Video Article

Aqueous Droplets Used as Enzymatic Microreactors and Their Electromagnetic Actuation

Huschyar Al-Kaidy[1], Kai Kuthan[1], Thomas Hering[1], Nils Tippkötter[1,2]

[1]Institute of Bioprocess Engineering, University of Kaiserslautern

[2]Bioprocess Engineering, University of Applied Sciences, FH Aachen

Correspondence to: Nils Tippkötter at tippkoetter@fh-aachen.de

URL: https://www.jove.com/video/54643
DOI: doi:10.3791/54643

Keywords: Bioengineering, Issue 126, Microfluidics, lab-in-a-drop, magnetic, superhydrophobic, nanoparticles, liquid marbles, actuation platform, enzymatic microreactions

Date Published: 8/28/2017

Citation: Al-Kaidy, H., Kuthan, K., Hering, T., Tippkötter, N. Aqueous Droplets Used as Enzymatic Microreactors and Their Electromagnetic Actuation. *J. Vis. Exp.* (126), e54643, doi:10.3791/54643 (2017).

Abstract

For the successful implementation of microfluidic reaction systems, such as PCR and electrophoresis, the movement of small liquid volumes is essential. In conventional lab-on-a-chip-platforms, solvents and samples are passed through defined microfluidic channels with complex flow control installations. The droplet actuation platform presented here is a promising alternative. With it, it is possible to move a liquid drop (microreactor) on a planar surface of a reaction platform (lab-in-a-drop). The actuation of microreactors on the hydrophobic surface of the platform is based on the use of magnetic forces acting on the outer shell of the liquid drops which is made of a thin layer of superhydrophobic magnetite particles. The hydrophobic surface of the platform is needed to avoid any contact between the liquid core and the surface to allow a smooth movement of the microreactor. On the platform, one or more microreactors with volumes of 10 µL can be positioned and moved simultaneously. The platform itself consists of a 3 x 3 matrix of electrical double coils which accommodate either neodymium or iron cores. The magnetic field gradients are automatically controlled. By variation of the magnetic field gradients, the microreactors' magnetic hydrophobic shell can be manipulated automatically to move the microreactor or open the shell reversibly. Reactions of substrates and corresponding enzymes can be initiated by merging the microreactors or bringing them into contact with surface immobilized catalysts.

Video Link

The video component of this article can be found at https://www.jove.com/video/54643/

Introduction

Technical applications with micro reactions are predominantly carried out in predefined microchannel chips. These systems are widely established and comprehensively described in the literature (inter alia [1,2,3]). In 2011, the turnover of microfluidic technologies worldwide totaled 6.2 billion euro [4]. In contrast, the use of freely movable micro reactor compartments was previously only examined and published to a limited extent. The most common method for moving aqueous micro droplets is electrowetting [5]. Other methods for the motion of drops on surfaces are based on electric fields [6], magnetic force [7] or acoustic actuation [8]. Due to their unfavorable surface to volume ratio, these droplet-based microreactor systems are exposed to strong evaporation effects. Thus, the drop motion is usually established as a liquid two-phase system, where the upper phase has a high boiling point protecting the aqueous phase from evaporation. Nevertheless, this approach involves a high risk of contaminating the reaction droplet by uncontrolled diffusion. This is a significant obstacle for the technical establishment of the mentioned systems.

Recent work is concerned with non-adherent liquid-solid phase transitions. A highly effective approach is the use of superhydrophobic surfaces, allowing the formation of spherical aqueous droplets. An extension of this reaction concept is the use of micro reaction compartments with a superhydrophobic surface or shell, which may for example consist of polytetrafluoroethylene (PTFE) particles [9]. Their contact angles on surfaces are usually in the range of 160° (depending on the surface roughness). The spherical compartments thus provide minimal resistance to movement on a surface and simultaneously provide protection against water evaporation.

