Neutral-Carrier-Based Ion-Selective Microelectrodes Design and Application A Review

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At present, neutral-carrier- and ion-exchanger-based ion-selective liquid membrane microelectrodes are available for the measurement of H^+ , Li^+ , Na^+ , K^+ , Mg^{2+} , Ca^{2+} , NH_4^+ and Cl^- . Fundamental aspects, design features and limitations of microelectrodes are discussed and examples for their application in physiology are given. The most important characteristics are summarized in view of intra- and extracellular physiological measurements. The selectivity coefficients K_{ij}^{Pot} with respect to the most common ions are compared to calculated values $K_{ij,max}^{Pot}$ required for measurements in intra- and extracellular samples without significant interference. For intracellular microelectrode studies, these required values are fully met for H^+ , K^+ , Mg^{2+} , Ca^{2+} and Cl^- , while in the case of extracellular measurements H^+ , Na^+ , K^+ and Ca^{2+} can be assayed adequately.

Keywords Microelectrodes, neutral carriers, ion selectivity, liquid membranes, potentiometry

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1 Introduction

In the present review, carrier-based ion-selective microelectrodes will be covered (for a recent review see ref. 1). As early as 1949, the term microelectrode has been used by physiologists for fine-tipped micropipettes (tip diameter $<1\ \mu m$) for intracellular studies.² Although such devices with inner diameters as small as $100\ \text{\AA}$ were known in 1967³, more recently amperometric electrochemical sensors much larger than $1\ \mu m$ have been called ultramicroelectrodes.⁴ Obviously, the terminology has become somewhat chaotic (see also ref. 5).

The first successful intracellular determinations with ion sensors were potentiometric pH measurements in vacuoles carried out in 1927 by using a platinum-based hydrogen electrode (tip diameter probably *ca.* $5\ \mu m$).⁶ In 1954⁷, pH-selective glass electrodes with tip diameters of about $80\ \mu m$ were used for studies in large cells. Later, capillaries of Na^+ - and K^+ -selective glass were

pulled to fine tips⁸ and this type of microelectrodes was the first tool for the potentiometric determination of Na^+ and K^+ activities in cytoplasm.^{8,9} In the following years, different designs of glass microelectrodes were developed: Insulated H^+ -selective glass electrodes, double-barrelled sensors and recessed-tip microelectrodes (diameter $<1\ \mu m$).¹⁰⁻¹² In the late sixties, Orme used an electrically charged ionophore selective for Ca^{2+} and Mg^{2+} as well as an ion exchanger for Cl^- developed by Orion Research Inc. The exchangers were dissolved in an organic solvent and filled into glass capillaries with very fine tips (diameter $<1\ \mu m$)¹³, thus being the first liquid membrane microelectrodes used. In 1971, Walker described an improved ion exchanger for Cl^- as well as one for K^+ (sensor tip diameter $0.5-1\ \mu m$).¹⁴ A very important step towards the successful use of liquid membrane microelectrodes was the silanization of the glass walls of the micropipettes for stabilizing the lipophilic membrane phase in the very tip and for additional insulation.¹⁴ Only one year later, Khuri¹⁵

developed the first double-barrelled liquid membrane microelectrode. It made a detection in small cells with high spatial resolution possible.

Although the use of chemical sensors based on neutral molecules acting as ion carriers (the macroretro- lides nonactin/monactin or the antibiotic valinomycin) was reported in 1966¹⁶, the successful application of these carriers in liquid membrane electrodes¹⁷ and in microelectrodes was described much later.^{1(ch. 7), 18} In 1975, the first synthetic neutral carrier which induced sufficiently high selectivity for intracellular Ca^{2+} assays (ETH1001, (–)-(R,R)-*N,N'*-[bis(11-ethoxycarbonyl)-undecyl]-*N,N',4,5*-tetramethyl-3,6-dioxo-1,8-octanedi- amide) was prepared¹⁹ and in 1976, neutral-carrier- based microelectrodes for Ca^{2+} and K^+ were describ- ed.^{20,21} Three years later, a Na^+ -selective liquid mem- brane microelectrode using the neutral carrier ETH 227 (*N,N',N''*-triheptyl-*N,N',N''*-trimethyl-4,4',4''-pro- pylidintris-(3-oxabutyramide))²² and liquid membrane pH microelectrodes with 3-hydroxy-picolinamide as charged carrier were described.²³ The sodium salt of nigericin²⁴, *p*-octadecyloxy-*m*-chlorophenylhydrazon- meso-xalo-nitrile²⁵ and tridodecylamine (TDDA)²⁶ were suggested as H^+ carriers in liquid membrane pH microelectrodes.

