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Review

Potential of levitated drops to serve as microreactors for biophysical measurements

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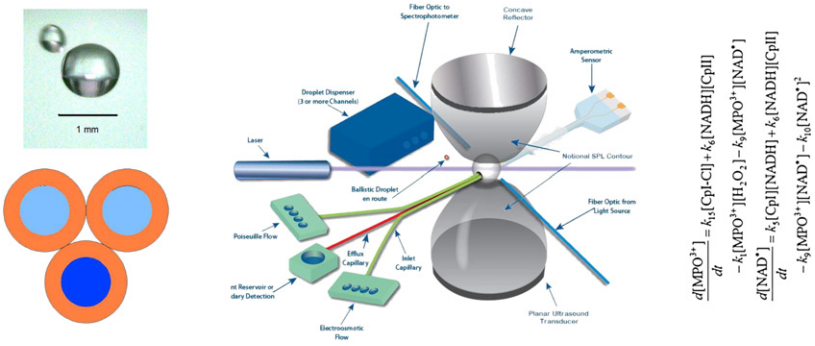
HIGHLIGHTS

- Ultrasonically-levitated drops show promise as reactors for studying biochemical kinetics.
- Such drops are most interesting for systems in which free radicals are reactants or products.
- Fluid handling and diagnostics are sufficiently developed for near-term application.
- Rate constants $\leq 1\,s^{-1}$ (first order) and $\leq 10^6\,M^{-1}\,s^{-1}$ (second order) should be measurable.
- Complex dynamics such as chaotic oscillations should be well-suited for study in levitated drops.

GRAPHICAL ABSTRACT



$$\frac{d[MPO^{2+}]}{dt} = k_{11}[CpI\text{-}Cl]+k_4[NADH][CpII] - k_5[MPO^{2+}][H_2O_2]-k_6[MPO^{2+}][NAD^{\bullet}]$$

$$\frac{d[NAD^{\bullet}]}{dt} = k_5[CpI][NADH]+k_4[NADH][CpII] - k_6[MPO^{2+}][NAD^{\bullet}]-k_{10}[NAD^{\bullet}]^2$$

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ABSTRACT

Microreactors are desirable for exploring chemical and biological processes, as reactant consumption is minimal and safety issues are easily managed. Levitated drops are a class of microreactors for which mixing is continuous and solid/liquid interfaces are absent or of lesser importance than in channeled microfabricated flow reactors. Thus, reactant adsorption or wall catalysis possibly of importance in ordinary microfluidic systems is absent in levitated drops. Transport of gaseous reactants or products is facile. Levitated drop microreactors are amenable to batch or continuous flow study of biochemical reactions. The possibility of studying oscillatory enzyme-catalyzed reactions in drops is apparent. This review explains the physics and chemistry of levitated drop microreactors and describes practical aspects of their design, fabrication, implementation, and optimization. Such considerations as drop evaporation, thermal control, protein behavior at the gas/liquid interface, and observation with spectroscopic and electrochemical probes are discussed.

Contents

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1. Introduction: the need for microfluidic kinetics measurements

Rates of protein association/dissociation, rates of enzyme-catalyzed reactions, and the dynamics of networks of reactions are central characteristics of living systems. Progress in understanding biochemistry has paralleled the ability to make reliable rate measurements. From the early work of Michaelis and Menten [1], through the development of stopped- and continuous-flow methods [2–4], the parallelism of centrifugal analyzers [5,6], and on to development of network measurements [7], comparatively large amounts of protein were required for rate studies. As the number of purified proteins has increased, the number of structure/function measurements needed to characterize these compounds has skyrocketed, while the amounts of many interesting compounds have in many instances been quite limited. Obtaining milligrams of, e. g., peroxidase from plant roots is easy, but obtaining micrograms of other enzymes, e.g. post-translationally-modified variants or mutants of kinases, may be challenging or impossible. Since diffusion-limited reaction rates are proportional to concentration, not protein mass, confining precious samples to small volumes increases rate while minimizing reactant consumption. Thus, the advent of microfluidic measurements [8–14] was widely hailed as important for progress in understanding biochemical dynamics. Microfluidics led to highly-parallel measurements, microfabricated sensor arrays, and advances in cytometry [15–22]. Even synthesis involving toxic intermediates or producing libraries of compounds has benefitted from the microfluidic revolution [23,24]. In response to these developments, a Flow Chemistry Society is newly formed [25].

Small samples imply the need to measure small amounts of material, and thus the need for low detection limits. Fluorescence can be used to detect single molecules, and nanoelectrospray mass spectrometry can be used for detecting femtomolar quantities of some proteins (nanomolar concentration in <1 μL of sample) [26]. It is thus feasible to characterize biochemistry in single cells or single organelles. However, organelles are quite complicated assemblies, and cells even more so. Biological dynamics are widely recognized as an example of emergent behavior [27,28], consistent with but not necessarily uniquely predicted by lower-level phenomena. To understand such aggregate phenomena as morphogenesis, immune response, or learning, one must not only identify the chemical species involved in these phenomena but also understand the connectivity and dynamics of the networks of which those species are a part [29–31]. Before the spatiotemporal dynamics can be well-characterized, rates of mass-action-limited reactions must be known. Reactions in homogeneous solution are inevitably different from those on surfaces or embedded in membranes. In part this is due to cooperativity or catalysis, in part to molecular orientation, and in part to rates of mass transfer. Distinguishing the influence of various processes requires that a baseline rate under some simple circumstances be established. The hypothesis underlying this review is that reactions in homogeneous solution are more readily understood and used as a point of reference than are reactions on surfaces or in membrane-bound sites.

2. Why now-common microfluidic systems are inappropriate for some measurements

The strengths of microfluidics typically focus on low reactant consumption, diffusion-limited mixing, low mass detection limits for analytical measurements, and safety. An equally important characteristic is that microfluidic systems have a higher ratio of surface area to volume (S/V, cm^{-1}) than do macroscale systems. This means that interactions between reactants and surfaces, perhaps of limited importance in macroscopic systems, can be dominant interactions in microfluidics. This can be shown by an order-of-magnitude calculation. Suppose one starts with a 1 μM solution. Further, suppose that the material of which the walls of a reactor are composed has 1 binding site for the reactant every 100 nm^2, quite plausible for proteins. For arithmetic convenience, model two limiting circumstances as cubes (even though flow systems come in a variety of shapes, the same scaling issues apply). For a cube 0.5 cm on a side, one has a volume of 1/8 cm^3, a surface area of 1.5 cm^2, and $S/V = 12\ cm^{-1}$. This size is typical of a single syringe in a stopped-flow kinetics system or a 96 well plate. The solution contains 1.25×10^{-10} moles. If the wall sites are saturated with reactant, there are 1.5×10^{12} molecules on the surface or 2.5×10^{-12} moles. About 2% of the molecules are adsorbed on the wall. A microfluidic system might have characteristic dimensions ~0.1 mm or smaller. Volume is $\sim10^{-6}\ cm^3$, surface area is $6\times10^{-4}\ cm^2$, and $S/V = 600\ cm^{-1}$. Now there are sufficient wall sites to scavenge all the molecules originally in solution. One typically tries to prevent such adsorption by coating the microfluidic system with, e.g., polyethylene glycol [32], bovine serum albumin [33], or poly-L-lysine [34]. If adsorbed species were inactive, wall scavenging would lead to underestimation of rate constants (for a particular observed rate, the actual reactant concentration would be less than the formal concentration, so k_{obs}[reactant, formal] $= k_{true}$ [reactant, actual] implies $k_{true} > k_{obs}$).

However, adsorption alone may not inactivate molecules. Indeed, supported catalysts are nothing but adsorbed molecules on a solid substrate. It has long been known that free radicals are catalytically annihilated on, e.g., quartz surfaces. The scaling equations for the case of pure diffusional mass transfer were reported by Noyes [35].

Two additional situations may further complicate the use of microfabricated microfluidic systems for study of kinetics. In some instances, gasses are reactants, intermediates, or products of reaction. While some materials, such as polydimethylsiloxane (PDMS), are oxygen-permeable, equilibration of gasses with surroundings is most facile when there is a solution/gas free surface. In most microfluidic systems, care is taken to avoid such surfaces in order to fully

contain reactant streams. Thus, controlling gas uptake and release is difficult (although the specific combination of PDMS and O_2 does lend itself to some interesting engineering [36]). Secondly, long-term use of a microfluidic device is impractical. Disposability is typically considered an asset, as cross-contamination can be prevented. However, if surface passivation is required, seasoning of a surface may, asymptotically, provide reproducibility. Such seasoning, in some instances, may be due to biofilm formation, so one faces a no-win choice: either select a one-use device (but have little confidence in the control of surface chemistry) or use the same device multiple times (but perhaps have a biofilm or other modified surface whose character is poorly understood [37]).

To fully assess the potential of microscale reaction technology, advantages and limitations of the macroscale batch and microscale reactors must be identified. A Continuously-Stirred Tank Reactor (CSTR) is the model for an ideally mixed macroscale reaction vessel, where one or more reactants are continuously flowed into a well-mixed, homogeneous reactor, and the output flow contains the reaction product, intermediates, and any unreacted input species. Microscale reaction systems involving microfluidics have advantages over macroscale reaction systems including reduction of sample and reagent volumes, and potentially reduction in reaction times to the extent that small spatial dimensions speed mixing and kinetics. For reactors used to detect limited amounts of analytes, precise flow and mixing control may improve sensitivity and precision [38]. However, the development and design of a microfluidic system requires more than simple scale down from a macroscale reactor system. There are differences in fluid behavior, in the relative importance of convection and diffusion, in the effects of viscosity, in the relative importance of bulk and surface properties, as well as differences in heat and mass transfer. Conversely, scale-up may be challenging, as the surface area to volume ratio drops as volume increases (typically ~ volume$^{1/3}$). In addition to changes in surface area to volume ratio, mixing times may change. If the mixing patterns change within a batch reactor system, there may be convectively-driven concentration fluctuations in the macroscopic system not found in the microfluidic system.

For the sake of brevity and focus, conventional microfluidic systems will not be further reviewed here. Further, "droplet" will be used to mean a sub-microliter bolus of fluid, particularly one that may merge with a larger fluid bolus. A "drop" will be an isolated bolus, regardless of size, or the larger of two boli that may interact. While a number of cited sources refer to "microdroplets," we deprecate use of the term.

3. Existing approaches to systems with high surface area/volume ratios; pendant drops, sessile drops, and drops in flowing fluorocarbon oils

Drops may abut any form of matter. While liquid/gas interfaces are of primary interest to the authors, significant progress has been made in liquid/liquid and liquid/solid interfaces for drops and their surroundings. Shear flow in confined channels allows formation of aqueous drops in fluorocarbon oils [20,22,39–47]. Individual drops are on the order of picoliters in volume, and can be generated at the rate of several thousand per second. Because of the small size of the drops, diffusive mixing and approach to homogeneity can occur nearly instantaneously. For small molecules in aqueous solution, with diffusion coefficient ~10^{-6} cm^2 s^{-1}, characteristic mixing time is 0.5 s, as a 10 pL drop has a radius ~10^{-3} cm and $d = \sqrt{2Dt}$ where d is diffusion distance, D is the diffusion coefficient, and t is the characteristic time. Flowing streams of such drops can be observed at steady state, allowing continuous flow kinetics experiments. Single cells or organelles can be encapsulated in such drops, allowing measurement of the distribution of individual unit behavior without requiring immobilization. Further, because fluorocarbon liquids typically can dissolve high oxygen concentrations and are not miscible with water, gas transport is facile and wall adsorption typically inconsequential. So long as experiment duration is compatible with flow channel length and fluid velocity, such systems are attractive. If one wishes to mix solutions, allow equilibration, and then add additional reactants, such design is possible, though not necessarily easily or flexibly accomplished.

