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Solid Contact Potentiometric Sensors Based on Host-Tailored Molecularly Imprinted Polymers for Creatine Assessment

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Potentiometric sensors for organic ions are in principle suitable for quick in situ determinations. A basic point in the improvement of these sensors is to discover particular receptors. In this examination, the probability of developing solid state potentiometric sensors with molecularly stamped polymers (MIP) as a recognition element was researched. Preparation of molecular imprinting polymers (MIP) as synthetic receptors for creatine and implemented in potentiometric sensors based on theepoxy-graphite matrix as a conductive solid contact. These MIPs are depending on the utilization of methacrylic acid (MAA) and 2-vinyl pyridine (2-VP) as utilitarian monomers. Sensors based on (MAA) and (2-VP) exhibited a stable response over a linear range $9.0x10^{-6}$-10^{-2} and $1.0x10^{-5}$-10^{-2}mol L^{-1}creatinecation with slopes of 57.1±0.2 and 54.1±1.2 mV $decade^{-1}$ and detection limits of 7.0×10^{-6} and 6.0×10^{-6}mol L^{-1} in 10^{-2}mol L^{-1}citrate buffer solution has pH 3, respectively. It was worth noting that the developed membrane sensors exhibited good selectivity towards creatine over many organic and inorganic cations, as well as some additives encountered in the pharmaceutical preparations and so these sensors, were successfully used for determination of creatine. The createdsensors were also utilized effectively for the quantification of creatine in serum as biological fluids. The resultsobtained were in great concurrencewith the information got by the measure spectrophotometry.

Keywords: Creatine, Molecularly imprintedpolymers, potentiometric sensors

1. INTRODUCTION

Obtaining electrochemical transducers have an access for ruggedness, robustness and low-cost effectiveness is becoming now of great importance in a growing number of situations [1]. Development of new schemes that permitsrapid, low-cost, on-site chemical analysis is referred as vanguard analytical approaches [2].In these new schemes, performance characteristics, such as linearity, stability, selectivity, limit of quantifications, etc., must be corresponded with the

requirementfor rapidity, expenditureand naivety. Under these new needs, other techniques like potentiometry, are resuming new rate.Potentiometric sensors have alsobecome the criterion approach for quantifying several analytes. Moreover, the evolution of solid contact-based sensors during the last few decades has created a "silent uprising" [3] that have led to stronger finements of the quantification limit (QL), wide application range and naivety of the sensor fabrication, implementation and automation. The need for new materials to constructtransducers as well as the use of mass manufacturing techniques is decisive to further reduce the expenses of thesetransducers. For this cause, recent breakthroughs in electronics can be considered as a key for finding new materials and procedures to manufacture burly, firm and extremely inexpensive potentiometric transducers [4].

Similar to bio-recognition systems and molecular recognition approachhas been studied deeply for the manufacture of burlytransducers with long-term stability and high selectivity [5].Utilizing the biomimetic ability of host-guest concept with well-defined cavities can be considered as a one of these methodological.One of the most impressive developments in layoutman-made host molecules has been achieved with molecularly imprinted polymers (MIPs). This planning has been introduced effectivelyto the improvement of chemically sensitive recognition layers. The selective enrichment of an analyte in the sensitive recognition layer changes the surfaceby a physicochemical way. Various detectable signals may be recorded to follow this process of incorporation into the polymersuch as mass, fluorescence, electrical resistance or capacitance. The most favorable transducers, however, areachieved with microelectronic detectors, such as quartz-crystal microbalance (QCM), surfaceacoustic wave (SAW) [6], afield-effect transistor (FET) [7],potentiometry [8-13]; and spectroscopy [14].

Chronic hemodialysis can be considered as a life-savingmedication for peoplehavekidney function failures.It should be strongly controlled because of thestrong correlation between patients' morbidity/death-rate and efficiency of this treatment. As the main aim for effective hemodialysis to removeall toxins from thepatient body, controllingof this removal process is the best approach forcorrectness and effectiveness medical estimate of the clinical therapy.

