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Development and Analysis of Bis[(Benzo-15-Crown-5)-4-Methyl] Pimelate Based Potassium Coated Wire Ion Selective Electrode.

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ABSTRACT

The conventional indirect ISE Sensors made with Valiynomycin are having loss and drift in the membrane potential and need calibration once in every two to three hours. The major issues faced in these electrodes were problems of stability, drift, life and interference due to higher protein content in samples resulting in pseudo-hypometry (false low measurements). This paper presents development and analysis of Poly Vinyl Chloride (PVC) matrix based coated wire Ion Selective Electrode (ISE) sensor for potassium which overcomes the above problem. The Coated Wire Ion Selective Electrode (CWISE) is used as sensor for the measurement of potassium concentration in human Blood samples. A PVC membrane solution is prepared by dissolving 5% Carboxylated PVC, Poly (Vinyl Chloride-co-vinyl Acetate-co-Vinyl Alcohol), O-Nitro phenyl Octyl Ether (2-NPOE) and the sensing element Bis[(benzo-15-crown-5)-4-methl] pimelate using the solvents Tetrahydrafuran and Cyclohexanone. The membrane solution is coated directly on silver wire incorporated in a PVC substrate fabricated as a flow through cell avoiding the intermediate filling solution and the developed sensor is evaluated for selectivity, response time, detection limit, stability and life. It is observed that the sensor is found to be excellent in selectivity and also of longer life. The selectivity coefficient against other Cations like Sodium was found to be $1.5x10^{-3}$. The drift in Membrane potential was found to be less than 1mv /hour which proved a higher stability and suitability of its application for Instruments using direct ISE principle. The developed sensor was proved to have reduced effects of pseudohypometry.

Keywords: CWISE, potassium Ion Selective Electrode, Membrane potential, direct ISE.

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INTRODUCTION

Potassium ion concentration is of clinical importance Nephrology and Neurology. Patients undergoing Dialysis due to renal failure need a continuous monitoring of Potassium level in blood and Urine. Ion selective Electrode (ISE) technique is the replacement of ancient Flame Photometry methodology of Electrolytes analysis. Conventionally used ISEs are mostly based on indirect potentiometry where either there is an internal filling solution between the membrane and the contact wire or the samples are diluted with diluents of specific ionic strength [1]. The membrane potential reaches the Instrument after its transport through this intermediate electrolyte solution between the membrane and the wire contact. In this case there are more chances for loss in the membrane potential resulting in drift. Due to these reasons, the indirect potentiometry need calibration once in every two to three hours and they need dilution for samples with higher concentrations of potassium. Also errors and interferences in measurement due sample dilutions and protein content in samples (Pseudo-hypometry) may occur. The success of any ISE is in selecting the membrane composition. The major issues in ISE are Life of the sensor, drift in membrane potential and interference due to other cations and anions. The drift is caused due to the presence of protein in the sample and deterioration of ionophore. The life of the ISE sensor is affected mainly due to the leaching out or deterioration of the ionophore due to its water soluble property and poor adherence of membrane to the metal surface. Previous studies show a better life for the membranes based on Carboxylated Poly Vinyl Chloride instead of conventional PVC [2,3]. Blood samples contain protein. So in order to reduce the induced effect of protein content (Pseudo-hypometry) in test samples we choose Poly Vinyl Chloride and Vinyl Alcohol [4]. A mixture of Carboxylated PVC and Vinyl Chloride – Vinyl Alcohol in 70% and 30 % can be expected to fulfill our requirement of long life and reduced effect due protein presence. We have chosen Bis [(benzo-15-crown-5)-4-methyl] pimelate as the sensing element which has been proved to give better life time for electrodes and having reduced deterioration or leaching effects. O-Nitro Phenyl Octyl Ether was been proved to be the best plasticizer than any other plasticizer for the electrodes with crown ether ionophore [5-7]. For reducing the interferences due to anions like chloride, nitrate etc., potassium tetrakis (p-Chlorophenyl) borate is included in trace quantity [8]. The solvent Cyclohexanone is selected in addition to the generally used Tetrahydrofuran (THF) as preferred for CWISE [9]. This enables better adherence of membrane to the metal surface due to it slower evaporation.

This paper explains the development and property study of Potassium CWISE. In the developed ISE sensor the ISE membrane is coated directly on the contact silver wire thereby avoiding the intermediate filling solution between the silver wire and the membrane [10]. As there is no drift or loss of membrane potential the developed sensor needs Calibration once in a day and it can measure high value samples and urine directly without any dilution. The sensor can sense the ionic concentrations directly without any intermediate solution so we call this technique as Direct Potentiometry or Direct ISE. Fixed interference method is used to evaluate the selectivity coefficient of the developed sensor as recommended by IUPAC (International Union of Pure & Applied Chemistry) [11,12].