Recent advances with pressure driven microfluidic devices allow creation of a droplet in the channels of a microreactor, allowing control of discrete volumes of liquid [48–50]. Such "digital microfluidics" or "droplet microfluidics" can use a variety of methods to create and manipulate drops or droplets, allowing flexibility in the system configuration. By manipulating device geometry through T-junctions [51–53], flow focusing [49,50], acoustic [54–59], or electrochemical methods, such as electrowetting [39,60–64], control of each independent drops through a series of micro-plates creates microreaction vessels where the drops can be mixed and analyzed. Electrowetting is the modification of the wetting behavior of a drop on an electrode due to a direct or alternating polarity electric field. If an electric field is applied non-uniformly, then a surface energy gradient is created which can be used to manipulate the position, shape, or velocity of a droplet sandwiched between two plates [60,61,65]. Droplet microfluidics by electrowetting have many biological and chemical applications [48] including the use of droplets for glucose sensing [66], DNA sequencing [67], and sample purification [68].

A microfluidic device utilizing surface acoustic waves (SAWs) has the advantage of manipulating the drop on a hydrophobic surface without the use of pumping systems, due to the fluid being transported on the device by the SAW [54]. Typically, the SAWs are micrometer to nanometer electroelastic waves, which are excited by an interdigitated transducer, and then propagate along the surface of a piezoelectric substrate, causing fluid movement driven by propagation of the wave [54,57]. With the use of one or two interdigital transducers, either a single drop or multiple drops can propagate along an electrode-free platform, with the potential to individually heat or mix the droplet along the way [69]. Such freedom allows the drop to be a transport device for biological material, such as a protein. Qi et al. have shown that atomization using SAWs makes it possible to extract bovine serum albumin (BSA), ovalbumin, and yeast cells on a paper microfluidic device [70]. In addition, microfluidic devices utilizing self-assembled monolayers (SAMs) can also be applied for purification of proteins for mass spectrometry applications [69,71,72] or as a means of droplet formation for drug delivery systems [73].

The main advantage of using a drop-based system over a continuous flow system is the reduction of contact with solid surfaces, also reducing adsorption and adhesion problems [38]. However, there are some disadvantages with microfluidic devices, such as difficulties in detection due to reduced sample volume and signal, difficulties in control of the fluid flow due to inhomogeneous surface composition, temperature, buffer characteristics, hydrodynamic effects, and cost of materials, depending upon the type of device [74,75]. There are also disadvantages to reaction conditions due to substrate interaction with the walls of the reaction vessel such as protein or enzyme adsorption [76], biofilm formation [37], or reactivity of the walls or substrate due to radical scavenging [77] and gas permeation. As already noted, if S/V is high enough, adsorption may significantly deplete some reactants, particularly proteins, from bulk solution, leading to confusion concerning mass transfer of species leading to reaction. Other issues encountered in reactor systems involving enzymes and enzyme entrapment include membrane fouling, protein concentration gradients, and decreases in pump performance with time [78].

4. Electrostatic, magnetic, and ultrasonic levitation

Complete avoidance of liquid/solid interactions can be achieved through drop levitation. At the price of creating a gas/liquid interface, adsorption and surface reactions are eliminated. One may introduce

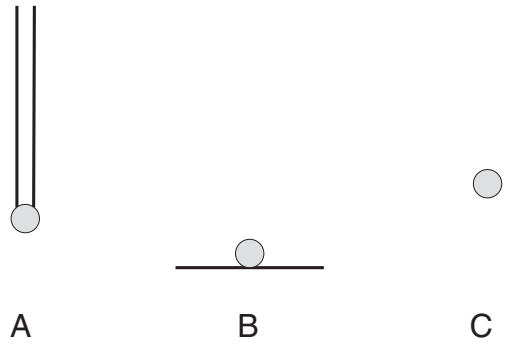

Fig. 1. Drops with gas/liquid interfaces. A. Pendant. B. Sessile. C. Levitated.

solid surfaces to such drops, allowing demonstration of interface effects, but such introduction is optional. The three types of drops with gas/liquid interfaces are sketched in Fig. 1.

The potential for levitated drop reactors (LDRs) for use as microanalysis systems was recognized no later than 1996 [79]. As with the flowing drop and digital microfluidic devices, sample volumes are reduced, and mixing conditions are controlled, but unlike the microfluidic microreactors, reactions in a levitated drop are not contained by the walls of a reaction vessel [79]. A small *S*/*V* is advantageous for biological reactions, especially in those involving proteins, which are likely to behave differently at an interface than in bulk solution. However, the limited amounts of material available for many biomolecules mitigate in favor of small volumes, and thus large *S*/*V*. Levitation of a drop can be achieved by optical [80,81], diamagnetic, aerodynamic, electrostatic or electromagnetic [82–85] or acoustic [86–92] levitation. A levitated drop is a unique reaction cell, as it has no solid walls, has facile exchange of gaseous reactants with the surroundings, and has a renewable surface, so applications of a levitated drop can include, but are not limited to, windowless optical cells, micro-liquid handling reaction cells, renewable gas samplers, and sample introduction interfaces [93–95]. These applications are an indication of the wide range of applicability of levitated drop microreactors to biological and chemical problems. Barnes et al. have shown that detecting single molecules is possible in picoliter drops [96], demonstrating much "room at the bottom" [97] for sample preparation and analysis in a levitated drop.

Sample preparation and sample introduction have been demonstrated using both electrostatic and acoustical levitation techniques. Bogan and Agnes have shown that droplets with a net charge can be created 'on demand' and levitated, as well as ejected, from an electrodynamic balance (EDB) for further purification and analysis [98]. As is the bane for many measurement techniques, when only limited sample volumes are available, minimum mass detection levels are achieved via preconcentration. Here, Bogan and Agnes utilized the evaporation of the droplet to concentrate the peptide leucine enkephalin and reduce the sample volume, as small spot sizes are needed for the technique they were using — matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). After levitation, the drops are ejected from the EDB onto a MALDI plate for analysis by TOF-MS [98]. They further used this combined levitation and analysis of charged drops on larger peptide and protein molecules to create μm spot sizes for UV-MALDI and found a decrease in detection limits for peptides and proteins, with nanoliter amounts of sample needed [99].

Chung et al. have also developed a levitator with electrostatic levitation to grow protein crystals in a containerless environment [82]. They grew lysozyme crystals in both a non-rotating and a horizontally rotating drop to test the effects of rotation on crystal growth. As the crystals grew larger, the rotation speed of the drop was not enough to keep the crystals in bulk solution, and it was noted that the crystal shapes depended upon rotation speed. Thaumatin crystals were also grown in rotating drops and compared with those of the lysozyme crystals, and were found to have differing crystal shapes under similar drop conditions.

Liu and Dasgupta have demonstrated a drop in a pendant drop system consisting of an organic phase and an aqueous phase, which shows that sample purification can be done by extraction in a levitating drop [100]. Other applications include the study of samples with liquid membranes [100]. Petersson et al. have also shown that sample enrichment for capillary electrophoresis can be performed in an acoustically-levitated drop [101]. They used a mixture of amino acids in ethanol to exploit ethanol's volatility in order to increase the concentration of the amino acid mixture. When the amount of time necessary for evaporation to generate the desired enrichment had passed, the sample was removed from the levitator and analyzed by CE.

However, drop removal from the levitator does not have to occur in order to analyze the sample. In fact, for many cases, *in stilla* sample detection would be preferred so as not to disturb or disrupt the reaction system, as in the case of a biological system, in order to retain sample integrity. (Note: In [92], we used *in stillo*. Here, we use the correct, feminine, gender in the Latin, *in stilla*.) A study by Weis and Nardozzi demonstrated that the rate of an alkaline phosphatase-catalyzed hydrolysis reaction in a levitated drop was similar to that in bulk, and the levitation process does not change enzyme activity, at least for this enzyme [90]. Experiments in drops do, however, permit measurement of reaction rate below 0 °C, as drops freeze only at sub-zero temperatures. Sample detection methods must maintain sample integrity. Detection in a levitating drop can be accomplished in numerous ways, including Raman spectroscopy, absorbance, fluorescence, and light scattering [102]. Laser techniques are useful for determining drop characteristics such as composition, diameter and temperature [103]. Kaiser et al. used Raman spectroscopy to study coated drops and compared the spectra of a levitated drop with that of the sample in a cuvette [81]. All of the major sample vibrational components were present both in the levitated drop spectrum and the cuvette spectrum, plus a nitrogen peak due to mixing of air appeared in spectra of the levitated drop, but not from bulk solution. Esen et al. used Raman spectroscopy to analyze the progress of a polymerization reaction in a levitated drop [80], showing the advantages of coupling Raman spectroscopy with levitated drops to track a chemical reaction.

Fluorescence and absorption measurements are also quite useful to study reactions in a levitating drop. With the use of fiber optics, analyte concentration can be measured directly or via spectral changes monitored during, e.g., titration in a drop [104]. Laser-induced fluorescent measurements were used to study changes in intensity as a result of evaporation or a change in pH in a levitated drop [88]. In this study by Santesson et al., as the droplet evaporated, the fluorescence intensity ratio for two excitation wavelengths but a fixed emission wavelength increased, demonstrating how drop evaporation can skew fluorescence or absorption measurements. The intensity ratio was stabilized when the levitated drop volume was controlled by the addition of droplets to minimize the effects of evaporation. The pH studies were performed by the addition of acetic acid by a droplet generator to a levitating drop and simultaneously monitoring the fluorescence signal of the fluorophore. These studies demonstrate how levitating drops can be stabilized in an acoustic field, allowing the study of biochemical reactions on a system through the addition of external reactants. Work on drop positional and shape stability has been an on-going focus of research [105–113].

Work by our group also showed the promise of using an LDR as a biological microreactor. An acoustically levitated drop was used to measure kinetic data from a reaction between NADH and pyruvate catalyzed by lactate dehydrogenase (LDH) to produce lactate [114]. The reactants were introduced into the levitator by a capillary network, and the drop was levitated at the end of the capillaries, minimizing the surface interaction with the capillary. As the NADH and

pyruvate react in the presence of LDH, the fluorescence from NADH decays, following Michaelis–Menten kinetics.

Work to date only begins to show the possibilities for the application of a levitated drop reaction system to biological problems. Since mixing in the drop is continuous and can be diffusional or convective, the levitated drop as a microreactor is a well-stirred system, potentially useful for study of enzyme kinetics. This contrasts with common stopped-flow methods, where greater amounts of enzyme are required. Continuous flow methods, e.g. with droplets in microfluidic systems employing fluorocarbon oils to keep aqueous droplets from contacting walls, use smaller volumes per drop, but rapidly exceed the volume of solution needed in levitators. If 1 μL is employed in a levitated drop, then 10^5 10 pL drops would consume the same volume of enzyme solution. For droplets generated at 1 kHz, this is ~100 s of measurement. In an LDR, not only is solid-surface chemistry not a factor, but neither is interaction with the fluorocarbon oil. If gases are substrates or products for the enzyme, transport from the surrounding medium into the drop or from the drop into the surrounding gas is facile. Access by multiple diagnostics simultaneously may also be easier, though to date this is more of an advantage in principle than in practice.

