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Short communication

The effect of the evaporation rate on electrochemical measurements with paper-based analytical devices and its minimisation by enclosure with adhesive tape

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ABSTRACT

In this work, the effect of liquid evaporation on electrochemical measurements with electrochemical paper-based devices (ePADs) is studied. The time evolution of cyclic voltammograms of ferricyanide is used to study the effect of liquid evaporation under different conditions of relative humidity and air currents. Moreover, the enclosure of ePAD channels with adhesive tape is evaluated in terms of minimising the evaporation, as well as increasing the sensitivity in the electrochemical determination of horseradish peroxidase in long-term assays.

1. Introduction

Paper has long been used as a substrate in chemical analysis. However, the concept of microfluidic paper-based analytical devices (PADs) was first introduced in 2007 [1]. Two years later, Dungchai et al. introduced paper-based devices for electrochemical detection i.e. electrochemical PADs (ePADs) [2]. Since then, research into ePADs has been extensive because they offer a quantitative detection system with better performance than PADs in terms of sensitivity, selectivity and detection limits. Electrochemical detection also has other interesting advantages such as the possibility of miniaturization, allowing the development of low-cost and portable equipment for on-site measurements, as well as access to a wide range of well-established electroanalytical methods. On the other hand, self-powered fluid transport by capillarity, high surface area/volume ratio, capacity for reagent storage or requirement of small sample volumes are inherent ePAD characteristics that provide new electroanalytical opportunities across many fields of interest [3–6].

Unlike conventional microfluidic devices, where fluids are fully enclosed within glass or plastic channels, (e)PADs contain channels patterned into paper which are usually open at the top and bottom. This exposes them to external risks such as contamination, fluid leakage onto any surface in contact with the channels and/or liquid evaporation. The latter has been studied in relation to the relative humidity (RH) in the air because this can modify the capillary flow into the paper [7]. Hence, the solvent evaporation rate increases at low RH values, slowing down or even stopping the flow rate when evaporation prevails over the capillary force. Some authors have reported this effect for water in chromatographic paper grade 1 which is particularly relevant for extended channels (> 30 mm) [8–10]. Several solutions have been proposed to prevent or minimise the evaporation, mainly based on sealing or covering the channels with impermeable layers such as poly(ethylene terephthalate) films [9], toner [10] or adhesive tape [11]. On the other hand, evaporation can be intentional in some applications. Using adequate (e)PAD designs, the liquid carrier can be passively [12] or actively [13] evaporated from prefilled channels, in such a way that the liquid sample flows by capillarity to compensate for the evaporation and the target analyte is accumulated at the detection area, thus improving the limits of detection. However, between these two opposite cases, there are a lot of applications in which the evaporation rate has not been managed and its effect on the analytical signals ignored.

This work presents some studies on the influence of the evaporation rate on electrochemical measurements with ePADs and the result of using adhesive tape to enclose the detection areas. The effects of the environmental RH and air currents on the ePAD signals are estimated by the time evolution of cyclic voltammograms of ferricyanide. Finally, adhesive-tape enclosed ePADs are evaluated for the amperometric determination of horseradish peroxidase crosslinked with glutaraldehyde in a bovine serum albumin matrix, using different reaction times and ferrocyanide/hydrogen peroxide as the enzymatic substrate.

