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Data availability

The data supporting this article have been included as part of ESI
Comparative analysis of a bulk optode based on a valinomycin ionophore and a nano-optode in micelles with Pluronic F-127 for the quantification of potassium in aqueous solutions

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Abstract

In this work, two types of optical sensors were prepared for the quantification of potassium: the bulk optode (BO) and nano-optode (NO). The BO was prepared using three main components: the ionophore valinomycin, the ion exchanger tetrakis(4-chlorophenyl) potassium borate (K-TCPB), and the chromoionophore ETH 5294 (CHI). The optimal composition was found to be in a ratio of [1:1:1]. The NO was prepared by miniaturizing the BO through sonication in surfactant Pluronic F-127. The working range for the linear calibration model of BO was from 10⁻⁶ to 1.0 M K⁺ with a LOD_BO = 0.31 µM, meanwhile for NO was from 10⁻⁴ to 1.0 M K⁺ with a LOD_NO = 30.3 µM. Both optodes were tested for selectivity towards K⁺ in the presence of alkaline and alkaline earth ions, with a selectivity coefficient > 1.0. Furthermore, precision and stability studies of BO and NO were performed for three levels of K⁺ concentrations, being 10⁻⁶, 10⁻³, 1.0 M for BO and 10⁻⁴, 10⁻², 1.0 M for NO, showing a good homogeneity of the NO in the whole concentration range. However, an excessive variability was obtained for BO at 1.0 M K⁺. Therefore, the NO represents a potential tool for quantification of K⁺.

Highlight

• "BO and NO show linear calibration for K⁺ with wide concentration ranges."
• "NO outperforms BO in selectivity, precision, and stability over time."
• "Optimal composition [1:1:1] ratio for both optodes enhances K⁺ quantification."
• "NO, with Pluronic F-127, enables K⁺ quantification with stability up to 48 hours."

Keywords: bulk optode, nano-optode, potassium, valinomycin, ionophore.

1. Introduction

Industrialization and human activities have caused environmental pollution that affects the quality of living organisms [1]. Experts have recommended monitoring both nutrients and pollutants in water, soil, and air samples to control environmental damage...
Developed countries have increasingly imposed stricter regulations on agricultural sectors. The control and monitoring of hazardous materials have become an important issue in a world where the need for information is almost instantaneous. Applied chemistry should develop accurate and precise quantification methods to meet the needs of a scientific field in constant growth. At the same time, increasing environmental restrictions are forcing the development of methods that are recyclable, easy to use, and inexpensive.

Chemical sensors have been extensively studied and are powerful tools for quantifying analytes of interest [3]. One type of optical sensors are optodes [4], which are formed by introducing a compound with an optical property onto ion-selective electrodes (ISE) [5]. Ionophore selective optodes (ISO) are based on an ionophore capable of selectively detecting a specific ion and a chromoionophore capable of modifying some optical properties in direct response to the concentration of a specific ion [6]. The reported optodes quantify gases such as $\text{O}_2$ and $\text{CO}_2$ [7], hydrogen ions (pH) [8], metal cations such as $\text{Na}^+$ [9], $\text{Ca}^{2+}$ [10], and heavy metals such as $\text{Ag}^+$ and $\text{Hg}^{2+}$ [11], $\text{Pb}^{2+}$ [12] and $\text{Cu}^{2+}$ [13], among others. Optodes have gained interesting competitive features such as selectivity in the presence of competitive ions, no need for sample pretreatment, low cost as well as fast response and portability for field measurements [4,14].

Optodes are chemical sensors that rely on changes in optical properties, such as reflectance, transmittance, absorbance, and fluorescence. They can be classified into two categories based on their size scale: bulk-optodes (BO), which operate at the micro and macroscopic level, and nano-optodes (NO), which operate at the nanometer scale. The typical composition of optodes consists of three active components, an ionophore for ion recognition, a chromoionophore as an optical indicator, and an ion exchanger as a charge-balancing additive [15]. The ionophore is an ion carrier capable of selectively and reversibly recognizing the target ion [16]. Valinomycin is a macrocycle with an internal cavity suitable for the entry of potassium ions [17]; this ratio is key to the high selectivity of the optodes and is easily tuned by switching to another specific ionophore [18,19]. Selectivity is a critical feature of an optode, due to the high stability of the complex between the valinomycin and $\text{K}^+$ [20]. The chromoionophore is an ion transporter similar to an ionophore but responsible for the analytical signal, acting as an optical indicator proportional to the concentration of the analyte [21]. Its optical properties are due to its chemical structure and degree of protonation [22,23], which is balanced by imine-amine
tautomerism [24,25]. The ion exchanger is a lipophilic salt that acts as an ionic additive to facilitate the transfer of ions, such as the analyte, from the solution to the optode membrane [26].

