Magnetic Liquid Marbles: Toward "Lab in a Droplet"

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Liquid marbles exhibit great potential for use as miniature labs for small-scale laboratory operations, such as experiment and measurement. While important progress has been made recently in exploring their applications as microreactions, "on-line" measurement of the components inside the liquid still remains a challenge. Herein, it is demonstrated that "on-line" detection can be realized on magnetic liquid marbles by taking advantage of their unique magnetic opening feature. By partially opening the particle shell, electrochemical measurement is carried out with a miniaturized three-electrode probe and the application of this technique for quantitative measurement of dopamine is demonstrated. Fully opened magnetic liquid marble makes it feasible to detect the optical absorbance of the liquid in a transmission mode. With this optical method, a glucose assay is demonstrated. Moreover, when magnetic particle shell contains low melting point material, e.g., wax, the liquid marble shows a unique encapsulation ability to form a rigid shell after heating, which facilitates the storage of the non-volatile ingredients. These unique features, together with the versatile use as microreactors, enable magnetic liquid marbles to function as a miniature lab (or called "lab in a droplet"), which may find applications in clinical diagnostics, biotechnology, chemical synthesis, and analytical chemistry.

1. Introduction

Droplet-based microfluidics, which transfers liquid in the form of individual droplets, shows superiority in manipulating small amount of samples and reagents when compared to microchannel-based fluidics.[1] Without the need for complex channel networks, pumps, tubing, or microvalves, droplet-based microfluidics offers several attractive features such as high flexibility, low cost, and versatility and reconfigurability for diverse applications. In droplet-based microfluidics, the droplet can be actuated by various forces, including electrowetting-on-dielectric (EWOD),[2] dielectrophoresis,[3] surface acoustic wave,[4] magnetic force,[5] and mechanical force.[6] The simplest droplet microfluidic is a droplet supported by a superhydrophobic surface. Despite the droplet in this way is easy to access, it is susceptible to liquid evaporation, and a humidified chamber may be required to overcome the evaporation issue.[1b,7] More commonly, droplet is immersed in immiscible fluid, e.g., silicone oil, to prevent evaporation and also reduce the force required for droplet actuation. Such oil-immersed systems, however, have drawbacks[1d] including the potential for the unwanted liquid-liquid extraction of analytes into the surrounding oil,[8] the inability of using oil-miscible liquids (e.g., organic solvents), and the inconvenience of integrating some on-chip detection or analysis techniques.[9]

Liquid marbles are droplets wrapped by a flexible particulate shell made of particles which are non-wettable to the liquid.[10] Recently, shaped solid blocks, which are millimeter-size and after soaking with water, coated with colloidal particles were also added to the category of "liquid marbles".[11] The particle shell functions to retain the liquid droplet in a nearly spherical shape, allowing the liquid marbles to sit stably on solid (or even liquid) surfaces without spreading, and it also helps to reduce droplet evaporation.[12] Liquid marbles behave like a soft solid and can be set in motion easily because of their extremely low adhesion to the substrate. These great features make liquid marble very promising for use as an alternative droplet-based microfluidic platform to bare droplet and oil-immersed systems. The main advantages of the liquid marble system include: 1) reduced droplet evaporation as compared to bare droplet systems; 2) less possibility of cross-contamination and more accessibility of the droplets than oil-immersed systems; 3) no need of complicated microelectrodes fabrication as compared to EWOD; and 4) low cost and convenience for rapid prototyping.

Apart from offering controlled liquid manipulation and transportation,[13] liquid marbles are promising for their potential use as a miniature lab, where small-scale laboratory operations, such as experiment and measurement, can be performed. Previously, liquid marbles have been proven to be a flexible microreactor functioning to incubate various chemical and biological processes,[14] including catalytic degradation of methylene blue,[14c] synthesis of graphene/Ag nanocomposite,[14d] cultivation of microorganisms,[14e] and production of cancer-cell spheroids[14f] and embryoid bodies from embryonic stem cell.[14g] The reaction processes carried out and the product produced in liquid marbles, however, have to be monitored and analyzed in "off-line" mode, in which fractions have to be collected and analyzed outside the liquid marble. In addition, liquid marbles have also been used as sensors for sensing gas[15] and organic contaminants in water.[16] These sensing systems, however, only showed qualitative sensing ability. "On-line" quantitative detection of analytes in liquid marbles has

