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Chemical and Biological Microassays in Freely Suspended Droplets on Novel Fluidic Chips

Principal Investigator: Orlin D. Velev

*Department of Chemical Engineering, North Carolina State University
Raleigh, NC 27695*

Abstract

We developed the principles of a platform for manipulation of freely suspended micro- and nanoliter droplets for micromanipulation and microassays. The liquid transport in such chips requires very low energy input due to the lack of microchannels or solid walls present in conventional microfluidic devices. Water droplets float on the surface of a denser perfluorinated liquid and are driven by alternating or constant electric fields created by addressable arrays of electrodes immersed in the oil. The ability to manipulate droplets from suspensions, and to observe the dynamics of particles inside them revealed charge and polarization effects that affect the dynamics of the droplets and thermal gradient effects that lead to separation of the particles into either the top or bottom part of the droplet. These effects can be conducive to carrying rapid agglutination assays, as they allow pre-concentration of the particles. We demonstrated a few chemical precipitation reactions inside the droplets and observed the dynamics of live cells suspended and transported within the droplet microcarriers. The results provide insights on the potential use of the droplet microfluidic platform to parallel assays on the microscale.

May 2004

Summary of the Results

Publications

- O. D. Velev, B. G. Prevo and K. H. Bhatt, *Nature*, **426**, 515 (2003). On-chip manipulation of freely suspended droplets.

Presentations at scientific meetings, conferences and invited seminars

- Particles 2004 conference, Miami, Florida, April 2004.
- MRS Fall Meeting, Boston, MA, December 2003.
- AIChE Annual Conference, San Francisco, CA, October 2003.
- Gordon Research Conference on Supramolecules and Assemblies, Andover, NH, June 2003.
- AIChE Annual Conference, San Francisco, CA, October 2003.
- Department of Chemical Engineering, Princeton University, NJ, April 2004.
- Department of Chemical Engineering, University of California at Santa Barbara, CA, October 2003.
- Army Research Laboratory (ARL), Maryland, December 2003.

Publicity (coverage in the media)

- *Analytical Chemistry*, an ACS journal, highlights the *Nature* droplet microfluidics paper, February 2004.
- *Science Daily*, "Researchers Manipulate Tiny, Floating Droplets on A Chip", news on the droplet microfluidics work, December 2003.
- *Nanobiotech News*, a national weekly newspaper, "Researchers Create Virtual Multiwell Plate that Could Analyze Live Cells and Molecules", December 2003.
- *Technology Research News* and *Technology Review* (MIT), "Chip Uses Oil to Move Droplets", December 2003.
- *Newswise*, "Researchers Manipulate Tiny, Floating Droplets on a Chip" press release on the *Nature* droplet microfluidics paper, December 2003.
- *Small Times*, a weekly nanotechnology report, "Researchers Devise Slick Lab-on-a-Chip", December 2003.
- *Innovations Report*, a German technology alert highlights the *Nature* droplet microfluidics paper, December 2003.

- *Sensor Technology Alert*, published by the Technical Insights division of Frost & Sullivan highlights the *Nature* droplet microfluidics paper, December 2003.
- *NCSU press release* on *Nature* droplet microfluidics paper, December 2003.
- *Nature News Service* "Chemists shrink beakers into drops", highlights of the 2003 Annual ACS Meeting in New York (out of nearly 7000 papers presented) September 2003.

PI awards partially based on these results

- Sigma Xi Faculty Research Award (Sigma Xi NCSU Chapter), 2004

Financial report

- The financial status of this project generated by the NCSU accounting system (all funds spent according to the STIR rules) is attached at the end.