In the last few years, many new ion-selective liquid membrane microelectrodes, especially based on neutral carriers [ETH 1907 (4-nonadecylpyridine) selective for

H^+ (acid range)²⁷, ETH 157 (*N,N'*-dibenzyl-*N,N'*-diphen- yl-1,2-phenylenedioxydiacetamide) for Na^+ (extracellu- lar)²⁸, nonactin/monactin for NH_4^+ ²⁹, ETH 5214 (*N,N''*- octamethylene-bis(*N'*-heptyl-*N'*-methyl-methylmalonam- ide) for Mg^{2+} (intracellular)³⁰, ETH 129 (*N,N,N',N'*-tetra- cyclohexyl-3-oxa-1,5-pentanediamide) for Ca^{2+} (intra- cellular, extracellular)³¹, Mn(III)TPP for Cl^- (intracel- lular)³²] have been described.

Here we will focus on potentiometric sensors (ion- selective microelectrodes) with tip diameters of about 1 μm and a liquid membrane¹³ as the ion-selective membrane (see Fig. 1).

2 Design features for liquid membrane microelectrodes

For the successful measurement of ions in an intracel- lular/extracellular environment a well-balanced opti- mization of all the electrode characteristics relevant for the application should be discussed rather than the superiority of a single property. Such relevant prop- erties are the selectivity, detection limit, useful lifetime of the sensor, stability and response time.

2.1 Selectivity, detection limit

The Nikolskii-Eisenman equation describes the emf of a potentiometric microelectrode cell assembly:

$$\text{emf} = E_c + E_D + s \log \left[a_i + \sum_{j \neq i} K_{ij}^{\text{Pot}} (a_j)^{z_i/z_j} \right] \quad (1)$$

$$s = 2.303 RT / z_i F = 59.16 \text{ mV} / z_i (25^\circ \text{C}) \quad (2)$$

where

E_c , a constant potential difference, depends on the reference electrodes and on the temperature (independ- ent on sample solution)

E_D is the liquid-junction potential difference gener- ated between reference electrolyte and sample solution (by adequate choice of the reference electrolyte often sufficiently independent of the sample solution)

R : gas constant, $8.314 \text{ J K}^{-1} \text{ mol}^{-1}$

T : absolute temperature, K

F : Faraday equivalent, $9.6487 \times 10^4 \text{ C mol}^{-1}$

z_i, z_j : charge numbers of the primary ion I and the interfering ion J, respectively (in units of the proton charge)

a_i, a_j : activities of the primary ion I and the interfer- ing ion J, respectively, in the sample solution (mol l^{-1})

K_{ij}^{Pot} : selectivity factor, i.e., a measure of the prefer- ence of the sensor for the interfering ion J relative to the ion I. For an ideally selective membrane electrode all K_{ij}^{Pot} would equal zero. Although the dimension of the selectivity factor is $(\text{mol/l})^{1-z_i/z_j}$, K_{ij}^{Pot} is commonly referred to molarity concentrations (mol/l). Assuming representative intracellular and extracellular concentra- tion ranges (see Table 1 in ref. 33), the required selec- tivity factors are calculated with Eq. (3):

$$K_{ij, \text{max}}^{\text{Pot}} = \frac{a_{i, \text{min}}}{(a_{j, \text{max}})^{z_i/z_j}} \cdot \frac{p_{ij}}{100} \quad (3)$$

Fig. 1 Single-barrelled microelectrode. Left: scanning electron micrograph of a silanized micropipette (tip diameter 0.27 μm). Right: schematic cross section.

Table 1 Tolerable selectivity factors, $\log K_{ij,\max}^{\text{Pot}}$, for extra- and intracellular determinations (1% error, worst case) of the measured ion I^i in the presence of interfering ions J^j (see ref. 33)

I^i	$\log K_{ij,\max}^{\text{Pot}}$ for J^j													
	Extracellular							Intracellular						
	H ⁺	Li ⁺	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	HCO ₃ ⁻	H ⁺	Li ⁺	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	HCO ₃ ⁻
H ⁺	0		-8.5	-7.1	-7.7	-7.8		0		-7.5	-8.4	-8.0	-6.3	
Li ⁺	3.1	0	-4.3	-2.8	-3.5	-3.6		2.1	0	-3.2	-4.2	-3.7	-2.1	
Na ⁺	4.4		0	-0.6	-1.2	-1.3		2.8		0	-3.5	-3.1	-1.4	
K ⁺	2.8		-3.6	0	-2.8	-2.9		6.0		0.6	0	0.2	1.8	
Mg ²⁺	8.9		-3.9	-0.9	0	-2.4		9.0		-1.7	-3.5	0	0.7	
Ca ²⁺	9.3		-3.6	-0.6	-1.9	0		4.7		-6.1	-7.9	-7.0	0	
Cl ⁻							-1.4							-1.3

with

$K_{ij,\max}^{\text{Pot}}$: highest tolerable value of the selectivity factor

$a_{i,\min}$: lowest expected activity of the ion I^i to be measured

$a_{j,\max}$: highest expected activity of the interfering ion J^j

p_{ij} : highest tolerable error in the activity a_i due to interferences from a_j (in %).