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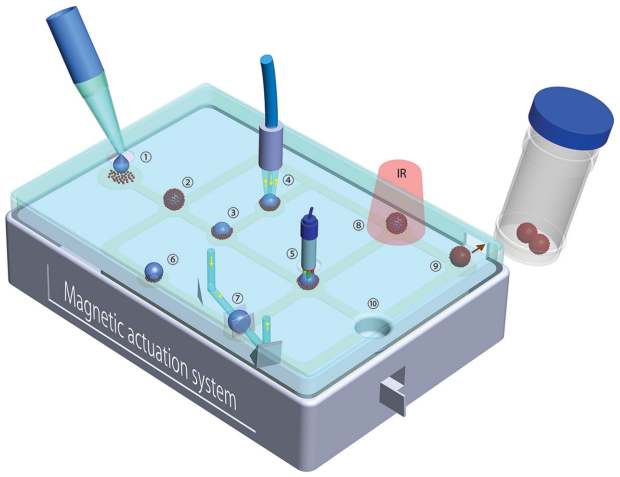

Figure 1. Schematic illustration of "on-line" detection and sample or reagent encapsulation based on magnetic liquid marbles: 1–2) liquid marble preparation; 3) magnetic opening; 4) reflection-mode optical detection; 5) electrochemical detection; 6) fully open liquid marble; 7) transmission-mode optical detection; 8–9) IR heat-induced encapsulation; and 10) disposal hole.

little been reported, until very recently, when Ling et al.[17] used plasmonic liquid marbles as a platform for quantitative detection of methylene blue and coumarin with surface-enhanced Raman spectroscopy (SERS).

The main difficulty for "on-line" quantitative detection of liquid marbles comes from the particle shell, which prevents detection probe from accessing the liquid droplet. In the previous work, we have proven that the particle shell can be opened and closed reversibly when the shell layer is made of magnetic particles (the liquid marbles are also referred to as "magnetic liquid marble").[13a] Depending on the intensity of the external magnetic field, magnetic liquid marbles can be opened partially, or even fully, allowing the liquid surface to be exposed to ambient environment on demand. This unique feature opens up opportunity of accessing liquid droplet for "on-line" detection of its components.

In this work, we demonstrate the "on-line" detection of liquid components in magnetic liquid marbles using electrochemical and optical approaches, which are the two most common detection techniques used in microfluidics. Based on these novel features, we further demonstrate that biological assays (e.g., glucose assay) can be performed in magnetic liquid marbles. In addition, we also show that the particle shell of magnetic liquid marbles can be hardened to preserve the samples/reagents, simply by introducing low melting point hydrophobic particles into the powder shell and a heat treatment. These demonstrations, as schematically illustrated in **Figure 1**, together with the versatile use of liquid marbles in the field of microreactors, will greatly promote the use of magnetic liquid marble as a new droplet-based microfluidics (or called "lab in a droplet").

2. Results and Discussion

Taking advantage of the magnetic opening feature of magnetic liquid marble,[13a] we prove the "on-line" detection of the liquid in the following sections. We first performed voltammetry by using a miniaturized three-electrode to probe dopamine in the liquid of a partially opened liquid marble. Such an electrochemical measurement is important to diagnose neurological disorders in brain functions. Subsequently, we demonstrated quantitative, optical absorbance detection on a fully opened liquid marble. With this optical method, a glucose assay was performed in liquid marble. Finally, we used wax-containing magnetic particles to prepare liquid marbles and hardened the particulate shell to encapsulate the reagent through a heat treatment.

2.1. Electrochemical Detection

On-line electrochemical detection has been widely used in other microfluidic systems because of the ease fabrication of electrodes, the high sensitivity, and the ability to perform both qualitative and quantitative test.[18] In this work, a three-microelectrode probe was prepared with an Ag/AgCl wire (203 μm diameter) and two platinum wires (254 μm diameter). The electrode tips can be immersed in the droplet of an open liquid marble for electrochemical measurement. As shown in **Figure 2**, a magnetic liquid marble containing dopamine solution (1.0 μM) was moved by a magnet bar positioned below the glass slide toward the three-microelectrode probe (images 1–3). Once it reached the target location, the magnet was moved upward to open the liquid marble (image 4). The three-electrode probe was then moved downward and immersed into the droplet for electrochemical measurement (images 5–6). After the electrochemical signal (image 7) was recorded, the probe was moved out of the droplet (image 8). The liquid marble was then closed by moving the magnet downward, and finally moved out of the measurement area (images 9–12).

For further measurement, cleaning of the three-electrode probe is crucial to avoid carry-over contamination. Herein, magnetic liquid marbles containing blank PBS solution (0.1 M PBS, pH 7.2) were used to clean the electrodes after each detection. The procedure for transferring blank PBS liquid marble and probe immersion was the same as the electrochemical detection. **Figure 3**a shows the square-wave voltammograms for successive measurements performed on liquid marbles of dopamine sample (0.5 μM in PBS), blank PBS (1st) and blank PBS (2nd). For the first blank PBS liquid marble, the oxidation current of dopamine still existed, while for the second one, the oxidation current completely disappeared. This result suggests that the probe can be completely cleaned using two blank PBS magnetic liquid marbles. The square-wave voltametric responses for three measurement cycles, as shown in Figure 3a, indicate the high reproducibility of this washing process.