MATERIALS AND METHODS

The chemicals required for the membrane i.e Poly (Vinyl Chloride) 5% carboxylated, Poly (VinylChloride-co-vinylAcetate-co-VinylAlcohol), Potassium tetrakis (p-Chlorophenyl) borate (KTPB) and O-Nitro phenyl Octyl Ether (O-NPOE) are purchased from Sigma-Aldrich. The solvents Tetrahydrofuran (THF <0.2% water) and Cyclohexanone are purchased from Fischer Scientific. Low melting point agrose is purchased from SRL research laboratories for reference gel formation. Sodium Chloride, Potassium Chloride and Sodium Bicarbonate salts from Qualigens Fine Chemicals.

Preparation of calibrators and Test solutions

About 7.455g of potassium Chloride is dissolved in one liter of double distilled water. This solution contains 100 mmol/L of potassium (0.1 M) and is called as the stock solution. Likewise stock solutions are prepared for 1.0Molar Sodium Chloride, 1.0 Molar solutions of Sodium Bicarbonate. The process of dilution is used to prepare the calibrators with 4.0 and 10.0 mmol/L of potassium concentration. The same procedure of dilution is used for preparing the other test solutions required at various concentrations for the evaluation of the developed sensor. The dilution process is followed as per the relation (1),

$$V_1 \times C_1 = V_2 \times C_2 \text{ .... } (1)$$

Where V_1 and V_2 are initial and final volume of solutions and $C_1$and C_2 are the corresponding potassium ion concentrations. Electrolyte solution is prepared with 250 mmol/L of Sodium, 15 mmol/L Potassium, 100 mmol/L of Bicarbonate and 150 mmol/L of Chloride ions using Sodium Chloride , Potassium Chloride and Sodium Bicarbonate salts. These concentrations are selected just above their normal ionic contents in human blood. Normal values of these ions in human blood are sodium 130-150 mmol/L, potassium 3.2-5.5 mmol/L, Chloride 75-110 mmol/L and Bicarbonate 25-40 mmol/L. The design plan consists of two stages namely 1) development & Evaluation of potassium coated wire ISE (Flow through Cell) and 2) Development and evaluation of automated Potassium Analyzer using Potassium CWISE.

Development and Evaluation of CWISE

Machined PVC Substrate

The PVC rod of 8mm diameter is selected and machined as shown in Figure.1 for preparing the potassium and Reference ISE. The PVC machined probe of potassium ISE is having a straight 1mm hole from the top to bottom with a small projection of 4mm length, outer diameter of 3mm and an inner diameter of 2mm. In the reference ISE probe it is drilled with a 3mm hole without any projection at the bottom.

Preparation of Potassium ISE probe

A silver wire (99.9% silver 0.5mm) is inserted in to the Potassium ISE probe and pasted at the tip using fast curing epoxy adhesive (Fevitite Rapid). The tip is dried for 30minutes. The silver wire at the tip is cut and scratched with surgical blade. Silver loaded epoxy purchased from RS Components is taken and equal quantity of Part A and Part B are mixed and then packed above the scratched tip of the silver wire [13]. Application of silver epoxy is to increase the surface area in which the membrane is to be coated. The silver epoxy is allowed to dry for 24 hours. The Silver epoxy packed area is then drilled from the bottom with 2mm drill so that a hole of 2.5mm depth is created. Thus the left out silver epoxy will be of 1 to 1.5mm above which the membrane will be coated. A Teflon sleeve of 0.5mm inner diameter and sufficient length is then inserted from the top of the silver wire in order to prevent the wire from rusting due to moisture. The probe is then packed with a cap and pasted with epoxy adhesive in such a way that the wire does not shake. The silver wire is then cut with sufficient length properly insulated and connected with a point insert connector with proper insulation.

