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Final Report
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# Development of Microdevice Solid-Phase Purification Utilizing Dual Pressure/Electro-Elution for Concentration and Enhanced Recovery of DNA

As previously described in Chapter 2, DNA extractions in microdevices are typically carried out in a pressure-driven mode using a standard syringe pump to control flow through the device. These solid phase extractions provide the benefits of reproducibility and high extraction efficiency, while also yielding highly purified, PCR-ready DNA in a reasonably small volume, typically e 5-10 µL. For evidentiary samples containing a relatively small amount of DNA, this volume reduces the overall concentration of DNA (per µL) that can be used in subsequent PCR reactions, therefore, compromising the ability to amplify DNA from these sources. In addition, as with all solid phase extraction protocols, a small amount of DNA is irretrievably lost to the solid phase, thus lowering overall extraction efficiencies and reducing the ability to recover small amounts of DNA from low copy number samples. These problems are further exacerbated when microfluidic SPE is integrated with downstream processes (such as PCR) because typical volumes of PCR microchambers are on the order of hundreds of nanoliters. Under these conditions, the volume incompatibility between SPE and PCR becomes of critical concern. Accordingly, it was imperative that this incompatibility be addressed, ideally with a microfluidic device design that permits continued use of the

optimized syringe pump-driven SPE described throughout this thesis. Although there may be other solutions to this incompatibility problem, most would involve changing the purification method to accommodate a smaller elution volume. In order to maximize what had already been accomplished with this solid phase, we envisioned a device that employed an electrophoretic elution step, one that worked in concert with the syringe pump-driven flow, to elute the DNA while concentrating it in a smaller volume. Although electrophoresis has been utilized for decades as a method to manipulate nucleic acids, there are numerous challenges associated with the electrokinietic retention of DNA in a flowing stream.

Consequently, the research presented in this chapter describes the evaluation of the concept of exploiting an electric field during the DNA elution phase of the SPE to enhance recovery of DNA and, subsequently, provide a more concentrated sample for downstream genetic analysis. A preliminary design for a glass microdevice capable of electro-solid phase extraction (eSPE) and dual pressure/electro-elution is described. Following the evaluation of a number of device designs, the optimized device, containing platinum electrodes, allows for continuous, syringe-driven flow to be accomplished, while a low voltage electric field is applied. Using this device, a typical solid phase extraction (sample load, protein wash, DNA elutions) using pressure-driven flow is accomplished, with the electric field imposed during the final elution step to trap DNA as it exits the device. The development of this device represents the first step towards addressing the volume incompatibility between the DNA purification and PCR amplification domains in integrated microfluidic devices.

*1.1 Microchip Solid-Phase Purification Background*

**1.1.1 DNA Mobilization in Electric Fields**

DNA molecules, negatively-charged in most buffer systems due to the phosphate groups in the backbone, have been manipulated using electric fields for separation purposes for the better part of half a century. Slab gel electrophoretic systems [1-6], capillary [7-9], and now microchip [10-17] have all exploited these inherent properties of DNA to sort fragments based on length in the presence of sieving matrices. In addition to these separation techniques, the charge on DNA has been utilized to electro-elute the molecules from polyacrylamide or agarose gels, [18-21], allowing successful recovery on intact DNA fragments for additional genetic analysis. Due to its charge, DNA will migrate towards the anode in the presence of an electric field [22], and this characteristic can be exploited to effectively mobilize and/or localize DNA - consequently, this presents a means for precisely controlling the placement and position of DNA in microfluidic flow systems. This approach to DNA manipulation will be exploited in the research presented in this chapter with the specific goal of enhancing the effectiveness of the DNA extraction in microdevices.

In recent research, Park et al [23] have demonstrated the concentration of DNA in an electric field for purification of post-amplification PCR products prior to sequencing. With their method, DNA was captured in a gap junction in a flowing stream of solution in a macrosystem [23]. This group demonstrated that, with the appropriate strength of field, DNA could be held in place while other contaminating solutions were removed in a wash step. Spring boarding from this, a microfluidic eSPE device was designed so that electric field could be applied in a channel downstream from the solid phase extraction

bed during syringe pump-driven extraction (**Figure 1A and 1B**), allowing for concentration of DNA in the PCR chamber of the device during DNA elution (**Figure 1B**). This design should not only allow for concentration of DNA in a smaller volume, but should also allow for the localization of the eluted DNA within the PCR chamber for subsequent amplification in an integrated microfluidic system (as described in Chapter 3 and 4). Additionally, as shown by Park et al. [23], this would allow for any residual contaminating reagents associated with the DNA purification procedure (i.e., isopropanol) to be removed, thus rendering the DNA more suitable for PCR amplification.

*1.2 Reagents and Experimental*

**1.2.1 Reagents**

Tris(hydroxymethyl)aminomethane (Tris) and 2-propanol were obtained from Sigma (St. Louis, MO). EDTA was purchased from Amresco (Solon, OH), while the 2-(4-morpholino)-ethane sulfonic acid (MES), and guanidine HCl utilized in the loading buffers were purchased from Fisher (Fairlawn, NJ). YO-PRO was obtained from Molecular Probes (Eugene, OR). *Taq* polymerase (5.0 units $\mu L^{-1}$), buffers, dNTPs, and other reagents for standard DNA amplification were purchased from Fisher (Fairlawn, NJ, USA). Fluorescein-labelled lambda DNA was obtained from Mirus, Bio (Madison, WI). All solutions were prepared in Nanopure water (Barnstead/Thermolyne, Dubuque, IA, USA).

**1.2.2 Instrumentation**

All extractions were carried out using either a single Harvard Apparatus model 22 syringe pump (Harvard Apparatus, Holliston, MA) or a SP120CE-300 syringe pump(WPI, Sarasota, FL) with a 250 µL Hamilton gas-tight syringe (Hamilton, Las Vegas, NV) was utilized. The syringe was connected to the inlet reservoir of the microdevice via PEEK™ tubing and Nanoport mini-tight fittings (Upchurch, Scientific, Oak Harbor, WA, as previously described.

