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Miniaturizing the miniature: Liquid droplets as miniscule reactors

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Abstract

Even though microreactors are performing an increasing number of chemical and biological reactions better than conventional macroreactors, their weaknesses are being observed and overcome. A recent method is to conduct reactions inside liquid microdroplets. Each droplet contains all the reagents and catalysts required and the conditions are controlled to promote the most favorable processes. By populating a microreactor with millions of microdroplets, each performing as a complete reactor or sub-reactor, it is possible to distribute different reactions at different rates. Then the droplets may be fused or fissioned as required so that complementary processes are brought into contact with one another at the appropriate points in time. The present article provides an overview of the width and potential of this new and exciting technology across chemical and biological applications.

Keywords: Microdroplet reactors, Chemical reactions, Biological processes, Key features, Future directions

Introduction

Their many advantages over conventional large (macro-scale) reactors have promoted growing preference for micro-reactors for the production of many specialty chemicals, biological products and other applications such as on-line analysis and diagnostics through integrated lab-on-a-chip technologies. The rapid growth and diversity of applications of micro-reactors is illustrated by demonstrated uses in on-line process optimizations (Garcia et al. 2006), optical resolution of racemic mixtures (Honda et al., 2007), vesicles for controlled and targeted delivery of drugs (Modi and Pandya 2011), the production of hydrogen by steam reforming of methane or methanol (Arzamendi et al. 2009), the growth of cardiac tissue (Iyer 2008) and automated high-precision micro-total analysis of reaction mixtures (Auroux et al. 2002).

These applications cover different reactor configurations (Doku et al. 2005) and different morphologies of reaction media (Shchukin and Sokhurukov 2004), thereby enhancing the potential and the versatility of microreactors. Some significant features that have driven the growth of microreactors are their large surface-to-volume ratios, which enable high rates of heat exchange with the environment, the ability to implement sophisticated and accurate control policies, and the predominantly laminar flow, which preserves the integrity of sensitive cells and bio-molecules (Patnaik 2011).

However, microreactors have some weaknesses too. Scale-up is a major impediment. In view of the long and narrow tubes and the often complex channel geometries sometimes employed (Fujiwara et al. 2007), increase in scale essentially involves stacking many tubes in parallel. This increases the cost and makes it difficult to ensure equal distribution of reactants and equal rates of heat transfer across all tubes. A second limitation is the laminarity of the flow, which does not provide sufficient mixing when this is desired. Thirdly, since the fluid is in a dispersed phase on a local level but continuum descriptions apply at a macro-level, there are resulting problems such as maintenance of a constant hydrodynamic pressure or control of surface charges (Dubois et al. 2006).

To overcome these limitations, microreactors are giving way to droplet reactors, where each liquid droplet functions as an independent reactor. Thus the microreactor is miniaturized further by discretizing it into miniscule segments. Apart from enabling discrete control policies to be employed, droplet microreactors have the ability to perform a large number of reactions, distributed among the droplets, without increasing the size or complexity of the equipment. This alleviates the scale-up problem of conventional microreactors. Unlike the continuum approach of microreactors, droplet-based microfluidics allows for independent control of each droplet, thus creating greater manouverability and more accurate control (Fair 2007). Droplet-based systems also avoid large dead volumes and frequent obstruction of the channels that are associated with the intricate channel topologies of microreactors (Dubois et al.

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2006). Benefits such as these have favored increasing interest in these miniature reactors; some major applications and issues have been discussed in recent books (Tian and Finehout 2009; Lin 2011; Day et al. 2012). The present communication provides an overview of two major classes of applications that are of interest to chemical and biological engineers, followed by a brief discussion of future prospects for research and applications in this exciting and rapidly growing area.

Chemical Applications

A major area of applications of droplet microreactors is in the measurements and control of complex chemical reactions. One reason for this choice is that such systems contain networks of reactions that proceed at different rates, and the control of individual droplets enables the rates to be controlled separately and dynamically. Additionally, recent advances in droplet generation allow highly monodisperse droplets (<3% polydispersity) to be produced at extremely rapid rates (up to 20,000 per second (Kobayashi et al. 2008)), thereby enabling higher production capacities per unit volume of reactor than are possible in equivalent standard microreactors.