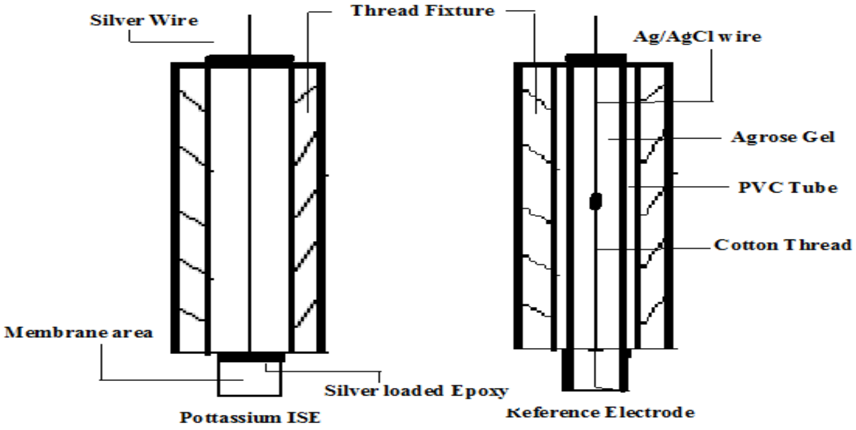

Figure 1: The ISE Modules of potassium and Reference PVC rod

Membrane Preparation and Coating

The membrane solution is prepared according to the recommendations of CWISE i.e a membrane solvent ratio of 1:3. The Membrane is prepared with 33% of membrane elements and 67% of the solvent. The membrane components are chosen in the ratio 1:33:66, ionophore, PVC and Plasticizer (O-NPOE) respectively [14, 15]. The additive salt Potassium tetrakis (p-Chlorophenyl) borate (KTPB) is added in trace quantity i.e less than 10% by weight of ionophore. The total content of PVC is chosen to be 70% Carboxylated PolyVinyl Chloride and 30% Poly (Vinyl Chloride-co-vinylAcetate-co-Vinyl Alcohol). The Membrane components weighed are dissolved in 3 times by membrane weight of solvents, THF (70% by weight) and Cyclohexanone (30%by weight). Generally THF is used as a solvent for ISE. THF is fast evaporating than Cyclohexanone. The slow evaporation of Cyclohexanone enables a slow curing of the membrane with assured adherence and firm coating on the metal surface. The prepared membrane container is packed with firm cork to avoid evaporation and allowed to dissolve completely for 4-5 hours. The completely dissolved potassium membrane solution is then taken in a glass syringe or a micropipette. The Potassium ISE probe is then fixed on a rotating platform with the tip facing upwards. The platform is rotated coaxially at 5rpm. Very small drop of the membrane solution was dispensed on the silver epoxy area. Each drop is allowed to cure for at least 1 hour. Care is taken so that the membrane thickness should not reach outside the tip level.

Reference probe preparation

Ag/AgCl reference [16, 17] probe is the best reference half-cell noted for stability and longer term survivability and prepared using the following procedure. The reference probe is prepared first by inserting a PVC tube of 3mm outer and 1mm inner diameter. The tube is projected to 4mm at the bottom and pasted with epoxy adhesive so that it is fixed firmly with the side walls of the PVC machined reference probe Silver wire (0.5mm 99.9%) of sufficient length is taken. The tip of the wire (10mm length) is chloridized by soaking in 10 % HCl for 15 minutes and 10% $FeCl_3$ for 15 minutes. After washing with distilled water it is then dried properly. A cotton thread of sufficient length is tied to the middle of the chloridized tip and the chloridized area is twisted well. The silver wire with thread knot is then inserted from the bottom of the PVC tube in such a way that the thread knot and the chloridized area is well above the middle of the reference probe. This ensures stability of the reference potential.

Preparation of Agrose gel

Low melting point agrose is used to form the reference gel. About 2g of agrose is mixed with 20ml of the prepared reference electrolyte solution and 10ml of Ethylene Glycol in a conical Flask. Ethylene Glycol helps in the maintenance of moisture of the reference gel during storage. The mixture is allowed to boil in a water bath. Agrose melts at about 50-60 degree C. Once completely melted the gel is aspirated into the reference PVC tube using a syringe and allowed to cool. Gel is formed inside the PVC tube surrounding the silver wire and cotton thread. The bottom tip of thread is cut and pasted to the side of the PVC tip. The top side of the tube is fixed firm with epoxy adhesive. The probe end is packed with a cap after cutting the silver wire with sufficient length. The wire is then insulated properly and connected with an insert point connector. The Ag/AgCl reference probe is ready for use. It contains approximately 10 mmol/L of potassium apart from other ions sodium, bicarbonate and chloride. Before its first usage both the probes are pre-conditioned by soaking in a standard solution containing 4 mmol/L of Potassium for 1 hour. The probes so developed are termed as Potassium and reference half-cells. Both the Potassium and reference probes together are called as the Potassium ISE.

RESULTS AND DISCUSSION

The evaluation of the developed potassium ISE is studied for selectivity coefficient, linearity, detection limit, response time, stability and its life.

