# Ionophore-based optical sensor for urine creatinine determination

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**Abstract**

Creatinine is a metabolite present in urine samples, which concentration when analysed is used to diagnose and monitor kidney performance. That is why it is needed the development of new sensors that permits it analysis obtaining accuracy results in a short period of time. An optical disposable sensor for monitoring creatinine levels in urine is described. The system, based on a new aryl-substituted calix[4]pyrrole synthetic receptor, has an unusual co-extraction scheme. Due to the low pKa values of creatininium (pKa 4.8), a careful selection of a lipophilic pH indicator that works in acid medium is required. The sensor components were optimized and the new sensor displays a good response time to creatinine (approximately 3 min) over a wide dynamic range (from $1 \cdot 10^{-5}$ to $5 \cdot 10^{-2}$ M). Moreover, the optical selectivity coefficients obtained for creatinine over common cations present in urine meet the requirements for real sample measurements. With a good sensor-to-sensor reproducibility (RSD, 5.1-6.9 % in the middle of the range), this method provides a simple, quick, cost-effective and selective alternative to the conventional methodology based on Jaffé's reaction.

**Keywords:** Creatinine; Sensor; Disposable optical sensor; host-guest chemistry; biofluids

## 1. Introduction

The determination of creatinine in urine and blood is among the most requested clinical tests worldwide. As a metabolic by-product of the muscles that is toxic for the cells, creatinine must be transported by the bloodstream and eliminated through renal filtration. For this reason, the levels of creatinine in blood ($0.4 \cdot 10^{-5}$-$1.1 \cdot 10^{-5}$ M) and urine ($0.3 \cdot 10^{-3}$ - $2.5 \cdot 10^{-3}$ M) are used by doctors to calculate the glomerular filtration rate (GFR), a value that is universally used to diagnose and monitor kidney performance. Thus, at a time when social and demographic changes are sharply increasing kidney-related conditions [1], the development of new tools for the determination of this substance is highly relevant.

The current approaches for the determination of creatinine have some important complications. Jaffe's reaction, which has been (and still is) the most used approach for more than a century, is a kinetic method with significant interferences. The same is true for bioassays, which –either by colorimetric or electrochemical detection– require the use of multiple enzymes and present some interference issues. The gold standard –isotope dilution gas chromatography– is not applicable in routine assays. As a result, studies in laboratories in different countries show disappointing levels of variability with serious medical implications [2].

To overcome some of these issues, alternative colorimetric and electrochemical sensors for creatinine have been proposed, but in most cases they have significant drawbacks when dealing with real samples [3,4]. More recently, an ionophore for creatinine has been reported by some of us, and its application in an ion-selective potentiometric sensor with the ability to quantify creatinine in urine and serum samples was demonstrated [5,6]. The improvements on the limits of detection allow for sample dilution, avoiding the typical matrix interferences of real samples. These results encourage further exploration of the use of this ionophore with alternative detection schemes.

Ionophore-based optical sensors (optodes) rely on the combination of ionophores and indicators present in lipophilic environments in different formats [7]. The selective extraction of a charged analyte along with a reference ion (typically proton) either by ion-exchange, in case of cations, or coextraction, in case of anions, triggers the optical signal. For the most part, molecular absorption (but also molecular emission), changes in the refractive index [8] or colour [9] are used. The signal is usually obtained under equilibrium, although kinetic conditions –with dynamic or exhaustive change [10] approaches– can be used. Most of the optodes developed so far have focused on the detection of inorganic

cations and, to a lesser extent, anions. Very few examples of optodes for organic ions or ionisable organic compounds have been reported and, to the best of our knowledge, none of them have been validated with real samples [11-16]. On the one hand, there are very few selective receptors for this kind of target based on host-guest chemistry [17,18]. Indeed, once integrated in the sensors, the limited selectivity commonly hampers real application in biological fluids, environmental samples, and so forth. On the other hand, a challenge results from the type of signal output, i.e. the availability of indicators capable of protonation/deprotonation at suitable pH conditions. Indeed, most of these systems rely on ammonium-based moieties, whose acidity constant dictates the range of pH working conditions. In short, the detection of organic cations with this type of indicator in acidic media is a highly challenging task. In the case of creatinine, for example, the low pKa (4.8) requires working in very unusual conditions when considering the typical ion-exchange scheme for cations. At present, no such system has been characterized and reported to work with real samples.