To study the influence of liquid marble size on electrochemical signals, magnetic liquid marbles of different sizes (10–40 μL) were used for the square-wave voltammograms. It was found that the peak current was almost unchanged with the liquid marble size (Figure S1, Supporting Information). Here we should point out that magnetic liquid marbles of smaller size, e.g. as small as 350 nL, can be prepared (Figure S2, Supporting Information). Although the current three-electrode system could be too large to be immersed into such a small droplet, it would

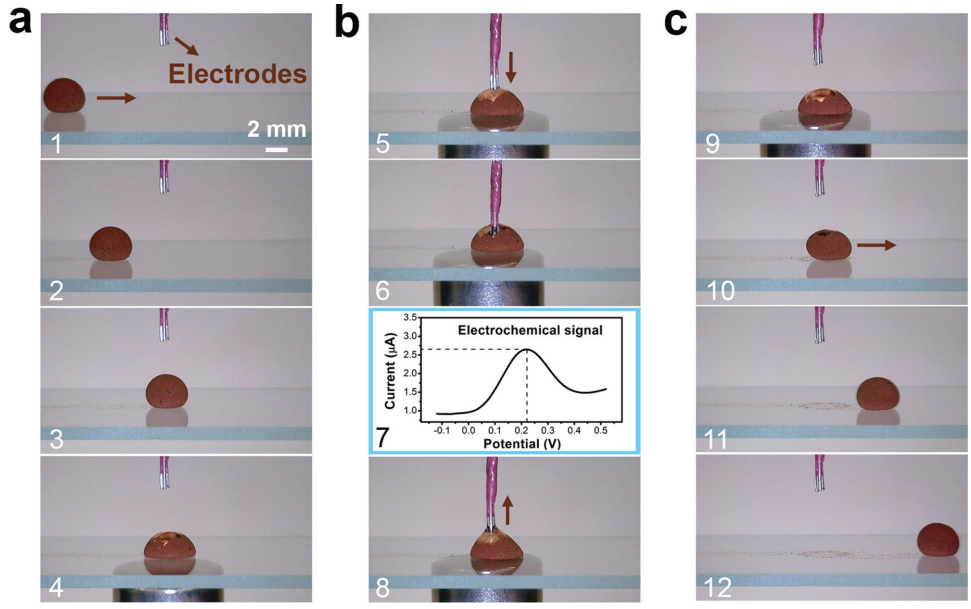

Figure 2. Electrochemical detection. a) Transport (1–3) of a magnetic liquid marble on glass substrate and its opening (4) with a magnet (10 mm diameter × 12 mm length). b) Moving three-electrode probe into the droplet (5–6), recording signal (7), and moving probe out of the droplet (8). c) Closing (9–10) and moving away (11–12) liquid marble after detection.

be feasible when the electrode size is further reduced. In the following, 20 μL liquid marbles were used for quantitative detection of dopamine. It should also note that electrochemical test often takes a few seconds. In such a short time, solvent evaporation from the open surface of a liquid marble is very small. Therefore solvent evaporation has a negligible effect on the concentration of analyte and electrochemical detection of liquid marbles.

As shown in Figure 3b, well-defined peaks were obtained for the oxidation of dopamine with a concentration range of 0–1.0 μM. The peak current was linearly proportional to the dopamine concentration. Figure 3c shows the calibration plot of current as a function of the concentration. The correlation coefficient was 0.997. Such a linear relationship indicates the capability to conduct reliable quantitative electrochemical measurements on magnetic liquid marbles. The limit of detection (LOD) was calculated as 39.8 nM, which is comparable to some reported LOD values of electrochemical dopamine detection, and it is also acceptable for the determination of dopamine in real samples of human serum.[19]

2.2. Transmission-Mode Optical Absorbance Detection

Optical absorbance spectroscopy is another technique commonly used for microfluidic on-line analysis, as it offers many benefits including nondestructive operation, fast response, and capability for multiple sensing.[1c] Although partial opening of a magnetic liquid marble allows the optical detection of the inside liquid in a reflection mode,[20] optical transmission measurement of the droplet has been impeded by the surrounding particles. As shown in **Figure 4**b, the light beam irradiated on a liquid marble was scattered by the surface particles (image 1) and as a result the transmission was zero across the entire wavelength range.

To conduct transmission-mode detection, we tried to fully open the magnetic liquid marble. As shown in Figure 4a, when the magnet positioned below the glass substrate approached the liquid marble very closely, the surrounding particles moved toward the substrate, enabling the exposure of the droplet. It should be noted that even when the liquid marble was fully opened, the droplet can still be moved around (Movie S1, Supporting Information), by magnetically controlling the particles aggregating between the droplet and the substrate. The substrate used to support the liquid marble was a cover glass with a thickness of 0.13–0.16 mm. To prevent the droplet from spreading, the glass surface was hydrophobilized. The intensity of the magnetic field at the lower surface of the cover glass was measured to be 0.04 T.