5. Batch vs. continuous flow approaches

For drops to be useful microreactors, one must have clean, simple, reliable means to introduce reactants and obtain homogeneous solutions. Both delivery and mixing present challenges. As can be found in any introductory text on reactor design [115], instantaneous mixing is an ideal only approximated in reality. Chapter 4 of [115] cogently explains the idealizations for reactors with and without draw-off of reactor contents. If influx volume balances efflux volume, and homogeneity is maintained, one has a continuous flow stirred-tank reactor (CSTR). If there is no efflux and the reaction is initiated instantaneously in some manner (injection of catalyst, addition of an initiator, instantaneous addition and mixing of one reactant), one has a batch reactor. If reactant is continuously added but there is no efflux, one has a semi-batch reactor. If there are evaporative losses of solvent precisely balanced by reactant solution influx, one can tune a semi-batch reactor to be constant volume, and thus to have constant catalyst and intermediate concentration. While not common industrially, such tuned, semi-batch methods have been employed in research (see, e.g. for a macroreactor, [37,116–119]).

5.1. Bolus introduction of reactants to drops

A simple model of injecting a droplet into a drop has much in common with shooting a projectile from one point to another. With appropriate initial velocity, a droplet will intersect a drop with sufficient kinetic energy to overcome the surface tension, merge with the drop, and mix rapidly. In practice, precision aiming of droplets is difficult. Santesson et al. show trajectories of piezoelectrically-launched droplets spiraling into a drop [102]. They used a piezoelectric droplet launcher, so the only forces guiding the droplet are gravity and acoustic pressure. Of late, we have been using electrostatically-launched droplets, with alternation of droplet charge to ensure that the droplet is electrostatically-attracted to the growing drop [120,121]. As explained earlier for picoliter drops, diffusional mixing time increases as the square of drop radius. For a 5 μL, spherical drop with $r \sim 0.11$ cm, diffusional mixing requires $t > 5 \times 10^3$ s, about 1.5 h. Observation of microliter drops in transmitted light with nanoliter NaOH droplets merging with HCl/phenolphthalein drops gives spontaneous mixing times of ~3–5 s, an improvement of 3 orders of magnitude. By modulating ultrasound intensity, sub-second mixing times are achievable. While kinetics as fast as that observable with stopped-flow mixing [122,123] appears to be unreachable, observation of reactions with ~1 s half-lives is within reach. For second-order reactions with ~μM concentration of each reactant, rate constants up to $10^6\ M^{-1}\ s^{-1}$ should be measurable. Obviously, if fluorescence of reactants or products gives low detection limits for these species, lower concentrations may be employed, with corresponding increase in the maximum measureable rate constant.

5.2. Drops as continuous flow reactors

While not entirely free of solid-liquid interfaces, pendant drops may have the majority of their surfaces as gas–liquid interfaces. Continuous flow, with influx and efflux balanced, makes such drops CSTRs. Our early attempt at making a 5 μL CSTR [114] failed, as we only had passive controls available to balance the flows. A servo control to maintain drop volume would likely solve this problem. The availability of inexpensive CMOS cameras and machine vision software (e.g. LabView drivers and virtual instruments) that can rapidly report drop size may assist in developing such a servocontroller. Note that hydrophobic coating of capillaries is essential to prevent wicking of drops up the side of the capillary bundle. We have found CYTOP® to work well, but any sufficiently hydrophobic coating should work. The capillary bundle needs to be positioned so that the drop is just below an acoustic node, ensuring that the drop is supported by the acoustic field, not surface tension attachment to the capillaries. Mixing time for 2.5 μL s^{-1} influx to a 2.5 μL drop has been observe to be ~2 s, indicating that convective mixing is overwhelming diffusional transport as desired. For a drop to operate as a CSTR, the residence time in the drop must be significantly greater than the mixing time. For a 5 μL drop, 2 s mixing time, one may operate the system as a CSTR for residence times of 10 s or greater and flow rate of all reactants combined below 0.5 μL s^{-1}. Such slow flows may most easily be maintained by using Poisseuille flow through capillaries of suitable length. Given:

$$Q = \frac{\pi \Delta P r^4}{8 \eta L} \tag{1}$$

where Q is flow rate ($cm^3\ s^{-1}$), ΔP is pressure drop across the capillary (typically, gauge pressure on the feed solution in dyne cm^{-2}, as the drop is at ~1 atmosphere), L is capillary length (cm), r is capillary radius (cm), and η is solution viscosity (Poise) [124]. Because η depends on solution composition and temperature, it is the least reliable of the parameters in the equation. For distilled water ($\eta = 0.01$ Poise), capillary length 1 m, capillary radius 50 μm $= 5 \times 10^{-3}$ cm, and pressure 28 psi $= 2$ atmospheres $= 202.6$ kPa $= 2.0 \times 10^6$ dyne cm^{-2}, resulting in $Q = 5 \times 10^{-4}\ cm^3\ s^{-1} = 500$ nL s^{-1}. Two inflow capillaries running at 500 nL s^{-1} would give a residence time of 5 s for a 5 μL drop, so one sees that headspace pressures can be quite modest and flows readily slowed to speeds compatible with residence times of many minutes (e.g. for radius 10 μm, all else constant, flow slows by a factor of 625, giving $\tau \sim 1$ h).

5.3. Mixing in drops; convection and diffusion

Levitated drops are sufficiently large that diffusion is inadequate to maintain homogeneity on a time scale commensurate with many biochemical events. Fortunately, the no-slip boundary condition at the interface between phases ensures that there will be circulation in any levitated drop, since acoustic waves are traveling waves in air. Even though standing waves are spatially static, they have a time-dependent amplitude at all points save nodes, and thus provide time-dependent, spatially inhomogeneous pressure on the gas–liquid interface, ensuring that convection occurs. Circulation in drops has been studied in non-reacting systems [111,112,125–134]. Flow and homogeneity have also been modeled in spherical drops in which first-order reactions occur that consume gas diffusing through the gas/liquid interface [135]. For drops of static shape, levitated on-axis in a cylindrically-symmetric acoustic field, the center of the drop is stationary for small drops, but vortices can form in large drops. Circulation

proceeds at speeds that increase monotonically to the surface of the drop. Under one set of conditions for a 30 μL drop [132], maximum velocity at the drop equator was 80 mm s^{-1}. Since drop diameter was approximately 4 mm, this is approximately 6 rotations per second, and flow is laminar. While shear (velocity increases along a radius) ensures that mixing is more rapid, azimuthally, than diffusion, radial mixing is also accelerated. If the drop moves off-axis, there is a break in axisymmetry, and oscillations in drop shape increase in frequency, but are ~50–100 Hz for a few mm departure from the axis of levitation [111]. Drop shape can be driven into oscillation by modulation of the acoustic field magnitude. In such cases, the drop shape oscillates with a radian frequency ω_l of

$$\omega_l^2 = \frac{\sigma l(l-1)(l+2)}{\rho a^3} \tag{2}$$

with l the oscillation mode number ($l=1$ for cylindrical symmetry, $l=2$ for the lowest non cylindrical mode, etc.), a is the radius of a spherical drop of the same volume as the levitated drop, σ is surface tension, and ρ is density. As water has a surface tension of about 72 dyne cm^{-1} at 25 °C, one obtains a frequency ~110 Hz for the lowest frequency mode a 5 μL drop. Mixing by driving drop distortion thus requires several tens of milliseconds at best. Yarin has studied how low-amplitude ultrasound perturbation of the appropriate frequency can drive drops into high-amplitude oscillation, thus optimizing convective mixing [136]. One anticipates that drop homogenization may be driven to occur in times well below 0.5 s, but not below 0.05 s. Stopped flow mixing times (~5 ms) are not likely to be reached with levitated drops, but mixing times well below those obtained by manual mixing should be easily obtained.

6. Problems facing adoption of levitated drops as microreactors

Just as adoption of microfluidics for routine use in biophysical research has required considerable time, so adoption of levitated drops has been a slow process. Aside from the limitations posed by the lack of turnkey systems, what technical problems limit the wide-spread adoption of levitated drop microreactors?

6.1. Positional stability

Levitating drops is easy. Levitating drops such that they are positionally stable within a few microns over a period of tens of minutes is more difficult. Any deviation from cylindrical symmetry, and any non-random modulation of the waveform driving the ultrasound transducer initiates drop motion or distortion. This complicates spectroscopic monitoring of the drop, as optical signals are modulated at the frequency of drop precession or oscillation. Because drop shape is a function of ultrasound power and the ratio of acoustic power to gravitational force, drop position subtly drifts as solvent evaporates. If droplets are injected into drops, conservation of momentum requires that the combined drop + droplet will be pushed off the levitator axis and that momentum must be dissipated before the drop returns to the levitator axis. Mixing is a dissipative process; drop orbital decay to the levitator axis is a slower dissipative process. If drops are to be used to study rapid reactions, it will be necessary to monitor drops while they are still orbiting the levitator axis after droplet injection. In principle, one can use piezoelectrically-steerable mirrors, servo-controlled to point the optical axis at the drop, to allow stable measurement even as the drop moves. In practice, we know of no implementation of such a strategy to date. Fig. 2 shows that an orbiting drop can be tracked by a 2-mirror system if fluorescence is to be monitored, but must be tracked by a 3-mirror system if absorbance is to be monitored (in both cases assuming that light is focused onto the drop rather than using diffuse illumination).

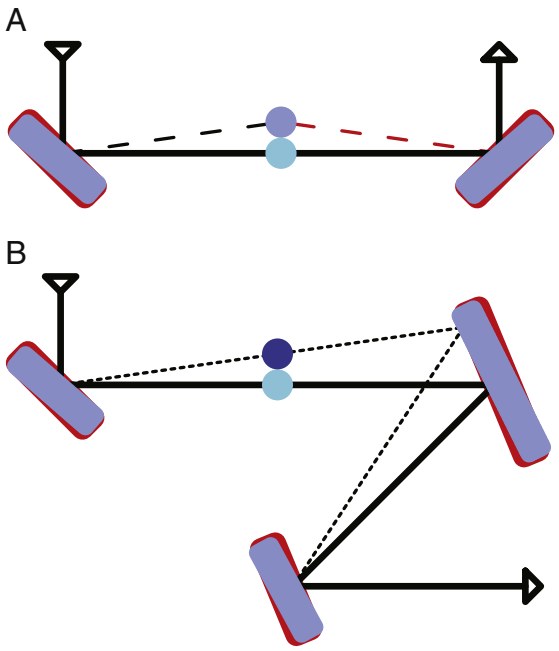

Fig. 2. Compensating for drop motion to allow spectroscopic monitoring in a moving drop. Inset A: fluorescence can be monitored using 2 tracking mirrors. Inset B: Absorbance monitoring requires 3 tracking mirrors. Aqua circle represents drop centered on the levitator axis; dark blue drop is the offset drop. Dotted lines show how the optical axis must move to maintain alignment, and the light blue mirrors show how rotation of the mirrors maintains the alignment.