Field was applied to the device using a dual polarity high-voltage power supply built in-house using two Spellman high-voltage sources (Hauppauge, NY, USA). An argon ion laser (Model LS200, Dynamic Laser, Salt Lake City, UT, USA) was used for excitation with a conventional confocal detection setup (16× objective, 1-mm pinhole). Emission was collected with a PMT (Hamamatsu, Bridgewater, CT, USA) through a 515-nm bandpass filter (Omega Optical, Brattleboro, NY, USA). The instrument and data acquisition were controlled through a LabVIEW application.

For microchip separations, amplified samples were analyzed on a Bio-Analyzer 2100 (Agilent Technologies) using DNA 500 kits according to the manufacturer's instructions.

### 1.2.3 Microchip Fabrication and Solid Phase Preparation

Borofloat glass (Telic Company, Valencia, CA) bottom plates for the microdevices were fabricated using standard photolithographic techniques, with channel dimensions of 1.0 cm to the weir, 200 µm deep and 425 µm wide. The PCR and reference chamber was 200 µm deep, elliptical, with radii of 1.5 and 0.375 mm. The design had the same dimensions and features as the SPE-PCR portion of the fully-

integrated device described in Chapter 4, as depicted in (**Figure 1**). Access holes were drilled at both ends of the channel using 1.1 mm diameter diamond-tip drill bit (Crystallite Corp., Lewis Center, OH). A borofloat glass cover plate was cut to fit the device and the cover plate and etched plate thermally bonded. The distance from the top of the weir to the cover plate was approximately 5-20 µm. Platinum electrodes were inserted into PEEK™ tubing and into drilled reservoirs in the positions noted in **Figure 1** using Nanoport fittings. Replaceable silica beads (5-30 µm) were used as the solid phase for all extractions using the eSPE device.

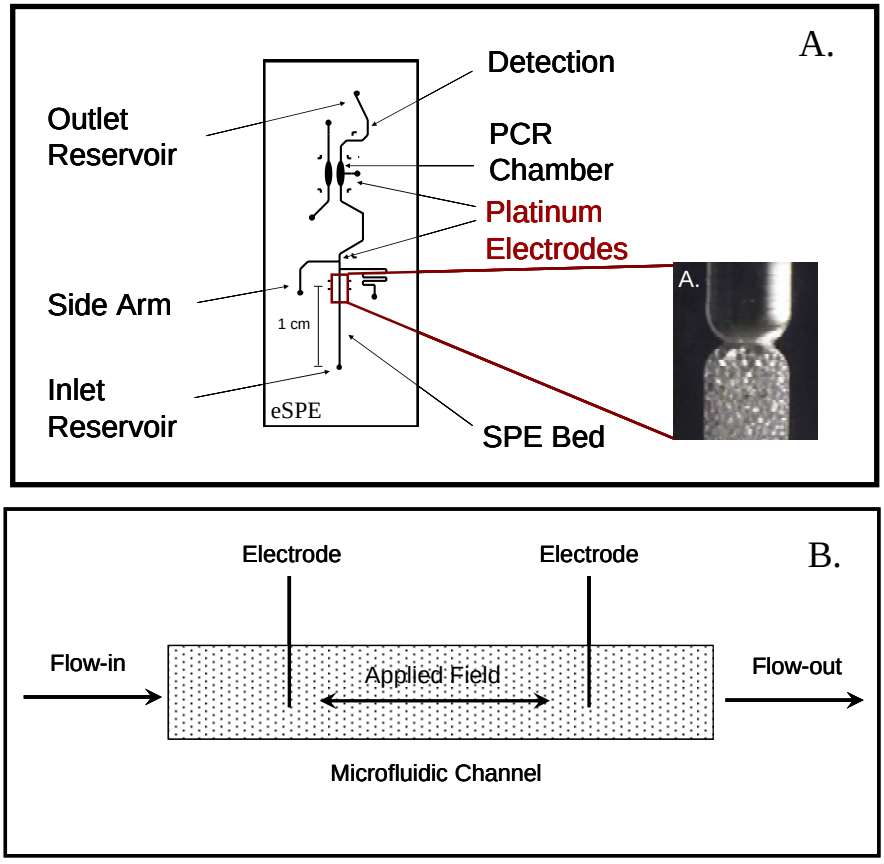


**Figure 1:** (**A**) The eSPE device design. Electrodes adjacent to the PCR chamber and just downstream of the SPE bed allow for application of field during the elution phase of the DNA extraction to concentrate eluting DNA. The side arm enables the addition of PCR reagents for future integration of PCR amplification. (**B**) Side view schematic of the microfluidic channel with inserted electodes, allowing both flow and field mobilization of DNA to occur simultaneously.

### 1.2.4 Solid Phase Extraction

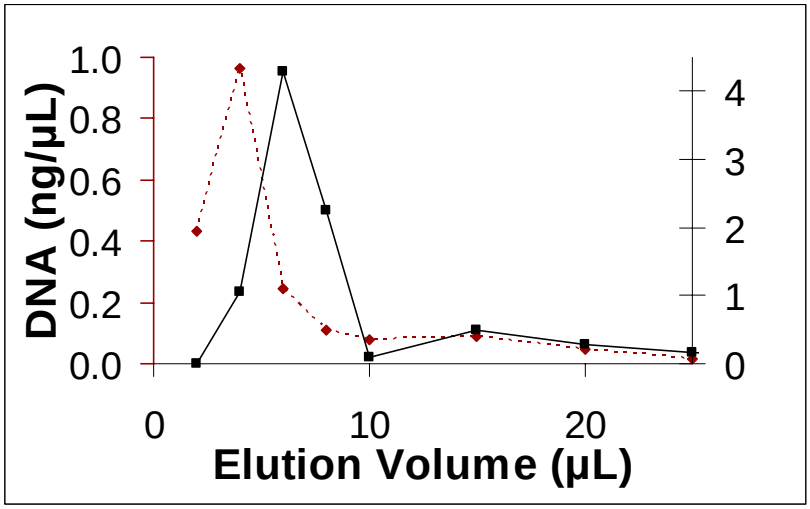


**Figure 2:** The elution of DNA from a silica-based micro solid-phase extraction (µSPE). The DNA, as determined fluorescently (red trace) elutes in approximately 10 µL. PCR amplification, however, is only possible with the latter 6-8 µL, a result of contaminating isorpropanol from the protein removal step of the extraction.

