatial resolution. Finally, photolithographic patterning of polymeric membranes is probably, due to their analogy to photoresists, among all techniques the most compatible with microelectronic processing.

UV photopolymerization of organic polymeric layers for electrochemical sensors has been first described by researchers at the University of Twente, using polyHEMA as hydrophilic [6.1] and polysiloxane as hydrophobic materials [6.2]. An excellent overview of the Twente work is given in [6.3]. In our laboratory, we have adapted their processing to new materials and have optimized it for the patterning of very thick layers up to 600 μm .

As described in chapters 4 and 5, two different polymeric layers are deposited for the fabrication of the present blood gas sensors: a hydrophilic layer ("hydrogel", HG), in which the internal electrolyte of the sensors is entrapped, and a hydrophobic layer, which serves as the gas permeable membrane (GPM). The HG is anchored to the transducer surface, whereas the GPM is partly attached to the underlying HG and partly to the transducer surface. For the 2nd Design, a third polymeric layer, having a thickness of 500 μm , defines the flow-through channel of the device.

Due to the complexity of polymer chemistry, a trial and error method was used in order to optimize the composition of the monomer solution. Over 250 different acrylamide monomer solutions have been tested, concerning the geometrical resolution of the corresponding polymeric structures, the adhesion to the substrate, the adhesion between HG and GPM, the swelling behavior, and other parameters.

6.2 UV photopolymerization technique

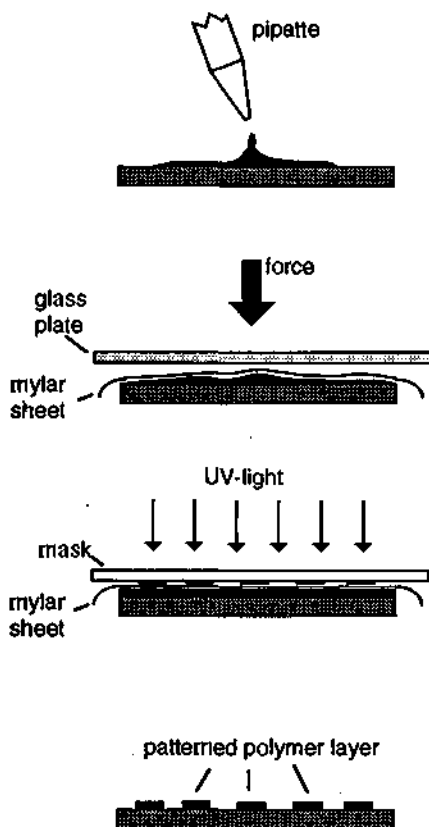


Fig. 6.1 Process steps for the photolithographic patterning of polymeric layers.

In Fig. 6.1, the deposition technique used for the patterning of the polymeric layers is shown. The processing starts with a chemical pretreatment (silanization) of the transducer surface, in order to improve the adhesion of the polymeric layer. Then, depending on the desired thickness of the layer, a corresponding volume of the monomer solution is cast on the wafer. The monomer solution is covered with a 12 μm thick Mylar® sheet (polyethylene terephthalate), which has a low oxygen permeability. Thus, it excludes oxygen from the polymerization reaction (oxygen inhibits the free radical polymerization). Furthermore, the Mylar sheet protects the mask from becoming dirty. With a glass plate pressed onto the Mylar sheet, the monomer solution is leveled in order to obtain a homogeneous monomer layer. The wafer is then transferred onto a mask aligner, and exposed to UV light through a photomask. After the polymerization, the unexposed parts of the monomer solution are removed by using a solvent.

6.3 Polyacrylamide hydrogel layer

6.3.1 Introduction

Hydrogels may be described as crosslinked hydrophilic polymers which swell by incorporating water to an extent that ranges from 10 % to greater than 99 % by weight. There are two reasons for using such a semi-solid electrolyte layer instead of a liquid electrolyte: one is related to the ease of fabrication and storage of the sensor; the other is in view of pressure and flow artefacts, in particular for the pO_2 sensor, which are reduced for a semi-solid hydrophilic layer because the polymer is fairly resistive against mechanical deformation.

As discussed in chapter 3, the hydrogel and the gas permeable membrane are decisive for the sensitivity, selectivity and response time of the pO_2 and pCO_2 sensor. In particular the diffusion coefficients and the Henry's law constants of O_2 and CO_2 are important. On the other hand, the substrate adhesion and the swelling behavior of the HG play a key role concerning the stability and the lifetime of the device. The requirements for the HG can be summarized as follows:

- 1) to take up the highest possible percentage of electrolyte solution;
- 2) to maintain a constant humidity;
- 3) to limit the thickness variations induced by humidity uptake or pressure changes in the sample;
- 4) to have a good adhesion to the substrate and to the GPM
- 5) to be highly permeable for O_2 and CO_2

Polyacrylamide gels (pAA) can have an extremely high water content (>95 %), which results in a gas solubility and diffusion close to that of an aqueous solution. This fact renders them attractive for the present application. Furthermore, the biocompatibility and the thrombogenicity of pAA is described as being similar to that observed with polyHEMA [6.4], which is a widely used polymer for biomedical applications. In addition, in pAA gels a high amount of salt can be dissolved.

Synthetic gels of polyacrylamide can be prepared by polymerization of an aqueous solution of the monomer acrylamide (AA) containing a small percentage of a crosslinking agent, usually N,N'-methylene bisacrylamide (BIS) [6.5]. The polymerization occurs only in the presence of free radicals. These radicals can be produced chemically in the presence of persulphate ions, or in the presence of a photoinitiator when exposed to UV light. Any compound that may act as a free radical trap will act as a polymerization inhibitor. Thus, to obtain reproducible polymerization rates it is necessary to control the concentration of oxygen in the monomeric mixture.