Response Time and Nernstian Response

Response time is nothing but the time required for the developed sensor to produce stable potential when changed from one concentration to the other. The recorded time to reach a stable potential in each measurement of calibrators were found to be between 3-6 seconds. So the developed sensor was evaluated also to have a good response time. The Nernst response of the developed Potassium ISE is verified by the two point calibration with two standards of different concentrations of potassium ions. The standards with of 4.0 mmol/L and 10.0 mmol/L Potassium ion concentrations are prepared by diluting 0.1 M potassium chloride stock solution. The electrode was dipped in both the solutions and the potentials were noted after attaining a stable voltage using a digital Voltmeter. The values are recorded in Table.1

Table 1: Calibration potentials of developed Potassium ISE

Calibrator	Potential mV
4.0 mmol/L	-32.73
10.0 mmol/L	-8.70

The slope of the calibration is calculated using the Nernst equation as shown in equation (2),

$$E = E_0 + S \log (K) \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where E_0 is the standard reference half-cell potential, S is the slope of calibration and K the activity of potassium ion concentration. The slope is then calculated from equation (3).

$$\text{Slope} = (E_2 - E_1) / \log (K_2/K_1) \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where E_1 & E_2 are the potentials developed by the Potassium ISE for the corresponding concentrations of concentrations 4 and 10 mmol/L of potassium (K_1 &. K_2).

The calibration curve which is shown in Figure.2 well agrees with Nernstian response [20]. This is called as two point calibration since we choose two calibrators. The Slope of calibration was calculated using equation (3) is arrived to be 60.4 mV/decade which correlates well with the slope of those Potassium electrodes developed using Valinomycin.

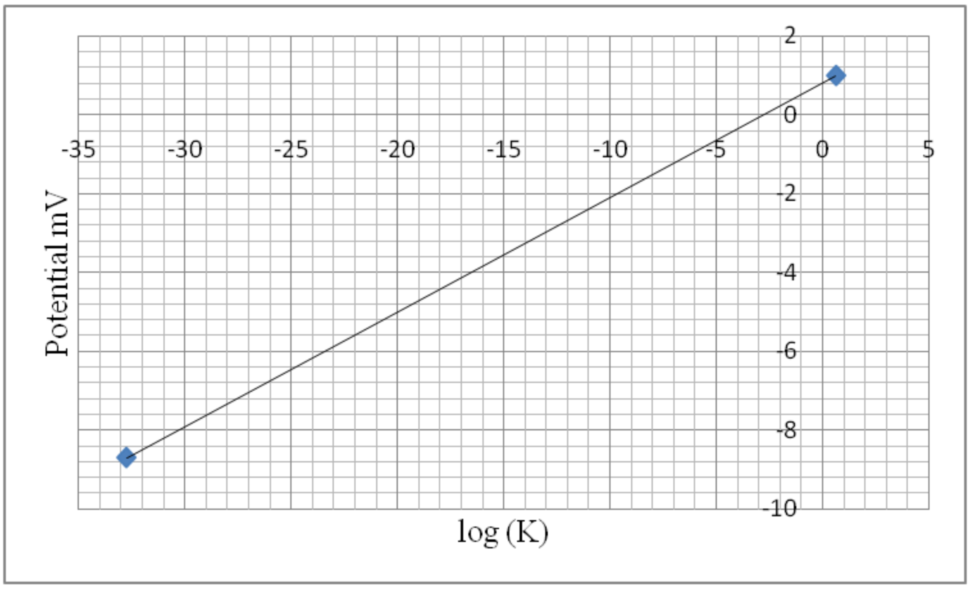

Figure 2: Calibration Curve

Detection Limit, Linearity and Selectivity Coefficient

The Potassium CWISE is evaluated for selectivity coefficient over sodium ion which is the major constituent (130-150 mmol/L) in human blood sample compared to other interfering ions like Lithium and Ammonium. As per the recommendations of IUPAC, Fixed interference method [18, 19] of mixed solutions is used to determine the selectivity coefficient of potassium in the presence of sodium. The probes are dipped into different solutions of varying potassium ion concentrations varying from 0.1-400.0 mmol/L and fixed sodium ion concentration (200mmol/L). The potentials of potassium ISE with respect to the reference probe are recorded using digital Voltmeter along with the corresponding time in seconds for the potential to attain stability when changed from one concentration to the other using a digital timer and the results are recorded. The limit of detection is the lowest and highest level of concentration of the potassium that the developed ISE can detect or sense.

A graph plotted with Potential of potassium ISE against logarithmic activity of potassium concentrations under fixed sodium concentration shows a linear straight line as shown in Figure.3. From the graph we could conclude that the developed sensor could detect the potassium ion concentrations from 0.3-300 mmol/L. The deviations in the curve at potassium ion concentrations of 0.3 and 300 mmol/L explain the lower and higher detection limits of the developed sensor. The curve also proves a good linearity of the sensor from 0.3 – 300 mmol/L.