These advantages are exemplified by numerous applications in different areas of chemistry and chemical engineering. For the kinetic characterization of enzymatic reactions, Hsieh et al. (2006) demonstrated millisecond resolution binding kinetics using molecular beacons. Such high sensitivities were achieved through the accumulated fluorescence of thousands of droplet microreactors, resulting in high signal amplifications. Gong et al. (2007) and Song and Ismagilov (2003) achieved similar resolutions for other systems, the former for the nucleation kinetics of thermoresponsive microgel particles and the latter for intra-cellular RNase A.
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Laval et al. (2007) employed droplet microreactors profitably to screen the solubilities of different chemical compounds at different temperatures. Much faster screening was possible than by traditional methods. Their studies and the others cited above address some critical areas of drug development.

Shum and coworkers (2009) preferred droplets from a different perspective. Their attention was on the synthesis of hydroxyapatite (HAp). Conventional methods such as solid-state reaction, co-precipitation, sol-gel and surface-templated methods result in the agglomeration of final powders that cannot be easily prevented. Thus it becomes difficult to produce HAp with large surface area by microfuidic emulsification. While their application required a double emulsion, comparable precision and control of droplet size and product properties are also possible for multiple emulsions (Lee and Weitz 2008).

An interesting variant on the applications discussed above is that of Dubois and coworkers (Dubois et al. 2006). They employed a droplet-based, open digital microfludic lab-on-a chip with task-specific ionic liquids to perform solution-phase synthesis of organic compounds. The negligible volatility of ionic liquids enables their use as droplet microreactors in the presence of air, thus making it easier to operate them, and still allows easy scale-up, thereby avoiding a major bottleneck of conventional microreactors.

Chan et al. (2005) presented a similarly innovative application. They used nanoliter-size octadecene droplets flowing through a microcapillary at 240-300^{0}C with cadmium and selenium precursors. This produced CdSe nanocrystals of extremely small but uniform sizes. The method allowed precise control of crystal properties by varying the temperature, droplet:carrier viscosity ratio and residence time. These accurately tailored nanocrystals provide an excellent source of quantum dots for electronic devices.

Table 1. Illustrative list of droplet microreactor applications for biological and chemical processes.

Application	System	Method	Reference
Amplification of genomic DNA	*Haemophilus parahaemolyticas*	ePCR*	Williams et al. (2006)
Calcein production and GFP expression	Cell-sized phospholipid coated droplets	Fluorescence microscopy	Hase et al. (2007)
DNA/RNA analysis	Blood samples	ePCR	Pan et al. (2006)
Encapsulation of macromolecules and cells	Polystyrene beads; mitochondria	W/O/W double emulsion	He et al. (2005)
Enzyme kinetics	Luciferase activity	W/O/W double emulsion	Liau et al. (2005)
Generation of lipid vesicles	Phospholipids	Water-lipid emulsion	Tan et al. (2006)
Genetic variations in a DNA population	Blood samples	ePCR	Dressman et al. (2003)
Genome sequencing	*Mycoplasma genitalium*	ePCR	Margulies et al. (2005)
Protein crystallization	Lysozome; Ferritin; Catalase; Insulin	Membrane-bound emulsion	Hansen et al. (2006)
Selection of enzyme libraries	Ebg, a low β-galactosidase activity enzyme	IVC*/FACS	Mastrobattista et al. (2005)
Selection of proteins and peptides for binding	Glutathione-S-transferase	IVC	Yonezawa et al. (2004)
Synthesis of ceramic materials	Hydroxyapatite	W/O/W double emulsion	Shum et al. (2009)
Synthesis of functional reaction networks	Oxidation of Co^{3+}	Liquid slugs	Gerdts et al. (2004)
Synthesis of monodisperse nanoparticles	Silica gel	Liquid slugs	Khan et al. (2004)
Synthesis of organic molecules	Nitration of benzene; Fluorination of aromatics	IVC	de Mas et al. (2003)

*ePCR = emulsion PCR; IVC = *in vitro* compartmentalization

Biological Applications

Biological and biotechnological processes comprise a second large body of droplet reactor applications. Many of these applications have been inspired by the idea of cellular compartmentalization, i.e.

the visualization of a cell as being composed of a finite number of chemical compartments which interact coherently to sustain metabolic processes (Jacquez 1985).

