Lab on a Chip

PAPER

that could measure the levels of various gaseous analytes in air.[21–24] We now demonstrate the fabrication and use of custom pencils for depositing a variety of reagents onto microPADs for detecting analytes in solution.

We first developed a method of fabricating reagent pencils. We then demonstrated that we could use the pencils to deposit reagents onto microPADs, and that once aqueous samples were added to the microPADs, the reagents dissolved from the pencil trace into solution and became available to react with the target analyte, just as reagents deposited from solution would.[25] After characterizing the dissolution of reagents from pencil traces, we studied the effect of the pencil fabrication process on the activity of the enzyme horseradish peroxidase (HRP). We then used reagent pencils to prepare microPADs for conducting a quantitative colorimetric assay for glucose. And, finally, we studied the shelf life of the enzymes HRP and alkaline phosphatase (ALP) deposited *via* pencil to see if the technique led to any significant stabilization of sensitive reagents.

Experimental section

Please see the ESI† for additional experimental details and a list of all reagents and equipment.

Fabrication of reagent pencils

We fabricated the reagent pencils in four steps (Fig. 1). A mixture of 75% poly(ethylene glycol) methyl ether (PEGME) and 25% graphite powder by mass was first pulverized and mixed manually using a porcelain mortar and pestle. Reagents in concentrations up to 15% w/w were added to the PEGME-graphite matrix, and the resulting mixture was further pulverized and blended by hand using an agate mortar and pestle. Approximately 0.75 g of the resulting mixture was then formed into a pellet by adding the mixture to a manual pellet press in five small portions and, after each addition of material, compressing the material for 5 seconds. The maximum force applied to the material was approximately 25 kN. The pellets had a final length of 19 mm and diameter of 6.31 mm. The pellets were loaded into mechanical pencil holders to facilitate their use as pencils.

Fabrication of paper-based devices

MicroPADs were fabricated by wax printing.[26] The pattern for each microPAD was drawn in AutoCAD, and was then printed onto cellulose chromatography paper (grade 1 Chr) using a solid-ink printer. The paper was baked in a convection oven for 15 minutes at 145 °C and was then cooled to room temperature under ambient conditions. The devices were stored in plastic petri dishes until they were used.

Determination of the mass of reagent pencil deposited on paper

A 4.0 cm by 4.0 cm square of chromatography paper was cut out and its initial mass was obtained. A pencil trace was deposited over the entire surface of the paper using a pencil composed of PEGME and graphite with no added reagents. The final mass of the paper was obtained, and the difference in mass was used to determine the amount of reagent pencil deposited on paper. This process was repeated by five different users in triplicate. The users were allowed to practice prior to the experiment and aimed to achieve uniform and consistent coverage of the paper with the pencil.