For all extractions, newly filled microdevices were conditioned with 6 M GuHCl (prepared in 1X TE, pH 7.9 and 100 mM 2-(4-morpholino)-ethane sulfonic acid (MES), pH 4, final pH of 6.1), for 10-15 minutes at a flow rate of 250 µL/h, to ensure removal of unreacted species and to prepare the silica bed for extraction. Prior to each subsequent extraction, the bed was removed. The basic extraction protocol consisted of the three pressure-driven wash steps, as previously described.

*1.3 Solid Phase Purification with Dual Pressure-Electro Elution*

**1.3.1 eSPE Device Design**

As discussed in Chapter 3 for integrated device design that contains both SPE and PCR domains, purified DNA is eluted from the solid phase in approximately 5-10 µL, directly into a PCR chamber that had a volume ranging from 500 nL-1000 nL. As a result of this volume discrepancy, only a minor fraction (<10%) of the DNA eluting from

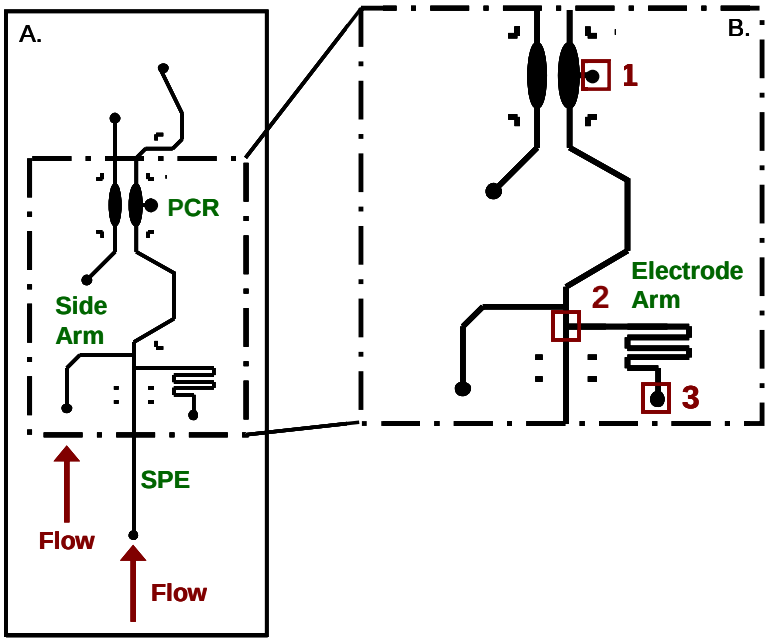


**Figure 3:** The eSPE device design showing solution flow (**A**) and electrode placement , numbered 1-3 (**B**). Electrodes are placed in position 1 and in position 2 or 3, as indicated for each experiment.

the solid phase is utilized in the subsequent PCR amplification. In addition, and as highlighted in **Figure 2,** PCR amplification is only efficient with the latter 6-8 µL of eluting DNA from the SPE bed, as a result of isopropanol contaminating the early fractions during the protein removal step of the extraction. Consequently, we attempted the design and development of a device to address these issues, specifically, one that would exploit the electrophoretic retention of DNA within the device to: 1) concentrate the eluting DNA in a smaller volume and 2) retain the DNA in the PCR chamber of the device while removing contaminating isopropanol.

A microdevice similar in design to the fully-integrated device presented in Chapter 4 except for the slightly-modified SPE and PCR domains was fabricated for these experiments. It contained a 1 cm long SPE bed and an elliptical chamber fluidically-connected, without valves, to the SPE domain, for PCR amplification. In addition, it has a side arm for the addition of PCR reagents and the arm utilized in the MGA for disposal of waste materials is utilized as the electrode arm on the eSPE design.

With this design, electrodes can easily be placed in multiple different configurations; however, the arrangement used in this chapter is a dual electrode set-up, with one electrode placed in position 1 and a second electrode placed in either position 2 or position 3, as depicted in **Figure 3**. Importantly, this design allows for the application of field during the elution phase of the purification and the durable, reproducible, and facile placement of electrodes within the channel of the device, while maintaining uninterrupted, pressure-driven solution flow. In addition, it also allows for multiple electrode configurations to be explored without redesign (and refabrication) of the device.

### 1.3.2 Evaluation of Device Design

*1.3.2.1 Evaluating Electrode Placement*

The first step in evaluation of the proposed design was to discern the most appropriate placement of the electrodes to generate an optimal electric field for DNA capture. Two configurations were evaluated, the first with electrodes at positions 1 and 3 (**Figure 3**), the second had electrodes positioned at points 1 and 2. The first configuration would keep the electrode at position 3 out of the solution stream, while the second configuration would place the electrode at position 2 within solution flow. Using these two configurations, rudimentary experiments were carried out to simply determine whether a constant current would be maintained with a constant applied voltage. Constant current over the period of time required to perform the elution step of the extraction process (2-3 mins) would indicate a steady, controlled capture field, while serious deviations in the current may indicate deleterious buffer depletion effects or Joule heating. Under electric fields, the movement of ions (the positive ions to toward the

cathode, the negative ions to the anode) in buffers can eventually result in depletion of that buffer [24], when all of the ions have migrated to their respective electrode and a drop in current is observed. In addition to this drop in current, the pH at each reservoir following depletion, as a consequence of ion migration, is either highly basic or highly acidic. Depletion is especially problematic and evident in systems where small volumes (such as microfluidic devices) are utilized [24]. Consequently, careful consideration and monitoring of depletion effects in the eSPE system was necessary. (see above) First, the microdevice with electrodes in the channel at positions 1 and 3, was filled with 1X TE buffer (typical DNA elution buffer) in a manner certain to not introduce any air bubbles to the device. Following filling, the electrodes were connected to the power supply and a constant voltage of 96 V was applied (chosen based on prior experimentation; data not

