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# Serum/plasma potassium monitoring using potentiometric point-of-care microanalyzers with improved ion selective electrodes

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ABSTRACT

Different causes can trigger imbalances on homeostatic mechanisms between intracellular and extracellular compartments resulting in abnormal blood potassium concentrations (hypo or hyperkalemia). This can lead to serious consequences, even a life-threatening situation. Early diagnosis, treatment and follow-up are essential to minimize critical impacts in patients. Bedside determination of blood potassium is not accessible in all health care centers or in all emergency departments and far less common in this kind of centers in emerging countries. We have therefore proposed a portable, economic and long-lifetime potentiometric point-of-care (POC) analytical microsystem to deal with this question. It is a continuous flow microfluidic platform, made of cyclic olefin copolymer (COC), which combines microfluidics and a detection system based on the potentiometric technique containing a potassium selective electrode with a novel composition of polymeric membrane, which improves lifetime. Its size is smaller than a credit card and shows a linear range of Nernst calibration equation from 1 to 26 mM $K^+$, a detection limit of 0.16 mM $K^+$, a satisfactory repeatability and reproducibility, and an analysis frequency of 20 samples $h^{-1}$, requiring only 25 μL as sample volume. Moreover, lifetime is as long as 9 months by intensive use. All these features comply with medical requirements. Human serum samples were analyzed with the developed device and the obtained results were compared with those provided by two methods: ICP-OES and another using ion selective electrodes. No significant differences were observed, demonstrating the suitability of the developed POC microanalyzer for bedside health applications.

## 1. Introduction

Potassium ion plays an important role in cell activity. Its regulation between intracellular and extracellular compartments relies on several homeostatic mechanisms [1]. Due to different causes, such as kidney dysfunction, metabolic disorders, intake of some medicaments, dehydration and type 1 diabetes among others, these mechanisms are altered, causing abnormal blood potassium concentrations called hypokalemia (<3.5 mM $K^+$) or hyperkalemia (>5 mM $K^+$) [1–3]. In general, light hypokalemia is well tolerated in healthy people, but when it becomes more severe, it can cause generalized weakness, lassitude, constipation, muscle necrosis and paralysis, being life-threatening in patients with cardiovascular disease [2]. Otherwise, manifestations of hyperkalemia include diarrhea, abdominal pain, myalgia, flaccid paralysis of extremities and, even, fatal arrhythmias when severe cases [1]. Rapid and effective treatments are readily available in order to increase or decrease blood potassium concentration such as drugs administration or hemodialysis, respectively. The faster the diagnosis and treatment, the lesser the irreversible or life-threatening consequences for patients.

There are several clinical equipment to determine potassium ion mainly in serum or plasma samples such as ion chromatography (IC), flame photometry, inductively coupled plasma optical emission spectrometry or mass spectrometry (ICP-OES or ICP-MS), or potentiometry using ion selective electrodes (ISE) [4–10]. However, despite these analytical techniques fulfill some of the conditions to take adequate diagnosis in a clinical laboratory environment, they show some constraints such as long turnaround time (TAT), lack of portability, high cost, need of skilled professionals, limited working range or strict sample pretreatment requirements. These drawbacks restrict the possibility of blood potassium control to diagnose or follow-up illness related to hyper/hypokalemia cases out of hospitals or for bedside monitoring in urgent episodes in emergency departments. In addition, their high cost makes them inaccessible for implementation in health centers in emerging countries. Taking all these reasons into account, robust, simple, economic, miniaturized and automatic point-of-care (POC) devices with short TAT, low consumption of sample and reagent, and low waste

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generation are required to permit accurate bedside potassium determination in serum, plasma or even in whole blood, besides of conventional routine analysis out of reference hospitals.

There are some POC analyzers or similar approaches on the market, which overcome some of the mentioned limitations. They are mainly based on colorimetric and potentiometric techniques [11–17]. However, currently they show some weaknesses such as high equipment cost, insufficient portability, high energy consumption, too large sample and reagents volume consumption, non-optimal level of precision and accuracy (only valid for screening procedures), still too long analysis time, need for frequent recalibration processes or short working linear ranges.

In order to solve some of these drawbacks and fulfill the required features, in this work a robust, economic, miniaturized, automated and user friendly potentiometric continuous flow POC analytical microsystem, made of cyclic olefin copolymer (COC), is developed for the monitoring of serum/plasma potassium concentration. The developed POC device has smaller dimensions than a credit card and integrates microfluidics and detection system in a single substrate.

