Automated Liquid–Liquid Extraction by Pneumatic Recirculation on a Centrifugal Microfluidic Platform

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Supporting Information

ABSTRACT: In this technical note, a liquid–liquid extraction technique was performed using pneumatic liquid recirculation on a centrifugal microfluidic device. Non-contact pneumatic pumping enabled a multi-cycle liquid–liquid extraction process using aqueous iodine in a potassium iodide solution and hexadecane while requiring a minimal amount of space on the device. The extraction process was completely automated on the device following sample introduction and required only 50 s for each extraction cycle. The pumping rate achieved during liquid recirculation was $120 \pm 10 \mu$L/min. A recycling process such as the one demonstrated would be difficult to implement in a conventional centrifugal microfluidic system.

Considerable effort in the field of analytical instrumentation has been focused on the design of microfluidic devices that allow classical analytical operations while maintaining processing times and costs at a minimum. The miniaturization of analytical devices also grants a significant environmental and economical advantage as lower sample, solvent, and reagent volumes are necessary.

A desirable goal of such miniaturization is the development of micro total analysis systems (μTAS) which are capable of nearly autonomous analysis of samples. μTAS devices have considerable potential to be used for environmental in-field analysis and point-of-care medical diagnostics while minimizing personnel and instrumental costs.

Microfluidics brings the challenge of finding an efficient method for directing liquid transport within its systems. Conventional microfluidic μTAS devices presently do not show great potential for portability as they typically rely on external pumps or electrochemical methods to direct liquid flow. In addition, electrochemical-based microfluidic systems are applicable only to conductive fluids. However, centrifugal microfluidic (CM) devices make use of centrifugal force for flow control and, as such, do not require any direct external connections to the microfluidic platform. These devices, which most often take the general form of compact disks (CDs), can be made portable, have very low power requirements, and allow for a high degree of parallelism as centrifugal force acts in a radial fashion. One significant constraint of conventional CM devices is that the amount of usable area for microfluidic structures is restricted due to the unidirectional flow of liquid. This renders conventional CM platforms unable to perform a large number of sequential operations.

Liquid–liquid extraction (LLE), a common multi-step sample preparation process, involves cycles consisting of addition of extractant to the sample, mixing of the two immiscible phases, and extraction of the desired phase. These cycles must be repeated several times to maximize the yield of the extraction process, which is especially critical for quantitative analytical applications. To date, CM devices have been incapable of efficiently implementing such a process as conventional CM design has limited capacity for recirculation. Overcoming this limitation requires the use of a complementary pumping technique to permit recirculation of liquid on the device while it is spinning. Several recirculation techniques have been suggested previously. Garcia-Codero has described a recirculation method based on an interplay of capillary and centrifugal forces, which requires stopping the disk to allow capillary forces to bring the liquid back through a hydrophilic channel. This involves the use of hydrophilic surface treatments on the CM device, which complicates the fabrication process and brings into question the longevity of some treatments such as oxygen etching. Lammertink has described the use of electroosmosis to cause recirculation of droplets in microfluidic channels, which requires integrated platinum electrodes and greatly limits the types of liquids that can be used in the system. A similar electroosmotic process has been described by Wang, which still retains the same chemical compatibility issues (i.e., a conduction solution). Abi-Samra has described a thermo-pneumatic pumping method that uses pressure created from the thermal expansion of air contained inside a ventless chamber to pump fluid back to the center of a disk, allowing for potential recirculation. However, this technique requires low rotational frequencies and heating of the isolated air to
temperatures that may volatilize some organic solvents. Furthermore, the pumping rate was shown to be relatively low, rendering it less attractive for multi-cycle operations.

In previous work, we have demonstrated a non-contact pneumatic pumping technique that can pumps fluids against centrifugal force, precisely control flow switching, and provide valveless fluid transfer. This technique relies on non-contact pumping by compressed air directed through a device’s vent holes. The resulting process creates air pressure and directs liquid flow as required, allowing a myriad of possibilities and breaking traditional CM limitations.

As an extension to the capabilities provided by non-contact pneumatic pumping, the method has been adapted here to perform rapid recirculation of a demonstrative sample during a two-cycle automated LLE extraction on a CM platform. The pneumatic recirculation technique can be used on disposable (i.e., single-use) or reusable CM platforms. This CM LLE technique based on pneumatic recirculation demonstrates the extended operational capabilities granted by pneumatic flow control.