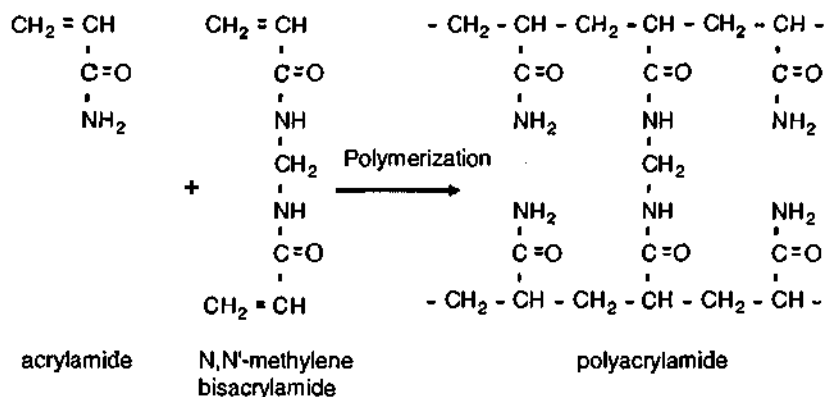


Fig. 6.2 Vinyl polymerization of the monomer acrylamide together with the bifunctional crosslinking agent N,N'-methylene bisacrylamide

In Fig. 6.2, the polymerization reaction of AA and BIS is schematically depicted. AA alone can form only linear chains of the monomer because it has one single acryl group, whereas BIS with its two acryl groups can link two monomer chains together. The amount of the crosslinker BIS determines the density of these links, and therefore physical properties of the gel as the pore size, the density, elasticity and mechanical strength.

6.3.2 Diffusion of gases through the polymer

The diffusion coefficient (D_e) and the Henry's law constant (H_e) of O_2 and CO_2 in the hydrogel are important for the $p\text{O}_2$ and $p\text{CO}_2$ response time and the $p\text{O}_2$ sensitivity. It is therefore a design goal for the HG to achieve both a high D_e and a high H_e . In the literature, only few data can be found on D_e and H_e of O_2 and CO_2 in pAA. Therefore, we have compared in an experimental study the gas diffusion in our pAA gels, in polyHEMA gels, and in aqueous solution. The study is based on the validity of eqn. (3.32), according to which the switch-on response of the $p\text{O}_2$ sensor during a short time interval after the start of polarization is given by:

$$I(t) \cong n F A H_e \sqrt{\frac{D_e}{\pi t}} p_s$$

Therefore, if the switch-on response is plotted as a function of $1/\sqrt{t}$, a linear curve should result, whose slope is proportional to $\sqrt{D_e}$ and H_e . Because all other variables are known, it is possible to determine these two material constants. Fig. 6.3 shows a comparison of the switch-on currents after a step

change from open circuit to -600 mV for a gold electrode with an area of 0.085 mm². The gold electrode is in one case bare, in the second case covered with a pAA layer, and in the third case covered with a polyHEMA layer. The pAA is polymerized from an aqueous solution with 30 % (wt.) AA, and 1 % (wt.) BIS, whereas the polyHEMA is polymerized from an ethyleneglycol solution with 60 % (wt.) HEMA and 1 % (wt.) tetraethyleneglycol dimethacrylate (TEGDMA) [6.6].

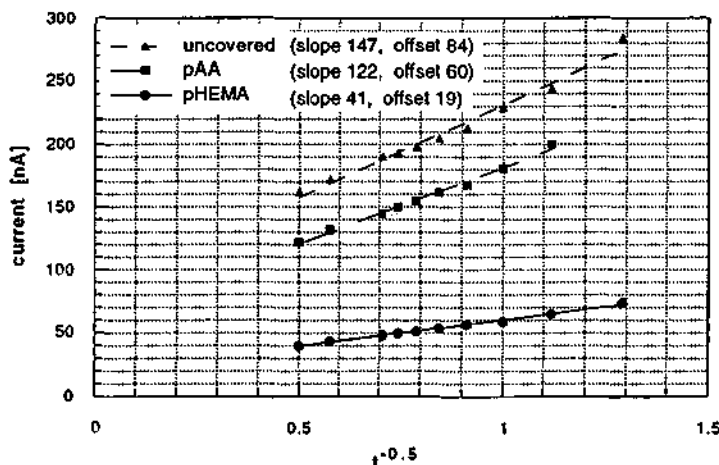


Fig. 6.3 Plot of the switch-on current as a function of $1/\sqrt{t}$. A gold electrode with an area of 0.085 mm², and a Metrohm RE (SCE) and CE (Pt) are used, immersed in an air saturated 1 M KCl solution. The gold electrode is in one case bare, in the second case covered with a pAA layer, and in the third case covered with a polyHEMA layer. The potential is switched from open circuit to -600 mV in order to perform O₂ reduction. The current in the first 4 s after the start of polarization is recorded. Before switching on the potential, the electrode is immersed in the solution for 1 h in order to establish an equilibrium pO₂ in the gel.

