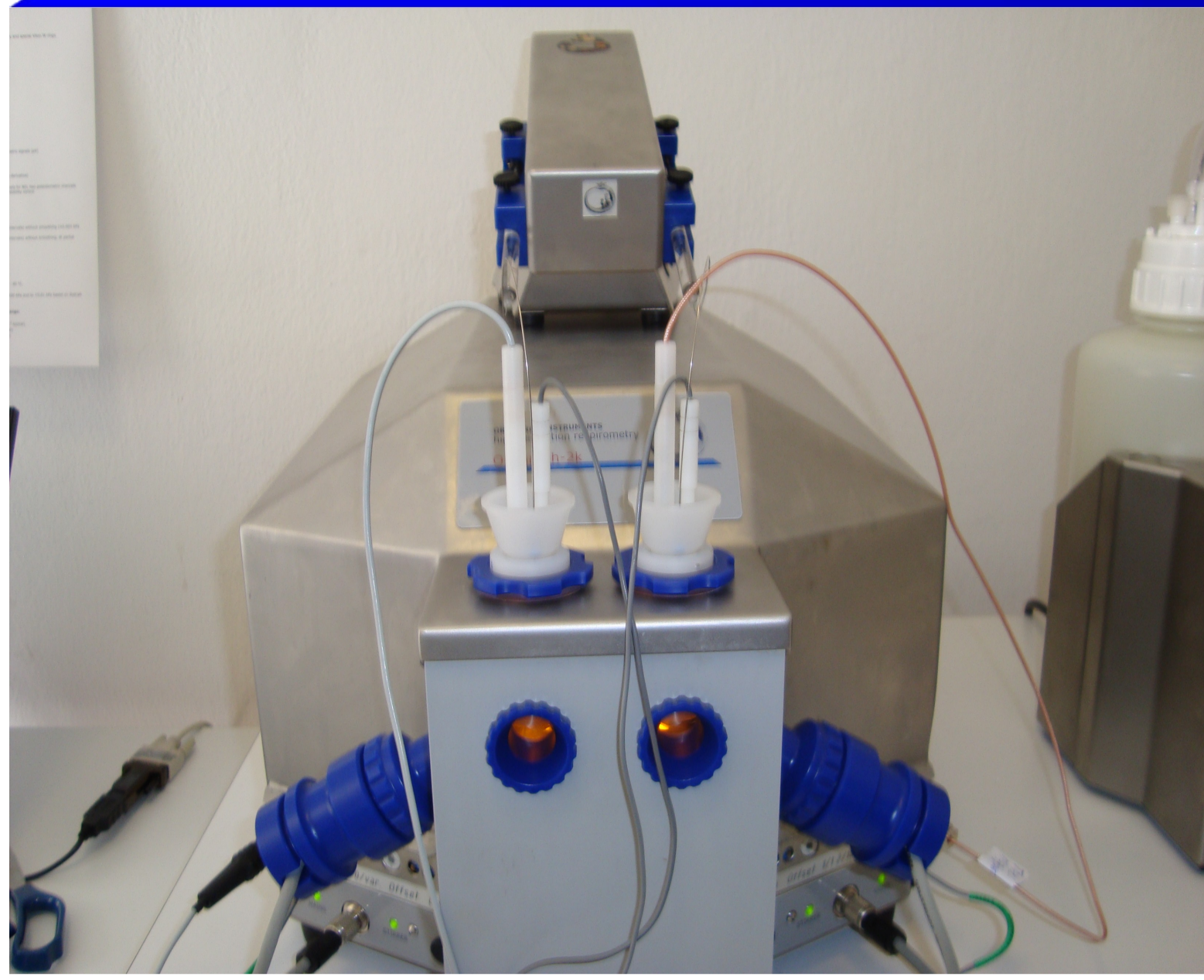
