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RESEARCH ARTICLE

Optimization of Extraction Parameters of Reverse Iontophoretic Determination of Blood Glucose in an Artificial Skin Model

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Abstract: ***Background*:** Reverse İontophoresis (RI) is one of the promising non-invasive technologies. It relies on the transition of low magnitude current through the skin and thus glucose measurement becomes possible as it is extracted from the surface during this porter current flow.

***Objective*:** This paper deals with the development and optimization of an RI determination method for glucose. CE dialysis membrane based artificial skin model was developed and the dependence of RI extraction on various experimental parameters was investigated.

***Methods*:** Dependence of RI extraction performance on noble electrodes (platinum, silver, palladium, ruthenium, rhodium) was checked with CA, CV and DPV, in a wide pH and ionic strength range. Optimizations on inter-electrode distance, potential type and magnitude, extraction time, gel type, membrane MWCO, usage frequency, pretreatment, artificial body fluids were performed.

***Results*:** According to the optimized results, the inter-electrode distance was 7.0 mm and silver was the optimum noble metal. Optimum pH and ionic strength were achieved with 0.05M PBS at pH 7.4. Higher glucose yields were obtained with DPV, while CA and CV achieved almost the same levels. During CA, +0.5V achieved the highest glucose yield and higher potential even caused a decrease. Glucose levels could be monitored for 24 hours. CMC gel was the optimum collection media. Pretreated CE membrane with 12kD MWCO was the artificial skin model. Pretreatment affected the yields while its condition caused no significant difference. Except PBS solution (simulated as artificial plasma), among the various artificial simulated body fluids, intestinal juice formulation (AI) and urine formulation U2 were the optimum extraction media, respectively.

***Conclusion*:** In this study, various experimental parameters (pretereatment procedure, type and MWCO values of membranes, inter-electrode distance, electrode material, extraction medium solvents, ionic strength and pH, collection medium gel type, extraction potential type and magnitude, extraction time and *etc*) were optimized for the non-invasive RI determination of glucose in a CE dialysis membrane-based artificial skin model and various simulated artificial body fluids.

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1. INTRODUCTION

According to the World Health Statistics 2018 Report [1], published by the World Health Organization which provides health statistics for its 194 member states, the estimated number of deaths that occurred because of non-communicable diseases (NCDs) was calculated to be 40 millions. There were four main NCDs groups that reported the majority of the estimated total deaths. These were, in descending order, cardiovascular diseases (totally 17.7 million deaths, which formed 45% of the sum); followed by various types of cancers (total 8.8 million corresponding to 22%); respiratory diseases of various chronic types (total of 3.9 million deaths indicating 10% of the sum); and finally type 1 and type 2 diabetes (totally 1.6 million deaths (4%). For the diagnosis and management of diabetes mellitus (DM), the mean value of the fasting plasma glucose level is a golden standard as indicated in the same report. It is estimated that the prevalence of DM will increase almost up to 600 million people by 2030 [1]. In 2017, according to another report published by the International Diabetes Federation [2], worldwide, about 415 million adults were diagnosed with diabetes.

Thus diabetic complications and disorders seem to be one of the main issues of health policies worldwide, for today and most probably for tomorrow as well. While fighting this

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virtual war, there is only one true indicator present, the blood glucose level ($mg.dL^{-1}$).

Determination of glucose level is an old issue [3], the idea of building an enzyme electrode (biosensor) for measuring glucose levels was first implemented by Clark and Lyons in 1962 [4]. Till today, an ongoing progress is witnessed based on the fruitful efforts in the field [5]. Besides conventional invasive approaches, non-invasive glucose determination is another difficult task for glucose sensing platforms and expressive research works have been concentrated for decades to actualize this purpose [5].

For non-invasive glucose monitoring, there are eleven technologies present in R&D or at marketing level [5-9]. Mainly spectroscopic techniques are the common promising solutions for non-invasive approaches [5-9]. These spectroscopic approaches use the physical interactions of light in various body fluids. Based on the physical properties monitored during these interactions, there are six common approaches, namely; photoacoustic spectroscopy, Fourier transform IR spectroscopy, spectropolarimetry, Raman spectroscopy, and finally spectroscopic optical coherence tomography [5, 6, 8]. Regarding the transdermal approaches, GlucoWatch Biographer, introduced by Cygnus, Inc., was the first biomedical device that was commercially available owning to noninvasive sensing technology based on transdermal approach for glucose quantification. Soon before its release, it was approved by FDA on August 26, 2002 [10]. The design of this biomedical device resembled a wristwatch and its technology was based on the extraction of the interstitial fluid by RI. Despite its innovative design, it never reached satisfactory sale numbers. This might be due to some problematic facts observed essentially because of the nature of RI, namely; the long time needed for equilibrium and warm up, poorer accuracy in comparison to conventional invasive solutions, increase in the irritation and sensitivity of the epidermis, uncomfortable feeling due to excess sweating. Independently of these and other problems, significant works were carried out and important advancements were achieved for developing and progressing the idea of monitoring glucose with a non-invasive biomedical device. Despite all these efforts, as is known, a fully accurate, precise and reproducible non-invasive device, capable of monitoring glucose, has not been commercialised yet. According to a recently published report, there are currently five strategies under development for commercial non-invasive glucose monitoring for diabetes [11]. These strategies are classified as; minimally invasive systems, light-based methods, earlobe clips, smart contact lenses and sweat patches. RI is classified as a minimally invasive system.

Working principle of RI is the transition of a low magnitude current through the epidermis and glucose measurement is possible because glucose is extracted through the epidermis surface during this convective flow. Some research groups dominated the RI research area since the late 1990s. Guy *et al*. [12-18] followed by Connoly *et al*. [19-25] were the research groups that mainly described the basic principles of this method. They focused on various experimental parameters that directly or indirectly affected the RI phenomenon for various analyte groups. Additionally, Connolly *et al*. [20, 21] and some other research groups [26, 27] even designed portable and programmable RI devices.

In the current study, various experimental parameters were optimized for the RI determination of glucose in a CE dialysis membrane-based artificial skin model and various simulated artificial body fluids. Findings and experimental data proved to be novel. To the best of our knowledge, there is no paper present in the literature discussing the same experimental parameters in detail.

2. MATERIALS AND METHODS

2.1. Chemicals

Methylcellulose (36718) with 4000cPs viscosity was obtained from Alfa Aesar, carboxymethyl cellulose (419273-1KG) was obtained from Sigma-Aldrich, sodium carboxymethyl cellulose was obtained from a local source; Doğa İlaç Hammaddeleri Tic Ltd, hydroxypropylmethylcellulose (Methocel ID34767) was obtained from Colorcon Ltd. Potassium chloride (12636-1KG), and sodium chloride (31434-5KG) were purchased from Sigma-Aldrich Inc, while calcium chloride (1.02387.1000), magnesium chloride hexahydrate (5832.1000) and D(+)-Glucose (1.08337.1000), were obtained from Merck KGaA. Dipotassium hydrogen phosphate (60356) and potassium dihydrogen phosphate (60230) were received from Fluka Inc. Sodium hydroxide was purchased from Sigma-Aldrich (06230) and hydrochloric acid (fuming 37%) was obtained from Merck KgaA (1.00317.2501). Silver nitrate was obtained from Carlo Erba Reagents (423955). Atomic spectroscopy standard solutions of rhodium (207411-100ML), and ruthenium (207446-100ML) were obtained from Sigma-Aldrich, while palladium (76035) and platinum (80964) were purchased from Fluka.

Water used for any scientific purpose was ultrapure water, that has a total organic compound level (TOC) of <10ppb and a total ion resistance of >18,2MΩ.cm, and was produced freshly by the Millipore MilliQ Gradient A10 system.

Cellulose ester (CE) VISKING dialysis membrane with 12000 Daltons MWCO value was obtained from the Medicell Membranes Ltd while 14000 and 2000 Daltons MWCO membranes were purchased from Sigma-Aldrich (D9527-100 FT Cellulose membrane and ALDRICH D7884-10FT Benzoylated membrane, respectively). All these membranes were used as artificial skins.

2.2. Instrumentation

All electrochemical processes, including electrodeposition and RI extraction steps, were performed with Bioanalytical Systems Inc (West Lafayette, Indiana, USA) BAS100B/W electrochemical workstation and PalmSens BV (Randhoeve, Houten, Netherlands) the electrochemical sensor interface used in combination with CH8 8-channel multiplexer. During RI procedures extracted, glucose samples were simply quantified by the blood glucose meter named Accu-Chek Active of the Roche Diagnostics.

2.3. Procedure

2.3.1. Electrode Design

All printed circuit board (PCB) electrodes were printed on 1mm thick conventional copper (Cu^0) circuit boards. There are two sets of four electrodes printed on the copper circuit board. Four electrodes of each set are designed as extraction electrode (ExE), Ag/AgCl reference electrode (RE), auxiliary electrode (AUX), and working electrode (WE), respectively. The detailed dimensional scheme is provided in the supplements section (*Supplement 1*). The ExE and WE have circular diameters of 13 and 8mm and their corresponding theoretical areas of 52.13, 50.24mm^2, while RE and AUX have 9.52 and 9.01 mm^2, respectively. The exact active surface areas of each section (ExE, WE, RE and AUX) of the PCB electrodes were determined with chronocoulometry [28] after fabrication for each batch. The application sheet used for this purpose is also provided in supplements section (*Supplement 2*) and the corresponding mean area values (n=10) were 53.30±0.71, 51.48±0.63, 11.82± 0.39, 9.58±0.16 for ExE, WE, RE and AUX, respectively. The relevant experimental data and corresponding chronocoulometric graphs are provided as supplementary materials (*Supplements 3-7*).

Freshly printed circuit board sheets (containing printed PCB electrodes) were kept in 3M aqueous HCl solution (containing 3% w/w H_2O_2) for 10 minutes to get rid of unprinted excess copper layers and finally rinsed with ultrapure H_2O for 30 seconds and dried under N_2 gas flow for 3minutes.

Later, the ExEs, of these dried PCBs, were modified by electrodeposition of silver from basic aqueous solution as first reported by Natarajan *et al.* [29]. Briefly, ExE was immersed in a 250mL aqueous solution, prepared by dissolving $AgNO_3$ (8.75g), $(NH4)_2SO_4$ (37.5g), citric acid (1.0g), $FeSO_4$ (0.375g) and NH_3 (up to pH 10.0), and a current magnitude of 0.5mA/cm^2 was achieved by applying -2.0V (*vs.* Ag/AgCl) at 25°C for 1 hour. Freshly coated ExE was rinsed in ultrapure water for 5 seconds. A schematic drawing of the PCB electrodes (Fig. **S1** in Supplement 1), and SEM images of plain and modified surfaces (Fig. **S2** in Supplement 1) are provided as supplementary material.

2.3.2. RI Extraction Cell Design

The dimensions and schematic view of the designed cell are provided in the *supplementary material (*Supplement 1*)*. The cell has two sections; a collection chamber with 17mL volume (Fig. **S3A** in Supplement 1) and a reservoir of 6mL (Fig. **S3B** in Supplement 1). The pictures of the manufactured cell in disassembled and assembled forms are also given (Fig. **S3C** in Supplement 1). Pictures of the benchtop view of the whole RI extraction system (Fig. **S4A** in Supplement 1) and a closer look (Fig. **S4B** in Supplement 1) of the simultaneous RI extraction performed in eight independent channels are also provided as supplementary materials.

2.3.3. RI Extraction Methods

The reservoir of the cell was filled with PBS (of 0.05M and pH 7.4) solution containing a desired concentration of glucose (usually 200mg.dL^{-1}) and the CE dialysis membrane with desired MWCO (usually 14kD) was used as the artificial skin. The collection chamber was filled with the desired cellulose gel (usually 4g/300 mL MC). Different electrochemical methods, continuous Constant Potential (CA), Differential Pulse Varying Potential (DPV) and Cyclic Varying Potential (CV) techniques, are used for RI, the technical specifications of which are summarized in Table **S1** provided in the supplementary material. A sampling of MC gel from collection chamber was performed by pipetting out 20 µL gel and extracted glucose samples were immediately quantified.

2.3.4. pH Study

The contents of the buffers used in the pH study are summarized in Table **S2**, and provided in the supplementary material. In the first option, only the pH value of the collection chamber was optimized. The reservoir of the cell was filled with PBS (of 0.05M and pH 7.4) solution containing 200 mg.dL^{-1} glucose and a CE dialysis membrane with 14kD MWCO were used as the artificial skin. The collection chamber was filled with MC gel (4g/300 mL), that was prepared with pH 1; 2; 3; 4; 5; 6; 7; 7.4; 8; 9; 10; 11; 12 and 13 buffers. In the second option, the pH values of both the reservoir and the collection chamber were optimized. Thus both the MC gels and 200 mg.dL^{-1} glucose solution were prepared with pH 1; 2; 3; 4; 5; 6; 7; 7.4; 8; 9; 10; 11; 12 and 13 buffers. In both the options, CA was performed at +2.0V (*vs.* Ag/AgCl) and 25°C for 3 hours. Just after the RI extraction, sampling of MC gel from collection chamber was performed by pipetting out 20 µL gel and extracted glucose samples were immediately quantified.