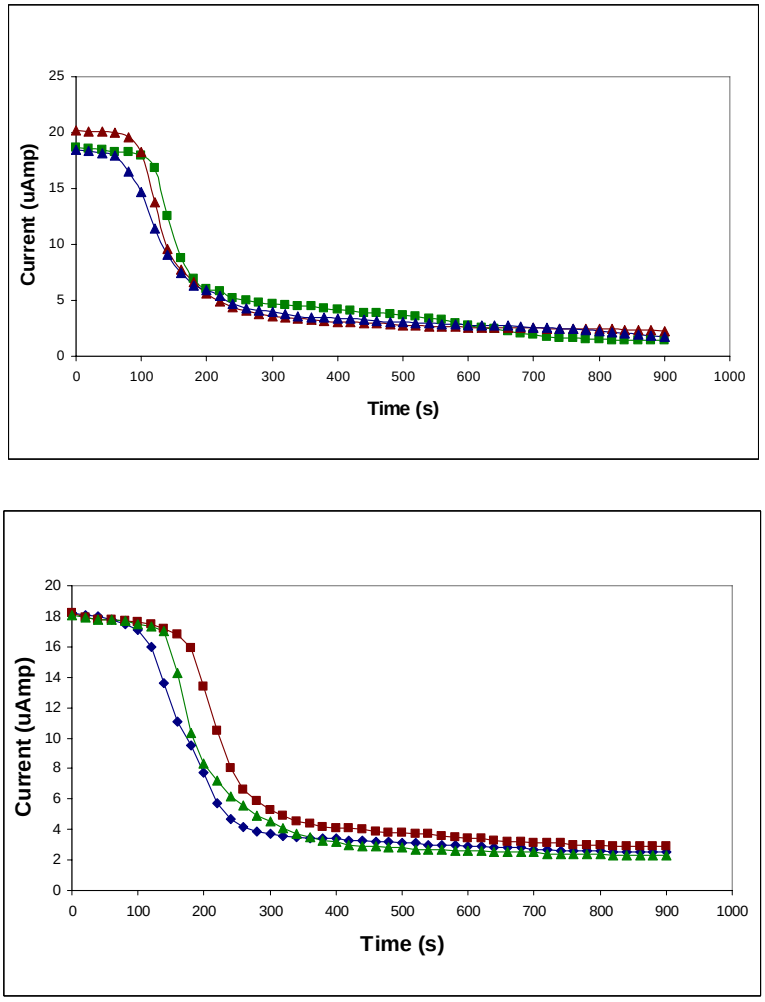


**Figure 4:** Buffer depletion experiments accomplished with (**B**) and without (**A**) flow of 1X TE buffer through the device. Note that although there is aslightly longer (approximately 50 s) before the severe drop in current is noted, the drop (indicative of buffer depletion) still occurs rapidly after application of field.

shown). Current was monitored and recorded every 20 s for 15 minutes and, following replacement of the buffer in the channel, the experiment repeated (*n*=3). The triplicate results, depicted in **Figure 4A**, show that just after 100 s, current dropped severely. This was indicative of buffer depletion occurring in the channel, a result that might not be unexpected as a result of the small volumes present in the channel. If this is, indeed, buffer depletion, the effect should be minimized or eversed by replenishment and/or replacement of the buffer in the system. The same experiment was repeated (*n*=3), electrodes in positions 1 and 3, with the syringe pump flowing 1X TE buffer at a rate of 250 µL/hr through the device (the same flow rate used for DNA extraction). As depicted in **Figure 4B**, there is only nominal change in the results, with current still dropping off severely except after 150 s - slightly later than the experiment lacking flow. This delay is likely due to some replenishment of buffer as a result of solution flow, however, the buffer in the electrode arm (see **Figure 3B**) cannot be adequately replaced with simple flow through the device, due to the fact that it is sealed. As such, the buffer in the electrode arm is irreversibly depleted during application of field, as before. The observed

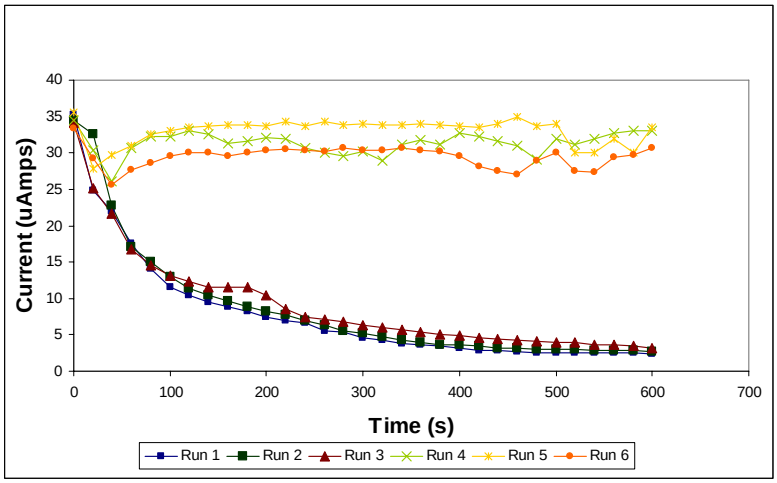


**Figure 5:** Results of buffer depletion experiments with and without flow of 1X TE with the electrodes in positions 1 and 2. Note that the current remains constant when flow is applied.

buffer depletion effects would prohibit effective DNA capture and, as a result, alternative electrode configurations were explored.