When the light beam irradiated on the droplet of an open liquid marble (Figure 4b, image 2), however, the transmission was still zero across the entire wavelength range. The reason is that there exist both reflection and refraction at the curved air–water interface. The light direction is totally changed by refracting on the outer surface of the droplet, and then reflecting on the inner surface, followed by exiting the droplet with refraction as well.

To realize transmission-mode detection, the path route of light beam was controlled by flattening the droplet with two hydrophobic glass slides (Figure 4b, image 3). Figure 4c schematically illustrates the setup, where the light beam strikes the contact area of the glass slide with the droplet at normal incidence, pass through the droplet, and reach the read probe for detection. With this method, the characteristic absorption of acid blue 25 at 615 nm was successfully detected for a magnetic liquid marble containing 8 mM aqueous solution of acid blue 25 (Figure 4b). Besides qualitative test, this transmission-mode method is able to provide high-precision quantitative measurement.

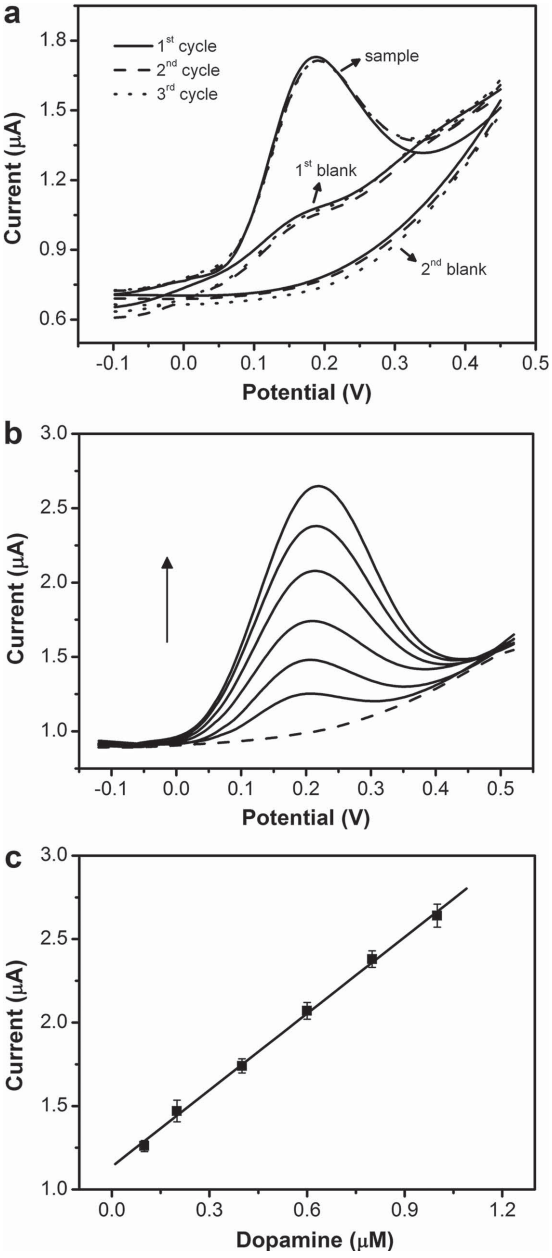

Figure 3. Dopamine detection. a) Square-wave voltammograms for successive measurements performed on liquid marbles of dopamine sample (0.5 μM), blank (1st) and blank (2nd), showing that the electrodes were completely cleaned using two blank liquid marbles. Three measurement cycles were conducted to demonstrate the reproducibility. b) Square-wave voltammograms of liquid marbles containing different concentrations of dopamine (ascending along *y*-axis): 0, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 μM. c) Calibration plot of current as a function of the dopamine concentration. Solid black squares are mean values; error bars represent the standard deviation; the solid line represents a linear fit with regression equation: $y = 1.1 + 1.5x$ ($R^2 = 0.997$, $n = 6$).