Recent research on optical sensors for potassium has largely focused on studies of fluorometric detection systems [27–32] and sensors used in biological samples [33–37]. Some investigations involve the use of paper supports or PEG-type surfactants with high selectivity [38,39]. Solvatochromic dyes have been proposed as a replacement for chromoionophores in recent studies [40]. This approach uses smaller sample volumes that do not require pH buffers [41] and has been successfully applied to hydroponic crop monitoring systems [42]. Some publications have used radiometric signals instead of the "α" signal. More recently, applications have been developed to capture images directly from cell phones and to analyze signals from digital cameras [43,44].

BO consists of a lipophilic film dissolved in tetrahydrofuran (THF) supported on a plasticized polymer. In contrast, NO is a miniaturized version of BO with active organic components encapsulated in micelles of an amphiphilic polymer. These micelles are stable in aqueous media [45]. NOs offer advantages such as small sample volumes, ease of preparation without organic solvents, and the ability to perform real-world measurements. Comparatively, NOs have a larger surface area and better ion diffusion coefficients, resulting in higher signal stability, however, some challenges such as pH cross-response, low photostability, and lack of ionophores for anions need to be improved [4].

In this study, a comparative analysis of two optodes is discussed, namely the classical BO and its miniaturized NO. The redesigned NO was enhanced through a design of experiments procedural approach, focusing on key factors such as calibration model, optode composition, as well as buffer pH condition. The experimental procedure validated the methodology based on variables such as selectivity, sensitivity, precision, and stability. In summary, this research explores the emergence of optical sensor, NO compared to BO, outlining their respective strengths and weaknesses, with a specific emphasis on stability for in-situ measurements.
2. Experimental

2.1. Reagents and Materials

All chemicals were used as received. The starting materials were as follows: Valinomycin, ≥98% (TLC); polyvinyl chloride (PVC), high molecular weight; tetrahydrofuran (THF), anhydrous, ≥99.9%, inhibitor-free; Pluronic F-127 powder, BioReagent, suitable for cell culture, Tris (hydroxymethyl) aminomethane ACS reagent, ≥99.8%, all of them were supplied by Sigma-Aldrich. Bis (2-ethylhexyl) sebacate (DOS), Selectophore™, ≥97.0%; 3 Octadecanoylimino 7 (diethylamino) 1, 2 benzophenoxazine, 9 (diethylamino) 5 (octadecanoylimino) 5-H-benzo[a]phenoxazine (Chromoionophore I, CHI or ETH 5294); Potassium tetrakis (4-chlorophenyl) borate (K-TCPB), Selectophore™, ≥98.0% were supplied by Sulpeco. Potassium chloride, ACS reagent, 99.0-100.5%; sodium acetate, ACS reagent, ≥99.0% were purchased from Merck.

Solutions of 20 mM TRIS-HCl buffer pH 7.0-9.0 were prepared with HCl (ACS reagent, 37%) and 20 mM acetate-acetic buffer pH 5.0-7.0 with glacial acetic acid (Merck, ReagentPlus®, ≥99%). Milli-Q type 1 water (18.2 mS/cm) from Millipore (Billerica, MA, USA) was used as the solvent for the preparation of all solutions.