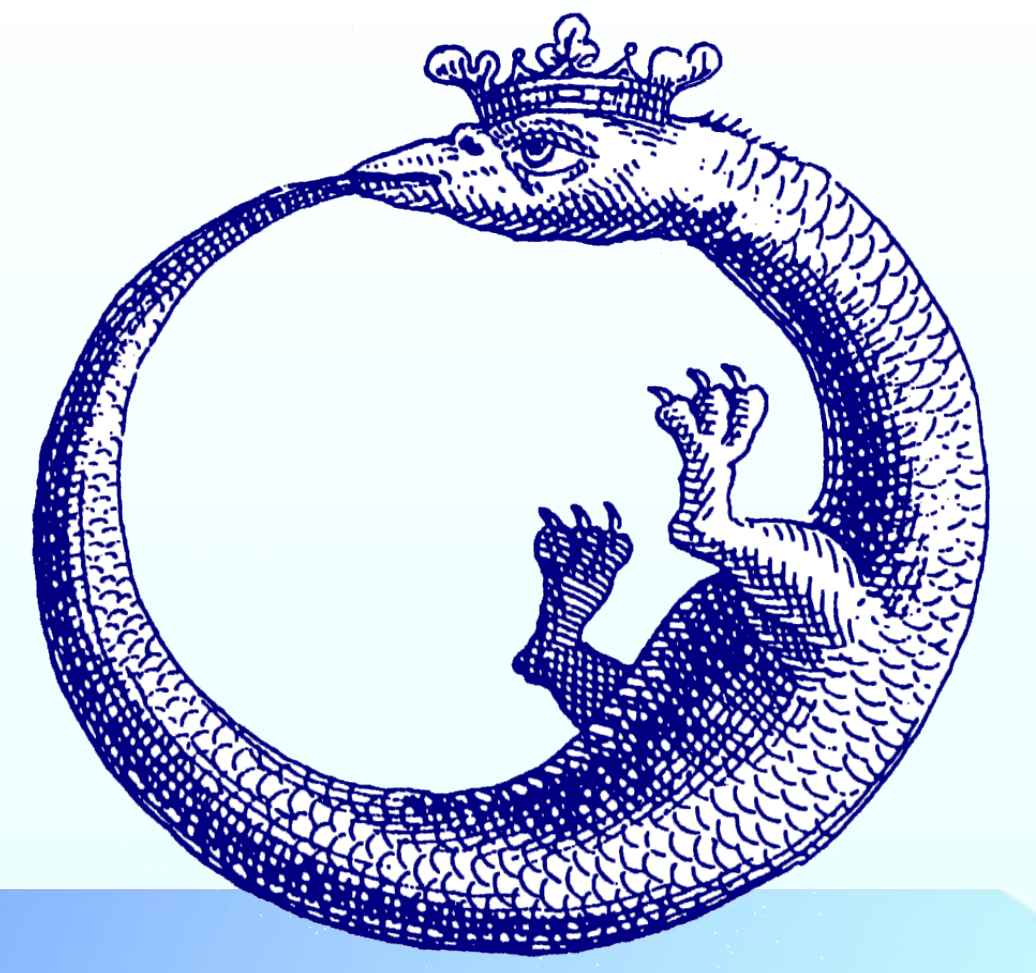