2.3.5. Ionic Strength Study

The dependence of RI extraction performance on ionic strength was studied with seven different ionic formulations by using CA at +2V (*vs.* Ag/AgCl) for 3 hours. The formulations of interest were as follows;

1). 0.05M PBS (0.136g KH_2PO_4 and 0.696g K_2HPO_4 in 100mL), 2). 0.005M PBS (0.0136g KH_2PO_4 and 0.0696g K_2HPO_4 in 100mL), 3). 0.5M PBS (1.3600g KH_2PO_4 and 6.9600g K_2HPO_4 in 100mL), 4). 0.005M PBS - 0.045M NaCl (0.0136g KH_2PO_4, 0.0696g K_2HPO_4 and 0.2633g NaCl in 100mL), 5). 0.005M PBS - 0.045M KCl: (0.0136g KH_2PO_4, 0.0696g K_2HPO_4 and 0.3355g KCI in 100mL), 6)0.005M PBS - 0.045M $CaCl_2$ (0.0136g KH_2PO_4, 0.0696g K_2HPO_4 and 0.4995g $CaCl_2$ in 100mL), 7). 0.005M PBS - 0.045M $MgCl_2$ (0.0136g KH_2PO_4, 0.0696g K_2HPO_4 and 0.91485g $MgCl_2$ in 100mL).

While performing RI extraction, four parallel extraction cells were employed for each buffer, each using VISKING 14kD MWCO dialysis membrane which was previously stored in PBS (of 0.05M and pH 7.4) for 1h. Collection chambers were filled with MC gel (prepared by dissolving 8g of MC in 600 mL 0.05M pH 7.4 PBS), while reservoirs were filled with 200 mg.dL^{-1} glucose in PBS of 0.05M and pH 7.4).

2.3.6. Gel Types Study

During gel type study, MC Gel (8g/600mL) was prepared as follows; briefly first 600mL of acetate buffer (of 0.06M at

pH 5.0) was simply prepared by mixing 357.0 mL of 0.1M CH_3COOH and 643.0mL of 0.1M $CH_3COOHNa.3H_2O$ and diluting the mixture to 2L with ultrapure H_2O. The pH of this mixture was then set to pH 5.5 with the addition of an appropriate amount of 0.1M NaOH by using a pH-meter. Next 8g of MC was weighed to a 1L beaker and approximately 200 mL 0.06M acetate buffer (pH 5.5) solution was heated to an average of 80-90°C and added slowly in 20 mL portions to weighed MC at 400rpm magnetic stirring. Then the remaining (about 400mL) acetate buffer solution (kept at room temperature) was added again slowly in 20 mL portions and mixed over the initial mixture for 5 minutes. Other gel types (HPMC, CMC and NaCMC) were also prepared similarly. To fill the reservoirs, glucose solutions at 3 different concentrations (50, 100 and 200 $mg.dL^{-1}$) were prepared with PBS (of 0.05M at pH 7.4) and ExE electrodes were placed within 7mm distance while the extraction duration was 3hours.

2.3.7. Membrane Type Study

Cellulose Ester (CE) dialysis membranes with 2000, 12000 and 14000 Daltons MWCO values (VISKING) were used as the artificial skins. The dependence of RI extraction performance on the membrane type was investigated by performing RI extraction simultaneously in eight parallel RI cells for each membrane type (n=8), while the reservoir was filled with 200$mg.dL^{-1}$ glucose solution (in PBS of 0.05M at pH 7.4) and the collection chambers were filled with MC gel (8g/600mL in 0.06M acetate buffer at pH 5.5). RI extraction electrodes were placed within 7mm distance to each other while the extraction duration was 180 minutes (3 hours)

2.3.8. Membrane Condition Study

CE dialysis membrane (12000Daltons MWCO) was used as artificial skin. The dependence of RI extraction performance on the membrane usage frequency was investigated by performing RI extraction simultaneously in three parallel RI cells for each membrane condition type (n=3), while the reservoir was filled with 200$mg.dL^{-1}$ glucose solution (in PBS of 0.05M at pH 7.4) and the collection chambers were filled with MC gel (8g/600mL in 0.06M acetate buffer at pH 5.5). There were three experimental groups for membrane condition such as; brand new (n=0), once used (n=1) and used more than ten times (n>10). RI extraction electrodes were placed at 7mm or less distance to each other while the extraction duration was 180 minutes (3 hours).

2.3.9. Membrane Pretreatment

The dependence of extraction performance on membrane pretreatment procedure was investigated by five experimental groups and a control group. CE membrane of 12kD MWCO, cut in 63mm×45mm segments, was used while the reservoir was filled with 200 $mg.dL^{-1}$ glucose solution (in PBS of 0.05M at pH 7.4) and the collection chambers were filled with MC gel (8g/600 mL in 0.06M acetate buffer at pH 5.5). In the control group, there is no treatment at all. While in the first experimental group, the CE membrane was dipped in PBS solution (of 0.05M at pH 7.4) for 1minute. In the second and third groups, CE membrane was stored in the same PBS solution at 25°C for 1 and 24 hours, respectively. In the fourth and fifth groups, the CE membrane was stored in 0.05M PBS (pH 7.4) at 80°C for 1 and 24hours, respectively. RI extraction electrodes were placed at 7mm distance to each other while the extraction duration was 180 minutes (3 hours).

2.3.10. Extraction Electrode (ExE) Material Study

As explained in the Electrode Design subsection, in default, ExEs were coated with silver by electrodeposition [28]. To compare the performance of ExEs with other nobel metals rather than silver, ExEs were coated with some other metals too [30-33]. Platinum, palladium, ruthenium and rhodium electrodeposited electrodes were prepared simply by immersing in 100ppm acidic aqueous (5% w/w HCl) solutions of the corresponding noble metal and applying -2.0V (*vs.* Ag/AgCl) for 1hour. CE dialysis membrane (12000 Daltons MWCO) was used as artificial skin. The dependence of RI extraction performance on the ExE material was investigated by performing RI extraction simultaneously in five parallel RI cells for each nobel metal (n=5), while the reservoir was filled with 200 $mg.dL^{-1}$ glucose solution (in PBS solution of 0.05M at pH 7.4) and the collection chambers were filled with MC gel (8g/600 mL in 0.06M acetate buffer at pH 5.5). RI extraction electrodes were placed within 7mm distance to each other while the extraction duration was 180 minutes (3 hours)

3. RESULTS AND DISCUSSION

3.1. Effect of Inter-Electrode Distance

The dependence of glucose yield of the RI extraction on the distance between the two ExEs is displayed in Fig. (**1**). Four different distance levels were studied; 1.0, 2.50, 7.0 and 10.0 mm. The RI cell reservoir was filled with 0.05M PBS solution (pH 7.4) containing 200 $mg.dL^{-1}$ glucose and a constant potential of +2.0V (*vs.* Ag/AgCl) was applied at 25°C for 3 hours. After the extraction period, the collection chamber was removed immediately and 20 µL of the MC gel (4g/300 mL) was sampled and dropped on the measurement strip of the blood glucose meter while the measured glucose concentration was written down to the logbook of the experiment.

Regarding the findings of the experiment, some interesting results were obtained. 1.0 mm distance had an average yield of 22.33 and 29.67 $mg.dL^{-1}$ for anode and cathode, respectively. While for 2.50mm distance as expected due to increasing distance the average yields dropped down to 16.33 and 16.50 $mg.dL^{-1}$. Unexpectedly, the optimum electrode distance was found to be 7.0 mm as the average yields were up to 39.33 and 32.33$mg.dL^{-1}$. Later on, with 10.0 mm distance, again the yield levels dropped down to 18.67 and 27.00 $mg.dL^{-1}$. When Standard Deviation (SD) values (expressed as error bars) of the multiple readings (n=6) were taken into account, interestingly, the most reproducible values were obtained with 2.50 mm distance while its glucose yield was the lowest. In the same surprising manner, the highest glucose yield was obtained with 7.0 mm distance while its reproducibility was relatively the worst. There are not so many RI papers dealing with the optimization of the

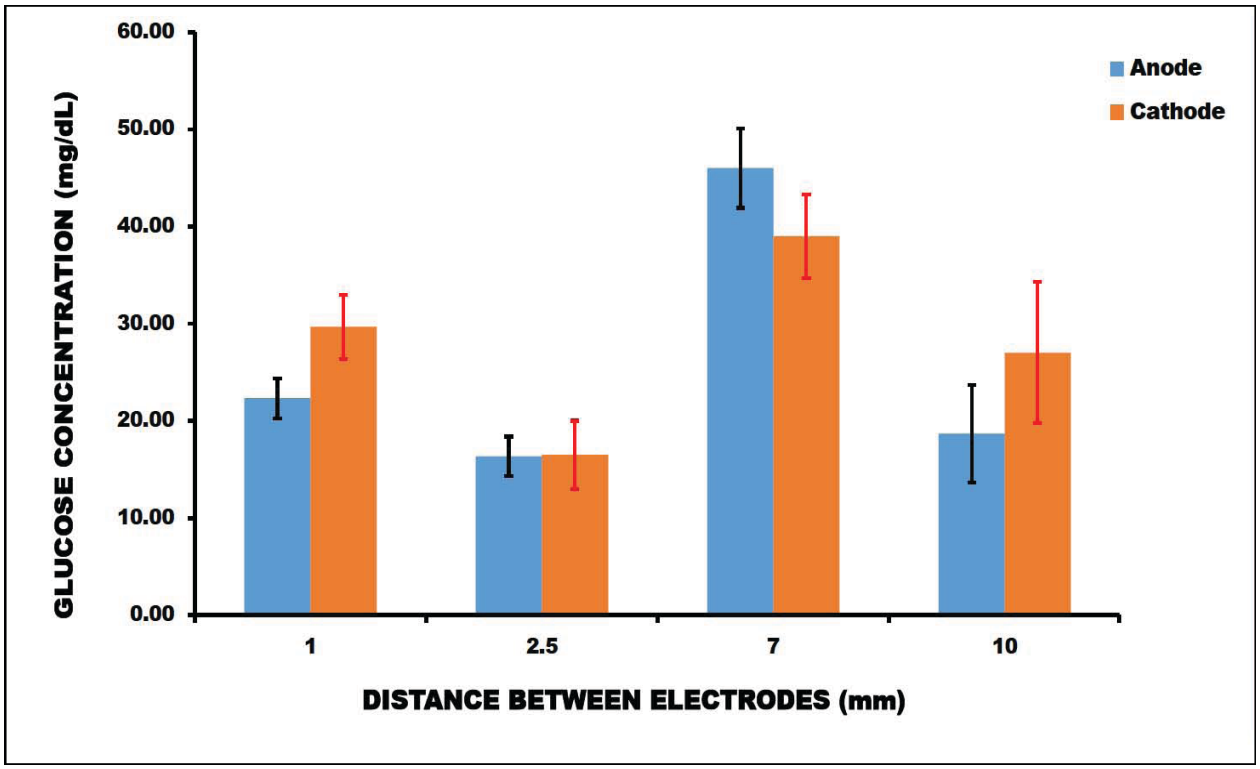

Fig. (1). The dependence of glucose extraction on the distance between electrodes of extraction (anode and cathode). Conditions as in Experimental section.

distance between anode and cathode. In a student's paper from Cornell University [34], the authors have described a definition of "inter-electrode distance" which defines the distance between the ExEs (anode and cathode). In this paper, inter-electrode distances were studied in the range of 1 to 6mm with 0.5mm increments. Their findings showed that the extracted glucose levels were more or less the same (varying around 0.98 mol/m^3) and this implied that inter-electrode distance was an insignificant parameter for the optimization of RI performance. Connolly *et al*. once also defined an electrode distance as 11mm in one of their RI papers [22, 24] and this distance was used in the entire experiment. Connolly *et al*. in another paper [23] described an inter-electrode distance of 23mm where they employed screen-printed electrodes for RI. Wang *et al*, in their two separate papers [35, 36], employed printable tattoo based RI electrodes and according to the scales in the electrode pictures, the inter-electrode distance may be described as 10mm. These papers define similar inter-electrode based on our findings. On the other hand, Mahe *et al*., in their paper dealing with acetylcholine (Ach) iontophoresis (not RI), evaluated three different inter-electrode distances of 5, 10 and 15 cm [37]. The distances they employed were almost 10 folds higher than the ones optimized in this paper and the mean of distances usually described in the literature. Their paper dealt with the transdermal iontophoresis of Ach rather than RI of Ach, thus their main goal was to administer a drug substance rather than extracting it. To achieve this goal, they probably targeted a wider surface area and a bigger electrode distance to form a more efficient flux thus evaluated almost 10 folds higher inter-electrode distances.

3.2. Dependence on Potential Technique

The influence of the operating potential technique on the extraction efficiency of the RI cell is summarized in Fig. (**2**). Three different techniques types were employed as, continuous Constant Potential (CA), Differential Pulse Varying Potential (DPV) and Cyclic Varying Potential (CV). The experimental details of these techniques are summarized in Table **S1** (supplementary material). Except these techniques, polarity (+/-) switching was also studied through CA and no significant difference was observed in the glucose yield (not shown). Through the techniques used, the highest glucose yield was observed with DPV, followed by CA and CV. The reproducibility of the yield is almost the same with CA (average yield of 29.81 and 26.33 mg.dL^{-1}) and DPV (35.13 and 31.08 mg.dL^{-1}) while the lowest with CV (29.50 and 29.75 mg.dL^{-1} for anode and cathode, respectively).