The microscale processing and analysis of samples containing chemical and biological agents require manipulation of microscopic volumes of liquids. Microfluidic chips promise to revolutionize many areas of analytical chemistry, chemical engineering, pharmaceuticals and biotechnology. However, the present lab-on-a-chip systems with prefabricated microchannels operate more as factories with permanently rigged pipes rather than as flexible laboratories. They are not well suited for microscale operations where multiple liquid samples are transported and mixed in arbitrary volumes and combinations. Conventional microfluidic chips with channels are also poorly suited for handling liquid suspensions containing solid particles, droplets, biological cells, or proteins. Particles or droplets may aggregate, coalesce or adhere to the walls and clog the channels. Proteins and lipids adsorb strongly onto many surfaces, while bulky DNA molecules may be delayed for entropic reasons (1). Living cells may adhere to channel walls and can be disrupted by valves or pressure build-ups.

Our project reported here is the first to achieve on-chip electric field driven manipulation of freely suspended droplets. A few techniques for manipulation of small droplets on solid surfaces by the use of interfacial tension gradients (2-4), vibration and light (5-7), electrowetting and dielectrophoresis (8-16), or scanning force microscopy (17) have been proposed and demonstrated earlier. However, any system where the droplets are in contact with solid walls has inherent drawbacks due to adsorption and fouling similar to those in conventional chips with microchannels. Solid particles, proteins or live cells are likely to pin the droplets and disrupt the chip operation by adhering to the solid surfaces. Additionally, the omnipresent contact angle hysteresis of droplets on solid surfaces increases the power dissipation.

We report here the results of the ARO-supported experiments on liquid-liquid microfluidic system for manipulation of micro- and nanoliter droplets in the freely-suspended state. This allows flexible microfluidic transport without contact with any solid surface, avoiding many of the problems with present lab-on-a-chip devices, and adding new functionality. The droplets, which may be from water or hydrocarbons, are suspended on the surface of perfluoromethyldecalin (PFMD). Perfluorinated hydrocarbons (F-oil) are inert, benign liquids with low dielectric permittivity that are more dense than water, hence the water droplets float partially submerged below the oil surface (18). Our group (18-20) and others (21-22) have earlier demonstrated that microdroplets suspended in organic and fluorocarbon oils have vast potential as compartments for synthesis of new classes of structured particles.

The key to using freely suspended droplets as microscopic carriers and reactors on fluidic chips is to develop tools for their precise and flexible manipulation, transport and mixing. The suspended droplets in our experiments were driven by alternating (AC) and/or constant (DC) electric fields applied via arrays of addressable electrodes immersed in the F-oil phase below

them (Figure 1A). The electrostatic patterns created by the electrodes allow controlled droplet motion along predetermined paths or freely in any direction. The electrodes and the electrical leads were fabricated on two-sided printed circuit boards that have the electrode patches on one side and the connecting leads on the other (Figure 2). The electrode boards were immersed inside a small dish with PFMD. Water droplets of volume 500-1000 nL were dropped by micropipette and floated suspended at the F-oil/air surface without contact with the electrodes. The droplets typically contained suspensions of micro- and nanoparticles in order to mimic the use of the common agglutination bioassays based on antibody-covered latex microspheres. In a few experiments we also used yeast cells suspended in phosphate-buffered saline (PBS) suspension. The droplets were driven with AC or DC voltages in the range of 200-600 V. The AC frequencies were in the 50-5000 Hz range. All electrodes that were not switched to the high voltage source were grounded.

First, we studied the mobility of microdroplets on fluidic chips with linear tracks (e.g. Fig. 3A,B), matrixes and mixers (e.g. Fig. 4A-D) in order to understand and optimize the electrostatic forces operating in the chips. The effect of the basic system parameters on the droplet mobility and the results are summarized in Table 1. The most reliable manipulation of water droplets was achieved by symmetric AC fields. The application of spatially inhomogeneous AC fields on particles in suspension leads to the so-called dielectrophoretic (DEP) force, F_{DEP} , which acts in the direction of the gradient of the squared electric field, ∇E^2 , and is described by the expression (23,24)

$$\vec{F}_{DEP} = 2\pi \varepsilon_1 \text{Re}[K(w)] r^3 \nabla E^2$$

where r is the radius of the particle, ε_1 is the dielectric permittivity of the media, and K is the Clausius-Mossotti factor, the effective polarizability of the particles. Dielectrophoresis has been used for manipulation of particles, biological cells and for the assembly of microscopic biosensors and microwires (25-26). The sign and magnitude of the DEP force depend on the real part of K , in this case, the effective droplet polarizability. The water droplets on our chips were always attracted along the field gradient to regions of high field intensities, as they have much higher dielectric permittivity and conductance than the F-oil.