Creatineis considered as an amino acid derivative which is prepared from glycine, L-arginine, and S-adenosylmethionine in the kidneys, liver and pancreas.It is a valuable marker for muscle damage and it is routinely determined and quantified in clinics and biomedical laboratories. The normal amount of creatine in serum is less than 22.2 μg mL^{-1}, butits concentration may increase to a value higher than 149.2μg mL^{-1} during muscle disorder [15]. Also, it has a great publicity due to its ability to enhance the physical performanceof the human body. This publicity is coming from its conversion into phosphocreatine inside muscles in a reversible pathway with adenosine triphosphate (ATP). When muscle concentrations minimize the supply of ATP, the phosphocreatine can re-phosphorylate adenosine diphosphate (ADP) to complete the supply of ATP.

Different analytical methods have been described for the quantification of creatine in biological samples based on: high performance liquid chromatography (HPLC) [16,17], gas chromatography(GC) [18], electrospray tandem mass spectrometry (ESI-MS/MS) [19], liquid chromatography-tandem mass spectrometry [20,21], infrared spectroscopy [22], capillary zone electrophoresis [23,24], UV/Vis spectrophotometry [25, 26] and bioluminescence [27].These approaches require costly hardware and solvents not ordinarily utilized for quick routine examination and not relevant in little facilities.

Strategies in light of electroanalytical methods are likewise accessible taking into account the utilization of either potentiometric or amperometric biosensors. Potentiometric sensors are one of the electrochemical ion selective sensors that relate the activity of a target ion to a detectable electrical potential as the measured signal.These sensors are coupled with reference ones under near-zero current conditions. Over the last fifty years, potentiometric sensors have included into routine analytical tools, with detectors for more than 100 analytes. Compared with other analytical techniques, these types of sensors have some unique properties, such as miniaturization, facility of working, portability and cheap. In the past few years, potentiometric sensors have made agreatadvance indeveloping the detection limit (LOD), exploring new membrane materials, proposing new sensing concepts through developing deeper theoretical research about potentiometric responses [28].The classical inner solution electrodes suffer from various drawbacks such as the drying out of the inner filling solution, temperature alterations and the pressure range [29]. The elimination of the internal filling solution from conventional potentiometric selective membranes gives rise to solid-contact ion-selective electrodes (SC-ISEs). Solid-contact ion-selective electrode is an asymmetrical sensor, where the membrane is introduced on one side in contact with a solidcontact or a solid transducer layer instead of an inner solution and on the another side the membrane will be in contact with the sample.

Potentiometric bio-sensing for quantitative estimation of creatine has been reported [30-33].A bi-enzymatic reactor sandwiching two enzymes, urease and creatinase and then the potentiometric signal was recorded with ammonia gas sensor[30-31,33]. The other one described only a mono-enzyme sensor for creatine-glass electrode sensitized by creatine kinase[32].Advantages and limitations of these sensors are presented in Table 1.The amperometric detection is based onabi-enzyme system that converts creatine in to sarcosine and finally sarcosine to glycine [34-37]. All of the above-mentioned approaches have the disadvantages of non-reproducibility and low storage stability. Although direct potentiometry has found many applications in pharmaceutical, biomedical and clinical analysis [38-40], no such methods are available for direct determination of creatine.

In this study, novel solid contact potentiometric sensors based on man-tailored host receptors were prepared, characterized and applied for the fast determination of creatine are described. The sensing membranes incorporate synthetic biomimetic receptors, based on the use of 2-vinyl pyridine and methacrylic acid as functional monomers. Execution qualities of both sensors reveal low location limit, high affectability, great selectivity, quick reaction, and application for precise determination of creatineinbiological fluids and pharmaceutical arrangements.

