

# Ion selective optodes: from the bulk to the nanoscale

Xiaojiang Xie · Eric Bakker

Received: 12 October 2014 / Revised: 8 December 2014 / Accepted: 13 December 2014 / Published online: 22 January 2015  
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**Abstract** This review describes recent advances in the miniaturization of ion selective optodes into microscale and nanoscale sensors. The topics include a comparison between film-based and miniaturized ion optodes, equilibrium and exhaustive detection modes, recent preparation methodologies and applications of microscale and nanoscale ion optodes, criteria for the design of optode sensors, and other future perspectives.

**Keywords** Nanosensor · Ion selective optodes · Microsphere · Nanosphere · Optical sensor

## Introduction

The quantification and monitoring of ionic species is a ubiquitous challenge encountered in but not limited to environmental science, biochemical research, food chemistry and clinical diagnostics [1–5]. Among other techniques, such as electrochemical methods, atomic (flame) emission spectrometry (AES), mass spectroscopy, atomic absorption spectrometry (AAS), fluorimetry, and capillary electrophoresis (CE), ion selective optical sensors provide a number of advantages with low cost and high versatility and compatibility with other assays [6–13]. A variety of optical ion sensors have been discovered, including fluorescent molecular probes, surface modified nanomaterials, and biologically expressed proteins [14–19].

It is unlikely that one type of sensor will dominate the entire field because each has its advantages and limitations. For instance, when applied to intracellular imaging, many synthetic molecular probes may leach, sequester, and have a limited loading concentration, but they are bright and easy to handle. The delivery can be noninvasive as well. Autofluorescent protein-based genetically encoded indicators can be expressed *in vivo* at specific locations. In contrast, molecular probes cannot differentiate cell types or cellular compartments. However, the varieties of the fluorophores for autofluorescent proteins are quite limited and their brightness needs improvement. Functionalized nanomaterials can overcome some of the disadvantages mentioned above, but delivery of large nanoparticles can be quite invasive and their biotoxicity/biocompatibility can be problematic.

Ionophore-based ion selective optical sensors, also called ion selective optodes (sometimes simply optodes) take advantage of the excellent selectivity of readily available ionophores (ion receptors). The working principle is shown in Scheme 1. Compared with other types of optical ion sensors, such as organic fluorescent molecular probes, the recognition of the analyte does not directly result in an optical signal change from a reporter covalently attached to the binding moiety. Instead, the recognition part and the optical reporter are two separate entities. An ionophore is used for the ion recognition, whereas a lipophilic pH indicator (also called chromoionophore) acts as the optical reporter. As the analyte level in the sample increases, the hydrogen ion concentration in the sensor decreases on the basis of ion-exchange. Therefore, the analyte activity can be detected indirectly by monitoring the hydrogen ion level in the sensor.

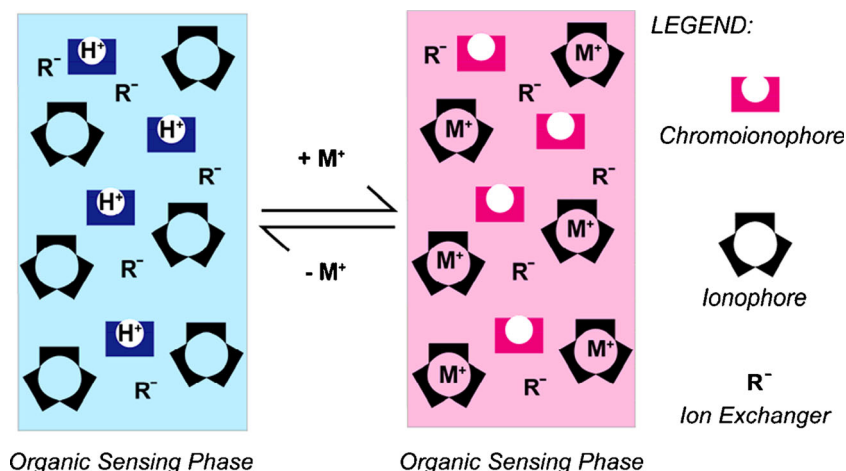
Ion selective optodes are versatile optical sensing tools that emerged from the field of polymeric membrane ion selective electrodes (ISEs) and are attractive for their sensor specificity because of the selective ionophores directly adopted from their ISEs counterparts. Moreover, the use of a separate

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Published in the topical collection *Direct Optical Detection* with guest editors Guenter Gauglitz and Jiri Homola.

X. Xie (✉) · E. Bakker (✉)  
Department of Inorganic and Analytical Chemistry, University of Geneva, Quai E.-Ansermet 30, 1211 Geneva, Switzerland  
e-mail: xiaojiang.xie@unige.ch  
e-mail: eric.bakker@unige.ch

**Scheme 1** Schematic illustration of how an ion selective optode containing a lipophilic pH indicator (chromoionophore), an ion receptor (ionophore) and an ion exchanger ( $R^-$ ) works with constant pH condition. The three sensing components are embedded in an organic hydrophobic environment that remains electrically neutral. Increasing the  $M^+$  level will result in a decrease in the protonation degree of the chromoionophore (and vice versa), which can be optically detected



chromoionophore simplifies the signal transducing mechanism between the recognition of the target ion and the optical signal output. Several reviews have appeared in the past that emphasized different attributes, such as sensor response mechanism, materials, and potential applications [1, 20–25]. Recently, there has been a growing interest in micrometer- and nanometer-sized ion selective optodes that allow one to apply them to reduced sample volumes. In this review, we highlight recent advances in ionophore-based ion selective optodes associated with their miniaturization and summarize the criteria and possible issues likely to be met to realize and utilize such miniaturized sensors. Other optical sensing platforms, such as molecular probes and functionalized colloidal nanomaterials, are not within the scope of this review.