Therefore, considering the importance of the determination of creatinine, we anticipated that the use of the novel ionophore in a system with optical detection would be highly significant. This work proposes the use of the new synthetic receptor for creatinine as ionophore in an optode. The results show the feasibility of building a new disposable device for the determination of creatinine in urine. Optimization of the analytical parameters and the signal treatment allows for the detection of creatinine with excellent sensitivity and limits of detection.

## 2. Experimental

### 2.1. Reagents and materials

All reagents used were analytical-grade and were obtained from Sigma-Aldrich (Sigma-Aldrich Química S.A., Madrid, Spain). Doubly deionized water (18.2 MΩ cm resistance) was obtained from a Milli-RO 12 plus Milli-Q station (Millipore, Bedford, MA, USA). Aqueous creatinine standard solutions were prepared by proper dilution of a 0.5 M stock solution of creatinine hydrochloride. 1.0 M stock solutions of the following species were also used: $K^+$, $Na^+$, $NH_4^+$ as chlorides. 0.1 M Acetic acid/sodium acetate buffer at different pH values were prepared from acetic acid and NaOH. 0.1 M HCl was used for the activation of the membranes. Poly(vinylchloride) (PVC; high molecular weight), 4′,5′-dibromofluorescein octadecyl ester (ETH7075), o-nitrophenyloctylether (NPOE), potassium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (KTFPB),

tridodecylmethylammonium chloride (TDMAC) and tetrahydrofuran (THF) purchased from Sigma Aldrich were used to make the sensing film. The ionophore calix[4]pyrrole for creatinine was synthesized in our labs [5].

### 2.2. Instruments and software

Sensing membranes were prepared by spin-coating using WS-400Bz-6NNP/LITE equipment from Laurell Technologies Corporation (PA, USA). The characterization of the creatinine-selective sensing membranes was done with a Hewlett Packard diode array spectrophotometer (model 8453; Norwalk, CT, USA). The film thickness of membranes was measured with the Dektak XT™ Stimulus Surface Profiling System contact profilometer (Bruker Corporation, Coventry, UK).

### 2.3. Membrane preparation

The membrane cocktail was prepared from a batch of 31.9 mg of PVC (31.5 wt. %), 63.9 mg (63.0 wt. %) NPOE, 3.2 mg (3.2 wt. %) of ionophore, 0.9 mg (0.9 wt. %) of ETH7075, and 1.5 mg (1.5 wt. %) of KTFPB all dissolved in 1 mL of freshly distilled THF. KTFPB was selected because is a non-competitive counter-ion for the creatininium cation [5]

The sensing membranes were casted by dropping 15 μL of cocktail on a 14 mm x 40 mm x 0.5 mm thick polyester sheet on the spin-coating device spinning at 190 rpm. Then, the membranes were dried in THF atmosphere. The sensing layer appears as a solid and homogeneous yellow circular film 10 mm diameter and 4 μm thick and contains 37.1 mmol·kg$^{-1}$ in ionophore, 12.4 mmol·kg$^{-1}$ lipophilic pH indicator and 17.4 mmol·kg$^{-1}$ lipophilic salt. After these membranes are dried they are ready to be used.

### 2.4. Measurement setup

The membrane is first activated by immersion in a 0.1 M HCl solution for 3 min, acquiring a yellow colour (due to the positively charged species of ETH7075) and then is immersed in a 0.01 M acetic acid/acetate buffer at pH 3.8 for 3 min. After this step, the absorbance ($A_{H_2I^+}$) at 455 nm is measured. Thereafter, the membrane is equilibrated for 3 min with standards or sample solution containing creatinine buffered at pH 3.8 and the absorbance ($A_x$) at the same wavelength is measured again. Finally, the membrane is introduced in 0.05 M creatinine solution for 3 min to obtain the limiting absorbance ($A_{HI}$) of the neutral form (orange colour) of the indicator. All these measurements were carried out at room temperature.