The selectivity coefficient is calculated using the modified form of Nicolsky-Eisenman equation,

$$K_{A\,B}^{Pot} = a_A / (\,a_B)^{ZA\,/ZB} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Where a_A is the activity of potassium in the intersection of the extrapolation of the linear portions of the curve, a_B is the fixed activity of Sodium ion. ZA and ZB are the charges on the potassium and sodium ions. The graph shows a very good linearity from lower level of 0.5 mmol/L to 75 mmol/L with a slight extrapolation near 40 mmol/L. Taking this to be $a_{A,}$ from the equation (1) we arrived at the selectivity coefficient of potassium developed ISE to be equal to 1.5×10^{-3} which less than 1 and means that the developed electrode is best selective to potassium than sodium. The linearity level is too high up to 300mmol/L which is well above the normal values of Potassium in Blood samples. This property of the sensor enables the possibility of direct measurement of potassium ion concentration even in the urine samples.

Life of the Electrode

The life of the developed potassium ISE is studied by the observed drift in the membrane potential with respect to time. The electrode if dipped into a solution containing a known and constant concentration of potassium ion (4.0mmol/L). The potentials of the electrode were recorded at different time intervals It seems that there is more change in potential initially at the rate 42 mv/hour and after 30 minutes it came down to around 1 mV/hour. The trend of drift or change in potassium ISE potential is shown in Figure.4. This drift in the membrane potential is mainly due to leaching out of ionophore from the surface of membrane and a shift in the standard reference electrode potential. The changes in the slope and detection limit over the period of time reveals the life of the electrode [21].

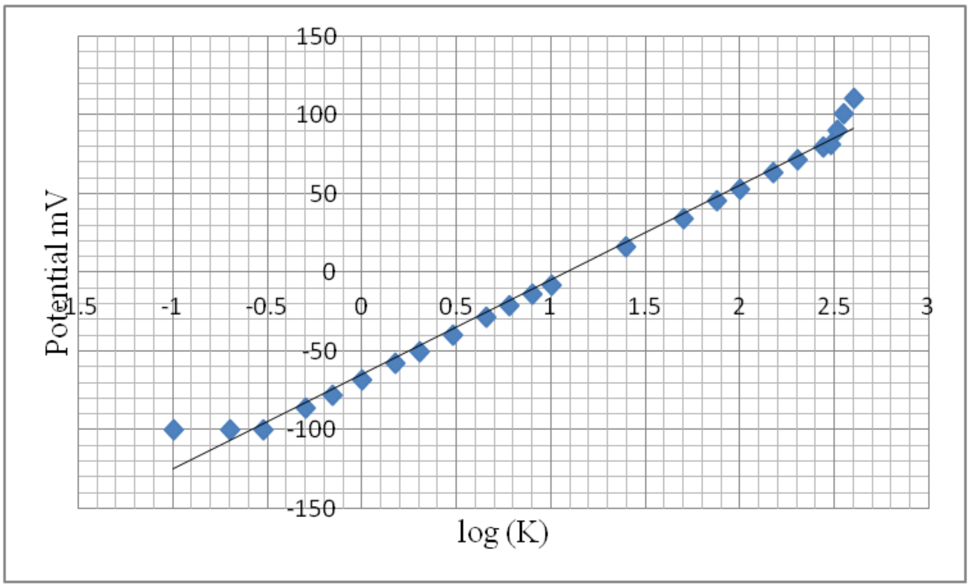

Figure 3: Log a(K^+) vs Potential Curve

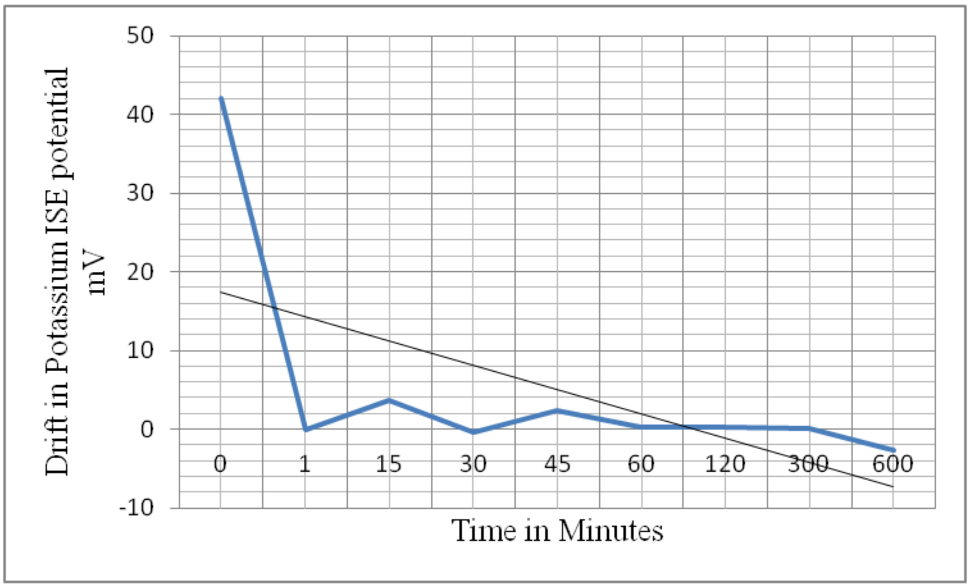

Figure 4: Trend of Change in Potassium ISE potential per hour at constant concentration.