6.2. Environmental stability and heat transfer

Two separate phenomena are dependent on temperature, humidity, and pressure in levitated drop reactors. One is the chemistry under study in the drop, and the other is the levitation and evaporation of the drop. Levitation requires that the acoustic transducer be at mechanical resonance, and that the transducer/reflector gap also be in resonance. Such resonance occurs when the transducer/reflector gap supports an acoustic standing wave. The speed of sound in a gas has the same relationship to wavelength and frequency as for any linear, longitudinal wave:

$$\lambda \upsilon = c \tag{3}$$

where λ is wavelength, υ is frequency (typically in Hertz), and c is the speed of sound in the gas.

While one may use planar transducers and reflectors [90,137,138], it is typically more efficient to use at least one concave component (typically the reflector, but both transducer and reflector may be concave) [92,110,113,139]. For planar components, resonance occurs for a transducer/reflector spacing H such that m is an integer and

$$2H\upsilon = mc \tag{4}$$

For non-planar components, m is a non-integer constant which may be found by numerical modeling or by empirical observation.

For 5 °C<T<30 °C, the speed of sound in air as a function of pressure, temperature, [CO_2], and humidity is known and fit to a smooth, polynomial function [140]. It is thus possible, in principle, to servo-control a levitator to always be in resonance. Our experience is that such control is easier in principle than in practice. For υ~20 kHz, the resonator air spacing must be precise within 0.01 mm (nominal acoustic wavelength ~17.2 mm). Because the reflector is concave, finding a reference zero spacing is challenging. However, once the right spacing is found, the acoustic field in practice does conform to theory; drops orbit in a toroidal acoustic potential minimum near

the planar transducer and are positionally stable on the resonator axis at nodes higher in the resonator [110,111,139,141]. Temperature control is readily achieved for temperatures above ambient, and ultrasonic humidification or chiller/heater humidification can adjust relative humidity.

Humidity influences the rate of drop evaporation, and temperature is obviously critical in carrying out kinetics experiments. As we have not performed kinetics experiments lasting more than 1 min as yet, it is unclear whether evaporation is going to be an important issue. The rate of droplet evaporation for solute-free drops is well-known as a function of drop size, temperature, and humidity [142]. While one might expect simple evaporative behavior as one sees e.g. in droplets subject to heating in reactive flows [143],

$$D^2 = D_0^2 - kt \tag{5}$$

where D is instantaneous drop diameter, D_0 is initial diameter, t is time, and k is a constant dependent on temperature, solution composition, and gas composition, in fact the situation is much more complicated. Yarin [142] starts from the approximation that

$$\frac{d}{dt}\frac{D^2}{D_0^2} \sim k, \tag{6}$$

but quickly moves to the more complicated situation where circulation in the levitated drop and ultrasound-correlated transport in the gas phase makes Eq. (5) an oversimplification. Drops of interest for biophysical measurement will doubtless have solutes, surfactants, and proteins contaminating the gas-liquid interface, and thus will alter evaporation rates [144]. Our chosen means to deal with evaporation is simply to have a droplet launcher with distilled water available, and to monitor drop size. Should the drop shrink sufficiently, we launch a few tens of nanoliters of distilled water to re-grow the drop.

Of greater concern is maintenance of thermal equilibrium. The support gas bathes the levitated drop, but evaporation will cool the drop, and reactions may either heat or cool the drop depending on the endo- or exothermicity of the reaction. Heat transfer from gases to liquids is inefficient. Circulation, or acoustic streaming in the drop, enhances heat transfer compared to an otherwise similar situation in static air. Rather than attempt to model this complex situation, perhaps it is best to compute the maximum endo- or exothermicity yielding less than 1 °C temperature change in the absence of rapid heat transport i.e. for adiabatic conditions. Given the 1 calorie/gram degree or 4.184 J/g degree heat capacity of water, a reaction in a 5 μL drop would need to produce or consume no more than 20 mJ. If a reactant is present at 1 mM concentration, 5 μL contains 5 nmol; if a reaction produces or consumes 100 kJ/mol, temperature control absent heat transfer will be adequate by better than 1 order of magnitude even for instantaneous reaction. While such approximate calculations are reassuring, measurements of drop temperature as a function of time would be preferable. Fluorescence measurements have proven useful for droplets in flames [145–151], but the small temperature changes expected for biochemical reactions may prove technically challenging to follow spectroscopically.

6.3. Continuous flow vs. instantaneously-initiated reactions

Two limiting cases for studying kinetics come from continuous flow systems and pulsed or (approximately) delta function-excited systems. If reactants are provided continuously to a system, then for plug flow (same velocity everywhere across the flow system lumen), the time at which a reaction is observed is the distance downstream divided by the linear flow velocity. If flow is laminar, the velocity profile across the lumen is parabolic, so time to any given position is radially-dependent and concentration dispersion is influenced by Aris–Taylor diffusion [152]. Except for electro-osmotic flow, where there is a slip-plane between the moving solution and the conduit wall, plug flow is only a good approximation in packed tubes (common in chromatography) or in turbulent flow. Both limiting cases present problems for kinetics measurements, although continuous flow experiments date to 1923 [153] and interrupted, or stopped-flow methods to the 1940s [2,4,154,155]. For biophysical measurements, instantaneous mixing/delta function excitation is convenient, as initial rate measurements can be exploited to measure rate constants, determine the appropriateness of simple rate laws, or be employed to measure the concentration of enzymes or substrates. For studying complex reaction dynamics or asymptotic, long-term behavior, continuous flow is more appropriate. To what extent can either limiting case be realized with levitated drops?

If droplet-borne reactants are ballistically-launched into a levitated drop, using piezoelectric or electrostatic droplet launchers, drop volume becomes the sum of pre-existing drop and launched droplet almost instantaneously. Because the impingent droplet has linear momentum, conservation of momentum ensures that the merged drop will move away from the levitator axis; in turn, the merged drop has angular momentum and begins to orbit the levitator axis. If impingent droplet momentum is high enough that the levitated drop moves outside the region of adequate sound pressure, the combined drops may fall, ending any utility of the system. Our experience with electrostatically-launched drops in air has been that speed is ~1 m s^{-1}, and displacement is not a problem for launched droplets ~10% of the volume of the originally-levitated drop. Nilsson has shown videos of piezoelectrically-launched droplets spiraling into a levitated drop. In the latter case, finding $t = 0$ is difficult, but drop displacement is not a problem.

As noted in §5.1, once drops have merged, they are not immediately well-mixed. Obtaining rapid mixing in microfluidic systems of any type is challenging. Diffusion in aqueous solution can homogenize over a distance of only ~10 μm in 1 s. While some improvement over current performance may occur, we expect that mixing time ~0.1–1 s is as rapid as is likely to be feasible in ~μL collisionally-merged levitated drops.

Continuous flow, on the other hand, allows for steady flow and controlled residence time to be achieved. Enclosed flow reactors are attracting attention for some types of synthetic work, among the reasons for which is the ability to control temperature and mixing due to the high S/V ratio [156,157]. Perhaps the previously-cited work on pendant drops is the best precedent work to using ultrasonically-levitated drops as continuous flow reactors [93–95,100]. However, in pendant drops, mixing is due only to diffusion and streaming from injection of new reactant and withdrawal of drop content. Not only is mixing time a function of flow rate, but so is the rate of approach to homogeneity. In an ultrasonically-supported drop, ultrasound-induced circulation augments mixing and homogenization. It is not sufficient, in most cases, to simply create homogeneity and expect it to be maintained; spontaneous pattern formation can occur in any system where different components diffuse at different rates so that no single value of the Damköhler number [158] is appropriate for comparing the rate of chemical reaction to mass transfer [115,159,160]. Thus, the stream driven by ultrasound makes a levitated drop a better-mixed, more-nearly-ideal CSTR than a pendant drop of the same size, fed at the same flow rate. To date, we know of no examples of levitated drops used as CSTRs but have plans to do so.

6.4. Mass, momentum, and thermal mixing

When droplets are injected into drops, mass, momentum, and energy of course must be conserved. If the kinetic energy of an impinging droplet is too great, secondary droplets may be ejected from the original drop, carrying with them reactants and solvent. We have found (article in preparation) that a 700 nL droplet impinging on a 3 μL drop at ~1 m s^{-1} does not produce secondary droplets, but it does cause distortions in drop shape 180° from the point of entry, suggesting that higher impinging speed for drop and droplet

of these sizes may well lead to release of secondary, or spalled, droplets.

Conservation of momentum means that when a ballistic-launched droplet merges with a levitated drop, the combined drops will move in the direction of the center of mass of the pair of drops. For the example started in the previous paragraph, the initial speed of the merged drop is ~0.19 m s^{-1}. Viscous drag and the potential well created by the sound field both act to slow the merged drop. We have seen the lateral motion of the drop halt over a distance of a few millimeters and in <10 ms. In the process of slowing, both momentum and energy dissipate into several modes. The merged drops have angular momentum with respect to the levitator axis, and so start orbiting that axis. Frictional losses in the drop convert linear momentum into oscillations in drop shape and, eventually, heat. If all the kinetic energy in the entering droplet were converted to heat, the temperature rise would be approximately 23 μK. Thus, so long as the droplet and drop are initially at the same temperature, they will remain so. Drop motion actually damps over an extended period. While we see mixing over a period of a few seconds, the drop continues to orbit the axis at a radius greater than the drop radius for >5 s. One thus expects that measurements of rapid kinetics will require tracking mirrors to keep the drop aligned with collection optics. While we have started designing such a tracking system, and there are analogies in adaptive optics for astronomy and defense applications, we do not yet have an example assembled.

6.5. Protein behavior at gas/liquid interfaces

Proteins are, to varying degrees, hydrophobic. At an air–water interface, one would thus expect the hydrophobic portions of the molecules to favor the air side of the interface, with hydrophilic portions remaining in solution, away from the interface. Such behavior was first suspected in the 19th century and understood in structural terms no later than 1938 [161]. Graham and Phillips showed that the structural changes at the gas/liquid interface took place over a period of hours, and that the kinetics were dependent on the specific chemistry of each protein [162,163]. Microscopic details awaited the availability of intense collimated X-ray sources, e.g., the work of Gidalevitz, Huang, and Rice [164]. Sum-frequency generation now provides a convenient means of characterizing interfacial protein films [165–169]. Whether denaturation at the gas/liquid interface is a significant problem will likely depend on experiment duration, rigidity of any enzyme active site, and the hydrophilicity of the protein. One may expect that some proteins will misfold upon first exposure to the interface, while others will be inert to rearrangement over hours to days. In advocating the exploration of drops for studying biochemical processes, we have clearly gambled that enough proteins will be sufficiently stable that useful results will be obtained. The relative freedom from radical-radical annihilation at the gas–liquid interface is in competition with protein denaturation as influences on the validity of experiments. In unpublished experiments, we found that lactate dehydrogenase and horseradish peroxidase maintain the same activity in 10 μL sessile drops over a period of 1 h as they have in bulk solution. Alcohol dehydrogenase, however, loses half its activity in 15 min. Our work is direct to study of myeloperoxidase, as this enzyme is known to generate and consume free radicals (e.g. [170–183]), and the enzyme is positively charged at biological pH.