The extent of creatinine recognition is measured by the degree of protonation of the pH indicator (1-α). Since the indicator can exist in three forms ($H_2I^+$, HI and $I^-$) but the recognition process only involves the positively charged ($H_2I^+$) and neutral (HI) forms, an experimental value (1-$\alpha_{eff}$) was used instead. Under the working conditions (pH 3.8), 1-$\alpha_{eff}$ can be calculated from the absorbance values measured at 455 nm of membranes equilibrated in buffer ($A_{H_2I^+}$), in $5 \cdot 10^{-2}$ M creatinine ($A_{HI}$) solution and in creatinine containing the problem or standard ($A_x$) according to eq. 1.

$$1 - \alpha_{\text{eff}} = \frac{A_x - A_{HI}}{A_{H_2I^+} - A_{HI}} \qquad \text{(equation 1)}$$

*2.5. Analysis of real samples*

Urine samples were obtained from healthy volunteers and were filtered before analysis. In order to evaluate the interferences and the matrix effects, samples were diluted at 1:50; 1:100; 1:150 and 1:200 ratios with the working buffer solution and measured in triplicate, selecting 1:150 dilution for creatinine determination in urine. The creatinine level predicted from the optode membrane was compared to that of Jaffé's method used as a reference [19].

## 3. Results and Discussion

The sensing mechanism of this system is based on a host-guest interaction, where the creatininium ion is recognized by the ionophore present in the membrane and –to preserve the electroneutrality– a proton is exchanged with the lipophilic pH indicator (HI), producing a change on the absorption spectrum. Thus, HI acts as an optical transducer of the recognition event. Since the pKa of creatinine is around 4.8 [20,21], the pH of the solution must be well below this value to maximize the concentration of the creatininium ion. Therefore, beyond the lipophilicity, the pH indicator must also have a pKa value low enough to work under these acidity conditions. For this reason, ETH7075 was selected as indicator, since it can show both acidic and basic properties [22,23] and has lower $pK_a$ value than other lipophilic pH indicators [24]. With the incorporation of ionic sites of different charges in the membrane, such as tetraphenyl borate or quaternary ammonium salts, positively charged $H_2I^+$ (yellow colour membrane) or negatively charged $I^-$ species of the indicator (pink colour membrane) can be generated (Fig. 1). In this work, the inclusion of tetraphenyl borate salt in the membrane makes the equilibrium between $H_2I^+$ and HI (yellow and orange membrane colour, respectively) possible.

Figure 1

Assuming that the membrane contains (indicated as barred species) the ionophore (L), the pH indicator ($H_2I^+$) and the lipophilic anion ($R^-$), the reaction with creatininium ions ($HC^+$) in solution proceeds to form the $HCL_p^+$ complex with p stoichiometry, as represented in eq. 2:

$$\overline{pL} + \overline{H_2I^+} + \overline{R^-} + HC^+ \leftrightarrow \overline{HI} + \overline{HCL_p^+} + \overline{R^-} + H^+ \qquad \text{(equation 2)}$$
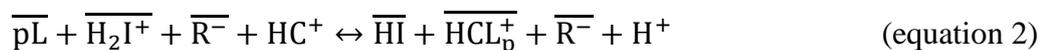
The experimental parameter used to describe the behaviour of the sensor is the deprotonation degree $\alpha$ ($[HI]/I_C$) measured by the normalized absorbance measured at 455 nm. Through an ion-exchange constant $K_{exch}$, this 1-$\alpha_{eff}$ experimental value is related to the analytical concentrations of ionophore $C_L$, pH indicator $C_I$, and lipophilic salt $C_R$, as well as the activities of creatininium and $H^+$ in aqueous medium according to eq. 3 [8].

$$K_{exch} = \frac{1}{a_{HC^+}} \left( \frac{a_{H^+} \cdot \alpha_{eff}}{1-\alpha_{eff}} \right) \frac{C_R - (1-\alpha_{eff})C_I}{(C_L - p(C_R - (1-\alpha_{eff})C_I))^p} \qquad \text{(equation 3)}$$

The ionophore used in this work is an aryl-substituted monophosphonate-bridged calix[4]pyrrole (L), recently reported by some of us as a creatininium receptor. It includes a functionalized aromatic polar cavity that allows for multiple host-guest interactions with a creatininium ion based on hydrogen-bonding, CH–$\pi$, $\pi$–$\pi$, and hydrophobic interactions resulting in a good fit considering the size and bond complementarity [5,6].