The equilibrium position of the trapped droplets with respect to the electrodes depended on the pattern of the energized electrodes. When the electrodes were connected in sequences of two energized and two grounded ones, the droplets migrated to the gap between the energized and non-energized electrodes (Figure 1B). This positioned them in closest proximity to the area of highest field intensity. The same was observed when single electrodes at the ends of a row were energized; in that case the droplets positioned themselves above the single area of high

intensity between the first and second electrode (Figure 3A). However, when single electrodes inside rows or matrixes were energized, the droplets positioned themselves exactly above them, balancing the attractive forces from the two gaps of high intensity (Figures 1C and 3B). As long as the field was present, the droplets were firmly held in place and resisted attempts to displace them by air currents, liquid convection, or by physically agitating the chip for many hours. Thus, we have proven that the chips can be used for long-term storage and manipulation of water droplets. The trapped droplets could be moved easily by consecutively switching on and off the voltage to the electrodes in the tracks (Figures 3A and B). The maximal speed at which the droplets moved in AC mode was approximately proportional to E^2 , which is in correspondence with the above formula for F_{DEP} (Figure 5). The frequency was not found to be a controlling parameter, as the polarizability of the droplets would be weakly dependent on the frequency in the range studied. The power dissipation in the experiments was extremely low as the currents through the cell were smaller than the capacitance leaks in the circuit. We estimate that the energy required to transport freely suspended droplets ($\approx 1 \times 10^{-9}$ J/cm for typical sizes and velocities studied here) is more than two orders of magnitude lower the energy required to move the same volume of liquid as drops on solid surfaces or in microfluidic channels.

The droplets could also be moved by applying constant electrical voltages. Water droplets in DC fields were expected to behave similarly to those in AC fields of the same amplitude, as the polarization effects with static fields are similar to the ones at low AC frequencies. The droplet response to DC fields, however, was found to be very different, pointing out a variety of unexpected charge and polarization effects. Typically the droplets responded very strongly to DC fields by either moving rapidly away from the energized electrode, or by being strongly attracted towards it. The velocity of droplet motion and the range of the interactions were about two times larger than the AC-driven effects at the same voltage range, e.g. speeds as high as 2.0 mm/s were measured for 750 nL droplets driven with a negative voltage of 500 V. This points out that the droplets possess significant charge and/or dipole moments and thus respond by Coulombic repulsion or attraction. Furthermore, charging and/or re-charging effects were observed at combined AC+DC voltages. Because of these re-charging effects, the use of DC fields was found to be more difficult to control. The use of DC field however allowed manipulation of hydrocarbon oil droplets, which could be used in encapsulation of the water ones.

The liquid-liquid chips described here could become a versatile experimental tool and an easy-to-implement technological platform for microdroplet transport, mixing, and chemical and biological microassays. This potential was demonstrated in a variety of experiments:

Controlled parallel transport of many droplets. Multiple droplets can readily be transported and manipulated on chips with a large number of addressable electrodes. This holds promise for developing massively parallel assays, which may hold many miniscule samples of biological agents. We moved simultaneously on parallel tracks up to 20 droplets from suspensions of same or different nanoparticles (Figure 3AB). Finally, chips with two-dimensional matrixes of individually addressable electrodes allowed independent positioning and moving in arbitrary direction, and mixing of droplets of various compositions. Combinatorial analysis can be performed by moving large numbers of nanoliter droplets in computer-controlled sequences and analyzing the results on a droplet-by-droplet basis.