6.6. Detection

Microreactors are useful only if the species produced or consumed in the drop can be monitored. Methods employed to date include optical absorption [184,185], fluorescence [90,114], chemiluminescence [114], Raman scattering [80,81,186], mass spectrometry [187–189], X-ray diffraction [190], capillary electrophoresis [11], and liquid chromatography [11]. The last of these typically employ the distil end of the injector or capillary to "swallow" the drop, and the injected portion of the drop is analyzed in a manner similar to the common uses of the separation techniques. We are developing microprobes, so we can do amperometric measurements in drops [191]. For reviews of earlier measurements, see the review by Santesson and Nilsson [102].

6.7. Automation and flexibility: speed of sound, dimensions

When our group became interested in drop levitation, we found that little attention was paid in the literature to automating the process of setting the transducer resonance frequency and matching the air gap to the transducer for mutual resonance. This meant that at the start of each workday and after any significant changes in temperature, humidity, or atmospheric pressure, manual adjustments were necessary to maintain levitation. Such temperamental behavior is aggravating, and dissuades anyone focused on applications rather than levitation from using acoustically-levitated drops for biophysical measurements. We have been developing servocontrols that are still not completely reliable. While knowledge of the dispersion relationship for humid air [140] certainly helps, the zeroing of the transducer/reflector spacing is challenging for non-planar reflectors, and the levitated drops influence the optimum spacing, particularly for high Q, well-tuned resonators [138]. We continue efforts in this area. Adoption of servocontrol, automated injection of reactants, and automation of optical alignment are developments that will be essential if drop levitators are to become part of the standard armamentarium of the biophysicist or biochemist.

7. Chemical oscillations and levitated drops

Our motivation in devising levitated drop reactors was to allow study of oscillatory biochemical reactions. Such reactions require a network of interacting species, with feedback and feed-forward dynamics to couple fundamental reactions into a non-steady-state network. Characterization of such networks requires that either transient oscillations be monitored while the system is far from equilibrium, or that the system be maintained far from equilibrium by continuous feed of reactants and removal of products [192,193]. As has already been noted, continuous feed conflicts with the constraints of many biological problems where the amounts of protein or nucleic acid available may be severely constrained. How might levitated drops help study such systems? We will suggest two scenarios for study of myeloperoxidase as referenced in §6.5:

1) Semi-batch reactor: the only experiment in which myeloperoxidase catalyzed an oscillating reaction was that reported by Brasen et al. [194]. They carried out the reaction in a quartz cuvette 1 cm^2, buffer volume 1.2 mL. Myeloperoxidase was estimated by optical absorption to be 1.5–2 μM, or approximately 2 nmol. With a molecular weight of 1.46×10^5 Da, this is 300 μg. This heroic experiment, one which would rapidly bankrupt even well-funded groups if carried out once daily, resulted in 8 transient oscillations in $[O_2]$ and concentration of various enzyme redox forms over a period of 2 h. The surface area to volume ratio was (approximately) 5 cm^{-1} of liquid/solid interface and 1 cm^{-1} of liquid/gas interface. Free radical recombination at the solid/liquid interface may have been significant [37], while oxygen transfer was limited to 1/6th of the total surface area. Suppose that, instead, the reaction had been carried out in a 5 μL levitated drop. Surface area is 0.14 cm^2, all liquid/gas interface. Surface area to volume ratio is 28 cm^{-1}, with no solid surfaces at which radicals can recombine. Instead of enzyme absorbance at 360 nm being ~0.6 and ~0.1 at other wavelengths shown in Fig. 3 of [194], the absorbance

would drop to 0.06 and 0.01, well within limits for useful measurement on the relevant time scale. Further, only 1.5 μg of enzyme would have been required, a reduction of two orders of magnitude in cost. A limitation of this arrangement is that no Clark oxygen electrode would be present, so monitoring [O_2] would need to be performed either by a fluorescence lifetime measurement or by microfabricating a Clark electrode, reintroducing a small solid/liquid interfacial region. Mass transfer between gas and liquid phases would be facile, and the drop would be well-stirred on the time-scale of the reaction. Damped reaction from consumption of substrates would be anticipated, but *any* semi-batch oscillatory reaction eventually damps.

2) Continuous flow stirred reactor. To sustain oscillations, one must work in an open system. A drop levitated on the end of a set of capillaries, operated as a well-mixed reactor with controlled residence time [78] but with total volume 5 μL would permit observation of sustained oscillations while also allowing introduction of perturbing substrates or inhibitors. Again, facile diffusion of O_2 from the surrounding gas would be anticipated. While some radical recombination on the capillary ends would be expected, this would be less intrusive than in a quartz cuvette. Using open-bore capillaries would mean that enzyme would be removed from the drop and so would need to be continuously re-instilled. For a residence time of 10 min (about the duration of a single oscillation), enzyme consumption would be 9 μg h^{-1}, permitting several days of experiments with the amount of enzyme consumed in the Brasen et al. [194] experiment. If dialysis membrane could be used to cap the efflux capillary so that enzyme could be retained in the drop while small molecules could still be removed without excessive pressure drop, then enzyme consumption would be no greater than in the semi-batch reactor.

From the standpoint of economy, freedom from reaction artifacts, and optical/diagnostics access, levitated drops hold great promise for the study of complex biochemical dynamics.

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References

[1] L. Michaelis, M.L. Menten, Die Kinetik der Invertinwirkung, Biochemische Zeitschrift 49 (1913) 333–369.
[2] B. Chance, The kinetics of the enzyme-substrate compound of peroxidase, Journal of Biological Chemistry 151 (1943) 553–577.
[3] B. Chance, The enzyme-substrate compounds of horseradish peroxidase and peroxides. II. Kinetics of formation and decomposition of the primary and secondary complexes, Archives of Biochemistry 22 (1949) 224–252.
[4] Q.H. Gibson, L. Milnes, Apparatus for rapid and sensitive spectrophotometry, Biochemical Journal 91 (1964) 161–172.
[5] D.W. Hatcher, N.G. Anderson, GeMSAEC: new analytic tool for clinical chemistry total serum protein with the biuret reaction, American Journal of Clinical Pathology 52 (1969) 645–651.
[6] N.G. Anderson, Basic principles of fast analyzers, American Journal of Clinical Pathology 53 (5) (1970) 778–785 (Pt. 2.).
[7] J.W. Frazer, D.J. Balaban, J.L. Wang, Simulation as an aid to experimental design, Analytical Chemistry 55 (1983) 904–910.
[8] D.J. Harrison, A. Manz, Z. Fan, H. Luedi, H.M. Widmer, Capillary electrophoresis and sample injection systems integrated on a planar glass chip, Analytical Chemistry 64 (1992) 1926–1932.
[9] A. Manz, D.J. Harrison, E.M.J. Verpoorte, J.C. Fettinger, A. Paulus, H. Lüdi, H.M. Widmer, Planar chips technology for miniaturization and integration of separation techniques into monitoring systems: capillary electrophoresis on a chip, Journal of Chromatography A 593 (1992) 253–258.
[10] D.J. Harrison, K. Fluri, K. Seiler, Z. Fan, C.S. Effenhauser, A. Manz, Micromachining a miniaturized capillary electrophoresis-based chemical analysis system on a chip, Science 261 (1993) 895–897.
[11] K. Seiler, D.J. Harrison, A. Manz, Planar glass chips for capillary electrophoresis: repetitive sample injection, quantitation, and separation efficiency, Analytical Chemistry 65 (1993) 1481–1488.
[12] S.C. Jacobson, R. Hergenröder, L.B. Koutny, R.J. Warmack, J.M. Ramsey, Effects of column geometry on the performance of microchip electrophoresis devices, Analytical Chemistry 66 (1994) 1107–1113.
[13] S.C. Jacobson, R. Hergenröder, L.B. Koutny, J.M. Ramsey, High speed separations on a microchip, Analytical Chemistry 66 (1994) 1114–1118.
[14] S.C. Jacobson, R. Hergenröder, L.B. Koutny, J.M. Ramsey, Open channel electrochromatography on a microchip, Analytical Chemistry 66 (1994) 2369.
[15] S.N. Krylov, E.A. Arriaga, Z. Zhang, N.W.C. Chan, M.M. Palcic, N.J. Dovichi, Single-cell analysis avoids sample processing bias, Journal of Chromatography. B, Biomedical Applications 741 (2000) 31–35.
[16] S.N. Krylov, D.A. Starke, E.A. Arriaga, Z. Zhang, N.W.C. Chan, M.M. Palcic, N.J. Dovichi, Instrumentation for chemical cytometry, Analytical Chemistry 72 (2000) 872–877.
[17] M. He, J.S. Edgar, G.D.M. Jeffries, R.M. Lorenz, J.P. Shelby, D.T. Chiu, Selective encapsulation of single cells and subcellular organelles into picoliter- and femtoliter-volume droplets, Analytical Chemistry 77 (2005) 1539–1544.
[18] Y. Chen, G. Xiong, E.A. Arriaga, CE analysis of the acidic organelles of a single cell, Electrophoresis 28 (2007) 2406–2415.
[19] R.D. Johnson, M. Navratil, B.G. Poe, G. Xiong, K.J. Olson, H. Ahmadzadeh, D. Andreyev, C.F. Duffy, E.A. Arriaga, Analysis of mitochondria isolated from single cells, Analytical and Bioanalytical Chemistry 387 (2007) 107–118.
[20] D.T. Chiu, Cellular manipulations in microvortices, Analytical and Bioanalytical Chemistry 387 (2007) 17–20.
[21] J.C. Gadd, C.L. Kuyper, B.S. Fujimoto, R.W. Allen, D.T. Chiu, Sizing subcellular organelles and nanoparticles confined within aqueous droplets, Analytical Chemistry 80 (2008) 3450–3457.
[22] D.T. Chiu, R.M. Lorenz, Chemistry and biology in femtoliter and picoliter volume droplets, Accounts of Chemical Research 42 (2009) 649–658.
[23] P. Watts, S.J. Haswell, The application of microreactors to organic synthesis, Chemical Society Reviews 34 (2005) 235–246.
[24] T. Wirth, Microreactors in organic synthesis and catalysis, John Wiley and Sons, New York, 2008, p. 297.
[25] Flow Chemistry Society, http://www.flowchemistrysociety.com/index.php2011.
[26] X. Sun, R.T. Kelly, K. Tang, R.D. Smith, Ultrasensitive nanoelectrospray ionization–mass spectrometry using poly(dimethylsiloxane) microchips with monolithically integrated emitters, Analyst 135 (2010) 2296–2302.
[27] B.D. Aguda, Emergent properties of coupled enzyme reaction systems. 1. Switching and clustering behaviour, Biophysical Chemistry 61 (1996) 1.
[28] S.B. Kraines, M. Koyama, R. Batres, An emergent simulation modeling approach for discovery of knowledge on phenomena in chemical systems, Journal of Chemical Engineering of Japan 39 (2006) 1010–1027.
[29] H. Jeong, B. Tombor, R. Albert, Z.N. Oltvai, A.-L. Barabási, The large-scale organization of metabolic networks, Nature 407 (2000) 651–654.
[30] A.-L. Barabasi, Z.N. Oltvai, Network biology: understanding the cell's functional organization, Nature Reviews Genetics 5 (2004) 101–113.
[31] E. Almaas, Z.N. Oltvai, A.-L. Barabasi, Metabolic networks: structure and utilization, in: S. Vaidyanathan, G.G. Harrigan, R. Goodacre (Eds.), Metabolome Analyses, Springer, New York, 2005, pp. 243–264.
[32] S.H. Emami, R. Solovey, T.E. Hogen-Esch, Peroxide-mediated crosslinking of poly(ethylene oxide), Journal of Polymer Science Part A: Polymer Chemistry 40 (2002) 3021–3026.
[33] B. Sweryda-Krawiec, H. Devaraj, G. Jacob, J.J. Hickman, A new interpretation of serum albumin surface passivation, Langmuir 20 (2004) 2054–2056.
[34] M.W. Kamande, K.A. Fletcher, M. Lowry, I.M. Warner, Capillary electrochromatography using polyelectrolyte multilayer coatings, Journal of Separation Science 28 (2005) 710–718.
[35] R.M. Noyes, Wall effects in photochemically induced chain reactions, Journal of the American Chemical Society 73 (1951) 3039–3043.
[36] S.M. Mitrovski, R.G. Nuzzo, A passive microfluidic hydrogen–air fuel cell with exceptional stability and high performance, Lab on a Chip 6 (2006) 353–361.
[37] D.D. Lewis, M.L. Ruane, A. Scheeline, Biofilm effects on the peroxidase-oxidase reaction, The Journal of Physical Chemistry. B 110 (2006) 8100–8104.
[38] J. Berthier, Digital microfluidics in today's microfluidics, Micro-Drops and Digital Microfluidics, William Andrew Publishing, Norwich, NY, 2008, pp. 1–5.
[39] H. Song, D.L. Chen, R.F. Ismagilov, Reactions in droplets in microfluidic channels, Angewandte Chemie International Edition 45 (2006) 7336–7356.
[40] M.R. Bringer, C.J. Gerdts, H. Song, J.D. Tice, R.F. Ismagilov, Microfluidic systems for chemical kinetics that rely on chaotic mixing in droplets, Philosophical Transactions. Series A: Mathematical, Physical, and Engineering Sciences 362 (2004) 1087–1104.
[41] B. Zheng, J.D. Tice, R.F. Ismagilov, Formation of droplets of alternating composition in microfluidic channels and applications to indexing of concentrations in droplet-based assays, Analytical Chemistry 76 (2004) 4977–4982.
[42] D.L. Chen, L. Li, S. Reyes, D.N. Adamson, R.F. Ismagilov, Using three-phase flow of immiscible liquids to prevent coalescence of droplets in microfluidic channels: criteria to identify the third liquid and validation with protein crystallization, Langmuir 23 (2007) 2255–2260.
[43] C.J. Kastrup, M.K. Runyon, E.M. Lucchetta, J.M. Price, R.F. Ismagilov, Using chemistry and microfluidics to understand the spatial dynamics of complex biological networks, Accounts of Chemical Research 41 (2008) 549–558.