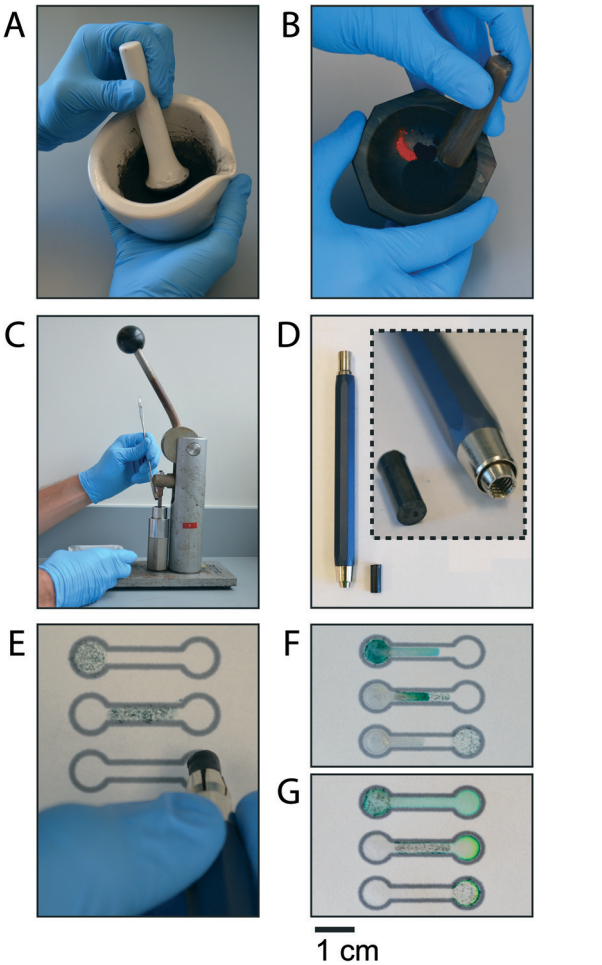


Fig. 1 Fabrication and use of reagent pencils. A) The matrix for the pencil composed of 75% PEGME and 25% graphite is pulverized. B) Reagents (blue and yellow dye) are added to the matrix, pulverized and mixed. C) The resulting mixture is added to a pellet press and pressed into a pellet. D) The pellet is loaded into a mechanical pencil holder. E) The reagent pencil is used to deposit reagents on microPADs in three distinct areas: the sample zone, channel and test zone. F) When water is added to the sample zone of the devices, it dissolves the reagents from the pencil traces and transports them into the test zone. G) The final appearance of the reagents in the test zone depends on the location of the pencil trace on the device.

Characterization of reagent delivery from the pencil traces

To characterize the dissolution of reagents from a pencil trace on a microPAD and the delivery of the dissolved reagents to a test zone on the device, we prepared a device with a sample zone, channel, and test zone that all had the

same surface area. A reagent pencil containing 15.0% w/w Erioglaucine (blue dye) was then deposited in the sample zone, channel or test zone of the devices by filling in the desired area of the device with a pencil trace. A PEGME-graphite pencil containing no dye was also tested as a blank to correct for the background signal. After depositing the pencil traces, deionized (DI) water (15 μL) was added to the sample addition zone of the devices. The DI water dissolved the dye from the pencil trace and transported it into the test zone *via* capillary wicking. The devices were dried under ambient conditions for 30 minutes, and the intensity of the color in the test zones was measured *via* digital image colorimetry (DIC).

Digital image colorimetry (DIC)

The results from the experiments with blue dye as well as the colorimetric assays were analyzed *via* DIC.[25,27,28] Upon completing a test, the devices were scanned and the resulting images were analyzed in ImageJ 1.46r. First, the images were split into the three color-channels; red, green and blue. For all tests except for the ALP assays, the green and blue channels were discarded, and the red channel was inverted. From the inverted red channel image, the mean color intensity of the entire test zone was measured using a microarray profile plugin.[25,29] The mean intensity values were then analyzed in Excel and Kaleidagraph. For the ALP assays, the blue and red channels were discarded, and the green channel was inverted and analyzed.

Effect of pencil fabrication on the activity of HRP

To determine if the process of fabricating reagent pencils had an effect on the activity of HRP, samples were saved at three points during the fabrication of reagent pencils containing 0.5% w/w HRP: i) before the enzyme was added to the PEGME-graphite mixture as a control sample, ii) after the enzyme had been mixed with the PEGME and graphite in the agate mortar, and iii) after the HRP-PEGME-graphite mixture had been pressed into a pencil core. Each sample was dissolved in 1XPBS to achieve a theoretical concentration of 0.58 U mL^{-1}. Graphite was removed from samples *via* centrifugation. An additional control sample containing HRP and PEGME was also prepared. The concentration of active HRP in each solution was then determined using an absorbance assay with 2,2′-azino-bis(3-ethylbenzothioazoline-6-sulfonic acid) diammonium salt (ABTS) as the electron donor dye substrate (Fig. S1†).[30,31] Two pencil cores were further analyzed by determining the activity of HRP in samples from the top, middle and bottom of the pellet.