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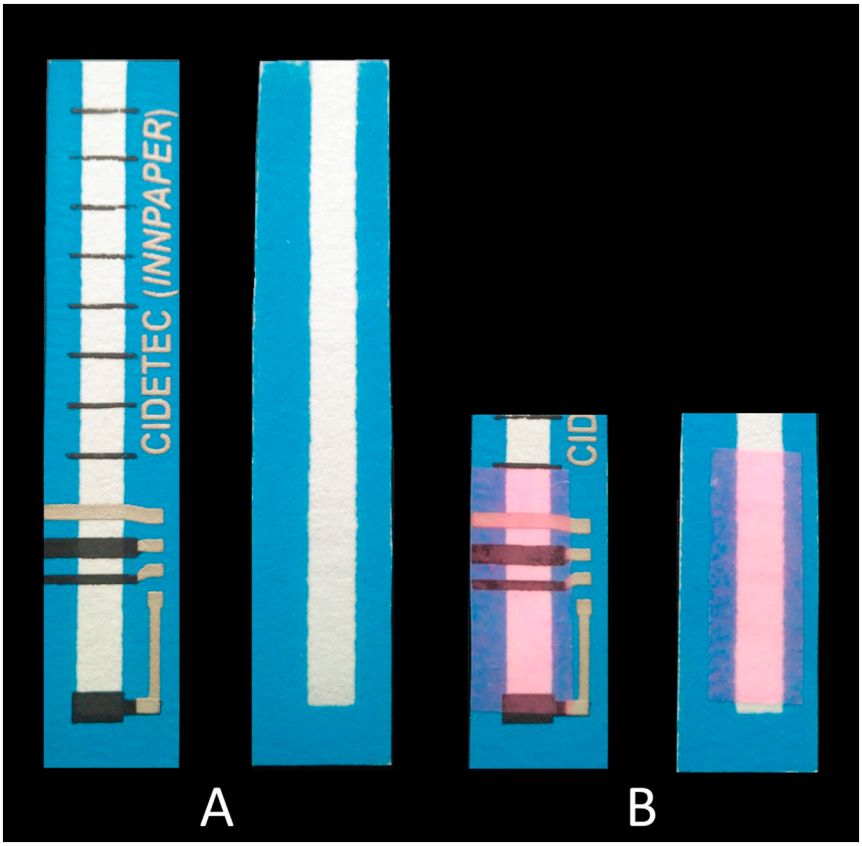

Fig. 1. Front and back of the (A) uncovered and (B) adhesive-tape enclosed ePADs. The tape is highlighted in pink for the picture. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Experimental

2.1. Reagents, materials and apparatus

Peroxidase from horseradish type II (HRP) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich. Other chemicals were also obtained from Sigma-Aldrich. They were of analytical grade and used without any further purification. All solutions were prepared in 150 mM NaCl, 10 mM phosphate buffer pH 7.0 (PBS). The HRP solutions were prepared in 0.2% BSA and PBS. The HRP substrate solution was 25 mM potassium ferrocyanide and 1 mM H_2O_2 in PBS. The HRP activity for ferricyanide was 57 ± 1 U·mg^{-1}, determined by spectroscopy (data not shown).

Scotch Magic Tape, Whatman chromatography paper 4 from GE Healthcare Life Sciences and other materials (described elsewhere [14]) were used to construct the ePAD. An Iviumstat potentiostat from Ivium Technologies and a home-made four-pin connector were used to carry out the electrochemical measurements. A HD200 thermo-hygrometer from Kimo, a Synergy HT Multi-Mode Microplate Reader from BioTek, an ultrapure water synthesis A10 Purification System from Millipore and a pH meter GLP2 from Crison were also employed. A polystyrene foam box with a soaked paper towel inside was used to create an atmosphere with high RH. The thermo-hygrometer probes and electrode connections were introduced through small holes in the back of this box. Compressed air was used for the active evaporation study. The different values of RH tested during the experiments were 98% (inside the polystyrene box), 45% (laboratory conditions) and 2.5% (air flow).

2.2. ePAD fabrication

The fabrication of the ePAD has been described elsewhere [14]. Briefly, Ultraswitch UVSW diluted with UVV6 was used to pattern hydrophobic barriers in chromatography paper 4 by screen printing. After drying in the UV oven, graphite and silver/silver chloride layers were successively printed and cured in the convection oven (120 °C, 5 min each). Fig. 1A shows the front and back sides of the ePAD. The hydrophobic walls delimited a rectangular microfluidic channel of 5 × 65 mm. The electrode system consisted of four electrodes arranged perpendicularly to this channel i.e. an Ag/AgCl pseudoreference electrode (1.5 mm wide) and three carbon electrodes: working (2 mm wide), counter (1 mm wide) and trigger (2 mm wide). The latter electrode was located at the end of the channel and was used to detect the arrival of liquid [14]. Eight additional carbon marks, separated by a distance of 5 mm along the microfluidic channel, were also printed as reference lines to facilitate the use of the ePADs. Afterwards, a strip of adhesive tape (23 mm long) was stuck onto each side. Each tape strip was located 2 mm from the end of the channel. Finally, the ePAD was cut out in such a way that the final channel length was 30 mm with a 5 × 5 mm open area for the sample to enter (Fig. 1B).

2.3. Enzyme immobilization

1 µl of HRP in 0.2% BSA solution was deposited on the back of the working electrode and dried at 37 °C for 5 min. Then, 1 µl of 0.1% glutaraldehyde was deposited at the same place and dried at 37 °C for 5 min. Finally, the adhesive tapes were stuck on and the ePAD was cut down.

