Published in final edited form as:

Anal Chem. 2014 December 16; 86(24): 11981–11985. doi:10.1021/ac503751a.

Evaporative Concentration on a Paper-Based Device to Concentrate Analytes in a Biological Fluid

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Abstract

We report the first demonstration of using heat on a paper device to rapidly concentrate a clinically relevant analyte of interest from a biological fluid. Our technology relies on the application of localized heat to a paper strip to evaporate off hundreds of microliters of liquid to concentrate the target analyte. This method can be used to enrich for a target analyte that is present at low concentrations within a biological fluid to enhance the sensitivity of downstream detection methods. We demonstrate our method by concentrating the tuberculosis-specific glycolipid, lipoarabinomannan (LAM), a promising urinary biomarker for the detection and diagnosis of tuberculosis. We show that the heat does not compromise the subsequent immunodetectability of LAM, and in 20 min, the tuberculosis biomarker was concentrated by nearly 20-fold in simulated urine. Our method requires only 500 mW of power, and sample flow is self-driven via capillary action. As such, our technology can be readily integrated into portable, battery-powered, instrument-free diagnostic devices intended for use in low-resource settings.

Graphical Abstract

We report here a method to concentrate a target analyte from hundreds of microliters of a biological fluid on a paper-based platform. Our method of concentration relies on applying localized heat to a paper strip, while using the wicking properties of the paper to drive flow

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The authors declare no competing financial interest.

of the biological fluid containing the target analyte. This technology may be (1) used as a sample preconcentration step to improve the sensitivity of downstream detection methods; (2) performed without centrifuges and other expensive laboratory equipment; and (3) readily adapted to a battery-powered, portable platform. To our knowledge, this is the first report of using heat on a paper device to enrich for a clinically relevant analyte of interest from a biological fluid.

Development of our technology was motivated by the need for a simple and low-cost point-of-care (POC) device to detect and diagnose tuberculosis (TB) in resource-limited settings. Despite being a largely curable disease, 8.6 million people were infected with TB and 1.3 million died of the disease in 2012 alone.¹ The TB epidemic remains uncontrollable due in large part to low detection rates, since undiagnosed patients are more likely to spread the disease. Currently, the most widely used methods for detecting and diagnosing TB are sputum smear microscopy, bacterial culture, and chest radiography.² These methods require physicians or trained technicians to perform, and results can take weeks to obtain. Moreover, these methods are only available in centralized laboratory facilities that are usually located in urban settings, where only 40% of suspected TB cases reside.³ The remaining 60% of suspected TB cases are in rural areas, where medical attention is initially provided at rural health clinics. The resources in these clinics are extremely limited; there are no reliable, low-cost diagnostic tests that can provide a microbial or molecular confirmation within even a few weeks. As a result, this underserved population may go undiagnosed and untreated, spreading the disease to others in their community and further exacerbating the global crisis. There is an urgent need for a simple-to-use, low-cost, rapid, and accurate POC TB diagnostic test.⁴

Our approach toward developing a POC TB diagnostic is to detect mycobacterial antigens that are present in the urine of persons infected with TB to serve as a biomarker for the presence of the *Mycobacterium tuberculosis* (MTb) bacterium.⁵ Compared to other clinical specimens (e.g., sputum, blood), urine is easy to collect from both adults and children, is less likely to be variable in sample quality, contains fewer bacterial contaminants, and is safer to handle.⁶ The most promising biomarker for TB diagnosis is lipoarabinomannan (LAM), an 18-KDa glycolipid found on the outer cell wall of MTb. This biomarker is released from metabolically active or degrading mycobacteria and is believed to enter the circulation and is subsequently filtered into the urine.⁶ Therefore, detection of urinary LAM provides an easily acquirable diagnostic sample.

LAM detection for TB diagnosis is achievable provided LAM can be detected at very low concentrations. Diagnostic tests for LAM are currently available but are not sufficiently sensitive without a preconcentration step, which entails ultrafiltration via centrifugation.^{7–9} We therefore developed a method that would simplify the concentration of LAM directly from urine without centrifugation. Since our ultimate goal is to translate this technology into a portable device that could be used at the POC in resource-limited environments, we developed our technology (1) on a platform that is compatible with miniaturization and (2) to be performed without the need for sophisticated lab equipment (e.g., centrifuge).

