Enhanced sample pre-concentration by ion concentration polarization on a paraffin coated converging microfluidic paper based analytical platform

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A. T. K. Perera,^{1,2,a)} Dinh-Tuan Phan,^{3,a)} Sanam Pudasaini,¹ Yu Liu,⁴ and Chun Yang^{1,b)}

AFFILIATIONS

¹School of Mechanical and Aerospace Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798

²Interdisciplinary Graduate Programme, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798

³Institute for NanoBioTechnology, Johns Hopkins University, Baltimore, Maryland 21218, USA

⁴School of Civil and Environmental Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798

^{a)}**Contributions:** A. T. K. Perera and D.-T. Phan contributed equally to this work.

^{b)}**Email:** mcyang@ntu.edu.sg

ABSTRACT

Microfluidic paper-based analytical devices (μ PADs) represent a modest and feasible alternative for conventional analytical methods. However, the inadequate sensitivity of these devices limits the possible applications of μ PADs. In this scenario, inducing ion concentration polarization (ICP) on μ PADs has shown promise to overcome this limitation by preconcentrating the analytes of interest. Here, we report a μ PAD implementing ICP using an off-shelf Nafion[®] membrane as the perm selective membrane. Two types of devices with a geometrical configuration of a straight channel converging at the middle connecting to circular reservoirs at the end of channels were fabricated. The devices are comprised of a single input channel and an absorption channel. The Nafion membrane is attached to the absorption channel of the device, which is encased by heating with paraffin films at both sides to lower the electro-osmotic flow generated by an applied DC electric field that is needed for ICP. The field induced ICP enables obtaining a maximum concentration factor of more than 2000 folds for fluorescein sodium salt solution on the μ PAD. Also, since evaporation of the sample solution was reported to be of great influence on the concentration factor, we analyze the effect of sample solution evaporation on sample preconcentration. Furthermore, our reported fabrication method for μ PAD can lower the fabrication cost down to 0.3 USD. This device shows the potential to be developed for serving as a diagnostic and environmental monitoring platform.

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I. INTRODUCTION

Use of microfluidic chip devices in sample analysis is taken to a new era with microfluidic paper-based analytical devices (μ PADs) for analytical chemistry applications such as separation of small molecules like protein and amino acids.^{1–4} μ PADs are manufactured using paper as the substrate material. μ PADs possess numerous advantages, including low material, fabrication and operation costs, simple and rapid fabrication processes, fluid handling by passive capillary wetting through porous structure, porous structure supporting effective immobilization of reagents through physical adsorption,

compatibility with various other analytical methods (namely, colorimetric, electrochemical, or electrochemiluminescence), easy storage and transport, disposability, and, above all, the simple and rapid detection capability of devices.^{2,3,5–10} Paper can be found ubiquitously and is available in a wide variety of forms from simple printing paper to highly engineered papers with specific features.³ Among these, Whatman filter papers have been used in most applications while nitrocellulose, paper towels, ITW Technicloth, and other paper types are also recognized as important paper substrates.^{1,7,9,11} Until now, most of these developed μ PADs are designed barely for the

detection of analytes. Also, most of the devices proposed the use of colorimetric detection as a common qualitative and quantitative detection technique in which the μ PADs are coated or predeposited with detection arrays on specific portions of the paper to serve as detection zones.^{3,6,7,9} Hence, in most situations, the limit of detection (LOD) depends on factors such as reactivity/binding of analytes with the detection array, absorption characteristics, color decay, etc. Some devices are reported for the use of electrochemical sensing and electrochemiluminescence as the method of detection for inorganic, organic, or biological sample analysis.^{8,12,13}

Concentration of analyte samples prior to analysis is a common practice in enhancing the output signals on microfluidic platforms. Several techniques, stacking methods like isotachopheresis (≈ 1000) and field amplified sample stacking, gradient focusing methods like isoelectric focusing and electric field gradient focusing, and other techniques such as electrophoretic and electrokinetic trapping are just to name some.^{14–18} Most of these techniques inherit their own pros and cons. For the stacking methods, it requires longer channels for the concentration of larger volumes, and limitations in changing velocity limit the obtainable concentration factor (CF), whereas during the field focusing methods, each of them require specific conditions, for instance, the isoelectric focusing can be applied to analytes that only have an isoelectric point. In the analyte trapping techniques, electrokinetic trapping is known to be a better performing technique, which depends on ion concentration polarization than the other techniques in that category.¹⁸