Aqueous drops coated with micro sized PTFE particles may maintain their spherical shape up to a diameter of around 2 mm. At higher volumes, the hydrophobic shell is usually not completely closed anymore [10]. The influence of other shell materials and the expansion of the field of application of the liquid marble to nonpolar solvents was implemented by Gao and McCarthy by using ionic liquids [12]. For the formation of hydrophobic particle-based shells, so far particle diameters in sizes of 10 nm- 30 µm have been described [11,14,16]. New studies showed that hydrophobic nanoparticles as shell material are of even better use than that of microparticles [13]. First stability studies confirmed an increase in stability when the particle size is reduced from *ca.* 600 nm to *ca.* 100 nm. This likely results from the denser particle distribution around the aqueous sphere [15].

The protection of aqueous reaction compartments by a hydrophobic shell and their designation as liquid marbles was first described in 2001 by Aussillous *et al.* and Mahadevan *et al.* [17,18]. Since then, few applications of these defined reaction compartments have been described.

For example, a gas sensor based on liquid marbles [19] and a detection method for water contamination based on an optically qualitative basis have been developed [20]. The authors distinguish the advantages of high reaction rates and the low consumption of chemicals of their micro reaction systems. Recent publications deal with the production of pH-sensitive liquid marbles [16] or the representation of 'Janus particles' with two different coatings of different functionality. For example, Bormashenko *et al.* could synthesize a microreactor with shells made of Teflon and semiconducting Carbon Black [21]. Furthermore it was demonstrated that microreactors can efficiently and convenient synthesize polyperoxides by absorbing external oxygen as comonomer through the permeable gas-liquid interface [24]. In another approach the shell of silica-particle-based liquid marbles provide the reactive substrate surfaces to regulate the classical silver mirror reaction [26]. Current problems for research and development in the field of hydrophilic-core-hydrophobic-shell droplets are the particle size adjustment, the reproducible production of monodisperse droplets, the wettability of surfaces and the effect of a second hydrophilic shell on the micro reaction compartments [22], as well as a better control of the droplet trajectories, *e.g.* for the development of continuous microPCR-systems [4].

A magnetic actuation of these microreactors offers the advantage of relatively high movement ranges and a good selectivity of the force when working in biochemical systems. When using hydrophobic magnetite particles, they fulfil both the function of the magnetic force transmission to the movement of the microreactors, as well as the function of a hydrophobic shell. The magnetic movement of droplets with magnetic particles inside a droplet was postulated for the first time in 2006 by Lehmann *et al.* [23] and Shikida *et al.* [25], who used manually moved permanent magnets as actuators for the mobilization of a single droplet. Another approach to move a small amount of liquid was realized by Zhao *et al.*, who used the hydrophobic Fe_3O_4 particles as magnetic shell. The shell of the magnetic liquid marble was opened on the upper side of the drop by a vertical reverse magnetic field [27]. Based on this concept, Xue *et al.* were able to develop particles which form a microreactor with a surface tension of 20.1 dyne cm^{-1} [28]. Lin *et al.* fabricated novel cellulose-based micro/nano hierarchical spheres with both superparamagnetism and superhydrophobicity which provide god stability for magnetic liquid droplet transportation and manipulation [31].This was so far only released as a proof-of-principle study and not used for any application. The magnetic and electrical control of the liquid marbles is currently pursued in first approaches. Zhao *et al.* in 2010 [15] and Zhang *et al.* 2012 [29] were able to develop a droplet manipulation by the manual (hand-operated) movement of a permanent magnet beneath core-shell droplets. Bormashenko *et al.* [11] achieved the acceleration of a ferromagnetic liquid marble to a speed of 25 cm s^{-1} by approaching a neodymium magnet. The above mentioned principle studies were carried out exclusively by the manual movement of a small permanent magnet. As a next development step, Zhao *et al.* were recently able to estimate the required magnetic flux density for the movement of magnetic liquid marble by varying the distance of a permanent magnet [30]. For a reaction control comparable to that of common lab-on-a-chip systems, it seems inevitable to provide the means of automated control of the discrete liquid volumes. To satisfy this need, we developed a new control system based on variable field gradients to fixate, move and open the magnetic microreactors.