It can be observed that the slope is the highest for the bare electrode, immediately followed by the pAA gel. The slope for the polyHEMA gel is much smaller than for the pAA gel. The results of this study indicate that the slope is 1.2 to 2.4 times smaller for the pAA gel than for aqueous solution. If it is assumed that the solubility of oxygen is approximately the same in the pAA gel than in aqueous solution, the value of D_e can be estimated to $3.0 \cdot 10^{-6}$ to $9.0 \cdot 10^{-6}$ cm²/s (D_e in aqueous solution is $1.8 \cdot 10^{-5}$ cm²/s). Furthermore, the value D_e of the pAA gel is estimated to be 5 to 10 times higher than the one for a polyHEMA gel, which has a lower water content.

These estimations are based on the assumption that the value of H_e remains constant in solution, pAA and polyHEMA, which is not completely correct. Davies and Tighe [6.7] indicate that D_e and H_e of a hydrogel are only dependent on the water content in the polymer because the entrapped water acts as a transport medium for the dissolved gas. This fact is confirmed by the present measurements.

In theory, the curves in Fig. 6.3 should cross the origin. However, for the bare electrode and the one with the pAA gel, the offset is fairly high, which indicates that the switch-on current is not purely diffusion limited. Therefore, the obtained results have to be interpreted carefully, and can only indicate a certain range of D_e and H_e .

6.3.3 Geometrical definition

For on-wafer polymeric membrane patterning by means of photolithography, an important parameter is the lateral resolution which determines the number of sensors that can be realized per wafer. There is a need for a high "contrast" in polymerization between the exposed and the unexposed regions. This ensures an easy development and a sufficient geometrical resolution and results in a clean sensor surface at the unexposed regions after the development. We have observed that the contrast of the UV polymerization depends strongly on the concentrations of the photoinitiator and of its catalyst. In addition, the geometrical resolution is dependent on the thickness of the (monomer) layer because the effect of light scattering increases with the thickness of the layer. Furthermore, the viscosity of the monomer solution is also an important factor: if the viscosity is too low, then the monomer solution tends to slowly flow during the exposure to UV, resulting in a smeared pattern. There are several ways to increase the viscosity of the monomer solution, e.g. by using a viscous solvent for the monomer solution, for example polyethyleneglycol or glycerol. We have tried to dissolve high molecular compounds in the solution, such as polyvinylpyrrolidone or hydroxyethylcellulose. However, a general problem is the compatibility between the added substances and the unpolymerized / polymerized acrylamide. The best way in this respect is to dissolve linear chains of polyacrylamide in the monomer solution, which are commercially available with different chain lengths. This allows to adjust the viscosity precisely, without having any compatibility problems.

Fig. 6.4 shows a photograph of a polymerized hydrogel layer of 100 μm thickness, in which 4 holes of each 30 μm x 30 μm are opened (not polymerized). This pattern demonstrates that, obviously, the lateral resolution is in the order of 10 μm .

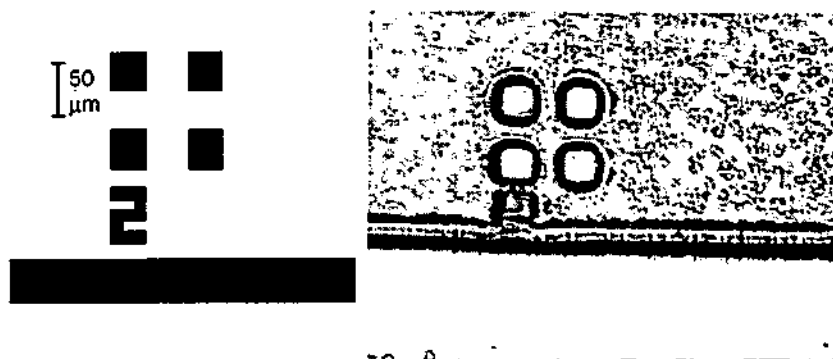
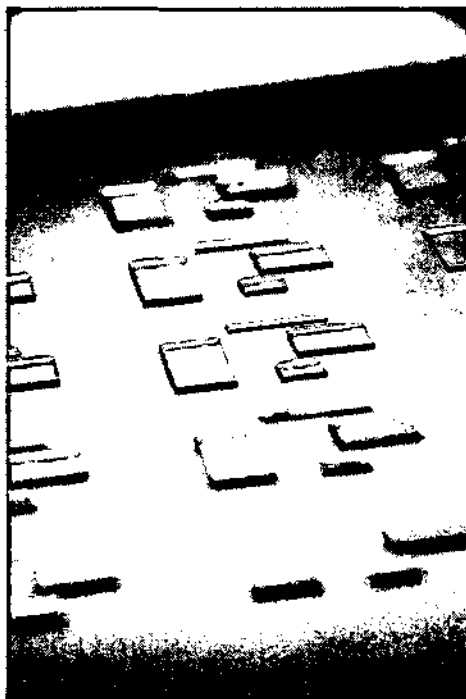


Fig. 6.4 Left side: Pattern on the mask: black areas are dark fields, white areas are transparent on the mask
Right side: Photograph of the corresponding pattern, realized with a 100 μm thick HG. The geometrical resolution is in the order of 10 μm

Fig. 6.5 shows hydrogel layers of 100 μm thickness, deposited and patterned onto a bare silicon wafer. As can be seen, the geometrical resolution is more than sufficient for the sensor layout.

Fig. 6.5 Photograph of polyacrylamide hydrogels with a thickness of 100 μm which are deposited onto a bare silicon wafer. The pattern of the hydrogels corresponds to the 1st Design.



