# Pre-storage of liquid reagents in glass ampoules for DNA extraction on a fully integrated lab-on-a-chip cartridge

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***Received 11th December 2009, Accepted 24th February 2010***
***First published as an Advance Article on the web 17th March 2010***
**DOI: 10.1039/b926139g**

Self-containing, ready-to-use cartridges are essential for mobile Lab-on-a-Chip (LoaC) systems intended for Point-of-Care (POC) use. Up to now, a common weak point in many LoaC developments is the need to dispense liquid reagents into the test cartridge before or during processing of the assay. To address this issue we have developed an efficient method for fusing liquid reagents into glass ampoules, which are then sealed into a centrifugally operated cartridge. For on-demand reagent release, the ampoules are disrupted through the flexible lid of the cartridge. Upon centrifugation, 98.7 μL out of 100 μL (CV = 2.5%) of the pre-stored contents are released into the microfluidic system. No liquid loss is observed for ethanol and $H_2O$ stored for 300 days at room temperature. Frozen storage is possible without damage to the ampoules. Applicability of this concept is demonstrated by performing a LoaC integrated DNA extraction after 140 days of reagent pre-storage. DNA yield from 32 μL of whole blood was up to 199 ng, which is 77% of an off-chip reference extraction. The presented approach allows the improvement of existing LoaC cartridges where pre-storage of liquid reagents was not implemented yet.

## Introduction

MEMS based microfluidic platforms represent a promising technology for the rapid point-of-care detection of various microbial and (bio-)chemical compounds.[1,2] Expectations on LoaC systems are triggered by ongoing advances in microfabrication-,[3–6] fluidic-, and biological assay development[7–9] on one hand, and are challenged by changing market requirements on the other hand.[10]

Lateral Flow (LF) test devices are an example of a low-cost mobile LoaC platform with on-board storage of all required dry reagents.[11] Analysis is performed fully automated without the requirement of any user interaction or trained personnel.[12,13] The sample is passively transported by capillary action inside the LF chip.[14] However, due to the lack of liquid system reagents and a low number of microfluidic unit operations,[15] LF tests are restricted to clinical- and home-care applications of low fluidic complexity, such as qualitative immunochromatographic tests.[16,17]

Actively driven LoaC devices, such as pressure- or centrifugally operated systems, perform laboratory handling steps by microfluidically controlled unit operations.[15] These microfluidic toolboxes allow the design of LoaC systems for the efficient automation of analytical processes in a specific, sensitive, and quantitative manner.[18] Compared to common LF test devices, these more versatile systems often require additional liquid reagents for more complex analytical assays. This may comprise ethanol-based buffers or aqueous solutions with high salt concentration, *e.g.* for DNA extraction.[19] Furthermore, rehydration buffers are required to solve dry reagents such as lyophilized enzymes or dried oligonucleotides, which are *e.g.* required for Polymerase Chain Reaction (PCR).[20]

Many fully integrated LoaC systems that have been published[21–25] do not consider on-chip storage of required liquid reagents. As a matter of fact, practical solutions for liquid pre-storage are essential for self-containing microfluidic cartridges and thus portable POC.[1] These systems run analytical protocols on a LoaC device in truly integrated and automated fashion. As a consequence, trained personnel are no longer required, human handling errors are reduced, which both leads to a more efficient assay workflow.

Previous research work on liquid pre-storage for LoaC systems has been published either as a single unit operation or offering an integrated solution.[26–29] Individual shortcomings of this research work include high vapour transmission rates,[27,28] incomplete liquid release,[26,27] and complex release mechanisms.[29] Therefore, these concepts are problematic for real-world applications.

In general, liquid reagents can be stored either within the diagnostic cartridge or within the analyzer. The latter approach is not an appropriate solution for low-cost handheld devices since additional storage- and dispensing units need to be implemented into the instrument, which are also prone to (cross-)contaminations. Therefore, in this work we focused on a liquid storage concept which can be applied to any disposable LoaC cartridges. In our approach liquid reagents are fused into glass ampoules and sealed into the cartridge. The hermeticity and chemically inertness of glass prevent the evaporation and

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degradation of the encapsulated liquids. The simple but reliable release mechanism guarantees a fast and defined opening of the glass ampoule and a complete transfer of its content into a microfluidic system. Applicability of this approach is demonstrated by performing a LoaC integrated DNA extraction after 140 days of reagent pre-storage.