When this calculated value for the required selectivity is actually achieved with a membrane, it means that even in the worst case the error made in the activity of a_i due to the interference of a_j is less than the set value p_{ij} . The required $\log K_{ij}^{\text{Pot}}$ values in Table 1 are calculated for $p_{ij}=1$, i.e., for a maximum tolerable error of 1%.

When lowering the activity of the primary ion I , deviations from the linear response (emf vs. $\log a_i$) and finally constant emf values are observed owing to the interference from ions J of the same charge sign (Eq. (1)). The activity of I at the transition point between linear response and constant emf is the detection limit of the sensor.³⁴ For Ca²⁺-selective microelectrodes, detection limits below 10⁻⁹ M Ca²⁺ (background: 125 mM K⁺) have been obtained with $\log K_{Ca,K}^{\text{Pot}}$ of -7.2.³¹

Liquid membrane electrodes based on charged sites generally show permselectivity for ions of opposite charge. In the case of no selective interaction between sites and counterions (classical ion exchangers), the selectivity of the sensor is dictated mainly by the extraction behaviour of the solvating membrane medium. Therefore, the following monotonic selectivity sequence is obtained for membrane electrodes based on classical cation exchangers (e.g., tetraphenylborate in a nitro-aromatic solvent):

and analogously for classical anion exchangers (e.g., quaternary ammonium salts in appropriate solvents):

where R^+ and R^- are lipophilic organic ions.

For liquid membranes with a selective interaction between charged site (charged ion carrier) and counterion, the potentiometric selectivity depends, in a rather complicated way, on both the ion-extraction selectivity of the membrane solvent and the ion-binding selectivity of the charged ionophore. For example, the same ligand [bis-(2-ethylhexyl)phosphate] is used in certain Ca²⁺-selective electrodes (membrane solvent: dioctylphenylphosphonate) and in divalent ion sensors with comparable selectivities for Ca²⁺ and Mg²⁺ (solvent: 1-decanol).³⁵ Neutral-carrier-based membrane electrodes make use of the outstanding inherent ion selectivity of certain natural and synthetic ionophores. Neutral ion carriers as a class of ionophores are electrically neutral lipophilic complexing agents having the capability of binding ions reversibly and transporting them across organic membranes by carrier translocation. To some extent, neutral carriers can be tailored for a given application. For design features of ionophores for ion-selective electrodes see ref. 36. The selectivity pattern of a given microelectrode may also be influenced to a certain extent by temperature changes³⁷, by applying a bias current³⁸ and by changing the tip diameter.³⁹

2.2 Sensor lifetime

Besides mechanical defects, electrical leakage pathways, severe surface contaminations and membrane poisoning, the lifetime of liquid electrodes is dictated, to a large extent, by the loss of membrane components (ion carrier, plasticizer solvent for membrane components, additives) into the sample solution. This affects membrane characteristics such as selectivity and membrane resistance. Through adequate membrane technology, the required concentrations of membrane components may be maintained in the membrane phase for at least the desired time period (for a more extended discussion of lifetime limitations see ref. 40). The lifetime of microelectrodes is, however, most frequently dictated by mechanical defects, surface contamination and electrical leakage. Lifetimes of up to one month have been reported.²² The use of quartz capillaries instead of pyrex glass for micropipettes is claimed to drastically increase the lifetime (up to 10 weeks when

stored in air).⁴¹ To make microelectrodes applicable to turgid plant cells even with tough cell walls, Felle has described a treatment with PVC.⁴²

2.3 Stability of the sensor signal

The major source of short-term instabilities probably originates in the high impedance of microelectrodes. Owing to the presumably wide range of intracellular activities (see Table 1 in ref. 33), the demands for potential stability and reproducibility are somewhat less stringent for intracellular than for extracellular (clinical) use. Normally, the drift and noise of the microelectrode cell assembly are in the range of 0.3 mV/h and 0.2 mV, respectively.

2.4 Response time of sensors

The response time of ion-selective membrane electrode cell assemblies depends on a great number of parameters.⁴³ The response times of microelectrodes with high internal resistance (due to the relatively low concentration of charge-carrying species and the small membrane area (*ca.* 10^{-8} cm²)) never reflect the dynamic mass transfer towards the membrane. Instead, they are characteristic of the resistance-capacitance time constant of the measuring circuit or of the ion-selective microelectrode itself. An important design feature,

therefore, is a low electrode impedance. Currently, this is realized by an appropriate design of the composition of the membrane phase⁴⁴ and/or by geometrical means (*e.g.*, coaxial electrodes).⁴⁵

3 Available microelectrodes

3.1 H⁺-selective microelectrodes

Different neutral-carrier-based membrane compositions for pH microelectrodes have been described, the most preferred ion-selective components being tridodecylamine (TDDA) and ETH 1907.^{26,27} Treatment of the TDDA-based membrane phase with CO₂ to eliminate sensor failure is not necessary if the additive sodium tetraphenylborate (NaTPB) is replaced by the more lipophilic potassium tetrakis(4-chlorophenyl)borate (KTPClPB). Due to the high selectivity over alkali ions usually no significant interference occurs in typical intra- as well as extracellular ion backgrounds (see Table 1). Under extreme intracellular conditions, K⁺ may interfere slightly when using the carrier ETH 1907. The selectivity coefficients for sensors with ETH 1907 and TDDA are given in Table 2. In certain cases, the pH microelectrodes were claimed to respond to CO₂.⁴⁶⁻⁴⁹ As mentioned in ref. 1 (p. 211), the interference can