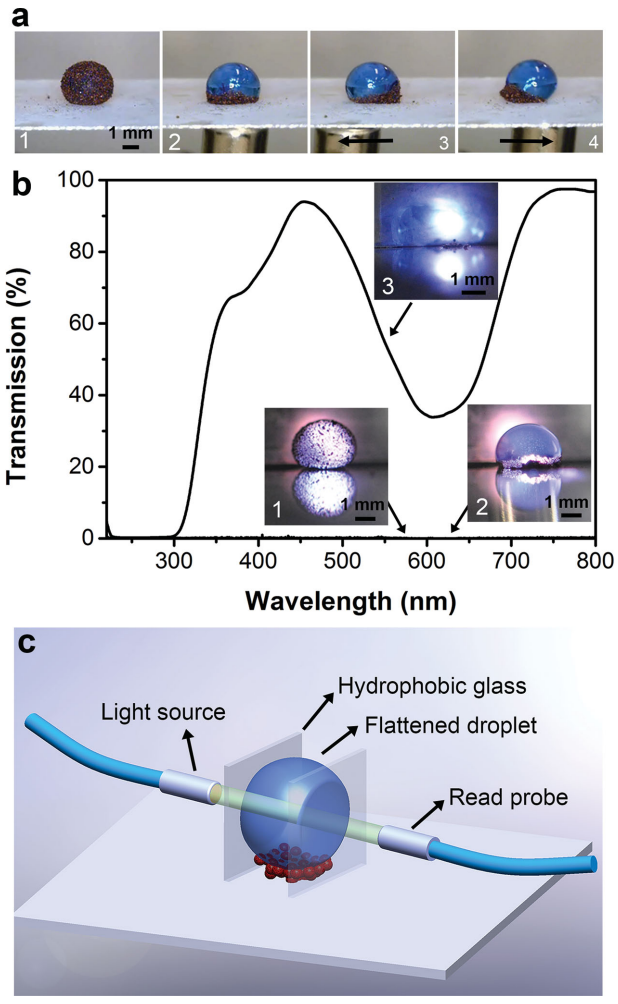

Figure 4. Optical absorbance detection. a) Fully opening of a magnetic liquid marble and its moving around with a magnet (4 mm diameter × 20 mm length) (also see Movie S1, Supporting Information). b) Transmission spectra of a liquid marble (1), the droplet of an open liquid marble (2), and the squashed droplet of an open liquid marble between two parallel hydrophobic glass slides (3). Droplet is 8 mM aqueous solution of acid blue 25. Insets show the photo of light beam irradiation on each object. c) Schematic illustration of the transmission detection of a flattened droplet.

As a demonstration, a glucose assay was performed in the magnetic liquid marble. To ensure the same path length for each measurement, a spacer with a thickness of 1.5 mm was placed between the two glass slides. The glucose assay is based on a colorimetric enzyme-kinetic method, where the reaction between glucose and the glucose detection reagent results in the formation of a violet colored quinoneimine, which has an absorbance peak at 545 nm. The formation rate of the colored quinoneimine is dependent on the glucose concentration. **Figure 5**a shows the absorbance at 545 nm as a function of time for various glucose concentrations from 1 to 10 mM. Time $t = 0$ in the graph corresponds to the time instant of 15 s after mixing the sample and the detection reagent. The slope of these curves obtained by linear fit corresponds to the formation rate of the colored quinoneimine. Figure 5b plots the formation rate as a function of the glucose concentration. As indicated by the solid line, the plot has an excellent linearity in the range of 0–6 mM. The correlation coefficient was 0.9988. The linear range of this assay is comparable to that reported for colorimetric glucose assay (0–5 mM).[21]

It is known that the level of glucose in normal body is 3.5–5.3 mM, and the conventional LOD is 0.5 mM with

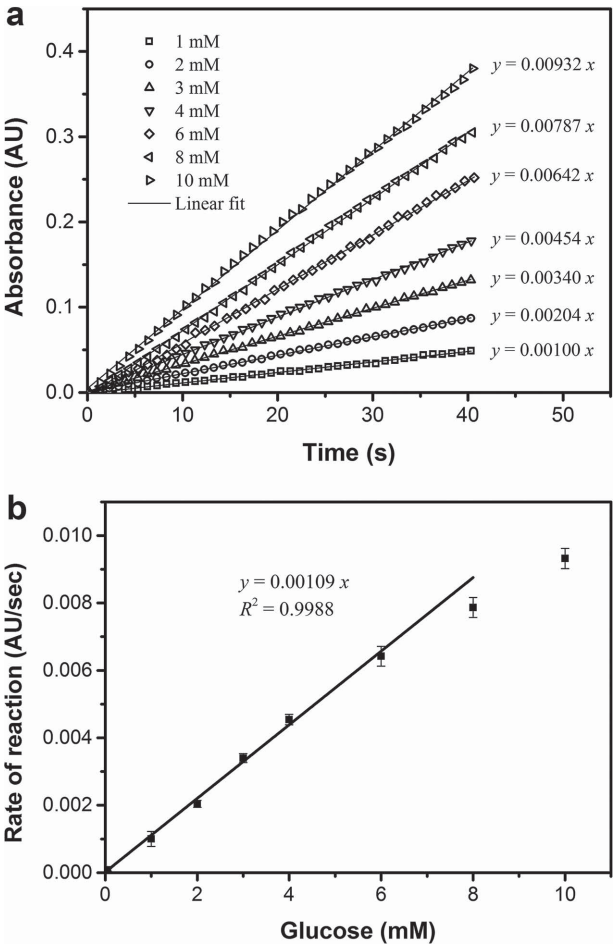

Figure 5. Glucose assay. a) Optical absorption at 545 nm as a function of time for glucose concentrations from 1 mM to 10 mM. Solid lines are linear fits to the data. b) Calibration plot of reaction rate as a function of glucose concentration. Solid black squares are mean values; error bars represent the standard deviation; the solid line shows the linear region (0–6 mM).

colorimetric methods.[21,22] The linear range of our liquid marble system spans the glucose levels of normal body (3.5–5.3 mM) and hypoglycemia (below 3.5 mM). The LOD is as low as 0.162 mM. When glucose level is higher than 6 mM, the data point deviated from linearity by leveling off, but it may be possible to detect higher glucose levels in diabetics by diluting the sample.