Protocol

1. Hydrophobization of Magnetic Nanoparticles

1. For the synthesis of the hydrophobic magnetic particles, add 0.85 g $FeCl_3$ hexahydrate (3.14 mmol) and 0.30 g $FeCl_2$ tetrahydrate (1.51 mmol) to 200 mL water/ethanol solution (4:1 v/v).
2. To this mixture, add 0.20 mL 1H,1H,2H,2H-Perfluorooctyltriethoxysilane (PFOTES) (5.23 mmol) with vigorous stirring by a magnetic stirrer (500 rpm). Carry out the synthesis in an inert gas atmosphere (N_2) by using a round-bottom flask with a cork in order to prevent the secondary oxidation of the magnetite particles.
3. Adjust the solution drop wise with an ammonium hydroxide solution (1.5 M) to a pH of 8 (pH determined by a pH meter). Stir the solution for 24 h by using a magnetic stirrer.
4. Separate the particles magnetically from the solution by placing the flask on a bar magnet (neodymium cuboid magnet 40 x 20 x 10 mm, adhesive force 25 kg). Pour out the solution while keeping the magnet attached to the bottom of the flask.
5. Wash the particles three times with the water/ethanol solution while using a bar magnet as described in 1.4). Dry the particles at 60 °C for 24 h (yield *ca.* 0.43 g).
6. For analyzing the particles, use a Scanning Electron Microscope according to manufacturer's instructions.

2. Fabrication of Microreactors

1. Grind all the dried particles slightly using a glass pestle and subsequently place all of them directly in a weighing pan (46 × 46 × 8 mm, polystyrene).
2. Pipette 10 µL reaction solution (composition as described in 5.1) onto all the particles and move the weighing pan slightly in a circular way for approximately 10 s (particle mass for a 10 µL microreactor: *ca.* 3.2 x 10^{-7} kg [33]). Store the remaining particles (particles which did not self-assemble around the reaction solution) at room temperature for further applications.
3. For measuring the contact angle of the microreactor, build a 5 µL microreactor with water as described in 2.2), place it on a teflon film and analyze the contact angle by using an optical contact angle measurement device according to manufacturer's instructions.

3. 3D-printing of the Coil Bodies

1. Design the double coil bodies with a height of 16 mm (one chamber), a diameter of 10 mm and an inner diameter of *ca.* 4 mm by using a CAD software according to manufacturer's instructions.
2. Print the coil bodies with a 3D printer according to the manufacturer's instructions by using materials such as polylactide filament. Wrap the bodies with a 0.08 mm copper wire to achieve 4,500 windings by using a computer controlled winding machine.

4. Fabrication of the Actuation Platform

1. Arrange the double coils in a matrix (*e.g.* 3 x 3 matrix) on an electric board with a Peltier element underneath, screw on the double coils and connect them to a control via ribbon cable (**Figure 3**).
2. Depending on the desired application, add an iron core (32 mm height, 4 mm diameter) or a neodymium magnet (12.5 mm height, 4 mm diameter, 1,035 kA m^{-1}) to the coil body to gain a stronger magnetic field.
3. To finish the platform place a plate, preferably quartz glass, with a maximum height of 1 mm on the coil matrix.
4. Place a microreactor on the surface of the platform.
5. To withdraw the upper particles from the reaction solution and thereby open a microreactor activate a coil with a neodymium magnet inside by using the control mentioned in 4.1). To close the microreactor again deactivate the coil.
6. To merge two microreactors which are initially *ca.* 10 mm apart use neodymium magnets as described in 4.2). Lift up the magnet in the required coil bodies by activating the coils for *ca.* 25 s to open one microreactor (required distance between microreactor and magnet lies about 12 mm) and to move the other one to the same position on the platform.
7. To cool down the reaction solution in the microreactor on the platform surface and to decrease the coil temperature switch on the Peltier element positioned beneath the coil matrix as described in 4.1).

5. Enzymatic Reaction by Merging Microreactors

1. Dissolve horseradish peroxidase at a concentration of 0.1 µg mL^{-1} in potassium phosphate buffer (0.1 M, pH 6.5). Dilute the substrate, 10-acetyl-3,7-dihydroxyphenoxazine (10 mM in dimethylsulfoxide (DMSO)) with potassium phosphate buffer (0.1 M, pH 6.5) to a concentration of 200 µM.
2. Use 10 µL of each of these solutions to build two microreactors as described in 2.2). Merge the two microreactors by the mean of magnetic forces (neodymium cylinder magnet: 12.5 mm x 4 mm, 1,035 kA m^{-1}) as described in 4.6) at 25 °C.
3. Detect the reaction by positioning a fluorescence probe (excitation wavelength: 570 nm, emission wavelength: 585 nm) *ca.* 10 mm directly above an opened microreactor before the merge.