Table 2 Selectivity factors, $\log K_{ij}^{\text{Pot}}$ of available microelectrodes

I ⁱ	Ion-selective component	$\log K_{ij}^{\text{Pot}}$ for J ^j							Ref.
		H ⁺	Li ⁺	Na ⁺	K ⁺	NH ₄ ⁺	Mg ²⁺	Ca ²⁺	
H ⁺	TDDA	0		<-12.7	<-10.7				26,27
	ETH 1907	0		-9.7	-8.7				27
Li ⁺	ETH 149	-0.5	0	-1.4	-2.3		-2.7	-0.6	1,33
	ETH 1810 ^b	1.0	0	-2.3	-2.6	-2.5	-4.0	-2.7	59,60
			0	-2.5 ^d					59,60
Na ⁺	dodecylmethyl-14-crown-4 ^b		0	-2.4 ^d	-2.2 ^d	-3.4 ^d	-5.3 ^d	-4.3 ^d	61
	ETH 227	-0.8	0.4	0	-1.7		-2.4	0.2	22
				0	-2.3 ^d				22
	ETH 157	-1.2	-1.7	0	-0.4		-3.4	-1.3	28
K ⁺	NAS ₁₁₋₁₈ glass ^c	1.1		0	-2.4		<-5.3	<-3.7	66
	valinomycin (low impedance)	-3.7	-4.1	-3.9	0	-1.5	-5.8	-4.9	68
	ion exchanger (classical)	-1.9	-2.3	-1.7	0	-0.5	-2.4	-2.1	68
NH ₄ ⁺	nonactin/monactin	-2.2	-3.6	-2.0	-0.6	0	-4.4	-4.2	29
Mg ²⁺	ETH 1117	2.8	0.1	-1.1	-1.4	-0.1	0	1.1	72
	ETH 5214	1.5	-1.2	-2.2	-2.3	-1.8	0	0.8	30
Ca ²⁺	ETH 1001						-3.3	0	20,82
		-0.1 ^d		-5.5 ^d	-5.4 ^d		<-4.9 ^d	0	20,82
	ETH 129	-2.5	-3.1	-3.6	-3.9	-3.6	-4.9	0	31
				-5.8 ^d	-7.2 ^d		-6.7 ^d	0	31

a. Determined by the separate solution method in unbuffered 0.1 M chloride solutions. b. Macroelectrode (Philips model IS 560^{59,60}, and IS 561 or Orion model 92.⁶¹ c. Haber-type bulb (diameter 3–10 mm). d. Obtained by the fixed interference method (for details see the corresponding references).

TDDA, tridodecylamine; ETH 1907, 4-nonadecylpyridine; ETH 149, *N,N'*-diheptyl-*N,N'*,5,5-tetramethyl-3,7-dioxo-1,9-nonanediamide; ETH 1810, *N,N*-dicyclohexyl-*N,N'*-diisobutyl-*cis*-cyclohexane-1,2-dicarboxamide; ETH 227, *N,N,N'*-triheptyl-*N,N,N'*-trimethyl-4,4',4''-propylidintris-(3-oxabutylamide); ETH 157, *N,N'*-dibenzyl-*N,N'*-diphenyl-1,2-phenylenedioxydiacetamide; ETH 1117, *N,N'*-diheptyl-*N,N'*-dimethyl-1,4-butanediamide; ETH 5214, *N,N'*-octamethylene-bis(*N'*-heptyl-*N'*-methylmethylmalonamide); ETH 1001, (–)-(R,R)-*N,N'*-[bis(11-ethoxycarbonyl)undecyl]-*N,N'*,4,5-tetramethyl-3,6-dioxo-1,8-octanediamide; ETH 129, *N,N,N,N'*-tetracyclohexyl-3-oxa-1,5-pentanediamide.

be eliminated by saturating the internal filling solution of the electrode (citrate buffer) with CO_2 or by carefully testing and selecting the electrodes.⁵⁰ No sensitivity to CO_2 was claimed in refs. 51 to 53 (for more information see ref. 1 (p. 211)).

The electrode properties of the two types of neutral-carrier-based sensors mentioned above are quite similar, the membrane resistance corresponding to ca. $4 \times 10^{10} \Omega$ (tip diameter $1 \mu\text{m}$, filling height $100\text{--}300 \mu\text{m}$, see Fig. 1). The slope of the electrode response function for sensors based on TDDA and ETH 1907 is $57.7 \pm 0.5 \text{ mV}$ (SD, $n=4$) and $56.7 \pm 1.0 \text{ mV}$ (SD, $n=6$), respectively. The response time t_{90} of the sensors is $\leq 5 \text{ s}$.^{26,27} The neutral carrier ETH 1907 shows a lower acidity constant ($\text{p}K$) than TDDA;⁵⁴ therefore, the linear dynamic response range is shifted to lower pH values (TDDA: $\text{pH} > 12$ to 5.5; ETH 1907: pH 9 to 2).^{26,27,54} With these two types of microelectrodes, pH measurements over the whole relevant physiological pH range are possible.