2. MATERIALS AND METHODS

2.1. Apparatus

All potentiometric measurements were recorded by means of pH/mV meter model 720/SA (Orion, Cambridge, MA, USA). Ag/AgCl doubl ejunction reference electrode (Sentek, UK) filled with (1.0 mol L^{-1}) lithium acetate was introduced as a counter to complete the electrochemical cell. The output signal was transferred to analterationpoint re-connected to one of six directions out, each with anelectrical connector for adaptation to thesensormodule. The selective membraneswere introduced on

an epoxy-graphite matrix as aconductive solid contact. The electrochemical cell arrangement is: Graphite contact |Creatine selective membrane| test solution || CH_3COOLi salt bridge || Ag/AgCl(3.0 mol L^{-1}KCl). A combination glass electrode (Sentek, UK) was introduced for all pH measurements. AJenway UV-Visiblespectrophotometer (Model 1601) was used for all subsequent spectrophotometric measurements under the recommended conditions of the standard method [41].

Table 1. Response characteristics of some potentiometric Creatine sensors

Sensing material	Immobilized enzymes	Linear Range, mol L^{-1}	Slope mV $decade^{-1}$	Detection limit mol L^{-1}	pH range	Buffer used	Interferes	Ref
Nonactine/ PVC-COOH	urease and creatinase	1.0 $x10^{-4}$- 3.0 x 10^{-2}	29.6	$1x10^{-5}$	6.0–8.0	1 mmol L^{-1} TRIS	NH_4^+ (NR), Urea (NR)	[30]
Nonactine/ Palmitic acid	urease and creatinase	$1.0x10^{-5}$- 1.0 x 10^{-3}	49.2± 0.3	$1x10^{-5}$	7.0	5 mmol L^{-1} TRIS	K^+(NR) , Na^+ (NR), Ca^{2+}(NR)	[31]
Glass electrode	Creatine kinase	$4.2x10^{-4}$ - 3.3x 10^{-3}			8.0-9.0	$2x10^{-3}$ mol L^{-1} ATP + 10^{-4} mol L^{-1} $MgSO_4$	Ascorbic (NR), uric (NR), creatinine (NR), Na^+ (NR), creatinine (NR).	[32]
Nonactine/ PVC-COOH	urease and creatinase	$1.0x10^{-4}$- 1.0 x 10^{-2}	50±0.8	$3x10^{-5}$	6.0–8.5	10 mmol L^{-1} TRIS	K^+ (-1.5), Na^+ (-2.9), Ca^{2+}(-3.48), Fe^{3+}(-3.5), Ascorbic (-3.2), uric (-3.3), creatinine (-3.2)	[33]
Nonactine/ Palmitic acid			35±0.3	$2x10^{-5}$			K^+ (-1.9), Na^+ (-2.6), Ca^{2+}(-3.27), Fe^{3+}(-3.12), Ascorbic (-3.4), uric (-3.2), creatinine (-3.7)	

NR= Not reported

2.2. Reagents and materials

Creatine monohydrate, creatinine, trisodium citrate and citric acid were obtained from Sigma (St. Louis, MO). Ethyleneglycoldimethacylic acid (EGDMA), methacrylic acid (MAA), 2-vinyl pyridine (VP), acetonitrile, potassium tetrakis (4-chlorophenyl)borate (TpClPB),tetrahydrofuran and benzoyl peroxide (BPO) were supplied by Fluka (Ronkonkoma, NY). The creatine solutions were prepared from standard 10^{-1}mol L^{-1}creatine solution by serial dilutions. All solutions used during the measurements were buffered with 10^{-2}mol L^{-1} citrate buffer solution at pH 3.

2.3. Polymer synthesis

The following items were mixed together and introduced to a glass tube: 5 mmol of (2-VP orMAA) as a functional monomer, 30 mmol of (EGDMA) as acrosslinker, 0.07 g of (BPO) as an initiator, 3 mL of (acetonitrile) as a porogenic solvent and 1 mmol of (creatine) as the template molecule. The mixture was sonicated for 10 min, purged with nitrogen gas for 10 min to expel dissolved oxygen and then sealed under vacuum. The polymerization process was implemented in a

water bath at 60 ^{o}C for 30 min. The bulk polymer was then removed from the glass tube and placed in an oven tillcomplete dryness. The dried polymer was grounded into fine particles and sieved through a 35-45 µm sieve. By these procedures, particles of 25-35 µm were collected. For non- imprinted micro-beads (NIP) itwas prepared as mentioned above with the exclusion of the template the procedure. Extraction of the template was doneusing a 9:1 (v/v) methanol: acetic acid solution and then washed with de-ionized water. All synthesized polymers (MIP/2-VP, NIP/2-VP, MIP/MAA and NIP/MAAwas implemented in potentiometric sensors for creatine assessment.