Representative Results

The shell particles have a diameter of around 640 nm. The magnetizable nanoparticles enclosed in this fluorosilane shell particles have diameters in a range between 22 nm and 37 nm. A 5 µL microreactor with water as a liquid core had a contact angle of around 160°.

The force needed to move a 10 µL microreactor as described above is 1.34 ± 0.08 µN. **Figure 1** shows the electromagnetic force of a coil with 4,500 windings of copper wire and an inner iron core powered with 58 mA. The force distribution is estimated by a finite elements model (FEM) to determine the required coil properties to move a microreactor. As can be seen in **Figure 1**, the magnetic force conducted by the described coil is strong enough to move a microreactor to a distance farther than 10 mm away from the coil center.

The magnetic force to open a microreactor equals 0.85 ± 0.05 mN which is much higher than the force needed to move the droplet. As the magnetic force induced by the coil with the iron core within (**Figure 1**) is not strong enough to open a microreactor, a neodymium magnet was used in the coils. By powering the double coils in alternating current flow directions, the permanent magnet can be moved towards or away from the platform. Thereby, the microreactor can be opened or closed magnetically. If two microreactors with a completely intact shell lie side by side they do not merge as the surface tension inhibits their fusion. Therefore at least one must be opened.

Figure 2 shows the Michaelis-Menten kinetics resulting from merging a 10 µL microreactor containing peroxidase with another 10 µL microreactor containing the corresponding substrate (n = 3). By Lineweaver-Burk linearization the calculated K_m value for the reaction within the microreactor is 86.85 µM ± 10.95 µM, the v_{max} value lies at 378.8 nmol L^{-1} s^{-1} ± 115.6 nmol L^{-1} s^{-1}. As the K_m value is in good correspondence with that given in literature, 81 ± 3 µM [32], it can be assumed that the enzymatic reaction within the small-scale microreactor with the hydrophobic shell material is not influenced with respect to affinity.

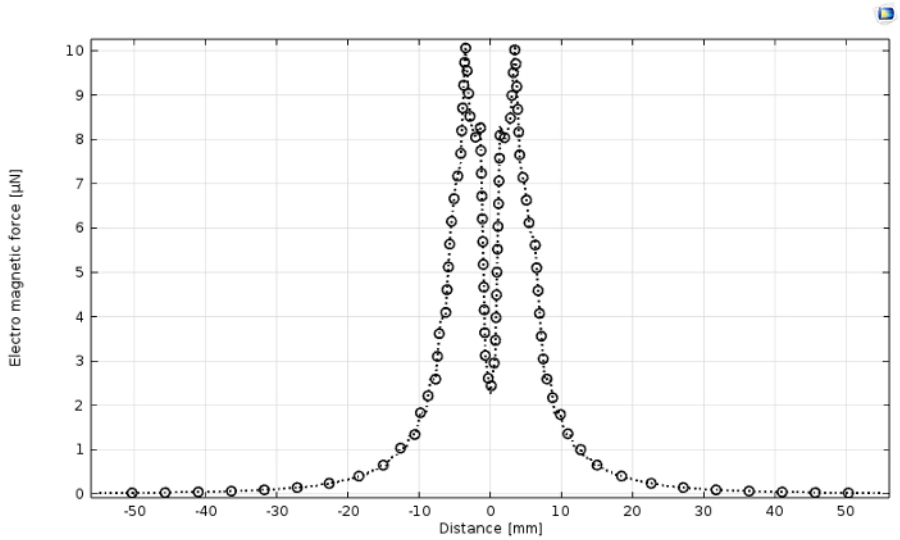

Figure 1: Electromagnetic force simulation of a coil with 58 mA and 4,500 windings of copper wire in dependence of the distance of the microreactor to the coil center determined by FEM. The coil diameter is 10 mm, the diameter of the iron core is 4 mm. Please click here to view a larger version of this figure.