When error bars are taken into account, the most reproducible results were obtained with Constant Potential (CA), thus CA was chosen as the optimum potential technique for the rest of the experiments of the project. In the current literature, the effect of electrochemical parameters of RI process on extraction performance is studied in units of varied applied current rather than applied potential. Depending on the electrode active area and the experimental model, there are various potential and current values reported. In their paper, Connolly *et al*. [20, 21] designed a programmable RI device which had a current-waveform generator and was capable of applying bipolar extraction DC current constant or in pulses.

In these papers, they have employed +36V constant potential to obtain a 300 µA DC current for RI extraction. Tak-

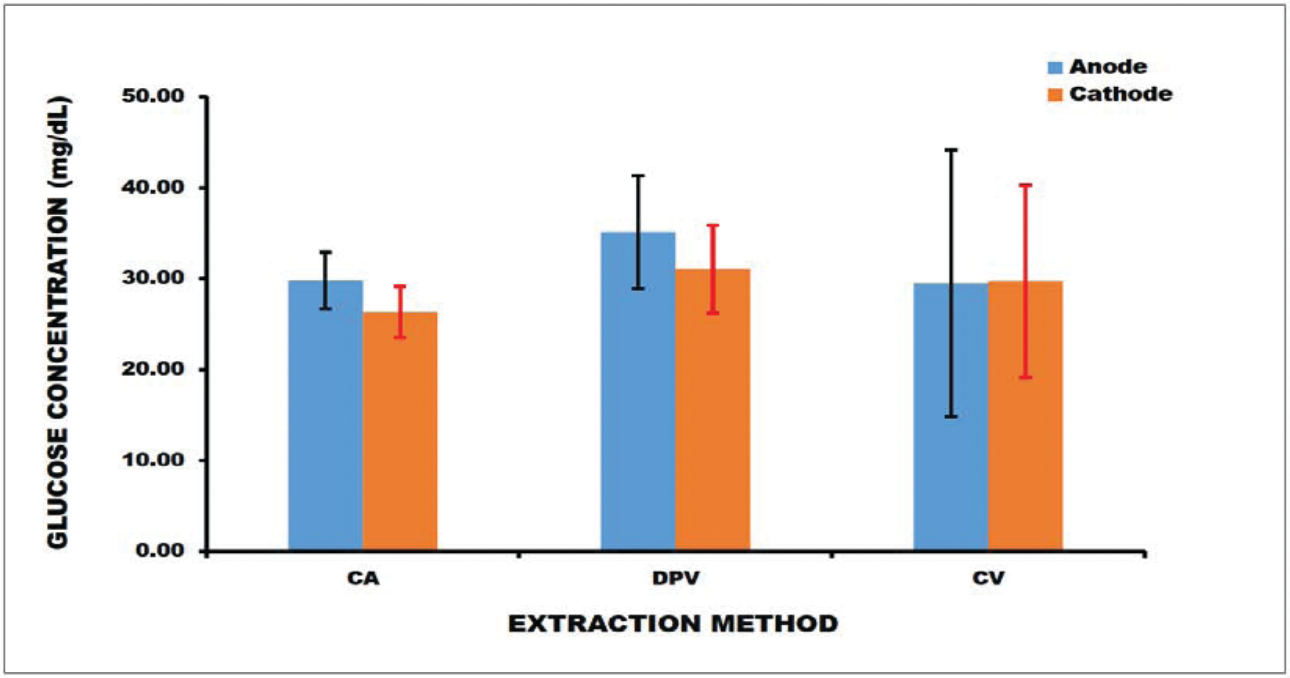

Fig. (2). Effect of potential type on the extracted glucose level. Continuous Constant Potential (CA), Differential Pulse Varying Potential (DPV) and Cyclic Varying Potential (CV). Conditions as in Experimental section.

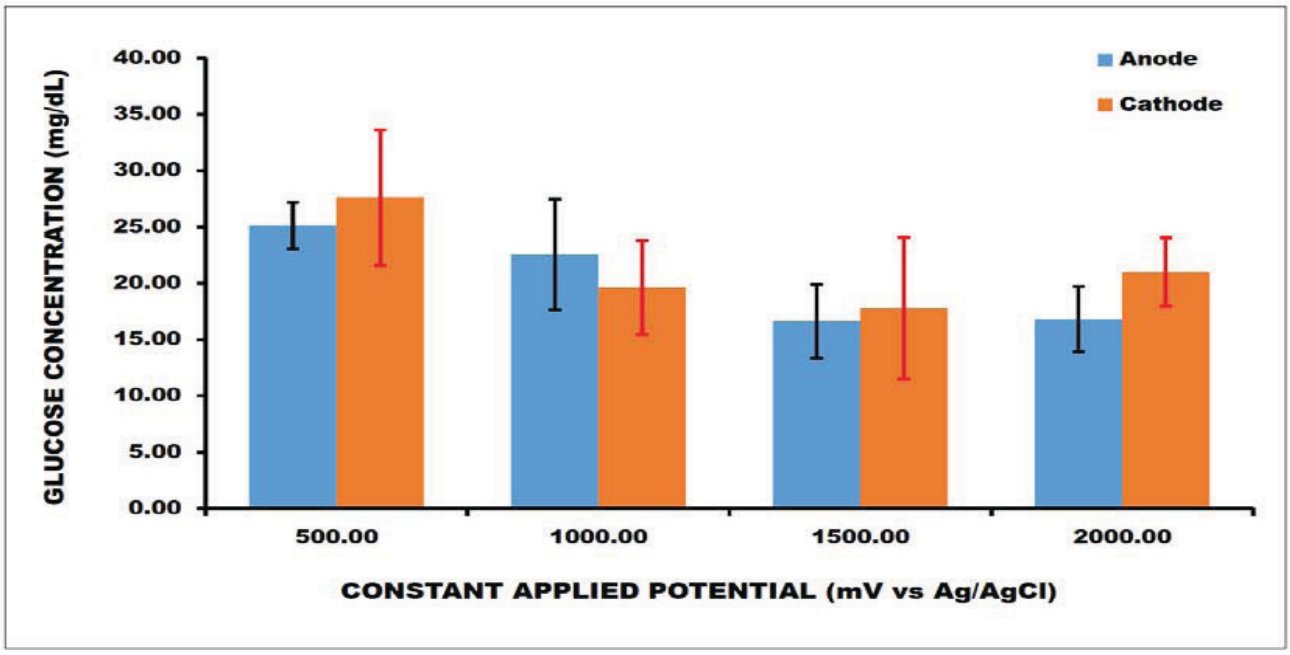

Fig. (3). Dependence of extracted glucose level on the magnitude of constant operating potential. Conditions as in Experimental section.

Shing Ching *et al*. [27] also designed and developed a programmable RI hardware having a voltage booster which is able to control the applied potential in the range 10-300 V for generating constant currents of 10-300 μA with an error of 1% in different programmable waveforms. They have tried direct current, biphasic current, pulsed biphasic current, either with or without rest intervals. Another programmable RI device was developed by Tokmakci *et al*. [26], which was capable of applying a range of 0-15mA DC current by 1mA steps within time 0-59 minutes.

3.3. Effect of the Potential Magnitude

The magnitude of the constant operating potential (CA) does not have a significant effect on the glucose yield of the RI extraction. Fig. (**3**) summarizes the dependence of the glucose yield on the operating potential.

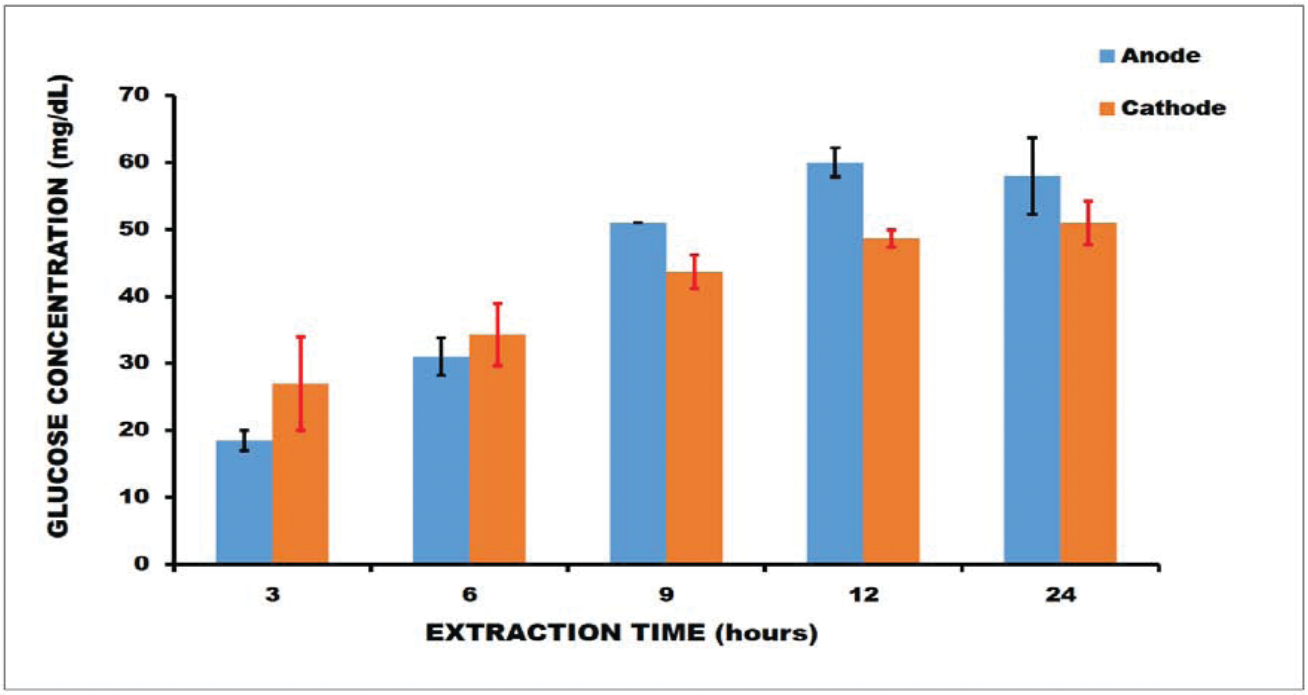

Fig. (4). Continous monitoring of RI extraction of glucose for 24h. Conditions as in Experimental section.

The initial potential of +500mV was increased in 500 mV steps until +2000mV. The initial glucose levels extracted at +500 mV were 25.13±2.10 mg.dL^{-1} and 27.63±6.0 mg.dL^{-1} for anode and cathode, respectively. When the potential raised up to +1000mV, glucose yields decreased to 22.56± 4.91mg.dL^{-1} and 19.63±4.16 mg.dL^{-1} for anode and cathode, respectively. When the potential went another 500mV increase anodic to +1500 mV, the glucose yields decreased even more to 16.63±3.29 mg.dL^{-1} and 17.81±6.28 mg.dL^{-1} for anode and cathode, respectively. Finally when the potential was set to +2000 mV with an increase of final 500mV step, the glucose yield reached an equilibrium at 16.81± 2.90 mg.dL^{-1} and 21.0±3.02 mg.dL^{-1} for anode and cathode, respectively. When precision was taken into account, repeatability was the highest with +2000 mV while it was the lowest with +1500 mV. As in current literature, the effect of electrochemical parameters of RI process on extraction performance was studied in units of varied applied currents rather than applied potential, therefore it will not be suitable to compare our findings with literature.

3.4. Daily Extraction Performance

The overall RI extraction period was monitored for 24 hours as can be seen in Fig. (**4**). The reservoir of the cell was filled with PBS (of 0.05M at pH 7.4) solution containing 200 mg.dL^{-1} glucose and a CE dialysis membrane with 14kD MWCO was used as the artificial skin.

The collection chamber was filled with MC gel (4g/300 mL) and a constant potential (CA) of +2.0V (*vs.* Ag/AgCl) was applied at 25°C for 24 hours. A sampling of MC gel from collection chamber, at 3rd, 6th, 9th, 12th and 24th hours, was performed by pipetting out 20µL gel and immediately replacing it with another 20µL portion of fresh MC gel (4g/300 mL). Sampled 20µL gel was simultaneously dropped on the blood glucose meter to determine the glucose content in mg.dL^{-1} unit. At 3rd, 6th, 9th, 12th and 24th hours, glucose levels of 18.5±1.50, 31.0±2.82, 51.0±0.00, 60.0± 2.16 and 58.0±5.71 were measured, at the anode. At the cathode, sampling at the very same hours yielded glucose levels of 27.0±6.97, 34.3±4.64, 43.7±2.49, 48.7±1.24, and 51.0±3.26, respectively. During the sampled 24h period, the extracted glucose levels increased in a linear trend up to the 12th hour, after which the extracted glucose concentration leveled up to a constant plateau. Thus the initial extraction time necessary to obtain reproducible extraction yield is 12 hours. After 12 hours of extraction, glucose yield is reproducible for 12 hours till the 24th hour. Guy *et al*. [12] also reported similar findings while monitoring iontophoretic delivery of nafarelin for 24h. After 12h, the iontophoretic transport of mannitol and nafarelin (1 mg.mL^{-1}) reached a plateau while similarly, the linear increase in the delivery of nafarelin leveled up at 12h and stayed constant till 24h. In another paper Guy *et al*. [38] performed RI on porcine ears of premature neonates for 5h to extract theophylline and caffeine. This paper does not provide information about the RI extraction performance after 5 hours, though a linear increase in extracted analyte concentration was observed in the first 5h for theophylline and caffeine, respectively. Mannitol RI extraction was monitored for 25h in another paper by Guy *et al.* [14]. In this paper, RI extraction was performed by cutting off the extraction current at the 2nd hour and monitoring process till the 25th hour. Till the 2nd-hour, the extraction performance increased linearly while starting from the 3rd hour, extraction significantly slowed down, but kept increasing linear till the 25th hour.