### Ion optode mechanism

In 1982, Charlton et al. introduced a  $K^+$  assay where valinomycin-doped test strips were used to detect  $K^+$ -based on the co-extraction of  $K^+$  and an anionic dye that was incubated beforehand in diluted samples [26]. This anionic dye, erythrosine B, provided a colorimetric reading for the sensor strip. This formed the first sensor concept that closely resembled modern ion optodes. However, the field of ion optodes did not grow rapidly until 1989, when Morf et al. proposed to add a lipophilic pH indicator to the sensing phase instead of mixing dye molecules with the sample [27]. This lipophilic pH indicator was named chromoionophore. The same group subsequently reported neutral carrier-based optodes for ammonium and calcium ions, representing the first successful examples [28, 29].

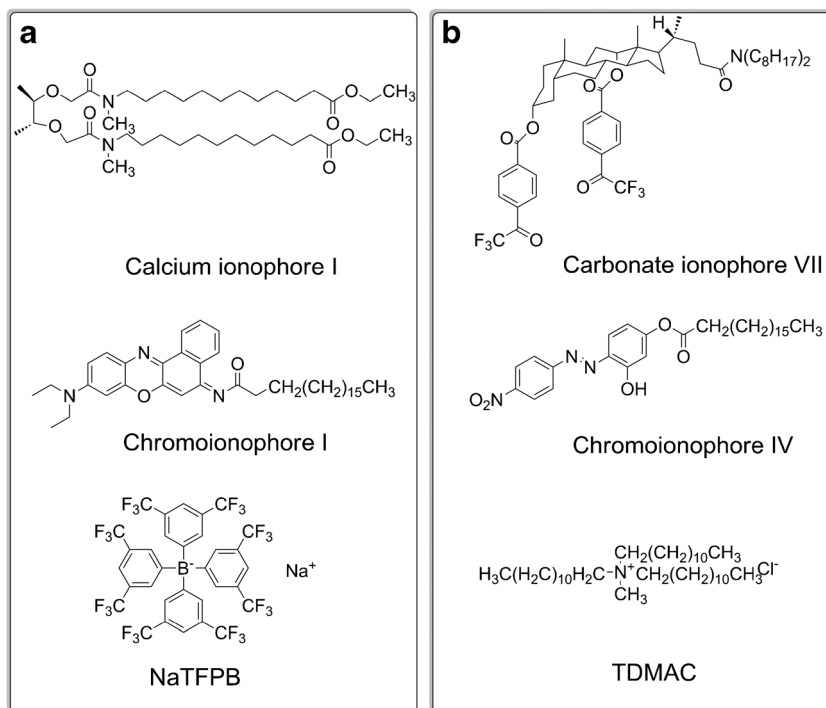
Today, classic film-based ion optodes contain the following materials: an ionophore, an ion exchanger, a chromoionophore, and a nonreactive hydrophobic polymer or solvent matrix. The actual combination of the materials is dependent on the target ions and can differ from case to case.

Exhaustive combinations for cationic sensor and anionic sensor have been listed in the literature and will not be repeated here [25]. For a cationic optode sensor, such as for  $Na^+$ ,  $K^+$ , and  $Ca^{2+}$ , the sensing components typically include an electrically neutral chromoionophore, a cation exchanger, and an ionophore, which is mostly neutral as well. To detect anionic species such as  $NO_2^-$ ,  $NO_3^-$ ,  $CO_3^{2-}$ , and  $Cl^-$ , a charged chromoionophore, an anion exchanger, and a neutral ionophore are combined. Figure 1 shows the chemical structures of the required sensing components for a  $Ca^{2+}$  selective optode [28] and a  $CO_3^{2-}$  selective optode [30] as examples.

It is known that cation-selective optodes respond to the ratio of the analyte activity and the proton activity, while anion-responsive optodes respond to the product of the analyte activity and the proton activity [21]. The reason for this is that the chromoionophores used in these sensors made protons inevitably the reference ions in all these sensing schemes. Wolfbeis and co-workers investigated ion optodes modified with polarity sensitive dyes in the absence of chromoionophore [31–33]. This approach resembles Charlton's work in 1982, but the polarity sensitive dyes used are more lipophilic than erythrosine B. Voltage sensitive dyes were recently incorporated in ionophore-based ion selective nanospheres to act as reference ions [34]. In this case, the reference ions are no longer protons but the electrically charged voltage sensitive dyes. The voltage sensitive dye partitions between the organic sensing phase and the aqueous sample phase, the process of which being governed by thermodynamic equilibria.

Fluorescence intensity and absorbance are by far the two major readout modes for ion optodes. However, other techniques, such as luminescence decay time, refractive index, or surface plasmon resonance (SPR) have been successfully applied. For instance, Freiner et al. proposed a waveguide setup where the refractive index of an ion optode film changes upon analyte binding [35]. Suzuki and co-workers reported on a SPR sodium ion sensor using an ion optode membrane film

**Fig. 1** Chemical structures and abbreviations for the sensing components of a  $\text{Ca}^{2+}$  selective optode (reference [28]) and a  $\text{CO}_3^{2-}$  selective optode (reference [30])



[36], Werner and co-workers presented a potassium ion sensor based on the ion-exchange principle and using fluorescence decay time as readout [37].

### Ion selective microspheres and nanospheres

While film-based ion optodes can be applied to fiber optics [38], flow cells [28], and waveguide devices [39], they are too bulky to operate in a small amount of volume such as living cells. The emergence of miniaturized ion optodes in the form of microspheres and nanospheres can help tackle this challenge. At the same time, the miniaturized particles can be interrogated in a parallel fashion and become potential chemical bio-imaging tools.

