

Bio Currents Research Center Tutorial

Response Times

The material below has been adapted from several primary papers and reviews originating from the BRC. These should be referred to in publications and for the original source materials.

References:

1. *Smith, P.J.S., Sanger, R.S. and Messerli, M.A. (2007) Principles, Development and Applications of Self-Referencing Electrochemical Microelectrodes to the Determination of Fluxes at Cell Membranes. In: Methods and New Frontiers in Neuroscience. Ed. Adrian C. Michael. CRC Press. Ch. 18: 373-405.*
2. *Messerli, M.A., Robinson, K.R. and Smith, P.J.S. (2006) Electrochemical sensor applications to the study of molecular physiology and analyte flux in plants. In: Plant Electrophysiology - Theory and Methods. Ed. Alexander G. Volkov. Springer-Verlag. 73-107.*

General properties of micron-sized sensors (<10 μm) are that they reach equilibrium relatively quickly, have greater spatial resolution, and are much less disturbing to the local chemical environment compared to macroelectrodes. The response times of ion-selective microelectrodes can be so fast that the electronics of the system slow the measured response³. For example, the response time of potentiometric electrodes will depend on their ability to supply enough charge to the voltage-sensing node in order for it to reach its steady state value. Low input impedance of the amplifier and stray capacitances in the circuit will draw more charge than an ideal system therefore slowing the response time of the system. Micron-sized, ion-selective sensors have been characterized with high resistances, 1-20 $\text{G}\Omega$ ³. Amplifier input impedances of 10^5 - 10^6 $\text{G}\Omega$ are typically used to prevent a significant voltage drop across the voltage-sensing node. With an ideal amplifier, why are the electrodes so fast? Diffusion through the stagnant aqueous layer at the surface of the electrode defines the response time of the sensors when the ion-selective membrane is equilibrated with the salt of an ion to which the electrode responds⁴. A brief theoretical analysis will put this in perspective. In a diffusion-limited system, how quickly will an analyte diffuse from the edge to the center of the microelectrode?

For a 4 μm diameter sensor, K^+ , for example, would require only 0.3 ms ($t = L^2/6D$ for diffusion in 3 dimensions, $D_{\text{K}^+} = 2.0 \times 10^{-5}$ cm^2/s). For comparison, it would take 21 s to diffuse from the edge to the center of a 1 mm diameter sensor. Most analytes that have been measured with self-referencing systems have diffusion coefficients in the range of 10^{-4} - 10^{-6} cm^2/s indicating that the electronics will be the primary factor limiting the response time of the measurement under relatively ideal conditions.