2.4. Sensors Design

The polymeric material was used to dope PVC-membrane selective electrodes. Thus, the sensitive membranes were provided by shuffling 190 mg of PVC, 350 mg of *o*,NPOE as a plasticizer, and varying amounts (14-60 mg) of the sensing polymer (Table 2). A 5 mg of TpClPB as an anionic excluder was introduced to other membranes to study the effect of anionic additives on the performance characteristics of the sensors. The mixture was stirred until the PVC was well dabbled, andsprinkled in 3.0 mL THF; the uniformity of the dispersion was guaranteed by continuous agitation on a magnetic stirrer. These membranes were introduced ona conductive supports have either conventional or tubular shapes [42-44].

Table 2. Potentiometricfeatures of creatine sensors

Parameter	MIP/MAA			MIP/2-VP			
	2.5 %	**5%**	**10%**	**2.5%**	**5%**	**10%**	**10% MIP/2-VP+ 30 mol % TPB^-**
Slope, mV decade^{-1}	55.9±0.9	55.3±0.6	57.1±0.2	43.3±0.8	53.2±1.1	54.1±1.2	57.1±0.6
Correlation coefficient, r (n=5)	0.997	0.999	0.998	0.998	0.999	0.999	0.999
Linear range, mol L^{-1}	$1.0x10^{-5}$-$1x10^{-2}$	$1.0x10^{-5}$-$1x10^{-2}$	$9.0x10^{-6}$-$1x10^{-2}$	$2.0x10^{-5}$-$1x10^{-2}$	$1.0x10^{-5}$-$1x10^{-2}$	$7.0x10^{-6}$-$1x10^{-2}$	$7.0x10^{-6}$-$1x10^{-2}$
Detection limit, mol L^{-1}	$8.0x10^{-6}$	$9.0x10^{-6}$	$7.0x10^{-6}$	$1.0x10^{-5}$	$9.0x10^{-6}$	$6.0x10^{-6}$	$6.0x10^{-6}$
Working range, pH	2.8-4.5	2.8-4.5	2.8-4.5	2.8-4.5	2.8-4.5	2.8-4.5	2.8-4.5
Response time, s	10 - 20	10 - 20	10 - 20	10 - 20	10 - 20	10 - 20	10 - 20
Accuracy, %	96.3	99.2	97.8	97.5	98.2	99.1	98.6
Repeatability (Cw_v), %	0.7	0.8	0.4	0.6	0.7	0.5	0.7
Between day-variability (Cv_b), %	1.2	1.3	1.6	1.2	1.1	0.8	0.4

Although ‘‘coated-wire’’ configurations have been correlated to random drifting potentials, this graphite-based solid contact has been used for long [45] with negligible drift. It has also the advantage of enabling future miniaturization because it uses a solid contact instead of using an internal reference solution. Membranes were soaked overnight in a $1x10^{-3}$mol L^{-1}creatine solution before use and were kept in the same solution when not in use.

2.5. Recommended procedure

2.5.1 Direct potentiometry

The proposed transducers were examinedby transferring 2.0 mL aliquots of 10^{-2} – 10^{-7}mol L^{-1} aqueous solution of creatine to 50 mL beaker containing 20 mL of 5 mmol L^{-1} citrate buffer solution at pH 3. The sensors were introduced in the solution and coupled with the reference electrode. The potential signals were monitored after complete stabilization to ± 1.5 mV and then wererecordedas a function of log [$Creatine^{+}$]. The calibration plots were taken for subsequent quantification of obscure creatine concentrations.