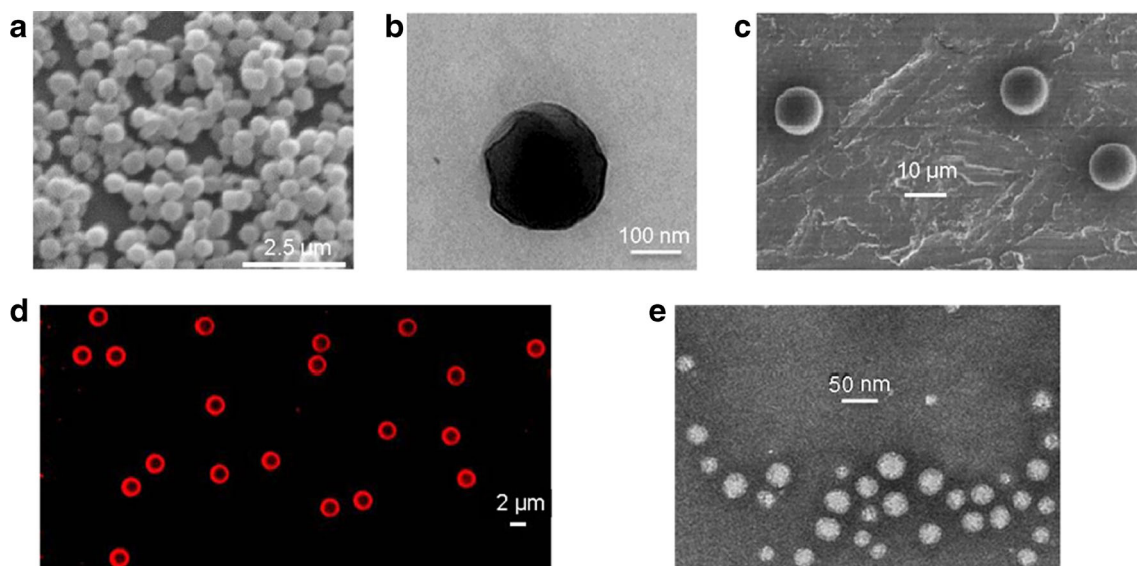
Kopelman and co-workers reported on nanosensors that were coined PEBBLEs (probes encapsulated by biologically localized embedding) [40–45]. Using inert, cross-linked acrylamide as a matrix, PEBBLE sensors can incorporate water soluble ion indicators such as rhod-2 and Calcium Green to detect ions without much interference from pH, and has proven to be valuable in the study of a number of intracellular analytes. However, the leaching of the hydrophilic indicator dye remains an issue and must be corrected for in practice. Hydrophobic PEBBLEs have been demonstrated for  $\text{K}^+$  (Fig. 2a),  $\text{NO}_2^-$ , and  $\text{Cl}^-$  and applied in live C6 glioma cells and rat conceptuses [43, 46]. The particles exhibited an average diameter from 300 to 700 nm. However, the usable lifetime of the sensors was limited to several hours because of leaching of the sensing materials (i.e., chromoionophore,

ion exchanger, and ionophore). Moreover, the preparation was time-consuming and the sensing components had to be adsorbed by the nanospheres after they had been cross-linked. This makes it difficult to produce multicomponent sensor particles with known and optimized compositions.

The situation was improved later on by Clark and co-workers who demonstrated sodium-selective polymeric nanosensors with an average diameter of ca. 120 nm (Fig. 2b) and a lifetime in solution of roughly 1 wk [47]. The nanospheres were prepared under sonication. The core was made up of classic ion optode materials, whereas the surface was covered with PEG-lipid molecules to offer stabilization. The nanosensors were successfully applied in isolated cardiomyocytes to visualize sodium and potassium flux across cell membrane, as shown in Fig. 3 [48, 49].

The same group also reported on biodegradable optode-based  $\text{Na}^+$  selective nanosensors using polycaprolactone as matrix [50]. The nanosensors were prepared by a solvent displacement method followed by centrifugation and washing. These nanosensors exhibited an average diameter of ca. 260 nm and lifetime of at least 2 wk at physiological temperature. When in the presence of lipase, the nanosensors degraded within 4 h at the lipase concentrations found in the liver but still were present after 3 d at the lipase levels found in serum. Polymer-free nanospheres with an average diameter of ca. 250 nm were later reported through a similar methodology [51].

In addition, Tohda and Gratzl fabricated ion selective microscopic beads for  $\text{Na}^+$  and  $\text{K}^+$  by a spray drying method [52–54]. Together with microbeads modified with glucose oxidase and those containing just reference dye, the ion



**Fig. 2** Microscopic images of different miniaturized ion selective optodes. **a** SEM image of  $K^+$  selective PEBBLE sensor adapted from reference [43]; **b** TEM image of  $Na^+$  selective nanosensor adapted from reference [47]; **c** SEM image of plasticized PVC microspheres adapted

from reference [59]; **d** confocal fluorescence image of surface modified polystyrene microparticles adapted from reference [71]; **e** TEM image of  $Na^+$  selective nanospheres adapted from reference [72]. Copyright: American Chemical Society