Table.2 shows that the change in slope and the detection limit as observed over the period of 300 days. The change in the slope is from 61.5 to 56.9 mV/decade and detection limit from 290 to 175 mmol/L during the period of observation. This explains that the deterioration or leaching of ionophore from the membrane surface could be considered to be very low and the electrode is able to maintain the linearity even after 10 months. So we could expect a minimum life time of a 12 to 18months for the developed sensor.

Table 2: Slope and detection limit of the developed Electrode over the period of time.

No of days	Slope mV/decade	Detection limit
1	61.5	225
5	61.1	225
10	60.9	225
30	59.8	225
45	59.9	210
60	59.3	200
90	59.8	200
120	60.2	200
180	58.9	175
240	57.8	175
300	56.9	175

Evaluation of the developed Sensor with Conventional Instruments

The developed sensor was also evaluated for its quality and agreeability with the conventional instruments. The blood samples are collected from various patients in a hospital and the samples are prepared to get the serum. The best sample type for any Electrolytes measurement is Blood serum than the plasma. Serum is the purest form where as plasma contains some additives called anticoagulants. The collected blood samples are allowed to clot in a test tube for 30 minutes and then centrifuged to about 1000 rpm. The clear pale yellow solution got as the supernatant is called the serum. The results of potassium ion concentrations of human blood serum samples with the developed sensor were compared with those results obtained from Systronics Flame-photometer and Beckman Coulter Auto-analyzer (Au480). From Table.3 it is observed a correlation of results between the developed and conventional instruments. The statistical analysis from the Figures.5 and 6 shows a correlation coefficient of 0.83 between the developed and conventional instruments Flame Photometer and Au480.

Table 3: Comparative Results of Potassium ion concentration between developed and conventional Instruments

Sampe no	Developed Sensor	Flame-photometer Systronics	Beckman AU480
1	3.51	3.47	3.51
2	3.23	3.08	3.03
3	3.42	2.85	2.79
4	4.66	4.72	4.56
5	4.31	4.02	4.21
6	6.90	6.61	6.72
7	4.12	3.15	2.98
8	5.52	5.64	5.57
9	4.13	4.08	4.11
10	4.75	2.97	2.85
11	3.56	2.32	2.48
12	6.46	6.23	6.51

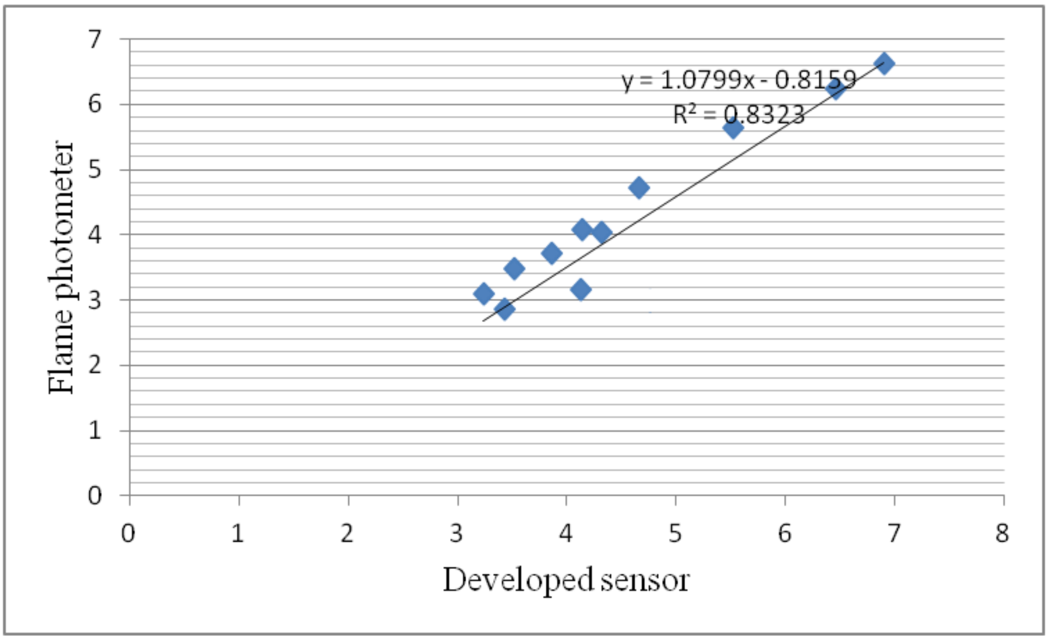

Figure 5: Correlation curve of results between developed Sensor and Flame Photometer.