2.5.2. Creatineassessment in human serum

Human serumsampleswere collected from volunteers and analyzed within 4hours of extraction. 9 mL portions of absolute ethanolwereintroduced to the blood samples, thoroughly mixed and centrifuged at 4000 rpm. The supernatant liquid was introduced to a 20 mL beaker and then evaporated at 50◦C till dryness and then reconstituted in deionized water. A 9 mL of 5 mmol L^{-1}citrate buffer solution was introduced and then transferred to 25 mL measuring flask, completed to the mark by the buffer solution. A 10 mL aliquot of the sample solution was transferred to a 25 mL beaker and then the electrochemical set-up wasplaced in that solution, and the emf readings were registered. The amount of creatineis expressed as [$Creatine]^{+}$and is calculated from the constructed calibration graph.

3. RESULTS AND DISCUSSION

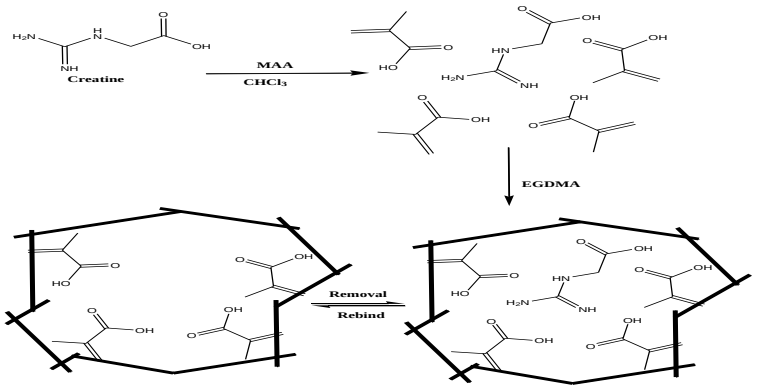

Figure 1. Schematic representation of non-covalent imprinting.

The molecular imprinting approach isan interesting idea thatprovides an applicable alternative technology. It uses highly stable polymers haveselective recognition sites that are appropriated to the morphology and functionality of the template. Arranging the polymerizable monomers around a template can achieve the so-called "imprinting effect" which is preferably the analyte itself or an analogmolecule with appropriate geometry and interaction sites. The template molecule is removed from the polymer after the polymerization process is finished.Recognition sites with an appropriate geometry to the original template are imprintedin the polymer backbone. Fig. 1 shows agraphical representation of the imprint approach.

3.1. Response characteristics of creatine selective sensors

Two types of creatine selective sensors based on solid contact electrodes were potentiometrically evaluated. The electrodes were manufactured using (MIP/MAA) and (MIP/2-VP) as electroactive materials for determination of creatinecation. The potentiometric sensitivity and linearity depend significantly on the amount of membrane composition. Thus the effect of the percentage of the electroactive material added was investigated and the results are presented in Table2. The amount of MIP microparticles was increased from 2.5 to 10%. This change conducted to a raise uptothe limit of detection and sensitivity. Overall, the use of 60.0% *o*,NPOE plasticizer and 30.0% PVC as a polymeric matrix in presence of 10% MIPparticles led tothe best sensitivity, with a slope of 57.1±0.2 and 54.1 ±1.2 mV decade^{-1}, over a linear range 9.0x10^{-6} to 1.0x10^{-2} and 7.0x10^{-6} to 1.0x10^{-2}mol L^{-1} and limit of detection 7.0x10^{-6} and 6.0x10^{-6}mol L^{-1} for MIP/MAA and MIP/2-VP respectively. Typical potentiometriccurvesfor all sensors are shown in Fig. 2. Aneffective response and better selectivity towards creatine could be noticed with MIP/MAA more than MIP/2-VP membrane based sensors.