The same experiments were repeated with the electrodes in configuration two, with one electrode in position 1 and the second electrode in position 2, directly in the flowing solution. **Figure 5** shows the results obtained with this new electrode configuration with the first three runs essentially replicating the data from **Figure 4**, i.e., when no flow is initiated through the channel, the current drops almost immediately (runs 1-3). However, when flow is generated during application of voltage (runs 4-6), the current remains reasonably constant for the 6 minute duration of the test. The stability of the current with this electrode configuration appeared adequate for the duration of time needed for successful elution of DNA from the silica bed. As a result of these preliminary experiments, this electrode configuration was used in all future experimentation with this device.

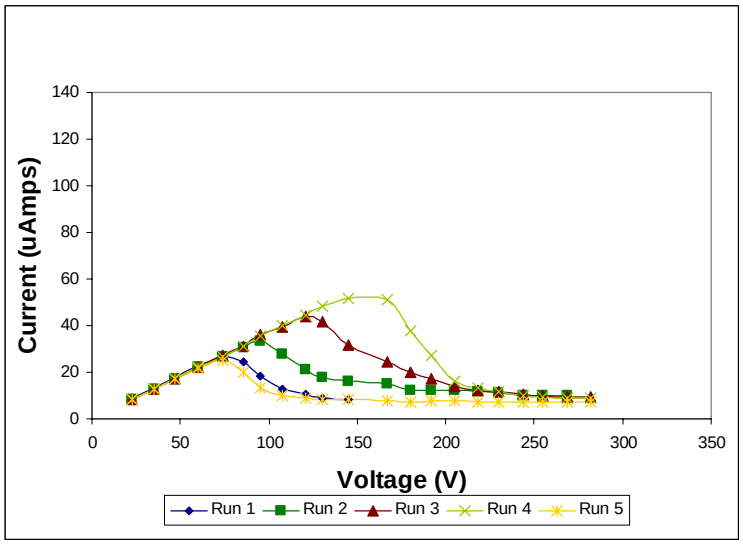


**Figure 6:** Ohm's law plot without flow of 1X TE, using the eSPE device.

### *1.3.2.2 Determining Optimal Field Strength for DNA Capture*

Upon defining an electrode configuration within the device that minimized buffer depletion, it was imperative to determine the maximum voltage that could be applied to the device without Joule heating occurring with this buffer system. Under high electric fields, the current, also known as Joule heat, can overcome the system's capability to dissipate it, resulting in problems with sample stability and bubble formation (due to hydrolysis) within the channel [24]. Joule heating is, simply, the increase in temperature of a conductor as a result of resistance to an electrical current flowing through it [24]. Consequently, see above it is important to determine the maximum voltage that can be utilized with the particular buffer system without excessive Joule heating effects. This can be defined by an Ohm's law plot. Ohm's law states that, within an electrical circuit, the current is directly proportional to the potential difference applied across them [24]. Mathematically, V = IR, where V is the voltage, I is the current, and R is the resistor [24]. Consequently, by monitoring the current generated in the system as increasing voltage applied, deviation from linearity can be monitored and that deviation (an increase in current) defines the point where Joule heating occurs [24]. Ohm's law plots in a variety of buffers have shown that power (voltage times current) should not exceed 1 W/m, for optimal performance in capillary-based systems [24]. Ohm's law plots were generated using 1X TE buffer for the electrode configuration having electrodes at positions 1 and 2 with and without buffer flow - the voltage applied was increased (12-13 V) every 15 s and the current monitored. As shown in **Figure 6**, the current is only stable through roughly the first minute (up to 74 V applied, $n$=5). This is likely due to buffer depletion effects, rather than Joule heating, as a distinct drop off in current is seen.

The experiment was repeated with syringe pump-driven flow at 250 µL/hr through the device, to simulate actual extraction conditions. **Figure 7** details the results of this experiment. Note that the effects of Joule heating and buffer depletion only become apparent at fields higher than 150 V/cm, indicating that with flow through the system during elution, higher field strengths can be applied without the deleterious effects of Joule heating occurring. Run 1 and 3 appear to have deviations due to Joule heating (rise in current from linear), while runs 2, 4, and 5, seem to show drops, or drops followed by rises, in current consistent with buffer depletion effects or a combination of both. Consequently, field strengths lower than 150 V/cm were utilized with all future experimentation with this buffer system.