## 2. Experimental

### 2.1. Materials and reagents

Different grades and thicknesses of COC layers, obtained from Tekni-Plex (Erembodegem, Belgium), were used to fabricate the miniaturized POC device: 8007 and 6013 foils of 25 and 400 μm thick, respectively. As a conductive support for the ion selective electrode (ISE) polymeric membrane, a carbon ink (ELECTRODAG PF-407A, Acheson, France) was used. A screen-printable Ag/AgCl paste (C2030812P3, Gwent, Pontypool, UK) was used for the Ag/AgCl reference electrode fabrication. Valinomycin, Bis(2-ethylhexyl) sebacate (DOS), potassium tetrakis(4-chlorophenyl)borate (KTClPB), polyvinyl chloride (PVC) and tetrahydrofuran (THF), all from Sigma Aldrich (Germany), and a poly(butylene sebacate) (PBS) Paraplex G-25 100%, from Hallstar (Illinois, USA), were used to prepare the potassium polymeric membrane.

All reagents used for the development and characterization of the POC analytical microsystem were obtained from Sigma Aldrich (Germany) being of analytical grade. Potassium standard solutions were prepared by successive dilutions from a stock solution of 100 mM $KNO_3$. In order to set at a constant value the reference electrode potential, a 100 mM KCl solution was prepared. A solution simulating a serum/plasma matrix [18] was used as a conditioning solution to keep pH and ionic strength buffered. It consists of 1.3 mM sodium dibasic phosphate ($Na_2HPO_4$), 29 mM sodium hydrogen carbonate ($NaHCO_3$), 150 mM NaCl and 0.03 mM $KNO_3$ set to pH 7.4 with hydrochloric acid.

Reference certified materials (calibrators and bovine and human serum controls) and lyophilized human serum samples for POC microanalyzer validation were provided by Biosystems S.A (Barcelona, Spain).

### 2.2. Fabrication of the POC microanalyzer

The POC microanalyzer was fabricated following a multilayer microfabrication procedure described in detail elsewhere [19–21]. It is based on the thermal lamination under pressure of different microstructured COC layers presenting two different glass transition temperatures (Tg): COC 8007 foils with a Tg of 75 °C used as sealing layers and blocks of two previously laminated COC 5013 layers with a Tg of 130 °C, (a sealing foil is used between them), used as structural layers containing all the micromachined structures of the device. Microanalyzer design, structures machining, electrodes integration and final lamination are the main steps carried out to fabricate the POC microanalyzer.

CAD-type software was used for designing the POC microanalyzer structure. It is composed of three parts (Fig. 1-A): a block (a) that contains the liquid inlet and outlet ports facing up and the reference electrode facing down, a block (b) containing the microfluidic structures (a bas-relief micromixer and detection channel, where a cavity for the ISE membrane deposition is further perforated) and a block (c) containing the conductive support of the ISE. Microchannels dimensions are 0.4 mm × 0.3 mm (W x D), detection channel dimensions, where the potassium selective electrode is integrated, are 2 mm × 0.3 mm (W x D) and the electrode cavity dimensions are 1.8 mm diameter. Final microanalyzer dimensions are 40 × 30 × 2.4 mm. Total POC microanalyzer weight and dead volume is 3g and 58 μL, respectively.

A computer controlled Protomat C100/HF micromilling machine (LPKF, Germany) was used for the microfluidic structures machining onto the COC layers.

For the reference electrode, Ag/AgCl paste was screen-printed using the DEK 248 screen-printer machine (DEK, Spain), being cured for 30 min at 80 °C. For the integration of the conductive support for the potassium selective electrode, a carbon ink was used following a previously optimized procedure [22,23]. It involves refilling with ink the bas-relief machined on the COC layer (Fig. 1 A c) and then curing it for 30 min at 80 °C. Immediately, b and c blocks were laminated. In order to complete the ISE, a novel and optimized potassium selective polymeric membrane was prepared containing in 3 mL THF, 2% Valinomycin, 84% PBS, 0.5% KTClPB and 13.5% PVC. The resulting liquid mixture was deposited inside the electrode cavity by successive depositions of 1 μL, every 5 min, until the cavity was filled. This protocol guaranteed the proper membrane formation avoiding bubble entrapment.