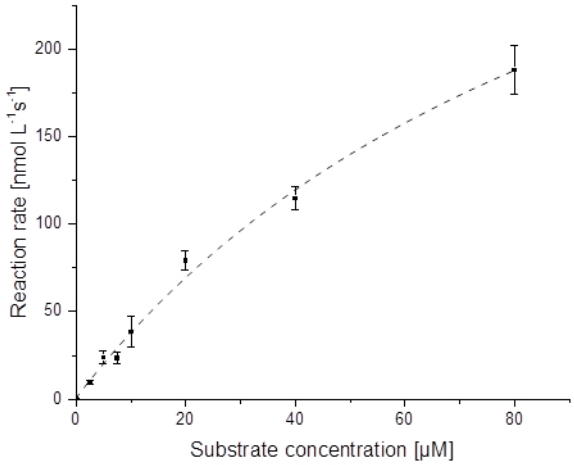

Figure 2: Michaelis-Menten kinetics of peroxidase measured by merging two 10 µL microreactors. The temperature is 25 °C and the buffer used is potassium phosphate (0.1 M) with a pH of 6.5. Three repetitions were carried out.

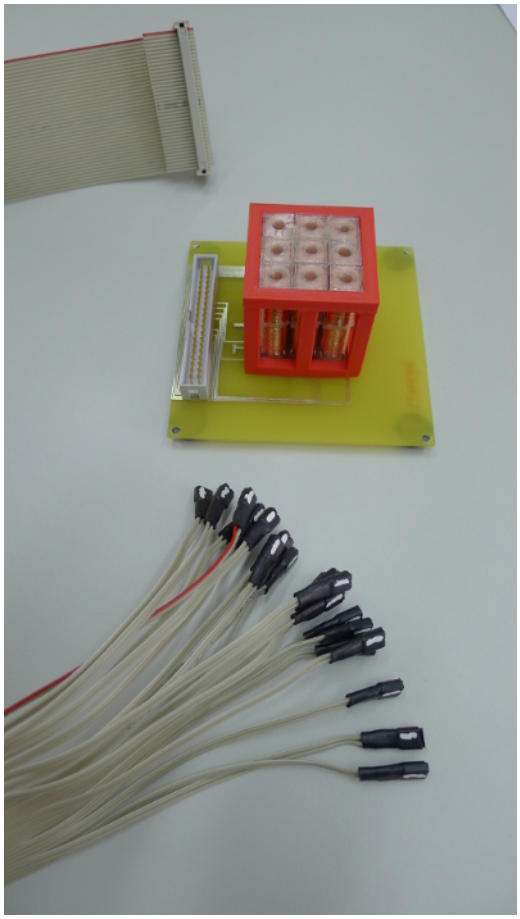

Figure 3: Actuation platform. The actuation platform consists of a 3 x 3 matrix of double coils. One coil has 4,500 windings, a height of 16 mm (one chamber), a diameter of 10 mm and an inner diameter of *ca.* 4 mm. The height of the coils is predefined by the height of the used neodymium cylinder magnet. The diameter was chosen because previous studies showed that this is a reasonable distance for moving a microreactor with this kind of magnet. The number of the windings and the current was determined by FEM. Please click here to view a larger version of this figure.

Discussion

For the successful use of microfluidic technologies, it is important to move the reaction volume corresponding to the requirements of the biotechnological synthesis and analyses. The actuation platform presented here makes it possible to move microfluidic droplets by magnetic force. The movement can be performed freely in two dimensions on a planar surface of a reaction platform by enclosing the liquid drop with a magnetic superhydrophobic shell. Thus an alternative system to predefined microfluidic channels with complex flow control installations, as used in conventional microfluidic systems, to pass solvents and samples is introduced. The automated actuation of small reaction droplets thus is a significant simplification of the known lab-on-a-chip platforms. Furthermore, the platform makes it possible to reversibly open a microreactor to add and remove reaction samples. In combination with a dispenser system, this can result in a high degree of automated reaction control and is the first step for *in silico* designable microreactions in a virtual lab-in-a-drop. The main limitation of this technique is that the microreactors can only be built with small volumes (up to *ca.* 30 µL). A critical step in the protocol is the detection of the enzymatic reaction inside the microreactors because the fluorescence probe has to be adjusted properly. A further detection possibility may be UV/vis spectroscopy.