2.4. Electrochemical measurements

All electrochemical measurements were registered after dropping 30 µl on the entrance area and waiting for the liquid to advance as far as the trigger electrode (around 120 s). Two types of electrochemical measurements were carried out. Firstly, cyclic voltammograms of 5 mM

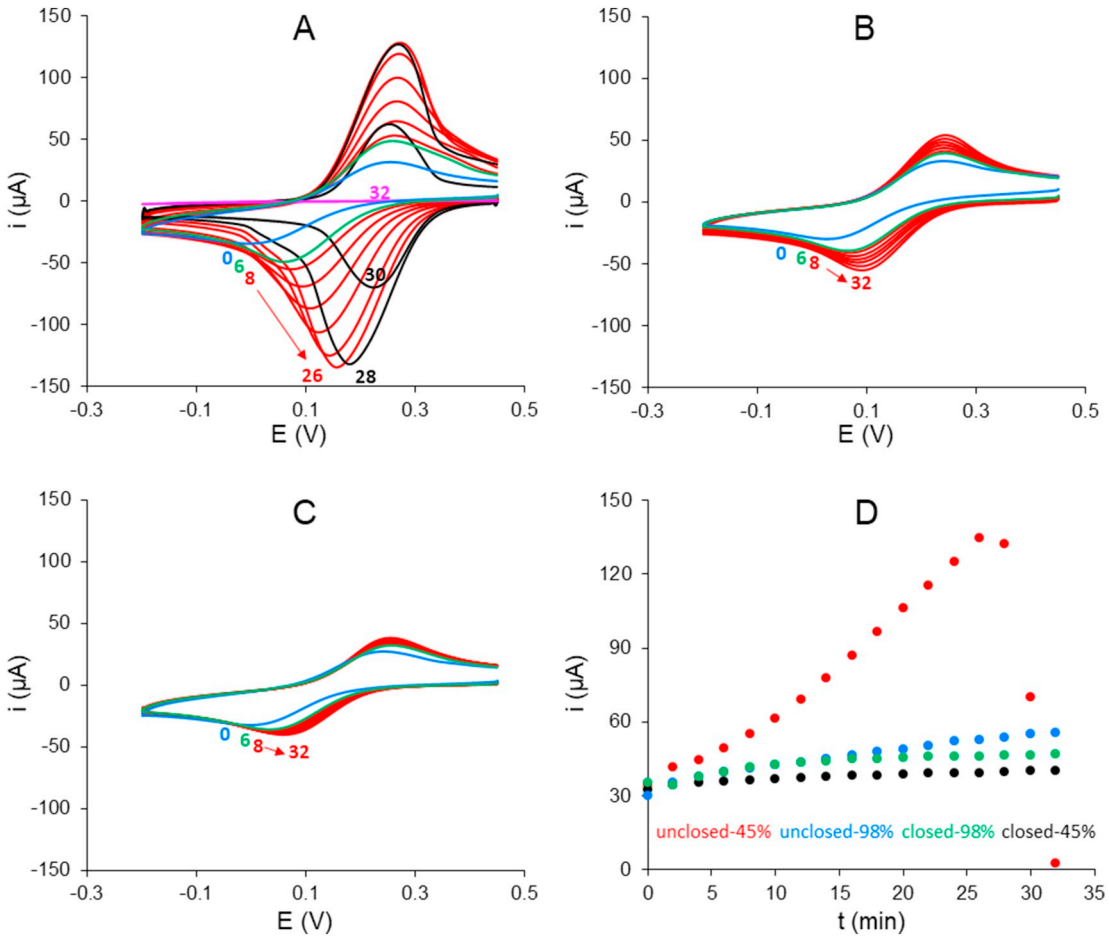

Fig. 2. Time evolution of the cyclic voltammograms registered for ferricyanide with (A) an unclosed ePAD at 45% RH; (B) an unclosed ePAD at 98% RH; (C) an adhesive-tape enclosed ePAD at 45% RH; and (D) time evolution of these peak currents. Numbers inside graphs indicate time (minutes).

