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Development and evaluation of an electrochemical biosensor for creatinine quantification in a drop of whole human blood

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ABSTRACT

An electrochemical biosensor for creatinine determination in a drop of whole human blood was developed and applied to the determination of creatinine in real clinical samples. It is based on the modification of a dual carbon working electrode with a combination of three enzymes: creatinine amidohydrolase (CNN), creatine amidinohydrolase (CRN) and sarcosine oxidase (SOX). Electrochemical transduction is performed using horseradish peroxidase (HRP) and potassium hexacyanoferrate(II) as mediator. A drop of human blood is enough to carry out the measurements by differential chronoamperometry where one carbon electrode detects creatine and the other both creatine and creatinine. The integrated differential signal obtained in the biosensor is linear with the concentration of creatinine in blood in the range 0.5–15 mg/dL and the enzyme-modified electrodes are stable for at least 3 months at 4 °C. The biosensor was lined to a reference method based on Isotope Dilution Mass Spectrometry (IDMS) with 50 real human blood samples and the results compared with those obtained by alternative routine techniques based on Jaffé method and an enzymatic method (Cobas 8000 Roche®, Crep2 Roche®). There were no significant differences between the creatinine concentrations found by the routine techniques and the developed biosensor.

1. Introduction

The measurement of the levels of creatinine in blood or urine is essential in clinical chemistry to evaluate renal, muscular, and thyroidal function. Creatinine is the metabolic end-product of creatine, which provides energy to muscular tissue, and it is eliminated from the organism in urine after glomerular filtration in the kidneys. Typical clinical levels of creatinine in serum are between 0.9 and 1.2 mg/dL but, depending on age and gender, could be marginally lower or higher. Creatinine levels higher than 1.7 mg/dL require clinical investigation and higher than 6 mg/dL indicate significant renal failure. Serum creatinine levels higher than 11.3 mg/dL have been found in certain renal, thyroidal, or muscular disorders. Values lower than 0.5 mg/dL indicate a reduction of muscular mass [1–3].

Routine procedures for the determination of creatinine in the clinical laboratory are usually based on the Jaffé spectrophotometric reaction or another colorimetric enzymatic reaction [2]. Additionally, creatinine can be measured by alternative analytical techniques such as Gas or Liquid Chromatography coupled to Mass Spectrometry. These alternative methods are expensive, time-consuming, require sample pretreatment and qualified personnel [1]. However, they are the basis of reference Isotope Dilution Mass Spectrometry (IDMS) methods employed for the preparation of serum reference materials and for the calibration of those routine techniques employed in the clinical laboratory [4].

Sensor and biosensor systems have been developed for the rapid determination of multitude of parameters in the clinical environment [5], including creatinine. Most creatinine biosensors described so far employ an electrochemical transducer and are based on non-enzymatic [6–11] or enzymatic reactions [2,3,6,9,10,12–17]. These systems provide several advantages over those methods employed in the clinical laboratory: they are simple, accurate and low-cost procedures which can

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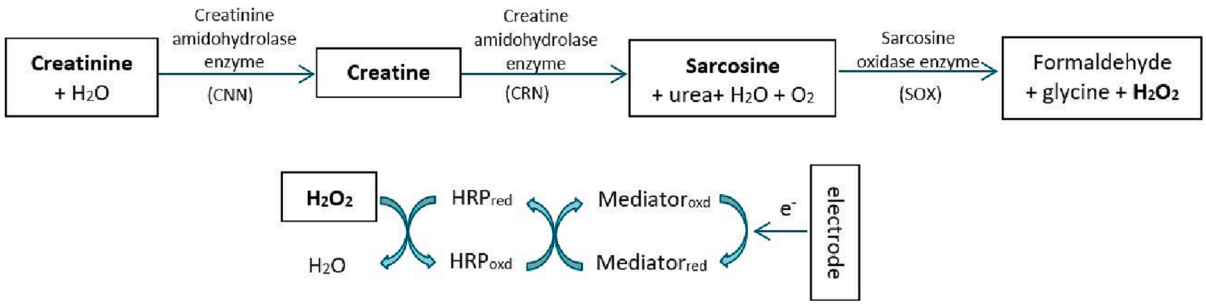

Fig. 1. Enzymatic reactions that take place in the determination of creatinine.

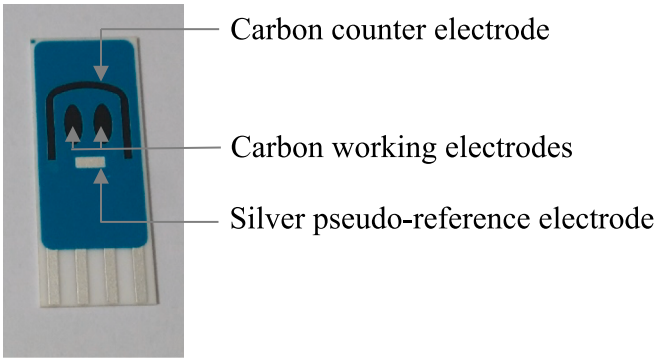

Fig. 2. Screen-printed electrode (SPE), DropSens.

Table 1
Levels found in serum for typical interferences in the determination of creatinine.

	n	Range	Mean	Median	Std. Dev.
Age	50	20–92	57.3	60.5	19.03
Urea (mg/dL)	50	23–381	84.48	67	63.13
Total protein (g/L)	19	32–77	65.37	67	10.08
Triglycerides (mg/dL)	24	71–278	133.3	125	52.7
Total bilirubin (mg/dL)	50	<1–31.3	<1	<1	———

be tailored to provide real-time detection in point-of-care applications [1–3].

The combination of three enzymes: creatinine amidohydrolase (CNN), creatine amidinohydrolase (CRN), and sarcosine oxidase (SOX) is the foundation of a very selective method for the detection of both creatine and creatinine. The first attempt at the clinical application of this enzymatic reaction using an electrochemical cell was published almost 40 years ago [18]. This enzymatic reaction is also the base of the first description of a point-of-care testing (POCT) device (from Radiometer Medical ApS) for creatinine determination in whole blood [19] and it is also the base of the most popular commercial POCT devices existing today [20,21].

Recently, Cánovas *et al* [22] reviewed existing point-of-care platforms for the determination of creatinine. They conclude that biosensors are the most promising alternatives for this task but also indicated that none of the reported sensors offer adequate selectivity in blood samples. Several of the creatinine biosensors commercialized in the last few years as POCT devices have been evaluated in the scientific literature [20,21,23–28]. For example, it is important to evaluate the glomerular filtration rate in patients undergoing injection of contrast media prior to medical imaging [20,21,23,24] and that can be achieved using POCT devices. In general, commercial POCT devices offer adequate performance [20,21] but might suffer from interferences, such as urea or creatine [25]. Several authors reported also problems from bias and

Table 2
Results for the validation of the LC-IDMS procedure.