Ion concentration polarization (ICP) is a successful method that allows preconcentration of charged samples. ICP, being an ionic transport phenomenon, occurs near an ion selective nanostructured membrane and is induced by the formation of an ion enrichment zone and an ion depletion zone, causing the ions in the solution to get accumulated at a narrow region at one side of the membrane. This is usually called the sample plug and can be used to preconcentrate dilute samples.^{19–22} Many researchers studied on this principle, and in almost all the designs the perm selective membrane was fabricated with different derivatives of Nafion: either off-shelf membranes or solutions of different concentrations.^{11,19–30} Among the designed devices, both μ PADs and conventional microfluidic platforms were developed with a substrate material like polydimethylsiloxane (PDMS). Phan *et al.* developed a continuous flow droplet based concentrator that uses the phenomenon of ICP for the concentration of samples, enabling a 100-fold concentration of a sample with droplet sizes ranging from 20 to 25 μm .³¹ During the studies with μ PADs, Yeh *et al.* obtained a maximum concentration factor (CF) of 944 folds within 30 min, which is believed to be the highest concentration factor reported.²² Recently, Gao *et al.* and Han *et al.* reported obtaining a CF of 50 for Rhodamine 6G and a CF of 40 for human serum, respectively.^{32,33} However, despite the advantages possessed by μ PADs implementing ICP, the fabrication techniques are still under development and their performance is dependent on manual operation and manipulation of conditions applied during operation.

Here, we present a μ PAD that enables enhanced sample preconcentration by using an off-shelf Nafion membrane with a design of converging and encased sample transporting channels, thereby achieving a maximum concentration factor of 2019 folds for the both side paraffin coated channel device. Also, the effects of evaporation and electro-osmotic flow (EOF) on sample preconcentration are

examined for one side coated and both side coated devices, showing the importance of channel encasement to attain a higher concentration factor while implementing ICP. Furthermore, the change of depletion layer length with an applied electric field is studied. Additionally, we report a convenient, flexible, and a less time-consuming fabrication process to manufacture μ PADs implementing ICP by direct cutting of paper, paraffin, and off-shelf Nafion membranes into desired pattern and stacking and assembling them by oven heating with an applied pressure.

II. WORKING PRINCIPLE

The phenomenon of ICP is implemented under an applied DC electric field such that ions (charged molecules) tend to move toward either a cathode or an anode. However, the presence of an ion selective membrane, Nafion, across the path allows flow of only cations through the membrane while preventing movement of anions. This leads to a charge imbalance in the system. Hence, ICP tends to form an ion enrichment region in the cathodic side of the nanostructured Nafion membrane and an ion depletion region at the anodic side to regain the charge balance in the system. During the creation of the depletion zone, a repulsive force acts on the ions trapped at the anodic side, preventing the movement of both cations and anions through the Nafion membrane. Thus, all ions get accumulated at a narrow region of the anodic side of the ion selective membrane, commonly known as the sample plug. In a converging channel, this trapping of ions at the anodic side gets more congested, leading to a denser and localized sample plug. Figure 1 shows a schematic illustration for the ICP phenomenon on a fabricated μ PAD. During our experiments, under an application of a DC electric field, where a positive voltage is provided to the sample inlet using the common electrode connected to the power supply and the cathodic connection is placed over the Nafion membrane, flow of sample gets restricted by the depletion force, and, therefore, the sample tends to be concentrated in the anodic side at a location closer to the Nafion membrane. The location of this sample plug is adjustable by variation of a DC electric field. Furthermore, the phenomenon of ICP can be influenced by the ionic strength and conductance of the perm selective membrane.^{19–21,34} However, it is not constrained by the hydrophobicity or binding characteristics of the molecules/ions or compound of interest.