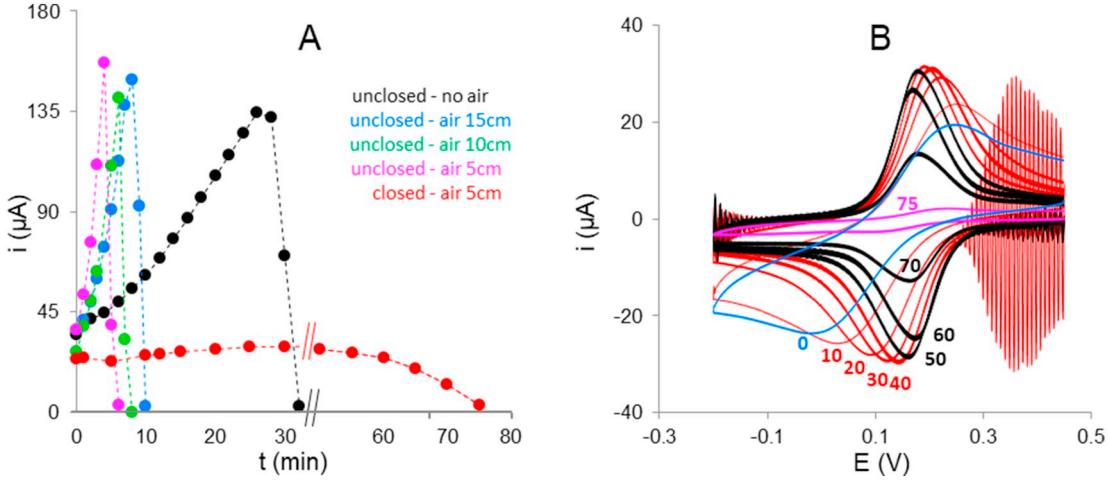

Fig. 3. (A) Time evolution of peak currents for ePADs at different distances from the air stream and (B) the cyclic voltammograms registered for ferricyanide with an adhesive-tape enclosed ePAD located 5 cm under the air stream. Air flow = 5 l·min^{-1}.

ferricyanide in PBS were registered between 0.45 and −0.2 V at 100 mV·s^{-1}, switching off the cell between successive scans. Secondly, amperometric signals were registered after applying 0 V for 5 s. Each point in the calibration curves was repeated three times, using one ePAD for each measurement. The limit of detection was estimated from the IUPAC recommendation, using the $3S_b/m$ formula where m is the slope of the linear range and S_b is the standard deviation of the background (signal without immobilized HRP) [15].

3. Results and discussion

The effect of RH on the electrochemical signals was studied at two RH values i.e. 45% and 98%. Fig. 2A presents the time evolution of the cyclic voltammograms of ferricyanide using an open ePAD at 45% RH. In this case, four well-defined periods could be distinguished. First, after the solution reached the end of channel, there was a moderate increase in the peak current for 5 or 6 min. This increment is related to

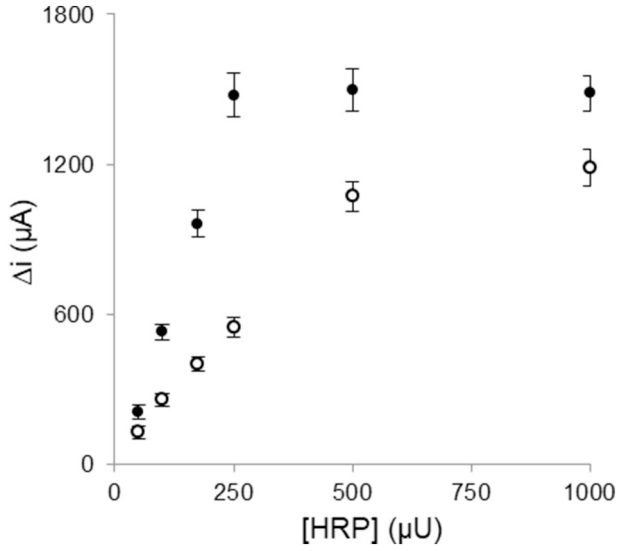

Fig. 4. Calibration curves for HRP immobilized using adhesive-tape enclosed ePADs after (○) 15 and (●) 30 min of reaction time. E_{app} = 0 V, t = 5 s, error bars n = 3.