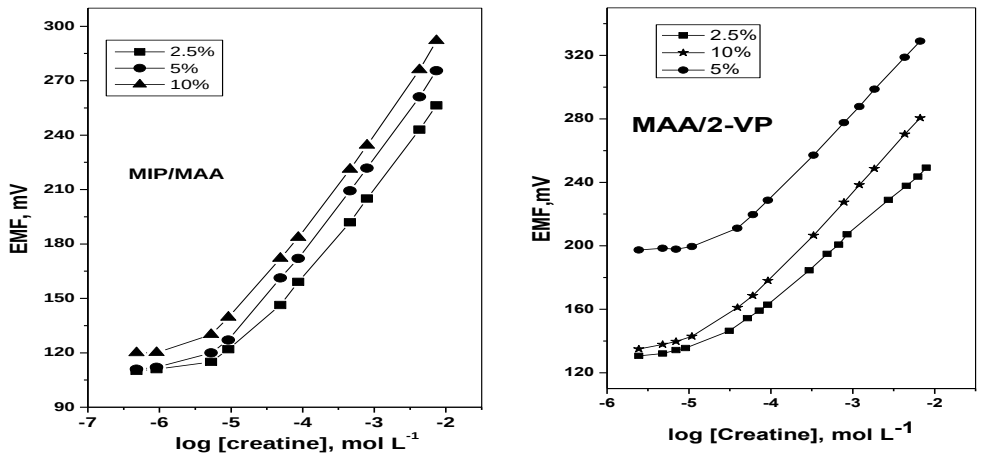

Figure 2. Potentialresponse towardscreatineusing MIP/MAA and MIP/2-VP membrane based sensors

One possible explanation for the response mechanism is that the ionic charge transfer occurring at the membrane during the binding of [creatine] $^{+}$ to the membrane MIP receptor sites produces an excess of surface charge. This creates an electric field that acts on the conducting electrons of the graphite solid contact. A potential change at the electrode is then developed, and the interface between

the membrane and the electrode acts as a condenser. They base onthe blocked interface between an ionic conductor (the membrane) and the electronic conductor, and thus they display limited stability (Fig. 3).

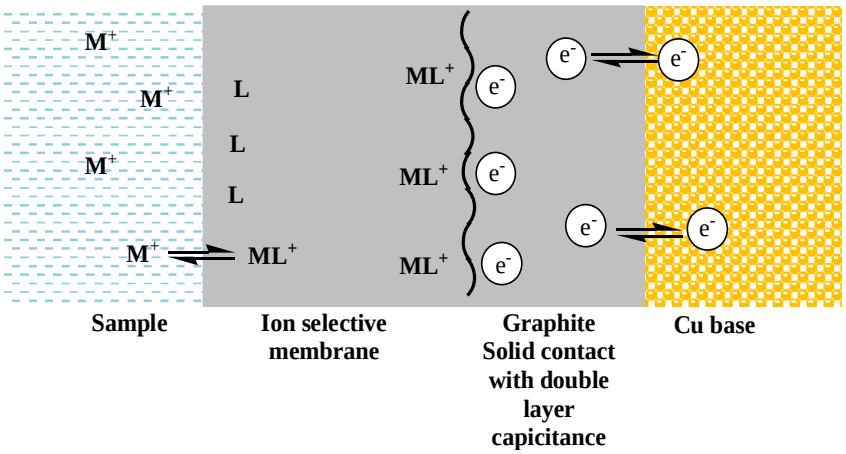

Figure 3. Schematic representation of all relevant interfaces within graphite solid contact ion selective electrode

The effect of adding 30 mol % TPB^- as an anionic additive to MIP/2-VP membrane based sensors caused a noticeableimprovement in the potentiometriccharacteristics. The slope improved to 57.1±0.6 mV $decade^{-1}$, linear dynamic range extended from 7.0 x 10^{-6} to 1.0 x 10^{-2}molL^{-1} and the limitof detection declined to 6.0x10^{-6}mol L^{-1}.

The time needed to attain a stable potential response within ± 1.5 mV using the suggested sensors in 10^{-6} to 10^{-2}mol L^{-1}creatine solutions with a rapid 10-fold rise in concentration was 30 s for theconcentration of ≥10^{-4}mol L^{-1} and 50 s for concentrations <10^{-4}mol L^{-1}. With all sensors checked, the limits of detection, times of response, linear quantification ranges and response slopes were found to be reproducible within ± 2% of their main values over 4 weeks interval.