Liquid membrane microelectrodes are increasingly taking the place of solid-state and glass membrane microelectrodes.^{1(p.217)} This is due to their much easier preparation, even of sensors with very small tip sizes and of multi-barrelled electrodes, as well as to the faster response. More information about a comparison of the two electrode systems is given in refs. 1 and 46. For their application during intracellular studies in ganglia of the medicinal leech see ref. 55.

3.2 Li^+ -selective microelectrodes

Physiological Li^+ activities typically lie in the micromolar range.^{1(p.217), 56(p.87)} To date, potentiometric sensors showing such low detection limits in extra- or intracellular media are not yet available. By using an adequate calibration of microelectrodes based on the neutral carrier ETH 149 (*N,N'*-diheptyl-*N,N',5,5*-tetramethyl-3,7-dioxo-1,9-nonanediamide) measurements of Li^+ concentrations around 1 mM in an intracellular background were successful.^{33,57} Such Li^+ levels are relevant in the treatment of manic-depressive psychosis.⁵⁸ Bulk PVC liquid membranes containing the neutral carrier ETH 1810 (*N,N*-dicyclohexyl-*N',N'*-diisobutyl-*cis*-cyclohexane-1,2-dicarboxamide)^{59,60} are suitable for Li^+ determinations in whole blood. This carrier exhibits higher selectivities over Na^+ , K^+ , Mg^{2+} and Ca^{2+} than ETH 149 (see Table 2 and Fig. 2). No microelectrodes based on ETH 1810 nor on dodecylmethyl-14-crown-4⁶¹ have been developed so far.

3.3 Na^+ -selective microelectrodes

Different membrane compositions have been studied for the measurement of intra- and extracellular sodium activities. With membranes based on the Na^+ carrier ETH 227, relevant intracellular determinations can be made²², while membranes containing ETH 157 or monensin are suitable for extracellular measurements of Na^+ activities only.^{1(p.232), 29, 62} Sensors based on ETH 227 as well as on ETH 157⁶³⁻⁶⁵ have to be calibrated in

appropriate solutions because of their limited selectivities over K^+ and Ca^{2+} (see Tables 1 and 2). The resistance of the microelectrodes are as follows: ETH 227 ca. $10^{10} \Omega$ (tip diameter $2 \mu\text{m}$, double-barrelled); ETH 157 ca. $3 \times 10^{10} \Omega$ (tip diameter $0.7 \mu\text{m}$). The response time strongly depends on the method of determination. Using ETH 227 response times t_{90} between 0.2 and $< 5 \text{ s}$ have been reported.²² For sensors with ETH 157 the response time t_{90} was $< 3 \text{ s}$.²⁸ The slope of the electrode function was found to be $53.0 \pm 2.5 \text{ mV}$ (ETH 227; SD, $n=3$). Interferences due to components such as inhibitors have to be checked carefully.^{1(p.230)} When compared with liquid membranes, the Na^+ -selective glass NAS₁₁₋₁₈⁶⁶ shows much better selectivities over alkali and alkaline earth ions but not over hydrogen ions (see Table 2). The same reasons mentioned for the H^+ -selective glass microelectrodes gradually lead to a replacement of glass by liquid membranes.