## Experimental

Glass is an excellent material for long-term stable liquid encapsulation due to its vapour barrier properties as well as chemical inertness towards biological and chemical compounds. As a material for the microfluidic cartridge cyclic olefin copolymer (COC) is chosen due to its unique properties such as optical transparency, moisture barrier, high water vapour resistance and temperature resistance compared to other typical polymers.[30]

### Functional principle and design

Liquid-filled glass ampoules are sealed into a microfluidic cartridge. For reagent release, the ampoules are disrupted by locally exerting mechanical force with a fingertip through the elastic lid of the cartridge. Released content is centrifugally displaced into the fluidic periphery through a filter structure inhibiting glass shivers from being forwarded into the fluidic system (Fig. 1).

Storage reservoirs holding 100 μL ampoules have dimensions of 16 mm × 5.1 mm × 5.1 mm. Filter structures consist of three quadratic (400 μm × 400 μm) microchannels connecting storage reservoir and fluidic system.

### DNA extraction cartridge

Genomic DNA is extracted from the blood of a healthy lab member within a fully integrated LoaC cartridge using components of a commercial DNA-extraction kit (QIAamp DNA Blood Kit Mini 250, Qiagen, Hilden, Germany). The extraction protocol given by the supplier has been previously transformed into microfluidic unit operations,[15] which are realized by a disk shaped microfluidic cartridge.[31] In this work, the existing cartridge design was complemented by embedding all required reagents into the modified disk. As depicted in Fig. 2, the self-containing cartridge comprises three glass ampoules, an inlet reservoir, a silica extraction matrix, and a liquid switching chamber guiding liquids—depending on the spinning direction—either to the waste reservoir or to the elution reservoir (purified DNA).[32] The glass ampoules contain 100 μL of buffers (2 times washing buffer, 1 time elution buffer) and are inserted into storage reservoirs, which are connected to the inlet reservoir *via* the microchannel filter structure for holding back glass shivers. For on-chip DNA extraction 32 μL of pre-lyzed blood (total volume including blood, lysis buffer, and ethanol: 96 μL) are inserted into the inlet reservoir of the fluidic cartridge. The inlet is then sealed by a self-adhesive foil and the rotational frequency protocol is started. After the lysate has seeped through the extraction matrix to bind the DNA, it is centrifugally driven radially outwards and deflected by the Coriolis force[33,34] into the waste reservoir. Then, the ampoules containing washing buffer are sequentially disrupted by finger-force while the disk is at rest. Upon consecutive rotation, the centrifugal force transfers released washing buffers through the extraction matrix washing away cell debris and proteins. These buffers are also deflected into the waste reservoir by the Coriolis force. As a last step, the ampoule holding the elution buffer is crushed and the spinning direction reversed. DNA bound to the extraction matrix is eluted and now deflected into the elution reservoir. The amount of DNA is measured using the DNA quantitation platform Quibit™ (Invitrogen GmbH, Karlsruhe, Germany).

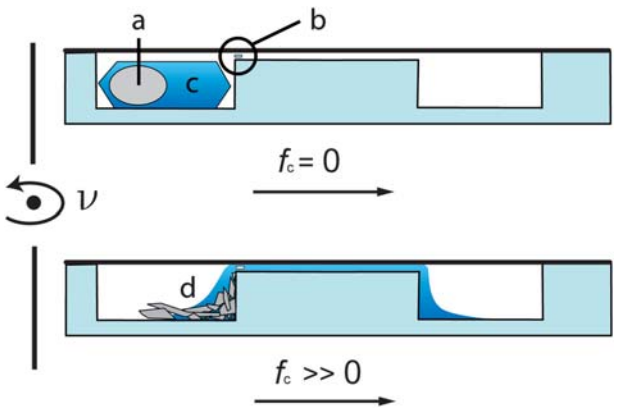


**Fig. 1** Schematics of storage and release within a centrifugally operated LoaC system: (a) enclosed air bubble, (b) filter structure consisting of 400 μM wide bars for retaining glass shivers, (c) glass ampoule, and (d) crushed glass ampoule.