ISE for TPP⁺ and High-Resolution Respirometry

OROBOROS INSTRUMENTS
high-resolution respirometry
Oxygraph-2k

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Features of the OROBOROS TPP ⁺ electrode	
ISE membrane:	+ simple mechanical mounting + optimized to increase signal stability
ISE membrane composition:	and minimize side effects by chemical substances such as ethanol and ADP
High quality inner electrode:	+ signal stability
PVDF electrode housing:	+ minimal O ₂ storage and diffusion
Integration into the O2k chamber :	+ minimal carry-over of inhibitors + minimal oxygen diffusion

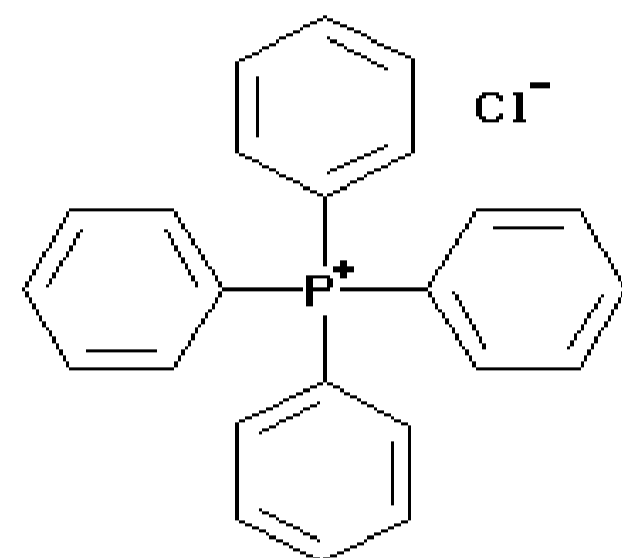


Figure 1: Set with 2 TPP⁺ and 2 reference electrodes

Development of a TPP⁺ electrode

Tetraphenylphosphonium (TPP⁺) accumulates in the mitochondrial matrix as a function of the mitochondrial membrane potential.

Tetraphenylphosphonium chloride



The TPP⁺ electrode is an ion selective electrode (ISE). The signal [V] of the TPP⁺ electrode is linearly dependent on the logarithm of free [TPP⁺].

The ISE system (OROBOROS INSTRUMENTS) consists of separate measuring and reference electrodes. The ISE are inserted through the stoppers into two chambers of the Oxygraph-2k and connected to the potentiometric channels of the O2k-MultiSensor system, for simultaneous recording of oxygen and TPP⁺.

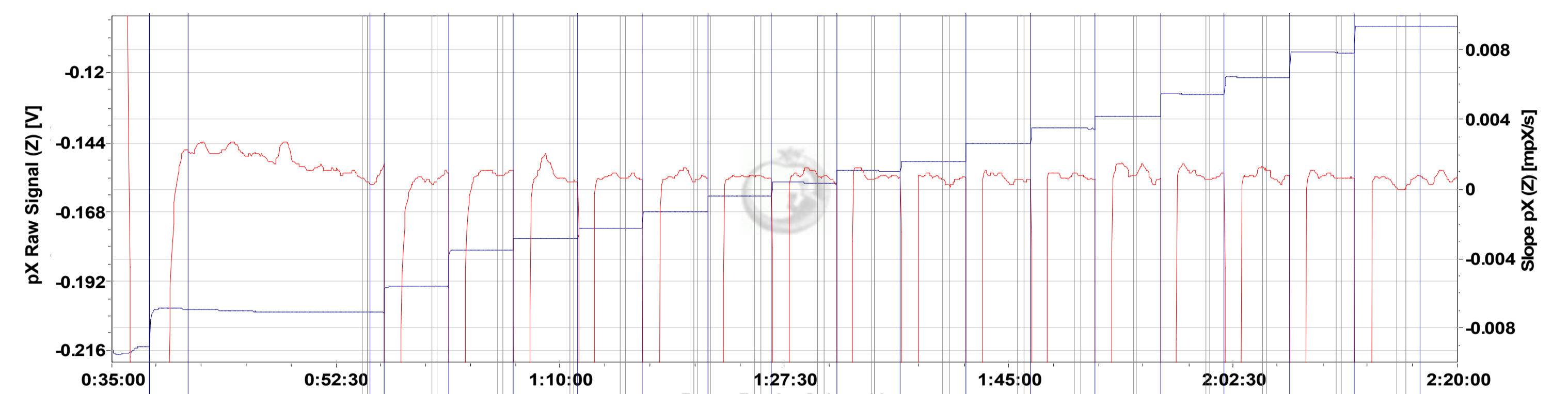


Figure 2: Calibration test of a TPP⁺ electrode performed in Oxygraph-2k chamber in MIRO6, 37°C with stirring speed 750 rpm. The calibration solution (1 mM TPPCl in 100 mM KCl) was injected automatically with TIP2k. A calibration range 0.1 -10 μM TPP⁺ was covered in 17 steps at 5 min intervals, using data recording intervals of 1 s.