Chemical reactions and precipitations in mixed droplets. Droplets from two separate transporters were coalesced in a 1:1 ratio at the track junctions and then the combined droplets were moved further along the tracks (Figure 4A-C). When the particles inside droplets were allowed to segregate to their tops prior to mixing, intermittent anisotropic clusters of particles formed on the surface of the newly combined droplets (Figure 4D). As the fluidic chips allow massive parallelization, they can be used for automated fabrication of functional micro- and nano-assemblies such as "supraparticles" with colloidal crystal structure (18). The chips allow carrying out chemical reactions and precipitation on the microscale, mimicking certain types of chemical agent assays. A variety of mixing and precipitation experiments on track and matrix chips were performed by controllably merging pairs of droplets of different solutions. Precipitation and color change reactions included the formation of Ca_3PO_4 , CaCO_3 and $\text{Fe}(\text{OH})_2$. The complex precipitation patterns inside the mixed droplets lead to the formation of crystal shell-like balls (Figure 4E). These particles are very clearly visible and allow more precise readout of the results of chemical microassays. Such shell-like crystalline particles might also be used as biomimetic capsules and are of interest to materials science.

Particle concentration and segregation inside droplets. One of the most important biological detection methods is the sphere agglutination assay, where the positive result is the clumping and binding of the spheres covered with immunoglobulin layers. This very simple and effective assay can not be implemented in any of the conventional microfluidic devices with microchannels or droplets moved on solid surfaces. In order to achieve this, we had to understand the phenomena and develop techniques for particle pre-concentration, mixing and manipulation inside the droplets. We found a range of important and interesting effects by observing the vertical distribution of the particles dispersed inside the droplets. Charged latex microparticles inside the droplets in seconds migrated and accumulated on the side of the droplet cap that protrudes above

the oil phase (Figure 6). In droplets containing more than a few volume percent of microspheres, colored diffraction from the concentrated particle phase directly below the droplet surface was observed, proving that the particles are concentrated to the point of colloidal crystallization (Figure 3C), even though the large bottom parts of the droplets are almost free of particles (confirmed by confocal scanning microscopy, Figure 3D).

We found that this particle accumulation on the surface could be attributed to liquid evaporation from the surface. When the evaporation was stopped by increasing the water vapor humidity inside the chamber, the particles did not separate. When a thick layer of dodecane was poured on top of the F-oil, so the droplets were immersed in media with uniform dielectric constant, the particles also remained uniformly dispersed in the droplets. The extreme speed of separation (compared with the evaporation rates) and its thoroughness suggest that there is an additional driving force to purely hydrodynamic separations. We are not aware of any report of such extraordinary particle re-distribution, structuring, and crystallization resulting from internal polarization of floating droplets. We are presently working on understanding the relative contributions of two possible phenomena for this effect: thermophoresis and thermal Marangoni effect due to different interfacial tension in the (cold) droplet surface and (comparatively warmer) droplet bottoms. This effect may be particularly important when droplets are used as carriers for micro- and nanoparticles and living cells, which may thus be separated and clearly visible on the top side. It also allows pre-concentration of particles and increasing the speed of biological immunoassays.

Encapsulation and manipulation of live yeast cells. One of the major advantages of our technique is the ability to encapsulate a small number of live cells and perform analyses that study the chemical toxicity or viral activity of water borne samples. We demonstrated the capability of the method to achieve this by manipulating and observing droplets containing yeast cells in PBS. Typically the cells precipitated on the bottom of the droplets. Experiments aimed at proving the cell viability and encapsulating single cells in nanoliter droplets are under way.

Encapsulation inside oil drops. The versatility of the method also allowed developing encapsulation techniques. We manipulated both water and hydrocarbon droplets on the chips, combining them in a 1:1 ratio. When a hydrocarbon-based surfactant such as sodium dodecyl sulfate was added to the water droplets, the balance of the interfacial tensions favors the complete engulfment of the water droplet in the hydrocarbon one. The water droplets became symmetrically encapsulated inside a liquid hydrocarbon shell (Figure 4F). Due to the absence of free interface and evaporation, the particles inside such encapsulated aqueous droplets did not re-

distribute vertically. These liquid-liquid capsules could be moved by both DC and AC fields. This encapsulation process can be used for protection of the water droplets from evaporation, for their long-term storage on the chip, and for the fabrication of (e.g. polymer) capsules.