In order to obtain a fully sealed monolithic device, a final lamination took place using a temperature-controlled customized press (Francisco Camps, Granollers, Spain) set at 6 atm of pressure and 105 °C.

Lastly, fluidic connectors were set over the corresponding liquid inlet

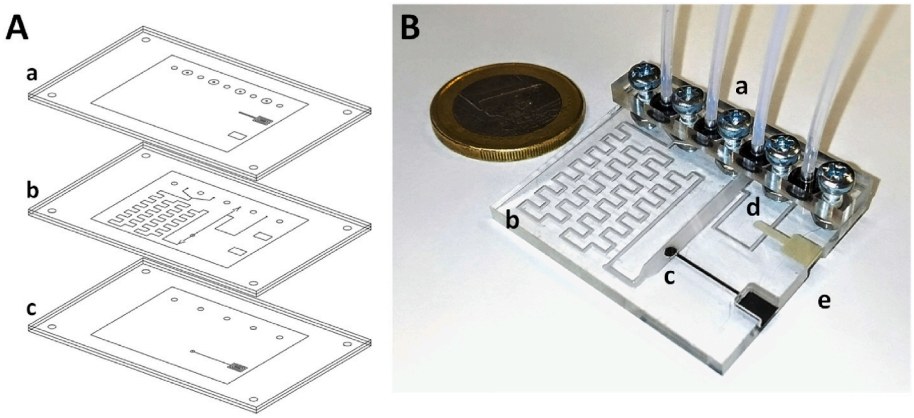


**Fig. 1.** A: Layered device design with top (a), middle (b) and bottom (c) blocks. B: Image of the POC microanalyzer; a) Liquid connections; b) Microfluidics; c) Detection microchannel integrating the ISE; d) Reference electrode; e) Electrical connections.

or outlet ports using a bracket.

### 2.3. Experimental setup

Fig. 2 shows the experimental setup used to optimize and verify the POC microanalyzer proper performance. It consists of a continuous flow setup composed by a 4-channel peristaltic pump (Minipuls 3, Gilson, US) operating with tubing of 1.14 mm internal diameter (Ismatec, Wertheim, Germany) made of Tygon and a Hamilton MVP injection valve (Reno, US). Tubing of 0.8 mm internal diameter (Scharlab, S.L., Sentmenat, Spain) made of Teflon was used to join the POC microanalyzer with all the external elements. A customized and miniaturized potentiometer (6016 4-electrodes, TMI, Barcelona, Spain) was used for the data acquisition and processing. Analytical information was transferred to a personal computer. As a future perspective, a fully automated and (semi) autonomous POC system will be easily implemented by using microvalves and micropumps instead, once the analytical potential of the POC analyzer is proven.

### 2.4. Analysis principle

Microfluidics inside the POC microanalyzer includes three liquid inlet ports (Fig. 2). The channels of the first two input ports converge at a junction point, where the injected samples or standard solutions into the carrier solution get mixed with the conditioning solution. This mixed stream is carried towards the detection channel, where the analyte is detected by the potassium selective electrode, and finally, it exits through the outlet port. With the aim of maintaining the reference electrode potential at a constant value, a 100 mM KCl solution was introduced at 100 μL $min^{-1}$ through the third inlet port flowing through the microchannel, where the reference electrode is located [24].

### 2.5. Samples preparation and comparison methods

With the purpose of validating the correct functioning of the developed POC microanalyzer, 8 reconstituted real human serum samples provided in lyophilized form by Biosystems S.A., and used in interlaboratory quality control studies (PREVECAL - International External Quality Assessment Program), were analyzed. Each sample was reconstituted with MilliQ water and stored in its bottle at 5 °C until analysis.