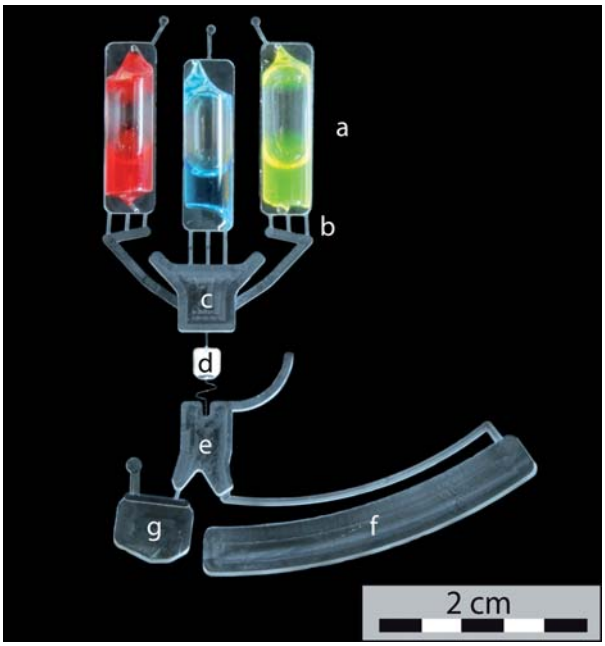


**Fig. 2** LoaC cartridge for on-chip DNA extraction featuring required buffers pre-stored in glass ampoules: (a) three glass ampoules containing buffers for DNA extraction (buffers are coloured for visualization purposes), (b) filter structures for retaining glass shivers, (c) inlet reservoir for introducing the sample, (d) DNA extraction matrix, (e) liquid switching chamber, (f) reservoir for waste, and (g) reservoir for the purified DNA.

### Fabrication of glass ampoules

The glass ampoules are fabricated from glass tubes (Borosilicate glass, Type 3.3, Hilgenberg, Malsfeld, Germany) of a length of 8 cm and an outer diameter of 5 mm featuring a wall thickness of 70 μm. A micro-bunsen burner (Hilgenberg, Malsfeld, Germany) is used for transforming glass tubes into liquid containing ampoules. The double-nozzle burner provides a thin blue flame (diameter ≈ 3 mm) with a maximum temperature of 1560 °C. For producing liquid-filled glass ampoules the centre of the glass tube is manually and slowly rotated in the flame until two vessels are obtained, which are closed at one side. Liquid samples are cooled down to the freezing point to minimize the impact of heat during the subsequent fusing process. Then, a volume of 100 μL is pipetted into the vessel and the upright liquid-containing vessel is brought into contact with the flame *circa* 8 mm above the liquid level. Upon shrinkage of the tube to about 50% of its diameter, the vessel is pulled out of the flame, cooled down to room temperature, and finally hermetically sealed by further heating. This repeated process of heating and cooling reduces the impact of the heat on the encapsulated liquid. The whole process is finished in about 30 seconds and results in ampoules of 15 mm ± 1 mm length holding 100 μL of liquid.

### Fabrication of microfluidic cartridge

Fluidic layouts are designed using the software tool AutoCAD® 2007 (Autodesk, München, Germany) and realized by a conventional CNC-milling machine (Mini-Mill 3 Pro, Minitech Machinery, Georgia, USA). The microfluidic channels are milled into TOPAS® COC 5013 disks (microfluidic ChipShop, Jena, Germany) with a diameter of 115 mm and a thickness of 6 mm. Liquid containing ampoules are inserted manually into a milled cartridge and sealed by a slightly elastic self-adhesive PCR film (AB-0558, Fisher Scientific GmbH, Schwerte, Germany).