### 3.1. Optimization of the response

### 3.1.1. Membrane optimization

An ionophore:lipophilic salt molar ratio (L:$R^-$ = 3:1) previously established in the study of the potentiometric sensor [6] was initially used. First, the influence of the pH indicator (HI) was studied. Membrane cocktails with L:HI:$R^-$ molar ratio 3:1:1, 3:2:1 and 3:3:1 were evaluated. The results show that an excess HI:$R^-$ ratio reduces the linear range, although the limit of detection (LOD) remains constant around $6 \cdot 10^{-5}$ M (Fig. 2).

Figure 2

After this, different lipophilic salt molar ratios, namely: 3:1:1, 3:1:1.25, 3:1:1.5 and 3:1:1.75 (L:HI:$R^-$) were tested (Fig. S1-S5). A 3:1:1.5 molar ratio gives the best results in terms of LOD ($1 \cdot 10^{-5}$ M) compared to the other compositions.

In this way, the optimized sensing membrane was prepared from a cocktail including 3.2 mg of ionophore, 1.5 mg of KTFPB, 0.9 mg of ETH 7075, 63.9 mg NPOE, and 31.9 mg of PVC in 1 mL of freshly distilled THF. The circular membrane prepared by spin-coating at 190 rpm is 4 µm thick across the membrane surface (Fig. S8).

### 3.1.2. Reaction parameters

One characteristic of ionophore-based optical sensors that include a pH indicator in the membrane is pH cross-sensitivity due to cations with a competitive ion-exchange process. In this work, the pH must be low enough to displace the equilibrium towards the generation of the creatininium ion. Thus, to study the influence of the pH, two experiments were performed using membranes equilibrated in a set of 0.01 M acetic acid/acetate buffers of different pH containing a constant concentration of 0.1 mM creatinine in one case and without it in the other. In the absence of creatinine (Fig. S6B), the absorbance at 455 nm corresponding to $H_2I^+$ species is constant until a pH around 4, and then decreases by deprotonation. In the presence of creatinine (Fig. S6A), the absorbance decreases until 4.25 and remains constant thereafter. A pH=3.8 was chosen as the optimum value, since the indicator is still completely in its cationic form and most of the creatinine is protonated (creatininium fraction at this pH of 90.9%, see Fig. S6C). Thus, at this pH, the signal only depends on the presence of creatininium.

The nearly constant value of absorbance from pH 4.25 onwards in the presence of creatinine can be explained by the combination of two opposite effects, the reduction of the ion-exchange due to the reduction in the amount of creatininium present and the displacement of the pH indicator equilibrium [22,23]. The selected pH (3.8) is in good agreement with the working pH used for the potentiometric sensor based on the same ionophore [6].

In order to evaluate the stoichiometry of ionophore:creatinine, the fit of experimental data with equation 2 for stoichiometric index p of 0.5, 1 and 2 was studied. The effect of the stoichiometry on the theoretical response curve is small, except for p = 2, hindering a clear distinction between 0.5 and 1 stoichiometric index (Fig. S7). Additionally, the summation of residual squares (srs) for the adjustment of experimental data to each theoretical model (eq. 3) (p:1; srs: 0.44; p:2; srs: 0.85; p:0.5; srs: 0.42) does not help to solve this issue. This difficulty evaluating the stoichiometry for the ionophore:creatinine complex has also appeared when studying the potentiometric sensor for creatinine based on the same ionophore [6]. The fit by least-squares of the experimental data in the maximum

slope zone of the response curve makes it possible to calculate a value of $0.7\pm0.3$ for $K_{exch}$, which suggests a 1:1 stoichiometry.