6.3.4 Adhesion to substrate

pAA does not adhere well to $\text{SiO}_2/\text{Si}_3\text{N}_4$ (or $\text{SiO}_2/\text{Al}_2\text{O}_3$) layers which cover 90 % of the transducer surface. In order to improve its adhesion, a pretreatment step of the transducer surface is performed prior to the deposition of the polymer. To establish a durable covalent binding of the polymer layer to the $\text{SiO}_2/\text{Si}_3\text{N}_4$, the surface is first treated with an appropriate silanization agent. Accordingly, the wafer is immersed for 10 minutes into a toluene solution containing 10 % (trimethoxysilyl)propyl methacrylate and 0.5 % water [6.8]. This solution is continuously stirred and held at 60 °C. After silanization, the wafer is rinsed with toluene and isopropanol and then dried under a N_2 stream. The methacrylate groups which are chemically bound to the Si_3N_4 or SiO_2 surface by means of the silyl groups will subsequently participate in the polymerization. This results in a polyacrylamide layer covalently linked to the substrate, as illustrated in Fig. 6.6.

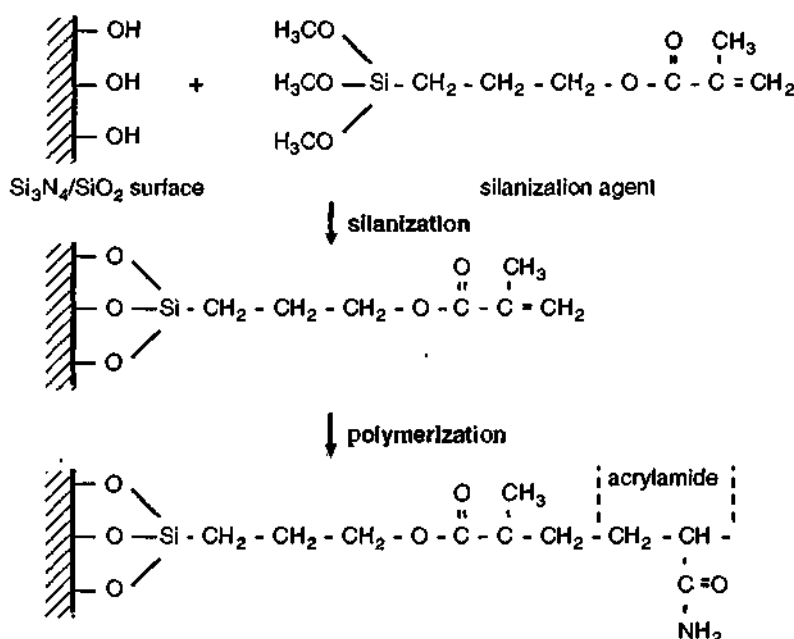


Fig. 6.6 Silanization of the $\text{Si}_3\text{N}_4/\text{SiO}_2$ surface by covalent attachment of (trimethoxysilyl)propyl methacrylate to surface $\text{Si}-\text{OH}$ groups, and subsequent covalent binding of acrylamide / bisacrylamide to the methacrylate groups. This is of course an idealized illustration. In reality, many binding variations are possible.

The following observations are made:

- 1) The best adhesion is achieved if the polymerization is performed immediately after the silanization. It is observed that the silanized surface degrades with time, in particular when the wafer is stored in solution (> 2 weeks). The effect is much less dramatic when the silanized surface is stored in air (> 1 month).
- 2) The covalent anchoring is the most efficient when polymerization at the interface surface - hydrogel is complete. An incomplete exposure to UV light can seriously diminish the adhesion because then the polymerization reaction does not entirely proceed to the wafer surface.
- 3) We believe that hexamethyldisilazane (HMDS), which is commonly used in IC processing as an adhesion promoter for photoresist layers, blocks the surface OH groups and renders them inaccessible for the silanization agent. Therefore, prior to the silanization, the $\text{Si}_3\text{N}_4/\text{SiO}_2$ surface is slightly etched in buffered hydrofluoric acid (BHF) to remove completely the HMDS groups introduced by the last photolithography step. Furthermore, the BHF dip guarantees a clean wafer surface, which is of course a key factor for the efficiency of the silanization.
- 4) The adhesion can be improved by a higher concentration of AA or BIS in the monomer solution. In this case, however, the physical properties of the HG and its water content will be different too.
- 5) By choosing a crosslinker with methacrylate or acrylate functional groups, which are more compatible with the silanized surface, the covalent links between the HG and the methacrylate groups at the surface are more easily established during the polymerization and this results in a better adhesion. Good results are obtained when using TEGDMA (Fig. 6.7), pentaerythritol triacrylate, or trimethylolpropane trimethacrylate, in combination with BIS.

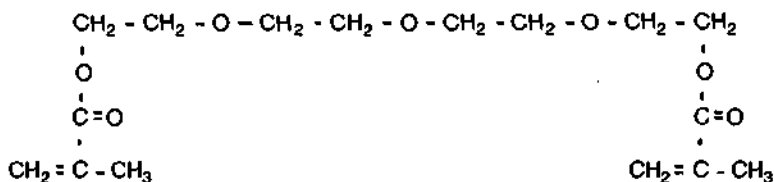


Fig. 6.7 Structure of the crosslinker TEGDMA (tetraethyleneglycol dimethacrylate), which can be used as additional crosslinker with two methacrylate functional groups.