III. METHODS

A. Device fabrication

The device was fabricated with a straight channel configuration, which complies with a sample inlet channel and an absorption channel with two reservoirs at both ends of the channels. The channels are converged as they meet at a junction in the middle of the device where a Nafion membrane is located. Two types of devices as one side paraffin coated and both sides paraffin coated were fabricated. For simplifying the fabrication process, each of the designs was prepared using Silhouette Studio, custom software designed for electronic craft cutting (Silhouette America Inc., Silhouette Cameo), and direct cutting was used as the fabrication technique. Whatman No. 1 quantitative filter paper, paraffin films, and Nafion membranes were all cut into a desired size and shape

FIG. 1. Schematic illustration of the working principle of implementing ion concentration polarization (ICP) on a fabricated microfluidic paper-based device (μPAD), EOF—electro-osmotic flow, EP—electrophoresis, F_{ICP}—depletion force created by ICP, F_{ca}—capillary force, which is due to a certain amount of the liquid sample left at the inlet reservoir under an applied electric field.

with the aid of the electronic cutter. In total, the μPADs consist of four layers: the top and bottom paraffin layers, the Nafion membrane layer, and the paper platform layer. An illustration of assembly of these layers is shown in three-dimensional view in Fig. 2(a). The overall dimensions of the device do not exceed 50 × 20 mm², indicating its suitability for on-site monitoring applications due to its compatible and smaller size. Figure 2(b) illustrates the fabricated

μPAD. The device comprises one in-built electrode, which runs over the Nafion junction to supply negative voltage, whereas a common electrode is used to supply a positive voltage.

The assembly of the components for the μPAD was done manually on a glass slide following several steps of stacking of layers and heating in an oven at 80 °C for a predetermined time. The oven heating was performed in two stages: during the first

FIG. 2. (a) Schematic of a three-dimensional view of the designed microfluidic paper-based device. (b) The device with a straight channel geometry. (c) The effect of heating time on the channel thickness. The bottom inset depicts the change of paraffin layer thickness with 2nd stage heating time; the top inset shows a comparison of the channel thicknesses for one side coated (2nd stage heating time—0 min) and both side coated (2nd stage heating time—3 min) devices.

stage, the bottom paraffin layer was adhered to the paper by heating for 5 min, whereas the second stage was used to assemble the top paraffin layer to encase the paper channel with the Nafion membrane. Here, the heating time was varied to study its effect on the channel thickness. During heating, paraffin films got melted and penetrated into the paper substrate, determining the thickness of the paper channel. This avoids the need of filter papers with different heights for fabricating paper channels with different thicknesses.^{1,3} Figure 2(c) illustrates the effect of the heating time on the channel thickness during the device fabrication. The inset figure provides a figurative comparison of a device, fabricated with the first stage heating that comprises only of a bottom paraffin layer, with a device with the second stage heating and a top paraffin layer. The channel thickness got reduced with the second stage heating. Graphical illustration shows that, with increasing the second stage heating time, the channel thickness is reduced gradually as the higher the heating time, the deeper the penetration of paraffin into the paper substance. However, the reduction of the channel thickness is affected by the limited thickness of paraffin, leading to a slower reduction rate of the channel thickness, further increasing the second stage heating time. The increment in the penetrated depth of wax into the paper is illustrated in the inset graph of Fig. 2(c). A detailed description for calculating the channel thickness is given in S1 in the [supplementary material](#). Considering the observations made during device fabrication and analyzing the factors that affect channel thickness and experimental conditions, we set 3 min as the optimal heating time in the second heating cycle for the both side paraffin coated device. To prevent the shrinkage of paraffin films and to maintain the alignment of paraffin film on top of filter paper during heating, a combination of paraffin film and filter paper was clamped in between two glass slides by using paper clips of a similar size. These clips maintain the alignment between different layers of the device. Aluminum (Al) foil was placed between the glass slide and the paraffin film to avoid sticking of paraffin films to the glass slide. During the second stage of heating, Al foil was placed only between the top paraffin layer and the glass slide, causing the bottom paraffin layer to stick on one of the glass slides. Furthermore, the use of a reusable anode electrode makes the fabrication process convenient and reduces the costs associated with fabrication and, most importantly, the process results in the fabrication of a disposable paper device in a convenient and a flexible manner within a short time. A detailed calculation of the fabrication cost and time can be found in S2 and S3 in the [supplementary material](#).

B. Materials

Whatman grade 1 qualitative filter paper with a pore size of 11 μm and a thickness of 180 μm (United Scientific, Singapore) was used as the platform of our μPAD in all experiments. Nanoporous Nafion perfluorinated membrane sheets were purchased from Alfa Aesar (Thermo Fisher Scientific Inc.) laboratory to serve as the perm selective membrane, and paraffin film (Parafilm "M") was used as a hydrophobic substance to determine the channel thickness.