[44] D.T. Chiu, Interfacing droplet microfluidics with chemical separation for cellular analysis, Analytical and Bioanalytical Chemistry 397 (2010) 3179–3183.
[45] D.T. Chiu, R.M. Lorenz, G.D.M. Jeffries, Droplets for ultrasmall-volume analysis, Analytical Chemistry 81 (2009) 5111–5118.
[46] D.E. Cohen, T. Schneider, M.B. Wang, D.T. Chiu, Self-digitization of sample volumes, Analytical Chemistry 82 (2010) 5707–5717.
[47] A.E. Sgro, D.T. Chiu, Droplet freezing, docking, and the exchange of immiscible phase and surfactant around frozen droplets, Lab on a Chip 10 (2010) 1873–1877.
[48] A. Huebner, S. Sharma, M. Srisa-Art, F. Hollfelder, J.B. Edel, A.J. Demello, Microdroplets: a sea of applications? Lab on a Chip 8 (2008) 1244–1254.
[49] S.Y. Teh, R. Lin, L.H. Hung, A.P. Lee, Droplet microfluidics, Lab on a Chip 8 (2008) 198–220.
[50] M.W.L. Watson, M.J. Jebrail, A.R. Wheeler, Multilayer hybrid microfluidics: a digital-to-channel interface for sample processing and separations, Analytical Chemistry 82 (2010) 6680–6686.
[51] A. Gupta, R. Kumar, Effect of geometry on droplet formation in the squeezing regime in a microfluidic T-junction, Microfluidics and Nanofluidics 8 (2010) 799–812.
[52] J.H. Choi, S.K. Lee, J.M. Lim, S.M. Yang, G.R. Yi, Designed pneumatic valve actuators for controlled droplet breakup and generation, Lab on a Chip 10 (2010) 456–461.
[53] A.R. Wheeler, W.R. Throndset, R.J. Whelan, A.M. Leach, R.N. Zare, Y.H. Liao, K. Farrell, I.D. Manger, A. Daridon, Microfluidic device for single-cell analysis, Analytical Chemistry 75 (2003) 3581–3586.
[54] A. Wixforth, Acoustically driven planar microfluidics, Superlattices and Microstructures 33 (2003) 389–396.
[55] M. Cecchini, S. Girardo, D. Pisignano, R. Cingolani, F. Beltram, Acoustic-counterflow microfluidics by surface acoustic waves, Applied Physics Letters 92 (2008).
[56] T. Franke, A.R. Abate, D.A. Weitz, A. Wixforth, Surface acoustic wave (SAW) directed droplet flow in microfluidics for PDMS devices, Lab on a Chip 9 (2009) 2625–2627.
[57] L.Y. Yeo, J.R. Friend, Ultrafast microfluidics using surface acoustic waves, Biomicrofluidics 3 (2009) (012002/012001-012002/012023).
[58] K. Sritharan, C.J. Strobl, M.F. Schneider, A. Wixforth, Z. Guttenberg, Acoustic mixing at low Reynold's numbers, Applied Physics Letters 88 (2006) (054102/054101-054102/054103).
[59] H. Nishiyama, R. Asari, Y. Inoue, A microreactor functionalized with acoustic wave effects and a liquid phase catalytic reaction, Physical Chemistry Chemical Physics 12 (2010) 5970–5973.
[60] M.G. Pollack, R.B. Fair, A.D. Shenderov, Electrowetting-based actuation of liquid droplets for microfluidic applications, Applied Physics Letters 77 (2000) 1725–1726.
[61] M.G. Pollack, A.D. Shenderov, R.B. Fair, Electrowetting-based actuation of droplets for integrated microfluidics, Lab on a Chip 2 (2002) 96–101.
[62] H. Moon, S.K. Cho, R.L. Garrell, C.J. Kim, Low voltage electrowetting-on-dielectric, Journal of Applied Physics 92 (2002) 4080–4087.
[63] P. Paik, V.K. Pamula, M.G. Pollack, R.B. Fair, Electrowetting-based droplet mixers for microfluidic systems, Lab on a Chip 3 (2003) 28–33.
[64] C.G. Cooney, C.Y. Chen, M.R. Emerling, A. Nadim, J.D. Sterling, Electrowetting droplet microfluidics on a single planar surface, Microfluidics and Nanofluidics 2 (2006) 435–446.
[65] J. Berthier, Electrowetting Theory, Micro-Drops and Digital Microfluidics, William Andrew Publishing, Norwich, NY, 2008, pp. 161–224.
[66] V. Srinivasan, V.K. Pamula, R.B. Fair, Droplet-based microfluidic lab-on-a-chip for glucose detection, Analytica Chimica Acta 507 (2004) 145–150.
[67] B.M. Paegel, R.G. Blazej, R.A. Mathies, Microfluidic devices for DNA sequencing: sample preparation and electrophoretic analysis, Current Opinion in Biotechnology 14 (2003) 42–50.
[68] A.R. Wheeler, H. Moon, C.A. Bird, R.R.O. Loo, C.J. Kim, J.A. Loo, R.L. Garrell, Digital microfluidics with in-line sample purification for proteomics analyses with MALDI-MS, Analytical Chemistry 77 (2005) 534–540.
[69] A. Renaudin, J.P. Sozanski, B. Verbeke, V. Zhang, P. Tabourier, C. Druon, Monitoring SAW-actuated microdroplets in view of biological applications, Sensors and Actuators B: Chemical 138 (2009) 374–382.
[70] A. Qi, L. Yeo, J. Friend, J. Ho, The extraction of liquid, protein molecules and yeast cells from paper through surface acoustic wave atomization, Lab on a Chip 10 (2010) 470–476.
[71] A. Renaudin, P. Tabourier, J.C. Camart, C. Druon, Surface acoustic wave two-dimensional transport and location of microdroplets using echo signal, Journal of Applied Physics 100 (2006).
[72] A. Renaudin, P. Tabourier, V. Zhang, J.C. Camart, C. Druon, SAW nanopump for handling droplets in view of biological applications, Sensors and Actuators B: Chemical 113 (2006) 389–397.
[73] A.S. Qi, J.R. Friend, L.Y. Yeo, D.A.V. Morton, M.P. McIntosh, L. Spiccia, Miniature inhalation therapy platform using surface acoustic wave microfluidic atomization, Lab on a Chip 9 (2009) 2184–2193.
[74] D. Mark, S. Haeberle, G. Roth, F. von Stetten, R. Zengerle, Microfluidic lab-on-a-chip platforms: requirements, characteristics and applications, Chemical Society Reviews 39 (2010) 1153–1182.
[75] S. Haeberle, R. Zengerle, Microfluidic platforms for lab-on-a-chip applications, Lab on a Chip 7 (2007) 1094–1110.
[76] E.S. Kirkor, A. Scheeline, Horseradish peroxidase adsorption on silica surfaces as an oscillatory dynamical behavior, The Journal of Physical Chemistry. B 105 (2001) 6278–6280.
[77] V. Lvovich, A. Scheeline, Simultaneous superoxide and hydrogen peroxide detection in peroxidase/NADH oscillator, Analytica Chimica Acta 354 (1997) 315–323.
[78] C.G. Hocker, I.R. Epstein, K. Kustin, K. Tornheim, Glycolytic pH oscillations in a flow reactor, Biophysical Chemistry 51 (1994) 21–35.
[79] E. Welter, B. Neidhart, Acoustically levitated droplets — a new tool for micro and trace analysis, Fresenius' Journal of Analytical Chemistry 357 (1997) 345–350.
[80] C. Esen, T. Kaiser, G. Schweiger, Raman investigation of photopolymerization reactions of single optically levitated microparticles, Applied Spectroscopy 50 (1996) 823–828.
[81] T. Kaiser, G. Roll, G. Schweiger, Investigation of coated droplets in an optical trap: Raman-scattering, elastic-light-scattering, and evaporation characteristics, Applied Optics 35 (1996) 5918–5924.
[82] S.K. Chung, E.H. Trinh, Containerless protein crystal growth in rotating levitated drops, Journal of Crystal Growth 194 (1998) 384–397.
[83] M. Przyborowski, T. Hibiya, M. Eguchi, I. Egry, Surface-tension measurement of molten silicon by the oscillating drop method using electromagnetic-levitation, Journal of Crystal Growth 151 (1995) 60–65.
[84] K. Schaefers, M.G. Frohberg, Electromagnetic levitation and its application in high temperature calorimetry, Thermochimica Acta 271 (1996) 75–84.
[85] E. Gorges, L.M. Racz, A. Schillings, I. Egry, Density measurements on levitated liquid metal droplets, International Journal of Thermophysics 17 (1996) 1163–1172.
[86] R. Eberhardt, B. Neidhart, Acoustic levitation device for sample pretreatment in microanalysis and trace analysis, Fresenius' Journal of Analytical Chemistry 365 (1999) 475–479.
[87] J.R. Gao, C.D. Cao, B. Wei, Containerless processing of materials by acoustic levitation, in: D. Schwabe (Ed.), Gravitational Effects in Materials and Fluid Sciences, 1999, pp. 1293–1297.
[88] S. Santesson, M. Andersson, E. Degerman, T. Johansson, J. Nilsson, S. Nilsson, Airborne cell analysis, Analytical Chemistry 72 (2000) 3412–3418.
[89] V. Vandaele, P. Lambert, A. Delchambre, Non-contact handling in microassembly: acoustical levitation, Precision Engineering - Journal of the International Societies for Precision Engineering and Nanotechnology 29 (2005) 491–505.
[90] D.D. Weis, J.D. Nardozzi, Enzyme kinetics in acoustically levitated droplets of supercooled water: a novel approach to cryoenzymology, Analytical Chemistry 77 (2005) 2558–2563.
[91] F. Priego-Capote, L. de Castro, Ultrasound-assisted levitation: lab-on-a-drop, TrAC 25 (2006) 856–867.
[92] C.R. Field, A. Scheeline, Design and implementation of an efficient acoustically levitated drop reactor for *in stillo* measurements, The Review of Scientific Instruments 78 (2007) 8.
[93] H. Liu, P.K. Dasgupta, A renewable liquid droplet as a sampler and a windowless optical cell. Automated sensor for gaseous chlorine, Analytical Chemistry 67 (1995) 4221–4228.
[94] H.H. Liu, P.K. Dasgupta, A liquid drop: a windowless optical cell and a reactor without walls for flow injection analysis, Analytica Chimica Acta 326 (1996) 13–22.
[95] H.H. Liu, P.K. Dasgupta, A liquid drop: what is it good for? Microchemical Journal 57 (1997) 127–136.
[96] M.D. Barnes, W.B. Whitten, J.M. Ramsay, Detecting single molecules in liquids, Analytical Chemistry 67 (1995) 418A–423A.
[97] R. Feynman, There's Plenty of Room at the Bottom, http://media.wiley.com/product_data/excerpt/53/07803108/0780310853.pdf1959.
[98] M.J. Bogan, G.R. Agnes, MALDI-TOF-MS analysis of droplets prepared in an electrodynamic balance: “wall-less” sample preparation, Analytical Chemistry 74 (2002) 489–496.
[99] M.J. Bogan, G.R. Agnes, Wall-less sample preparation of [mu]m-sized sample spots for femtomole detection limits of proteins from liquid based UV-MALDI matrices, Journal of the American Society for Mass Spectrometry 15 (2004) 486–495.
[100] H.H. Liu, P.K. Dasgupta, Analytical chemistry in a drop. Solvent extraction in a microdrop, Analytical Chemistry 68 (1996) 1817–1821.
[101] M. Petersson, J. Nilsson, L. Wallman, T. Laurell, J. Johansson, S. Nilsson, Sample enrichment in a single levitated droplet for capillary electrophoresis, Journal of Chromatography. B: Biomedical Sciences and Applications 714 (1998) 39–46.
[102] S. Santesson, S. Nilsson, Airborne chemistry: acoustic levitation in chemical analysis, Analytical and Bioanalytical Chemistry 378 (2004) 1704–1709.
[103] A. Omrane, S. Santesson, M. Alden, S. Nilsson, Laser techniques in acoustically levitated micro droplets, Lab on a Chip 4 (2004) 287–291.
[104] O. Rohling, C. Weitkamp, B. Neidhart, Experimental setup for the determination of analytes contained in ultrasonically levitated drops, Fresenius' Journal of Analytical Chemistry 368 (2000) 125–129.
[105] A.V. Anilkumar, C.P. Lee, T.G. Wang, Stability of an acoustically levitated and flattened drop — an experimental-study, Physics of Fluids. A, Fluid Dynamics 5 (1993) 2763–2774.
[106] C.P. Lee, A.V. Anilkumar, T.G. Wang, Static shape and instability of an acoustically levitated liquid-drop, Physics of Fluids. A, Fluid Dynamics 3 (1991) 2497–2515.
[107] A.L. Yarin, M. Pfaffenlehner, C. Tropea, On the acoustic levitation of droplets, Journal of Fluid Mechanics 356 (1998) 65–91.
[108] J.R. Lister, A.B. Thompson, A. Perriot, L. Dechemin, Shape and stability of axisymmetric levitated viscous drops, Journal of Fluid Mechanics 617 (2008) 167–185.
[109] C. Shen, W. Xie, B. Wei, Digital Image processing of sectorial oscillations for acoustically levitated drops and surface tension measurements, Science China Physics, Mechanics and Astronomy (2010) 7 (ASAP-online).
[110] C.L. Shen, W.J. Xie, B. Wei, Parametrically excited sectorial oscillation of liquid drops floating in ultrasound, Physical Review. E, Statistical, Nonlinear, and Soft Matter Physics 81 (2010) (046305/046301-046305/046306).
[111] C.L. Shen, W.J. Xie, Z.L. Yan, B. Wei, Internal flow of acoustically levitated drops undergoing sectorial oscillations, Physics Letters A 374 (2010) 4045–4048.
[112] E.H. Trinh, R.G. Holt, D.B. Thiessen, The dynamics of ultrasonically levitated drops in an electric field, Physics of Fluids 8 (1996) 43–61.