### 3.2. Analytical characterization

The response of the ionophore-based optical sensors has the usual sigmoidal shape adjusting the data set to a Boltzmann (eq 4). The fit of the experimental data to the Boltzmann equation provides a $R^2 = 0.987$, as shown in Figure 3. The calculated limit of detection is $1\cdot10^{-5}$ M, obtained as the intersection of the linear calibration function adjusted in the maximum slope zone and a linear function adjusted in the minimum slope zone at low analyte concentration (Table 1).

$$1 - \alpha_{eff} = \frac{A_1 - A_2}{1 + e^{\frac{\log[creatinine] - A_3}{A_4}}} + A_2 \qquad \text{Equation 4}$$

Figure 3

The reproducibility of the measurement was evaluated at two different creatinine concentrations, located in the maximum slope zone of the calibration function, $5\cdot10^{-4}$ M and $5\cdot10^{-5}$ M. Coefficients of variation of 5.1 and 6.9% (n=5) were obtained, respectively.

Table 1

The influence of different interfering species typically found in urine was studied using the separate solutions method. This method is typically used to study selectivity in this type of sensors [8] and it is carried out obtaining a calibration for each interferings considered (Figure S10), obtaining the $K_{exch}$ for each one. Finally, equation S1 is applied to obtain the Log $K_{C, j}$ for creatinine and the considered interfering, being this value a measurement of the difference between the $K_{exch}$ of the analyte and the interfering. Seven different interfering solutions containing different concentration of interfering species from $1\cdot10^{-6}$ to 0.1 M. The $K_{exch}$ calculated for interferents (Table 2) are, at least, three orders of magnitude lower than that obtained for creatinine (0.7). This indicates that the selectivity coefficient obtained by the separate solution method in terms of log $K_{C,j}$ is always lower than -3. Finally, considering that the maximum tolerable error ($P_{IJ}$) in urine is around 10% [25], the required selectivity coefficient for 1, 10 and 100% was calculated (Table 2). In the case of $K^+$ and $Na^+$, the error is under 10% and in case of $NH_4^+$ and creatine it is even below 1%. The selectivity coefficient found for $K^+$ and $Na^+$ are similar to the ones found by the same ionophore in an ISE [6], but in the case of $NH_4^+$ and creatine,

the selectivity coefficients are better when using the same ionophore included in an optode.

Additionally, we studied the potential interference of other compounds usually present in urine as are aspartic and glutamic acids. In both cases we found no answer at their maximum physiological value in urine ($1-\alpha_{eff} = 0.91$ for $2 \cdot 10^{-3}$ M in aspartic acid and $1-\alpha_{eff} = 0.99$ for $2 \cdot 10^{-3}$ M in glutamic acid). We attribute the selectivity of the sensing membrane towards creatinine due to both the calix[4]pyrrole ionophore and the permselectivity of the membrane by including an anionic lipophilic salt. At the working pH both aspartic and glutamic acids have negative net charge.

A comparison table of recently developed creatinine determination methods (see Table S1), shows some analytical parameter, as well as type of measurement and recognition. We observe that the detection limit and dynamic range are similar to other methods previously published, that are mainly electrochemical methods, and in terms of response time, permits a faster analysis than other optical methods.

Table 2

### 3.3. Analysis of real samples

The direct analysis of urine samples is affected by unspecific interferences. Thus, considering the good sensitivity of the proposed sensor, the influence of the dilution factor on the prediction of creatinine was studied. Optimum values for 1:150 dilution factors were found (Table S2), since lower dilutions (1:50 and 1:100) affect the predicted values due to the matrix effect and higher dilution (1:200) makes the creatinine concentration too low and out of the dynamic range of the sensor. Analysis of real urine samples yields recovery values from 81 to 112% (Table 3). High creatinine concentration values were tested by spiking samples at 15 mM level, obtaining recoveries in the range of 85 to 113% (Table S3).