Day	CreatinineLevel 1 (mg/dL)	CreatinineLevel 3 (mg/dL)
1	0.75	6.81
2	0.73	6.81
3	0.74	6.80
4	0.74	6.75
5	0.74	6.81
6	0.75	6.80
Assigned value	0.771	6.81
Accepted range	0.636–0.907	5.87–7.74

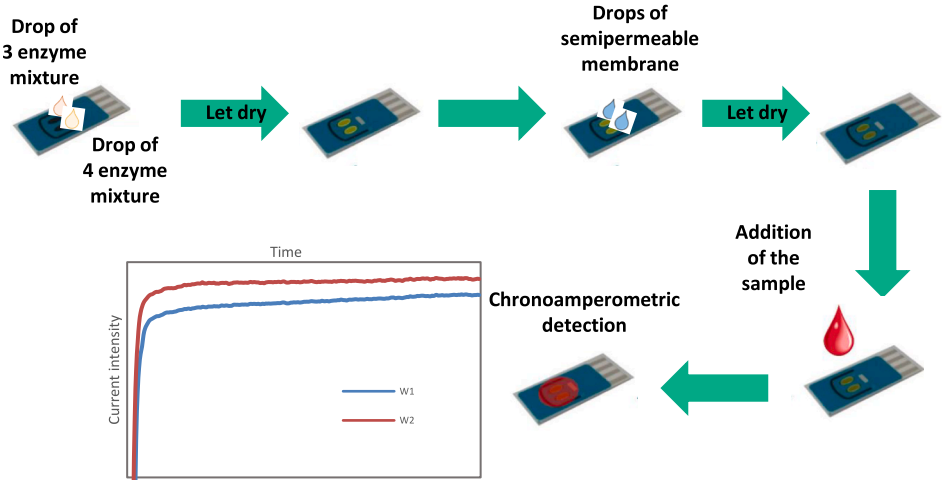

Fig. 3. Scheme of the preparation of test strips and measurement of the blood samples.

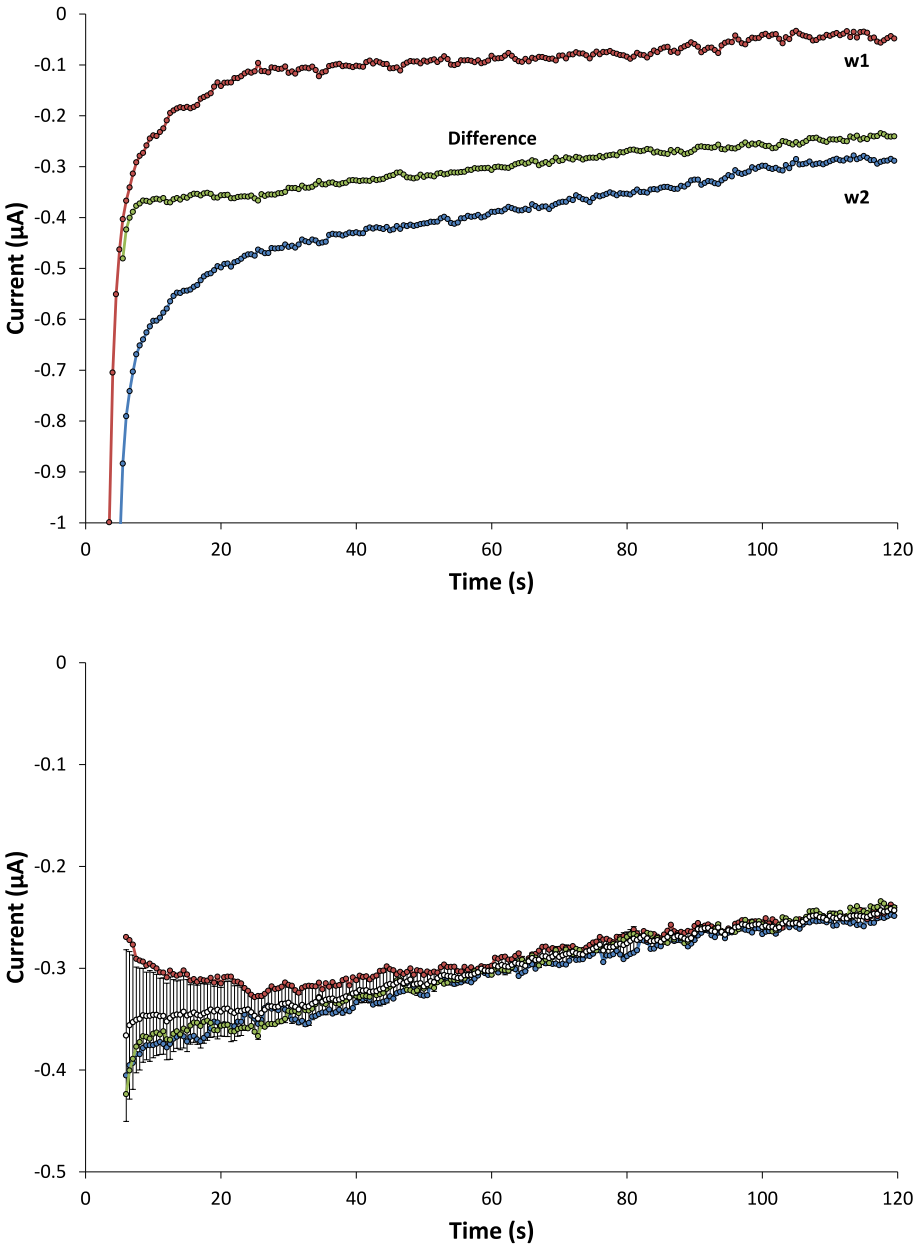

Fig. 4. A) Chronoamperogram obtained for a real human blood sample at w1 and w2 and the measured differences between both electrodes. B) Differences measured for three different electrodes (green, blue and red dots) using the same sample and average of the differences (white dots, error bars correspond to1SD).

imprecision [26–28]. In a recent paper, Dalton *et al* [27] reported that commercial POCT devices require calibration to reference IDMS methods, and this is not always realized [28].

In this work, we have developed a dual-electrode creatinine electrochemical biosensor for whole blood analysis based on the well-known enzymatic reaction shown in Fig. 1. In the first electrode, CRN, and SOX were immobilized, while in the second electrode CNN, CRN and SOX were present. So, creatine is measured in the first electrode while the sum of creatine and creatinine is measured in the second electrode. The electrode response for human blood was lined to a reference LC-IDMS procedure [29] and the results obtained compared with the routine creatinine measurement methods used in the clinical environment. The development, evaluation, and validation of the biosensor for future point-of-care applications is here described.