## Experimental Section

### Reagents

An iodine solution of concentration 10 g/L (A&C American Chemicals, Montreal, QC, Canada) in 5% aqueous potassium iodide (Fischer Scientific, Nepean, ON, Canada) served as a clearly visible aqueous sample and hexadecane (Caledon Laboratories Ltd., Georgetown, ON, Canada) served as the organic extractant. An aliquot of 25 μL of the iodine solution and two 38 μL volumes of hexadecane were used during the experiment. Iodine was chosen as the demonstration amphipathic dye due to its highly visible color change from orange in an aqueous solution to purple in hexadecane. Several other organic solvents including benzene, carbon tetrachloride, and dichloromethane were investigated as potential extractants, but they were determined to be incompatible with the polycarbonate-based platform.

### Device Fabrication

A CM device was assembled using the rapid prototyping procedure established by Kido. The schematics of the device’s different layers were designed with the use of SolidWorks 2005 (SolidWorks Corp., Concord, MA). The device consists of five layers (Figure 1), with layers 1 and 5 being polycarbonate DVDs (Figure 1a, e). Layer 3 (Figure 1c) was a polycarbonate CD and layers 2 and 4 (Figure 1b, d) were double-sided adhesive cutouts (FLEXmount DFM-200-Clear V-95 150 poly V-95 400, FLEXCon, Spencer, MA) used to hold the device together. The device’s vent, injection and alignment holes, chambers, and channels were milled into the CD and DVD layers with the use of a computer numerically controlled (CNC) 2D milling machine (QuickCircuit 5000, T-Tech Inc. Norcross, GA). The adhesive layers were cut by xurography with a cutting plotter (CE300-Mk2-60, Graphtec America, Inc., Santa Ana, CA). All layers were precisely aligned with alignment holes present on each disk, pressed together, and laminated with a cold laminator (Jet Mounter ML25, Drytac, Concord, ON, Canada). The cutouts were modeled to be matched to the device’s chambers, channels, and valves so as to be well aligned with the core of the device. During assembly, a fused silica capillary valve with an inner diameter of 75 μm (Polymicro Technologies, Phoenix, AZ) was manually embedded into the device with commercially available epoxy glue as described by LaCroix-Fralish.

### Device Design

The LLE device’s design can be found in Figure 2. The channels milled in this device had a depth of 0.7 mm while the chambers had a depth of 1.4 mm. Two chambers (6 mm × 4.5 mm) near the top of the disk serve as extractant reservoirs. The central extraction chamber is designed with a wider arced central part that allows it to efficiently mix both phases and a narrower funnel-shaped part to allow for settling of the denser phase after the extraction process. The liquid then passes through an adhesive cutout based capillary valve developed by LaCroix-Fralish into a metering chamber (8 mm × 5 mm) in which the phase separation occurs. The aqueous phase is directed after extraction to the bottom pumping chamber through a 75 μm fused silica capillary valve from which it is pumped by pneumatic recirculation back into the extraction chamber for the cycle to be repeated. The 75 μm inner diameter capillary valve was chosen to allow liquid flow when desired while ensuring that the burst frequency remained high enough to retain liquid in the metering chamber reproducibly. The hexadecane containing the extracted iodine is collected in a circular extractant recovery chamber (5.5 mm radius). Each chamber has a vent hole (0.5 mm radius), and the extractant injection and extraction chambers both have injection holes (0.5 mm radius). All other channels have widths of 1 mm.
Experimental Configuration. The centrifugal apparatus consisted of a base, servo motor, and strobe system described in detail by Duford14 with the addition of a color digital camera (GRAS-14SSC-C, Point Gray, BC, Canada). This entire system is synchronized on a single computer with the use of the LabVIEW software (LabVIEW 8.6, Developer version, National Instruments, QC, Canada) and automated with cycle settings that are preset by the user. This method enabled the acquisition of high-speed digital strobe images of single cells on the device during operation allowing one to follow liquid flow during an extraction run. In addition, two previously described non-contact pneumatic pumping configurations9 were used for controlling liquid flow. A schematic of the experimental configuration can be found in Figure 3.