The silanization can be performed only on surfaces which have OH sites. Therefore, on Pt, Ag and AgCl surfaces, no covalent anchoring is possible. A. van den Berg et al. [6.9] describe a method where the Pt surface is oxidized electrochemically or by means of an oxygen plasma, in order to introduce OH groups onto the Pt surface prior to silanization. The disadvantage of this method is that the catalytic activity of the Pt surface is probably modified, and that plasma processes have to be applied at the end of the fabrication, which can affect the threshold voltage of the ISFETs. This is the reason why this technique is not applied in the present work. Instead of this, the adhesion problem of HG on Pt, Ag and AgCl layers is reduced by choosing a sensor geometry where the metallic layers are arranged only in thin bands. Between the bands, the HG is firmly and durably anchored to the $\text{Si}_3\text{N}_4/\text{SiO}_2$ surface.

6.3.5 Adhesion between hydrogel and gas permeable membrane

The adhesion of the GPM to the underlying HG needs to be strong in order to avoid the loosening of the GPM, which has to withstand external (hydrostatic pressure) and internal mechanical stress (swelling of the HG). Furthermore, the forming of an electrolyte film between the HG and the GPM has to be prevented. An insufficient adhesion between the HG and the GPM is normally noticed already during the fabrication: the mechanical stress induced by the swelling of the GPM during its development in xylene tears the GPM into pieces.

The GPM is deposited partly onto the hydrogel and partly onto the sensor surface. The adhesion of the GPM to the sensor surface is ensured by the silanization which has been finished prior to the polyacrylamide deposition. Similar to that chemical binding, a chemical link between the GPM and the HG has to be ensured. Fig. 6.8 shows that a covalent binding of the polyacrylamide to the methacrylate functional groups of the GPM crosslinker is possible. However, due to the constraints of the fabrication

sequence, it is particularly difficult to optimize the chemical link at the interface HG / GPM.

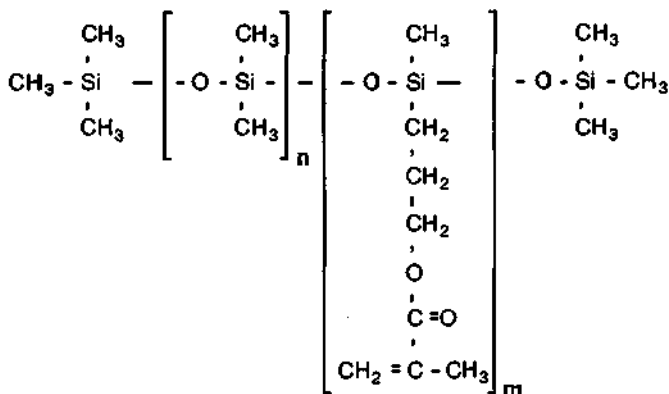


Fig. 6.8 Structure of the polysiloxane polymer used as gas permeable membrane: it is a copolymer containing 97-98 % dimethyl siloxane (n) and 2-3 % (methacryloxypropyl)methyl siloxane (m). The methacrylate functional group of the latter compound can covalently bind to acrylamide or bisacrylamide.

The chemical attachment of the GPM to the HG is only possible if active acryl groups are still available in the pAA gel during the polymerization of the GPM. Therefore, the HG should only be partially polymerized prior to the deposition of the GPM. Of course, as mentioned above, this limits the adhesion of the pAA to the substrate. Therefore, a trade-off has to be found concerning the time of UV exposure during the patterning of the pAA. First, the exposure time should be long enough to guarantee a sufficient attachment of the HG to the substrate and, second, it should not be too long to ensure that still active acryl groups are available in the pAA.

Several ways to circumvent this problem have been tried:

- 1) By choosing very similar polymerization rates for both the HG and the GPM, a better link at the interface is obtained. Riboflavin-5'-phosphate and camphorquinone are rather slow photoinitiators, and their application requires an UV exposure time of more than 10 min at a light intensity of 10 mW/cm². In contrast, the photoinitiator 2,2-dimethoxy-2-phenyl-acetophenone (DMPAP), which is used for both HG and GPM, leads to a short and compatible polymerization time in the range of 20 to 60 s for layers up to 100 µm. DMPAP was tested in the 1 to 5 % (wt.) concentration range without addition of a catalyst. For riboflavin-5'-phosphate (0.5 % wt.) and camphorquinone (1 % wt.),

the catalyst N,N,N',N'-tetramethyl ethylenediamine is used at a concentration of 0.5 % (v/v). The catalyst is necessary to increase the lifetime of the free radicals.

- 2) We tried to introduce new active acryl groups into the structured pAA layer, after the patterning of the HG. This can be done by immersing the wafer, prior to the patterning of the GPM, into an aqueous solution containing monomeric AA and BIS. It is expected that the AA and BIS would diffuse into the polymer network and later participate in the polymerization reaction during the exposure of the GPM, thereby forming a chemical link to the polysiloxane. However, the experiments indicate that the polymerized pAA does not take up these acryl compounds since we observe no favorable effect on the adhesion, neither with AA and BIS nor with other crosslinkers.
- 3) The choice of the crosslinker for the HG also has an effect on the adhesion at the interface to the GPM. A strong adhesion is obtained when a crosslinker with methacrylate groups is used presumably because polysiloxane has also methacrylate functional groups. The adhesion is particularly improved by TEGDMA due to a special effect. TEGDMA is soluble in the water / glycerol acrylamide monomer solution, but during the polymerization TEGDMA precipitates and builds a thin layer on top of the pAA gel. This interfacial layer, which is not removed during the development, acts as an intermediate adhesion layer for the GPM by formation of chemical bonds. This is of course only possible if the pAA is not exposed too long to the UV light.
- 4) To circumvent the trade-off for the UV exposure time of the pAA, two different crosslinkers are incorporated in parallel into the AA monomer solution: TEGDMA as a fast crosslinker that is responsible for the attachment of the pAA to the sensor surface, and N,N'-Diallyltartardiamide as a slow crosslinker which remains predominantly active during the exposure of the HG and serves later for the chemical link to the GPM. This method allows a longer UV exposure for the pAA gel, while still having a good adhesion to the GPM. However, the resulting effect is lower than expected.
- 5) Another idea is to modify the surface of the polymerized acrylamide gel by means of a post-treatment after the patterning of the HG, similar to the procedure proposed by [6.10]. Accordingly, methacrylate groups are chemically bound to the pAA surface in an analogous process as for the silanization of the wafer surface. The attached functional groups later participate in the polymerization reaction during the UV exposure