2. Experimental section

2.1. Reagents and materials

The enzymes creatinine amidohydrolase, creatine amidinohydrolase, sarcosine oxidase and horseradish peroxidase were prepared by dissolving the enzymes in PBS buffer 0.1 M, pH = 7.5, prepared using tablets purchased to Oxoid (Hampshire, UK).

$^{13}C_1$-labelled creatinine with 99% enrichment, purchased from Sigma-Aldrich (St. Louis, MO, USA) and $^{13}C_2$-labelled creatine, in-house synthesized at the University of Oviedo, were used as tracers for the quantification of creatine and creatinine by IDMS.

Acetonitrile (Optima ™ LC-MS Grade) and methanol (Optima ™ LC-MS Grade) from Fisher Scientific, ammonium formate (LiChropur, ≥99.0%) trifluoroacetic acid (Reagent Plus, 99%) and formic acid (puriss, p.a., >98%) from Sigma-Aldrich from Merck were used to prepare mobile phases. Ultra-pure water was obtained from a Purelab Flex 3

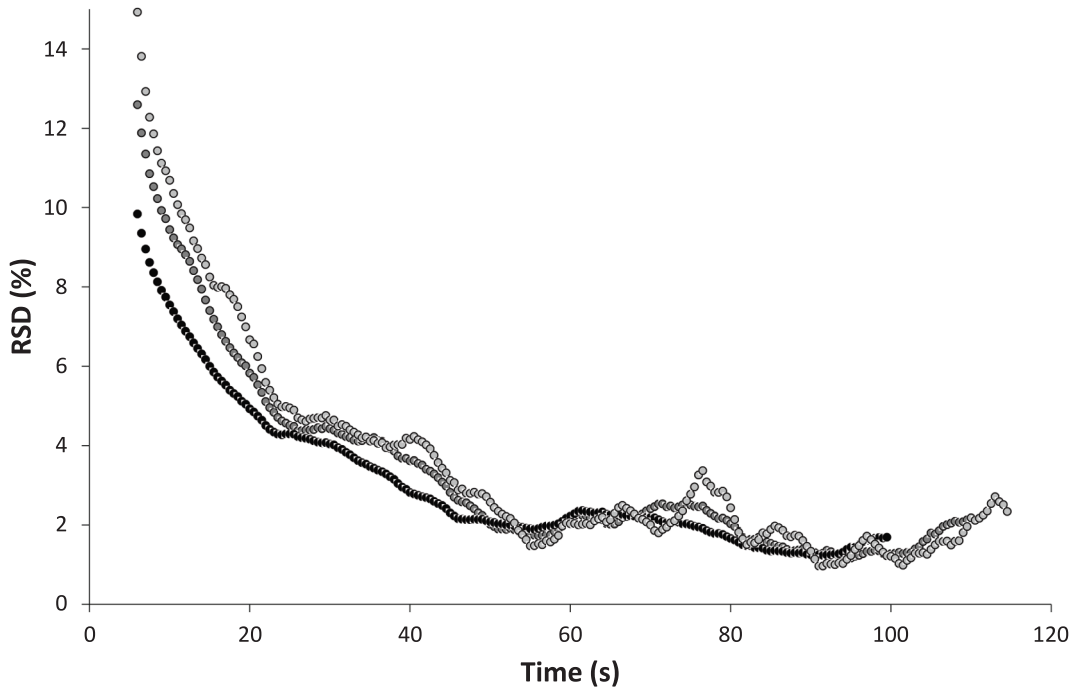

Fig. 5. Relative standard deviation of the integrated signals (peak areas) for three replicates of the same sample using 5 s, 10 s and 20 s integration times (light grey, grey and black dots respectively).

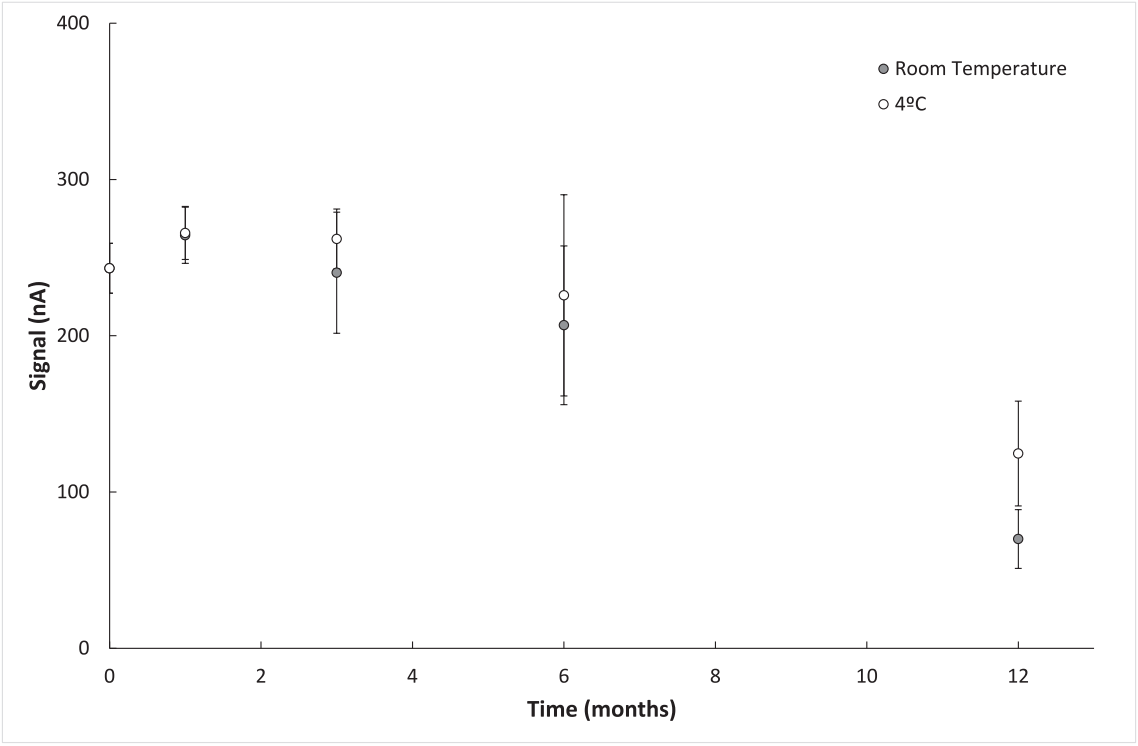

Fig. 6. Stability of the electrodes during 12 months after production.

water purification system (Elga Labwater, Lane End, UK).