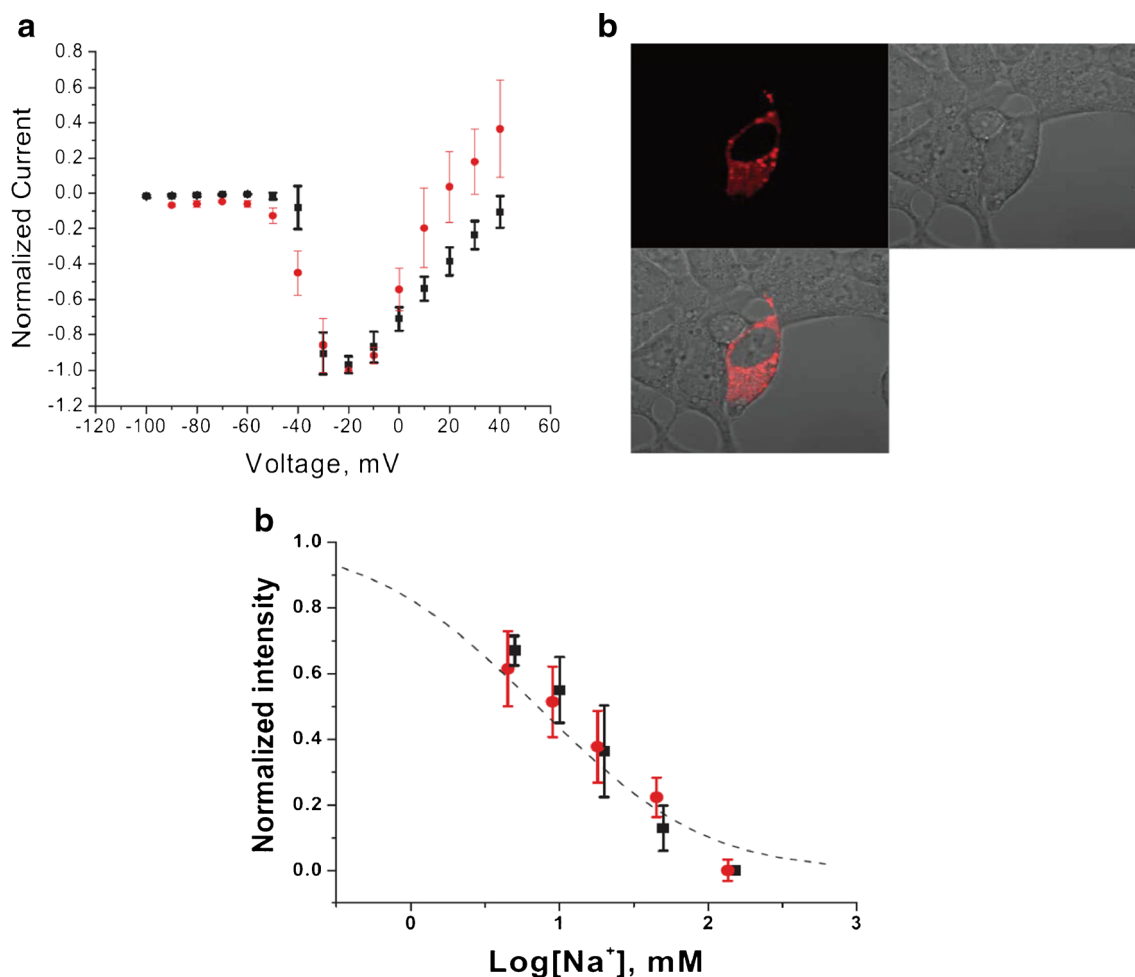
selective beads were assembled into a plastic bar coined “sliver sensor” 100 to 300  $\mu m$  wide and 1 to 1.5 mm long, and inserted under the skin. It was shown that this optical sensing array could successfully monitor pH,  $Na^+$ ,  $K^+$ , and glucose in the interstitial fluid in vitro. Michalska and co-workers recently reported that ion optode components can be loaded into cross linked poly(maleic anhydride-alt-1-octadecene) self-assembled micelles [55]. Carboxylate groups were formed after hydrolysis of the anhydride groups on the copolymer, which could function both as surfactant and ion exchanger. Loading the nanospheres with chromoionophore I alone resulted in a pH sensitive system.

Earlier, Seitz and co-workers derivatized lightly cross-linked polymer microspheres that swell and shrink as a function of analyte concentration [56]. Ionophores were incorporated into poly(4-hydroxy-3-nitro)styrene microspheres to demonstrate that ion binding accompanied by loss of protons causes the polymer to swell. The particles exhibited diameters from 0.3 to 3  $\mu m$ . Although they are not ion optode-based microspheres, the ion exchange process is quite similar to ionophore-based ion selective optodes.

Bakker and co-workers reported on ion selective microspheres that function as film-based optodes and that were prepared using a sonicating particle caster. The microspheres exhibited monodispersity and average diameter on the order of several micrometers (Fig. 2c). Based on this platform, microsensors with selectivity toward  $Na^+$  [57–59],  $Ag^+$  [60],  $K^+$  [57, 61–64],  $Ca^{2+}$  [57],  $Pb^{2+}$  [65],  $NO_2^-$ , [46]  $Cl^-$ , [46, 57], and  $PO_4^{3-}$  [66] were successfully fabricated. The multiplexed flow cytometric sensing of blood electrolytes in physiological samples using these fluorescent ion optode microspheres was

demonstrated [67]. Although PEG was used to stabilize the microspheres, they were still quite prone to coagulation owing to their relatively large size. The excessive amount of aqueous waste generated during the fabrication had to be removed to concentrate the microspheres.

Approaches based on commercial templates were also reported. Quantum dots were incorporated into ion selective optode materials to make both microspheres and nanospheres [68, 69]. Quantum dots can serve as an additional label for these sensors because they are rather inert. The excitation and emission are tunable by changing their size. They exhibit a wide excitation range, narrow emission bandwidth, and good photostability. Such core-shell structures were made of a quantum dot core, surrounded by a second polymeric coating of the sensing components and a third layer of amphiphilic molecules. At the same time, the fluorescence output may become complicated owing to spectral overlap and possible inner filter and FRET-based energy transfer processes. Silica particles were also reported as template [70]. Since the surface of the commercial silica particles was hydrophilic, silanization was required before doping the core with ion optode sensing components. The particles obeyed optode theory, albeit with rather long response times of ca. 10 min. On the other hand, the surface of nonmodified polystyrene particles is highly hydrophobic and may be utilized to make optode-based microsensors. We recently reported that the simple adsorption of ion exchanger, chromoionophore, and valinomycin on the surface of commercial polystyrene particles resulted in a functional optode sensor [71]. As shown in Fig. 2d, the modification is only limited to the surface because diffusion within polystyrene is extremely slow.