It should be mentioned that although the optical and electrochemical detection technologies used in this work are based on bench-top laboratory equipment including electrochemical analyzer, light source and spectrophotometer, these external equipment could be miniaturized and integrated based on previous studies,[1c,23] which have realized the miniaturization and integration of the detection equipment on other types of droplet-based microfluidic devices.

2.3. Sample/Reagent Encapsulation

As a new droplet-based microfluidic platform, magnetic liquid marble possesses unique feature to encapsulate sample/reagent after chemical reaction or analysis by hardening the particulate shell. This feature can find applications in some biochemical assays, where it is required to preserve the samples after analysis, for instance, for follow-up assessment or biobanking and archiving. This encapsulation feature is not achievable by bare droplet or oil-immersed systems. To demonstrate the encapsulating ability, wax-based particles were used to prepare liquid marbles. The particles surrounding the droplet can be bound together by melting and then solidifying (via cooling) the wax. Wax is known to be chemically inert and has been used for microfluidic valves[24] and reagent storage[25] because of its easy handling. After being melted, wax can self-organize to form a thin uniform film over aqueous solutions, thus keeping separate from the reagent of interest and allowing the reagent for further use.

When a liquid marble prepared with wax/Fe_3O_4 nanoparticle powder was placed on glass substrate, nevertheless, we found that the wax/Fe_3O_4 liquid marble collapsed (**Figure 6**a, image 3) when it was heated by infrared radiation (halogen

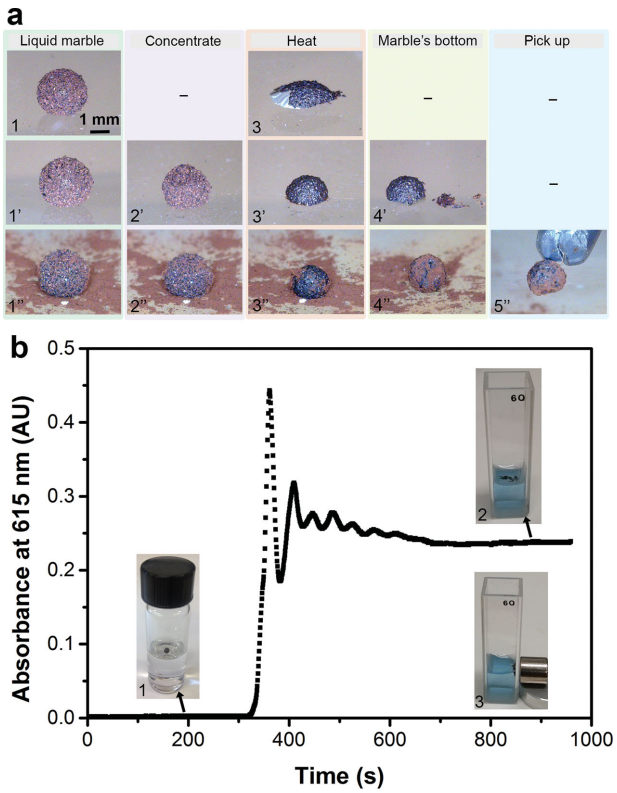

Figure 6. Reagent storage and recovery. a) Liquid marble (8 μL) prepared with a fine wax/Fe_3O_4 nanoparticle powder (1:1.8, w/w) and its morphology after concentration and heating: (1, 3) on glass substrate without concentration; (1′–3′) on glass substrate; and (1″–3″) on a bed of highly hydrophobic Fe_3O_4 nanoparticles. 4′ and 4″ show the adhesion with glass substrate and Fe_3O_4 nanoparticles, respectively. Image 5″ shows the picking up of fused liquid marble through lifting a microfiber with tweezers (the fiber was fixed on marble surface during wax melting). b) Optical absorbance at 615 nm as a function of time during the reagent recovery process by heating the fused wax/Fe_3O_4 shell floating on water surface. Inset 1: floating of the fused shell on water surface. Inset 2: retrieval of the encapsulated reagent (acid blue 25). Inset 3: collection of the collapsed wax/Fe_3O_4 shell.

lamp, 500 W). The collapse can be ascribed to both the decrease in the particle size and the increase in the inter particle distance due to wax melting.[15c] To make the particles more compact (i.e., decrease the inter particle distance), the liquid marble was left in ambient environment for 20 minutes for droplet evaporation. This process led to the sample concentration and the decrease in droplet size from 8 μL to 5 μL (images 1′ and 2′). The packing of particles at the water/air interface were observed using an optical microscope. After concentration, the inter particle distance decreased (Figure S3, Supporting Information). While the concentration process effectively prevented the liquid marble from collapsing during heating (image 3′), the wax/Fe_3O_4 shell was found to stick to the glass substrate due to the adhesion between the melted wax and the glass (image 4′).