Liquid Assayed Multiqual Levels 1 and 3 serum controls for creatinine determination were provided by Bio-Rad Laboratories (Hercules, California).

2.2. Instrumentation

Creatinine biosensor

The biosensor was developed at Healthsens S.L. on disposable screen-printed electrodes (SPEs) from Metrohm DropSens S.L. (Oviedo, Spain) containing two carbon working electrodes, a silver pseudo-reference electrode and a carbon counter electrode printed on a ceramic

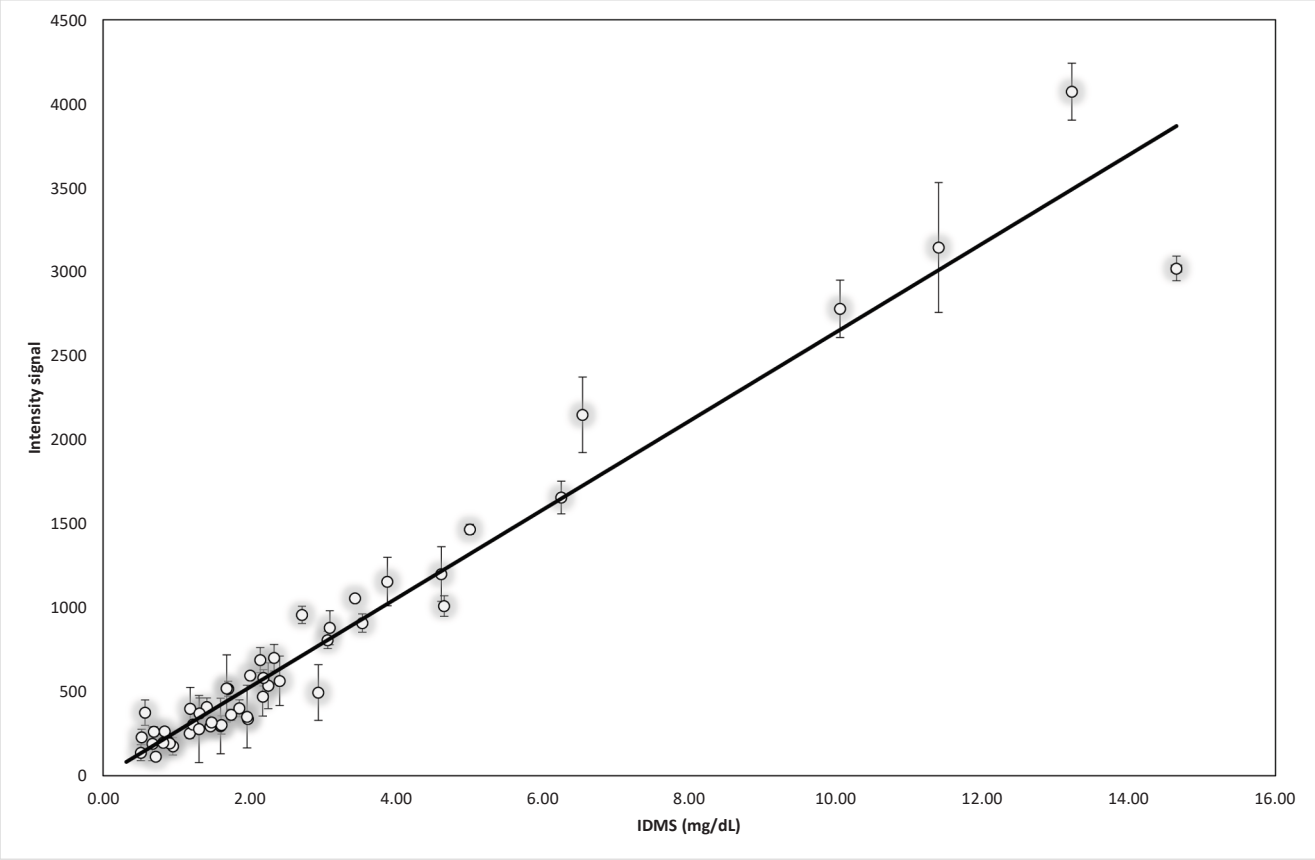

Fig. 7. Relationship between signal area in blood (nA) vs. creatinine concentration in serum, determined by IDMS (mg/dL). The uncertainties of IDMS results (n = 3) are smaller than the points.

substrate (2.5 × 1.0 cm) as shown in Fig. 2. A dielectric screen covers the whole surface except for the electrodes allowing the independent modification of the electrode surfaces, as required. Chronoamperometric experiments were conducted on a bipotentiostat model μStat400 (Metrohm DropSens S.L.) controlled by DropView 3.0 software.

Clinical routine techniques

The autoanalyzer Cobas 8000 (Roche Diagnostics) in combination with creatinine module Cobas c 702 was employed for the determination of creatinine based on the blank-compensated, kinetic Jaffé reaction without deproteinization. Enzymatic creatinine was determined on a Cobas 6000 autoanalyzer (Roche Diagnostics) using a module Cobas c 501. These measurements were performed in the Biochemical Laboratory of the Central University Hospital of Asturias (HUCA) and are IDMS calibrated.

Isotope dilution liquid chromatography tandem mass spectrometry

A chromatographic system Agilent 1290 Infinity II (Agilent Technologies, Santa Clara, CA) was coupled to a triple quadrupole mass spectrometer Agilent 6460 with an electrospray (ESI) ion source. All measurements were done on positive ion mode. Chromatographic separation of creatine and creatinine was carried out by 2D Liquid Chromatography. The UPLC separation on the first dimension was carried out using a reverse phase column Zorbax RRHD Eclipse Plus C18 (3.0 × 50 mm, 1.8 μm particle size, 95 Å pore size, Agilent Technologies). On the second dimension a Zorbax 300-SCX (50 mm × 2.1 mm i.d., 5 μm particle size) from Agilent Technologies was employed. Samples were homogenized on vortex mixer model FB 15,024 (Fisher Scientific, Hampton, NH) while all standard solutions were prepared gravimetrically on an analytical balance model MS205DU (Mettler Toledo, Zurich, Switzerland). Reference measurements were done at the University of Oviedo.