Colorimetric glucose assay

Paper-based devices with a sample zone, a reagent zone, a test zone and a waste zone all connected by a single channel were fabricated. A reagent pencil containing 10.0% w/w enzymes (5.0% w/w GOx and 5.0% w/w HRP) and a reagent pencil containing 15.0% w/w ABTS were prepared. ABTS (~20 μg) was deposited in the sample zone, and the enzymes (~0.2 U of HRP and ~0.9 U of GOx) were deposited in the reagent zone of the devices. External calibration solutions (10 μL) containing glucose in concentrations ranging from 0 mM to 1.25 mM prepared in 1XPBS were added to the sample zone. The solutions wicked from the sample zone through the reagent zone and into the test zone where a blue-green color developed when glucose was present in the sample. A small amount of the solution, which did not contain a significant concentration of blue-colored ABTS, wicked into the waste zone (Fig. S2†). The devices were allowed to dry for 30 minutes, and the results were quantified by DIC. Nine replicates were performed for each concentration of glucose. The results from the external calibration solutions were used to prepare an external calibration curve that was fit in KaleidaGraph with the following rectangular hyperbolic equation:

$$y = \frac{C_1 x}{C_2 + x} \quad (1)$$

Two calibration standard solutions with concentrations of 0.25 and 0.80 mM glucose were also tested following the same procedure. The external calibration curve was used to determine the concentration of these samples in order to evaluate the accuracy and precision of the assay.

An identical set of experiments was also conducted using devices prepared with the same quantities of reagents deposited from solution. To prepare these devices, 2 μL of a 19 mM ABTS solution prepared in DI water was deposited in the sample zone, and 1 μL of a solution containing 230 U mL^{-1} HRP and 930 U mL^{-1} GOx prepared in 1XPBS was deposited in the reagent zone. The reagents were dried for 30 minutes under ambient conditions before the assays were performed.

Stability of HRP

The stability of HRP was monitored under five different storage conditions: i) deposited from a reagent pencil and stored on paper (pencil trace); ii) stored in a reagent pencil (pencil core); iii) deposited from solution and stored on paper (solution); iv) deposited from solution and stored on paper in the presence of trehalose (solution + trehalose); and v) stored dry (as supplied by the vendor) in a capped microcentrifuge tube under ambient conditions (dry storage). To evaluate the activity of the HRP for each storage condition, a total of 0.023 U of HRP was deposited into circular test zones with a diameter of 5 mm,[32] and a colorimetric assay was performed by adding 3 μL of 1-Step™ ABTS (a proprietary HRP substrate solution, Thermo Scientific) to each zone using the Mantis liquid dispenser. The reaction was allowed to proceed under ambient conditions for 30 minutes. The signal from each test zone was then quantified *via* DIC and normalized to the signal from the first day of the experiment. Eight replicates were performed for each test.

For the experiments involving pencil-based deposition, a reagent pencil containing 0.50% w/w HRP was used to fill each test zone with a pencil trace. A PEGME-graphite pencil containing no HRP was used as a blank to correct for the background signal. For the experiments involving solution-based deposition, 2 μL of HRP solutions with a concentration of 12 U mL^{-1}, prepared in 1XPBS, were deposited into each test zone and dried under ambient conditions. Trehalose was added to the HRP solution in a concentration of 0.17 M for the solution + trehalose experiments. Solutions of 1XPBS, both with and without 0.17 M trehalose, were also tested as blanks to correct for the background signal.

For the experiments where the HRP was stored on paper, the enzyme was deposited onto the devices on day 0 of the experiment. The devices were stored wrapped in aluminum foil under ambient conditions, and the colorimetric assays were performed on select days up to day 42. Since the signal for the HRP deposited from solution dropped to zero after 7 days, these tests were not continued after this day. On the initial day of the experiment, the solution-deposited enzyme was tested both before and after it dried on the paper.

For monitoring the stability of HRP stored in the pencil core, the reagent pencil was stored in an aluminum case under ambient conditions. On select days, the pencil was used to deposit HRP in test zones, and the colorimetric assay was performed immediately after deposition. For monitoring the stability of HRP stored as a dry powder, small portions of HRP were weighed out into amber-colored microcentrifuge tubes on day 0 of the experiment and were stored under ambient conditions. On select days, the HRP in one of the tubes was dissolved in 1XPBS. The solution was deposited into the test zones, dried for 30 minutes under ambient conditions, and the colorimetric assay was performed. The stability of HRP stored in the pencil core and as a dry powder was monitored for 63 days. The ambient temperature during the entire experiment for all storage conditions fluctuated between 18 °C and 24 °C, and the ambient relative humidity fluctuated between 27% and 56%.