To prevent the wax from sticking on the glass substrate, a bed of highly hydrophobic Fe_3O_4 nanoparticles was used (images 1″–3″). In this way, the wax/Fe_3O_4 shell could be easily lifted off the substrate although some Fe_3O_4 powder was observed to adhere on the bottom of the shell (image 4″). More interestingly, a microfiber could be fixed on marble surface during wax melting, and the wax/Fe_3O_4 shell can be lift through the fiber (image 5″), which seems to be impossible for ordinary liquid marbles. Herein, the way of using wax-based particles for formation of a rigid shell is novel. The binding agent, wax, participated in the liquid marble formation as part of the hydrophobic particles, which offers simpler and more cost efficient solution when compared to chemical vapor deposition technique[26] and superglue method[27] reported by other researchers.

The wax-based sample/reagent encapsulation method was further evaluated by adding a dye (acid blue 25) to the liquid. After fusing the wax/Fe_3O_4 shell, the hardened liquid marble could float on water surface. By heating the hardened liquid marble to a temperature above the melting point of the wax, the dye can be released. Figure 6b shows the optical absorbance at 615 nm (characteristic absorbance peak of acid blue 25) as a function of time during the recovery process. Before heating, the absorbance kept nearly zero, indicating that the fused wax/Fe_3O_4 shell was impermeable to water and there was no reagent leakage. After heating, the shell melted and the encapsulated dye was released. Correspondingly, the absorbance at 615 nm increased dramatically. The fluctuation of absorbance in Figure 6b was contributed to the unevenness of the solution concentration when the dye freely diffused. The recovery rate was calculated to be 93.5 ± 2.7% based on the optical absorption measurement (Figure S4, Supporting Information). This dye encapsulation and release process had a successful rate of 85% (17 out of 20). The failure cases were due to the buckling of liquid marble during evaporation, which can be improved by precisely controlling solvent evaporation. In addition, the collapsed wax/Fe_3O_4 shell and any loose magnetic particles can be collected with magnetic force (Figure 6b, inset 3), thus avoiding any interference on the reagent performance during further use.

Although the fused particle shell maintained its impermeable feature to liquid water after heating treatment, it was still permeable to air due to the presence of small voids in the shell (Figure S3 in Supporting Information). When stored in air, the liquid in the droplet become condensed and final dry, leaving the solid reagents within the shell. Also, the rigid liquid marble could be stored in low temperature condition (e.g., into dry ice or liquid nitrogen) before drying. Sealing the hardened liquid marbles in airtight vial is also an option of slowing down the solvent evaporation.

Since the encapsulation and storage involves heat and droplet evaporation, the storage may be less suitable for the chemicals which are sensitive to heat or salt concentration (e.g., enzymes). However, such a feature can find applications for encapsulating heat-resistant reagents, for instance, reagent pre-storage and on-time release for polymerase chain reaction (PCR),[25] where the DNA polymerase is thermally stable and the reagents can be released during the initial denaturation step.

3. Conclusion

We have demonstrated the new features of magnetic liquid marbles: "on-line" electrochemical/optical detection and sample storage. These sample detection and storage techniques together with the unique actuation and microreactor characteristics allow the development of liquid marble-based lab-in-a-droplet devices integrating automatic sample preparation, actuation, analysis, and sample packing/storage functions, which will open up the unique applications of magnetic liquid marbles especially in clinical diagnostics, biotechnology, chemical synthesis and analytical chemistry.

4. Experimental Section

Preparation of Highly Hydrophobic Fe_3O_4 Nanoparticles: Highly hydrophobic Fe_3O_4 nanoparticles were synthesized as described previously.[13a] Typically, 1.5 M aqueous NH_4OH solution was added dropwise to 200 ml water/ethanol solution (4:1, v/v) containing $FeCl_3{\cdot}6H_2O$ (0.85 g, 3.14 mmol), $FeCl_2{\cdot}4H_2O$ (0.30 g, 1.51 mmol) and tridecafluorooctyltriethoxysilane (0.20 mL, 5.23 mmol) under nitrogen protection and vigorous stirring until pH 8. After stirring for 24 h, the resulting precipitate was isolated from solution with a bar magnet, washed with water/ethanol mixture for three times and dried at 60 °C.