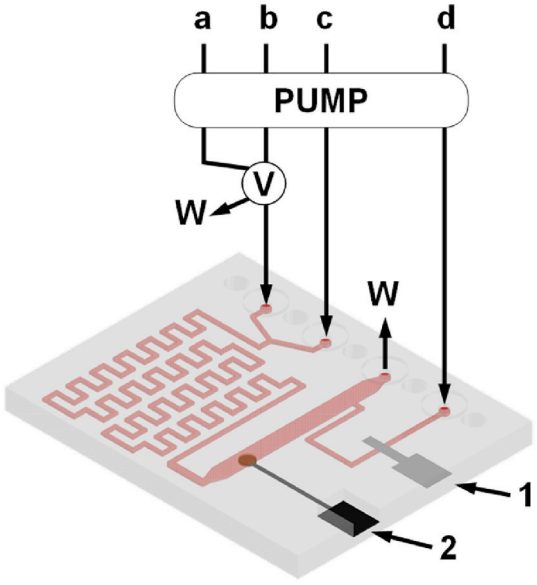


**Fig. 2.** Experimental setup, where: a) sample and standards; b) $H_2O$ as carrier solution; c) conditioning solution set at pH 7.4; d) 0.1 M KCl auxiliary solution; W: waste; V: Hamilton MVP injection valve; 1) reference electrode; 2) ISE.

The samples were analyzed in triplicate with the developed POC device without any previous treatment (filtration or dilution). The obtained results were compared with those got with the ICP-OES reference method (Optima 4300 DV, PerkinElmer Inc.) and those obtained by commercial ISE equipment (BA400 Analyzer, Biosystems S.A.). For the ICP-OES, samples were diluted in 0.05% (w/v) EDTA and 0.5% (v/v) NH3 to fit the concentrations to the linear range of the equipment and measured in triplicate. For the commercial ISE equipment, samples were analyzed in triplicate without any pretreatment.

## 3. Results and discussion

### 3.1. Optimization of the POC microanalyzer

The objective of the presented investigation was the development of an economic, robust, simple, selective and portable POC microanalyzer for serum/plasma potassium monitoring of patients with hypo/hyperkalemia episodes. The selected application requires fulfilling some specific needs, for instance sufficient working range that encompasses the pathological and physiological potassium concentrations, adequate stability of the signal baseline, minimum analysis time, minimum sample and reagents consumption, maximum automation potential, adequate lifetime and ease of use. Considering all this, different variables and parameters related to the POC microanalyzer performance were studied.

In order to favor the electrode cleaning between analyses, the ISE was placed in a straight channel. Dead volume was reduced in the sensor area by a small detection chamber, so the analysis time was diminished around 40% compared to other similar microanalyzers [23–25]. Besides, the likelihood of bubbles retention in the detection chamber was reduced dramatically, improving the robustness of the whole POC microanalyzer.

Lifetime of polymeric membranes used in ISE is an important factor often overlooked but crucial, especially to implement in practice. In most cases, potentiometric devices for measuring potassium ion use Valinomycin as a recognition element and DOS as a plasticizer [22,24,26–29]. This type of polymeric membranes have been extensively studied and show adequate analytical features for the intended applications, in particular for those that do not require intensive use of the electrodes. However, for applications where the ISEs are in wet conditions for long periods of time, their lifetime is considerably reduced, often being less than a couple of weeks. This forces to frequently replace sensors and apply a disposable concept, which increases the price of the equipment considerably and the maintenance needs. There are studies showing that the loss of the initial analytical features of ISEs over time (their sensitivity and selectivity in concrete) is due to the exudation of the components of the selective membrane towards the solutions in contact [26,30,31]. In this process, the plasticizer plays a very important role. Among other functions, it is the compound responsible for dissolving the rest of the membrane components: recognition element, additives and PVC. On the one hand, the stronger the plasticizer lipophilicity, the longer it remains in the polymeric matrix with the rest of the dissolved membrane components, and therefore, the longer the lifetime of the ISEs [32–34]. On the other hand, taking into account that components of polymeric membranes are gradually lost inevitably, the higher the proportion of a component in the sensor membrane, the longer the lifetime of the ISE. Therefore, different compositions of potassium selective PVC-membranes containing two different plasticizers, DOS (commonly used) and PBS (a plasticizer less used in the formulation of ISEs polymeric membranes but much more lipophilic than the previous one), were tested with ad hoc electrodes in batch conditions. Different ratios of other membrane components were tested as well. The different formulations and obtained results are shown in Table 1.