2.3. Subjects and sampling procedure

Venous blood from 50 adult patients was employed in the development of the creatinine biosensor. Samples were selected randomly from the creatinine levels found in routine testing carried out at the Clinical Biochemistry Laboratory of HUCA. Most of the samples arose from nephrology patients but samples from haematology and digestive patients were also included. The age range was between 20 and 92 years. The ranges and average levels found in serum for typical interferences in the determination of creatinine by the routine procedures are shown in Table 1. No interferences in the measurements by the dual-electrode sensor were detected by bilirubin or urea. The study protocol was approved by the Regional Ethics Committee (Comité Ético de Investigación Clínica del Principado de Asturias) with protocol number 178/18.

Blood samples were extracted at HUCA by venepuncture into test tubes containing EDTA. Those blood samples were measured for creatinine within 36 h of collection at Healthsens, in triplicate. Additional blood samples were extracted from the same individuals and sera was obtained. Those serum samples were analysed using the Cobas 8000 Roche® and the Crep 2 Cobas® analyzers at the Clinical Laboratory of the HUCA and using the reference IDMS method at the University of Oviedo. Please note that the biosensor provides creatinine measurements for total blood while the rest of the methods are for serum samples. So, the response of the biosensor (nA) for the different blood samples measured was lined with the IDMS results (in serum) for adequate results comparison.

Table 3
Results for the concentration of creatinine in 50 serum samples by the different measurement procedures. The results calculated for the biosensor are based on the calibration shown in Fig. 7. Data on the concentrations measured for creatine by IDMS are also included.

	Concentration of creatinine in serum (mg/dL)				Concentration of creatine in serum (mg/dL)
Sample	Biosensor	Jaffe	Enzymatic	IDMS	IDMS
1	2.61	2.06	2.15	2.15	0.57
2	0.52	0.52	0.53	0.52	1.01
3	11.91	10.42	11.1	11.40	6.51
4	1.00	0.81	0.84	0.84	0.57
5	3.62	2.60	2.73	2.71	1.02
6	2.26	1.92	2.05	2.01	6.68
7	0.95	1.15	1.17	1.18	0.96
8	10.52	9.50	9.62	10.06	1.34
9	1.12	1.56	1.62	1.60	0.26
10	3.33	3.02	3.29	3.09	0.21
11	0.43	0.69	0.72	0.72	0.43
12	0.87	0.68	0.42	0.53	0.94
13	1.14	1.62	1.61	1.62	0.33
14	1.15	1.20	1.23	1.23	0.72
15	1.55	1.48	1.43	1.42	1.48
16	1.28	1.99	2.00	1.98	0.66
17	2.66	2.33	2.40	2.33	0.48
18	2.03	2.16	2.23	2.25	0.60
19	4.37	3.86	3.92	3.88	1.70
20	3.82	4.52	4.69	4.65	0.31
21	0.66	0.92	0.95	0.95	0.38
22	1.96	1.65	1.75	1.71	2.00
23	1.11	1.39	1.47	1.47	0.46
24	1.51	1.17	1.21	1.19	0.73
25	0.78	0.84	0.89	0.89	1.06
26	1.33	2.01	1.98	1.97	0.17
27	0.72	0.69	0.69	0.67	1.51
28	1.78	2.13	2.22	2.18	0.70
29	4.00	3.34	3.46	3.44	0.52
30	6.27	5.95	6.09	6.25	3.34
31	1.20	1.52	1.50	1.48	0.51
32	0.73	0.89	0.92	0.91	0.38
33	2.14	2.40	2.42	2.41	0.20
34	1.52	1.86	1.87	1.86	0.45
35	1.37	1.71	1.78	1.75	0.49
36	0.99	0.61	0.69	0.69	0.45
37	3.06	3.05	3.14	3.07	0.30
38	3.44	3.57	3.62	3.54	0.30
39	1.41	1.35	1.34	1.32	0.76
40	11.43	13.42	13.93	14.65	0.39
41	1.87	2.96	2.87	2.94	0.27
42	1.05	1.28	1.31	1.31	0.86
43	1.97	1.67	1.71	1.69	0.29
44	2.20	2.13	2.18	2.19	0.50
45	0.75	0.84	0.83	0.82	0.77
46	4.55	4.38	4.55	4.62	3.70
47	8.13	6.70	6.41	6.54	0.79
48	1.42	0.52	0.57	0.57	1.96
49	15.42	12.32	12.70	13.22	0.46
50	5.55	4.76	4.92	5.01	0.35

2.4. Procedures

Preparation of the electrochemical biosensor

A solution containing creatinine amidohydrolase (CNN), creatine amidinohydrolase (CRN), sarcosine oxidase (SOX), horseradish peroxidase (HRP) and potassium hexacyanoferrate(II) trihydrate in PBS buffer 0.1 M pH = 7.5 (4 enzyme mixture) is deposited on one of the carbon working electrodes of the SPE. Then, a solution containing creatine amidinohydrolase (CRN), sarcosine oxidase (SOX), horseradish peroxidase (HRP) and potassium hexacyanoferrate(II) trihydrate in PBS buffer 0.1 M pH = 7.5 (3 enzyme mixture) is deposited on the other working electrode. After drying, a semipermeable membrane is deposited sequentially on each electrode and, finally, the disposable electrodes are ready to use. A batch of 170 disposable electrodes was prepared for this study.

Measurement of the blood samples by chronoamperometry

The modified screen-printed electrodes are set in the bipotentiostat and a drop of 50 μL of whole blood is deposited covering all four electrodes as shown in Fig. 3. The analytical signal was obtained by chronoamperometric detection on both working electrodes fixing a potential of −0.015 V during a total of 120 s with intervals of 0.5 s.

Measurements by IDMS

The procedure for the determination of creatinine in serum by IDMS was based on the double tracer procedure published previously [29]. Samples of ca. 0.2 g of serum were placed in 1.5 mL Eppendorf tubes and spiked with ca. 0.2 g of a mixed tracer containing $^{13}C_1$-creatinine and $^{13}C_2$-creatine at 10 μg/g levels and shaken manually for 1 min. The amounts of sample and tracers added were controlled gravimetrically. After that, 400 μL of methanol were added for protein precipitation and, after vortex mixing, the tubes were centrifuged at 15,000 g for 10 min. The supernatant was transferred to a new Eppendorf tube and evaporated to dryness. The solid sample was reconstituted in mobile phase A, for the first chromatographic dimension, centrifuged at 15,000 g for 20 min and filtered through a polyvinylidene fluorure membrane (13 mm diameter and 0.22 μm pore size) before its injection in the 2D-LC-MS/MS system. The certified serum standards (Liquid Assayed Multiqual, levels 1 and 3, BIO-RAD®) were treated in the same way as the samples. All real serum samples were measured in triplicate and the concentrations of both creatine and creatinine were determined.