2.2. Preparation of the potassium cocktail and synthesis of BO and NO

The potassium cocktail contains the active components used for the preparation of BO and NO, namely 2.7 mg of the potassium ionophore I, valinomycin (2.43 μmol), 1.2 mg of the ion exchanger as well as lipophilic salt tetrakis-4-chlorophenylpotassium borate, K-TCPB (2.42 μmol), and 1.4 mg of the chromoionophore I, ETH 5294 (2.40 μmol). The components were dissolved in 1.0 mL of THF to form a homogeneous solution called potassium cocktail, which was used as a starting point for the synthesis of BO and NO. The structural support of BO consists in 160 mg DOS plasticizer and 80 mg PVC polymer added into cocktail. Fixation of BO was performed with a 20 μL aliquot on a 10 mm x 20 mm x 0.25 mm glass plate fixed in a 12V DC 4000 rpm fan under constant motion until THF evaporated. For the preparation of NO, 100 mL of 1.0% Pluronic F-127 was added to 1.0 mL of a potassium cocktail in THF and the mixture was sonicated at 30 °C in a 40 kHz water bath until the organic phase was completely dissolved. Purification of NO included removal of THF by purging with compressed air until a clear blue solution of NO micelles was obtained. A summary scheme of the BO y NO sensor synthesis is shown in Figure S1.
2.3. Characterization and calibration of BO and NO samples.

The pH of the solutions was measured using a pH meter, model 8103 from Orion Ross (USA). Absorbance measurements were taken using a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific, USA) with 10 mm quartz cells. The chromoionophore I absorption profiles were used to characterize the BO and NO. The deprotonated state had a maximum peak at a wavelength of 529 nm, while the protonated state had peaks at 615 nm and 667 nm [46]. An isosbestic point was observed a 576 nm [47]. The morphology of NO was analyzed using a JEOL JSM-7500F scanning electron microscope (SEM) operated at 20 kV by applying a drop of NO and then subjecting it to vacuum drying and carbon coating. The hydrodynamic size distribution of NO and Pluronic F-127 0.1% micelle (without potassium cocktail) were determined by dynamic light scattering (DLS-Brookhaven 90 Plus) at 25 °C using a monochromatic laser (658 nm) in a cell with an optical path length of 1 cm. Various publications at approximately 1.0% concentration of surfactant showed an average diameter for micelles ranging from 40 nm to 100 nm [48]. In addition, the presence of colloidal particles (micelles) in a solution was confirmed by observing the Tyndall effect during light interaction.

The glass plate containing BO was calibrated by immersing it in standard solutions buffered with 1.0 - $10^{-6}$ M K\(^+\) in 20 mM TRIS buffer at pH 7.0 for 15 minutes until color stabilization. Since BO is solid, the glass plate was put in a 10 mm quartz cell with the sensing phase facing inward. The potassium solution was then added until BO was completely covered. For calibration of NO, a mixture of 2.0 mL of NO in Pluronic F-127 1.0% and 0.5 mL of standards between 1.0 - $10^{-4}$ M K\(^+\) buffered in 0.1 M TRIS buffer pH 7.0 was used, and the color stabilization time was set to 15 min. The maximum peak absorbance at a wavelength of 667 nm was used as the analytical calibration signal, representing the deprotonated state of CHI [49]. KCl was used as the potassium source, and a stock solution of 1.0 M K\(^+\) was prepared from it. Calibration standards for potassium concentrations were produced by diluting the stock solution.

2.4. Data analysis

The response of the optodes is typically described by a sigmoid model [50], as a graph of the correlation between the logarithmic of potassium activity as Log aK\(^+\) (X-axis) and the extent of protonation of chromoionophore, called "1-\(\alpha\)" (Y-axis). This parameter (1-\(\alpha\)) is expressed as the ratio of the protonated form of the chromoionophore to the total chromoionophore concentration. Experimentally, the value of "1-\(\alpha\)" can be
determined from the absorbances of both the protonated \( (A_{HC^+}) \) and deprotonated \( (A_C) \) forms (Equation 1). To obtain the value of \( 1-\alpha=0 \) for the deprotonated form, a 10 mM NaOH solution is used. For the protonated form, a 20 mM TRIS buffer solution at pH 7.0 is employed to obtain the value of \( 1-\alpha=1 \). The absorbance of an unknown sample \( (A) \) is also taken into consideration:

\[
1 - \alpha = \frac{[C-H^+]}{[C_c]} = \frac{A - A_c}{A_{HC^+} - A_C} \tag{1}
\]

2.5. Mechanism of detection

The detection of potassium ions by the optode is described as ion exchange [51]. The mechanism of detection of BO y NO is shown in Figures 1a y 1b respectively; its begins when the potassium ion enters the sensing phase of the optode and binds selectively to the valinomycin ionophore, allowing the release of a hydrogen ion from the CHI to maintain ionic charge neutrality within the optode [52]. The concentration of potassium ions is proportional to the loss of hydrogen ions, and the change in protonation state of CHI causes a color shift in optode. The sequential behavior in the detection mechanism (Equation 2) suggests a dependence between the ratio of the active components of the optode and the signal.

\[
K^{+}_{(aq)} + C-H^+_{(org)} + I_{(org)} \rightleftharpoons K-I^+_{(org)} + H^+_{(aq)} + C_{(org)} \tag{2}
\]
Figure 1. (a) Mechanism of $K^+$ detection in BO. (b) in NO. (c) Chemical structures of the starting reagents for the synthesis of BO and NO.