Table 3

**Conclusion**

This work reports on the implementation of a calix[4]pyrrole ionophore for the optical detection of creatinine in disposable format, being one of the first creatinine optical sensing membranes based on a ionophore-chromoionophore scheme The sensing membrane components as well as the working pH were first optimized to establish a suitable analytical performance. The sensor selectivity was assessed considering the common cations and species present in urine and the obtained coefficients allowed for a

maximum tolerable error between 1 and 10%. Based on the results in buffer, real sample measurements were achieved by proper dilution, making suitable recovery for biofluid detection possible. With the development of low-cost optical sensors based on paper and integration with a smartphone reader, the system developed here provides new opportunities as a decentralized analytical platform [26].

# References

1.  Bello, A. K.; Levin, A.; Tonelli, M.; Okpechi, I. G.; Feehally, J.; Harris, D.; Jindal, K.; Salako, B. L.; Rateb, A.; Osman, M. A.; Qarmi, B.; Saab, S.; Lunney, M.; Wiebe, N.; Ye, F.; Johnson, D. W. *Global Kidney Health Atlas: A report by the International Society of Nephrology on the current state of organization and structures for kidney care across the globe.;* International Society of Nephrology, Brussels, Belgium, 2017.

2.  Delanghe, J. R.; Cobbaert, C.; Galteau, M.; Harmoinen, A.; Jansen, R.; Kruse, R.; Laitinen, P.; Thienpont, L. M.; Wuyts, B.; Weykamp, C.; Panteghini, M. Trueness verification of actual creatinine assays in the European market demonstrates a disappointing variability that needs substantial improvement. An international study in the framework of the EC4 creatinine standardization working group. *Clin. Chem. Lab. Med.* **2008,** *46*, 1319.

3.  Buhlmann, P.; Badertscher, M.; Simon, W. Molecular recognition of creatinine. *Tetrahedron* **1993,** *49* (3), 595-598.

4.  Elmosallamy, M. A. F. New potentiometric sensors for creatinine. *Anal. Chim. Acta* **2006,** *564* (2), 253-257.

5.  Guinovart, T.; Hernandez-Alonso, D.; Adriaenssens, L.; Blondeau, P.; Martinez-Belmonte, M.; Rius, F. X.; Andrade, F. J.; Ballester, P. Recognition and Sensing of Creatinine. *Angew. Chem. , Int. Ed.* **2016,** *55* (7), 2435-2440.

6.  Guinovart, T.; Hernandez-Alonso, D.; Adriaenssens, L.; Blondeau, P.; Rius, F. X.; Ballester, P.; Andrade, F. J. Characterization of a new ionophore-based ion-selective electrode for the potentiometric determination of creatinine in urine. *Biosensors Bioelectron.* **2017,** *87*, 587-592.

7.  Xie, X.; Bakker, E. Ion selective optodes: from the bulk to the nanoscale. *Anal. Bioanal. Chem.* **2015,** *407* (14), 3899-3910.

8.  Bakker, E.; Bühlmann, P.; Pretsch, E. Carrier-Based Ion-Selective Electrodes and Bulk Optodes. 1. General Characteristics. *Chem. Rev.* **1997,** *97*, 3083-3132.

9.  Erenas, M. M.; de Orbe-Paya, I.; Capitan-Vallvey, L. F. Surface Modified Thread-Based Microfluidic Analytical Device for Selective Potassium Analysis. *Anal. Chem.* **2016,** *88* (10), 5331-5337.

10. Mistberger, G.; Crespo, G. A.; Bakker, E. Ionophore-based optical sensors. *Annu. Rev. Anal. Chem.* **2014,** *7*, 483-512.

11. Cash, K. J.; Clark, H. A. In vivo histamine optical nanosensors. *Sensors* **2012,** *12*, 11922-11932.

12. Chan, W. H.; Lee, A. W. M.; Wang, K. Design of a primary amine-selective optode membrane based on lipophilic hexaester of calix[6]arene. *Analyst* **1994,** *119*, 2809-2812.