Experimental Procedure. The operational principles of a single cycle of the LLE device can be found in Figure 4. The two extractant reservoirs were first filled to capacity with 38 μL of hexadecane. Then 25 μL of the iodine demonstration solution were injected into the extraction chamber (Figure 4a). The device was loaded onto the CM apparatus and spun clockwise at 250 rpm (revolutions per minute) for 5 s with a stream of air at 0.8 SCFM (standard cubic feet per minute) directed at the vent holes of the injection chambers to empty the first extractant reservoir into the extraction chamber (Figure 4b). At this velocity, only the top chamber empties into the extractant chamber as the air pressure is not sufficient to counter the centrifugal force experienced by the second injection chamber, which is more radially outward on the disk. The servo motor was then running “shake mode”15,16 at 90 rpm for 15 s during which a back and forth rotation motion of the motor provides thorough mixing of the two phases (Figure 4b, d). The spin rate was then increased to 800 rpm, rotating counter-clockwise for 10 s to burst the adhesive-based capillary valve and allow liquid flow into the metering chamber (Figure 4e). The design of the metering chamber is such that once it is half filled (~25 μL), all excess liquid flow is metered off into the extractant recovery chamber. When subjected to centrifugal force, the aqueous layer was pushed to the bottom of the metering chamber as it is the denser of the two liquids, filling it, forcing the organic phase to flow into the extractant recovery chamber (Figure 4f). After the extractant was collected in the recovery chamber (Figure 4g), the device was spun at 2600 rpm counter-clockwise for 10 s to burst through the fused silica capillary and direct the aqueous phase and a small amount of hexadecane remaining on the upper surface into the pumping chamber (Figure 4h). The spin rate was then decreased to 200 rpm counter-clockwise for 15 s and the first pneumatic system was activated for 5 s at 0.8 SCFM. At this lower rotational frequency, the second extractant chamber was emptied (Figure 4i) as the reduced centrifugal force at this lower rotational rate (as compared to 250 rpm) could be overcome by the pneumatic pressure. Finally, a second pneumatic flow was directed at the vent hole of the pumping chamber at 1.0 SCFM for 10 s to cause pumping of the remaining liquid (recirculation) back into the extraction chamber (Figure 4j). The metering and pump preparation parts of the cycle were then repeated as previously described.

The duration of the mixing process (15 s) was chosen to give a significant color change and kept consistent to facilitate the comparison of the extraction processes over both cycles. The disk rotation frequencies and air flow rates were optimized experimentally for this CM platform design.

![Figure 3](image1.png)

**Figure 3.** Experimental setup: (a) compressed air cylinders, (b) flow meters, (c) computer-controlled solenoid valves, (d) high-speed digital camera, (e) LLE device and (f) Servo motor. Parts a–c represent two completely independent pneumatic systems.

![Figure 4](image2.png)

**Figure 4.** Operational principles of a single cycle of the LLE device. Blue represents pure hexadecane, which in reality is a clear fluid, red represents the aqueous iodine sample, and purple represents hexadecane containing extracted iodine: (a) injection of sample and extractant onto the device; (b) addition of first extractant volume by pneumatic fluid transfer from top reservoir; (c, d) mixing of the two phases; (e–g) separation by metering; (h) transfer of aqueous phase into pumping chamber; (i) addition of second extractant volume by pneumatic fluid transfer from bottom reservoir; (j) pneumatic recirculation of aqueous phase back to the extractant chamber.
Technical Note

in the Supporting Information.

Figure 5. Strobed experimental images showing the operation of the LLE device over two cycles (aqueous iodine, orange; pure hexadecane, clear; iodine in hexadecane, purple): (a) addition of first hexadecane volume from top extractant reservoir, (b) mixing, (c) separation of the two phases by metering, (d) transfer of aqueous phase into pumping chamber, (e) addition of second hexadecane volume from bottom extractant reservoir, (f) pneumatic recirculation, (g) mixing, and (h) separation of the two phases by metering. High-resolution versions of each image are available in color in the Supporting Information.

Table 1. Average Red, Green, and Blue Pixel Intensities of a Line of 50 Pixels Taken from the Hexadecane Layer in the Extraction Chamber before and after Mixing/Extraction

<table>
<thead>
<tr>
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<th>before mixing</th>
<th>after mixing</th>
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<tbody>
<tr>
<td></td>
<td>red</td>
<td>green</td>
</tr>
<tr>
<td>1st extraction cycle</td>
<td>156 ± 4</td>
<td>168 ± 4</td>
</tr>
<tr>
<td>2nd extraction cycle</td>
<td>164 ± 7</td>
<td>178 ± 6</td>
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*The small standard deviations in pixel intensity indicate that the difference in color before and after extraction is significant, which was verified by a two sample $t$-test at a 99% confidence level.

Image Analysis. Digital image processing was applied to the experimental images obtained before and after the extraction processes with the use of a custom MATLAB program (MATLAB R2010a, MathWorks Inc., MA) to verify that an extraction had taken place in each cycle. A line of 50 pixels in width was selected from images of the extractant contained inside the extraction chamber. The average red, green, and blue (RGB) intensities were quantitatively compared before and after each extraction cycle.

RESULTS AND DISCUSSION

As shown in the experimental strobed images in Figure 5, the automated LLE process was completed as described in the Experimental Section. Valveless pneumatic fluid transfer caused the addition of extractant as desired in each cycle (Figure 5a–e). The extraction chamber allowed for efficient mixing of the two phases while preventing liquid from splashing out of the vent hole or through the pumping channel (Figure 5b–g). The funnel shaped bottom of this chamber permitted the aqueous phase to settle before metering ensuring that only the organic phase was collected.