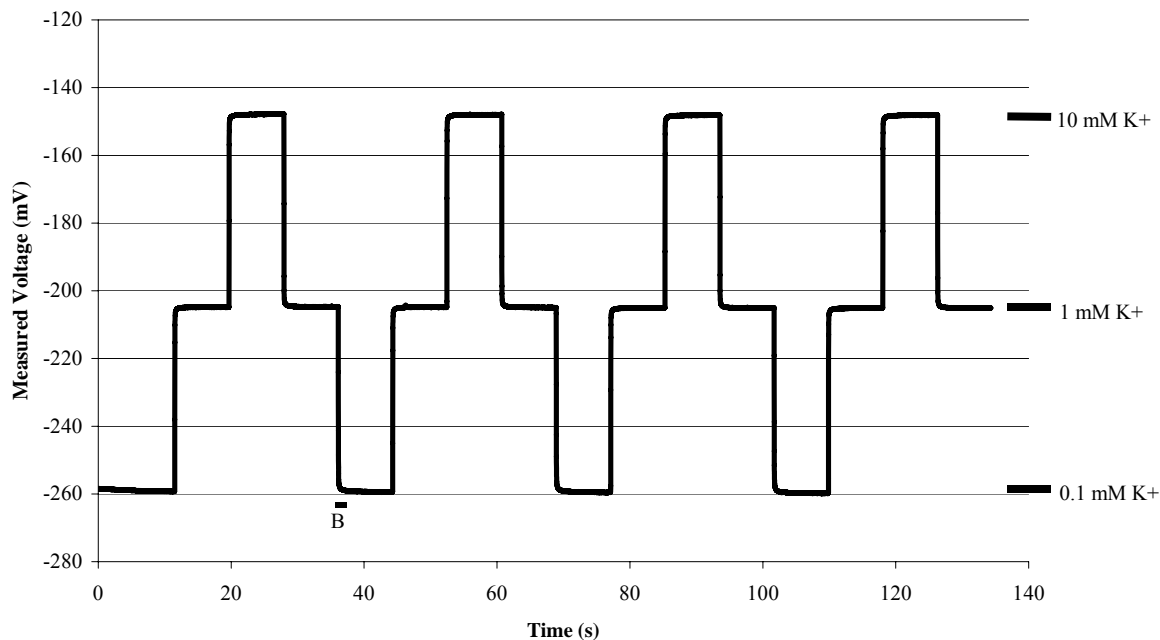


Figure 1A: Voltage response of a K^+ -selective electrode to step changes in K^+ concentration. A) The voltage response recorded by a K^+ -selective electrode as a simple medium containing 100 mM HEPES (pH 7.0) with 0.1, 1 or 10 mM KCl was passed over the tip with a rapid exchange flow system.

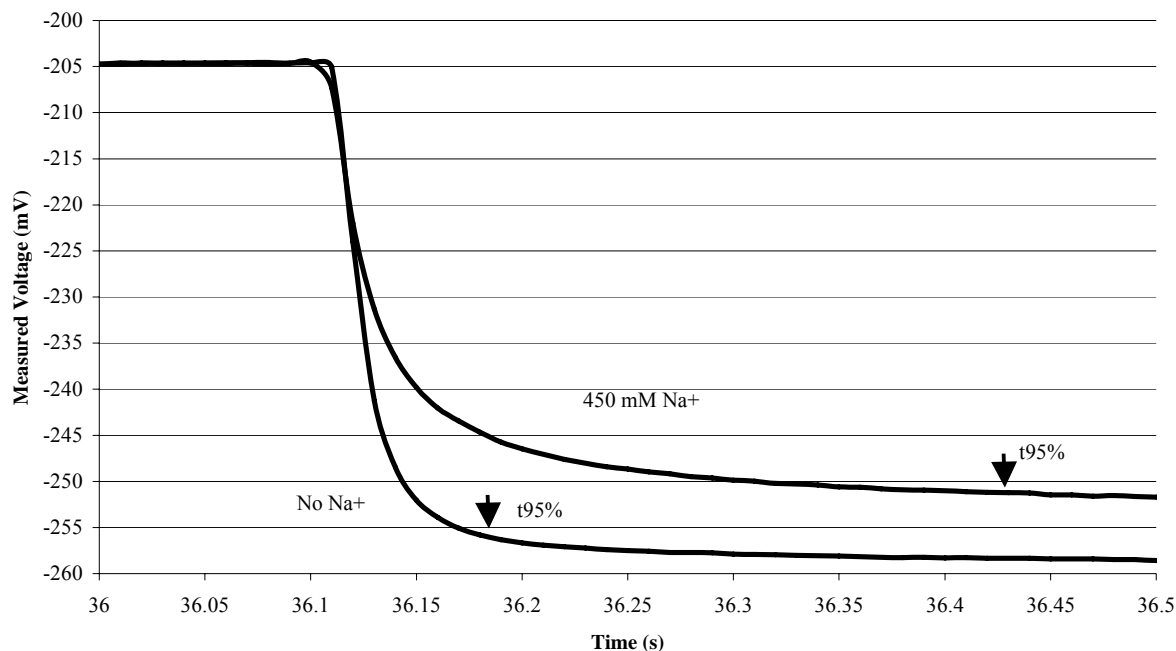


Figure 1B: An overlay of the magnified voltage response from 1 to 0.1 mM KCl, marked in (A) with a response under similar conditions in the presence of 450 mM NaCl. Na^+ is a characterized interferent of the K^+ -selective electrode used here. In the presence of Na^+ , the response time is nearly 3 times longer and causes the electrode to respond to this change in K^+ concentration 8% lower than optimal.