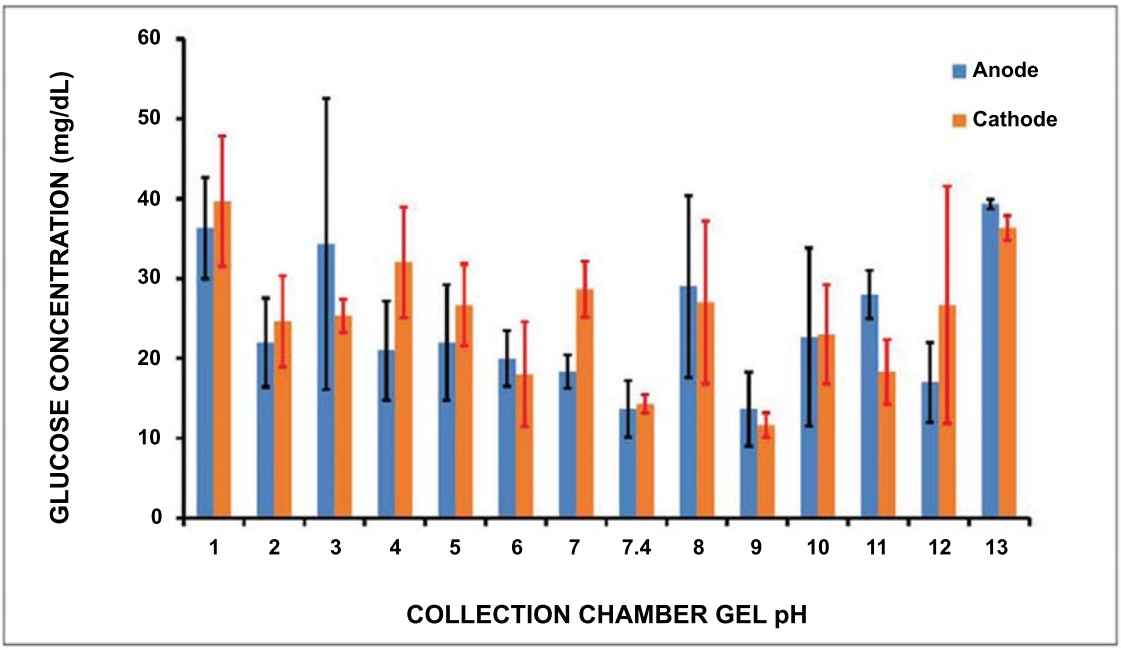

Fig. (5). Effect of medium pH of only collection chamber on the performance of glucose extraction. Conditions as in Experimental section.

3.5. Dependence on Medium pH

The dependence of extraction performance on collection chamber pH is displayed in Fig. (**5**). During this pH study, the reservoir of the RI cell was filled constantly with 0.05M PBS solution (pH 7.4) while the collection chamber was filled with MC gel of different pH values in the range 1-13 as described in Materials and Methods Section. The extraction yield was found to be the highest at pH 1 and 13 while to be the lowest at pH 7.4 and 9. The pH values with the highest yields should not be assigned as optimum pH values as pH 1 and 13 will be totally destructive pH values for the human epiderm. Regarding the extraction reproducibility, pH 7.4 displayed more precise glucose yields followed by pH 9. The lowest precision was observed with pH 3, 8, 10 and 12. The reproducibility observed with different pH values were expressed by error bars based on the standard deviation of multiple (n=3) readings.

The possible effect of simultaneously varying both reservoir and collection chamber pH on the success of glucose extraction is summarized in Fig. (**6**). During this pH study, the reservoir of the RI cell and the collection chamber were filled with MC gel of different pH values in the range 1-13 as described in Materials and Methods Section. The data presented display an almost similar trend to that of Fig. (**5**), indicating that varying reservoir pH does not have a significant effect on glucose yield of RI extraction. Similar to Fig. (**5**), the highest glucose extraction was observed with pH 1.0 while the lowest yields were observed with pH 7.4 and 9.0.

Most of the pH values displayed relatively high standard deviation values in comparison to 7.4 and 9.0. Thus although glucose extraction decreased, the most successful reproducibility was also observed with pH 7.4 and 9.0 similar to Fig. (**5**). There is only one paper, with extensive pH study data during RI process, published by Guy *et al.* [12]. They evaluated RI extraction performance of mannitol at only three pH values (4.0, 7.4 and 8.0) and reported that enhancement in the cathodal extraction efficiency was observed by increasing the pH while anodal extraction conversely suffered from the same situation. These findings are very much similar to our data for pH 5.0, 6.0, 7.0 and 7.4. We also provided data regarding the far acidic region below pH 3.0, three points at pH 1.0, 2.0 and 3.0 were monitored and parallel results were obtained for anodal extraction by a sharp decreasing from pH 1.0 to 3.0, surprisingly this trend was also observed with the cathode.

3.6. Dependence on Ionic Strength

The influence of the ionic strength of the reservoir on the performance of the RI glucose extraction was also studied. As can be seen in Fig. (**7**), seven different filling buffer solutions were designed for the reservoir.

The experimental procedure for the ionic strength study was summarized in Materials and Methods Section, briefly the buffers 1 to 3 were reserved for PBS buffers (pH 7.4) with the molarity of 5 to 500mM, while the remaining buffers (4 to 7) were designed with 5mM PBS buffers (pH 7.4) plus 45mM of four different cation chlorides. NaCl, KCl, $CaCl_2$ and $MgCl_2$ were employed for buffers 4 to 7, respectively. As can be seen in Fig. (**7**), increasing the PBS molarity from 5mM to 50mM does not have a very obvious positive effect on the extraction while increasing further to 500mM showed a negative effect on glucose extraction, thus it can be concluded that 5mM level may be easily used to prepare PBS (pH 7.4). Regarding the remaining buffers 4 to 7, neither monovalent cation chlorides nor divalent cations showed a positive effect on the glucose yield. Therefore it can be said that enriching the reservoir filling solution (5mM

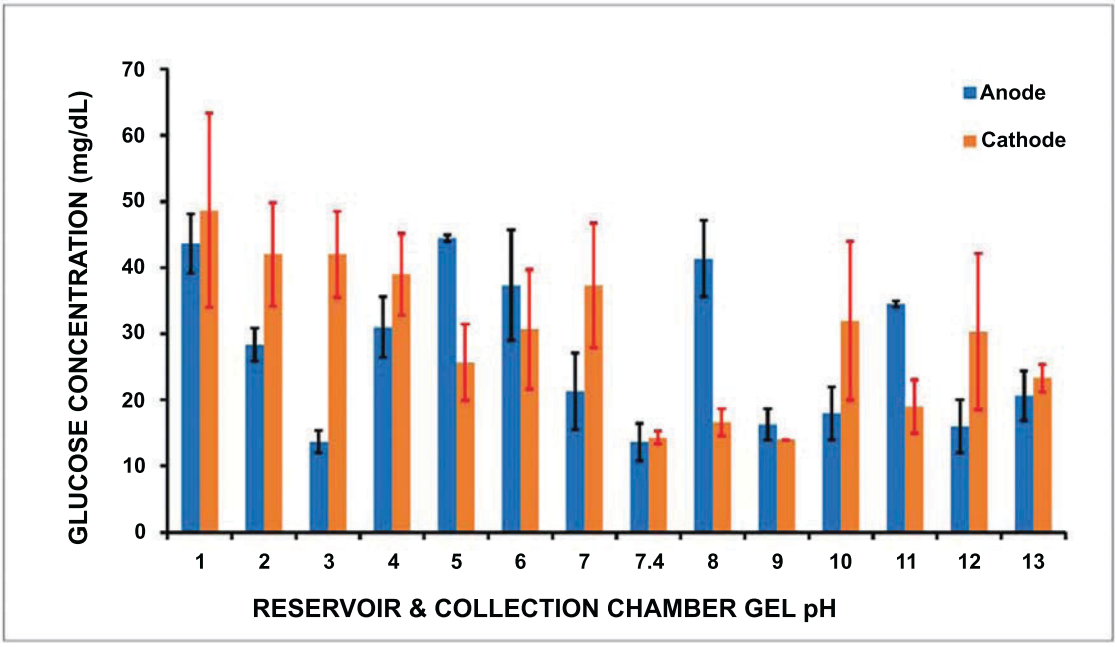

Fig. (6). Effect of medium pH of both reservoir and collection chamber on the performance of glucose extraction. Conditions as in Experimental section.

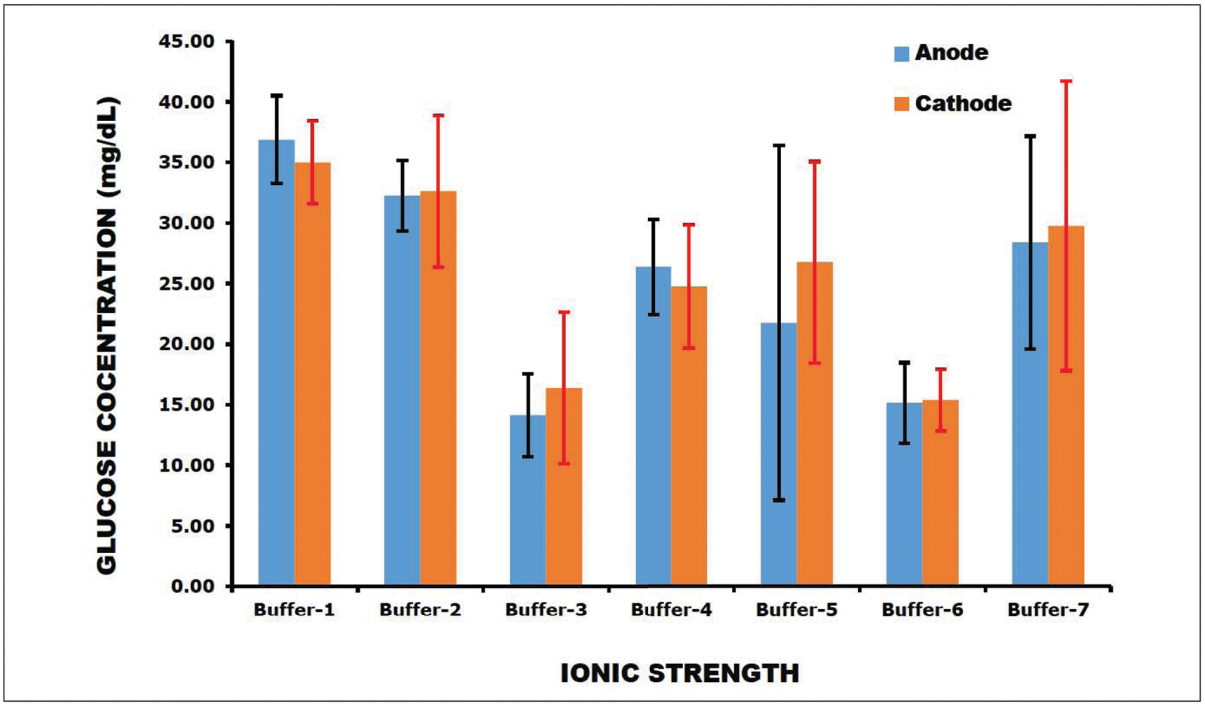

Fig. (7). The dependence of performance of glucose extraction on the varying ionic strength of the reservoir. Conditions as in Experimental section.

PBS of pH 7.4) with mono- or divalent cation chlorides will be an unnecessary procedure as it causes a decrease in glucose yields in the range of 15-50%. Therefore it can be concluded that the ionic strength of the reservoir is inversely proportional to the glucose yield of the RI extraction. In literature, somewhat similar results awere reported by Guy *et al.* [13]. This is the only paper present with extensive ionic strength data. In their paper focused on the parameters determining RI electroosmotic flow, they also stated that both cathodal and anodal extraction was enhanced by reducing the electrolyte ionic strength in the chambers of the electrode.