**Fig. 3** Intracellular sodium measurements using  $\text{Na}^+$  selective nanosensors from reference [48]. **a** IV response curve of whole cell patched HEK PN1 cells. Normalized sodium current using control internal filling solution (*black squares*) and internal filling solution containing sodium nanosensors (*red circles*). Data represent five

experiments plus standard deviation. **b** HEK PN1 cell loaded with sodium nanosensors using microinjection. Confocal image showing fluorescence (633/680 nm), bright field, and overlay. **c** Concentration calibration of nanosensors in vitro (*black squares*) and in cell-free solution (*red circles*). Copyright: National Academy of Sciences

The fabrication of most nanosphere or nanoparticle form ion optodes requires sonication, polymerization, centrifugation, dialysis, or similar procedures. These steps can be time-consuming and limit the mass production the sensors. Solvent displacement, also called precipitation, is a newly introduced method for the preparation of functionalized nanomaterials. Recently, Xie et al. introduced a new precipitation-based approach to yield ion selective nanospheres, which may alternatively be called nano-emulsions [72]. The fabrication of the nanospheres required one to only dissolve all the sensing components in tetrahydrofuran and subsequent mixing with aqueous solution. The nanospheres could be used immediately after the removal of tetrahydrofuran by purging with compressed air. No further laborious purification steps were required. These nanospheres exhibited typical average diameter of 40 to 100 nm with good monodispersity (Fig. 2e). It was demonstrated that the nanospheres still behave as film-based optodes. The nanosensors not only functioned as conventional

ion optodes but also enabled an exhaustive sensing mode that will be discussed below in more detail. The nanospheres were also used to introduce the concept of light controlled ion concentration perturbations [73]. When the chromoionophore is removed, they were also shown to serve as titration reagents that may potentially replace traditional water soluble chelators owing to their much reduced pH cross-response [74].

#### Detection mode: equilibrium and exhaustive

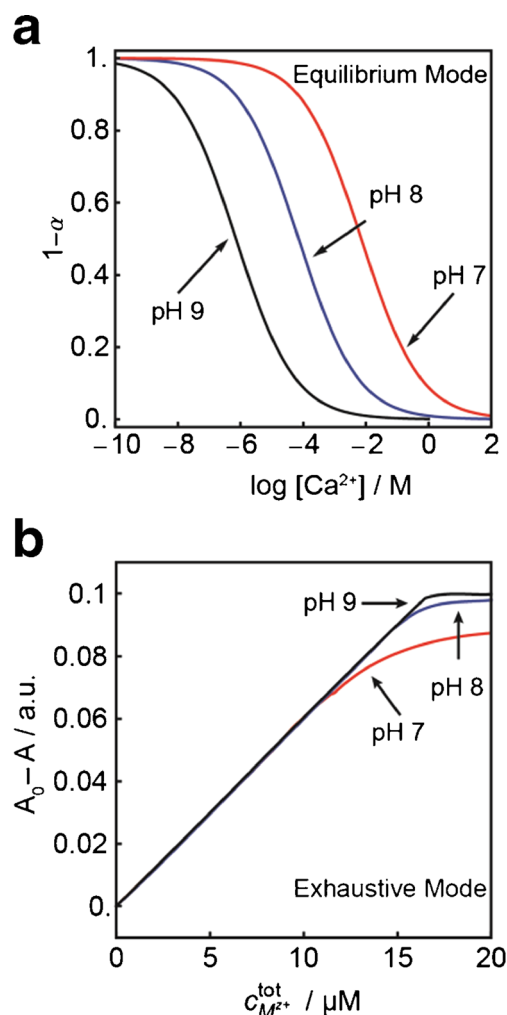
Conventionally, ion selective optodes are interrogated under equilibrium conditions. In other words, the sensing film or particles are in contact with the sample solution to allow for a passive partition of the analyte ions between the aqueous phase and the organic sensing phase until thermodynamic equilibrium is reached. The optical readout, typically absorbance or fluorescence, is recorded to calculate the degree of

protonation for the chromoionophore, which is then plotted against the logarithm of the analyte concentration (activity, more strictly). A sigmoidal calibration curve is obtained at fixed pH. In addition to passive sensing equilibration, the sensing process also can be triggered by light, as demonstrated by the development of photodynamic ion selective sensors for  $\text{Cl}^-$ ,  $\text{Ca}^{2+}$  and  $\text{Na}^+$  [6, 75, 76]. These sensors are normally doped with photoresponsive compounds such as a spiropyran or a photoacid generator. We refer to the publications cited above for a rigorous discussion of this sensing principle.

To obtain the theoretical ion optode response function, one must consider the phase transfer equilibrium of the extraction process, the charge balance within the sensing phase, the mass conservation and formation constants for the chromoionophore and the ionophore. Detailed derivations have been discussed in the literature. Here, the response function for a cationic analyte  $\text{M}^{z+}$  is shown in Eq. 1, where,  $a_{\text{M}^{z+}}^{\text{aq}}$  and  $a_{\text{H}^+}^{\text{aq}}$  are the activity of  $\text{M}^{z+}$  and  $\text{H}^+$  in the aqueous sample,  $K_{\text{ex}}$  is the overall phase extraction equilibrium constant,  $n$  is the complex formation stoichiometry for the ionophore,  $R_{\text{T}}$ ,  $\text{Ind}_{\text{T}}$  and  $L_{\text{T}}$  are the total concentration of ion exchanger, chromoionophore and ionophore in the sensing phase,  $1-\alpha$  is the degree of protonation of the chromoionophore. This response function should apply to both film-based and miniaturized particle-based ion optodes:

$$a_{\text{M}^{z+}}^{\text{aq}} = \frac{1}{zK_{\text{ex}}} \left( \frac{a_{\text{H}^+}^{\text{aq}} \alpha}{1-\alpha} \right)^z \frac{R_{\text{T}} - (1-\alpha)\text{Ind}_{\text{T}}}{\{L_{\text{T}} - z^{-1}n[R_{\text{T}} - (1-\alpha)\text{Ind}_{\text{T}}]\}^n} \quad (1)$$