The equilibrium constant of the valinomycin-potassium ion complex ($K_{eK^+}$), is determined by the activities of the components of the optode [52] (Equation 3). The equilibrium constant $K_{eK^+}$, can be expressed in terms of concentrations of the ionophore ($C_I$), chromoionophore ($C_C$), and ion exchanger ($C_E$) (Equation 4).

This model encompasses a neutral ionophore, such as valinomycin, which is bound to a $K^+$ cation. This results in the formation of a protonated chromionophore ($C_{-H^+}$), as shown in equation 2. In this case, we worked with equal molar concentrations of...
ionophore, chromoionophore, and ion exchanger. This specific case allows the simplification of the response function of an optode, which in turn enables the derivation of equation 4. The ratio of optimal concentrations between the ionophore and the ionic sites of the membrane is variable for each ionophore-analyte pair. This work assumes equimolar concentration, i.e. \([C_i]=[C_C]=[C_E] \) [53].

\[
K_e^{KI^+} = \frac{a_{H^+} [C]}{a_{K^+} [C-H^+]} \tag{3}
\]

\[
K_e^{KI^+} = \frac{a_{H^+} \alpha}{a_{K^+} (1-\alpha)} \frac{[C_E]-(1-\alpha)[C_C]}{[C_i]- [C_E]+(1-\alpha)[C_C]} \tag{4}
\]

Equation 4 can be simplified by a more simplified sigmoid calibration model is then obtained (Equation 5). This can be rearranged by applying logarithms to obtain a linear calibration model (Equation 6).

\[
\left(\frac{\alpha}{1-\alpha}\right)^2 = a_{K^+} K_e^{KI^+} / a_{H^+} \tag{5}
\]

\[
\log \left(\frac{\alpha}{1-\alpha}\right) = 0.5 \log a_{K^+} + 0.5 \log K_e^{KI^+} / a_{H^+} \tag{6}
\]

For both calibration models, the independent variable is the activity the potassium ion \((a_{k^+})\) calculated with the Debye Hückel equation [54] and the dependent variable is a function of “\(\alpha\)”. Some terms are constants such, as the hydrogen activity \((a_{H^+})\) due to the buffer-controlled pH, and the equilibrium constant \(K_e^{KI^+}\) can be determined by fitting the experimental data by the least-squares approximation method. [55]

2.6. Validation

Validation of an analytical method is the process of verifying that it meets the requirements for correct operation within the scope of its application [56]. Method validation includes parameters such as selectivity, sensitivity, precision, and stability.

Selectivity is the ability to show no signal in the presence of interfering ions other than \(K^+\); the study included \(Na^+\), \(Li^+\), \(Ca^{2+}\), and \(Mg^{2+}\) by the separate solution method [57]. A parameter widely used in optodes is the selectivity coefficient \((k_{K_+}^{opt})\), defined as the bias or horizontal distance at “\(1-\alpha=0.5\)” between the sigmoid curves of \(K^+\) and the interfering \(J^+\) [11]. A selectivity coefficient higher than one, i.e. that the \(J^+\) concentration gives a signal equivalent to 10% of \(K^+\), was determined as an acceptance criterion.
The sensitivity is the change in response of the optode to the smallest variation in K⁺, and the limit of detection is the minimum concentration of K⁺ large enough not to be confused with analytical noise [56]. The sensitivity calculation was performed from the standard deviation (SD) of a series of ten replicates (n=10) with a mean (x̄) of a potassium-fortified blank with a value close to the lower limit of the potassium concentration. For BO, a concentration of 10⁻⁶ M K⁺ was used, meanwhile for NO, 10⁻⁴ M K⁺. The criterion for calculating the limit of detection (LOD) was 3 times SD and for the limit of quantification (LOQ) was 10 times SD.