The development process of the platform showed that an electromagnetic coil with a diameter of 10 mm is sufficient for droplet movement. On the other hand, a double coil filled with air or an iron core is not able to induce the magnetic force which is needed to open a microreactor shell. Therefore, neodymium cores were chosen in the coils to perform this task. The resulting magnetic field gradients again can be varied by electromagnetical movement of the magnet vertical to the reaction platform. The pitch size of the platform is only limited by the control. The already existing control and software is designed and ready to use for a 10 x 10 matrix. The Peltier element is not needed for the described applications but may be necessary when the microreactor has to be fixed for a longer time and it will allow to cool down the reaction mixture above the platform surface.

In the future, the combination of the actuation platform with the liquid marbles can function as flexible lab-in-a-drop-systems for the implementation of screenings, fast optical analyses and complex enzyme cascade reactions with very small reaction volumes. In addition, the platform can be used for (bio-)chemical analyses such as PCR, electrophoresis or ELISA. Moreover, the screening of new, industrial relevant enzymes and aptamer amplifications are promising possibilities.

Disclosures

The authors have nothing to disclose.

Acknowledgements

The authors would like to acknowledge the DFG for the support.

References

1. Squires, T., & Quake, S. Microfluidics: Fluid physics at the nanoliter scale. *Rev. Mod. Phys.* **77** (3), 977-1026 (2005).
2. Song, H., Chen, D.L., & Ismagilov, R.F. Reactions in Droplets in Microfluidic Channels. *Angew. Chem. Int. Ed.* **45** (44), 7336-56 (2006).
3. Erickson, D., & Li, D. Integrated microfluidic devices. *Anal. Chim. Acta.* **507** (1), 11-26 (2004).
4. Zhang, Y., & Ozdemir, P. Microfluidic DNA amplification-A review. *Anal. Chim. Acta.* **638** (2), 115-25 (2009).
5. Ohashi, T., Kuyama, H., Suzuki, K., & Nakamura, S. Control of aqueous droplets using magnetic and electrostatic forces. *Anal. Chim. Acta.* **612** (2), 218-25 (2008).
6. Srinivasan, V., Pamula, V.K., & Fair, R.B. Droplet-based microfluidic lab-on-a-chip for glucose detection. *Anal. Chim. Acta.* **507** (1), 145-50 (2004).
7. Lehmann, U., Vandevyver, C., Parashar, V.K., & Gijs, Martin A. M. Droplet-Based DNA Purification in a Magnetic Lab-on-a-Chip. *Angew. Chem. Int. Ed.* **45** (19), 3062-7 (2006).
8. Guttenberg, Z.*, et al.* Planar chip device for PCR and hybridization with surface acoustic wave pump. *Lab Chip.* **5** (3), 308 (2005).
9. Al-Kaidy, H.*, et al.* Biotechnology and Bioprocess Engineering - From the First Ullmann's Article to Recent Trends. *ChemBioEng Reviews.* **2** (3), 175-84 (2015).
10. McHale, G., & Newton, M.I. Liquid marbles: principles and applications. *Soft Matter.* **7** (12), 5473 (2011).
11. Bormashenko, E., Pogreb, R., Bormashenko, Y., Musin, A., & Stein, T. New Investigations on Ferrofluidics: Ferrofluidic Marbles and Magnetic-Field-Driven Drops on Superhydrophobic Surfaces. *Langmuir.* **24** (21), 12119-22 (2008).
12. Gao, L., & McCarthy, T.J. Ionic Liquid Marbles. *Langmuir.* **23** (21), 10445-7 (2007).
13. Bhosale, P.S., Panchagnula, M.V., & Stretz, H.A. Mechanically robust nanoparticle stabilized transparent liquid marbles. *Appl. Phys. Lett.* **93** (3), 034109 (2008).