3.4 K^+ -selective microelectrodes

The K^+ -selective microelectrodes, based on ion exchangers¹⁴ or on valinomycin^{21,67,68}, show a nearly theoretical response to K^+ in aqueous solutions. As far as the selectivity is concerned, the valinomycin-based microelectrodes almost perfectly satisfy the needs for intra- and extracellular measurements (see Tables 1 and 2). Because of the early realisation of these highly selective liquid membrane microelectrodes, K^+ -selective glass microelectrodes have never played an important role.^{1(p.233)} The resistance of the original valinomycin-based sensor is very high ($5.0 \times 10^{11} \pm 1.9 \times 10^{11} \Omega$; SD, $n=4$, tip diameter $1 \mu\text{m}$)⁶⁸ as compared with that of the ion-exchanger-based microelectrodes (Corning 477413; $10^9\text{--}10^{10} \Omega$; tip diameter $0.5\text{--}1 \mu\text{m}$;¹⁴ see also sensors with KTpClPB in 1,2-dimethylnitrobenzene: $1.7 \times 10^9 \pm 0.7 \times 10^9 \Omega$; SD, $n=6$, tip diameter $1 \mu\text{m}$)⁶⁸. Modifications of the valinomycin-based membrane compositions showed a drastically lower resistance: $10^{11} \Omega$ (tip diameter $1.5 \mu\text{m}$)⁶⁷ and $1.2 \times 10^{10} \pm 0.9 \times 10^{10} \Omega$ (SD, $n=12$; tip diameter $1 \mu\text{m}$)⁶⁸. The response time t_{90} of the sensor was simultaneously reduced from 30 s to $< 1 \text{ s}$.⁶⁸ However, the ion exchanger-based sensors still show a faster response (rise time ca. 0.5 s ⁶⁹, $2\text{--}10 \text{ ms}$;⁷⁰ $t_{63}=40\text{--}150 \text{ ms}$ ⁷¹). Due to their low resistance and fast response the ion exchanger systems are still widely used^{1(p.240)}, although measurements are prone to interference from relatively lipophilic cations, such as acetylcholine (ACh) and tetraethylammonium (TEA) ($\log K_{\text{K}^+/\text{ACh}}^{\text{Pot}}=+3.6$; $\log K_{\text{K}^+/\text{TEA}}^{\text{Pot}}=+4.5$). In contrast, valinomycin-based electrodes show very high selectivities over these ions ($\log K_{\text{K}^+/\text{ACh}}^{\text{Pot}}=-3.6$; $\log K_{\text{K}^+/\text{TEA}}^{\text{Pot}}=-3.2$).^{21,68} In certain applications the concentration of these cations may even be determined utilizing ion-exchanger-based microelectrodes (see Table 9.19 in ref. 1). With the exception that fast transients in K^+ activity are to be measured, the use of microelectrodes based on neutral carriers is recommended.^{1(p.240)}

Li⁺ MICROELECTRODE (ETH 149) **Li⁺ MACROELECTRODE (ETH 1810)** **Na⁺ MICROELECTRODE (ETH 157)** **Na⁺ MICROELECTRODE (ETH 227)** **Mg²⁺ MICROELECTRODE (ETH 1117)** **Mg²⁺ MICROELECTRODE (ETH 5214)** **Ca²⁺ MICROELECTRODE (ETH 1001)** **Ca²⁺ MICROELECTRODE (ETH 129)**

Fig. 2 Ion selectivities of microelectrodes. The selectivity is shown as percentage contribution of the individual ions to the sum of the Nikolskii-Eisenman equation (1). Selectivity factors and ion activities are taken from Table 2 and ref. 33 (Table 1), respectively. The range for the primary ion was determined by keeping the measured ion at its typical activity while varying the activities of all interfering ions over the physiological range as given in ref. 33 (Table 1). The ranges for the interfering ions were obtained by maintaining the physiological activity of the primary ion at its typical value, at the same time keeping that of the interfering ion under consideration high and the activities of the other interfering ions low, and *vice versa*.

3.5 NH_4^+ -selective microelectrodes

The macrotetrolides nonactin/monactin are used as neutral carriers in a NH_4^+ -selective microelectrode developed recently. The application of this electrode implies the knowledge of the Na^+ and K^+ activities in the sample to be measured, because the selectivities in respect to these ions are relatively poor (see Table 2).²⁹ Therefore, a proper calibration or even a simultaneous determination of and chemometrical correction for these interferents is necessary. The resistance of these microelectrodes is $5.8 \times 10^{10} \pm 1.3 \times 10^{10} \Omega$ (SD, $n=6$; tip diameter $1 \mu\text{m}$; $200\text{--}300 \mu\text{m}$ filling height) and the response time t_{90} is $1\text{--}2 \text{ s}$. Since for intracellular measurements (kidney) the activities of NH_4^+ and of interferents differ over a wide range, possible interferences have to be carefully evaluated for every tissue.

3.6 Mg^{2+} -selective microelectrodes

Until very recently, the only available Mg^{2+} -selective microelectrode was based on the synthetic neutral carrier ETH 1117 (*N,N'*-diheptyl-*N,N'*-dimethyl-1,4-butanedi-*am*ide).⁷² For intracellular ion backgrounds, serious interference from K^+ has to be taken into account (Fig. 2). Even the selectivities in respect to Na^+ and Ca^{2+} are not adequate for a tolerated interference $<1\%$ (see Tables 1 and 2). For an appropriate calibration of the electrode, different possibilities have been described, such as the determination of Na^+ and K^+ in the tissue and calibration in the corresponding ion background⁷³⁻⁷⁸ or the simultaneous measurement of K^+ in the same sample and correction for its interference.⁷⁹ Recently, an improved Mg^{2+} -selective ionophore (ETH 5214)³⁰ has been developed. The optimized Mg^{2+} -selective microelectrode based on this neutral carrier