3.7. Effect of Gel Composition

Different gel types rather than Methyl Cellulose (MC) were also employed in the collection chamber. As visible in Fig. (**8**), among the other gel types used, only Carboxymethyl Cellulose (CMC) displays a significant positive effect on glucose yield and increases the glucose concentration while

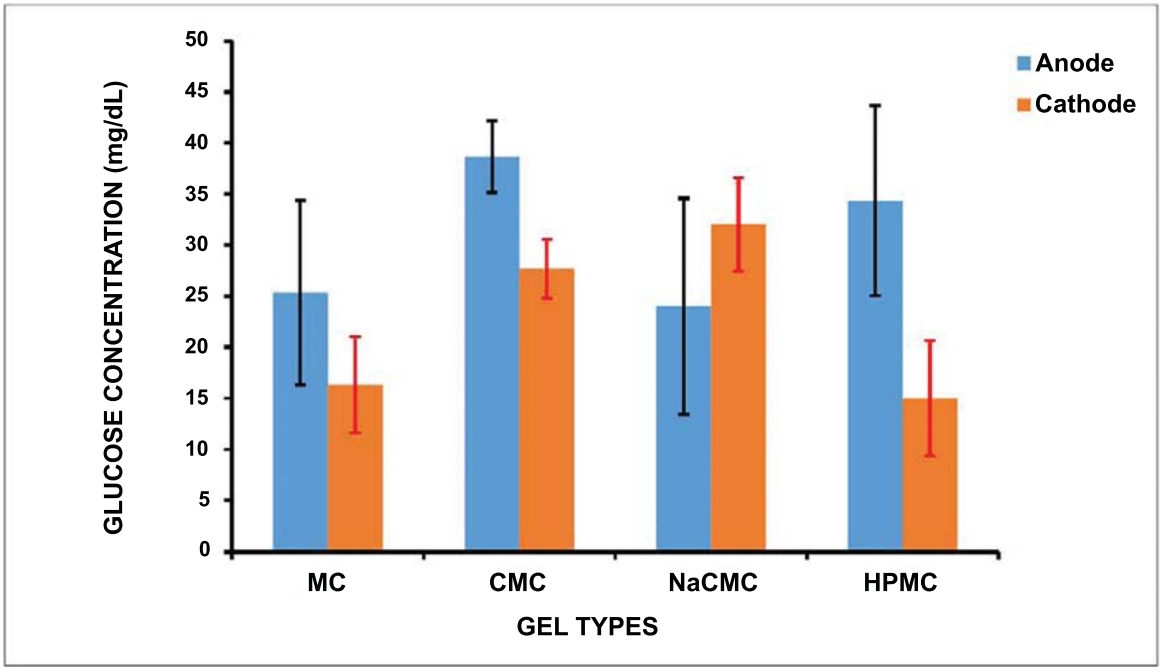

Fig. (8). Effect of collection chamber gel type on the performance of the RI extraction; MC stands for methyl cellulose, CMC for carboxymethyl cellulose, NaCMC for sodium carboxymethyl cellulose and HPMC for hydroxypropylmethyl cellulose. Other conditions as in Experimental section.

the remaining gels own relatively higher SD values (n=3) and because their reproducibilities were poorer, it was hard to say that they too followed the same trend. In literature, in-vitro RI experiments have been performed using MC gels [23]. Though in current literature, there is no record reported studying the effect of gel type on the performance of RI extraction, and it will not be very suitable to compare our findings with the study by, Shivakumar *et al.* [39], who in their paper, dealt with the iontophoretic delivery of diclofenac sodium, and sought for an imparting agent for viscosity by employing hydroxyethyl cellulose for this purpose.

3.8. Effect of Membrane MWCO Values

Cellulose Ester (CE) membranes were used as the artificial membranes during RI extraction. CE membranes with different Molecular Weight Cut-Off (MWCO) values were also employed to see the dependence of RI extraction success on the permeability of the membranes. With different MWCO values, expected results were obtained as can be seen in Fig. (**9**).

Increasing MWCO from 2kD to 12kD increased glucose concentration from 17.00±2.36mg.dL^{-1} to 22.58±5.51mg.dL^{-1} resulting in an +32.82% anodal extraction increase. Further increase from 12kD to 14kD increased the value from 22.58±5.51 mg.dL^{-1} to 28.19±5.51 mg.dL^{-1} resulting in a percentage increase of +24.85% anodal extraction. Increased cathodal percentages also showed pretty much the same trend. Thus it can be concluded that MWCO values are directly proportional to the glucose yield of the RI extraction. Employment of CE membranes in *in-vitro* RI experiment is common in literature. Connolly *et al.* [19, 22-24] in their papers, where they introduced a novel diffusion cell, used a CE membrane with a 500D MWCO, while Corish *et al.* [40] used a CE membrane with a 14kD MWCO. To the best of our knowledge, there is no record in literature, making a comparison of CE membranes with different MWCO values and analyzing their effects on RI extraction performance.

3.9. Effect of Membrane Usage Frequency

Any possible effect of membrane usage frequency on glucose yield of the RI extraction was also studied. As can be seen in Fig. (**10**), a new (n=0) CE membrane was compared with an old CE membrane used only once (n=1) and even with an old CE membrane used more than ten times (n>10).

Anodal extracted glucose levels were 32.50±3.53, 30.50±2.12 and 34.00±1.41 mg.dL^{-1} for n>0, n=1 and n=0, respectively. Regarding the cathodal levels, 21.50±0.70, 16.50±0.75 and 18.50±0.81 mg.dL^{-1} were achieved for the same frequency values. Based on the obtained experimental data, it may be concluded that the usage frequency of the CE membrane had no significant effect on the glucose yield of the RI extraction. There is no paper in the literature, making a comparison of CE membrane usage frequencies with their effects on RI extraction performance, usually, these membranes are used once [19, 22-24, 40]. Thus these findings provide some information missing in the literature about the use of CE membranes during the RI process.

3.10. Effect of Pretreatment Procedure

Different pretreatment procedures were tried to make a pretreatment optimization for the CE membrane used during RI extraction experiments. All experimental parameters tried

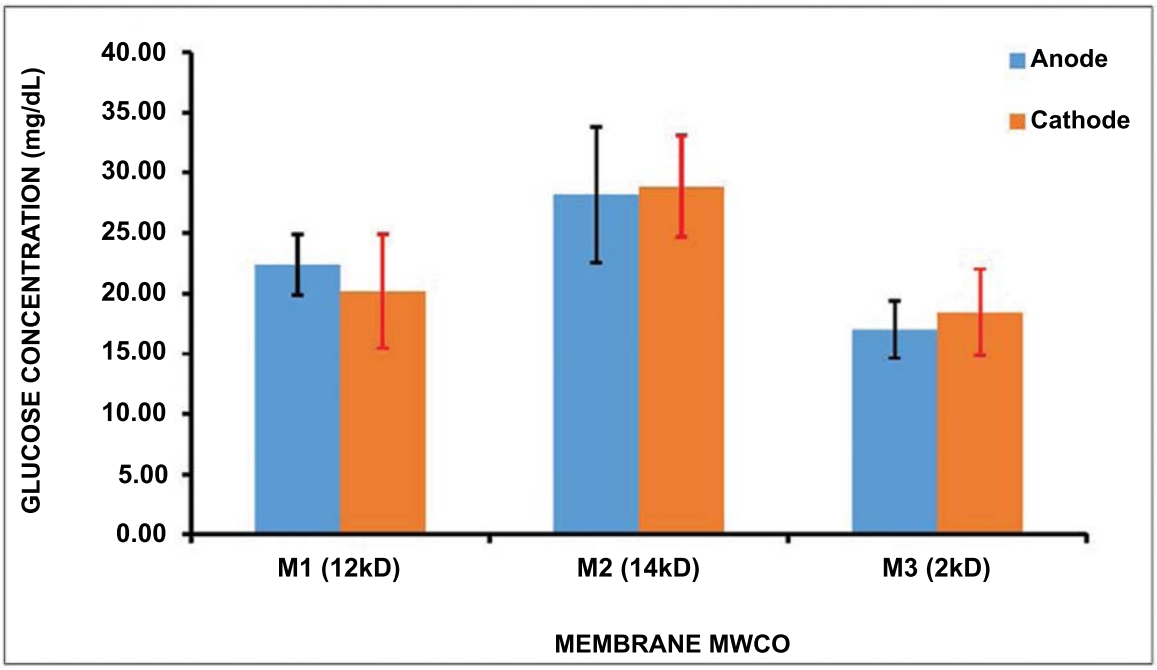

Fig. (9). Effect of membrane permeability of the glucose yield of RI extraction; cellulose ester membrane with a MWCO value of 12kD (M1), cellulose membrane with a MWCO of 14kD (M2) and benzoylated cellulose membrane with a MWCO of 2kD (M3). Conditions as in Experimental section.

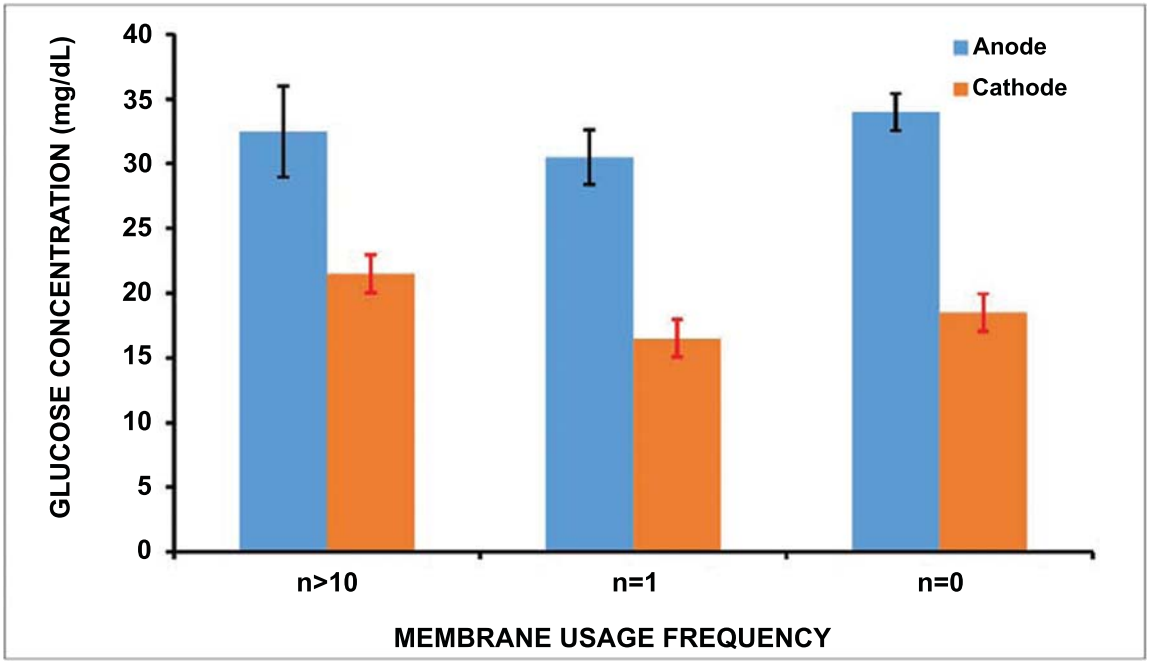

Fig. (10). Effect of usage frequency of the membrane on the glucose yield of the RI extraction. Conditions as in Experimental section.

for the optimization procedure and corresponding results are summarized in Fig. (**11**).

The experimental procedures used in this study are summarized in Materials and Methods section. Briefly, six experimental groups (n=3) were formed for 12kD CE membrane. The first four groups were studied in room temperature. In the first group, membrane was used as received, in the second group, membrane was dipped in ultrapure water for 3 minutes. In the third and fourth groups, the membrane was stored in ultrapure water for 1 and 24 hours, respectively. The procedures of the fifth and sixth groups were the same as of the third and fourth groups, except the temperature at 80°C. Storing the membrane in ultrapure water at room temperature for 24 hours seems to be the optimum pretreatment procedure as the highest glucose extraction yield was achieved. Surprisingly, repeating the same procedure at 80°C did not cause any difference in glucose extraction performance.

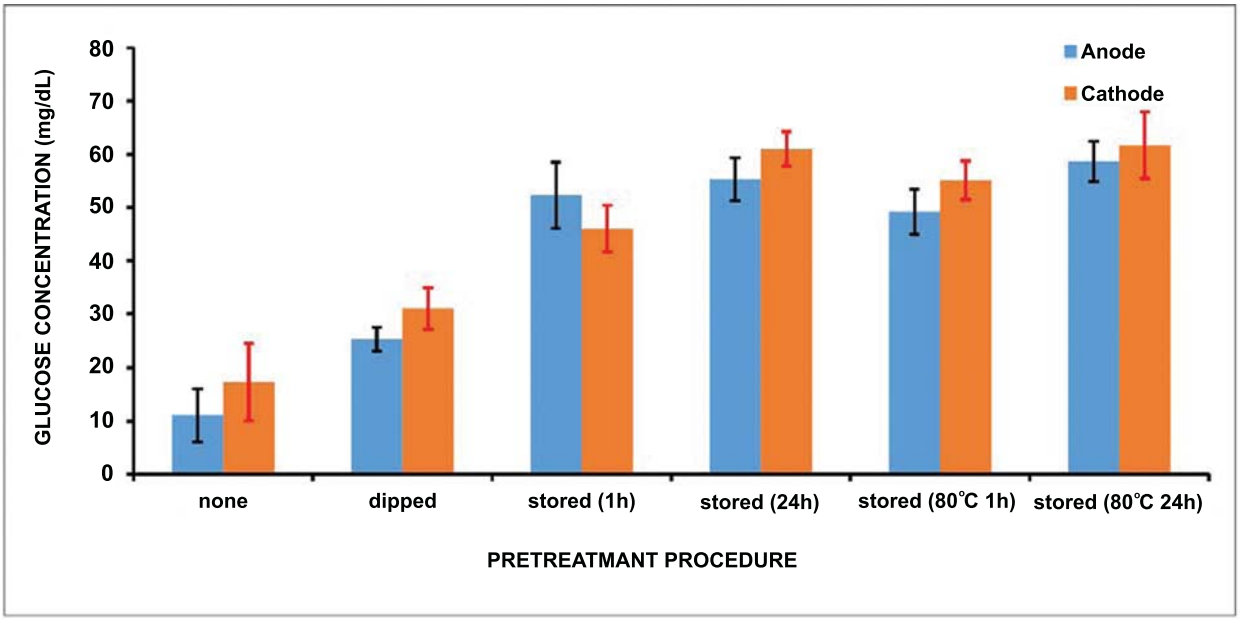

Fig. (11). Effect of membrane pretreatment choice on the performance of the glucose RI extraction. Conditions as in Experimental section.