	Raw signal @ 0 TPP ⁺ [mV]	Sensitivity @ 1-3 μM TPP ⁺ [mV/dec]	Critical [TPP ⁺] @ 40 mV/dec [μM]	Drift of raw signal [mV/s]
Mean ± SD	-196 ± 14	53 ± 5	0.7 ± 0.3	0.0007
n	96	99	82	99

Table 1: Typical performance parameters of OROBOROS TPP⁺ electrodes. Values are calculated from non-amplified signal. The critical sensitivity is defined at 40 mV/decade [mV/dec]. At this sensitivity, the critical [TPP⁺] was 0.7 μM. The absolute value of drift was measured in all calibration runs in the range 2-5 μM TPP⁺.

A long-term study over 3.5 months

To evaluate the long-term stability of 10 TPP⁺ electrodes without change of membranes

with rinsing procedure necessary for removal of inhibitors and uncouplers: water, 99% ethanol, water

Design

10 TPP⁺ electrodes were followed for 3.5 months after mounting a new membrane:

- one calibration run (Fig. 2) per electrode every 7-14 days
- 10 runs each electrode

Ethanol: 5 electrodes rinsed with water, 99% ethanol, water
Water: 5 electrodes rinsed with water only

Results

Calibration test	Rinsing group	Raw signal @ 0 TPP ⁺ [mV]	Sensitivity @ 1-3 μM TPP ⁺ [mV/dec]	Critical [TPP ⁺] @ 40 mV/dec [μM]	Drift of raw signal [mV/s]	Response time [s]
1 st	Ethanol (5)	-199 ± 10	51 ± 4	0.9 ± 0.5	0.0007	14 ± 3
	Water (5)	-213 ± 18 (3)	52 ± 4	1.0 ± 0.4	0.0006	16 ± 6
5 th	Ethanol (5)	-200 ± 14	56 ± 6	0.6 ± 0.3	0.0004	49 ± 10*#
	Water (5)	-199 ± 8	54 ± 3	0.7 ± 0.2	0.0002	28 ± 11
10 th	Ethanol (4)	-189 ± 18	54 ± 7	0.9 ± 0.4	0.0006	52 ± 19*
	Water (4)	-198 ± 12	55 ± 5	0.4 ± 0.2*	0.0003	36 ± 14*

Table 2: Performance parameters of TPP⁺ electrodes in the course of the study. Values shown as mean ± SD are calculated from non-amplified signal. **Ethanol:** electrodes rinsed with water, ethanol, water. **Water:** electrodes rinsed with water only, *P<0.05 vs. 1st run in the same group, #P<0.05 vs. water group in the same run.

Ethanol group: One electrode needed a new membrane after 7th run

Summary

Characteristics of the electrodes:

- High sensitivity: > 40 mV/dec for [TPP⁺] > 0.7 μM
- Low detection limit: < 0.2 μM TPP⁺

Long-term performance of the electrodes:

- The zero signal, sensitivity, and critical [TPP⁺] did not change significantly within 3.5 months (Table 2).
- The response time increased slightly in the course of the study, there is a trend of a faster increase in response times of ethanol-rinsed vs. water-rinsed electrodes (Figure 5).

Conclusions

- Improved performance parameters of the OROBOROS TPP⁺ electrode are the basis for application in mitochondrial physiology with high-resolution respirometry.
- The low detection limit allows for using low [TPP⁺] to avoid inhibition of mitochondrial respiration.
- Rinsing with ethanol is possible, the same membrane can be used on a TPP⁺ electrode for many experiments. The membranes can be changed at intervals to ensure high performance.