The metering process achieved an effective separation of the two phases with only a thin layer of hexadecane remaining with the aqueous phase (Figure 5c–h), because the 25 $\mu$L capacity of the metering chamber was a conservative estimate to ensure that the metering capacity is never exceeded during separation. With our platform, approximately 25 $\mu$L of extractant was recirculated with the aqueous phase as is done with traditional LLE methodology. This is typically needed to obtain the extractant without having it contaminated by the aqueous phase. Following its flow through the fused silica valve (Figure 5d) into the bottom chamber, the aqueous phase was successfully pumped by pneumatic recirculation (Figure 5e). The air pressure generated by the stream of compressed air efficiently pumped the aqueous sample back into the extraction chamber at a flow rate of 120 ± 10 $\mu$L/min. As the inlet into the pumping chamber was a fused silica capillary valve, the air stream did not travel back up to the metering chamber due to the small 75 $\mu$m sized diameter of the valve, allowing for precise flow control while requiring no additional features (chambers, channels, etc.) on the disk.

Digital image processing was used to demonstrate the extraction of iodine from the aqueous phase into the hexadecane phase in each cycle. A comparison of the pre- and post-extraction average RGB intensities of a 50 pixel wide line selected from images of the hexadecane in the extraction chamber is summarized in Table 1. As can be seen in Figure 5a, the pure extractant is a colorless liquid and the relatively high RGB values of its image correspond to the white background underneath the device. As shown in Table 1, there is a significant decrease across the entire RGB profile which was less prominent in the red after the extraction process. This distinctive change in the RGB profile of the solution confirmed our visual observations that the post-extraction hexadecane is darker and more pink-based in color than the pure compound, indicating the presence of iodine. The decrease in RGB values for both extraction cycles were similar as the aqueous solution of iodine was very concentrated, resulting in similar amounts of iodine being extracted during both mixing steps of the extraction cycles.

To quantitatively examine the variation, the standard deviations of the RGB intensities were calculated and found to be small in comparison to the mean values. The differences in pixel intensities before and after extraction were determined to be statistically significant at a 99% confidence level by a two-sample $t$-test. During our experiments, the color difference could be observed visually pre- and post- extraction and confirmed by the change in RGB profiles for both extractions.
cycles. The absolute differences between RGB values before mixing (Table 1) over the two extraction cycles can be attributed to the irreproducibility of the strobe lighting; however, this difference did not affect the comparison of pre- and post-extraction conditions as the RGB profiles were very different and clearly distinguishable.

The recirculation air flow rate used was determined experimentally to be the minimum required to pump the aqueous phase back to the extraction chamber. It is important to note that it can be arbitrarily adjusted upward provided that the overall force of the combined centrifugal force and air pressure applied to the valve of the destination chamber is less than the force required to burst it. This allows pneumatic recirculation to be used at a variety of rotational frequencies as the air flow can always be scaled accordingly. Furthermore, an increased air flow rate also allows for higher pumping rates, which could greatly reduce analysis times. This offers great flexibility for the experimenter in terms of disk designs and experimental conditions. The direct on-disk detection method proposed is not only convenient but minimizes the potential for analyte contamination. However, offline detection can also be carried out if desired by gently accessing the analyte through the top DVD layer.

As shown during these experiments, pneumatic recirculation can easily be combined with valveless pneumatic fluid transfer. The possibility of combining both techniques is valuable as this allows stand-alone operation of multi-cycle devices following the introduction of reagents on a disk. The non-contact nature of the pneumatic fluid control techniques makes these devices very portable and suitable for field use, bringing us one step closer to the creation of complete CM µTAS. These advantages make pneumatically enhanced CM platforms an attractive approach to chemical analyses, and an on-disk liquid–liquid extraction process could potentially be used to isolate environmentally important species such as polycyclic aromatic hydrocarbons (PAHs) that have a relatively low solubility in water.

While only two cycles were shown here, the entire extraction process can be repeated with more extractant chambers without the need for any additional system components. Valveless pneumatic fluid transfer and pneumatic recirculation require only a single pneumatic line each and can accommodate as many cycles as required depending on the particular operation or experiment. This work lays the foundation for the development of more pneumatic-centrifugal based platforms that are application specific, where extraction yields and extraction efficiencies can be suitably determined.

CONCLUSIONS

Pneumatic recirculation of liquid was successfully demonstrated with a two cycle liquid–liquid extraction, an operation not conventionally possible on traditional CM systems. The flexibility of pneumatic pumping is unparalleled as it does not require any additional design features and can be fine-tuned to the conditions of each experiment. A high pumping rate renders this technique capable of efficient fluid transfer, reducing the time required for analytical processes. Pneumatic recirculation offers the potential for CM devices to perform a variety of complex cyclical operations, making it an invaluable contribution to the pneumatic toolset.

REFERENCES


ASSOCIATED CONTENT

Supporting Information
Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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