Stability of ALP

A similar procedure to the one used for monitoring the stability of HRP was used to monitor the stability of ALP. A total of 0.0091 U of ALP was deposited into test zones with a diameter of 5 mm either *via* reagent pencil or *via* solution. For pencil-based deposition, a reagent pencil containing 0.50% w/w ALP was used. For solution-based deposition, 2 μL of ALP solutions with a concentration of 4.6 U mL^{-1} in 1XPBS were deposited into each test zone and dried under ambient conditions. The activity of the enzymes was determined by adding 3 μL of BCIP®/NBT-Purple Liquid Substrate System for Membranes (a proprietary ALP substrate solution, Sigma Aldrich) to the test zones using the Mantis liquid dispenser and allowing the reaction to proceed for 30 min under ambient conditions. The activity of the ALP was monitored over the course of 21 days with eight replicates for each condition.

Results and discussion

Fig. 1 depicts the fabrication of reagent pencils. The pencil cores could be fabricated in minutes once all the components were weighed out. We found that it was most convenient to prepare large batches of the PEGME-graphite mixture, and then use portions of the mixture to prepare pencil cores containing specific reagents. The PEGME served as both a filler material to dilute the reagents and as a binder to hold the reagents and graphite in the pellet. We selected PEGME as the main component for the pencils due to its solubility in water so that reagents added to the PEGME matrix would be released into solution once the polymer dissolved. The graphite improved the mechanical properties of the pencil cores and made the pencil traces visible on the devices to denote where reagents were deposited. The optimum ratio of PEGME to graphite was determined empirically by making pencil cores and testing their writing properties on paper.

We found that, on average, reagent pencils deposited 3.6 ± 0.7 μg mm^{-2} of the pencil core onto the surface of the paper (Table S1†). The magnitude of this area density is well-suited for the deposition of reagents onto microPADs because these devices typically require microgram quantities of reagents for an assay and tend to have surface areas of at least 40 mm^2. The reagent pencils could contain reagents in concentrations up to 15% w/w. We found using Erioglaucine disodium salt (blue dye, m.w. 793 g mol^{-1}) as a model reagent, that when we added reagents to the PEGME-graphite mixture in concentrations above 15% w/w, the pencil cores became brittle and crumbled easily when applied to paper. The maximum concentration of a particular reagent that can be added to a pencil core will likely depend on the properties of each reagent.

Most pencil cores could be stored under ambient conditions with no change in their physical properties. The PEGME-graphite mixture itself was not found to be hygroscopic. Cores containing certain reagents in sufficiently high concentrations, for example the pencil core containing 15% w/w Erioglaucine, appeared to absorb moisture from the air and soften over time. Storing these hygroscopic pencil cores in a desiccator eliminated this problem.

Fig. 1 illustrates the preparation of a pencil containing a mixture of blue and yellow dyes as model reagents. The dyes were deposited in the sample addition zone, channel or test zone of microPADs (Fig. 1E). When water was added to the sample addition zone, it dissolved the dyes from the pencil trace and transported them into the test zone, resulting in the appearance of a green color as expected for a mixture of blue and yellow dyes (Fig. 1F and G). The graphite, on the other hand, remained in its original position as a permanent record of where the reagents were deposited. For this reason, when conducting colorimetric tests with reagents deposited in the test zone of a device, we always imaged the bottom face of the device, opposite where the reagents were deposited, so that the graphite would interfere less with the signal from the assay.

Using the reagent pencil containing 15.0% w/w Erioglaucine, we studied the effect of the location of the pencil trace on the signal obtained in the test zone (Fig. 2). We obtained the highest signal when the pencil trace was located in the sample addition zone (Fig. 2D). The lowest signal was obtained when the pencil trace was located in the test zone. We believe this was due to two main reasons: i) the presence of the graphite in the test zone generated a large background signal even when imaging the bottom face of the devices, and ii) a significant coffee ring effect was observed in the test zone, which means the average signal measured from the test zone *via* DIC is underestimating the total amount of dye that dissolved into the water. These results suggest that, whenever possible, reagent pencils should be applied in the sample addition zone or channel to maximize the measured signal for colorimetric tests. The results also show that reagents can be deposited in the test zones of devices, but that a lower signal would be expected.