## Results and discussion

The glass ampoule pre-storage concept is investigated as a candidate for liquid reagent storage on the centrifugal microfluidic cartridge. The centrifugal microfluidic platform relies on forces and pseudo-forces occurring in a rotating microfluidic substrate. Typical microfluidic unit operations of this platform are reviewed elsewhere.[15,35–37] Fluidic experiments are performed on a centrifugal test stand, which monitors liquid movement with a high-speed CCD camera.[38]

### Opening mechanism

Force for ampoule disruption is exerted through the flexible lid of the disk by a finger. The mean force for crushing a glass ampoule (70 μm wall thickness) is measured with a push–pull device (Z010, ZWICK/Roell, Ulm, Germany) to be 9.0 N (CV = 32%, $n = 5$). Upon crushing, the ampoules implode due to the negative pressure inside relative to atmospheric pressure contributing to an entire liquid release. This is owed to the encapsulation process where warm air is enclosed in the ampoules, which contracts upon cooling down. Shortly after disruption, the released liquids remain in the storage reservoir. Upon rotation, the centrifugal force transfers the released content *via* the filter-structure into the downstream microfluidic system. During this step, forces of ~500 × $g$ occur at the radial position of the filter-structure. In comparison to that, this foil requires forces higher than 2500 × $g$ for delamination. Liquids have been successfully released in more than 20 tests. Optical inspection of the released content confirmed the exclusion of particles and contact between the adhesive foil and the substrate. To underline this statement, we complemented another LoaC cartridge for performing a genetic assay with reagent pre-storage.[39] Also here, no interference with the optical readout system occurred in the measuring cavities.

Sealing has not been investigated yet for suitability to long-term storage. Nevertheless, we do not expect a degradation of the adhesive since this foil is routinely used for sealing microtiter plates. At current experiments we use another sealing technique developed at our department[40] with a bonding strength factor ~2 higher than the used foil.

### Released volume

The total recovered volume from crushed ampoules is measured after 120 s of rotation at $f = 70$ Hz to be 98.7 μL out of 100 μL initial volume (CV = 2.5%, $n = 6$). Fig. 3 shows a glass ampoule holding 100 μL coloured water (left) and the crushed capsule with the released and transferred content (right). Release of 100% pre-stored liquid volume was not possible due to a pinning of droplets in the μL range within the storage reservoir. This is a general property of sub-μL volumes within microchannels and not specific to this storage concept.

### Long-term storage

Glass ampoules containing 100 μL $H_2O$ or ethanol are periodically weighed using a microbalance (SC2, Sartorius, Göttingen,

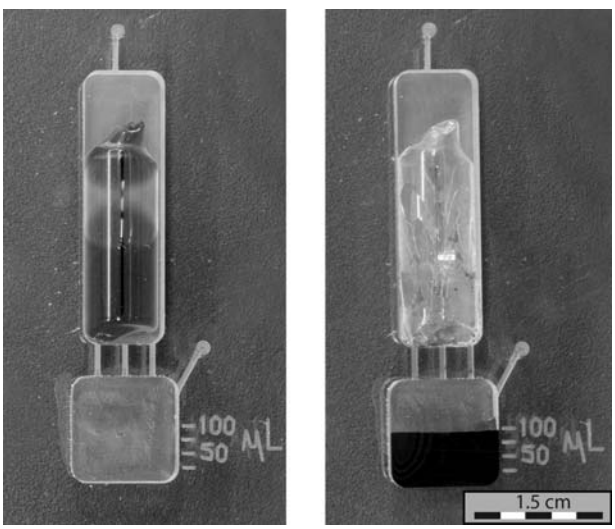


**Fig. 3** Storage and release structure on a centrifugally operated LoaC cartridge. Glass ampoule filled with 100 μL of black ink in a sealed storage reservoir (left). After exerting manual force through the flexible lid of the cartridge, released content is centrifugally transferred into the fluidic periphery. 98.7 μL out of 100 μL are transferred through thin microchannels, excluding shivers (right). Force for shivering is punctual exerted with a fingertip. Therefore, the upper part of the ampoule is still intact.