The response time of ISMs is governed by the ability to provide charge to the sensing node. This involves the availability of charge carriers at the surface of the electrode tip. Therefore, surface area of the ion-selective solvent, proportional to the square of the tip diameter, will affect response time. The presence of interferents will also slow response times. We have characterized the response times of micro electrochemical sensors with our system with different tip diameters, column lengths, and in the presence of an interferent. Figure 1A shows an example of the response times for a K^+ -selective electrode in a simple solution (100 mM HEPES pH 7 with 0.1, 1 and 10 mM KCl). Figure 1B shows an enlargement of the response time from 1 to 0.1 mM K^+ , indicated in Figure 1A, and an overlay under the same conditions with the addition of Na^+ , a known, albeit poor interferent. These 2-3 μm K^+ -selective electrodes respond to 95% of their new steady state value ($t_{95\%}$) in 41-77 ms depending on the change in concentration, line 3 Table 1. The electrode possesses $10^{3.9}$ selectivity for K^+ over Na^+ indicating that a lot of Na^+ must be present to interfere with K^+ detection. In the presence of 450 mM Na^+ (seawater levels) not only does the response time increase nearly 3-fold but the Nernstian response also decreases by 8%, Figure 1B. Both of these features are classic indications of an interferent^{4,5}. In Table 1 we give a listing of the response times of 3 of the potentiometric sensors and two of the amperometric sensors in defined media. Simple solutions were used in media 1 and 3 to show the effects on response time with changes in tip size and column length. The sensors remained stationary during the experiment, while 2-3 adjacent streams of media (1 mL/min) were rapidly positioned in front of the measuring electrode. The rapid exchange system was able to exchange streams in front of the measuring electrodes in less than 8 ms ($t_{95\%}$). These measurements describe the response time of the entire measuring system from the sensor through the electronics for 4 electrodes of each type. The response times are generally faster than 200 ms for electrodes with tips greater than 1 μm . A few trends can be distinguished:

- 1) longer column lengths give rise to longer response times,
- 2) 2-3 μm diameter pipette tips are faster than 1 μm diameter tips,
- 3) longer response times occur when measuring in lower concentrations of the primary ion
- 4) The response times of the H^+ electrodes do not vary as much as the K^+ or Ca^+ electrodes and are not as dependent on column length as the K^+ . This may be a result of the H^+ buffer present in solution.
- 5) The response time of the O_2 electrode is very fast as predicted by Schneiderman and Goldstick⁶.

Table 1

Electrode	Column length	Tip Size	Medium	Response times ($t_{95\%}$ msec) for concentration ranges			
				10-1 mM	1-0.1 mM	0.1-1 mM	1-10 mM
K ⁺	100 μ m	1 μ m	1	195 \pm 59	376 \pm 86	165 \pm 41	114 \pm 26
				369 \pm 91	516 \pm 86	247 \pm 54	191 \pm 46
	1000 μ m	1 μ m	1	41 \pm 3	77 \pm 4	53 \pm 4	44 \pm 3
w/ 450 mM Na ⁺	100 μ m	2-3 μ m	2	64 \pm 9	225 \pm 6	91 \pm 8	69 \pm 15
H ⁺	30 μ m	1 μ m	3	pH 6-7	pH 7-8	pH 8-7	pH 7-6
				209 \pm 12	220 \pm 15	214 \pm 27	202 \pm 13
	300 μ m	1 μ m	3	251 \pm 21	269 \pm 14	245 \pm 11	244 \pm 17
	30 μ m	2-3 μ m	3	135 \pm 23	130 \pm 32	126 \pm 30	124 \pm 35
Ca ²⁺	30 μ m		4	10-1 mM	1-0.1 mM	0.1-1 mM	1-10 mM
				58 \pm 9	81 \pm 10	48 \pm 7	53 \pm 10
Electrode		Surface area	Medium	Response times ($t_{90\%}$ msec) to different partial pressures.			
O ₂	n.a.	2 μ m ²	4	Air – N ₂ saturated		N ₂ – Air saturated	
				17.95 \pm 5.26		17.03 \pm 5.75	
NO	n.a.	30–40 μ m ²	4	Saline – NO bubbled		NO bubbled – Saline	
				49.63 \pm 13.21		61.00 \pm 14.33	

Table 1. Characteristics and response times determined for a variety of electrochemical microsensors under different conditions. All tip diameters and reactive areas are approximate.

Medium 1. 100 mM HEPES (pH 7.0) with 0.1, 1.0 or 10 mM KCl.

Medium 2. Medium 1 with 450 mM NaCl.

Medium 3. 100 mM MES (pH 6), 100 mM HEPES (pH 7,8) set with KOH

Medium 4. in mM, 120 NaCl, 5 KCl, 2 CaCl₂, 2 MgCl₂ 10 HEPES (pH 7.4)

Literature Cited:

3. *Ammann, D. (1986) Ion-selective micro-electrodes, principles, design and application. Springer-Verlag, Germany.*
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5. *Fleet, B., Ryan, T.H., and Brand, M.J.D. (1974). Investigations of the factors affecting the response time of a calcium selective liquid membrane electrode. Anal. Chem. 46:12-15.*
6. *Schneiderman, G., and Goldstick, T.K. (1978). Oxygen electrode design criteria and performance characteristics: recessed cathode. J. Appl. Physiol. 45:145-154.*