shows sufficient selectivity over H^+ , Na^+ and Ca^{2+} for Mg^{2+} determinations in an intracellular environment (see Fig. 2). The selectivity over K^+ is improved by about one order of magnitude as compared with that of the sensor based on ETH 1117 (see Table 2). With a typical intracellular ion background (10 mM Na^+ , 100 mM K^+ , $10^{-3} \text{ mM Ca}^{2+}$) the slope of the electrode response is $29.4 \pm 0.2 \text{ mV}$ (SD, $n=4$; 23°C), the detection limit $\log a_{\text{Mg}} -4.3 \pm 0.1$ (SD, $n=4$), the resistance $5.0 \times 10^{10} \pm 1.7 \times 10^{10} \Omega$ (SD, $n=18$) and the response time $t_{90} \leq 3 \text{ s}$.³⁰ Because of the poor selectivity especially in respect to Ca^{2+} (Tables 1 and 2), no extracellular Mg^{2+} measurements are possible. In PVC bulk macroelectrodes other ionophores were investigated as well, (e.g., cyclic octa- and decapeptides or the antibiotic X-14885A^{80,81}), but no reliable potentiometric Mg^{2+} determination in the extracellular environment without the use of chemometrics has been possible as yet.

3.7 Ca^{2+} -selective microelectrodes

The widely used Ca^{2+} -selective microelectrode containing the neutral carrier ETH 1001 satisfies the requirements for extracellular Ca^{2+} measurements.^{20,33,82} For intracellular studies K^+ and Mg^{2+} cause significant interference (see Fig. 2). Different changes in the original membrane composition were studied to get a Nernstian electrode response of sensors with small tip diameters ($<1 \mu\text{m}$) below $\text{pCa}=5$ (for an overview see refs. 82 and 83). The electrical resistance of this type of electrodes with tip diameters of $1\text{--}2 \mu\text{m}$ is *ca.* $2 \times 10^{10} \Omega$. PVC seems to be a suitable additive to improve the electrode response. However, it leads to response times in the range of seconds and a reproducible preparation of the sensors is difficult (see below). With the original

membrane composition and a special electrode design, the electrode resistance may be lowered by a factor of 12.5 and very fast response is obtained (time constant $\tau_c = 7$ ms (ref. 45)).

Recently, a new Ca^{2+} -selective microelectrode containing the neutral carrier ETH 129 has been developed.³¹ Figure 2 shows the high selectivity in respect to alkali and alkaline earth cations perfectly meeting the requirements for intra- and extracellular studies (see also Tables 1 and 2). The detection limit in Ca^{2+} -buffered solutions with an ion background of 125 mM K^+ is below $\text{pCa} = 9$ and the resistance is $3.8 \times 10^{10} \pm 2.8 \times 10^{10} \Omega$ (SD, $n=10$; tip diameter 1–1.5 μm ; filling height 50–100 μm).³¹ As the membrane components have to be dissolved in a solution containing PVC and additional THF to control the viscosity during the filling procedure, it is difficult to achieve a reproducible sensor quality. After the filling procedure, the THF evaporates and/or dissolves in the internal filling solution. This shrinking of the membrane may cause a recess at the very tip of the electrode. Therefore, the filling height should not exceed 200 μm (final filling height ca. 100 μm).^{84(p. 128)} Very recently, a method has been developed for inserting the internal filling solution after drying the membrane during 24 h.⁸⁵ Using the carrier ETH 129, the addition of PVC is not only necessary for small tip diameters as in the case of ETH 1001. Even for relatively large microelectrodes (tip diameter $>10 \mu\text{m}$) a super-Nernstian response below $\text{pCa} = 5$ and hence a poor detection limit are obtained without PVC. Due to the high selectivity and low detection limit special attention has to be paid to a proper calibration in buffered solutions.^{31, 86–88} Nevertheless, first applications of this membrane composition have been reported recently.^{89–94} In contrast to the carrier ETH 129 and ETH 1001, Ca^{2+} electrodes based on organophosphates show much poorer selectivities, especially in respect to Mg^{2+} ($\log K_{\text{CaMg}}^{\text{Pot}} = -1.9$).^{95, 1(p. 266)} They have not been used in realistic intracellular applications (see ref. 35).

3.8 Cl^- -selective microelectrodes

Until very recently, Cl^- -selective microelectrodes were based on classical ion exchangers (e.g., Corning 477913).⁹⁶ A Mn(III)porphyrin complex exhibits anion selectivities in PVC bulk membranes.⁹⁷ A new Cl^- -selective microelectrode based on a similar carrier (5,10,15,20-tetraphenyl-21*H*,23*H*-porphyrin manganese(III) chloride, Mn(III)TPP) has been developed.³² This Cl^- -selective microelectrode shows an improved selectivity in respect to HCO_3^- and some organic anions (lactate, acetate, isethionate, malate, oxalate and citrate) as compared to the Corning ion exchanger (see Table 2 and ref. 32). Intracellular Cl^- determinations seem therefore possible without interference from these ions. The relatively lipophilic anion SCN^- , present in high concentration in blood samples of smokers^{56(p. 81)} may cause some interference ($\log K_{\text{ClSCN}}^{\text{Pot}} = +3$).