Internal mixing in oscillating droplets. Assuring quick and uniform reaction is a major problem in other microfluidic chips due to the lack of easy procedures for efficient mixing of the reagents on the microscale. We observed rapid mixing of the contents of two droplets are merged into larger one (Figure 4B,C). We believe that this mixing is enhanced by the very low tangential resistance of the fluid boundaries and by the appearance of interfacial tension gradients that pull the droplet surfaces by Marangoni effects. An additional operation that allows even faster mixing and reaction could be implemented by oscillation of the droplets when the voltage supplied to the electrodes is of very low frequency (0.5-2 Hz). Due to the polarization effects, the droplets would quickly jump away and then back every time the voltage switches from negative to positive half-periods. This oscillation allows increasing the mixing speed further. This we believe that our method is well suited for microassays also due to the quick natural or forced mixing inside the fluid droplets.

In summary, as a result of this project we have demonstrated and understood the basics of the controlled transport and manipulation of free microdroplets containing particles and/or chemical reagents for applications in biological and chemical microassays. The technique is simple, robust, easy to implement in a technological platform, and to scale down in droplet size. These chips can be particularly well-suited for confining single living cells or genetic material into individual droplet containers, and performing biochemical reactions, precipitation assays, high throughput drug or toxin screening.

Table 1. Effect of the experimental parameters on the responsiveness and mobility of the suspended microdroplets.

Factor	Range studied	Effect on droplet responsiveness
AC field	Symmetric square waves	Moves the water droplets towards areas of highest field intensity (Fig. 1A,B).
DC bias	0 - 500 V	Attraction or repulsion, followed by re-charging. Moves both water and oil droplets. Very strong, but erratic.
AC amplitude	0 - 600 V	Increases (\uparrow) proportionally to E^2
AC frequency	50 - 5000 Hz	None
Droplet volume	500 - 1500 nL	\uparrow
Distance between droplet bottom and chip surface	0.01 - 0.5 mm	\downarrow
Electrolyte in water droplets	None added - 0.1 M	(\uparrow) Small
Fluorinated or non-fluorinated surfactant added in droplet	0 - 0.1 wt. %	None
Full immersion of the droplets in overlying dodecane layer	-	$\downarrow\downarrow$ Could lead to complete loss of responsiveness
Electrode geometry	Square or circular	Square electrodes more effective at shorter drop-electrode distance, circular at larger