Fluorescein sodium salt ($\text{C}_{20}\text{H}_{10}\text{NaO}_5$) and sodium chloride (NaCl , ACS reagent, $\geq 99.0\%$) were purchased from Sigma-Aldrich. Ultrapure type-01 de-ionized water was obtained from a PURELAB

Option-Q water purification system. All glassware was thoroughly washed with Iso Propyl Alcohol (IPA), rinsed with de-ionized water and oven dried prior to use. Fluorescein sample solution for preconcentration demonstration was prepared by mixing 80 μM fluorescein sodium solution with 100 mM sodium chloride solution at 1:1 volume ratio. A series of fluorescein sodium salt solutions with various concentrations of 500 mM, 100 mM, 1 mM, 100 μM , 1 μM , 500 nM, and 100 nM were prepared for the calibration purpose. Fluorescein sodium salt was dissolved in ultrapure de-ionized water for the preparation of 500 mM fluorescein sodium solution. A serial dilution was conducted from 500 mM to 100 nM solution using ultrapure de-ionized water as the dilution agent. An ARE heating magnetic stirrer was used for the preparation of solutions.

C. Experimental setup

The experimental setup consists of a high voltage power supply (Stanford research systems, Inc., Model PS350/5000V-25W), an inverted fluorescence microscope (Eclipse TE2000-E, Nikon), and a personal computer for data acquisition. A MIRO camera with Phantom Camera Control 2.14b was used in capturing fluorescence images acquired through the microscope, and an analysis of the images for several test parameters was done using ImageJ software. All videos were recorded using a Canon 300D digital camera, and the analysis was performed using ImageJ and Microsoft Excel software.

IV. RESULTS AND DISCUSSION

A. Device characterization

Prior to the demonstration of ICP for analyte preconcentration, it is essential to characterize the device. Hence, the capillary flow through the paper channel was studied as the initial step of characterization. Flow velocity through channels provides information on self-driven flow of solution due to capillary action and essentially the effect of converging channel for the sample preconcentration. Accordingly, to measure the flow of solutions through the channels of the one side paraffin coated and both side paraffin coated devices, 50 μl of 100 mM fluorescein solution was placed on each device's sample inlet. Converging straight channel geometry was used as the paper-based platform. The movement of solution through the channel was captured in a video, and frames extracted from the video were used for the calculation of the traveled distance of the sample with time by "ImageJ" software. Figurative and graphical representation of fluid front traveled distance vs time in both devices is shown in Figs. 3(a) and 3(b). Both illustrations show that, having a larger channel thickness, the one side paraffin coated device yields a higher velocity than that of the both side paraffin coated device. From the gradient of graphical representations, we can see that as the samples reach the converging region, there is a slight reduction in velocity; as the samples move out from the converging region, there is a slight increment in velocity. A graphical illustration for this converging-diverging section (10–15 mm) is provided in S4 in the [supplementary material](#) to clearly show this effect as Fig. 3(b) illustrates the fluid front movement with time for the whole channel length. It indicates that the effect becomes more significant for the both side paraffin coated device. The variation of velocity in the converging region proves that there is a squeezing

FIG. 3. (a) Images showing flow through the channel: scale bar = 10 mm. (b) Flow through the channel in terms of distance vs time. Gradients representing the velocity in each region are: 0.39 mm/s before the converging region and 0.29 mm/s at the diverging region. The dashed lines represent the converging diverging region in the device. (c) Electropherograms showing current vs time by using the current monitoring method for measurement of EOF. (d) Current vs voltage relationship on the μ PAD during ICP preconcentration of samples; Region (I)—Ohmic region; Ohmic conductance = $4.67 \mu\Omega^{-1}$, Region (II)—Limiting region, Region (III)—Overlimiting region; overlimiting conductance = $2.12 \mu\Omega^{-1}$.

effect on the fluid flow, making it to concentrate at a more localized region and thus improving the concentration factor. Furthermore, the steep gradient at the beginning of the flow in each device indicates a pressure induced flow due to a pressure difference between the sample inlet and the absorption reservoir.