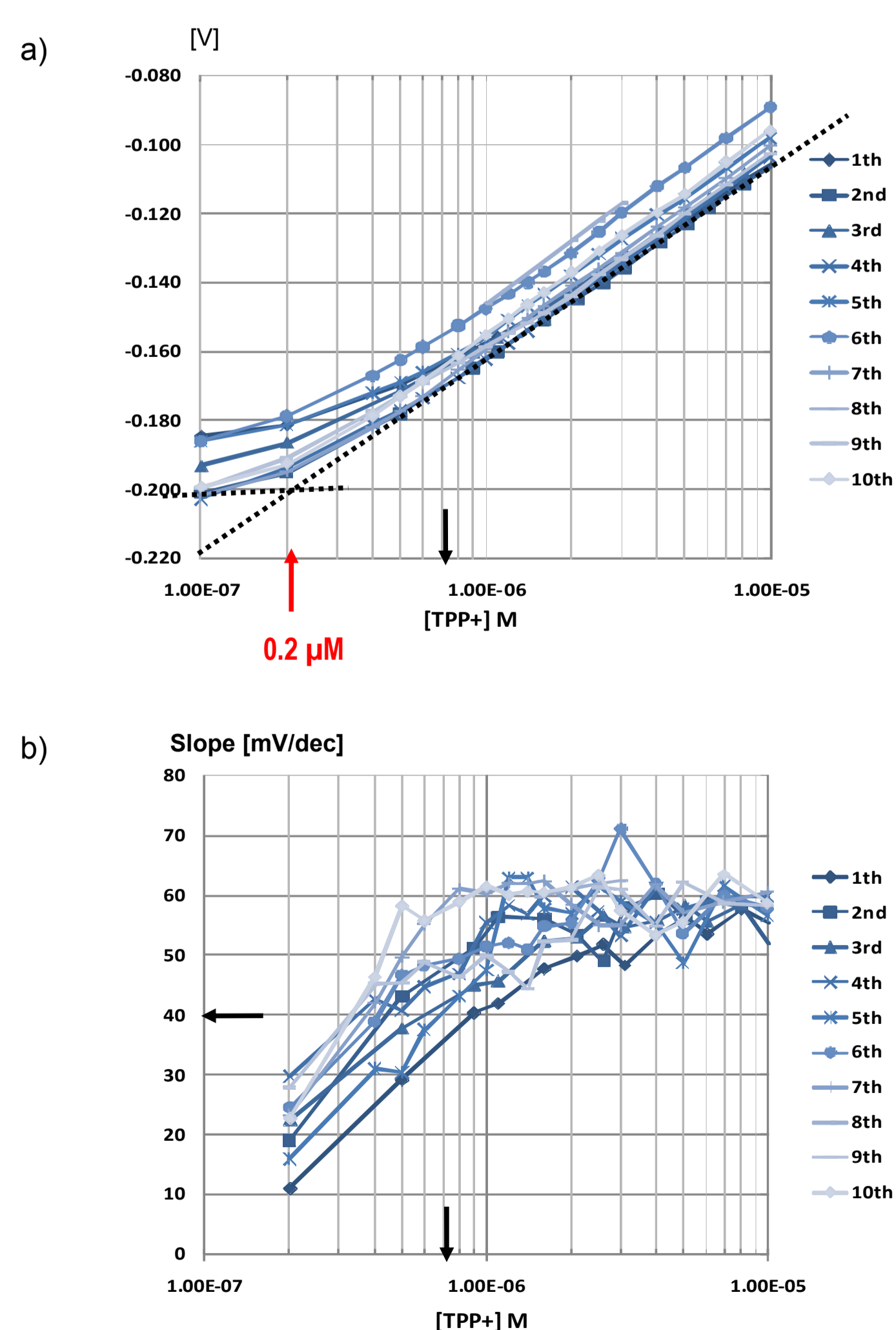


Figure 3: The performance parameters of a TPP⁺ electrode over 3.5 months. a) Non-amplified raw signal [V] as a function of log[TPP⁺]. The parameter of a linear fit slope [mV/dec] represents the sensitivity of ISE. b) Sensitivity [mV/dec] of non-amplified signal (calculated for each point) as a function of log[TPP⁺]. The red arrow shows the detection limit of 0.2 μM, the black arrows show critical [TPP⁺] of 0.7 μM (see Table 1).