Separation based on 2D-LC and detection by tandem MS

In this case a 2D-LC system was employed to facilitate the separation of creatine and creatinine from matrix components and reduce matrix suppression effects. The first dimension employs a reverse phase C18 column using a gradient separation with 0.1% (w/w) trifluoroacetic acid (TFA) in water as mobile phase A and 0.1% TFA in acetonitrile as mobile phase B. The second dimension employs an ion exchange column with 0.1% (w/w) formic acid and 20% (v/v) methanol adjusted at pH 2.8 as mobile phase A. Mobile phase B contains additionally 10 mM ammonium acetate and the pH was adjusted to 3.5. The flow rate was adjusted to 0.4 mL/min in both LC dimensions and the injection volume was 4 μL. The total time required per sample was 20 min including column reconditioning.

Tandem MS analysis was carried out by electrospray ionisation in positive ionisation mode using Selective Reaction Monitoring (SRM). The $[M + H^+]$ ion was selected as precursor ion for both compounds. The ionization source working conditions were 2500 V as capillary voltage, 0 V as nozzle voltage, 45 psi as nebulizer pressure, 9 L min^{-1} as drying gas flow rate and 250 °C as drying gas temperature. The sheath gas flow rate and temperature were 8 L min^{-1} and 400 °C, respectively. The fragmentor voltage was set at 115 V. For the determination of the isotopic distribution of creatine and creatinine the measurements were performed in SIM mode monitoring *m/z* 130.1 to 135.1 and 112.1 to 117.1 respectively. For the characterization of creatine and creatinine and their determination in samples, controls and CRMs the measurements were performed in SRM mode monitoring the transitions 132.1 → 90.1, 133.1 → 91.1, 134.1 → 92.1 and 135.1 → 93.1 with a collision energy of 8 eV for creatine and 114.1 → 86.1, 115.1 → 87.1, 116.1 → 88.1, 117.1 → 89.1 with a collision energy of 7 eV for creatinine.

Validation of IDMS results

The certified serum standards (Liquid Assayed Multiqual, levels 1 and 3, BIO-RAD®) were measured along with the serum samples during 6 measurement sessions. The standard deviations for triplicate analysis were always lower than 1%. The average results obtained during the 6 measurement sessions for the two reference materials are given in Table 2. As it can be observed, the agreement with the reference values is satisfactory.

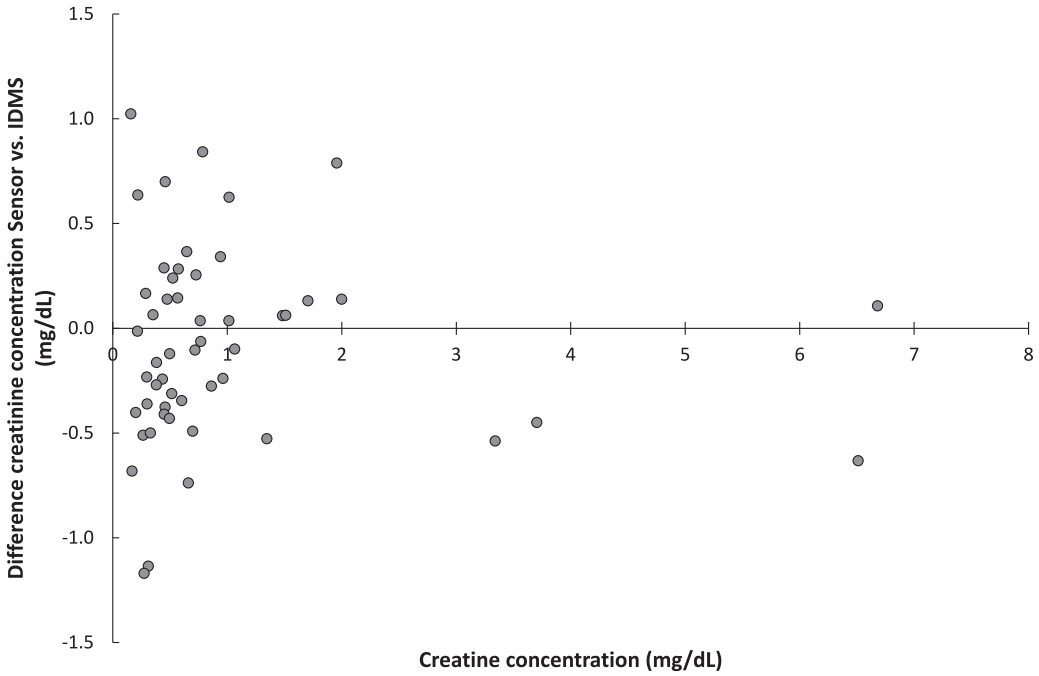

Fig. 8. Effect of the creatine concentration on the biosensor response for creatinine.