A second important observation from the results shown in Fig. 2D is that the uncertainties in the measured signals are relatively high, with relative standard deviations approaching 20%. For comparison, colorimetric assays performed on microPADs with reagents deposited from solution typically produce results with relative standard deviations in the range of 5–10%.[25] This increase in uncertainty was expected because it is difficult to achieve a uniform distribution of the reagents in the pencil core, and the amount of reagent deposited on the paper depends on how much force is applied to the pencil during deposition. Fortunately, at least in the context of colorimetric enzymatic assays, reagents are added to the devices in excess, and the signal is only a function of the concentration of the analyte (the limiting reactant). Therefore, the high uncertainty in the amount of reagent deposited on the devices will not necessarily increase the uncertainty in the final results of an assay.

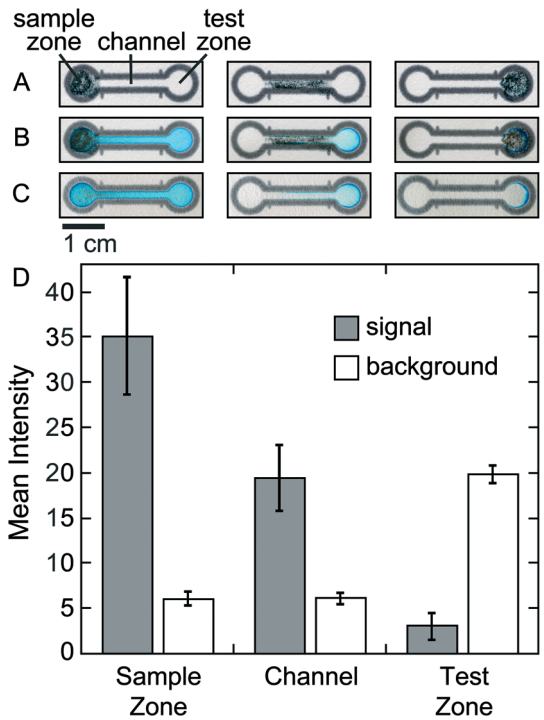


Fig. 2 Characterization of reagent delivery from pencil traces to the test zones of microPADs. A) Devices with blue dye deposited *via* pencil in the sample zone, channel or test zone. B) Top-view of the devices shown in A 30 minutes after adding water to each sample zone. C) Bottom-view of the devices shown in B, which were scanned and used to quantify the results. D) Bar graph of the background-corrected signal and background signal obtained from each device. Signals represent the mean of 9 replicates, and error bars represent one standard deviation from the mean. Background signals were obtained by performing the same experiment using pencils with no blue dye.