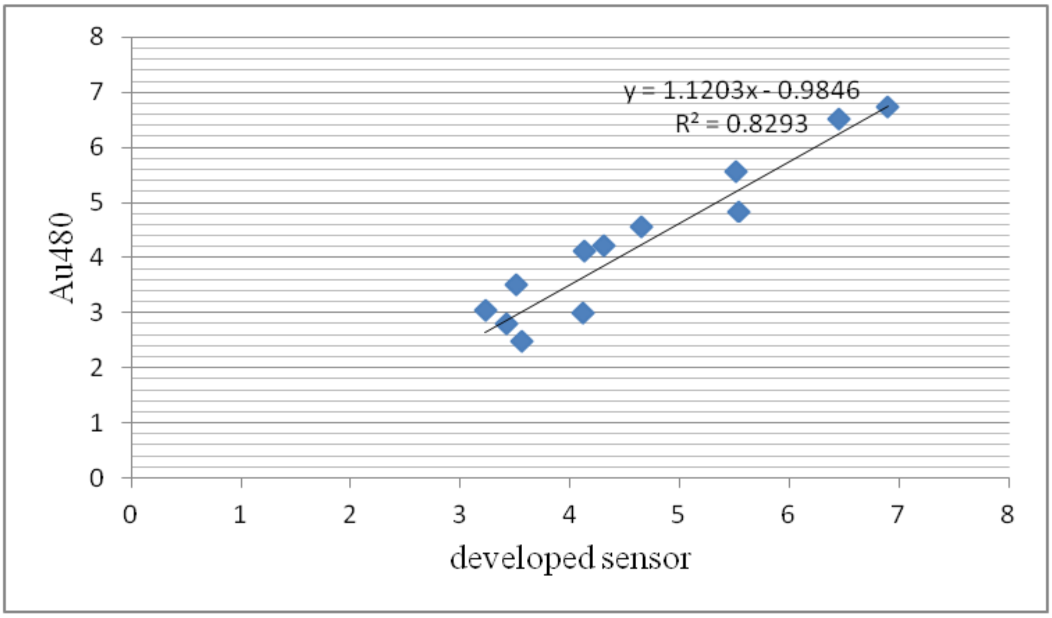

Figure 6: Correlation curve of results between developed Sensor and Beckman Au480

Assuming E_X as the potential of CWISE, the unknown concentration K_x of potassium in blood sample can be calculated in mmol/L using equation 2 and 3.

Pseudo-hypometric effects

From table 3 we observed that there was a large deviation in the values for sample numbers 3, 7, 9 and 10. When cross checked with the patient conditions we found those samples contain either higher protein or cholesterol i.e. hyperproteinemia or hypercholesterolemia. As explained earlier we could conclude that those low value measurements by other instruments were due to the interference of higher protein or cholesterol content in the samples. These low value samples were measures as normal values by the developed sensor. Thus the falls low measurement (pseudo-hypometry) due to protein and cholesterol was not observed in the developed sensor. On eliminating these deviating samples we could observe a correlation coefficient of comparison between the developed sensor and the conventional instruments was found to be 0.99 as shown in figure 7 and 8.