First of all, taking as reference the plasticizer and the proportion commonly used in potassium potentiometric membranes (DOS and 65%, respectively), different proportions of ionophore were evaluated. The

**Table 1**
Formulation and features for the potassium polymeric membranes used with batch electrodes. The amount of additive (KTClPB) is 0.5% in all the membranes. Linear range was from 0.01 to 30 mM. Each value with confidence interval is the mean of 3 replicates.

| Membrane | DOS (%) | PBS (%) | Val.[b] (%) | PVC (%) | Sensitivity (mv/dec.) | LoD (μM) | $\log K^{pot}_{K^+,Na^+}$ [a] | pH range | Lifetime dry (months) | Lifetime wet (months) |
|---|---|---|---|---|---|---|---|---|---|---|
| DOS 1 | 65 | - | 1.0 | 33.5 | 54 ± 4 | 12 ± 8 | −2.7 ± 0.5 | - | - | - |
| DOS 2 | 65 | - | 1.5 | 33.0 | 54 ± 3 | 8 ± 2 | −4.2 ± 0.4 | - | - | - |
| DOS 3 | 65 | - | 2.0 | 32.5 | 54 ± 2 | 2 ± 1 | −4.3 ± 0.1 | 2.5–10.0 | 1 | <1 |
| DOS 4 | 65 | - | 3.0 | 31.5 | 53 ± 2 | 20 ± 30 | −4.3 ± 0.2 | - | - | - |
| DOS 5 | 79 | - | 2.0 | 18.5 | 53 ± 4 | 5 ± 4 | −4.3 ± 0.8 | 2.5–10.0 | 3 | <1 |
| DOS 6 | 84 | - | 2.0 | 13.5 | 55 ± 3 | 3 ± 2 | −4 ± 1 | 2.5–10.0 | 3 | <1 |
| DOS 7 | 90 | - | 2.0 | 7.5 | - | - | - | - | - | - |
| PBS 1 | - | 65 | 2.0 | 32.5 | 52 ± 3 | 4 ± 3 | −4 ± 1 | 2.5–12.0 | - | - |
| PBS 2 | - | 79 | 2.0 | 18.5 | 56 ± 5 | 2 ± 2 | −4.0 ± 0.1 | 2.5–12.0 | >12 | 9 |
| PBS 3 | - | 84 | 2.0 | 13.5 | 56 ± 2 | 2 ± 1 | −4.1 ± 0.3 | 2.5–12.0 | >12 | 9 |
| PBS 4 | - | 90 | 2.0 | 7.5 | - | - | - | - | - | - |

[a] Potentiometric selectivity coefficient calculated with 150 mM $Na^+$.
[b] Val. = Valinomycin.

objective, apart from seeking to lengthen the lifetime of the membrane, was to find a better selectivity against $Na^+$ ion, which is the most interfering ion of the ISE present in the matrix of plasma or serum samples. As it can be seen, the membrane with 2% Valinomycin is the one that provided the best results regarding sensitivity, detection limit, repeatability and selectivity against $Na^+$. It should be noted that the membrane with 1.5% Valinomycin also provided similar results, but taking into account the factor of exudation of the components and the loss of analytical features over time, the highest percentage was chosen as optimal.

Once selected the percentage of ionophore, ISEs with membranes containing DOS or PBS as plasticizer in different proportions (65, 79, 84 and 90%) were evaluated. In this sense, those resulting from a mixture with 90% plasticizer (either DOS or PBS) had an excessively liquid consistency to be used as an ISE membrane. With the rest of the proportions, comparable results were obtained in terms of analytical features. The only appreciable difference was lifetime. In this sense, for membranes with DOS, the higher the plasticizer percentage, the longer the lifetime. In addition, at the same plasticizer percentage, membranes with PBS show much longer lifetimes than their analogs with DOS. This behavior can be seen both in electrodes stored dry and in electrodes immersed in water. In addition, ISEs with PBS have a wider working pH range than those with DOS, although the pH in the application studied is neutral and would not be a limiting factor. Finally, it was observed that ISEs with PBS membranes do not require prior conditioning with the analyte, so freshly prepared ISEs present a stable response from the first use, which is not the case of DOS-based membranes.

In this way, it was demonstrated that the use of a more lipophilic plasticizer, in this case a polyester sebacate (PBS), lengthens the lifetime of the electrodes significantly, presenting adequate analytical features and comparable to those shown by traditionally used potassium electrodes. For this reason, the following polymeric membrane composition was used to be evaluated in ISEs integrated in the microfluidic platform to develop the K+POC microanalyzer: 2% Valinomycin, 84% PBS, 0.5% KTClPB and 13.5% PVC.