The sensing process is associated with the redistribution of the analyte ions together with hydrogen ions between the sensing phase and the sample phase. For a cationic analyte, ion exchange between protons and the analyte occurs, whereas for an anionic analyte, coextraction of the analyte and protons takes place. Therefore, ion selective optodes, whether film- or particle-based, respond to changes in sample pH as well. The calibration curve at different pH values can be very different. For instance, for a calcium selective optode, changing the sample pH by one unit will shift the sensor response by two orders of magnitude (Fig. 4a). One advantage of this pH cross response is the ability to tune the sensor response range. For example, a sensor that responds to  $\text{Ca}^{2+}$  from  $10^{-6}$  M to  $10^{-2}$  M at pH 8 will do so in the range of  $10^{-4}$  M to 1 M at pH 7. On the other hand, the pH cross response is typically regarded as a major drawback of ion selective optodes. In practice, the sample pH must be controlled or measured at the same time. For sensing carbon dioxide, this problem is avoided by the direct sensing of carbon dioxide activity through the use of a coupled extraction of carbonate and hydrogen ions. This functions in contrast to the Severinghaus  $\text{CO}_2$  probe, which determines the  $\text{CO}_2$  level through indirect



**Fig. 4** **a** Theoretical equilibrium based  $\text{Ca}^{2+}$  ion selective optode response at different pH simulated using Eq. 1; **b** Theoretical exhaustive mode ion optode response at different pH

measurement of pH change in aqueous solution upon  $\text{CO}_2$  extraction [30].

Recently, we have shown that the pH dependence can be overcome by operating the ion selective sensors in what is called an exhaustive sensing mode [77, 78]. In this case, the sample analyte will ideally be completely consumed by the probes. The response will only depend on the total amount of the analyte and make the exhaustive sensors potentially calibration free. In a classic equilibrium-based sensing mode described by Eq. 1, on the other hand, the sample concentration is usually considered unchanged before and after exposure to the sensor.

The exhaustive detection mode can only work when there are more binding sites in the sensing phase than the amount of analyte in the sample. If a thin optode film is used, a thin layer of sample is preferred since large sample volumes will result in extremely long response times. Using suspensions of nanospheres or microspheres, on the other hand, may avoid this limitation because of a massively

increased surface to volume ratio and much smaller diffusion distances.

While the analyte is consumed during the exhaustive sensing process, the remaining analyte concentration should still be at equilibrium with the sensor. For the exhaustive mode to be valid, the remaining analyte concentration must be negligible compared with the initial concentration. Based on mass balance and partition equilibria of the analyte, a theoretical model has been put forward for exhaustive sensors based on absorbance measurements, shown in Eq. 2:

$$a_{M^{z+}}^{aq} = a_{M^{z+}}^{\bar{aq}} + \frac{1}{z} \frac{A_0 - A}{\epsilon b} \tag{2}$$

where  $a_{M^{z+}}^{\bar{aq}}$  represents the remaining  $M^{z+}$  activity in the aqueous phase, which follows Eq. 1,  $A_0 - A$  is the change in absorbance,  $\epsilon$  is the molar extinction coefficient for the chromoionophore, and  $b$  the optical path length for the nanosphere suspension. An important feature of the ion selective optodes working in this mode is that the response is linear with analyte concentration, as shown in Fig. 5 for protamine selective nano-optodes and calcium selective nano-optodes. The linear range is related to the amount of nanospheres used. A wider linear range occurs upon high loading of the

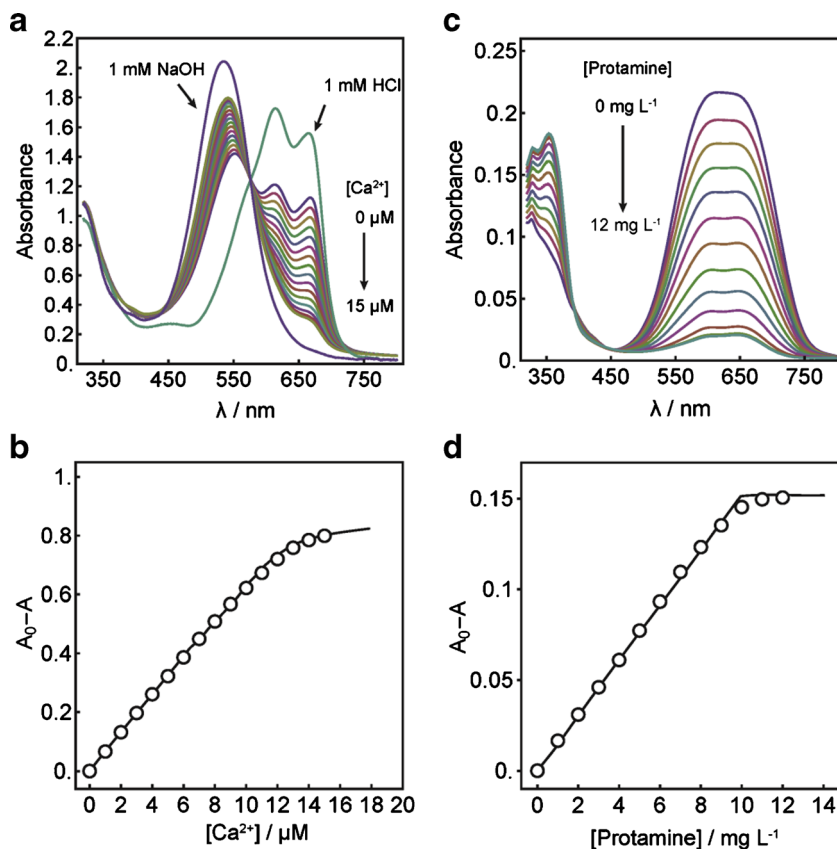
nanospheres. Compared with sigmoidal calibrations, a linear calibration curve is statistically much easier to handle.

The exhaustive sensing mode has, of course, its own limitations. In particular, it is not applicable to samples that cannot afford large concentration perturbations. For instance, exhaustive sensors are not suitable for interrogation of ion concentration in live cells because the massive consumption of the ions could potentially kill the cells. Even when the cells can survive such dramatic ion concentration perturbation, the outcome is likely biased because of possible compensation by the cells themselves. An equilibrium sensing mode is preferred in this case since the sample concentration will not be altered.