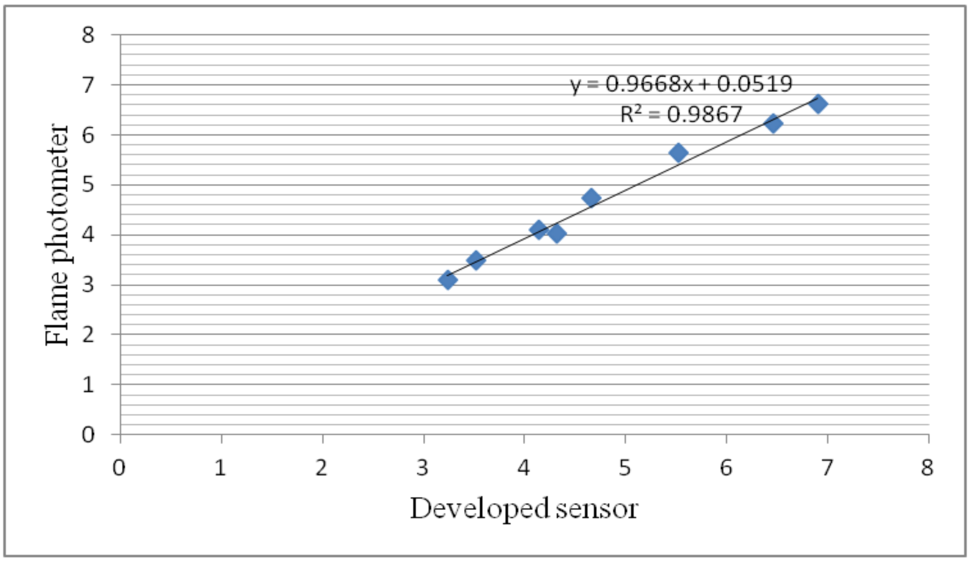

Figure 7: Correlation curve of results between developed Sensor and Flame Photometer eliminating low value samples

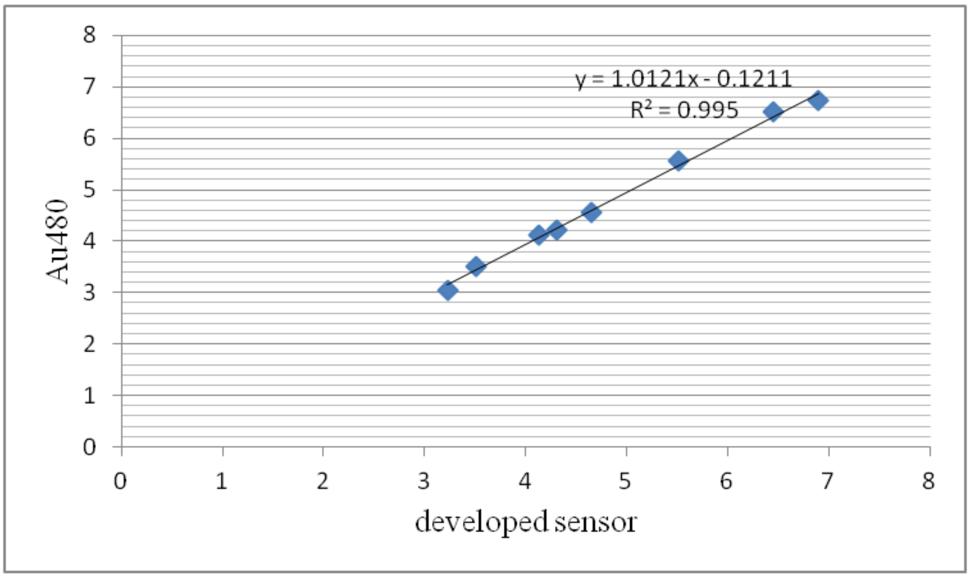

Figure 8: Correlation curve of results between developed Sensor and Beckman Au480 eliminatingt low value samples.

Check for Quality, Accuracy & Precision.

The worldwide accepted quality check procedure is to compare the target values of standard quality controls with allowed standard deviation. We have chosen Bio-Rad serum quality controls (Liquid Assayed Multiquals) Level 1, 2 and 3 [22]. The target values and ranges are specified in Table.4. The results obtained by running these quality controls as samples with the developed Sensor are recorded in Table.5. It is observed that the results were close to the target values as recommended by Bio-Rad without much deviation.

Table 4: Bio-Rad Liquid assayed Multiqual Quality control data Lot No.45650

Multiqual Liquid Assay	Target Potassium mmol/L	Range mmol/L
Level 1	2.41	2.17-2.65
Level 2	3.98	3.58-4.37
Level 3	7.7	6.93-8.47

Table 5: Bio-Rad Quality controls analysis using the designed sensor

Multiqual Liquid Assay	Potassium mmol/L (Developed Sensor)
Level 1	2.38
Level 2	4.11
Level 3	7.65

CONCLUSION

From the above analysis and study it is concluded that the Potassium CWISE developed is having low drift, high stability and good linearity. This property of the sensor allows it to be used for direct ISE measurement where there is no need for sample dilution. The higher stability allows us to design the instrument with once in a day calibration where we can save the cost of calibrators. Being very simple fabrication process it is very easy to make a set up for bulk production. The higher linearity and stability of developed sensor enables it to be used for direct measurement of potassium in Urine and dialysate fluids. Reduced leaching and reduced effect of protein content in samples ensures that the developed sensor eliminates the possibility of Pseudohypometey i.e. false low measurements. Pseudohypometey is unavoidable in cases of Flame-Photometer and Indirect ISE. The longer life assurance enable the Sensor can find application in Whole blood sample analysis which is important for ICU patients where there will insufficient time to prepare the serum. The statistical study showed a correlation coefficient of 0.99 in comparison of results of developed sensor and conventional instruments.

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