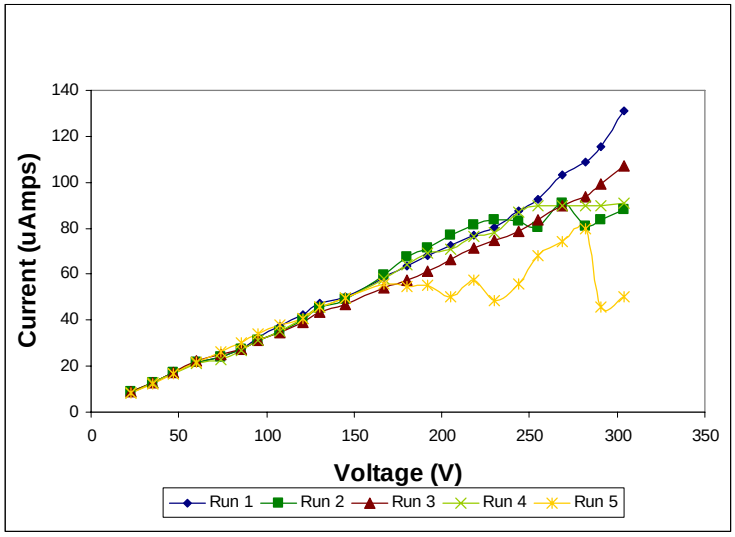


**Figure 7:** Ohm's law plot with flow of 1X TE buffer, using the eSPE device.

*1.3.2.3 Buffer Optimization*

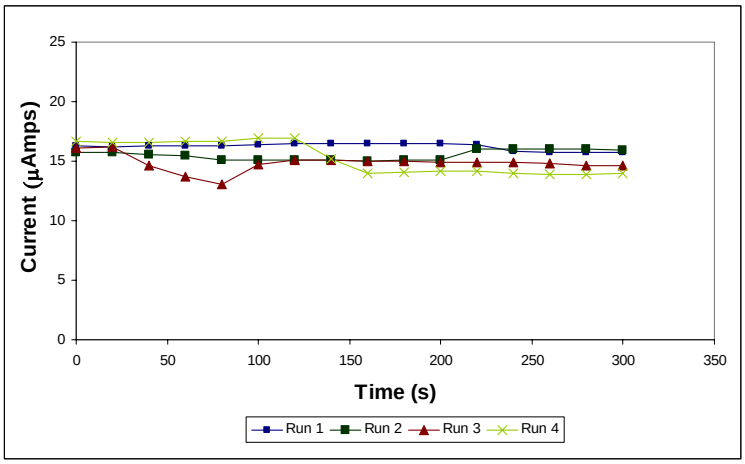


**Figure 8:** Consistent current was measured with Tris buffer and the applied voltage (1400 V) for up to 300 s (5 minutes), with no evidence of buffer depletion effects that could prove deleterious to DNA retention.

Based on the previous experimentation that showed that current was adversely affected when the field strength applied was higher than 150 V/cm, the buffer was adjusted slightly to allow for increased field strengths to be utilized, as early results (data not shown) indicated that higher field strengths promoted more efficient capture. Park et al [23] utilized a simple Tris buffer for their experiments, leaving out the EDTA present in the original buffer used in this work. As a result, Tris alone was evaluated with the eSPE device. Using the new buffer with flow in the channel at 250 µL/hr, a field strength of 1400 V/cm was applied to the device and the current measured. As shown in **Figure 8**, the current was stable for 5 minutes at this field strength and with this buffer, with no obvious effects of Joule heating, indicating that the new buffer would be appropriate for use in the system and would tolerate the high field strengths needed for retention.

### 1.3.3 Electrophoretic Retention and Control of DNA

*1.3.3.1 Evaluation of DNA Retention and Release*

Following initial evaluation of the device design, electrode placement, and buffer system, experimentation shifted towards the optimization of DNA capture and electrophoretic control of DNA. By flowing fluorescein-labeled DNA through the device