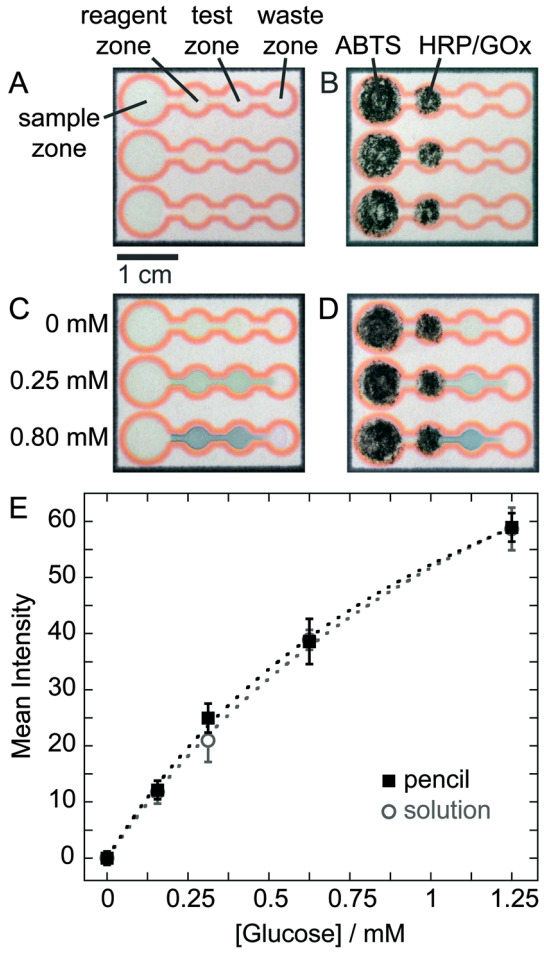
Since many colorimetric assays rely on enzymes, and pressure is known to be a potential enzyme denaturant,[10] we studied the effect of pressing HRP into a pencil core on the activity of the enzyme. A paired *t*-test of the results showed no statistically significant difference in the activity of HRP before and after it was pressed into a pencil core (p = 0.68, Table 1). We did observe large differences in the determined activities between trials with a relative standard deviation of 17%, which we attribute primarily to the uncertainty in weighing out small masses of HRP (Fig. S3†). The relative standard deviation for the activity assay performed in triplicate on the same sample solution was less than 3% (Table 1: trial 1). We were not able to find literature discussing the activity of HRP when mixed with graphite and/or polymers in solid form. In solution, HRP has been shown to have high barostability compared to other enzymes, but the effects of pressure on enzyme activity are also known to be dependent on temperature, time of treatment and the composition of the matrix solution.[33–36] During the fabrication of reagent pencils, the HRP was compressed for relatively short periods of time (~25 s total) at relatively high pressures (~800 MPa) and this treatment did not affect the activity of the enzyme. When comparing the activity of HRP in different regions of a single pencil core, we saw relative standard deviations of up to 7%, which further confirms that reagents are not uniformly distributed throughout the pencil cores (Table 1: trials 2 and 3; Fig. S4†).

Once we determined that HRP retained its activity after being pressed into pencil cores, we demonstrated that reagent pencils could be used to prepare functional

Table 1 Comparison of the activity of HRP before and after pressing it into a pencil core. The difference in activities between trials can be attributed to the uncertainty in weighing out small masses of HRP. Results represent the mean of three replicates, and the uncertainty is reported as one standard deviation from the mean. For trial 1, replicates were performed on a single sample. For trials 2 and 3, replicates were performed using three different samples taken from different locations of the PEGME-graphite-HRP mixture and the resulting pencil core. The activity of the HRP reported by the vendor was 67 U mg^{-1}

	Activity before pressing (U mg^{-1})	Activity after pressing (U mg^{-1})	% Yield after pressing
Trial 1	55.3 ± 1.5	53.2 ± 1.2	96.2 ± 3.4
Trial 2	70.0 ± 4.3	74.9 ± 4.4	107.1 ± 9.1
Trial 3	64.7 ± 4.1	64.8 ± 4.4	100.2 ± 9.3

microPADs capable of performing quantitative colorimetric assays using a glucose assay as a model test (Fig. 3).[5,25] The assay relies on a coupled enzymatic reaction, which ultimately results in the oxidation of ABTS from a colorless reduced form to a blue-green oxidized form that appears in the test zone of the devices.[25] The results for assays conducted on devices prepared using reagent pencils were virtually indistinguishable from the results for assays conducted on identical devices prepared with reagents deposited from solution (Fig. 3C–E), and there were no statistically significant differences between the results for two calibration standards that were tested using the different types of devices (Fig. 3F). The relative error and relative standard deviation for the 0.80 mM glucose calibration sample were both on the order of 10% for the devices prepared using reagent pencils, thus confirming that the uncertainty in the results of this assay are independent of the uncertainty in the amount of reagent deposited on the devices using pencils.