Other microscale technologies that have been developed specifically for the concentration of nanometer-scale species have relied on electrokinetic methods, nanofiltration, or evaporation-enhanced methods. Electrokinetic methods, such as isotachopheresis, require an electric field to induce sample flow and thus requires power supplies and complex electrical circuitry.^{10,11} In a step toward eliminating the reliance on bulky laboratory instruments, a recent report demonstrated isotachophoretic preconcentration on a paper-based device that could be powered by a small button battery.¹² Still, the primary limitation of these electrokinetic-based methods is that they are restricted to charged samples.

Nanofiltration sample concentration involves incorporating a porous monolithic component within a microfluidic path to capture and concentrate analytes that are larger than the pore size. Such nanoporous filters have been used to concentrate a range of target analytes (e.g., microbes, proteins, nucleic acids).^{13–17} However, pressure-driven or electrokinetic mechanisms are still needed to drive the flow of sample through the nanofilters and thus suffer from the limitation that sophisticated laboratory instruments are needed. Moreover, concentrated samples that are captured in the porous microfilters are often difficult to recover for downstream analysis, and clogging of the pores and the subsequent back-pressure buildup can effectively halt the concentration process.

We and others have also employed evaporation as a method to concentrate analytes within a liquid sample.^{18–20} This method uses evaporation of the carrier liquid and the resultant volume reduction to concentrate the analyte of interest. Walker and Beebe demonstrated evaporation-induced concentration using a simple device that contained a single straight channel with one inlet and one outlet port.¹⁹ Concentration of the target analyte occurred as the liquid carrier passively evaporated from the outlet of the prefilled channel. As the liquid evaporated, the liquid sample flowed from the continuously replenished inlet port toward the outlet port to compensate for liquid loss. Meanwhile, the target analyte accumulated and concentrated at the outlet port. Though easy to fabricate and simple to conduct, concentration by passive evaporation is extremely slow and impractical if large volumes of sample are needed to be processed. Therefore, to enhance evaporation, we and others used convection to drive air flow over the entire microfluidic channel containing the liquid sample, which was separated from the air flow channel by a semipermeable membrane. The device by Sharma et al. was able to process large volumes in a short amount of time (1 mL/min at 37 °C); however, the final concentrated volume was limited by the large total channel volume of the device.¹⁸ Our device overcame this limitation, for applications that required a smaller final volume of concentrated sample, by designing it with a meniscus dragging effect to concentrate the sample to a smaller volume at the end of the channel path.²⁰

The technology we describe herein overcomes many of the limitations that these other methods present. Compared to the electrodynamic and nanofiltration methods, our method demands less power to operate, does not require complicated microfluidic manufacturing methods, and is much simpler in design and therefore is more easily translatable to a miniaturized portable platform. In contrast to other evaporation-enhanced methods, our method utilizes heat to accelerate evaporation of the liquid carrier and the concomitant concentration of the target analyte. Unlike the aforementioned evaporation-enhanced

methods, which were developed in polymer-based microdevices, our method is performed on a paper-based substrate. Paper was selected for several reasons: (1) liquid samples can wick through the cellulose fiber network of the paper via capillary action, thereby abrogating the need for external pumps to drive fluid flow; (2) cellulose paper is thermally stable at temperatures up to 300 °C;²¹ (3) our sample concentration technology may be readily incorporated in-line with a paper-based, lateral flow assay design with sample input upstream and detection module downstream of our technology; (4) devices can be disposed of after use by incineration, thereby reducing hazardous waste; and (5) paper is inexpensive, thus making this technology easily translatable to a low-cost platform. Figure 1 schematically illustrates the principle of our method to concentrate samples by heated evaporation on a paper device.

We recently demonstrated the feasibility of our method using bromophenol blue (BPB) in deionized water.²² Here, we applied this technology to concentrate the clinically relevant TB biomarker, LAM, in simulated urine (Ricca Chemicals; Arlington, TX). LAM was concentrated as shown in the experimental setup illustrated in Figure 2.