the solution spreading inside the pores of the paper after the apparent channel filling. This fact has been reported previously [16] and can be associated with the broad pore-size distribution of chromatography papers and their different filling rates [17]. After that, there was a period of 20 min where the peak current increased linearly with time (4.69 μA·min^{-1}) and the difference in the peak-to-peak potential decreased. These two changes can be explained by the passive evaporation of water along the whole open channel which results in progressive ferricyanide preconcentration and reduction in the liquid volume. This signal evolution also indicates a change in the diffusion regime of the ferricyanide from semi-infinite linear to limited diffusion, i.e. from conventional to thin-layer voltammetry [18]. The third period was shorter, about 4–5 min, and was characterized by sharp drops in the peak currents and the peak-to-peak potential differences (≈0 mV). These changes are a consequence of the small amount of liquid remaining inside the paper and hardly making any contact with the electrodes. Finally, a fourth period was observed when this tiny contact was missed in the detection area and the signals could not be registered. An increase in the RH modifies the evolution of the ferricyanide signals with time for the open channel ePAD. Only two periods were differentiated at 98% RH during the first 30 min (Fig. 2B), i.e. the above-mentioned "total" channel filling and the subsequent ferricyanide preconcentration by passive evaporation. In this case, water evaporation from the paper was hindered because the water concentration in the air was close to saturation. The rate of the peak current increment was 0.66 μA·min^{-1}, seven times lower than the value obtained at 45% RH. The behaviour of the ePADs covered with adhesive tape (Fig. 2C) was similar to the open ePADs at 98% RH. However, two important differences should be highlighted: the rate of the peak current increment was much lower (0.11 μA·min^{-1}) and this value was the same at 45% and 98% RH (Fig. 2D). Therefore, enclosing paper channels with adhesive tape can be considered a simple way of introducing an impermeable layer at the paper/air interface and avoiding the effect of RH on electrochemical measurements made using ePADs.

Furthermore, the enclosure of ePADs with adhesive tape also provides protection against other factors that affect the evaporation rate, such as air currents. Fig. 3A presents the evolution with time of the peak currents in ferricyanide voltammograms measured with ePADs at 45% RH and under a perpendicular air flow (5 l·min^{-1}). The changes in the voltammogram for the open ePADs were identical to those observed previously with passive evaporation. The main difference was the acceleration of the evaporation by the proximity of the air jet to the paper surface (21.26, 30.3 and 40.1 μA·min^{-1} at 15, 10 and 5 cm respectively). For an adhesive-tape enclosed ePAD located at 5 cm under the air flow, the rate of the peak current increment was 0.17 μA·min^{-1} for the first 40 min, which is similar to the value obtained without air currents. Afterwards the peak current decreased slowly until the above-mentioned evidence of liquid shortage in the detection area appeared (Fig. 3B). This time evolution of the cyclic voltammograms is different from that observed for the uncovered ePADs (Fig. 2A). In this case, the liquid under the adhesive tapes remains initially unaltered while the solution is actively evaporated outside. When the surrounding paper is dried, the liquid in the covered area is progressively dragged out by capillary action but it is not evaporated inside. Therefore there is no preconcentration at the detection area and the peak currents do not increase significantly. It is also worth mentioning the current oscillations registered in some voltammograms. These current anomalies seem to be related to dryness at the detection area because they were only observed for enclosed ePADs after several minutes under the air flow (Fig. 3B). The shape and evolution of these temporal current instabilities during potential scans could be associated with complex non-linear dynamical behaviour [19,20].

Finally, the adhesive-tape enclosed ePADs were used for the amperometric detection of HRP immobilized in the paper channels. Different amounts of HRP were cross-linked with glutaraldehyde in a BSA matrix under the working electrodes. The enzyme was quantified by the ferricyanide generated from the ferrocyanide/H_2O_2 substrate. Fig. 4 shows the calibration curves obtained after different enzymatic reaction times. At 15 min, the calibration curve showed a linear relationship in the 50–500 μU range, good sensitivity (2.07 A·U^{-1}), a limit of detection of 26.9 μU and a repeatability of 7.5% (RSD calculated from signals of 250 μU HRP; n = 5). After 30 min, the linear range was shorter (50–250 μU), the repeatability (RSD = 6.8%; n = 5) and the limit of detection (12.63 μU) were similar, but a considerable increase in sensitivity was achieved i.e. 6.28 A·U^{-1}. On the other hand, the calibration curves measured with open ePADs suffered from evaporation and, although the sensitivity at 15 min was higher (3.73 A·U^{-1}), signals could not be registered after 30 min because the detection areas were dry (data not shown). Therefore, these results demonstrate that enclosure of ePADs with adhesive tape can be a practical solution for long-term assays, where passive or active evaporation would hamper or even prevent the measurements.

4. Conclusions

Electrochemical measurements with open ePADs are affected by active or passive evaporation of liquids. Enclosing their open channels with adhesive tape prevents the influence of external factors such as humidity or air streams. Moreover, this approach can be a good solution for long-term assays. These results will be used in future work that will be focussed on lateral flow assays based on enzymatic detection with ePADs.

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