[113] W.J. Xie, B. Wei, Dependence of acoustic levitation capabilities on geometric parameters, Physical Review. E, Statistical, Nonlinear, and Soft Matter Physics 66 (2002) (026605-026601 - 026605-026611).
[114] Z.N. Pierre, C.R. Field, A. Scheeline, Sample handling and chemical kinetics in an acoustically levitated drop microreactor, Analytical Chemistry 81 (2009) 8496–8502.
[115] J.B. Butt, Reaction Kinetics and Reactor Design, 2nd ed., rev. and expanded. ed. M. Dekker, New York, 2000.
[116] D.L. Olson, A. Scheeline, The peroxidase-NADH biochemical oscillator: experimental system, control variables, and oxygen mass transport, Analytica Chimica Acta 283 (1993) 703–717.
[117] D.L. Olson, A. Scheeline, The peroxidase-NADH biochemical oscillator. 1. Examination of oxygen mass transport, the effect of light, and the role of methylene blue, Journal of Physical Chemistry 99 (1995) 1204–1211.
[118] D.L. Olson, A. Scheeline, The peroxidase-NADH biochemical oscillator. 2. examination of the roles of hydrogen peroxide and superoxide, Journal of Physical Chemistry 99 (1995) 1212–1217.
[119] D.L. Olson, E.P. Williksen, A. Scheeline, An experimentally-based model of the peroxidase-NADH biochemical oscillator: an enzyme-mediated chemical switch, Journal of the American Chemical Society 117 (1995) 2–15.
[120] W.-C. Choi, E. Chainani, K.T. Ngo, A. Scheeline, O. Masha, Convective Mixing in Ultrasonically-levitated, Drops (in preparation).
[121] K.T. Ngo, W.-C. Choi, E. Chainani, A. Scheeline, Ballistics of Electrostatically-launched Droplets, (in preparation).
[122] N.E. Meagher, D.B. Rorabacher, Mathematical treatment for very rapid second-order reversible kinetics as measured by stopped-flow spectrophotometry with corrections for the cell concentration gradient, Journal of Physical Chemistry 98 (1994) 12590–12593.
[123] B.C. Dunn, N.E. Meagher, D.B. Rorabacher, Resolution of stopped-flow kinetic data for second-order reactions with rate constants up to 10^8 M^{-1} s^{-1} involving large concentration gradients, experimental comparison using three independent approaches, Journal of Physical Chemistry 100 (1996) 16925–16933.
[124] L.D. Landau, E.M. Lifshitz, Fluid Mechanics, Pergamon Press, 1959.
[125] E.H. Trinh, J.L. Robey, experimental study of streaming flows associated with ultrasonic levitators, Physics of Fluids 6 (1994) 3567–3579.
[126] H. Zhao, S.S. Sadhal, E.H. Trinh, Internal circulation in a drop in an acoustic field, Journal of the Acoustical Society of America 106 (1999) 3289–3295.
[127] Y. Abe, S. Yamada, K. Aoki, Study on internal flow and surface deformation of large droplet levitated by ultrasonic wave, Annals of the New York Academy of Sciences 1077 (2006) 49–62.
[128] E. Gross-Hardt, A. Amar, S. Stapf, B. Bluemich, A. Pfennig, Flow dynamics measured and simulated inside a single levitated droplet, Industrial and Engineering Chemistry Research 45 (2006) 416–423.
[129] Y. Abe, Y. Yamamoto, D. Hyuga, K. Aoki, A. Fujiwara, Interfacial stability and internal flow of a levitated droplet, Microgravity Science and Technology 19 (2007) 33–34.
[130] Y. Yamamoto, Y. Abe, A. Fujiwara, K. Hasegawa, K. Aoki, Internal flow of acoustically levitated droplet, Microgravity Science and Technology 20 (2008) 277–280.
[131] K. Hasegawa, A. Abe, A. Kaneko, Y. Yamamoto, K. Aoki, Visualization measurement of streaming flows associated with a single-acoustic levitator, Microgravity Science and Technology 21 (2009) S9–S14.
[132] Z.L. Yan, W.J. Xie, B. Wei, Vortex flow in acoustically levitated drops, Physics Letters A 375 (2011) 3306–3309.
[133] J. Li, P. Liu, H. Ding, W. Cao, Nonlinear restoring forces and geometry influence on stability in near-field acoustic levitation, Journal of Applied Physics 109 (2011) (084518/084511-084518/084515).
[134] Y.J. Lu, W.J. Xie, B. Wei, Multiple-lobed bifurcation of rotating liquid drops levitated by ultrasound, Journal of Applied Physics 107 (2010) (014909/014901-014909/014908).
[135] H. Watada, A.E. Hamielec, A.I. Johnson, A theoretical study of mass transfer with chemical reaction in drops, Canadian Journal of Chemical Engineering 48 (1970) 255–261.
[136] A.L. Yarin, D.A. Weiss, G. Brenn, D. Rensink, Acoustically levitated drops: drop oscillation and break-up driven by ultrasound modulation, International Journal of Multiphase Flow 28 (2002) 887–910.
[137] E.H. Trinh, Compact acoustic levitation device for studies in fluid dynamics and material science in the laboratory and microgravity, The Review of Scientific Instruments 56 (1985) 2059–2065.
[138] H. Hatano, Axisymmetric analysis of a tube-type acoustic levitator by a finite element method, IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control 41 (1994) 772–777.
[139] W.J. Xie, B. Wei, Temperature dependence of single-axis acoustic levitation, Journal of Applied Physics 93 (2003) 3016–3021.
[140] O. Cramer, The variation of the specific heat ratio and the speed of sound in air with temperature, pressure, and CO_2 concentration, Journal of the Acoustical Society of America 93 (1993) 2510–2516.
[141] M.A.B. Andrade, F. Buiochi, J.C. Adamowski, Finite element analysis and optimization of a single-axis acoustic levitator, IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control 57 (2010) 469–479.
[142] A.L. Yarin, G. Brenn, O. Kastner, D. Rensink, C. Tropea, Evaporation of acoustically levitated droplets, Journal of Fluid Mechanics 399 (1999) 151–204.
[143] N.C. Clampitt, G.M. Hieftje, Investigation into the mechanism of desolvation of sample droplets in flame spectrometry, Analytical Chemistry 44 (1972) 1211–1218.
[144] S. Borosa, M. Kareth, Petermann, Apparatur zur Untersuchung der Stofftransportmechanismen an Schwebenden Tropfen unter Erhöhten Drücken, Chemie Ingenieur Technik 84 (2012) 1–5.
[145] A.M. Murray, L.A. Melton, Fluorescence methods for determination of temperature in fuel sprays, Applied Optics 24 (1985) 2783–2787.
[146] H.E. Gossage, L.A. Melton, Fluorescence thermometers using intermolecular exciplexes, Applied Optics 26 (1987) 2256–2259.
[147] T.R. Hanlon, L.A. Melton, Fuel droplet temperature using a modified exciplex fluorescence thermometry system, Chemical Physics of Processes of Combustion (1990) (113/111-113/114).
[148] M.R. Wells, L.A. Melton, Temperature measurements of falling droplets, Journal of Heat Transfer 112 (1990) 1008–1013.
[149] T.R. Hanlon, L.A. Melton, Exciplex fluorescence thermometry of falling hexadecane droplets, Journal of Heat Transfer 114 (1992) 450–457.
[150] J. Zhang, L.A. Melton, Potential systematic errors in droplet temperatures obtained by fluorescence methods, Journal of Heat Transfer 115 (1993) 325–331.
[151] Q.-Z. Lu, L.A. Melton, Measurement of transient temperature field within a falling droplet, AIAA Journal 38 (2000) 95–101.
[152] R. Aris, On the dispersion of a solute in a fluid flowing through a tube, Proceedings of the Royal Society of London Series A 23 (1956) 67–77.
[153] H. Hartridge, F.J.W. Roughton, A method of measuring the velocity of very rapid chemical reactions, Proceedings of the Royal Society of London Series A 104 (1923) 376–394.
[154] B. Chance, The accelerated-flow method for rapid reactions. I. Analysis, Journal of the Franklin Institute 229 (1940) 455–476 (613-640).
[155] B. Chance, The accelerated-flow method for rapid reactions. II. Design, construction, and tests, Journal of the Franklin Institute 229 (1940) 737–766.
[156] J.P. McMullen, K.F. Jensen, Integrated microreactors for reaction automation: new approaches to reaction development, Annual Review of Analytical Chemistry 3 (2010) 19–42.
[157] C. Wiles, P. Watts, High-throughput organic synthesis in microreactors, Advances in Chemical Engineering 38 (2010) 103–194.
[158] D.F. Boucher, G.E. Alves, Dimensionless numbers, Chemical Engineering Progress 55 (1959) 55–64.
[159] A. Hunding, G.D. Billing, Spontaneous pattern formation in spherical nonlinear reaction-diffusion systems: selection rules favor the bipolar "mitosis" pattern, Journal of Chemical Physics 75 (1981) 486–488.
[160] K.B. Yatsimirskii, L.N. Zakrevskaya, P.E. Strizhak, E.V. Rybak-Akimova, New oscillation reactions and pattern formation in dioxygen systems, Chemical Physics Letters 186 (1991) 15–18.
[161] H. Neurath, H.B. Bull, The surface activity of proteins, Chemical Reviews 23 (1938) 391.
[162] D.E. Graham, M.C. Phillips, Proteins at liquid interfaces: I. Kinetics of adsorption and surface denaturation, Journal of Colloid and Interface Science 70 (1979) 403–414.
[163] D.E. Graham, M.C. Phillips, Proteins at liquid interfaces: III. Molecular structures of adsorbed films, Journal of Colloid and Interface Science 70 (1979) 427–439.
[164] D. Gidalevitz, Z. Huang, S.A. Rice, Protein folding at the air–water interface studies with X-ray reflectivity, Proceedings of the National Academy of Sciences of the United States of America 96 (1999) 2608–2611.
[165] J. Wang, S.M. Buck, Z. Chen, The effect of surface coverage on conformation changes of bovine serum albumin molecules at the air-solution interface detected by sum frequency generation vibrational spectroscopy, Analyst 28 (2003) 773–778.
[166] P. Sen, S. Yamaguchi, T. Tahara, New insight into the surface denaturation of proteins: electronic sum frequency generation study of cytochrome *c* at water interfaces, The Journal of Physical Chemistry. B 112 (2008) 13473–13475.
[167] G. Ma, J. Liu, L. Fu, E.C.Y. Yan, Probing water and biomolecules at the air–water interface with a broad bandwidth vibrational sum frequency generation spectrometer from 3800 to 900 cm^{-1}, Applied Spectroscopy 63 (2009) 528–537.
[168] L. Fu, G. Ma, E.C.Y. Yan, In situ misfolding of human islet amyloid polypeptide at interfaces probed by vibrational sum frequency generation, Journal of the American Chemical Society 132 (2010) 5405–5412.
[169] L. Fu, J. Liu, E.C.Y. Yan, Chiral sum frequency generation spectroscopy for characterizing protein secondary structures at interfaces, Journal of the American Chemical Society 133 (2011) 8094–8097.
[170] C.C. Winterbourn, A.J. Kettle, Radical–radical reactions of superoxide: a potential route to toxicity, Biochemical and Biophysical Research Communications 305 (2003) 729–736.
[171] V.F. Ximenes, S.D. Silva, M.R. Rodrigues, L.H. Catalani, G.J. Maghzal, A.J. Kettle, A. Campa, Superoxide-dependent oxidation of melatonin by myeloperoxidase, Journal of Biological Chemistry 280 (2005) 38160–38169.
[172] C.C. Winterbourn, M.B. Hampton, J.H. Livesey, A.J. Kettle, Modeling the reactions of superoxide and myeloperoxidase in the neutrophil phagosome. Implications for microbial killing, Journal of Biological Chemistry 281 (2006) 39860–39869.
[173] A.J. Kettle, R.F. Anderson, M.B. Hampton, C.C. Winterbourn, Reactions of superoxide with myeloperoxidase, Biochemistry 46 (2007) 4888–4897.
[174] A.J. Kettle, M.S. Coker, A.L.P. Chapman, C. Bishop, C.C. Winterbourn, It's a gas man! Ammonia chloramines and bacterial killing, Fifth International Human Peroxidase Meeting, Akaroa, NZ, 2007, p. 79.
[175] B.S. van der Veen, M.P.J. de Winther, P. Heeringa, O. Augusto, J.W. Chen, M.J. Davies, X.-L. Ma, E. Malle, P. Pignatelli, T. Rudolph, Myeloperoxidase: molecular mechanisms of action and their relevance to human health and disease, Antioxidants Redox Signaling 11 (2009) 2899–2937.
[176] D.L. Lefkowitz, J. Mone, S.S. Lefkowitz, Myeloperoxidase: the good, the bad, and the ugly, Current Immunology Reviews 6 (2010) 123–129.
[177] B.-H. Shao, M.N. Oda, J.F. Oram, J.W. Heinecke, Myeloperoxidase: an oxidative pathway for generating dysfunctional high-density lipoprotein, Chemical Research in Toxicology 23 (2010) 447–454.