For anodal extractions, dipping improved yield from 11.00± 5.02 to 25.33±2.24 mg.dL^{-1} corresponding to a shift of +130.27%. In a similar behavior, storing at 25°C for 1 hour escalated glucose level from 11.00±5.02 to 52.33±6.25 mg.dL^{-1} corresponding to a shift of +375.72% for anodal extraction. Storing for a longer period (24 hours) only made a slight difference as compared to storing for 1 hour, and raised glucose level to 55,33±4.02 mg.dL^{-1} at the anode. Storing at 80°C for 1 or 24 hours made only again slight differences in comparison to 1 or 24 hours at 25°C, and glucose levels were at around 49,18±4.24 and 58,67±3.80 mg.dL^{-1}, respectively at the anode. Regarding the cathodal extractions, 17.33±7.24, 31.00±3.82, 46.00±4.34, 61.00±3.26, 55.11±3.62 and 61.67± 6.26 mg.dL^{-1}, glucose was extracted with the same corresponding pretreatment procedures. In the literature, for in-vitro RI extraction purposes, CE membranes are usually treated in accordance to the manufacturer's recommendation [19, 22-24, 40] which includes only soaking the membrane in a large volume of pure water for fifteen minutes at 25°C and then rinsing it thoroughly in pure water [41]. Other pretreatment procedures are not discussed in the literature, thus the findings of this manuscript provide some missing information about the pretreatment procedures of CE membranes prior to the RI process.

3.11. Effect of Extraction Electrode Material

The dependence of RI glucose yield on the material of the extraction electrode is displayed in Fig. (**12**). Silver (Ag), platinum (Pt), palladium (Pd), ruthenium (Ru) and rhodium (Rh) were the noble VIIIB group transition metals used as the extraction electrode (fabricated by electrodeposition) for comparison. Metals were electrodeposited on both anode and cathode extraction electrodes and used during glucose extraction procedures. Extracted glucose levels were quantified by a blood glucose meter. Fig. (**12**), displays the effect of electrode material on the extraction performance in accordance with the extracted glucose levels using an average of multiple glucose readings (n=3) and the Standard Deviation (SD). The anodic average results and the corresponding SD levels were 24.42±4.75, 18.75±3.89, 22.25±2.47, 19.25± 6.01, 19.25±2.48 for Ag, Pt, Pd, Ru and Rh, respectively. Regarding the cathodic average results and the SD levels, 22.75±4.02, 23.00±2.12, 22.50±1.41, 18.75±5.30 and 19.75± 8.13 were obtained with Ag, Pt, Pd, Ru and Rh, respectively. In ligth of these findings, it can be concluded that Ag extraction electrode extracted the highest glucose levels in comparison to Pt, Pd, Ru and Rh. These metals displayed a response trend of Ag > Pt > Pd > Ru > Rh. These findings were parallel to literature.

Though there is no paper making a comparison of various noble metals simultaneously for RI extraction, there are some papers comparing Ag and Pt electrodes. In a paper, Kalia *et al*. [16] described Ag electrode as the most well-suited electrode for RI as it does not cause any sharp decrease in pH which is the case with Pt. Ag electrode has the considerable advantages; its electrochemical redox reactions occur at potentials lower than those where the electrolysis of water occurs.

When water is electrolyzed, two main processes take place are faced; first the protons generated at the anode, because of their relatively small dimension and relatively higher mobility, they may easily compete to carry charge and thus reduce the efficiency of RI extraction process, and second, the undesirable low pH observed in the compartment of anode may cause acidic skin sensitivity and it may have an unfavorable effect on the stability of the analyte.

Corish *et al*. [40] made a similar comment on the positive effect of Ag electrodes on the performance of RI based on the absence of anode generated aqueous proton and thus

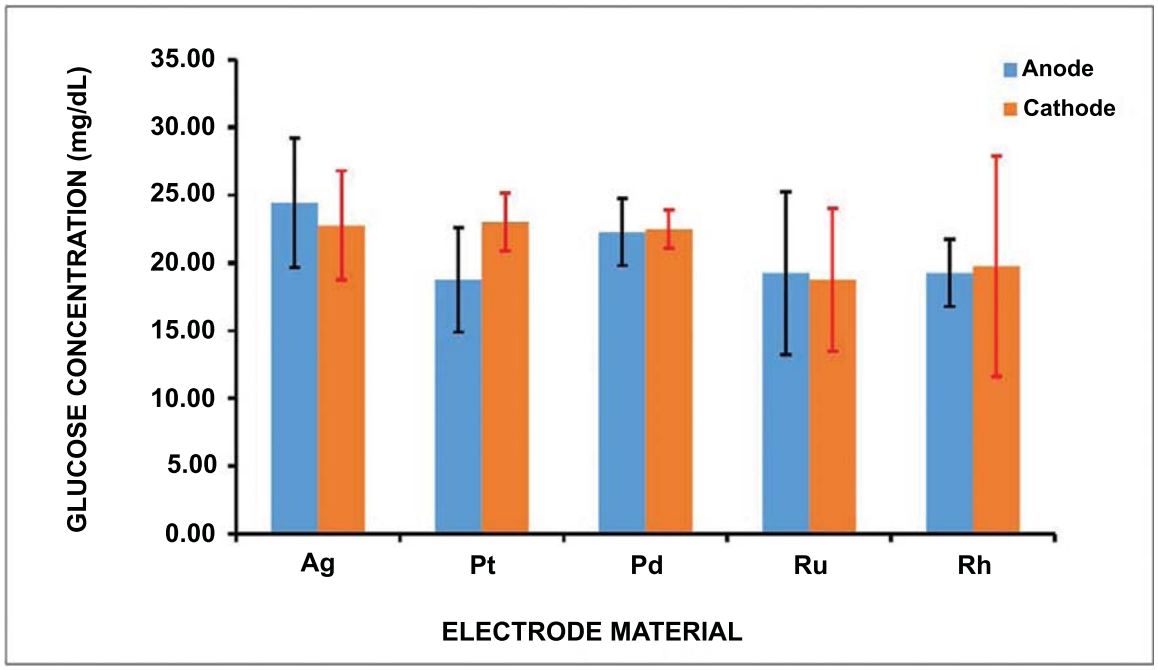

Fig. (12). The dependance of glucose yield of RI extraction on the electrodeposition of various transition metals on extraction electrodes. Conditions as in Experimental section.

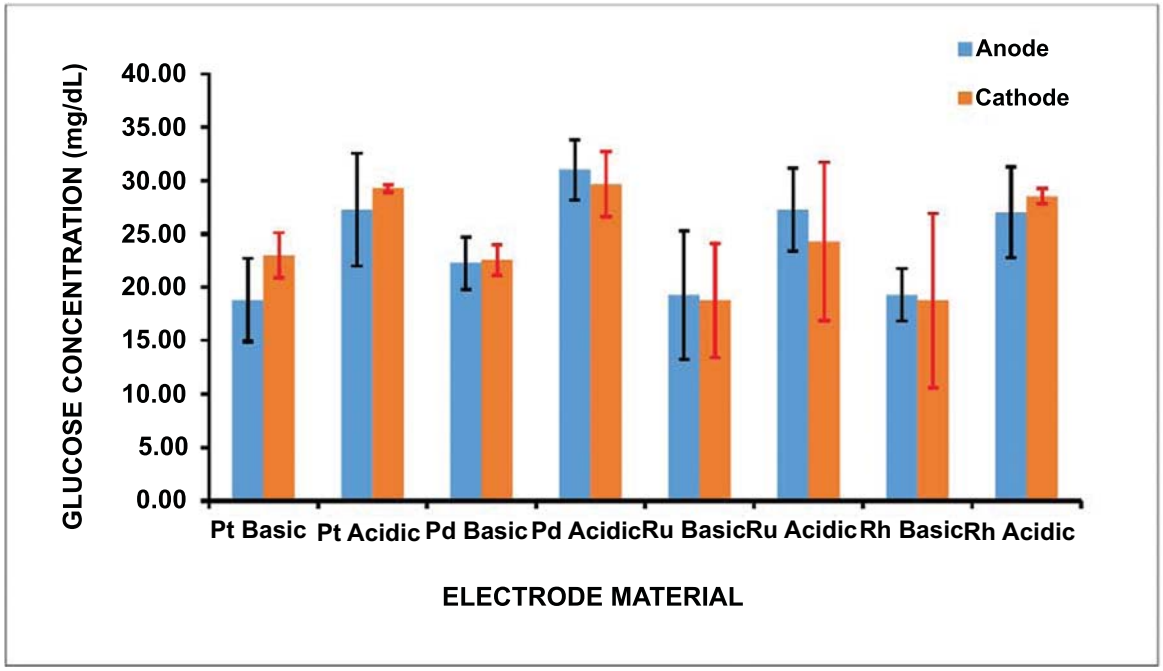

Fig. (13). Dependence of glucose yield of RI extraction on electrodeposition of various transition metals in acidic (pH 2.0) or basic (pH 10.0) medium. Conditions as in Experimental section.

ensured the stability of the analyte during RI process. Degim *et al*. [42] also highlighted the "anode generated proton" challenge when employing other electrodes like Pt rather than Ag for RI.

The effect of materials of both anodic and cathodic electrodes on extraction performance was also assessed by repeating similar experiments (Fig. **12**) in both acidic and basic environments and the results are summarized in Fig. (**13**).

When the electrodeposition process was performed in basic environment, glucose yields decreased approximately 30%. The exact significant anodic decrease ratios were calculated to be -31.19%, -28.23%, -29.36%, -28.71% for Pt, Pd, Ru and Rh, respectively. While cathodic decreases were

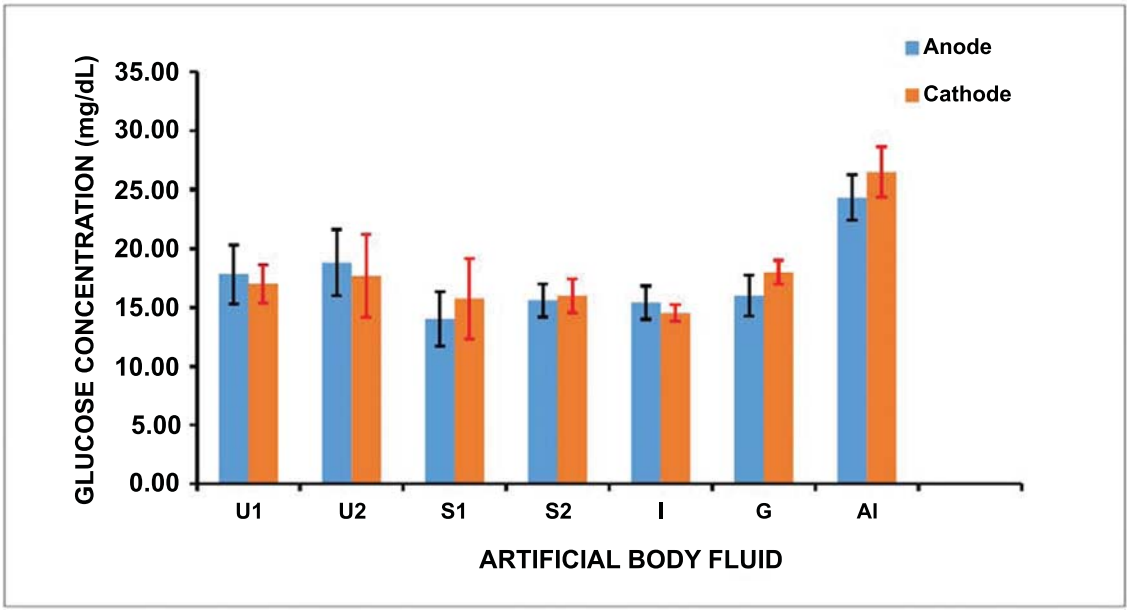

Fig. (14). Dependence of glucose yield of RI extraction on various simulated artificial body fluids; urines (U1, U2), salivas (S1, S2), intestine juices (I, AI) and gastric juice (G) used as the extraction medium. Conditions as in Experimental section.