14. Bormashenko, E., Balter, R., & Aurbach, D. Micropump based on liquid marbles. *Appl. Phys. Lett.* **97** (9), 091908 (2010).
15. Zhao, Y., Fang, J., Wang, H., Wang, X., & Lin, T. Magnetic Liquid Marbles: Manipulation of Liquid Droplets Using Highly Hydrophobic Fe 3 O 4 Nanoparticles. *Adv. Mater.* **22** (6), 707-10 (2010).
16. Fujii, S., Kameyama, S., Armes, S.P., Dupin, D., Suzaki, M., & Nakamura, Y. pH-responsive liquid marbles stabilized with poly(2-vinylpyridine) particles. *Soft Matter.* **6** (3), 635 (2010).
17. Aussillous, P., & Quéré, D. Liquid marbles. *Nature.* **411** (6840), 924-7 (2001).
18. Mahadevan, L. Non-stick water. *Nature.* **411** (6840), 895-6 (2001).
19. Tian, J., Arbatan, T., Li, X., & Shen, W. Liquid marble for gas sensing. *Chem. Commun.* **46** (26), 4734 (2010).
20. Bormashenko, E., & Musin, A. Revealing of water surface pollution with liquid marbles. *Appl. Surf. Sci.* **255** (12), 6429-31 (2009).
21. Bormashenko, E., Bormashenko, Y., Pogreb, R., & Gendelman, O. Janus Droplets: Liquid Marbles Coated with Dielectric/Semiconductor Particles. *Langmuir.* **27** (1), 7-10 (2011).
22. Bormashenko, E. Liquid marbles: Properties and applications. *Curr. Opin. Colloid Interface Sci.* **16** (4), 266-71 (2011).
23. Lehmann, U., Hadjidj, S., Parashar, V.K., Vandevyver, C., Rida, A., & Gijs, M. Two-dimensional magnetic manipulation of microdroplets on a chip as a platform for bioanalytical applications. *Sens. Actuator B-Chem.* **117** (2), 457-63 (2006).
24. Sato, E., Yuri, M., Fujii, S., Nishiyama, T., Nakamura, Y., & Horibe, H. Liquid marbles as a micro-reactor for efficient radical alternating copolymerization of diene monomer and oxygen. Chem. Commun. *(Cambridge, England).* **51** (97), 17241-4 (2015).
25. Shikida, M., Takayanagi, K., Inouchi, K., Honda, H., & Sato, K. Using wettability and interfacial tension to handle droplets of magnetic beads in a micro-chemical-analysis system. *Sens. Actuator B-Chem.* **113** (1), 563-9 (2006).
26. Sheng, Y., Sun, G., Wu, J., Ma, G., & Ngai, T. Silica-based liquid marbles as microreactors for the silver mirror reaction. *Angew. Chem. Int. Ed.* **54** (24), 7012-7 (2015).
27. Zhao, N.*, et al.* Self-organized Polymer Aggregates with a Biomimetic Hierarchical Structure and its Superhydrophobic Effect. *Cell Biochem. Biophys.* **49** (2), 91-7 (2007).
28. Xue, Y.*, et al.* Magnetic Liquid Marbles: A "Precise" Miniature Reactor. *Adv. Mater.* **22** (43), 4814-8 (2010).
29. Zhang, L., Cha, D., & Wang, P. Remotely Controllable Liquid Marbles. *Adv. Mater.* **24** (35), 4756-60 (2012).
30. Zhao, Y., Xu, Z., Parhizkar, M., Fang, J., Wang, X., & Lin, T. Magnetic liquid marbles, their manipulation and application in optical probing. *Microfluid Nanofluid.* **13** (4), 555-64 (2012).
31. Lin, X.*, et al.* Superhydrophobic magnetic poly(DOPAm-co-PFOEA)/Fe3O4/cellulose microspheres for stable liquid marbles. Chem. Commun. *(Cambridge, England).* **52** (9), 1895-8 (2016).
32. Glettenberg, M., & Niemeyer, C.M. Tuning of Peroxidase Activity by Covalently Tethered DNA Oligonucleotides. *Bioconjugate Chem.* **20** (5), 969-75 (2009).
33. Al-Kaidy, H., & Tippkötter, N. Superparamagnetic hydrophobic particles as shell material for digital microfluidic droplets and proof-of-principle reaction assessments with immobilized laccase. *Eng. Life Sci.* **16** (3), 222-30 (2016).