Preparation of Wax/Fe_3O_4 Powder: Paraffin wax (T_m = 53–57 °C) was used to prepare wax/Fe_3O_4 powder. Briefly, a mixture of wax and highly hydrophobic Fe_3O_4 nanoparticles (1:1.8, w/w) was heated in an oven at temperature above the wax melting point for 10 minutes. The mixture was naturally cooled down to room temperature under constant stirring, and then moved into a mortar. To enable the effective grinding, the wax and the mortar were cooled with liquid nitrogen. After grinding, a fine wax/Fe_3O_4 powder was obtained.

Formation and Manipulation of Magnetic Liquid Marbles: The obtained fine Fe_3O_4 or wax/Fe_3O_4 powder was placed evenly over the surface of a watch glass and a droplet of the analyte solution dispensed from the pipette tip was deposited onto the powder. As the droplet rolled around, the particles spontaneously attached onto the droplet surface owing to the tendency to minimize the surface free energy, thus forming a liquid marble. The as-obtained magnetic liquid marble was placed on a glass substrate. The movement of the liquid marble toward the target spot and its opening was realized by manually moving a permanent neodymium cylinder magnet positioned below the glass substrate. The magnetic field intensity was tested by a PASCO magnetic field sensor PS-2162.

Electrochemical Apparatus: Electrochemical measurement was carried out using a CHI 700D electrochemical analyzer. A three-microelectrode system was employed, with platinum wires (254 μm diameter, A-M Systems, Inc.) as working and counter electrodes and Ag/AgCl wire (203 μm diameter, A-M Systems, Inc.) as reference electrode. The electrode preparation was based on the previous report.[28] Briefly, each

electrode wire was insulated with a plastic tubing, and then the insulated three electrode wires were joined together with an additional plastic tubing. At the end of the electrode wires, nail polish was used to fix the position of the electrode wires, with 3 mm of bare wires being exposed.

Dopamine Detection: Electrochemical detection of dopamine was carried out using square-wave voltammetry, with a potential step of 4 mV, an amplitude of 25 mV, a frequency of 15 Hz, and a quiet time of 2 s. Dopamine solutions were prepared using 0.1 M phosphate buffer solution (PBS, pH 7.2) as the supporting electrolyte.

Optical Apparatus: For optical detection, two optical fibers (400 μm diameter) were used. One illuminates, and the other collects and transfers the signal to a USB4000 spectrometer. The light source was a DH2000-BAL device that combines deuterium and tungsten halogen lamps for an output in the wavelength range of 230–2000 nm. All these devices were purchased from Ocean Optics (Dunedin, FL). To control the path route of light beam, two hydrophobic glass slides were used to flatten the droplet of a fully open liquid marble. Briefly, a hydrophobic glass slide was fixed in front of the illuminating optical fiber. After the liquid marble was positioned and fully opened, the liquid drop was sandwiched between optical fiber which had a hydrophobic glass cover and another hydrophobic glass slide. Flat liquid surfaces were formed by gently compressing the liquid drop with the two parallel hydrophobic glass slides. In this way, the optical absorbance of the opened liquid marble can be measured. To ensure the same path length for each measurement, a spacer (thickness 1.5 mm) was placed between the two glass slides.

Glucose Assay: A colorimetric enzyme-kinetic method was used for the determination of glucose concentration based on previous report.[29] Briefly, reference glucose solutions of different concentrations were prepared by dissolving glucose in 10 mM PBS (pH 7.4). The constitution of the glucose detection reagent was: 4.5 U mL^{-1} glucose oxidase, 4.5 U mL^{-1} peroxidase, 4.5 mM 4-aminoantipyrine, and 7.5 mM *N*-ethyl-*N*-sulfopropyl-*m*-toluidine in 10 mM PBS (pH 7.4). Glucose and all the reagents were purchased from Sigma. For glucose assay, a liquid marble containing 12 μL reagent was put on a hydrophobic glass slide, moved to the front of the illuminating optical fiber, and then partially opened with a magnet positioned below the glass slide. To this open liquid marble, 6 μL glucose sample was added with pipette. The liquid marble was then moved around for mixing by manually moving the magnet. After 15 s, the well-mixed liquid marble was fully opened and the exposed droplet was flattened by two hydrophobic glass slides for optical absorbance measurement. The absorbance at 545 nm was recorded with time for at least 40 s. The rate of increase of absorbance at 545 nm was then plotted against the glucose concentration.

Limit of Detection (LOD): The LOD was estimated by measuring the response of a blank sample, and determined as the mean result of the blank sample plus three times the standard deviation (SD) (mean + 3 × SD). The detection for each concentration of test solutions was performed in triplicate. In glucose assay, glucose solution with a very low concentration of 0.05 mM was used for LOD calculation.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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