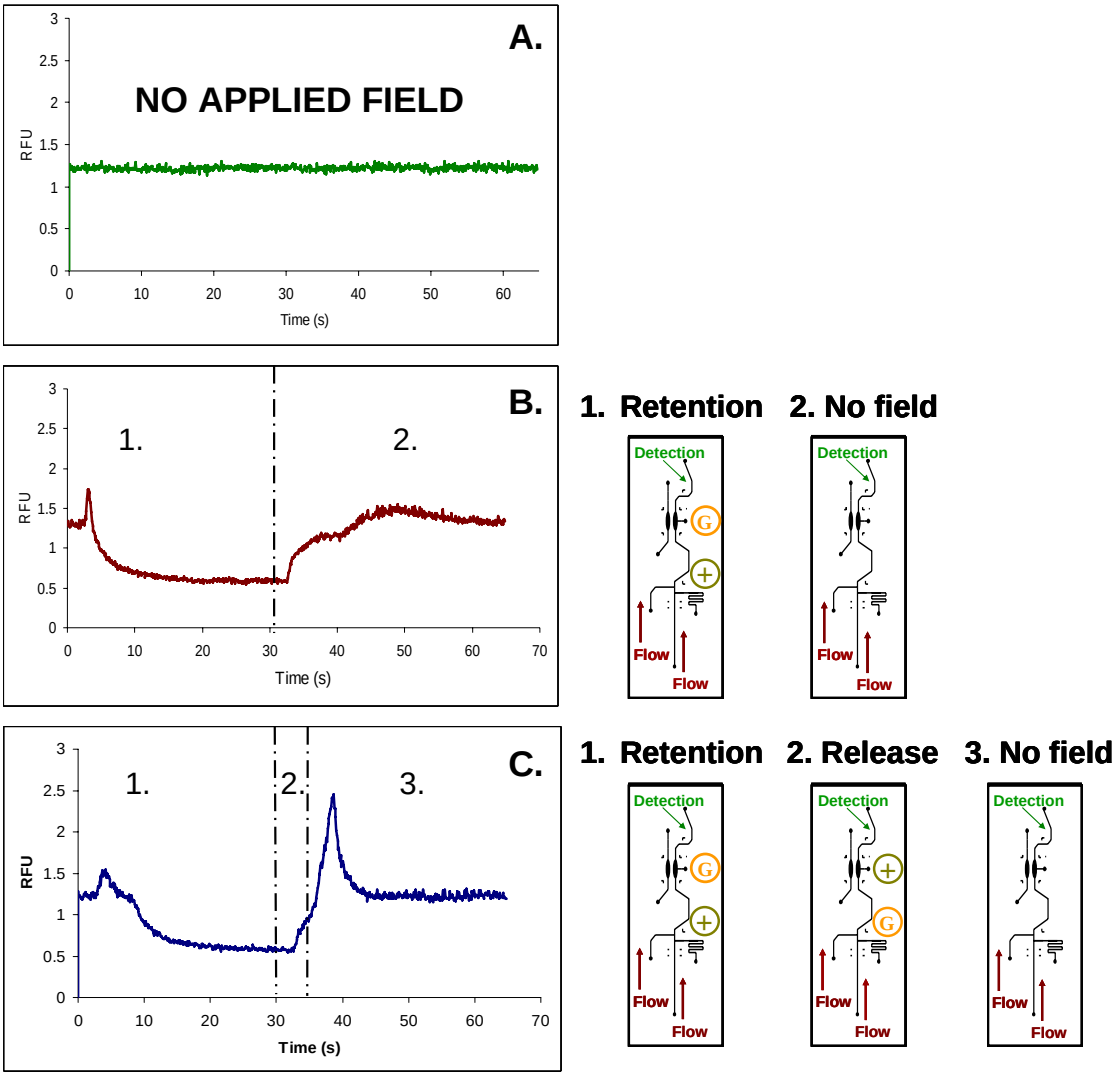


**Figure 9:** Determination of field parameters for DNA retention and release. By flowing fluorescein-labeled DNA through the device, DNA retention and release can be evaluated. Dashed lins indicate change in applied field. When no field is applied (**A**), a stable signal is detected. Applying a field results in a drop in signal, as seen in **B, Step 1**. Termination of field **(B, Step 2**) results in a slow release of DNA and the return of signal back to baseline. The addition of a short (5 s) field reversal (**Step 2, C**) results in the release of DNA in a smaller volume (higher concentration). **Conditions: Fluorescein labelled Lamda DNA (Mirus Bio, Madison, WI), 5 ng/µL in Tris buffer (100 mM, pH=8.0), was flowed through the SPE domain and Tris buffer (100 mM, pH=8.0) was flowed through the side arm at 250 µL/hr continuously. During step 1 of both B and C, 1400 V was applied for 30 s as depicted. In C only, the field was reversed for 5 s following the 30 s retention step. In 4A, 4B step 2, and 4C step 3, no field was applied. DNA was detected by excitation with a 488 nm argon ion laser and detection at 536 nm.**

and using laser-induced fluorescence (LIF) detection downstream of the electrodes, DNA retention and release could be monitored in real-time. In order to effectively employ LIF detection, a plexiglass manifold was built to position and then immobilize the device, similar to that constructed for the MGA device presented in Chapter 4. As depicted in **Figure 9**, when no field is applied (**A**), for 60 s (well below the time at which any buffer depletion or Joule heating effects would be seen) a stable signal of ~1.25 RFU is detected. Applying an electric field to the device during flow results in a slow, but steady, drop in fluorescence, presumably due to DNA retention in the PCR chamber (see **B, Step 1)**. Termination of field (**B, Step 2**) at 30 sec results in a slow increase in fluorescence back to baseline ~(1.25 RFU), presumably due to the release of DNA . With the goal of being to be able to release the DNA rapidly and in a small volume, these release kinetics observed in Figure 9B are, unfortunately, too slow - this would result in a broad elution peak volume (~4 µL in this case) that would not be beneficial for this microfluidic application. This problem was solved by the addition of a short (5 s) reversal of the electric field (**Step 2, C**) prior to DNA release, which resulted in the DNA being released in a smaller volume (~1.3 µL) and, hence, at a higher concentration. These first results were important as they were indicative of successful control and concentration of DNA within the eSPE device, all in the presence of flow with extraction-type flow rates. This three step process - retain, release, termination of field - was utilized for all future experiments because it provided DNA in a concentrated form, in a volume more compatible with typical microchip PCR protocols.

*1.3.3.2 Field Strength Optimization*

Following successful demonstration that DNA could be retained in the eSPE device using the three step electric field manipulation protocol, the magnitude of the applied field was evaluated, to ensure sufficient DNA capture was occurring. In order to accomplish this, the three step protocol was again applied to a constant flowing stream of fluorescein-labelled DNA, and fluorescence monitored at a detection point in the channel beyond the electrodes. Field strength was varied during the capture step (30 s) and release step (5 s), with the field terminated at 35 s for each run. **Figure 10** depicts the results of some of these experiments. At low field strengths, there is little DNA retention in the capture zone - however, as the field is increased, the increased retention of DNA is evident. At field strengths higher than 1400 V/cm, Joule heating and bubble formation become problematic, and retention is adversely affected (noted by direct observation of bubbles in the device channel and the inability to detect any DNA retained). These results indicate that 1400 V/cm is the maximum applied field that can be utilized with this buffer system and this will be the field applied in further experimentation with this device.

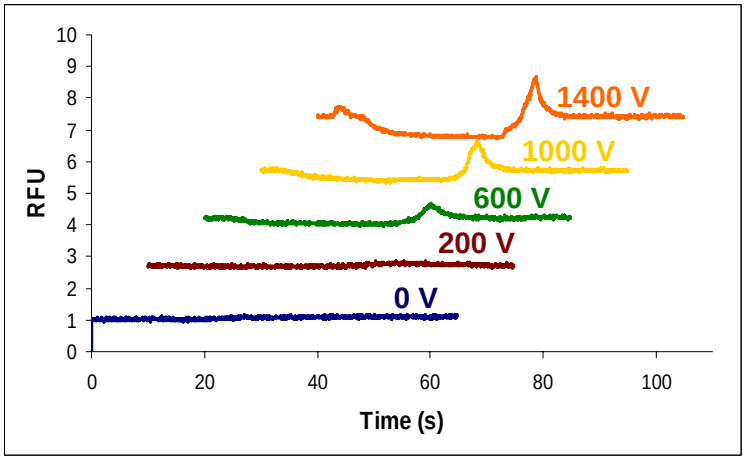


**Figure 10:** Optimization of applied field. Increasing the applied field results in a larger amount of DNA retained and release, as depicted. At lower voltages, DNA is not retained in the device sufficiently, while at higher voltages significant retention and release is observed. **Conditions:** DNA and buffer flowed through device at 250 µL/hr, continuously, as previously described. Applied voltage in both steps was the same, as indicated. Detection as previously described.