of the GPM, and therefore stabilize the anchoring of the GPM to the HG. For this purpose, linear chains of polyacrylamide with carboxyl functional groups are added to the HG monomer solution. The HG is then deposited and patterned, followed by the immersion of the wafer for 20 minutes in a toluene solution containing 10 % (v/v) (trimethoxysilyl)propyl methacrylate at 60 °C. This silanization agent is covalently bound to the carboxyl groups of the polyacrylamide chains, and thus provides a chemical link for attachment of the polysiloxane. Although the modification of the carboxyl group with the silanization agent is not stable in humid environment, we decided to try it. The basic idea appears to be promising, but it has not succeeded.

- 6) Another approach is to introduce a second pAA hydrogel layer, which is deposited on top of the first one, but is patterned with a shorter exposure time. The second pAA can bind to the first layer, while still ensuring a sufficient amount of free acryl groups to which the GPM can be bound. Again, the method was not successful. It is observed that the second pAA layer does not adhere to the first one if the UV exposure time of the first layer is relatively long. One could imagine that the monomer solution for the second layer diffuses into the patterned first layer after a contacting time of some minutes. During the following UV exposure of the second layer, both layers would be glued together. In reality, it is observed that the monomer solution does not diffuse into the pAA layer, even if the latter has previously been well hydrated.

All the experiments demonstrate that a binding between the HG and the GPM is possible only with a careful choice of the UV exposure time of the HG. The addition of a more compatible crosslinker (TEGDMA) or the use of a "retarded" crosslinker are tricks to make the range of the exposure time wider.

Furthermore, special attention has to be paid to the choice of solvent of the AA monomer solution. Because the HG is hydrophilic, and the GPM is hydrophobic, these membranes tend to build up a separation zone at their interface when they come into contact. It is likely that, in this case, the active acryl groups of the HG and the methacrylate groups of the GPM are separated, whereby a chemical link is hindered. Therefore, a phase separation between the two polymers has to be prevented by choosing an appropriate solvent. Favorable results are obtained when using ethyleneglycol or a mixture of glycerol and water. In the latter solvent mixture, however, TEGDMA is nearly insoluble and probably forms only a suspension. In addition, it seems that a relatively high amount of AA (30 % wt.) further prevents the phase separation between HG and GPM.

6.3.6 Water content and humidity

The water content mainly depends on monomer and crosslinker contents in the HG. According to the choice of the composition a gel ranging from a hard to a soft sponge-like material is produced. The hydrated polymer is generally somewhat softer after equilibration with aqueous solution. The experiments show that a sufficient water content together with an appropriate mechanical stability of the HG is achieved with an AA content of 30 % (wt.) and a BIS or TEGDMA content of about 1 % (wt.). Such a HG doubles its thickness if it is completely hydrated, in comparison with the thickness under dry conditions. The water content is therefore at least 50 %. No deterioration of the sensor behavior is observed when a higher monomer and crosslinker content is used, which may be necessary to limit the swelling of the gel. On the other hand, the essential feature concerning humidity is the capability of the HG to maintain a constant water content, and not the highest possible water content. This characteristic is more likely determined by the type of solvent used, and by the geometrical arrangement of the HG. As already discussed, the addition of glycerol to the monomer solution can contribute to a more stable humidity. The glycerol / water mixture can be up to 9 / 1, and in this solvent mixture still 30 % AA (wt.) can be dissolved.

6.4 Gas permeable membrane

The gas permeable membrane isolates electrically and chemically the hydrogel from the sample, and it protects the hydrogel and the underlying transducer from contamination. Therefore it has to be impermeable to water and ions. Furthermore, the GPM acts as a diffusion limiting membrane for the gases to be analyzed. To achieve a high sensor selectivity, there is a need for a selectively permeable membrane which is permeable only for O₂ and CO₂. Real membranes, of course, never exhibit such an ideal selectivity. Therefore, one has to live with the fact that any material to be used as GPM is, to some extent, permeable for all gases dissolved in blood. On the other hand, there are no gases dissolved in blood which could interfere with the pO₂ or pCO₂ sensor, except for some gases used in anaesthesia.

In order to obtain a fast response and a high sensitivity, the diffusion coefficient (D_m) and the Henry's law constant (H_m) of O₂ and CO₂ in the GPM should be high. For an adequate lifetime, a mechanically strong GPM is needed, which has a good adhesion to the substrate as well as to the hydrogel. A trade-off has to be made for the thickness of the membrane: a thicker membrane results in a slower response of the sensor, while a thinner membrane may cause leakage and membrane failure. Furthermore, because the measured sample is mainly in contact with the GPM, the GPM should not

be thrombogenic, in order to avoid any problems associated with blood clotting.