F

Technique	0.25 mM	0.80 mM
pencil	0.29±0.03	0.84±0.08
solution	0.28±0.06	0.89±0.06

Fig. 3 Comparison of a colorimetric glucose assay performed on devices prepared with reagents deposited from solution and reagents deposited from reagent pencils. A) Device with reagents deposited from solution. ABTS was deposited in the sample zone and a mixture of GOx and HRP was deposited in the reagent zone. B) Device with reagents deposited from reagent pencils. C and D) Devices 30 minutes after adding samples containing 0 mM, 0.25 mM and 0.80 mM glucose to the sample zones. The results appear as a blue-green color in the test zone. E) External calibration curves prepared using standard glucose solutions by measuring the intensity of the color in the test zones. Data points represent the mean of 9 replicates, and error bars represent one standard deviation from the mean. F) Results for the determination of the glucose concentration in two calibration samples. Results represent the mean of 9 replicates; the uncertainty is reported as one standard deviation from the mean.

It should be noted that the devices for the glucose assays were fabricated using reagent pencils that had been prepared eight months prior to conducting the experiments and had been stored under ambient conditions. The results from glucose assays performed when the pencils were freshly prepared can be seen in Fig. S5.† The results from these two sets of experiments suggest that enzymes and reagents like ABTS, which is sensitive to light and oxygen, are stable in the reagent pencils over long periods of time. We would like to emphasize that we are not proposing that this particular glucose assay will have commercial applications. We simply used this assay as a way of comparing the capabilities of devices prepared with reagents deposited *via* pencils to devices prepared with reagents deposited from solution because this assay has been studied in detail in the context of paper-based fluidic devices.[2,5,25]

The glucose test also demonstrates that multiple reagents can be deposited on a microPAD in close proximity with little risk of cross contamination. Reagent pencils can even be sharpened using pencil sharpeners to deposit reagents with higher resolution. Reagents could also be deposited on the two faces of the sample zone or channel to increase the amount of reagent or the number of reagents that could be deposited on a single device.

Our final experiments evaluated the stability of enzymes deposited on paper using reagent pencils (Fig. 4 and S6†). HRP and ALP were chosen as model enzymes because they are used commonly for signal amplification in diagnostic tests.[11,37] We compared the stability of HRP under five different conditions, including in the presence of trehalose, an enzyme stabilizing reagent that has been used previously with microPADs (Fig. 4A).[5] A relatively low concentration of HRP was used for this experiment so that differences in the stability of the enzyme would become apparent within a few days.

The signal for the HRP deposited *via* solution started high and then decreased rapidly to zero within 3 days, indicating that the enzyme had no detectable activity. Even on the first day of the experiment (day 0), the signal from the assay decreased by 45% as the HRP solution dried in the test zone, confirming that HRP was denaturing as a result of the method of deposition. The HRP deposited *via* solution in the presence of trehalose showed a slight improvement in shelf life, but the signal still decreased to zero within 7 days. We believe the main reasons why we observed a more rapid decrease in the activity of HRP stored on microPADs