The robustness of the procedure was conducted by checking the effect of pHalteration and time measuring the accuracy of the results. The pH effect on the potentiometric response of creatine transducers was checked with standard 1.0x10^{-4} and 1.0x10^{-3}mol L^{-1}creatine solutions over a 2-8 pH range. Adjusting the pH of the test solution was carried out using either nitric acid and/or sodium hydroxide solutions.The graphs of *E* (mV) versus pH (Fig. 4) indicated that the response of the sensors was constant over the pH range 2.8–4.5 for both MIP/MAA and MIP/2-VP membrane based sensors. A decrease in the potential response was noticed above pH 5may be due to the formation of neutral creatine in solution. Since the pKa of creatine is 3.4, pH values below the pKa resulted in ~ 99 % ionization (protonation) of creatine. On the other hand, the rise up in the potential at low pH (< 2.5) may be attributed to the response of the membrane to hydrogen ions. All subsequent measurements were measured for all sensors in 5 mmol L^{-1} citrate buffer solutions at pH 3. Under these conditions, creatine is now completely ionized and monitored as $[creatinium]^+$ ion.

The ruggedness of the proposed approach was also checked by doing the measurements using four different electrodes with two different devices on different days. A less than 1.0 % relative

standard deviation (RSD) was noticed for measurements in replicates during 3 days (n=10). The results showed that the suggested approach produced results with high precision and stability and offered several features. These are the low costin measurements, fast response towards creatine, high sensitivity, and good selectivity towards creatine over many organic ions, high accuracy and precision, no sample pre-treatment, minimum manipulation steps, and thepossibility to couple with automated systems.

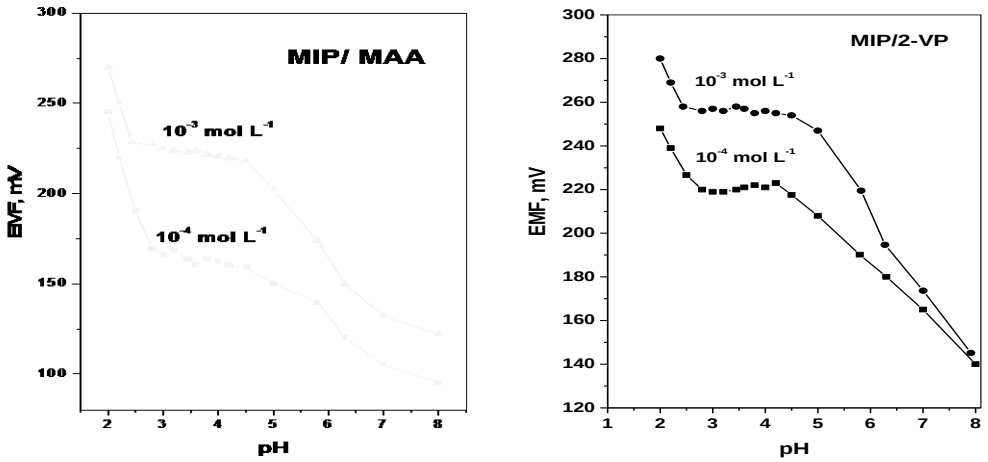

Figure 4. pHeffect on the potentiometric response of creatine membrane sensors

3.2. Sensors' selectivity

Selectivity coefficient values of the proposed transducers towards different cationic inorganic and organic species commonly associated in biological samples with creatine were verified using the so-called "two solution method (TSM)" [46]. The method, it bases on measuring potentials of a pure 10^{-5}molL^{-1}creatine solution, E_A, and a solution containing the same concentration of creatine mixed with 10^{-3}mol L^{-1} of the interfering species, E_{A+B}. The selectivity coefficientvalues weremeasured by substituting the value of the potential difference, $\Delta E = E_{A+B} - E_A$, into the following equation:

$$\log k_{A,B}^{pot} = \log a_A \left(e^{\Delta E Z_A F/(RT)} - 1 \right) / (a_B)^{Z_A/Z_B}$$

Where Z_A and $Z_B$are charge numbers of the creatine, *A*, and the interfering ions, *B*; a_A and a_B are the activities of the creatine, *A*, and the interfering species, *B*; *RT/F* is the slope of the calibration curve and $k_{A,B}^{pot}$ is the selectivity coefficient for creatine against the interfering ion.