*1.3.3.3 Timed Release of DNA*

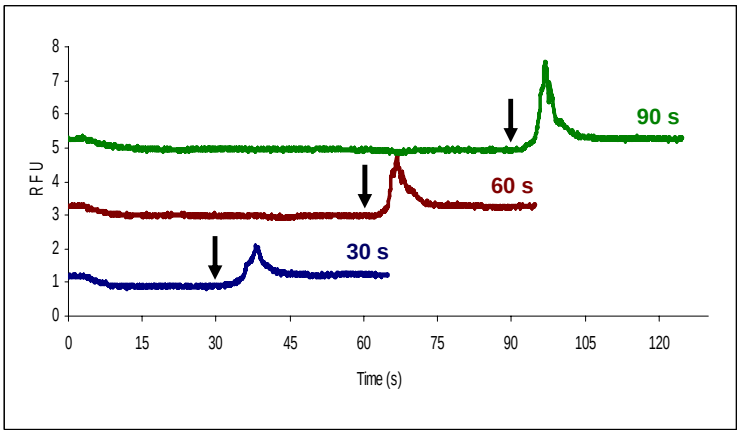


**Figure 11:** Precision retention and release of DNA from the eSPE device. Field was applied for varying lengths of time as indicated (arrows indicate where field is reversed for 5 s, followed by termination of field), while DNA (5 ng/µL) was flowed continuously at 250 µL/hr through the device. DNA eluted past the detector only when the reverse field release step occurred

In order to define that the voltage-induced retention of DNA was real (and not artifactual), it was important to show that the release of DNA could be temporally-controlled. In addition, control of DNA release from the capture zone of the device is critical to the precise manipulation of the concentrated, purified nucleic acids in the microfluidic system, and essential to the isolation of the DNA in the PCR chamber for amplification in integrated analyses. In order to ascertain this, experiments were carried out where the duration of retention step was varied. Again, utilizing the steady flow of fluorescein-labelled DNA, the first DNA retention step was varied from 30 to 90 s, while the release steps remained the same as previously described (5 s, at the same applied field as the retention step, 1400 V/cm). As shown in **Figure 11**, DNA is eluted past the detector only when the retention voltage is terminated and the reverse field release step is applied (arrows indicate where field is switched), demonstrating precise control over DNA retention in the device. In addition, the peak height increase observed as the DNA retention time was increased was consistent with the presence of increased DNA concentration, as would be expected. As a result of this and the other device

evaluation experiments presented in this chapter, it was determined that this design would sufficiently enable the capture and timed release of DNA during the elution step of solid phase purification.

### 1.3.4 DNA Purification with Electrophoretic Retention of DNA

Having demonstrated the effective concentration and controlled release of DNA in the eSPE device, the ability to trap DNA in the chip during the elution phase of a DNA purification was attempted. In order to evaluate this retention, fluorescein-labelled DNA was once again utilized so that LIF detection could be employed during the elution phase of the extraction. A mass of 25 ng (25 µL) of labeled DNA was loaded onto the silica bed in the device at a flow rate of 250 µL/hr. Following the loading step, a 5 minute wash with 80% isopropanol was performed with Tris buffer flowing through the side arm of the device in both the load and wash steps. Prior to the elution step, the platinum electrodes were connected to the power supply and the device aligned on the stage for fluorescence detection. Just

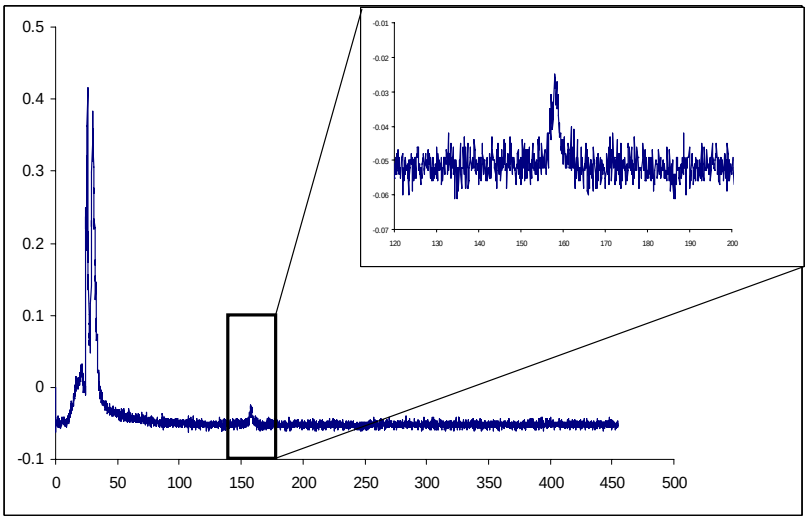


**Figure 12:** eSPE of fluorescein-labelled DNA. Note the presence of a large elution of DNA that passes through the field unperturbed; however a small amount of DNA (inset) is retained and eluted during the release phase.

preceding the commencement flow through the solid phase for elution, a field of 1400 V/cm was applied to the electrodes and held for 150 s. Following the cessation of field, a short, 5 s field reversal was applied to allow for release of DNA from the device. As depicted in **Figure 12**, there is a large signal at the beginning of the elution (0-50 s), representing the bulk of the DNA passing through the device unperturbed by the applied field. At ~155 s (**Figure 12, inset**), however, when the field was reversed and the DNA released, the presence of a small DNA elution peak was detected. Although this represents only a small fraction of the total eluted DNA, this was the first example of electrophoretically-retained DNA during the course of micro-solid phase purification. Likely, the field strength and/or surface area for capture were not large enough to allow for more complete capture of DNA. Clearly, further development of the device design and protocol are needed to ensure that the majority of the eluting DNA is retained; however, these preliminary results provide reasonable optimism that with proper evaluation and continued optimization, the complete capture, concentration, and retention of DNA can be achieved.

*1.4 Concluding Remarks*

A preliminarily microfluidic device was designed and optimized for the capture and concentration of DNA during the elution phase of purification, with the goal of retaining the entire mass of eluted DNA within the microfluidic device for eventual integration with PCR amplification and electrophoretic analysis. Although more effort is needed to fully realize the potential of this design, the ability to apply a field, retain and capture DNA with precision elution, while maintaining solution flow through the device

was demonstrated. The electrophoretic capture of DNA in a flowing stream in a microchannel represents a serious microfluidic challenge that, heretofore, has not been demonstrated in the litreature. Thus, it is important to recognize the steps this new research project has taken towards accomplishing this goal with the realization that more complete and thorough development of this device and methodolgy are still necessary.

*5.5 References*

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