13. Choi, M. M. F.; Wu, X. J.; Li, Y. R. Optode Membrane for Determination of Nicotine via Generation of Its Bromoethane Derivative. *Anal. Chem.* **1999,** *71* (7), 1342-1349.

14. Choi, M. M. F.; Chung, K. O. P.; Wu, X. Nicotine derivative optode membrane with nonactin as ionophore. *Talanta* **2002,** *56* (6), 1027-1038.

15. He, H.; Uray, G.; Wolfbeis, O. S. Enantioselective Optodes. *Anal. Chim. Acta* **1991,** *246*, 251-257.

16. Holy, P.; Morf, W. E.; Seller, K.; Simon, W.; Vigneron, J. P. Enantioselective Optode Membranes with Enantiomer Selectivity for (R)- and (S)-l-Phenylethylammonium Ions. *Helv. Chim. Acta* **1990,** *73* (5), 1171-1181.

17. Bell, T. W.; Hou, Z.; Luo, Y.; Drew, M. G.; Chapoteau, E.; Czech, B. P.; Kumar, A. Detection of creatinine by a designed receptor. *Science* **1995,** *269* (5224), 671.

18. Bühlmann, P.; Simon, W. Neutral hosts for the complexation of creatinine. *Tetrahedron* **1993,** *49* (35), 7627-7636.

19. Pizzolante, J. M. Stabilized alkaline picrate reagent for Jaffe creatinine determination. US4818703A, Apr 4, 1989.

20. Gao, J.; Hu, Y.; Li, S.; Zhang, Y.; Chen, X. Tautomeric equilibrium of creatinine and creatininium cation in aqueous solutions explored by Raman spectroscopy and density functional theory calculations. *Chemical Physics* **2013,** *410* (Supplement C), 81-89.

21. Grzybowski, A. K.; Datta, S. P. 31. The ionisation constant of the protonated form of creatinine. *J. Chem. Soc.* **1964,** (0), 187-196.

22. Mi, Y.; Green, C.; Bakker, E. Polymeric Membrane pH Electrodes Based on Electrically Charged Ionophores. *Anal. Chem.* **1998,** *70* (24), 5252-5258.

23. Mi, Y.; Bakker, E. Lipophilic Ionic Sites for Solvent Polymeric Membrane pH Electrodes Based on 4',5'-Dibromofluorescein Octadecylester as Electrically Charged Carrier. *J. Electrochem. Soc.* **1997,** *144* (2), L27-L28.

24. Qin, Y.; Bakker, E. Quantitive binding constants of H+-selective chromoionophores and anion ionophores in solvent polymeric sensing membranes. *Talanta* **2002,** *58* (5), 909-918.

25. Oesch, U.; Ammann, D.; Simon, W. Ion-selective membrane electrodes for clinical use. *Clin. Chem.* **1986,** *32* (8), 1448.

26. Lopez-Ruiz, N.; Curto, V. F.; Erenas, M. M.; Benito-Lopez, F.; Diamond, D.; Palma, A. J.; Capitan-Vallvey, L. F. Smartphone-Based Simultaneous pH and Nitrite Colorimetric Determination for Paper Microfluidic Devices. *Anal. Chem.* **2014,** *86* (19), 9554-9562.

Table 1. Analytical parameters of the sensing membrane for creatinine determination.

| Analytical Parameters | |
|:---:|:---:|
| Boltzmann equation | |
| $1 - \alpha_{eff} = \dfrac{A_1 - A_2}{1 + e^{\frac{\log[Creatinine] - A_3}{A_4}}} + A_2$ | |
| $A_1$ | 0.996 |
| $A_2$ | 0.030 |
| $A_3$ | -3.990 |
| $A_4$ | 0.457 |
| $R^2$ | 0.986 |
| Limit of detection | $1 \cdot 10^{-5}$ M |
| Dynamic range | $1 \cdot 10^{-5} - 5 \cdot 10^{-2}$ M |
| Intermembrane precision (n=5) | |
| $5 \times 10^{-4}$ M | 5.1% |
| $5 \times 10^{-5}$ M | 6.9% |