The analytical precision of the method was evaluated with the statistical parameter relative standard deviation (RSD), explained as the difference between the mean and the experimental value of K⁺, expressed as a percentage [58]. Ten replicates (n=10) were performed under the same measurement conditions of three levels of K⁺ for BO and NO. The acceptance criterion for precision was that RSD>RSD_Horwitz, where RSD_Horwitz is the maximum expected deviation for each concentration (C) level expressed as a percentage, this statistic is representative in calibrations with wide concentration ranges [59].

\[
DS = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n}}
\]

\[
RSD = \frac{DS \times 100}{\bar{x}}
\]

RSD_Horwitz \quad RSD_{Horwitz} = 2^{1-0.5 \log C}

Stability was studied as the variability of the signal over time at the same K⁺ concentration. Periodic measurements of the same optode were performed and the percentage recovery was calculated at three K⁺ levels for both optodes. This is done by keeping the optode immersed in the K⁺ solution between measurements and controlling external factors such as humidity, temperature, and external light sources to avoid variability. The acceptance criterion was set at the maximum time that the experimental deviation did not exceed the RSD_Horwitz tolerance.

3. Results and discussion

3.1. Optical properties of the optode and characterization

The UV-Vis spectra were used to quantify K⁺ ions, and the analytical response of the BO and NO showed absorption peaks at 529 nm (Figure 2a and 2b). These peaks correspond to the absorption of the deprotonated form of CHI, while absorption peaks at
615 nm and 667 nm correspond to the protonated form of CHI. Additionally, an isosbestic point was observed at 576 nm in all absorption spectra. These results are consistent with those reported in the literature for CHI [60]. With increasing concentration of K\(^+\), the degree of protonation of the chromoionophore gradually decreases, confirming the cation exchange mechanism.

**Figure 2.** UV-Vis spectra of (a) BO and (b) NO, at different potassium concentrations.

Figure S2 shows the size distribution of Pluronic F-127 micelles (without potassium cocktail) and NO obtained by DLS analysis, with a mean size of 82.2 nm (polydispersity index: 0.216) and 181.7 nm (polydispersity index: 0.196), respectively. The incorporation of potassium cocktail produces greater micelles. Figure S3 displays the
SEM image of the NO micelles, revealing spherical structures. The drying process required for measurement, caused agglomeration of the micelles in the images. The dark areas in the SEM micrographs are the result of the carbon coating used for non-conductive surfaces. The Tyndall effect observed in Figure S4 confirms the presence of a colloidal system formed by the NO micelles dispersed in water.

3.2. Composition of the sensing phase of the optode.

The composition of the active components of the optode is proportional to its analytical performance, increasing the signal obtained and improving sensitivity at low K$^+$ concentrations. In this section the ratios of concentrations of ionophore, ion exchanger and chromoionophore are studied. In preliminary tests, it was inferred that the concentration of the ionophore must be equal to or greater than that of the ion exchanger, which in turn must be equal to or greater than that of the chromoionophore, as expressed by the equation CI ≥ CE ≥ CC. Any deviations from this ratio result in the inability to maintain electroneutrality within the sensing phase.

The sigmoid curve shifted to the right with increasing valinomycin. Figure S5 shows that BO at a ratio of [3:1:1] achieved full protonation (1-α=0) at low K$^+$ and was poorly sensitive in the high K$^+$ range. However, for NO at higher valinomycin ratios, better sensitivity was obtained with a slope that spanned the entire range of K$^+$ (Figure S5a and S5b).

When the K-TCPB ratio was increased, the sigmoid curve shifted to the left, a behavior opposite to that of valinomycin increments. For both optodes at a ratio of [3:3:1], complete deprotonation (1-α=1) was achieved at higher K$^+$, resulting in narrower K$^+$ ranges. Therefore, the [1:1:1] ratio was chosen as the optimal ratio because it had a good slope and covered a wider K$^+$ concentration range.