Owing to the high resistance of microelectrodes based on Mn(III)TPP ($7.0 \times 10^{10} \pm 1.8 \times 10^{10} \Omega$; SD, $n=5$; after

48 h conditioning), care has to be taken to use proper silanization or glass capillaries with high specific resistance, especially when small tip diameters are used. In some cases, a certain pH interference is observed (at pH 7.2 ± 0.6 maximum uncertainty 3 mV).

The choice between the two Cl^- -selective microelectrodes depends on the application. Either better selectivity (Mn(III)TPP) or lower resistance and shorter response time (ion exchanger Corning 477913) may be asked for.

4 Application of microelectrodes

Different useful monographs describe in detail possibilities and limitations in the application of microelectrodes.^{1,98,99} In the last ten years, neutral-carrier-based ion-selective microelectrodes have become very popular (see Fig. 3). By now, most of the components are commercially available.¹⁰⁰ The advantage of neutral carrier- over classical ion-exchanger-based electrodes is the wide range of accessible ion selectivities. Although H^+ - and Na^+ -selective glass electrodes have remarkable selectivities which are fully adequate for intracellular applications, they have been largely replaced by systems based on neutral carriers, mainly because of their ease of production. Double-barrelled microelectrodes are very popular because they are increasingly applied to relatively small cells and vertebrate samples.¹⁰¹ Many different designs are available.^{1(ch. 6), 102} Of 326 published applications, more than half of the microelectrodes (53%) used in physiology since 1985 are double-barrelled (44% single-barrelled; 3% triple-barrelled).

5 Limitations of microelectrodes

At present, the smallest tip diameter reported for ion-selective microelectrodes (double-barrelled) based on neutral carriers is 0.089 μm (diameter of the ion-selective barrel around 0.05 to 0.07 μm).¹⁰³ The effective volume in which the sample ions are sensed is estimated to be ca. 10^{-19} l and ca. 10^{-15} l for microelectrodes with tip diameters of 0.06 and 1 μm , respectively. With the latter used as detectors in open tubular column liquid chromatography (3.5 μm inner column diameter), practical detection volumes of $<6.2 \times 10^{-12}$ l have been achieved.¹⁰⁴ Microelectrodes with submicron tips can now be used in relatively small cells. For example, single cells of frog skin epithelium with diameters $\leq 10 \mu\text{m}$ (cell volume: 0.5×10^{-12} l) have been successfully penetrated with Ca^{2+} microelectrodes of tip diameters $<0.18 \mu\text{m}$ (effective volume of measurement: ca. 10^{-18} l).¹⁰⁵

Measurements with the Ca^{2+} -selective microelectrodes of the type described here (for details see ref. 31) are therefore well conceivable in effective sample volumes of $<10^{-14}$ l. At a detection limit of 10^{-9} M Ca^{2+} this corresponds to $<10^{-23}$ mol of detected free Ca^{2+} ions.

Fig. 3 Number of published applications of various neutral-carrier-based microelectrodes per year as a function of their year of publication. For 1988 the number for the first five months was extrapolated.

Formally, this is equivalent to the detection of only a few single ions. A somewhat more detailed discussion, however, shows that such extreme measurements can only be performed if the Ca^{2+} in the detection volume is in equilibrium with an adequate Ca^{2+} buffering system.¹⁰⁶

Assuming at least one single free ion in the detection volume we obtain

$$V(l) \times c_{\text{DL}}(M) > 1.7 \times 10^{-24} \text{ mol} \quad (4)$$

where $V(l)$ is the detection volume in liters and $c_{\text{DL}}(M)$ is the detection limit of the sensor in mol l^{-1} . Obviously, a reduction in the detection volume (tip diameter) asks for an increase in the detection limit. The Ca^{2+} -selective microelectrode described here seems to be a rather good compromise.

The further miniaturization of ion-selective micro-sensors is generally limited by the extremely high electrical resistance of the bulk liquid membrane phase ($R_m = 10^9 - 10^{11} \Omega$). This requires the availability of highly developed operational amplifiers having input impedances Z of at least $10^{14} \Omega$ (i.e., $Z \gg R_m$) and input currents i in the fA range (i.e., $i \ll E/R_m$, where E is the emf of the cell). In addition, special experimental precautions are necessary for high-precision measurements.

Perhaps even more critical is the effective charge-transfer resistance R_s at the tip of the microelectrode,

which is related to the interfacial ion-exchange kinetics and is inversely proportional to the exchange current.^{107,108} For an exchange-current density of around $10^{-6} \text{ A cm}^{-2}$,¹⁰⁹ a liquid membrane microelectrode with a $1 \mu\text{m}$ tip diameter exhibits an extremely high surface resistance of $R_s > 10^{12} \Omega$ and would therefore behave as a polarized electrode even at a bias current flow of only 10 fA .¹⁰⁶ These polarization phenomena may lead to characteristic deviations from the Nernstian electrode response.¹⁰⁶ Similar effects have been ascribed earlier to electrical shunts arising along and/or through the glass wall of the capillary of microelectrodes.⁸⁷ Such insulation problems offer a further limitation to lower tip diameters and detection limits.

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