Hydrodynamic and chemical variables were studied with a procedure of univariate optimization. Adequate sensitivity, working range and baseline signal stability, maximum analysis frequency and minimum waste generation and, thus minimum sample and reagents consumption were some analytical features taken into account in order to define optimal values for each evaluated parameter. Table 2 collects all the tested variables, showing the studied intervals and the optimal values.

In order to simulate the sample matrix, a conditioning buffer solution similar to serum/plasma and blood in terms of concentration of phosphate, carbonate and sodium (the main interfering compound for the ISE) was used at pH 7.4 [18], obtaining adequate analytical features regarding linear range and sensitivity. As the required time for baseline recovery after each measurement depends on peak height, and they

**Table 2**
Optimization of hydrodynamic and chemical variables.

| Variable | Studied interval | Optimal value |
|---|---|---|
| Conditioning buffer solution: | | |
| $[HPO_4^{2-}]$ (mM) | - | 1.3 |
| $[HCO_3^-]$ (mM) | - | 29 |
| $[Na^+]$ (mM) | - | 150 |
| pH | - | 7.4 |
| $[K^+]_{background}$ (mM) | 0.01–0.05 | 0.03 |
| Injection volume (μL) | 25–250 | 25 |
| Channel flow rate (μL min-1) | 400–1000 | 650 |

turned to be high even with the lower potassium standard tested (1 mM $K^+$), a background of potassium ion was added to the conditioning solution to accelerate this phenomenon and improve response time. Different $KNO_3$ concentrations were evaluated from 0.01 to 0.05 mM $K^+$. A background potassium concentration of 0.03 mM was chosen, reducing analysis time by almost 15% (from 205 to 180 s) but keeping the rest of the analytical features.

Regarding hydrodynamic variables, injection volume of the sample was tested between 25 and 250 μL while flow rate of each stream (conditioning and carrier solutions) was studied between 400 and 1000 μL $min^{-1}$. Flow rate for the 100 mM KCl solution to keep constant the reference electrode potential was fixed at 100 μL $min^{-1}$. Best results were found using a sample injection volume of 25 μL and a flow rate of 650 μL $min^{-1}$ for each stream.

Lastly, in order to verify the interfering effects of the matrix of serum/plasma over the measurement process, a study of the potential interferences was performed. For this new potassium polymeric membrane formulation, the potentiometric selectivity coefficients ($\log K^{pot}_{i,j}$) of the potential interfering cations of Valinomycin ($Na^+$, $Mg^{2+}$ and $Ca^{2+}$) at the expected concentration in blood were calculated, using the fixed interference method [35]. Obtained values for $\log K^{pot}_{i,j}$ were −4.1, −4.8 and −3.2, respectively. Thus, at these concentrations they are not a significant interference. The interference of total proteins (measured as bovine serum albumin) was also evaluated, especially due to its lipophilic nature, obtaining a positive interference less than 5%.

In addition, the interference of different drugs used as medical treatments for different common diseases was evaluated. To do that, a bovine serum containing 3.9 mM $K^+$ was spiked with a concentration of each drug slightly higher than the maximum therapeutic level expected in blood [36]. In this sense, Fig. 3 shows the %RSD for the different drugs individually evaluated compared to the control solution. As it can be seen, in the event that the patient is under treatment with aspirin, a result with an underestimation around 15% could be obtained. With ibuprofen, imipramine and ethosuximide, it could be an underestimation between 5 and 15%. The rest of the evaluated drugs did not cause