[178] R.C. Allen, J.T. Stephens, Myeloperoxidase selectively binds and selectively kills microbes, Infection and Immunity 79 (2011) 474–485.
[179] M.J. Davies, Myeloperoxidase-derived oxidation: mechanisms of biological damage and its prevention, Journal of Clinical Biochemistry and Nutrition 48 (2011) 8–19.
[180] A. Klinke, C. Nussbaum, L. Kubala, K. Friedrichs, T.K. Rudolph, V. Rudolph, H.-J. Paust, C. Schröder, D. Benten, D. Lau, K. Szocs, P.G. Furtmüller, T. Meinertz, M. Sperandio, S. Baldus, Myeloperoxidase attracts neutrophils by physical forces, Blood 117 (2011) 1350–1358.
[181] K.D. Metzler, T.A. Fuchs, W.M. Nauseef, D. Reumaux, J. Roesler, I. Schulze, V. Wahn, V. Papayannopoulos, A. Zychlinsky, Myeloperoxidase is required for neutrophil extracellular trap formation: implications for innate immunity, Blood 117 (2011) 953–959.
[182] A. Parker, S.L. Cuddihy, T.G. Son, M.C.M. Vissers, C.C. Winterbourn, Roles of superoxide and myeloperoxidase in ascorbate oxidation in stimulated neutrophils and H_2O_2-treated HL60 cells, Free Radical Biology and Medicine 51 (2011) 1399–1405.
[183] A.J. Kettle, A. Maroz, G. Woodroffe, C.C. Winterbourn, R.F. Anderson, Spectral and kinetic evidence for reaction of superoxide compound I of myeloperoxidase, Free Radical Biology and Medicine 51 (2011) 2190–2194.
[184] C.R. Field, Developments in Analytical Chemistry: Acoustically Levitated Drop Reactors for Enzyme Reaction Kinetics and Single-Walled Carbon Nanotube-Based Sensors for Detection of Toxic Organic Phosphonates; Ph.D., University of Illinois at Urbana-Champaign (2009).
[185] R. Behrens, A. Scheeline, Ultrasonically Levitated Microreactors: Continuously Stirred or Shaken? FACSS, Rayleigh, NC, 2010.
[186] S. Biedasek, M. Abboud, H.-U. Moritz, A. Stammer, Online-analysis on acoustically levitated droplets, Macromolecular Symposium 259 (2007) 390–396.
[187] S.F.W. Bakhoum, G.R. Agnes, Study of chemistry in droplets with net charge before and after Coulomb explosion: ion-induced nucleation in solution and implications for ion production in an electrospray, Analytical Chemistry 77 (2005) 3189–3197.
[188] M.S. Westphall, K. Jorabchi, L.M. Smith, Mass spectrometry of acoustically levitated drops, Analytical Chemistry 80 (2008) 5847–5853.
[189] J. Leiterer, U. Panne, A.F. Thuenemann, S.M. Weidner, Container-less polymerization in acoustically levitated droplets: an analytical study by GPC and MALDI-TOF mass spectrometry, Analytical Methods 3 (2011) 70–73.
[190] J. Leiterer, F. Emmerling, A.F. Thuenemann, U. Panne, Structure analysis using acoustically levitated droplets, Analytical and Bioanalytical Chemistry 391 (2008) 1221–1228.
[191] A. Scheeline, E. Chainani, Z.N. Pierre, S.M. Markwell, Electrochemistry in an Acoustically-Levitated Drop, Pittcon Paper 160-6, Orlando, FL, 2010.
[192] R.J. Field, M. Burger, Oscillations and Travelling Waves in Chemical Systems, John Wiley and Sons, New York, 1985.
[193] I.R. Epstein, J.A. Pojman, An Introduction to Nonlinear Chemical Dynamics: Oscillations, Waves, Patterns, and Chaos, Oxford University Press, New York, 1998.
[194] J.C. Brasen, A. Lunding, L.F. Olsen, Human myeloperoxidase catalyzes an oscillating peroxidase-oxidase reaction, Archives of Biochemistry and Biophysics 431 (2004) 55–62.