-21.37%, -24.11%, -22.68%, -34.21% for Pt, Pd, Ru and Rh, respectively.

3.12. Real Sample Determinations

Real sample experiments were performed in various artificial simulated body fluids. The solutions for this purpose were prepared in accordance to the literature [43-45] and as urine solutions (U1 and U2), saliva solutions (S1 and S2), gastric juice (G), intestine juice (I) and Artificial Intestine juice (AI). When glucose quantification was carried out, following a 3h RI extraction, the extracted glucose levels were expressed as corresponding mean values and SD values in Fig. (**14**). RI extraction in artificial urine solutions yielded pretty much similar mean values of glucose such as 17.80± 2.50 and 18.80±2.83 $mg.dL^{-1}$ with anodes while 17.00±1.63 and 17.67±3.51 $mg.dL^{-1}$ with cathodes for U1 and U2, respectively. Following artificial body fluids, saliva solutions slightly decreased glucose levels than urine solutions. Comparing the two artificial saliva solutions with each other, pretty much similar glucose levels were obtained such as 14.00±2.31 and 15.60±1.41 $mg.dL^{-1}$ with anodes while 15.75±3.40 and 16.00±1.42 $mg.dL^{-1}$ with cathodes for S1 and S2, respectively. Intestinal juice was simulated again with two artificial body fluid solutions which displayed quite different glucose yields during RI extraction. Using the intestinal juice at pH 7.4 (I) ended up with an extracted glucose level of 15.40±1.42 and 14.50±0.71 $mg.dL^{-1}$ for anode and cathode, respectively.

Regarding the intestinal juice at pH 6.8 containing pancreatin enzyme (AI), glucose extraction yielded the highest levels of glucose as 24.33±1.92 and 26.5±2.12 for anode and cathode, respectively. The gastric juice at pH 1.0 containing pepsin enzyme (G) displayed similar glucose yields like S1, S2 and I as 16.00±1.73 and 18.00±1.00. When all simulated body fluids are taken into account, it can be concluded that the highest glucose yields are obtained when extracting from AI which is followed by U2 and U1, while all remaining simulated body fluids decrease glucose levels in comparison, but with more or less the same extraction performance. The highest glucose level extracted in AI may be explained with some penetration enhancers [46] such as polyvinyl alcohol, polyethylene glycol, polyvinylpyrrolidone and magnesium stearate, found in the commercial pancreatin enzyme source used. In a similar manner, relatively higher glucose levels with artificial urine samples U1 and U2 may also be explained with the presence of another penetration enhancer; urea in the formulations. These findings bring novelty to literature as there is no paper present in the literature performing RI in various simulated artificial body fluids.

CONCLUSION

A non-invasive RI determination method for glucose was developed and optimized experimentally. MC gel and CE membrane with 12000 Daltons MWCO combination was employed as default for most of the experiments. According to optimized results, Extraction Electrodes (ExEs) should be placed within 7.0mm distance and silver was found to be the optimum noble metal rather than platinum, palladium, ruthenium and rhodium for fabrication. The reservoir of the RI cell may be best filled with PBS solution of 0.05M at pH 7.4, and regarding the effect of ionic strength, various ionic contents and strengths were investigated in detail. Slightly higher glucose yields were obtained with Differential Pulse Varying Potential (DPV) method, while Constant Potential (CA) and Cyclic Varying Potential (CV) methods achieved almost the same levels. During CA, at +2.0V (*vs.* Ag/AgCl) more reproducible glucose yields were observed. Starting from the

3rd hour, glucose levels could be monitored continuously for 24hours. Carboxymethyl cellulose (CMC) gel (8g/600 mL in 0.06M acetate buffer at pH 5.5) served as the best collection media in the collection chamber of the RI cell. Pretreated CE membrane with 12kD MWCO served as the artificial skin model. Its pretreatment procedure deeply effected extraction performance, while its condition did not make any significant difference. Except the PBS solution (simulated as artificial plasma), among the various artificial simulated body fluids studied (urines, salivas, intestinal juices and gastric juices), intestinal juice formulation (AI) and urine formulation U2 were the extraction media in reservoirs with the highest yields, respectively. Experimental results were summarized and displayed as graphs, and supplementary materials, such as additional experimental data, SEM pictures, schemes, set-up pictures, excel calculations *etc*, have also been provided.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not Applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used in the study that is the base of this research.

CONSENT FOR PUBLICATION

Not Applicable.

AVAILABILITY OF DATA AND MATERIALS

All data explained can be found in the paper and the supplementary material.

FUNDING

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CONFLICT OF INTEREST

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this manuscript.

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Declared none.

SUPPLEMENTARY MATERIAL

Authors provide seven supplements, their names and contents are as follows;

Supplement 1. Word file containing Table **S1**, Table **S2** and Figs. **S1-S4**.

Supplement 2. PDF file of BAS Application Capsule 133.

Supplement 3. Excel file of ANSON Plots Data for AUX area calculations.

Supplement 4. Excel file of ANSON Plots Data for ExE area calculations.

Supplement 5. Excel file of ANSON Plots Data for RE area calculations.

Supplement 6. Excel file of ANSON Plots Data for WE area calculations.

Supplement 7. Excel file of ANSON Plots Data, SUMMARY of all area calculations.

Supplementary material is available on the publisher's website along with the published article.

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Supplementary Material

Optimization of Extraction Parameters of Reverse Iontophoretic Determination of Blood Glucose in an Artificial Skin Model

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TABLE S1. Technical specifications of the electrochemical methods used for RI extraction.

Electrochemical Method	Properties
CA	Constant potential of +500, +1000, +1500 and +2000mV (vs Ag/AgCl) were employed during optimization study for 3.3 hours (200 mimutes).
DPV	E_{begin}:0.0V, E_{end}: +2.0V E_{step}: 0.005V, E_{pulse}: 0.025V scan rate: 0.003V/sec t_{pulse}: 0.07 sec, t_{eq}: 2 sec One scan took 11.6 minutes, 17 successive scans were performed (total run time 197.2 minutes)
CV	$E_{vtx}1$: 0.0V, $E_{vtx}2$: +2.0V E_{step}: 0.005V, E_{start}: +2.0V scan rate: 0.010V/sec number of scans: 30 t_{eq}: 2 sec (total run time 200.5 minutes)

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TABLE S2. Preparation of the buffers used during the optimization of reservoir and collection chamber medium pH.

pH	BUFFER CONTENT	DILUTE TO
1.0	50mL 0.2M KCl + 134mL 0.2M HCl	200mL
2.0	50mL 0.2M KCl + 13.0mL 0.2M HCl	
3.0	982.3mL 0.1M CH_3COOH + 17.7mL 0.1M $CH_3COOHNa.3H_2O$	--
4.0	847.0mL 0.1M CH_3COOH + 153.0mL 0.1M $CH_3COOHNa.3H_2O$	--
5.0	357.0mL 0.1M CH_3COOH + 643.0mL 0.1M $CH_3COOHNa.3H_2O$	--
6.0	52.2mL 0.1M CH_3COOH + 947.8mL 0.1M $CH_3COOHNa.3H_2O$	--
7.0	100mL 0.1M KH_2PO_4 + 58.2mL 0.1M NaOH	200mL
7.4	100mL 0.1M KH_2PO_4 + 78.2mL 0.1M NaOH	
8.0	100mL 0.1M KH_2PO_4 + 93.4mL 0.1M NaOH	
9.0	100mL 0.025M $Na_2B_4O_7.10H_2O$ + 9.2mL 0.1M HCl	
10.0	100mL 0.05M $NaHCO_3$ + 21.4mL 0.1M NaOH	
11.0	100mL 0.05M $NaHCO_3$ + 45.4mL 0.1M NaOH	
12.0	50mL 0.2M KCl + 12mL 0.2M NaOH	
13.0	50mL 0.2M KCl + 132mL 0.2M NaOH	

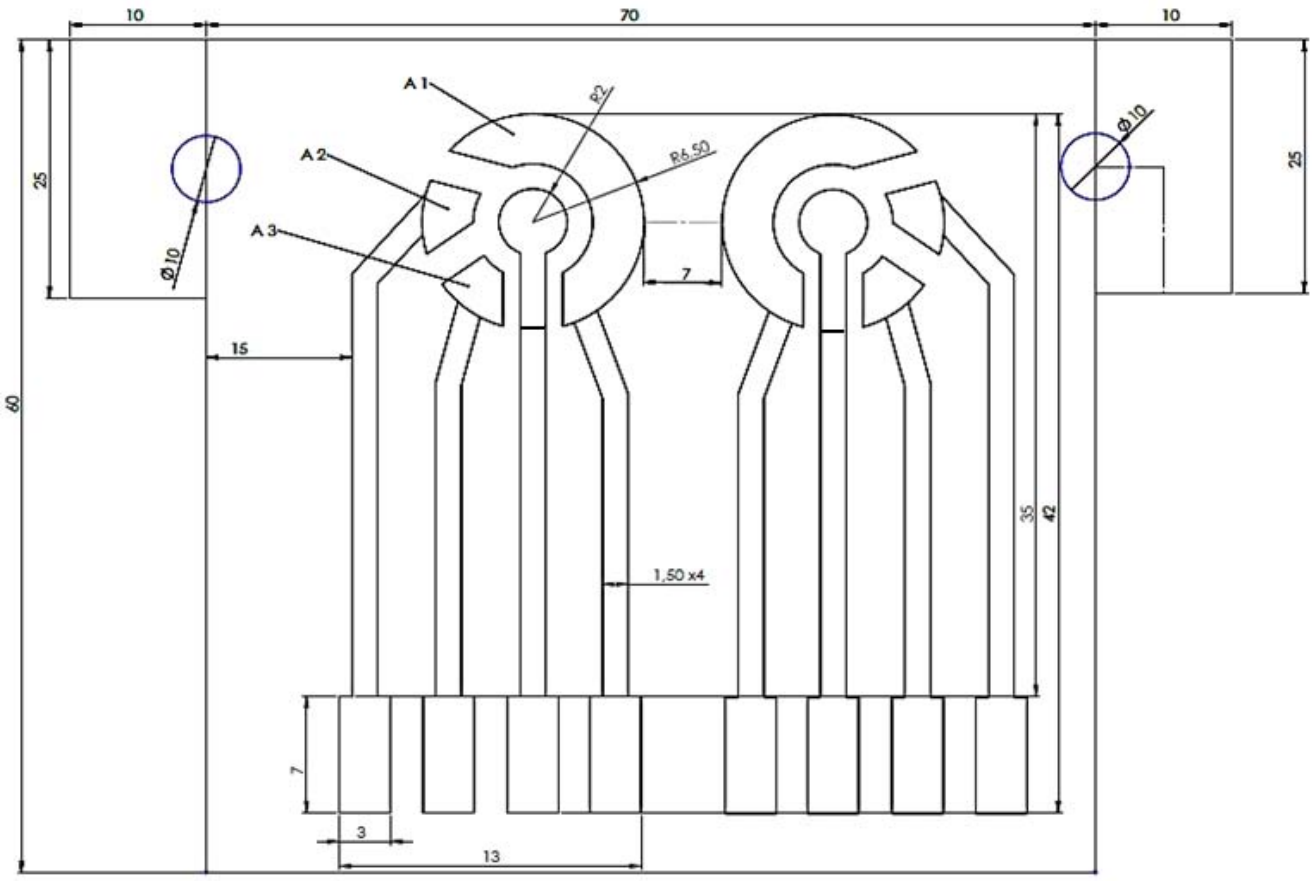

FIGURE S1: Physicial dimensions of the printed circuit board (PCB) three electrode system designed for RI extraction.