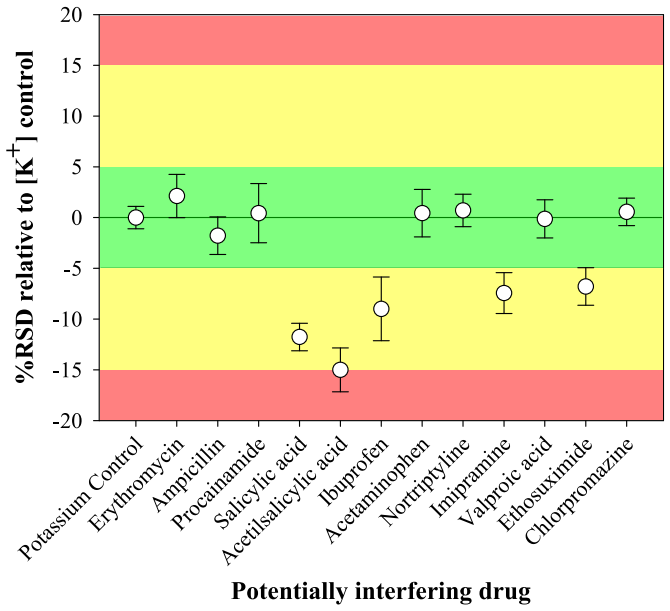


**Fig. 3.** Interference study with common drugs used in medical treatments using a bovine serum matrix. The potassium concentration was 3.9 mM. Green zone: no interfering effect with this drug; Yellow zone: interfering effect that could affect diagnostic decisions; Red zone: high interfering effect. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

significant differences at that high concentrations evaluated. With these results, it would be necessary to take into account whether the patient is taking certain drugs in order to make an appropriate clinical decision based on the potassium values obtained. It is also worth noting that blood samples should be collected in tubes that do not contain dipotassium EDTA as an anticoagulant, since this could lead to a considerable overestimation in the determination of the analyte. In these cases, by hospital protocol, the sample is usually taken in tubes with lithium heparin or disodium EDTA.

### 3.2. Analytical features

Different calibrations using standard solutions of potassium ($KNO_3$) from 1 to 26 mM were carried out to obtain the analytical characteristics of the proposed analytical device. Fig. 4 shows the signal recordings and

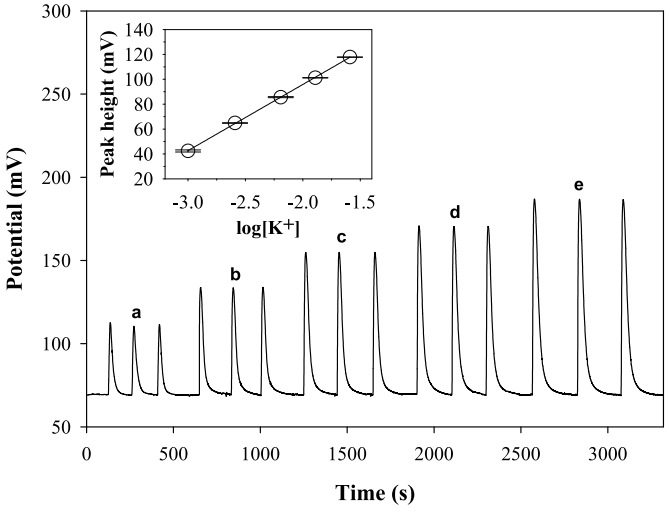


**Fig. 4.** Obtained recorded signal and calibration plot using potassium standard solutions of 1 mM (a), 3 mM (b), 6 mM (c), 13 mM (d) and 26 mM (e).

calibration curve for one calibration experiment. Nernst calibration equation (95% confidence, triplicates for each concentration) was $E = 202\ (\pm2) + 53\ (\pm1)$ log $[K^+]$ and $R^2 = 0.9997$. The working range was found to be from 1 to 26 mM $K^+$. According to IUPAC, the detection limit was 0.16 mM $K^+$ [37]. Repeatability was evaluated through 10 successive injections of two human serum controls of 2.8 and 6.6 mM $K^+$ to evaluate the POC microanalyzer operation at the extremes of the physiological potassium concentrations range in serum, plasma or whole blood. RSD of the peak heights were 2 and 1%, respectively. Control materials and calibrators (both of human and bovine serum) provided by Biosystems S.A. were analyzed to study method accuracy, achieving differences of less than 8%. Reproducibility of the POC microsystem was also evaluated through 8 calibrations carried out along 5 months. Mean slope and intercept obtained values were 54 mV $dec^{-1}$ and 206 mV with RSD values of 3 and 9%, respectively. So the intra and inter-day precision of the POC analytical device was confirmed. As showed previously, the POC microanalyzer lifetime was as long as 9 months in intensive use, being able to analyze 20 samples per hour with a total waste generation of 4 mL per analysis. All these evidences show the high robustness and the great potential of the POC system developed for the proposed application.

### 3.3. Human serum analysis

The developed POC analyzer was applied to the analysis of 8 different real human serum samples. Results are shown in Table 3.