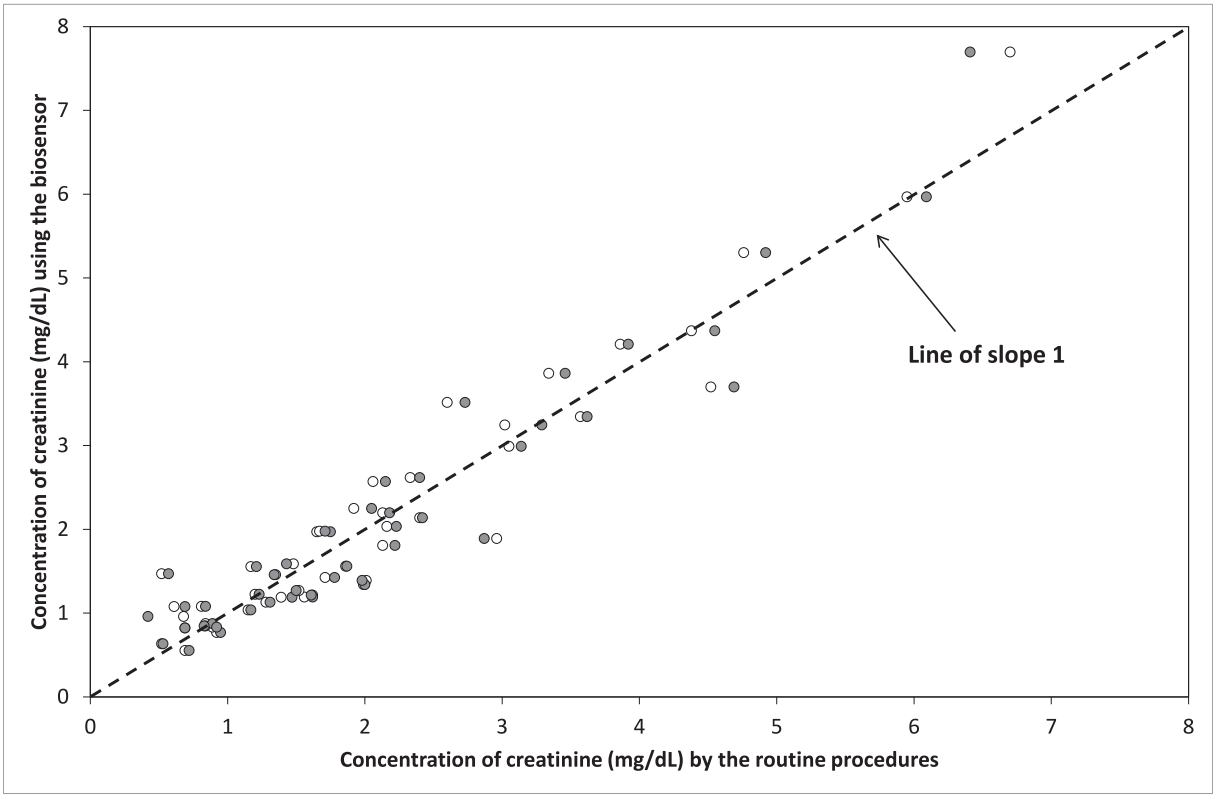

Fig. 9. Comparison of the results by the Jaffé (white dots) and the enzymatic (grey dots) methods with the biosensor results for 46 samples.

3. Results

3.1. Development and optimisation of the creatinine biosensor

The determination of creatinine using the enzymatic reaction shown in Fig. 1 is well described in the literature [1–3]. In our approach a dual working electrode configuration is employed in which creatine is detected in the first electrode (w1) and both creatine and creatinine are measured in the second electrode (w2). The difference between the currents measured in both electrodes (w1 and w2) corresponds then to the content of creatinine in the sample. Critical parameters in the optimisation of the biosensor were the actual composition of the three- and four-enzyme mixtures added to the electrode surfaces and the nature of the semipermeable membrane employed to cover the electrodes. This

Table 4
Statistical analysis of the plots shown in Fig. 8.

Linear-fit values	Cobas 8000 (Jaffé)	Crep2 Cobas (Enzymatic)
Slope	1.023 ± 0.045	1.010 ± 0.046
Intercept	−0.012 ± 0.114	−0.026 ± 0.117
Correlation coefficient	0.9599	0.9579
S y/x	0.43	0.44
95% Confidence Intervals		
Slope	0.933 to 1.113	0.919 to 1.101
Intercept	−0.239 to 0.215	−0.260 to 0.208
Is the slope significantly different from 1?	No	No
Is the intercept significantly different from 0?	No	No

information has been considered confidential by Healthsens S.L. and will not be discussed here.

As indicated in the Procedures Section, the analytical signal was obtained by chronoamperometric detection on both working electrodes fixing a potential of −0.015 V during a total of 120 s with intervals of 0.5 s. Of interest during the optimization of the creatinine biosensor was the reproducibility of the measurements when the same blood sample was added to different electrodes from the same batch. For example, Fig. 4A shows the chronoamperograms obtained at w1 and w2 for a human blood sample and the difference between both currents measured every 0.5 s. As it can be observed, the currents at w1 and w2 increased drastically during the first 10 s and increased more slowly after 20 s. The difference between w1 and w2 shows a linear reducing trend after 10 s into the measurement. Fig. 4B shows the chronoamperograms of the differences between w1 and w2 for a triplicate of the same blood sample measured on three different electrodes from the same batch.

As it can be observed in Fig. 4B, the average of the differences shows a large standard deviation at the beginning of the chronoamperogram but the signals converged after ca. 60 s. with relative standard deviations (n = 3) between 2 and 4%.

To improve signal stability it was decided to integrate the signals between two different times in the difference chronoamperogram using possible intervals of 5, 10 and 20 s. The plot of the relative standard deviation of the areas vs time for the different integration intervals is shown in Fig. 5.

As it can be observed, RSD under 2% were obtained both for 10 and 20 s integration times between 60 and 100 s after the beginning of the chronoamperogram and these measurement conditions were selected finally.

3.2. Stability of the electrodes

A new batch of the electrodes was prepared to evaluate their long-term stability. A standard solution of creatinine was measured in the same day of production (0 months) and after 1, 3, 6 and 12 months. The electrodes were divided into two groups and stored, on the one hand, at Room Temperature, and on the other hand, at 4 °C. 10 electrodes were measured each time with each condition. The results obtained are illustrated in Fig. 6. As it can be observed, the electrodes are stable at least three months both at room temperature and at 4 °C. However, the standard deviation of the signals for the different electrodes tested on the same day (n = 10) increased with time after production, particularly noticeable for the electrodes kept at room temperature at three months, so we decided to keep the electrodes refrigerated and use them within three months after production of the batch to assure signal stability and reproducibility.

3.3. Calibration of the biosensor and comparison with alternative techniques.

A series of 50 real blood samples were measured with the biosensor in triplicate and the integrated areas were plotted versus the concentrations measured for the same 50 samples in sera by the reference IDMS procedure. The results obtained are shown in Fig. 7. The response of the biosensor for 50 μL of blood (nA) is linear with the concentration of creatinine in serum between 0.5 and 15 mg/dL with a correlation