3.3. Effect of pH

Buffer solutions TRIS-HCl (pKa=8.06) [61] for pH 7.0-9.0 and acetate-acetic (pKa=4.75) [62] for pH 5.0-6.0 were used to study the optode response. The K$^+$ sensing mechanism of the optodes depends on the H$^+$ ion exchange of the medium. The sigmoid curves in Figure S6 show a shift for BO to lower K$^+$ with increasing pH, meanwhile in the case of NO the sigmoid curves for the same buffer do not show much variation and are independent of pH. Therefore, pH 7.0 was chosen as the optimal value with a good response over the whole K$^+$ range for both optodes.
3.4. Selectivity

Selectivity towards K⁺ of sensors was evaluated by measuring the response of the optode in the presence of other interfering alkali and alkaline earth metal ions. Calibration curves were performed for sodium (Na⁺), lithium (Li⁺), calcium (Ca²⁺) and magnesium (Mg²⁺) ions over the complete concentration range and, following the methodologies for BO and NO, measurements were performed in 0–20 mM TRIS buffer at a pH value equal to 7.0.

The results in Figure 3 show the sigmoid curves of the interferents and Table S1 shows the selectivity coefficients for the ions. The sigmoid curves of the interferents for both optodes are well separated from the potassium curve with selectivity coefficients > 1.

These results show that the use of valinomycin as an ionophore provides a good selectivity for the quantification of K⁺ without the need for a pre-treatment. The selectivity coefficient values of NO are higher than those of BO, so it is more selective. The micelle acts as a selective membrane that prevents interferences from entering the optode, so the effect of interferences on NO is less. These results are consistent with the interaction between valinomycin and ions of charge +1, since the ionic radius are similar to that of K⁺. For ions of charge +2, following the higher selectivity coefficients for these ions, probably no stable complexes were formed [47].
Figure 3. Sigmoid curves based on $1-\alpha$ for different interfering ions for (a) BO and (b) NO. The dashed line at $1-\alpha=0.5$ shows the distance between $K^+$ and the interfering ions, sigmoid curves were extrapolated for concentrations higher than 1.0 M.

3.5. Sensitivity

The results for sensitivity of sensors are shown in Table 1, obtaining a standard deviation value ($n=10$) for BO of $SD_{BO} = 1.05 \times 10^{-7}$ and for NO of $SD_{NO} = 1.01 \times 10^{-5}$.

From these data, the sensitivity of the method was calculated, then the limit of detection was estimated, for BO: $LOD_{BO} = 3.15 \times 10^{-7}$ M $K^+$ and for NO: $LOD_{NO} = 3.03 \times 10^{-5}$ M $K^+$, as well as the limit of quantification, for BO: $LOQ_{BO} = 1.05 \times 10^{-6}$ M $K^+$ and for NO: $LOQ_{NO} = 1.01 \times 10^{-4}$ M $K^+$. The optodes showed good accuracy at low concentrations,
for BO ($10^{-6}$ M K$^+$) and NO ($10^{-4}$ M K$^+$), with RSD$_r < 10\%$. In BO, the sensing phase is highly sensitive to variations. Surface factors, such as thickness and roughness, are challenging to control precisely. However, these factors can be resolved in NO, which is dispersed by the formation of micelles.

Table 1. Estimation of LOD and LOQ for BO and NO.

<table>
<thead>
<tr>
<th></th>
<th>Bulk optode</th>
<th>Nano optode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard deviation (SD)</td>
<td>$1.05 \times 10^{-7}$</td>
<td>$1.01 \times 10^{-5}$</td>
</tr>
<tr>
<td>Relative standard deviation (RSD$_r$)</td>
<td>9.52%</td>
<td>9.43%</td>
</tr>
<tr>
<td>Limit of detection LOD = 3 x SD</td>
<td>$3.15 \times 10^{-7}$ M</td>
<td>$3.03 \times 10^{-5}$ M</td>
</tr>
<tr>
<td>Limit of quantification LOQ = 10 x SD</td>
<td>$1.05 \times 10^{-6}$ M</td>
<td>$1.01 \times 10^{-4}$ M</td>
</tr>
</tbody>
</table>

3.6. Precision

In the case of BO, a tendency to increase variability was observed for 1.0 M K$^+$ with RSD$_r = 3.04$ % higher than RSD$_{\text{Horwitz}}$. On the contrary, NO showed values of RSD$_r = 1.26$ % for 1.0 M K$^+$, which are lower than RSD$_{\text{Horwitz}}$. The data are presented in Table 2. The different behavior of BO and NO with respect to RSD$_r$ is probably a consequence of the particle size and the extent of the particle-solution contact. The larger contact area between NO and K$^+$ achieves a more homogeneous sensing phase because of both NO and K$^+$ solution are in the same aqueous phase; on the other hand, BO loses the structural stability of PVC-DOS, due to a process of leaching of the plasticizer due to low lipophilicity and constant contact with the aqueous solution. In conclusion, NO is more accurate than BO, with a maximum RSD$_r$ of 1.91 % compared to 5.66 % obtained by BO.