Also, we observed a certain amount of sample liquid left in the inlet reservoir once the channel is fully wetted by capillary flow. This observation confirms that the sample volume of $50 \mu\text{l}$ is adequate and acts as an unlimited supply of liquid for the given channel length without drying off the channel during the process of implementing ICP. This is also further confirmed by the study on the effect of evaporation in Sec. IV B.

Electro-osmotic flow (EOF) of solutions within the paper channel is another important factor that affects device performance. If the EOF velocity within the channel is reduced, a localized concentration plug with a higher fluorescence intensity can be obtained.²² Hence, the effect of paraffin coating on EOF was measured by using the current monitoring method^{35,36} at a fixed voltage of 400 V. For this, two phosphate-buffered saline (PBS) buffer solutions with different concentrations that slightly differ from each other were used. Current was measured referring to standard procedures, and Fig. 3(c) shows the schematic of the basic procedure of EOF measurement for a microchannel together with the variation of current with time for devices with different

channel thicknesses. The calculation of EOF velocity is provided in S5 in the [supplementary material](#). It is seen that the one side paraffin coated device yields a higher EOF, which is 0.2 mm/s, while the both side paraffin coated device results in a lower EOF. Moreover, EOF gets reduced as the channel thickness is reduced. For both side coated devices, EOF velocities are 0.167 mm/s and 0.132 mm/s for the devices with second stage heating times of 3 min and 5 min, respectively. This reduction of EOF is due to the restricted movement of solutions through the limited thickness of the both side paraffin coated devices as well as by lower zeta potential of paraffin wax, and it can be explained by Smoluchowski equation^{37–42} as

$$u = (\epsilon\zeta/4\pi\eta)E, \quad (1)$$

where ϵ is the dielectric constant for the electrolyte, ζ is the Zeta potential, η is the viscosity, and E is the applied electric potential.

As the Zeta potential of system is reduced by both side coated wax, it leads to the lower EOF velocity of electrolyte.

Current vs voltage variation was studied as a measurable indication of implementing ICP. The measurements provide informative data to predict a working voltage range for a μ PAD to implement ICP. The general pattern of current vs voltage variation is shown in Fig. 3(d). In region I, initially the current

increases linearly with voltage following the Ohmic relationship and is hence called the Ohmic region. Gradually, the current reaches an almost stable value with increasing voltage since the current is affected by the presence of the depletion zone, thereby forming region II. Hence, this region is named the limiting region and identified as the voltage range where the phenomenon of ICP can successfully be implemented. Further increment of the applied voltage results in increasing current flow with the destruction of the depletion zone by electroconvection that occurs at relatively high voltages. Hence, region III is identified as the overlimiting region.^{21,22}

B. Effect of evaporation of solution on sample preconcentration

During sample preconcentration on paper-based devices, the forward flow of solution in channels is governed by capillary flow. However, some of the solution gets evaporated into the surrounding from the open reservoir and open channels, where applicable, reducing the volume of sample available for preconcentration. Hence, the droplet volume on the inlet reservoir in both, one side paraffin coated and both side paraffin coated devices, were analyzed experimentally.

The experimental setup consists of a solid stage for the device to reside, a long working distance lens attached to MIRO camera focusing on a predetermined spot on the stage to capture the droplet images, and an inverted Nikon 300D camera hanging over the device to capture flow through the channel. 50 μ l of 100 mM

fluorescein sodium solution was inserted into the inlet reservoir of the device with a micropipette. It should be noted that the inlet reservoir of the both side paraffin coated device comprises of an open area to the atmosphere for introduction of samples. Also, for the clarification of this phenomenon, evaporation of a droplet having the same volume on a nonabsorbing surface (glass) was also investigated. The analysis of the captured droplet images was performed with the aid of a "ImageJ-Low Band Asymmetric Drop Shape Analysis (LB-ADSA)" tool. During the analysis, it was assumed that the fluorescein sodium solution behaves similarly to water since a lower concentration aqueous solution of fluorescein sodium salt was used.