### Response time

The response time of ion optodes is generally given by the diffusion of all mobile species in the sensing organic phase and the aqueous sample phase. For plasticized PVC films with thicknesses of several micrometers, and with reasonably high sample concentrations, the limiting step is the diffusion of the sensing components within the film. In this case, assuming a mean diffusion coefficient for all mobile species and a film thickness of  $d$ , the time

**Fig. 5** **a** Absorption spectra of  $Ca^{2+}$  exhaustive nanosensors in response to various  $Ca^{2+}$  concentrations in 10 mM Tris-HCl buffer at pH 7.4; **b** Calibration curve for the  $Ca^{2+}$  nanosensors using the absorbance difference at 663 nm fit with theoretical curve; **c** Absorption spectra of protamine exhaustive nanosensors in response to various protamine levels in 10 mM tris-HCl buffer and 0.1 M NaCl at pH 7.4; **d** Calibration curve for the protamine nanosensors using the absorbance difference at 641 nm. Adapted from reference [78]. Copyright: American Chemical Society



required to achieve 95 % of the steady-state response  $t_{95}$  can be simply estimated through Eq. 3 [79]:

$$t_{95} = 1.13 \frac{d^2}{D_m} \quad (3)$$

where  $D_m$  is the diffusion coefficient in the sensing phase. On the other hand, if the analyte level in the aqueous phase is very low, the response time may become limited by mass transport in the aqueous phase. For instance, Lerchi et al. observed earlier that the response time of extremely sensitive optodes for transition metals is mainly dependent on mass transport in solution [80, 81]. The slow response at low analyte level can be overcome by using ion selective microspheres and nanospheres. As a result of the enhanced surface to volume ratio and spherical diffusion characteristics, mass transport is dramatically accelerated. For instance, Wygladacz et al. reported that for  $\text{Ag}^+$  selective plasticized PVC microspheres with an average diameter of 10  $\mu\text{m}$ , a response time of less than 15 min was found for  $10^{-9}$  M  $\text{AgNO}_3$  compared with a response time of 8 h in the case of film-based optodes [60]. For polyions such as protamine, the diffusion in the organic sensing phase can be quite slow. Previous film-based optical sensors for protamine based on ion exchange principles exhibited response times too long to obtain an equilibrium signal. Xie et al. showed that for protamine selective nano-optodes, the time to reach thermodynamic equilibrium was nearly instantaneous. Moving from a sensing film fixed to a support to freely moving suspended particles dramatically increases the mass transport efficiency [78].

### Selectivity

When ion optodes are miniaturized from the bulk to the nanoscale out of the same material, no dramatic change in the selectivity pattern is expected. The reason for this assumption is that the selectivity for ion optodes mainly depends on the ionophores. Without incorporating ionophores, the selectivity is determined by the thermodynamic equilibrium and in general follows the Hoffmeister series. It has been shown that for PVC-based ion selective microspheres, the selectivity pattern remains similar with film-based optodes based on the same material [61, 64, 65].  $\text{Na}^+$  selective nano-optodes incorporating quantum dots have shown similar selectivity for  $\text{Na}^+$  over  $\text{K}^+$  compared with PVC-based microspheres [69]. The selectivity for the decylmethacrylate-based  $\text{K}^+$  selective PEBBLE over  $\text{Na}^+$  is even slightly better than in plasticized PVC films [43].

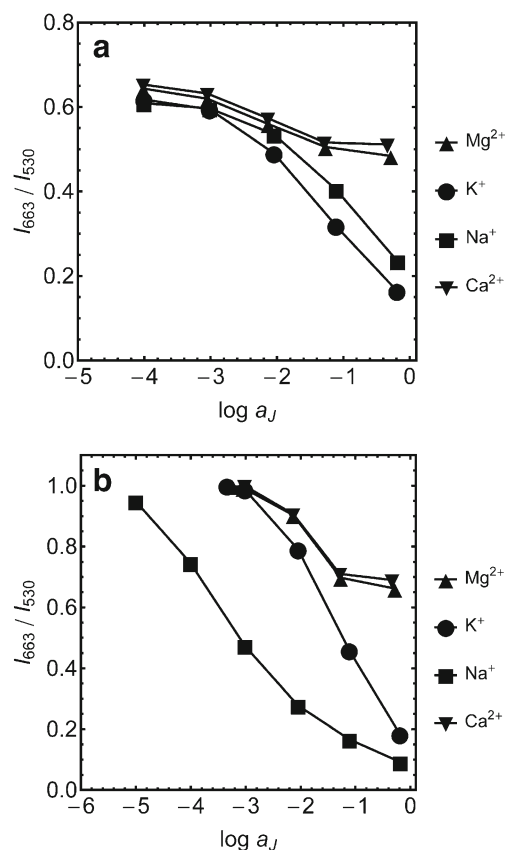
However, other factors may influence the selectivity pattern as well. Since the response function is directly derived from fundamental phase transfer and complexation equilibria, different microenvironments will affect the partition

equilibrium of the uncomplexed ions. For instance, as shown in Fig. 6, ionophore-free nanospheres reported by Bakker and co-workers showed a similar response to all ions [72]. Similarly, for nanospheres doped with additional ionophores, the response for all interfering ions was found to be nearly the same. These observations indicate that the nanosphere core forms a relatively polar environment compared with film-based optodes. We speculate that the higher polarity of the core is caused by the insertion of the surfactants, which is more polar than conventional plasticized PVC.

### Choice of materials

#### Surfactant

In order to realize micrometer size or nanometer size ion optodes, surfactants may be required to prevent the particles from coagulating. However, the presence of surfactant may also interfere with the sensing process. In particular, ionic surfactants such as sodium dodecyl sulfate (SDS) should be avoided since they may spontaneously extract together with cations into the sensor phase and repel anionic species.