Table 2. Estimated RSD$_r$ (repeatability) at various potassium concentrations and comparison with RSD$_{\text{Horwitz}}$. 
<table>
<thead>
<tr>
<th>Potassium concentration (M)</th>
<th>RSDr Nano-optode (%)</th>
<th>RSDr Bulk optode (%)</th>
<th>RSD Horwitz (%)</th>
<th>Horwitz (%)</th>
</tr>
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<tbody>
<tr>
<td>10^-6</td>
<td>5.66</td>
<td>16.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^-3</td>
<td>2.99</td>
<td>5.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>3.04</td>
<td>2.00</td>
<td></td>
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<tr>
<td>10^-4</td>
<td>5.66</td>
<td>8.00</td>
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<td>10^-2</td>
<td>2.99</td>
<td>4.00</td>
<td></td>
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<tr>
<td>1.0</td>
<td>3.04</td>
<td>2.00</td>
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</table>

3.7. Stability

The stability of the optode signal in contact with K⁺ reached up to 2 hours for BO and up to 48 hours for NO as shown in Figure 4.

In this study, it was found that at concentrations of 10^-6 and 10^-3 M K⁺, there was variability within the estimated tolerances for BO. After 30 minutes, an increase in concentration to 1.0 M K⁺ was observed, which was expressed as an increase in the percentage of K⁺ recovery. Additionally, it was observed that the structural stability of the PVC-DOS polymer decreased after 30 minutes, resulting in its migration to the aqueous phase. This led to a decrease in the organic NO sensing phase, which in turn required a smaller amount of K⁺ to achieve the maximum loading capacity of the micelles and produce a saturated signal.

In comparison, NO reaches up to 48, 24 and 6 hours for concentrations of 10^-4, 10^-2 and 1.0 M K⁺. The higher stability of NO is due to the fact that the use of a surfactant such as Pluronic F-127 improves the equilibrium constant of the valinomycin-K⁺ complex [63]. A higher interaction between valinomycin-K⁺, due to the miniaturization of the sensing phase, is responsible for maintaining the K⁺ output. Consequently, the optical density, optode lifetime and signal stability is better in NO compared to BO.
Figure 4. Optode response versus time at various K⁺ levels for (a) BO and (b) NO, with measurement conditions are 0 20 mM TRIS buffer pH 7.0, circled points exceed RSD<sub>Horwitz</sub>.

3.8. Calibration of K⁺

Figure S7 displays BO and NO optodes after 15 minutes of signal stabilization, with a transition from blue to reddish tones as the K⁺ concentration increased. Therefore, optodes exhibited a color change in response to changes in K⁺ concentration. In addition, it is shown the behavior of the optode under fully protonated (TRIS buffer) and deprotonated (10 mM NaOH) chromoionophore conditions.
The sigmoid calibration was compared with the linear one, the range of \( K^+ \) is narrow in sigmoid model. The results in Figure 5 were performed in triplicate over a range of \( K^+ \) concentrations from \( 10^{-6} \) to 1.0 M for BO and \( 10^{-4} \) to 1.0 M for NO. The values for both calibration models were calculated according to Equation 1 using the absorbance at 667 nm with solutions of 20 mM TRIS pH 7.0 (A_{HC^+}) and 10 mM NaOH (A_C). The \( K_e^{KI^+} \) was calculated from data and a Log Ke of -4.47 and -5.87 were obtained for BO and NO, respectively (see Equation S1 and S2 in the Supplementary Information).

**Figure 5.** (a) Sigmoidal calibration from Log a\( K^+ \) and 1-\( \alpha \). (b) Linear calibration from Log a\( K^+ \) and Log [\( \alpha/1-\alpha \)], the bars represent the RSD_{Horwitz} for each \( K^+ \) concentration.
Experimental results showed that the sigmoid model loses linearity at the extremes of the K\textsuperscript{+} range, justifying the change to the linear model with a good correlation coefficient (r\textsuperscript{2}>0.99) in all range of K\textsuperscript{+} concentration. The tolerance at each calibration point is given by RSD\textsubscript{Horwitz}.