In chapter 3, the following relationships have been derived that specify the influence of D_m , H_m and the thickness d_m of the GPM on the operational characteristics of the sensors:

For the pO_2 sensor:

- the sensitivity and the stirring effect are proportional to $\frac{H_m D_m}{d_m}$ (3.12)

- the response time is proportional to: $\frac{d_m^2}{D_m}$ (3.26)

The response time does not depend on the Henry's law constant. Accordingly, it is reasonable to choose a membrane material which has a high D_m , but a low H_m , to achieve a fast response and at the same time an acceptable sensitivity and stirring effect. This is the reason why polyethylene is mostly used for pO_2 sensors and preferred over teflon or silicone rubber, which have both a high D_m and a high H_m .

For the pCO_2 sensor:

The membrane material has no effect on the sensitivity, only on the response time:

1) if both the electrolyte and the membrane are limiting the diffusion:

- the response time is proportional to: $\frac{d_m^2}{D_m}$ (3.67)

2) if the membrane is predominantly limiting the diffusion:

- the response time is proportional to: $\frac{d_m}{H_m D_m}$ (3.68)

For the pCO_2 sensor, a high D_m and a high H_m of the GPM are desirable, and hence silicone rubber or teflon are usually the material of choice.

For both the pO_2 and the pCO_2 sensor, a small thickness d_m will have a great effect on the response time of the sensor.

In Tab. 6.1, values of D_m and H_m are given for several membrane materials that are commonly used for pO_2 or pCO_2 sensors.

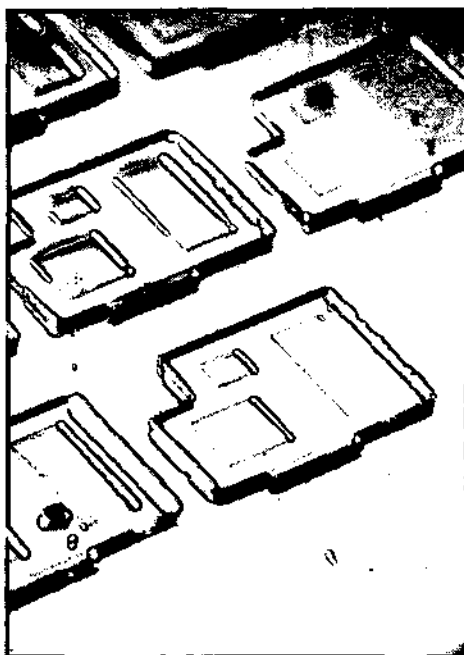
	D (O ₂) [$\frac{\text{cm}^2}{\text{s}}$]	H (O ₂) [$\frac{\text{mol m}^{-3}}{760 \text{ mmHg}}$]	D (CO ₂) [$\frac{\text{cm}^2}{\text{s}}$]	H (CO ₂) [$\frac{\text{mol m}^{-3}}{760 \text{ mmHg}}$]
Teflon (PTFE)	3.4·10 ⁻⁷ [6.11] 1.1·10 ⁻⁷ [6.13] 1.6·10 ⁻⁷ [6.15] 4.6·10 ⁻⁷ [6.16] 2.5·10 ⁻⁷ [6.17]	8.1 [6.11] 9.9 [6.13] 9.6 [6.14] 10.8 [6.17]	2.0·10 ⁻⁷ to 2.0·10 ⁻⁸ [6.18]	
polyethylene	9.0·10 ⁻⁷ [6.12]	0.29 [6.12]	8.0·10 ⁻⁷ [6.12]	15.6 [6.12]
silicone rubber	1.6·10 ⁻⁵ [6.12] 3.0·10 ⁻⁶ [6.14]	13.3 [6.12]	1.1·10 ⁻⁵ [6.12]	86.7 [6.12]
water	1.8·10 ⁻⁵ [6.12]	1.33 [6.14]	1.8·10 ⁻⁵ [6.12]	32.9 [6.19]
air	1.8·10 ⁻¹ [6.12]		1.4·10 ⁻¹ [6.12]	

Tab. 6.1 Diffusion coefficients and Henry's law constants for O₂ and CO₂ in different materials at 25 °C

There are several reasons why we have chosen polysiloxane as the membrane material. First, a material is needed which can be patterned by photopolymerization. This is not possible for teflon or polyethylene. Second, silicon rubber fulfills the demands concerning response time and sensitivity. Furthermore, it is known for its water resistance and its excellent mechanical properties. Lastly, it is a material which is widely used in medical applications and, correspondingly, its clinical applicability has been proven.

For the final preparation of the GPM (see Fig. 6.8), we use a commercially available polysiloxane containing 97-98 % dimethyl siloxane and 2-3 % (methacryloxypropyl)-methyl siloxane (Petrarch, Hüls America). As photoinitiator, we add 0.5 % (wt.) 2,2-dimethoxy-2-phenyl-acetophenone (Aldrich) [6.20]. The deposition is performed exactly as described for the polyacrylamide HG. After the UV exposure, which is in the range of 2 min for a light intensity of 10 mW/cm², the pattern is developed in a xylene bath, and finished by rinsing with isopropanol. Fig. 6.9 shows a photograph of a GPM, after UV exposure and development.

Fig. 6.9 Photograph of patterned polysiloxane gas permeable membranes which are deposited partly onto the hydrogel layers and onto the bare substrate. The thickness is 300 μm for the GPM and 100 μm for the HG. The pattern of the HG and the GPM corresponds to the 1st Design.