Briefly, the distal 1 cm tip of an 80 × 5 × 0.8 mm strip of chromatography paper was sandwiched between two custom-made aluminum plates and heated to 220 °C by a commercial resistive heater (OMEGA Engineering, Inc.; Stamford, CT). The temperature of the distal tip was monitored with a fine gage, bare wire thermocouple that was mechanically juxtaposed between the paper and heater. After concentrating, 1 cm sections of the strip were placed separately in a 0.6 mL microcentrifuge tube, with a hole punctured at the bottom, which was placed in a 1.7 mL microcentrifuge tube. The tube-within-a-tube was spun at 10 000*g* for 10 min to recover the LAM-containing simulated urine from the saturated paper. LAM was subsequently quantified via immunodot blotting using a polyclonal anti-LAM antibody (BEI Resources; Manassas, VA), an antirabbit secondary antibody (GE Healthcare Biosciences; Westborough, MA), and an enhanced chemiluminescent substrate (Thermo Scientific; Rockford, IL). The acquired chemiluminescent signal was quantified by the integrated density that was measured by ImageJ software for each sample using a fixed circular area that encompassed the largest sample on the immunodot blot membrane. The background integrated density measured from simulated urine was subtracted from the measured integrated density of each sample. LAM concentrations were then calculated using freshly prepared calibration standards that were spotted on the same nitrocellulose membrane as the nonconcentrated and concentrated samples. The degree to which LAM was concentrated, herein called the concentration factor, was quantified as the ratio of the concentration determined from the distal 1 cm tip to the concentration in the section located 5–6 cm from the distal end of the paper strip (i.e., Sample #6 in Figure 3). We also quantified the “concentration efficiency” to estimate the degree to which our method concentrates the analytes and subsequently recovers the concentrated sample. The “concentration efficiency” was defined as the following:

$$\text{concentration efficiency} = \frac{\text{analyte concentration factor}}{\text{volume concentration factor}}$$

where the volume concentration factor is the ratio of the total volume of liquid evaporated to the volume of the liquid recovered from the distal 1 cm tip of paper after sample concentration. To validate our protocol of spinning 1 cm sections of the saturated paper to recover the liquid from the paper for subsequent quantification, we cut the saturated paper strip into 1 cm sections and quantified the BPB concentration in all sections (Figure 3).

As expected, BPB concentration was highest at the heated distal tip (Sample #1). The BPB concentration in Sample #2 was the lowest, the reason for which remains unclear, but more importantly, the BPB concentrations in the proximal six sections of the paper strip were statistically comparable to the unconcentrated solution contained in the beaker. As such, the analyte concentration factor was quantified to be the ratio of the analyte concentration in Sample #1 to the concentration in one of the proximal sections, namely Sample #6, which had a comparable concentration to the original unconcentrated solution. More importantly, these data indicated to us that the analyte concentration that is quantified from the liquid recovered from our tube-within-a-tube spinning protocol would be accurate.

We first evaluated our concentration technology with LAM in deionized water. Similar to our initial feasibility experiments with BPB in deionized water, after 10 min of evaporative concentration, we achieved a concentration factor of approximately 20 (Figure 4A). The application of heat to enhance fluid evaporation did not appear to compromise the immunodetectability of LAM. This was expected since LAM is inherently thermally stable.⁷ Further, our method was able to recover a vast majority of the concentrated sample for subsequent detection, as indicated by the approximately 70% concentration efficiencies for both systems (Table 1).

We then evaluated our concentration technology with LAM in simulated urine. The volume of urine evaporated after 10 and 20 min was comparable to the volume of water evaporated after the same amount of time (Figure 4B). After 20 min, approximately 600 μ L of liquid was evaporated. Concentrating LAM in simulated urine for 10 min resulted in a 7-fold concentration of LAM, less than the concentration factor of LAM in water but not unexpected since urine is a more complex fluid. By increasing the concentration time to 20 min, we achieved an 18-fold concentration in LAM (Figure 5).

Since the simulated urine used herein was comprised solely of various salts (unlike real urine, which would also contain proteins), we believe the decreased concentration efficiency in the LAM/simulated urine samples compared to the LAM/water samples after 10 min of evaporative concentration (Table 1) was due to the concomitant concentration of salts that may have reduced the affinity between LAM and anti-LAM antibody. This would be expected for antigen–antibody interactions that are dominated by ionic interactions.²³ Despite this, LAM still remained immunodetectable even after concentrating the simulated urine, and the salts within, by 28-fold in volume (i.e., the *volume* concentration factor).