Potentiometric selectivities of the produced electrodes are correlated to the favorableinteraction between the recognition sites in the polymer with creatine ion in theacidic medium over many organic ions bearing basic nitrogen and inorganic cations. Selectivity values for a group of possible interfering ions are shown in Table 3.

The selectivity characteristics shown in Table 3 revealed that MIP/MAA ionophore is less permeable to various interferents than MIP/2-VP and hence it is more selective to creatine.

Creatine sensors based on immobilized enzymes [30-33] suffer from sever interference from NH_4^+, urea, creatinine and ascorbate. From all of the above, it can be seen that the proposed sensors show

superior sensitivity and selectivity behavior, and exhibit a better linear response range and lower limit of detection than many of these previously suggested sensors based on immobilized enzymes. Advantages and limitations of these previously reported sensors are presented in Table 1.

Table 3. Potentiometric selectivity coefficients ($k^{pot}_{creat,B}$) of MIP/MAA and MIP/2-VP membrane based sensors.

Interferent	$k^{pot}_{creat,B}$		
	MIP/MAA	**MIP/2-VP**	**MIP/2-VP+ 30 mol % TPB^-**
Creatinine	-1.23	-2.01	-1.10
Leuthin	-4.23	-4.12	-3.53
Aspartate	-4.35	-4.34	-4.01
Cysteine	-2.10	-1.51	-1.32
Glycine	-2.30	-2.34	-2.01
Lysine	-1.60	-2.10	-1.45
Serine	-3.82	-3.80	-3.42
Glutamate	-4.01	-3.66	-3.56
Oxalate	-3.62	-3.52	-3.34
Tartrate	-3.81	-3.90	-3.22
Citrate	-4.05	-3.89	-3.75
Na^+	-2.21	-2.01	-2.10
Mg^{2+}	-1.62	-1.52	-1.55
Ca^{2+}	-1.30	-1.50	-1.21
NH_4^+	-1.52	-1.84	-1.50
K^+	-1.52	-1.85	-1.12

Table 4. Creatinequantification in serum samples using creatinemembrane-based sensor

Sample	**Creatine, µg mL^{-1} ***		
	Spectrophotometry, [34]	**Direct potentiometry**	**Difference**
Serum	21.7±0.9 18.9±0.8 24.5±0.5	19.2±0.6 21.8±0.3 22.2±0.4	+2.5 -2.9 +2.3

* Average of six measurements

3.3. Analytical Applications

Creatine was assessed in different human serum samples collectedfrom volunteers. Table 4showed the results obtained using both the spectrophotometric procedure [41] and the proposed potentiometric approach. *F*-test revealed no considerabledifference at 95% confidence level between mean and variancesets of results for the proposed potentiometric approach and the standard spectrophotometric method. Quality control/quality assurance (QC/QA) of the proposed approach was

checked by daily creatine analysis running in duplicates over 3weeks and using the data obtained for *R* and *X* control charts construction[47].The distribution of measurements and range of quantification under investigation showeda statistical controlfor the results.

Verification of the proposed method for assessing creatine was achieved by calculating the range,linearity and sensitivity, thedetection limit, accuracy and precision, repeatability (CV_w),and between day-variability (CV_b).Data obtained on six batches (six measurements each) using the quality assurance standards [48] are shown in Table 2. These resultsprovided a high support for applying the suggested methods for quality control quantification of creatine.

4. CONCLUSION

The molecular imprinting approach was utilized to produce creatine host-tailored detectors for potentiometric assay. MAA or 2-VP was used as functional monomers to obtain different MIP materials as recognition sites. The proposed potentiometric membrane sensorsshowed good characteristics for the estimation of creatine quantity in biological fluids and pharmaceutical formulations. The construction of these sensors was simple, fast and reproducible. The sensors showed stable and good response features. This approach is cost-effective and presents the features of high accuracy, sensitivity, fast response and the use for direct assay of creatine without sample pre-treatment. Validation of the proposed method supported its use for quality control/quality assurance in the creatine production.

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