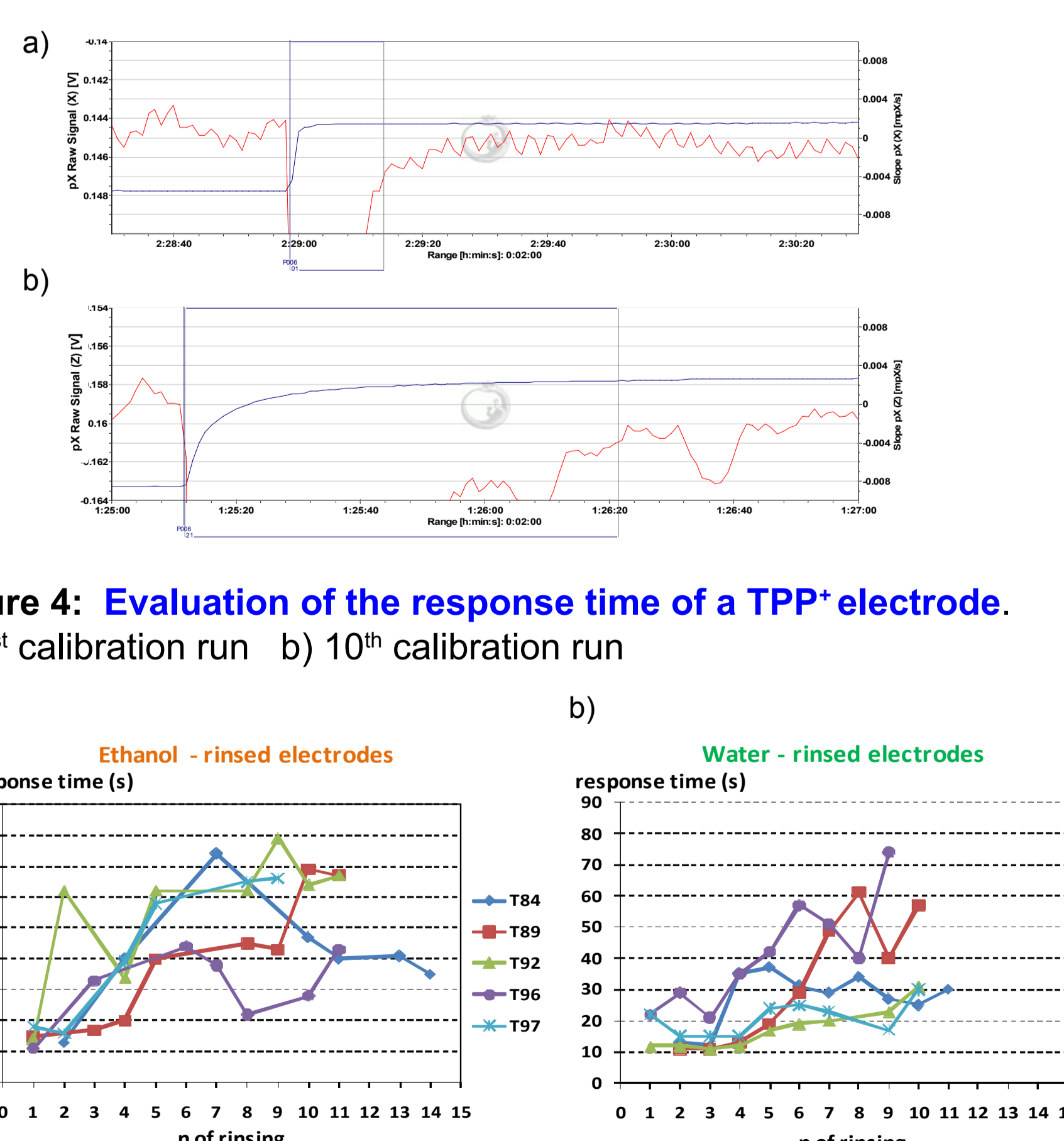


Figure 4: Evaluation of the response time of a TPP⁺ electrode. a) 1st calibration run b) 10th calibration run
Figure 5: The response time as a function of number of runs. a) electrodes rinsed with water, 99% ethanol, water b) electrodes rinsed with water only. The response time was defined as the time after which the slope of the raw signal achieved the value -0.004 mV/s (IUPAC recommendation [1]), measured at the calibration step from 0.8 to 1 μM TPP⁺. The number of data points for time derivation was 10.

References:

- Buck RP, Lindner E (1994) Recommendation for nomenclature of ion-selective electrodes (IUPAC recommendations 1994). *Pure Appl. Chem.* 66: 2527-2536.

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