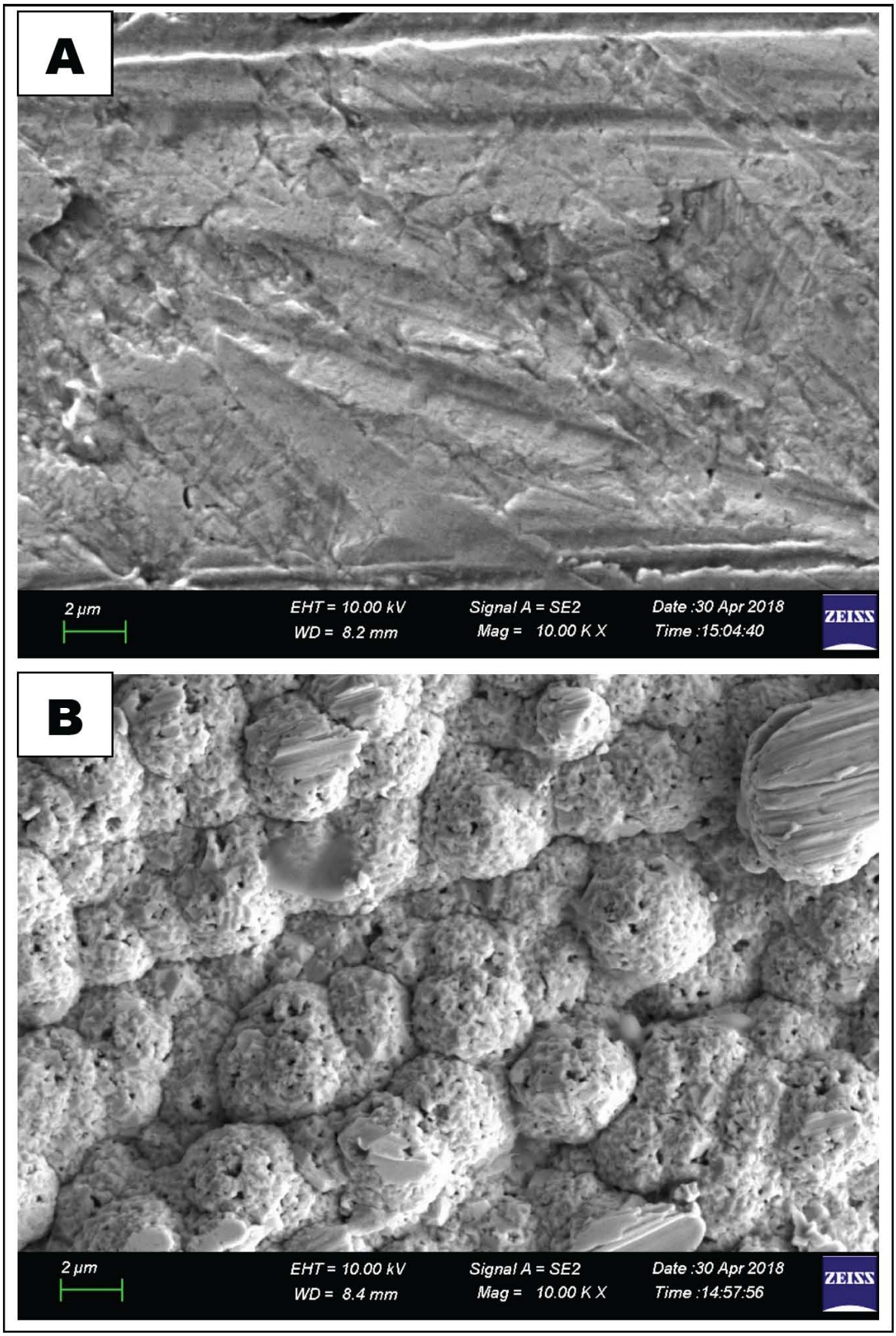

FIGURE S2: SEM images of bare (A) and silver electrodeposited (B) ExE.

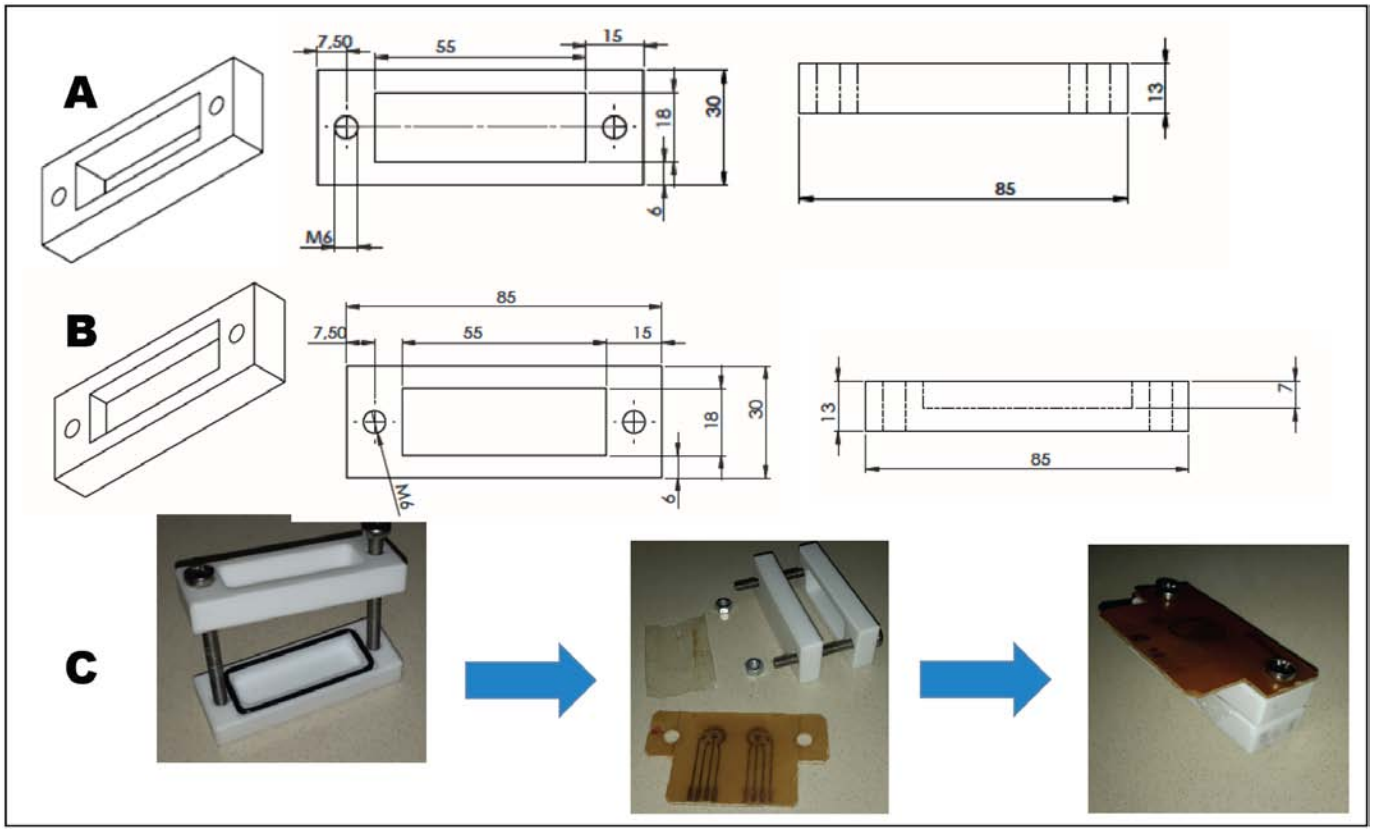

FIGURE S3: Physical dimensions of the collection chamber (A) and reservoir (B) of the RI extraction cell (C).

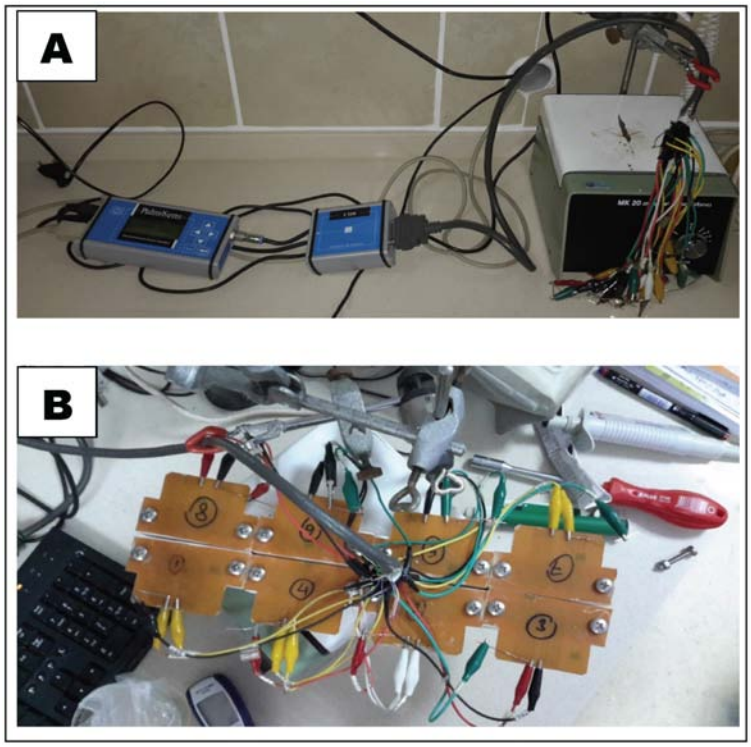

FIGURE S4: Benchtop view of the multichannel RI extraction system (A), composed of 8 independent RI cells (B).

CAPSULES
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May 1998

Determination of Electrode Area with Chronocoulometry

Key Terms
Chronocoulometry, Diffusion coefficient determination, Anson Plot, BAS 100 Series

Accurate knowledge of electrode area is necessary for many electrochemical experiments. Chroncoulometry is a technique commonly employed for the determination of electrode area. Ferricyanide is a well-characterized anion and will be used in this determination. The potential waveform for chronocoulometry is shown in Figure 1. The initial potential (where no electrolysis of ferricyanide occurs) and final potential (where complete reduction occurs) can be obtained from a cyclic votammogram (Figure 2). The chronocoulometric response (Figure 3) is the total charge passed (Q) vs. time (t) from initiation of the step.

The response is described by:

$$Q_t = \frac{2nFACD_o^{1/2}t^{1/2}}{\pi^{1/2}} + Q_{dl} + nFA\Gamma_o$$

where Q_{dl} is the capacative charge. Γ_o is the surface excess of reactant, and the other terms have their usual meaning. The diffusion coefficient (D) of ferricyanide is 7.6 x 10^{-6} cm^2 s^{-1} (1). A plot of Q vs. $t^{1/2}$ (Anson plot, Figure 4) transforms the data into a linear relationship whose slope is $2nFACD_o^{1/2}/\pi^{1/2}$. Note: Be sure to convert to appropriate units. The slope is reported as $\mu c/ms^{1/2}$.

The active area of the electrode determined from the presented data is 7.78 x 10^{-2} cm^2. The calculated radius of 0.157 cm is more accurate than the measured geometric radius of 0.15 cm.

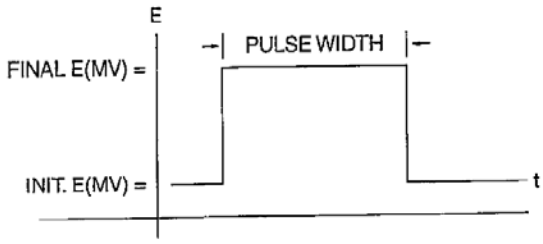

Figure 1. Potential excitation for chronocoulometry.

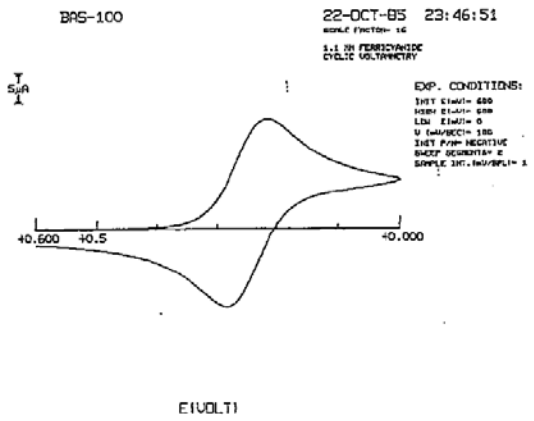

Figure 2. Cyclic voltammogram for the reduction of 1.1 mM ferricyanide in 0.1 M KCl pH 3 obtained at a glassy carbon electrode.

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1. *R.N. Adams, "Electrochemisty at Solid Electrodes," Marcel Dekker, New York, 1969, p. 219 (and references therein).*

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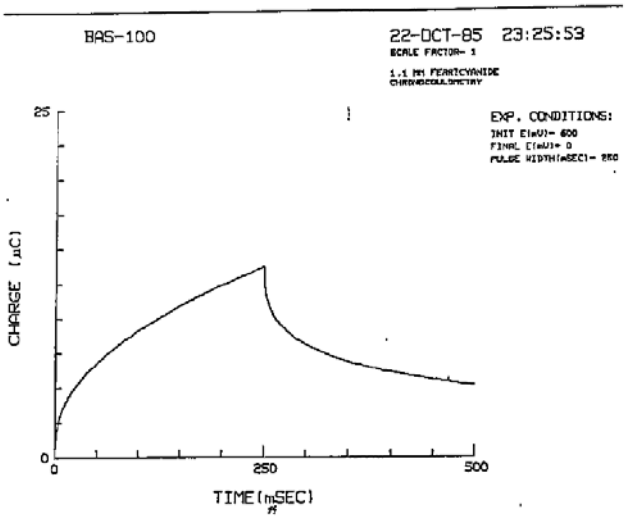

Figure 3. Chronocoulometric response for 1.1 mM ferricyanide.

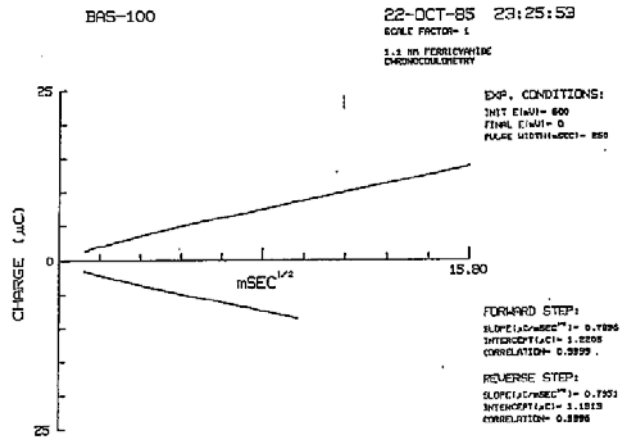

Figure 4. Anson Plot of data shown in Figure 3.