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**Table 1** Weight of $H_2O$ filled ampoules (mg)

| Time/days | Ampoule 1 | Ampoule 2 | Ampoule 3 | Ampoule 4 | Ampoule 5 | Error/mg |
|---|---|---|---|---|---|---|
| 0 | 163.32 | 140.74 | 177.57 | 156.31 | 173.66 | 0.01 |
| 70 | 163.33 | 140.75 | 177.57 | 156.31 | 173.65 | 0.01 |
| 140 | 163.31 | 140.72 | 177.56 | 156.30 | 173.63 | 0.01 |
| 210 | 163.33 | 140.73 | 177.57 | 156.33 | 173.64 | 0.01 |
| 300 | 163.34 | 140.75 | 177.57 | 156.31 | 173.65 | 0.01 |

**Table 2** Weight of ethanol filled ampoules (mg)

| Time/days | Ampoule 1 | Ampoule 2 | Ampoule 3 | Ampoule 4 | Ampoule 5 | Error/mg |
|---|---|---|---|---|---|---|
| 0 | 155.47 | 164.83 | 158.99 | 170.33 | 141.49 | 0.01 |
| 70 | 155.47 | 164.83 | 159.00 | 170.33 | 141.48 | 0.01 |
| 140 | 155.45 | 164.79 | 158.98 | 170.30 | 141.46 | 0.01 |
| 210 | 155.46 | 164.83 | 158.98 | 170.33 | 141.49 | 0.01 |
| 300 | 155.46 | 164.83 | 158.99 | 170.32 | 141.48 | 0.01 |

Germany) with an accuracy of 0.01 mg. Within this precision, >99.97% volume constancy for 10 fused ampoules (5 × $H_2O$, 5 × ethanol) is confirmed after 300 days for $H_2O$ (Table 1) and ethanol (Table 2). For some liquid reagents, storage conditions below the freezing point are required. To test the suitability of this concept for this requirement, ten ampoules containing 100 μL $H_2O$ have been kept at −20 °C for 60 days. No crushing of the ampoules occurred due to the volumetric expansion upon formation of ice crystals.

### DNA extraction

DNA from human blood was extracted with the microfluidic cartridge depicted in Fig. 2. The highest DNA yield (6.2 ng $\mu L^{-1}$ whole blood) obtained in six extractions was 77% compared to an off-disk reference extraction (8.0 ng $\mu L^{-1}$ whole blood) performed with the commercial DNA extraction kit. Ten capsules each of two washing buffers and one elution buffers from the DNA extraction kit have been encapsulated and have shown no detectable liquid loss after 140 days. The successful experiments demonstrate the suitability of this storage concept for performing LoaC-based assays.

## Conclusion

"*A major challenge for diagnostic applications is how to store wet […] chemicals in sealed reservoirs within the disposable package.*" (Huang *et al.*)[1]

This quote documents the relevance of liquid reagent storage concepts for mobile POC systems. This work presents a novel generic liquid storage concept useful for any LoaC cartridges. It is based on fusing liquid reagents into glass ampoules. We have shown that this concept features a defined and controlled liquid release and the outstanding possibility of long-term storage compared to state-of-the-art concepts.[27,28] DNA extraction in a fully integrated LoaC cartridge containing all required liquid buffers on-chip shows the applicability of our approach.

The leading advantages of our storage concept using glass as a material for encapsulation over others using *e.g.* polymers are the outstanding properties of glass, like moisture resistance and chemical inertness. Hermetic encapsulation of liquid reagents enables storage of liquids and lyophilized reagents on the same chip. Temperature sensitive substances can be gently encapsulated by introducing chilled or frozen reagents into the glass tubes to be fused. High-throughput processes for fabricating liquid-containing glass vessels are well established in the medical and pharmaceutical industry. Due to the price of ∼10 Eurocents per ampoule, they are well suited for disposable LoaC cartridges.

## Acknowledgements

We gratefully acknowledge financial support by the German Federal Ministry of Education and Research (project SONDE, grant number 13N10116).

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