No statistical differences were found from the graphical representation (n = 8; 95% confidence) of the results for the proposed method and the ICP-OES reference method: $[K^+]_{ICP\text{-}OES} = 1.01\ (\pm0.09)\ [K^+]_{POC} + 0.0\ (\pm0.5)$; $R^{2=}$ 0.9876, and in accordance with the paired *t*-test ($t_{calc} = 0.835$, $t_{tab} = 2.365$, $t_{calc} < t_{tab}$). Likewise, no statistical differences were found from the graphical representation (n = 8; 95% confidence) of the results for the proposed method and the commercial ISE: $[K^+]_{ISE} = 0.9\ (\pm0.2)\ [K^+]_{POC} + 0\ (\pm1)$; $R^2 = 0.9118$, and in accordance with the paired *t*-test ($t_{calc} = 0.605$, $t_{tab} = 2.365$, $t_{calc} < t_{tab}$). As it can be seen, the developed POC device provides excellent accuracy, and results are in most cases closer to those obtained by ICP-OES rather than the ones obtained with commercial ISE equipment. On the other hand, the POC confers better precision than the commercial ISE equipment and it is comparable the ICP-OES method.

The reliable obtained results confirm that the developed POC microanalyzer is appropriate for the serum/plasma potassium determination, being cheaper, easier to use, with high portability and wider working range and showing an extended lifetime compared to some other potentiometric microanalyzers previously reported or on the

**Table 3**
Serum potassium concentration values in mM (95% confidence; n = 3) obtained by the three methods.

| Sample | POC developed | ICP-OES | % error POC vs ICP | Commercial ISE | % error POC vs ISE |
|---|---|---|---|---|---|
| 1 | 6.7 ± 0.1 | 6.6 ± 0.1 | 1 | 7 ± 1 | −4 |
| 2 | 6 ± 1 | 6.6 ± 0.1 | −2 | 7 ± 1 | −9 |
| 3 | 3.9 ± 0.1 | 3.9 ± 0.1 | −1 | 4 ± 1 | −3 |
| 4 | 4.9 ± 0.1 | 5.0 ± 0.1 | −1 | 5 ± N/A[a] | −2 |
| 5 | 8.0 ± 0.4 | 7.89 ± 0.04 | 2 | 7 ± 1 | 14 |
| 6 | 3.3 ± 0.1 | 3.4 ± 0.1 | −4 | 3 ± 1 | 10 |
| 7 | 5.7 ± 0.1 | 6.2 ± 0.1 | −7 | 5 ± N/A[a] | 14 |
| 8 | 3.0 ± 0.2 | 2.8 ± 0.1 | 7 | 3 ± 1 | 0 |

[a] N/A: data not available.

market.

## 4. Conclusions

A low cost and miniaturized POC analytical microsystem prototype for serum/plasma potassium determination using potentiometry has been developed, characterized and used to analyze different human serum samples with successful results. It integrates in a single substrate all the microfluidics needed and the potentiometric detection system composed by a polymeric membrane ion selective electrode and a Ag/AgCl reference electrode. The novel polymeric membrane composition optimized lengthens considerably lifetime of the whole POC microanalyzer, making it much more attractive than current commercial potassium analytical devices. In addition, other features have been improved such as precision, repeatability, reproducibility, working range and limit of detection. Regarding consumables and maintenance, as well the consumption of reagents and samples as wastes generation, are very low. It has also a strong potential for automation in continuous mode, while showing a fairly fast analysis time. Furthermore, it is smaller and cost is much lower compared to common clinical laboratory equipment. From the obtained analytical features, it can be ensured that the proposed POC microanalyzer has the great potential for commercialization in clinical applications for plasma potassium bedside determination as well in reference hospitals as in health centers of emerging countries. Next steps are focused on the miniaturization of the whole experimental setup to obtain a more compact design and its validation for whole blood samples analysis.

## Credit author statement

Antonio Calvo-Lopez: Conceptualization, Microsystem design and fabrication, Methodology, Investigation, Formal analysis, Validation and Writing - Original Draft. Eva Arasa-Puig: Investigation, Formal analysis and Writing - Review & Editing. Julian Alonso-Chamarro: Conceptualization, Resources, Writing - Review & Editing, Supervision, Project administration and Funding acquisition. Mar Puyol: Conceptualization, Resources, Writing - Review & Editing, Supervision, Project administration and Funding acquisition.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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