The results provide the combined effect of evaporation and flow through the channel on volume reduction. Figure 4(a) shows the droplet volume reduction on the reservoir with time for different devices/surfaces, which are nonabsorbing, one side paraffin coated, and both side paraffin coated, while the inset figure shows the side view of a droplet captured by MIRO camera. This graph indicates that the volume reduction of the droplet on nonabsorbing surface follows a linear trend, whereas for both the paper devices, the initial rate of the volume reduction is higher and then a gradual reduction is observed, which is due to the combined effects of both evaporation and flow through channel toward the volume reduction of the sample droplet. This reduction follows a third order polynomial trend. The initial sudden volume reduction observed on paper-based devices is mostly supported by the dry and porous paper channel available for the flow as well as the pressure induced by the initial droplet volume on flow through channel

FIG. 4. (a) Droplet volume on the reservoir. The inset image shows a side view of the droplet on the reservoir: scale bar = 1 mm. (b) Volume of sample absorbed into the porous channel for both devices. (c) Comparison of flow through the channel with the combined effects of flow through the channel and evaporation on droplet volume reduction. (d) Volume loss due to the sole effect of evaporation.

rather than evaporation. As the time elapses, the volume reduction rate is reduced since the channel gets saturated with continuous flow, nonexistence of pressure induced flow, and reduced volume of the droplet remaining on the reservoir. Furthermore, the droplet volume on the one side paraffin coated device reservoir is greatly reduced than that on the both side paraffin coated device reservoir, indicating the increased evaporation from open channel geometry and increased flow through channel due to a larger channel thickness. Another interesting fact observed is that the volume reduction rate roughly doubles from the nonabsorbing surface to the both side paraffin coated device and again from the both side paraffin coated device to the one side paraffin coated device. This confirms that the presence of paraffin coating exerts a positive effect on minimizing the evaporation of sample solution from the reservoir, and volume reduction is the combined effects of both evaporation and forward flow of solution through the channel. The times taken for 50 μ l sample to completely dry on the nonabsorbing, both side paraffin coated and one side paraffin coated surfaces are approximately around 142 min, 65 min, and 24 min, respectively.

Since the analysis only provides the combined effects of evaporation and flow through the channel, a separate analysis was carried out to identify the contribution from flow through the channel toward volume reduction in the reservoir. For this purpose, the paper was considered as a porous medium and an area calculation was performed with the aid of ImageJ. Hence, the following simple mathematical formula of calculating pore volume in a porous medium was adopted to calculate the volume of sample transported through a channel,

$$V_p = \varphi \times V_b, \quad (2)$$

where V_p , φ , and V_b stand for the pore volume, porosity, and bulk volume, respectively.

For a paper-based device, V_b takes the form of

$$V_b = A \times t. \quad (3)$$

φ is defined as

$$\varphi = 1 - \frac{\rho_{\text{paper}}}{\rho_{\text{fibre}}}, \quad (4)$$

where A is the area of the device covered by the solution, t is the channel thickness, ρ_{paper} is the bulk density of paper, and ρ_{fibre} is the density of cellulose fibers used in filter paper. For Whatman No. 1 papers, ρ_{paper} and ρ_{fibre} take the values of 483 kg/m³ and 1540 kg/m³, respectively.⁴³

It is assumed that the porous channel becomes saturated during flow and there are no void spaces within the area wetted by the solution. The paper porosity does not affect the volume calculations based on our observation that part of the solution is absorbed immediately by the paper in the reservoir area once it is introduced to the sample inlet reservoir. In the calculations, the porosity of Whatman No. 1 filter paper-0.69 was used. Figure 4(b) shows the sample volume vs time for both devices. Clearly, the volume absorbed into the channel increases almost linearly with time for both devices. Also, it suggests the higher flow through the channel

in the one side paraffin coated device mainly due to its higher channel thickness. Figure 4(c) shows a comparison of the combined effects of evaporation and flow and the effect of flow through channel on the volume reduction in reservoirs of the two paper-based devices. The volume reduction on the single side paraffin coated device is greatly contributed by evaporation, thereby reducing the available volume of sample for preconcentration. This is one of the most important reasons that preconcentration on the single side paraffin coated device ends up at a lower concentration factor. Figure 4(d) depicts the volume loss due to the sole effect of evaporation for the two devices. The figure provides the cumulative volume evaporated with time. Initially, there is a significantly higher-volume reduction within a certain time in the one side paraffin coated device due to evaporation when compared to the both side coated device. Thereafter, the volume reduction on the one side coated device is not observed, indicating that the volume reduction is governed by flow through the channel. However, for the both side paraffin coated device, the initial volume reduction is supported by flow through the channel rather than by evaporation. The comparison of volume loss rate also implies that the top paraffin layer acts as a barrier for the sample evaporation and it greatly inhibits the evaporation of solution from the paper channel. It provides adequate time for the device to draw the sample into the channel prior to evaporation.