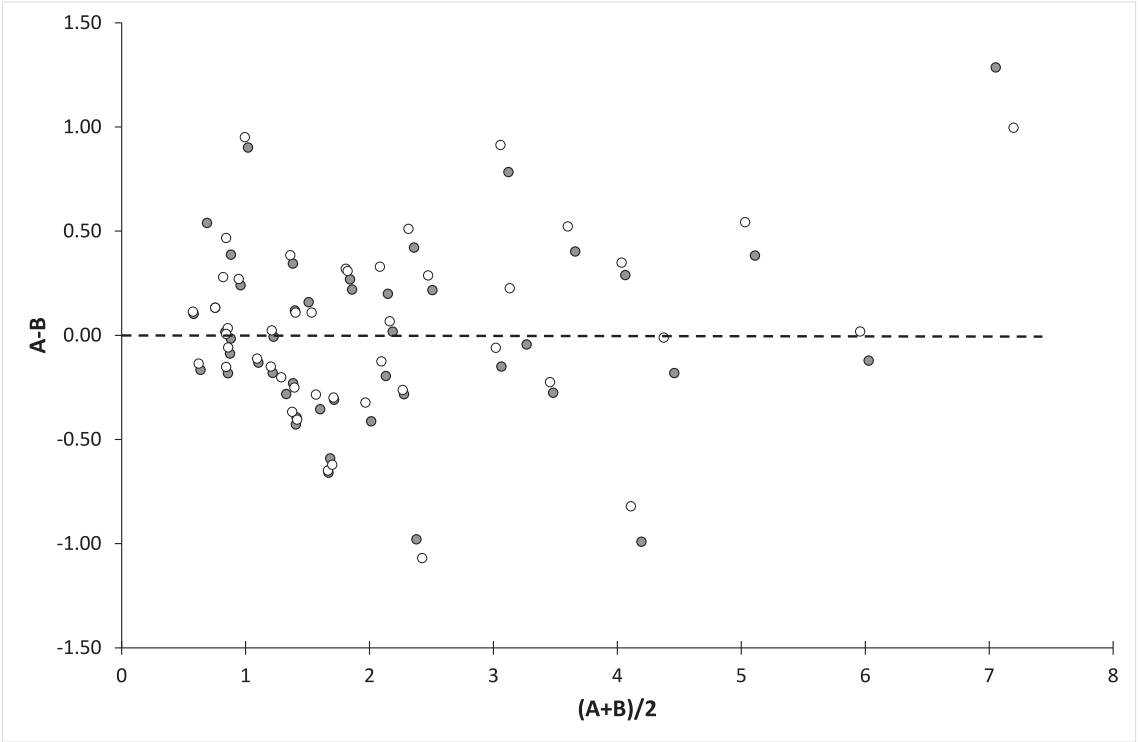

Fig. 10. Bland-Altman plot of the accuracy of biosensor vs. Cobas 8000 Roche® (white dots) and Crep2 Cobas Roche® (grey dots).

coefficient of 0.9747. The standard deviations of the signals seem not to be related with the signal levels, so a classical linear regression was applied. So, we employed this calibration line to obtain the concentrations of creatinine in sera for the 50 measured samples and compared the results with those obtained by the routine procedures Cobas 8000 (Jaffé) and Crep2 Cobas (enzymatic) in the clinical laboratory. The whole dataset for the found concentrations of creatinine in serum by the four alternative methods is shown in Table 3. We have included also in Table 3 the creatine results (mg/dL) obtained by the LC-IDMS procedure.

4. Discussion

4.1. Effect of creatine levels on the biosensor response.

It has been indicated that for some commercial POCT devices the levels of creatine posed and interference on the measured creatinine concentrations [25]. As the LC-IDMS procedure used here reported both the creatine and creatinine levels, it was possible to study the effect of creatine on the creatinine response of the biosensor. Fig. 8 shows the differences between the biosensor and the IDMS results as a function of the creatine levels in serum. As it can be observed, levels of creatine up to ca. 7 mg/dL were obtained by LC-IDMS and there seems to be no effect of creatine on the biosensor response demonstrating the lack of creatine interferences.

4.2. Correlation between the LC-IDMS results and the routine procedures.

The results found with the routine methods were compared with the LC-IDMS results using the well-known Bland-Altman plots [30]. Bland-Altman plots between IDMS and both Jaffé and the enzymatic methods (which can be prepared using the data given in Table 3) showed very good agreement except for the four high concentration samples (samples 3, 8, 40 and 49) and recommended its exclusion from the final comparison with the biosensor. After those samples were excluded the Bland-Altman analysis of the routine Jaffé method with reference to the LC-IDMS results showed that the bias was 0.04 mg/dL and the concordance limits were between −0.13 and 0.20 mg/dL with 42/46 samples (91%) within those limits. Similar results were obtained with the enzymatic method in comparison with the reference LC-IDMS procedure. These excellent results were expected as the routine methods carried out in the HUCA are IDMS calibrated.

4.3. Correlation between the biosensor results and the routine procedures.

After eliminating samples 3, 8, 40 and 49, the results were compared with those obtained by the routine procedures. The comparison results are shown in Fig. 9. The statistical summary of the comparison is shown in Table 4 and indicates that the biosensor provides results in agreement with both the Jaffé and the enzymatic methods.

The Bland-Altman results for the comparison of the biosensor with both routine procedures are shown in Fig. 10. The bias when comparing the biosensor with the Jaffé method in the automatic analyser Cobas 8000 Roche® was 0.04 mg/dL and −0.01 mg/dL when comparing with the enzymatic method in the Crep2 Cobas Roche® automatic analyser. So, there is no substantial bias using the biosensor. The concordance limits with the Jaffé method were between −0.80 and 0.87 mg/dL, with 89% of the samples (41/46) within those limits. The concordance limits with the enzymatic method were between −0.86 and 0.85 mg/dL, with again 89% of the samples (41/46) within those limits.

4.4. Comparison between serum and blood.

Creatinine is measured in blood by the biosensor and in serum by the other techniques so a small discussion on the possible differences should be carried out. The chronoamperometric measurement in blood is based on the diffusion of both creatine and creatinine though the semi-permeable membrane to the modified electrodes. The diffusion rates of creatine and creatinine could be influenced by the haematocrit levels (volume of red blood cells vs total blood volume) when analysing blood samples. As it can be observed in Fig. 4, after 60 s the signal tends to a plateau and a steady state diffusion is obtained. We think that, if different haematocrit levels are found in the samples, that would affect the diffusion rate during the first 60 s and all samples will reach the same steady state regardless of the haematocrit level. However, as no haematocrit measurements were made this discussion point will have to be left open for future studies.

5. Conclusions

An electrochemical biosensor for the direct determination of creatinine in a drop of human blood was developed. The biosensor is not interfered by creatine and has a linear response with the concentration of creatinine in serum between 0.5 and 15 mg/dL. The biosensor was lined to a reference method based on Liquid Chromatography Isotope Dilution Mass Spectrometry. The comparison of the biosensor with other routine procedures (Jaffé and enzymatic colorimetric methods) showed a lack of bias in the range 0.5 to 7 mg/dL creatinine and concordance limits of ca. ±0.85 mg/dL. So, this is the first step in the development of a Point-Of-Care creatinine measurement device based on this biosensor. Further improvements are required to reduce the concordance limits in the Bland-Altman plots to, ideally, ±0.2 mg/dL creatinine.

CRediT authorship contribution statement

Gabriel Álvarez Menéndez: Investigation, Writing – original draft, Visualization. **Olaya Amor-Gutiérrez:** Writing – original draft, Project administration. **Agustín Costa García:** Conceptualization, Project administration. **María Funes-Menéndez:** Investigation. **Catuxa Prado:** Conceptualization, Supervision. **Diego Miguel:** Investigation. **Pablo Rodríguez-González:** Supervision, Funding acquisition. **Adriana González-Gago:** Investigation, Supervision. **J. Ignacio García Alonso:** Conceptualization, Writing – review & editing, Funding acquisition, Project administration.

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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