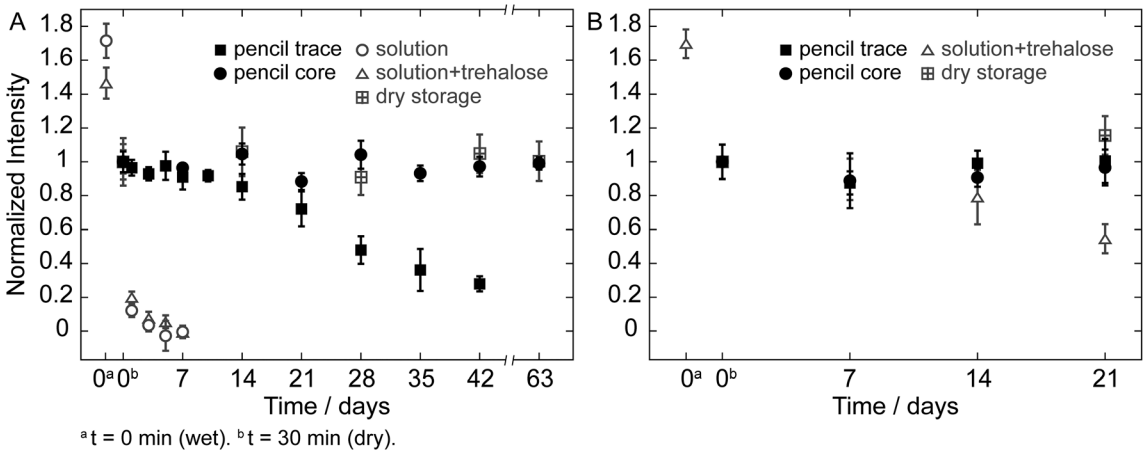


Fig. 4 Stability of HRP and ALP in reagent pencils. A) Comparison of the stability of HRP stored under various conditions either on paper (pencil trace, solution and solution + trehalose) or off paper (pencil core and dry storage), and deposited *via* reagent pencil (black data markers) or deposited *via* solution (grey data markers). B) Comparison of the stability of ALP stored under various conditions. Data points in both graphs represent the mean of 8 replicates, and error bars represent one standard deviation from the mean. The results for each storage condition were normalized to their respective mean signal intensities from day 0.

compared to previous reports showing a shelf life of weeks to months for enzymes stored on paper-based devices is that we used a relatively low concentration of HRP, the HRP was not stored in the presence of any other reagents such as other enzymes, proteins or salts; and the HRP was stored on untreated chromatography paper wrapped in aluminum foil but not protected from ambient humidity or temperature.[4,5]

The pencil-deposited HRP, on the other hand, delivered a relatively constant signal for 10 days (the fluctuations in the signal were due most likely to local variations in the concentration of HRP within the pencil core), and a detectable signal was still observed on day 42, indicating that some of the enzyme was still active. These results confirm that reagent pencils extend the shelf life of HRP deposited on paper compared to solution-based deposition, and suggest that pencil-based deposition of reagents is a promising approach for improving the shelf life of sensitive reagents on microPADs.

The signal for HRP stored dry under ambient conditions either as a powder (as supplied by the vendor) or in the pencil core remained constant even after 63 days. This result suggests the PEGME-graphite matrix is not imparting any special stabilizing effect on the HRP, but simply maintaining the stability that the enzyme already possesses in dry form. While many techniques have been developed for stabilizing enzymes,[4,8,10,11,38–40] reagent pencils stand out for their simplicity. Pencils can be prepared within minutes, stored under ambient conditions for months and then applied to a device at any time with no additional preparation. Since pencil-based reagent deposition is such a straightforward process, these results suggest that reagent pencils containing sensitive reagents could be transported into the field and added to devices at the point of care, thus resolving the problem of short shelf life for many paper-based assays.

To demonstrate that the stabilization effect of the reagent pencils is not unique to HRP, we also evaluated the stability of ALP deposited *via* reagent pencils (Fig. 4B). The signal for ALP deposited from solution decreased over the course of the experiment, albeit not quite as rapidly as the decrease in signal observed for HRP. ALP appears to be a more stable enzyme compared to HRP when stored on microPADs under ambient conditions. The signal for the ALP deposited *via* pencil remained constant for the duration of the experiment, thus confirming that enzymes deposited *via* reagent pencils are more stable on microPADs than enzymes deposited from solution. The signal for ALP stored in dry form, either as a powder (as supplied by the vendor) or in the reagent pencil, was also constant over the course of the experiment, which agrees with the results obtained for HRP.

Conclusions

We developed reagent pencils as a simple and solvent-free technique for depositing reagents onto membrane-based devices that leads to a longer shelf life for sensitive reagents compared to solution-based deposition and does not affect the accuracy or precision of the results of enzymatic colorimetric assays. While reagent pencils could be loaded readily into pen plotters to automatically deposit reagents onto devices for large-scale fabrication, the more compelling application of reagent pencils that we foresee involves small-scale, manual preparation of devices at the point of care. We envision a kit of reagent pencils—much like a box of colored pencils—that could be transported into the field along with generic paper-based devices. The device could then be customized in the field by applying the reagents required to detect a specific analyte of interest. We believe that this method of reagent deposition will lead to new opportunities in the field of point-of-care diagnostics, and we are currently exploring the use of other materials for the pencil matrix, improving the reproducibility of the deposition process and evaluating the types of reagents and types of assays that are compatible with this technique.

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