C. Preconcentration of sample by ICP

Figure 5(a) shows the graphical representation of sample concentration in the one side coated device, while Fig. 5(b) shows the same for the both side coated device. In the both side coated device, a maximum fluorescence intensity of 223.4 was attained within the preconcentration duration of 803 s, corresponding to a fluorescein concentration of 80.78 mM. This results in a CF of 2019, which is the highest reported up to date to the best of our knowledge. The initial concentration of fluorescein sodium salt in the solution used for the implementation of ICP was 40 μ M. A detailed calculation of the concentration factor determination is provided in S6 in the [supplementary material](#). As for the one side coated device, the observed CF was only 62.5, much lower than that of the both side coated device. The resulting higher concentration factor in the both side coated device can be attributed to the combined effects of a lower EOF and much smaller sample evaporation during the process; these resulted from both side paraffin coating and the effect of channel converging geometry in the preconcentrating region.

At lower concentrations, within the paper channel of the one side paraffin coated device, the fluorescein seems to saturate rapidly, giving rise to a higher intensity value for the same fluorescein concentration than that obtained for the both side paraffin coated device. This is supported by the higher channel thickness of the one side paraffin coated device, which allows the sample to move forward within the channel rapidly. However, the evaporation from the top surface of the one side paraffin coated device lowers down the maximum attainable fluorescence intensity of the device, whereas the both side paraffin coated device has an encased paper channel, thereby achieving the higher maximum fluorescence intensity.

FIG. 5. (a) Fluorescence intensity vs time for the one side coated straight channel geometry. The inset shows the calibration curve for the one side coated device. (b) Fluorescence intensity vs time for the both side coated straight channel geometry. The inset shows the calibration curve for the both side coated device. (c) Depletion layer length vs applied voltage. The depletion length scales as a quadratic power of applied voltage.

Since the concentration at the anodic side leads to the concentration band movement further toward the anode with changing voltages, a scaling analysis was conducted to determine the depletion length increment with voltage. Figure 5(c) shows the depletion length scales as a quadratic power of applied voltage. This, however, does not support Dukhin's prediction⁴⁴ that the depletion layer thickness decreases inversely with an electric field.⁴⁵ This scaling of the depletion length as a quadratic power of applied voltage provides a means of adjusting the system performance to obtain the concentration band of desired samples at a predetermined position on the anodic side of the channel through regulating the applied voltage within the limiting region.

The sample solutions of fluorescein sodium salt used during the experiment were mixed with NaCl. The addition of NaCl in 1:1 volume ratio to fluorescein sodium solution serves the purpose of increasing ionic concentration in solution and hence the rapid implementation of ICP to preconcentrate samples. The phenomenon behind this is that as the concentration of ions increases near the Nafion membrane, the creation of ion depletion zone is observed sooner than that at a lower ion concentration case, leading to a fast implementation of ICP.

V. CONCLUSION

In this study, we presented a convenient, flexible, and less time-consuming fabrication process for μ PADs using Whatman filter papers and laboratory paraffin films, thus reducing both fabrication time and cost significantly. The device fabricated following a featured design with encased converging channels at the middle has achieved significantly enhanced concentration factors of more than 2000 for the straight channel geometry. Our studies showed that this enhanced concentration factor is due to the reduced EOF, reduced evaporation of the sample, as well as the squeezing effect of the converging channel geometry. Furthermore, for the one side paraffin coated straight channel device, a concentration factor of 62.5 was obtained. The reduction of the concentration factor is greatly contributed by evaporation of sample through open channel geometry; hence, less sample is available for preconcentration. Also, we showed that the paraffin coating on either side of the paper channel serves as a channel thickness determining agent, a good hydrophobic barrier substance that reduces the EOF, and an encasing for the paper device, which minimizes the evaporation of sample. Additionally, we found that the depletion length in the

μ PAD scales with a quadratic power of applied electric voltage. Nonetheless, the present simply fabricated, inexpensive device shows a potential for a broader range of analytical applications from laboratory sample preconcentration, point of care diagnostics to environmental monitoring.

SUPPLEMENTARY MATERIAL

See the [supplementary material](#) for the detailed calculations presented in the study.

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