Optode characterization

Protocol

1. Prepare fresh optodes: PMMA optical fibre and connectors; coat ~10 cm of the central section of each optode with an appropriate ion-selective membrane. Let optode dry 5-30 min in air.
2. Condition optodes for ~ 30 min in a buffer solution that will be used for further measurements (BG buffer). Choose the buffer wisely, so that it contains no or the least possible amount of the interfering ions that could cause the optode response along with your target analyte (primary ion).
3. All the measurements with optodes and ion-selective electrodes (ISE) are carried in stirred solutions.
4. Once the conditioning is complete, record spectra of the optodes in BG ($\alpha = 0$), place them for 15-30 min in 0.01 M NaOH and once the response is stabilized, record the spectra of their spectra in base ($\alpha = 1$); the chromoionophore range is then calibrated.
5. Optode calibration is presented as response dependence on analyte concentration. Optode response is the degree of the chromoionophore protonation ($1-\alpha$) ranging from 0 to 1. The calibration is usually constructed using one of three ways described below.

5.1. Separate solution: in separate beakers prepare individual calibration solutions containing BG buffer and a known concentration of the analyte, e.g. 0.1; 0.01; 0.001; 0.0001; and 0.00001 M. Expose the optodes to each solution for 15 min or until a complete stabilization of their response, record their spectra, and corresponding a-values, repeat this for all other calibration solutions[1].

5.2. Addition: place optodes in a precisely measured volume of BG buffer, record their response at 0 concentration of the analyte. Add first portion of analyte stock solution to obtain the lowest desired concentration, wait for ~ 15 min or until the response is stable and record optode spectra. Repeat this to obtain at least 4-5 points for the calibration.

[1] If you choose to start from the most concentrated solution, pay attention to have the response very stable and steady at low concentrations to avoid its overestimation; if you choose to start with the least concentrated solution, pay attention to the response steadiness not to underestimate it at high concentrations, the response time could be slightly increased.

Calculating the concentrations, do not forget that the volume of solution increases with each addition. Example, V_0 = 200 ml:

V_{add}, µl	C_{add}, M	V_{tot}, ml	Log C
20	0.1	200.02	-5
180	0.1	200.2	-4
180	1	200.38	-3
1,800	1	202.18	-2
20,000	1	222.18	-1

Dilution: a method mostly suitable for automated calibration. Place optodes in a precise volume of most concentrated of the desired solutions and after stabilization (allow at least 10-15 min) record their response. Take out a precise volume of the solution (e.g. 180 out of 200 ml) and add the same volume of BG buffer (10-fold dilution). Repeat the measurement-dilution cycle as desired.