This concept led Tawfik and Griffiths (1998) to develop an *in vitro* compartmentalization system (IVC), in which each compartment is a microdroplet of water that contains all the ingredients for an experiment. Each droplet is sufficiently small so as to contain just one gene, and many such droplets are dispersed in a water-in-oil emulsion. Thus each compartment functions as an artificial cell thereby establishing the analogy. The microscopic sizes of the droplets makes it possible to disperse millions of them in a small amount emulsion (~ 10^{10} droplets in 50mL (Taly et al. 2007)), thus making IVC an efficient screening protocol for biological reactions.

The ability to create micro-size droplets that are functioning reactors enables their use for DNA applications such as DNA detection and PCR analysis (Pan et al. 2006). In view of its wide usages, PCR has been the dominant beneficiary of the new technology. Conventional PCR has drawbacks such as high heat mass, low heating and cooling rates, and the possibility of contamination (Ruano et al. 1990). Droplet-based DNA amplification and analysis offers several advantages such as reduced times for analysis, more precise control of initial molecular concentrations, high throughput rates and lower consumption of reagents as well as waste generation (Zhang and Xing 2010).

The success of emulsion PCR (ePCR) with microdroplets has led to variations to make it more versatile and effective. One example is the generation of an ensemble of microbeads, each carrying between 10^4 (Dressman et al. 2003) and 10^7 (Margulies et al. 2005) identical copies of the same template molecule. These emulsion drops mimic living cells in cloning single DNA molecules.

Yet another DNA application has been described by Vijay et al. (2006). They designed an electro-wetting displacement apparatus that performed sample preparation, microanalysis and the detection of specific, known DNA strands; it was applied to blood samples to identify potential genetic disorders.

The work of Dubois et al. (2006) in using ionic liquid droplets as e-microreactors has been described above. In a similar vein, Jung and Kang (2010) recently reported the use of charged droplets driven by Coulombic force as solution-phase reaction chambers for biological reactions. By varying the electrostatic force, coalescence of droplets could be regulated and even switched on or off. They applied the technique to monitor glucose concentration variations in the alkalization of phenolphthalein and the bioluminescence reaction of luciferase in the presence of adenosine triphosphate.

Weaver et al.'s (2011) study addresses the twin issues of glucose monitoring and blood sample analysis but indirectly. Rather than measure glucose *per se*, they followed the concentration of insulin in blood by using microdroplets as radio labeled probes to monitor insulin dysregulation *in vivo*. This method increased the accuracy and speed of analysis and reduced the amount of insulin-chelate vector and the reaction temperature. Droplet microreactors thus appear to have proven potential for on-line automated analysis of blood samples for different medical purposes.
Table 1 above summarizes some major applications of droplet microreactors in the chemical and biological processing areas.

Droplet Control

The presentation done so far should indicate that control of the sizes and properties of the (liquid) droplets is a pivotal feature that determines the performance of a microreactor. Therefore a number of studies have understandably focused on this aspect. Different types of devices are available to generate drops of different kinds at different rates and under different conditions; these have been described elsewhere (Lindemann and Zengerle 2008; Ben-Tzvi and Rone 2010) and are not within the scope of this overview. The focus here will be on the principles and some illustrative applications.