**Fig. 6** Selectivity for nanospheres comprised of CH1 and KTFPB in 10 mM pH 7.4 Tris-HCl buffer, without (a) and with (b) NaX inside the nanosphere reported in reference [72]. Copyright: American Chemical Society



Cationic surfactants such as hexadecyltrimethylammonium bromide (CTAB) can repel cations and promote co-extraction with anions. Nonionic surfactants, including polyethylene glycol, Pluronic F-127, Triton X-100, Tween 20, and Brij 30 have been used to successfully fabricate ion selective microspheres and nanospheres. These surfactants bear similar hydrophilic groups but differ in the hydrophobic parts. Since the hydrophobic part may insert into a nanoscale organic sensing core, the surfactant is likely to influence the polarity of the particle core and shift the apparent pKa of the chromoionophore in some cases [72]. Moreover, the hydrophilic chains of these nonionic surfactants are composed of poly(ethylene oxide), which is known to be biocompatible and beneficial for in vivo applications.

### Optical reporter

As for film-based optodes, the choice of chromoionophore for micro/nano-optodes depends on several variables. First, the chromoionophore has to be compatible with the instrumental detection mode. Most chromo-ionophores are suitable for interrogation under absorption mode, established for the family of Nile blue derivatives. They exhibit very high molar extinction coefficients but typically a less attractive quantum yield. Reducing the amount of dye when used in fluorescence mode or imaged under fluorescence microscope may pose a problem.

Recently other compounds have been reported as new chromo-ionophores [82, 83]. However, the palette of chromo-ionophores still needs expansion, especially for those with a high quantum yield. To design a chromoionophore, there are more factors to consider besides quantum yield, such as the pKa value, spectral range, molar extinction coefficient, photostability, lipophilicity, and so on. Together with the ionophore used, the pKa value will influence the response range of the sensor. Long wavelength absorption in the visible range and the near infrared region is preferred. Ratiometric dyes are also preferred because they provide a more accurate determination. In addition, the scope of chromoionophore should not be limited to organic dyes. Semiconductor fluorophores, metal ion complexes, and biologically expressed fluorophores are potential candidates as well. These materials easily provide higher quantum yields, better photostability, and narrow absorption and emission bands.

### Ion exchanger

The most commonly used ion exchangers are organic analogs based on tetraphenylborate (e.g., tetrakis[3,5-bis(trifluoromethyl)phenyl]borate, TFPB) and tetraalkylammonium salts (e.g. tridodecylmethylammonium, TDMA). Others, such as carboxylated poly(vinyl chloride) (PVC) and organic sulphonate, can also serve as ion

exchanger but may result in incomplete dissociation because of the relatively high pKa of these functional groups. Some of the hydrophilic groups of these compounds also can act as amphiphiles when they are used to make nanosensors. Ion exchangers are made very lipophilic to prevent leaching. The leaching from microspheres and nanospheres may be dramatically accelerated because of to the larger surface to volume ratio.

### Ionophore

Ionophores help stabilize the target ions and ensure the sensor selectivity. Nowadays, plenty of ionophores are available for cations that include nearly all main group and transition metal ions. For one target ion, one can even find several ionophores with different complex formation constants. Ionophores with higher complex formation constants can shift the sensor response to a lower concentration range and make them more sensitive.

Despite the huge success in developing cation selective ionophores, the advancement of anion selective ionophores is in comparison much less developed. Anions such as  $\text{SO}_4^{2-}$  and  $\text{PO}_4^{3-}$  are extremely hydrophilic and thus the complex formation constant for an ionophore must be sufficiently large to compensate the solvation energy difference between aqueous and organic phases. In addition, achieving high specificity is very difficult. Some so-called “anti-crown ether” compounds have been reported to be highly selective to chloride. However, for ions such as nitrate, sulfate, and phosphate, the interference from highly lipophilic anions such as perchlorate and thiocyanate remains a problem. Highly selective ionophores for fluoride have been reported [84]. Recently, salophen-based metal ion complexes have been proposed as ionophores for nitrite, salicylate, copper (II), and monohydrogen phosphate [85–90]. However, these compounds may be susceptible to hydrolysis. Although their lifetime in film-based sensor films can be prolonged by an increase in viscosity and film thickness, when loaded into nanospheres or microspheres, hydrolysis will be accelerated because of the increased surface to volume ratio. In addition, in contrast to most cation selective ionophores that remain optically silent in the visible range, most metal complex-based ionophores do absorb in this range and the spectrum can change upon binding with the anion of interest. This should also be taken into account when designing optical sensors.

### Matrix material

Most film-based ion optodes have adopted plasticized PVC as matrix materials since it is the major material used for ion selective electrodes (ISEs). For miniaturized microsphere and

nanosphere-based ion optodes, more versatile matrix materials are being explored, including polyacrylamide, polystyrene, polycaprolactone, decyl methacrylate, and polymer-free solvent as described above. The choice of matrix material will help to control the micro/nanosensor size, response time, selectivity, and biocompatibility.

### Future perspective

Despite a history of around 25 years for ion selective optodes, the field of miniaturized ion optodes is still young and rapidly growing. New and exciting concepts and extensions to conventional techniques are regularly introduced into the field, such as different detection modes and various sensor materials. Ion selective optode nanosensors and microsensors have opened new avenues in applied and academic research fields, including imaging in biological environmental samples. In the meantime, some aspects still remain unsatisfactory and require a radical improvement, such as the pH cross response, relative signal change, brightness, and photostability of the chromoionophore for interrogation in fluorescence mode, and selectivity of anion-specific ionophores. It is also important to discover more convenient and less laborious methods to fabricate miniaturized optode-based sensors. As these characteristics are being improved, ion selective optode sensors will likely become widely accepted and find their way into an increasing number of real-world applications.

**Acknowledgments** The authors thank the Swiss National Science Foundation (SNF) and the University of Geneva for financial support.

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