Table 2. $K_{exch}$ selectivity as log $K_{Creatinine,j}$ calculated using the separated solution method and $P_{Creatinine,j}$ required for the interferents present in urine at different tolerable errors (1, 10 and 100%).

| Interfering | $K_{exch}$ | Log $K_{Creatinine, j}$ | Required $P_{Creatinine,j}$ 1% | Required $P_{Creatinine,j}$ 10% | Required $P_{Creatinine,j}$ 100% |
|---|---|---|---|---|---|
| $K^+$ | $7.0 \cdot 10^{-4}$ | -3.0 | -3.3 | -2.3 | -1.3 |
| $Na^+$ | $2.5 \cdot 10^{-4}$ | -3.4 | -3.6 | -2.6 | -1.6 |
| $NH_4^+$ | $2.9 \cdot 10^{-5}$ | -4.4 | -2.6 | -1.6 | -0.6 |
| Creatine | $2.9 \cdot 10^{-6}$ | -5.4 | --- | --- | --- |

Table 3. Determination of creatinine in urine samples using the sensor and reference method (n=3).

| Sample | Sensor mM | Reference mM | Recovery (%) |
|---|---|---|---|
| 1 | 6.3±0.2 | 5.5±0.2 | 114 |
| 2 | 6.1±0.3 | 7.5±0.3 | 81 |
| 3 | 1.9±0.3 | 1.7±0.1 | 112 |
| 4 | 4.5±0.4 | 4.1±0.1 | 110 |

**Figure captions**

**Figure 1.** Absorption spectra of PVC-NPOE membranes containing 4′,5′-dibromofluorescein octadecyl ester (ETH7075). A: Membrane with Ionophore (3.2 wt. %) and KTFPB (1.5 wt. %) after equilibration with 0.1 M HCl; B: Same membrane after equilibration with 50 mM creatinine in pH 3.8 buffer; C: Membrane with ionophore (3.2 wt. %) and TDMAC (0.9 wt. %) after equilibration with 0.1 M NaOH.

**Figure 2**. Theoretical model and experimental data (n = 3) obtained using different molar ratios of L:HI:R$^-$: 3:1:1 (red data), 3:2:1 (green data) and 3:3:1 (blue data).

**Figure 3.** Calibration function of creatinine sensing membrane (n = 3).
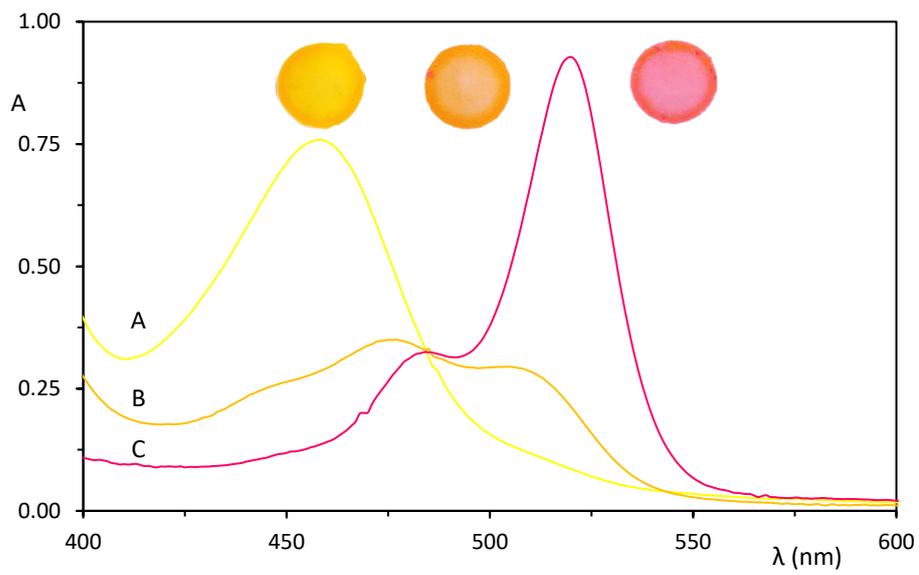
Figure 1

Figure 2

Figure 3