6.5 Polymeric flow-through channel

The first demonstration of a flow-through cell for multiple ions sensors is given by Sibbald et al. [6.21], who introduced the term "analyte-chopping analysis" for a system in which samples are passed through the sensor cell in alternation with a calibration solution. This is similar to the well known flow injection analysis (FIA). However, a FIA system uses non-equilibrium measurements and relies on the reproducible dispersion of the analyte boundaries into the carrier solution. In view of the physiological variation of the viscosity of blood, it is preferable to ensure that the sensor and the sample are in equilibrium (pCO_2) or steady state (pO_2) at the time of measurement, i.e. that the sample injection period exceeds the response time of the sensor. To build a flow-through cell meeting this requirement, Sibbald et al. [6.22] use a polyimide sealing layer on the substrate, where a flow-through cell cap is pressed onto. This glass cell cap has an internal volume of 30 μl . Tsukada [6.23] follows the same basic idea, but uses a photosensitive polyimide layer, thus excluding the complicated chemical etching of polyimide. In 1991, van der Schoot and van den Vlekert [6.24] [6.25][6.26] presented a flow-through cell where four independent ISFET chips are sealed to a glass flow channel by means of anodic bonding (450 $^{\circ}\text{C}$,

800 V). The bonding technique provides not only an excellent encapsulation but also a way to immobilize hydrogels and membranes. Special ISFET design is necessary to withstand the harsh bonding conditions and to provide a sufficiently flat chip surface.

The idea to deposit the flow-through channel directly onto the chip, as it is realized for the 2nd Design, has been proposed by van der Schoot for his miniaturized chemical analysis system. As material, the same polysiloxane mixture is used as for the GPM, and the deposition technique corresponds to Fig. 6.1.

6.6 Conclusion

By using photolithographic patterning, it is possible to reproducibly deposit the hydrogel and the gas permeable membrane directly on wafer level by means of photopolymerization. This deposition technique, compatible with IC technology, allows to process the sensor chip entirely on wafer, which is a necessary step in view of mass production.

By choosing carefully the composition of the monomer solutions and by a strict control of the UV exposure time, it is possible to deposit polymeric layers which fulfill the above mentioned demands. The most critical parameters are the adhesion of the HG to the substrate, and the adhesion between the HG and the GPM. Only a covalent attachment at these two interfaces can guarantee a sufficiently long operating lifetime of the sensor.

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7 Conclusion

Two generations of a silicon based pO_2 , pCO_2 and pH blood gas sensor for extracorporeal application have been designed, fabricated and characterized. The clinically well accepted electrochemical sensing principles have been chosen, realized in a planar and miniaturized form on a single silicon chip. The sensor chips are batch fabricated on 3-inch silicon wafers using integrated circuit (IC) technology. The inner electrolyte and the gas permeable membrane of the pO_2 and pCO_2 sensor have been replaced by thin, semi-solid polymeric membranes. These membranes are patterned on wafer level by photopolymerization which is an IC compatible processing. This processing technique, including the composition of the monomer mixtures, have been especially developed for the fabrication of this sensor, in order to guarantee a simple fabrication and a good performance of the sensors. The batch fabrication results in highly reproducible devices and needs no expensive manual processing.

The sensors have been tested in aqueous solutions and in transfusion blood, and no difference in the sensor behavior has been observed. In particular the devices of the 2nd Design exhibit good operational characteristics. The linearity of all three sensors is high, the response time t_{95} in the order of 1 to 2 min for a flow of 200 $\mu\text{l}/\text{min}$. The stability is $\pm 2\%$ /day for the pO_2 sensor, and the pCO_2 and pH sensor exhibit a drift of 1 to 2 mV/day. The three sensors can be operated simultaneously without any electrical interference. Furthermore, the cross-sensitivity between the three sensors is negligible. The device has an operational lifetime of more than 2 months. The results of the 2nd Design are promising for a future application of the sensor in a handheld analyzer or in a small bedside instrument.

In comparison with standard sensor technology used for commercial benchtop blood gas analyzers, the present sensor comes close to their performance, but cannot compete with them. One has to consider, however, that the classical technology is based on over 30 years of industrial experience, and has reached a high level of sophistication. We believe, that with some more investments in the development of the miniaturized device, the performance can still be improved considerably. Nevertheless, with regards to fabrication costs, sample size, reagent consumption and sensor size, the present device has distinct advantages compared to classical sensors. The performance in whole human blood is being investigated in April/May 1994.

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Biography

Philippe Arquint was born on August, 12, 1962 in Luzern, Switzerland. He received his diploma as electrical engineer from the Swiss Federal Institute of Technology (ETHZ), Zürich, Switzerland, in April 1987. His master thesis dealt with the hardware and software development for a miniaturized implantable hearing aid. The work was performed at the University Hospital in Zürich. From 1987 to 1990 he was employed by Hamilton Bonaduz AG, Bonaduz, Switzerland, where he was responsible for the software and partly for the hardware development of a clinical analyzer "ES300", a OEM product for Boehringer Mannheim, Germany. This instrument is able to process fully automatically ELISA tests (Enzyme Linked ImmunoSorbent Assays), which are commonly used in clinical analysis. Beside his job at Hamilton, he took evening courses at the Engineering School in Chur (HTL) in software engineering. In June 1990, he joined the Institute of Microtechnology (IMT) of the University of Neuchâtel, Switzerland, where he started to work in the group of Prof. de Rooij as a research assistant for the development of a blood gas sensor, based on silicon technology.

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