In summary, we report here a simple method to concentrate analytes of interest from within a biological fluid on a paper-based platform by nearly 20-fold in 20 min. In sharp contrast to previously reported concentration methods that were developed in polymer-based microfluidic devices, our technology enables analyte concentration that is wholly

independent of external instruments to drive the flow of fluid through the device (e.g., syringe pumps). As such, our technology may be readily integrated with downstream paper-based detection methods (e.g., lateral flow immunodetection assays) to form a fully integrated diagnostic device with sample in/answer out capabilities. Moreover, our technology can concentrate hundreds of microliters of sample down to tens of microliters, which is requisite for detection methods that require a small final sample volume (e.g., lateral flow immunodetection, surface enhanced Raman spectroscopy). By optimizing the parameters that affect analyte concentration (e.g., paper material, temperature, geometry, etc.), we expect to be able to process larger fluid volumes with higher levels of analyte concentration and shorter concentration times.

The particular clinical application reported herein is the concentration of the TB biomarker, LAM, from simulated urine, in order to enhance its downstream immunodetection for a more sensitive TB diagnosis. Since this technology would have its greatest clinical impact as a POC diagnostic device in resource-limited settings, we are currently adapting our technology to be battery-powered and portable. In our current setup, the calculated power requirement to heat the distal tip is 500 mW. Although we used a large heating strip for convenience (50.8×16 mm), to calculate the power required to heat the distal tip, we only used the actual area of the heating strip that was in contact with the sample (10×5 mm). Future embodiments will use smaller heating elements to match the size of the sample. Given the low power requirement of our method, we expect our technology to be readily adaptable to a battery-powered platform.

This technology is a first step toward providing an accurate TB diagnosis to millions of people who are currently undiagnosed or misdiagnosed due to the lack of a simple-to-use and rapid diagnostic test that can be used in resource-constrained settings. More importantly, our technology may be easily implemented for many other applications such as the diagnosis of other infectious diseases, surveillance of waterborne pathogens, or other clinical applications where concentration of a low-abundance, heat stable biomarker would greatly enhance downstream detection and ultimately save millions of lives.

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Figure 1. Schematic illustration of our evaporative concentration technology for sample concentration. When the liquid sample arrives at the heated region of the paper strip, the liquid carrier evaporates, thereby enriching for the target analyte.

Figure 2.

Evaporative concentration experiments entailed submerging the paper strip into a beaker that held the unconcentrated analyte-containing sample. The sample wicks toward the distal 1 cm of the paper strip, which is heated to 220 °C with a commercial resistive heater.

Figure 3.

Concentration profile of bromophenol blue (BPB) in deionized water recovered from 1 cm sections of the paper strip (Samples #1–8) after 10 min of evaporative concentration. Shown are average BPB concentrations obtained from triplicate samples. Sample P is the unconcentrated solution obtained from the beaker. An F -test was performed to determine the equality of variances between BPB concentrations in each section compared to that of Sample P. Then, a two-sample t -test assuming either equal or unequal variances (as determined by the F -test) was performed. The BPB concentrations in Samples #3–8 were statistically comparable to that in Sample P ($p > 0.05$).

Figure 4.

(A) The concentration factor of BPB in deionized water and LAM in deionized water after 10 min of evaporative concentration ($n = 3$). (B) Volume of simulated urine or deionized water (dH₂O) evaporated after 10 and 20 min of evaporative concentration ($n = 3$).

Figure 5.

(A) The concentration factor of LAM in simulated urine after 10 and 20 min of evaporative concentration ($n = 3$). (B) Representative immunodot blot used to quantify LAM before and after evaporative concentration for 10 min. Freshly prepared LAM standards in simulated urine were spotted on the same membrane as the concentrated and nonconcentrated samples. The non-concentrated sample was quantified using the 1 cm section located 5–6 cm from the distal end of the paper strip after sample concentration (Sample #6 in Figure 3), whose concentration was statistically comparable to the original unconcentrated solution.

Table 1

Concentration Efficiencies of BPB/Water, LAM/Water, and LAM/Simulated Urine after 10 or 20 min of Concentration

sample	concentration time, min	concentration efficiency, %	overall concentration factor
BPB/ water	10	68	19
BPB/ water	20	78	34
LAM/ water	10	72	21
LAM/ urine	10	51	7
LAM/ urine	20	65	18