A literature review on potassium optical sensors reveals various types of optical signals, including colorimetric and fluorescence methods [64] in Table S2. Compared to the method developed here, most optical sensors use valinomycin as a selective agent for K\textsuperscript{+}. This demonstrates high analytical selectivity. In this work, a colorimetric agent was used, specifically CHI, which is related to the exchange of H\textsuperscript{+} between phases as an analytical signal. The use of solvatochromic dyes (SDI - SDII) [65] implies the migration of these dyes out of the sensing membrane, which is a larger molecule compared to H\textsuperscript{+} and more difficult to control the exchange. The use of K-TCPB as an ion exchanger facilitates the migration of K\textsuperscript{+} in both directions of the optode, allowing for analytical reversibility. However, its sodium version achieves the strongest binding between valinomycin - K\textsuperscript{+}, which hinders the reuse of the optode. PVC, as an no polar polymeric membrane support, is inert but can degrade in permanent contact with aqueous solutions. Membranes made of PVC, PVA, or POT can improve physical stability in BO. The signal used for quantifying K\textsuperscript{+} with optodes is absorbance in UV-Vis spectroscopy. However, the use of solid supports such as paper-based optode devices (POD) [66], nylon membrane [40], or agarose [67] makes it incompatible with transmittance measurements. Therefore, reflectance measurement equipment is required [68], fluorescent methods were also reported [69]. Conversion to the linear calibration model allows for the full working range of K\textsuperscript{+} to be utilized, as opposed to the more limited range of 2 or 3 orders of magnitude [70].

4. Conclusions

This study introduces two optical sensors for the quantitative determination of potassium ions (K\textsuperscript{+}): a traditional bulk optode (BO) employing the ionophore valinomycin, and a miniaturized nano-optode (NO) supported by Pluronic F-127 surfactant. The NO micelles were formed through a straightforward ultrasound sonication process. The analyses showed spherical micelles with size to 181.7 nm. The optimal composition for BO and NO in a [1:1:1] ratio, included valinomycin (ionophore), K-TCPB (ion exchanger), and CHI (chromoionophore), respectively. Both optodes exhibited high selectivity for K\textsuperscript{+} over interfering ions, with NO demonstrating superior
selectivity due to the barrier effect of Pluronic F-127. Calibration studies revealed linear responses for both optodes, with BO covering a range of $10^{-6}$ to 1.0 M $K^+$ and NO from $10^{-4}$ to 1.0 M $K^+$. Sensitivity analyses yielded limits of detection (LOD) of $3.15 \times 10^{-7}$ M $K^+$ for BO and $3.03 \times 10^{-5}$ M $K^+$ for NO. Precision studies highlighted differences between BO and NO sensors, with BO showing decreased optical signals at high $K^+$ concentrations, while NO maintained consistent variability across the entire range. Stability assessments demonstrated prolonged signal stability for NO (up to 48 hours) compared to BO (up to 2 hours). Analytically, NO outperformed BO in selectivity coefficients, precision, stability, and independence from pH control during measurements. The unique structural features of NO, facilitated by Pluronic F-127, contribute to its enhanced analytical performance for $K^+$ quantification. The findings indicate that NO exhibits comparable performance to existing sensors, suggesting its potential as a reliable analytical tool for potassium ion determination.

A comprehensive analytical optimization of the $K^+$ quantification process for bulk-optode and nano-optode was conducted, with a particular focus on critical factors such as the ratio between the active components of the optode and the pH values of the aqueous medium associated with the analyzed sample. Conversely, the validation of an analytical method is essential to ensure the reliability of the results, in accordance with the international standards that regulate chemical laboratories [71]. The results obtained from a validated methodology are suitable for decision-making on real samples. Consequently, it is not only essential to develop an accessible and cost-effective analytical method, but it is also crucial to assess the efficacy of the methodology during the analytical validation process.

The combination of these two parameters—optimization and analytical validation—is crucial. Applied in the comparative study of bulk-optodes and nano-optodes is novel and suitable to obtain reliable analytical results from the point of view of selectivity, sensitivity, precision and stability.

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