Droplet control involves three main features: droplet generation, droplet fission and droplet fusion. Droplet dispensing devices operate on one of three main principles: electrowetting on dielectric (EWOD) or electrohydrodynamics (EHD) or dielectrophoresis (DEP). Briefly, EWOD controls the wettability of liquids on solid surfaces by using an electric potential; the electric energy across a thin dielectric film between the liquid and a conducting substrate is the controlling variable (Lee et al. 2002). EHD (also known as electro-fluid-dynamics) relies on the motions of ionized particles in electric fields and their consequent interactions with the surrounding fluid (Forbes et al. 2010). The operating principle behind DEP (Jones 2003) is that polarizable fluids will be attracted to regions of higher electric field intensity; so the liquid droplet should have a higher dielectric permittivity than its surrounding fluid. These methods have been compared by Zeng and Korsmeyer (2004).

Droplet fission provides a convenient way to scale-up the capacity of a reactor. Since each droplet contains all the reactants, splitting a droplet into two or more droplets effectively multiplies the capacity. Fission also enables the concentrations of the reactants to be controlled as required (Tan et al. 2004). There are basically two methods of fission. Active methods rely on external power or electrical control of the splitting mechanism; the method is versatile in the sense that it can also be used for transport, fusion, mixing and other fundamental fluidic operations (Teh et al. 2008; Fair et al. 2007). By contrast, passive fission utilizes shear forces created by channel design as the source of energy to split the droplets. This should suggest that passive droplet fission can be controlled by varying the flow rate of the continuous phase and the resistances in the channels. As might be expected, many configurations of channels are available, each with its advantages, disadvantages and applications.

While fission allows new droplets to be created, fusion or coalescence provides a means to perform reactions inside the droplets. As with fission, fusion too may be of active type or passive type. Passive fusion, like passive fission, operates by utilizing channel geometry and the flow rate to control the location of droplet fusion. Fidalgo and coworkers (2007) demonstrated droplet fusion by selective hydrotreatment of a segment of a microchannel. Similarly, controlled electrical energy is used to achieve active fusion. By sequentially turning on and off a series of electrodes, a droplet can be guided toward another droplet until the two coalesce (Zeng and Korsmeyer 2004). Tan and Takeuchi (2006) used channel design to bring the droplets together and parallel aligned electrodes to fuse them. Other variations to coalesce liquid droplets in a desired manner have also been described (Teh et al. 2008). What is important is the proper regulation of droplet fusion and fission such that reaction rates are optimized. This balance is not easy and few studies have addressed control of the relative rates of fission and fusion.

Conclusions and Future Directions

Droplet-based microreactiors offer many benefits and can be used for a wide range of biological and chemical processes. Their advantages over conventional microreactors have been outlined above. Although, or possibly as a result of, considerable research

has been focused on droplet microfluidics, many key issues still remain unresolved.

While the initial applications were based on water-oil (W/O) emulsions, recent developments of double emulsions (W/O/W) remove a major difficulty of the former. By separating oil and water from each other, agglomeration of droplets is avoided (Shum et al. 2009). The potential of IVC in DNA analyses through emulsion-based PCR opens new possibilities such as the direct monitoring of the actual activities of endogenous cellular enzymes and the generation of DNA-protein microbeads for functional genomics and proteomics (Griffiths and Tawfik 2006).

Although fusion of droplets allows the contents of two or more droplets to be combined, current techniques are not universal and do not offer the ease and control that is possible, for example, in the well of a microtitre plate (Taly et al. 2007). While expanding the scope of droplet microreactors, new applications also pose new challenges. Some examples are the requirement of new materials with special properties, techniques for surface patterning, and integration of different microfluidic platforms (Song et al. 2006). A promising development in this direction is the production of "Liquid Teflon" (Rolland et al. 2004).

The overarching challenge to the commercialization of microfluidic technologies is the development of a true lab-on-a-chip technology that is cost-effective and adaptable to different applications. Even though droplet microfluidics is in a nascent stage, initial research into chip-level integration has yielded promising results. For instance, Nisisako and Torii (2008) could produce monodisperse emulsion droplets on a large scale by an integrated microchip. Such studies point to a bright future for droplet-based microreactors (Day et al. 2012).

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