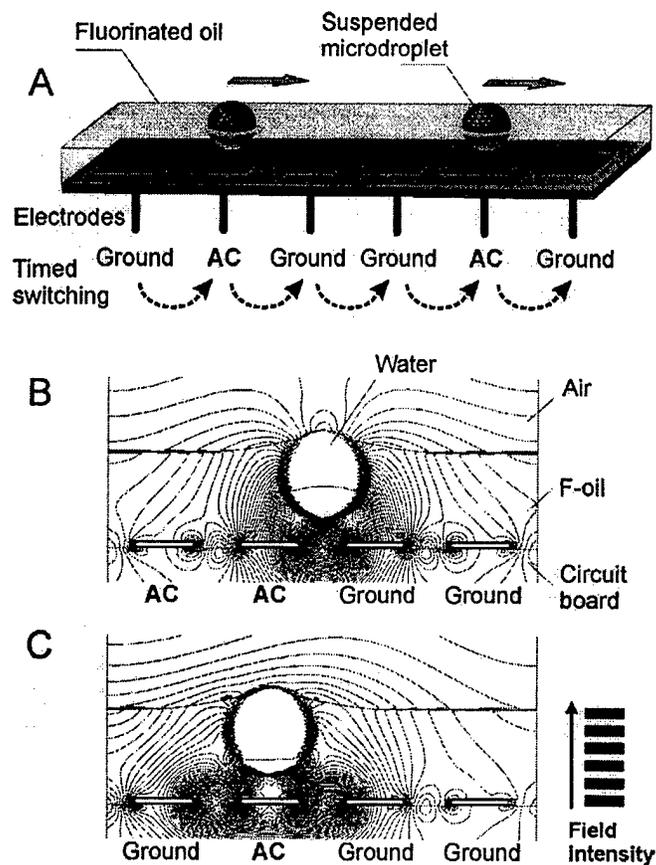


Figure 1. (A) Schematics of the operation of the dielectrophoretic transporter of microsuspended droplets. (B) Geometry of the system, equilibrium position of the droplet, and theoretically calculated intensity of the electric field, for symmetrically energized electrodes (the droplet and interface positions are approximately to scale). (C) The same for a single energized electrode in a row.

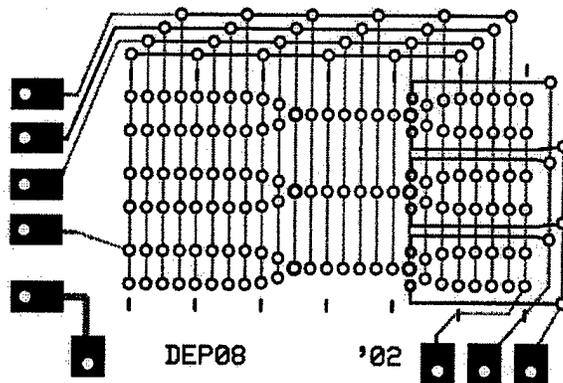


Figure 2. Example of the design of an electrode pattern where droplets are moved along tracks, mixed, and can be switched to different paths (cf. Figure 3A-C). The red and the green leads are situated on the top and bottom part of the chip, respectively, and are connected through the holes.

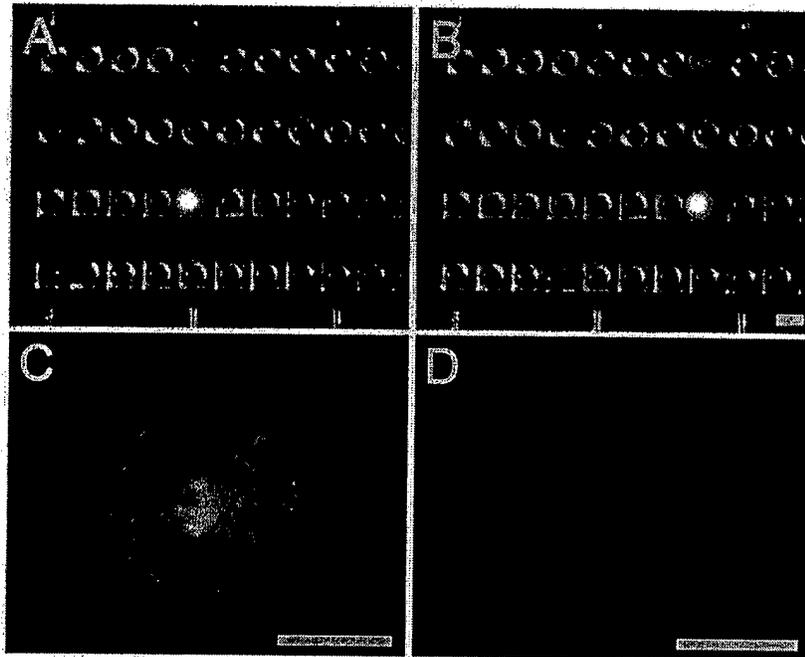


Figure 3. (A) Initial equilibrium positions of four droplets on arrays, where every fourth electrode starting from left is energized (droplets contain, top to bottom: fluorescent latex microparticles, gold nanoparticles, regular white latex, magnetic latex). Note the difference in the position of the droplets at the left end of the array (corresponding to Fig. 1B), and in the middle of the array (similar to Fig. 1C). (B) The same four droplets after three cycles of switching of the electrodes to the right. (C) Colloidal crystals formed on the upper surface of a droplet containing 20 wt.% of sulfate latex; the particles have crystallized because of the attraction to the top surface. (D) Confocal microscopy view from above of a 3D reconstruction of a droplet containing 0.2 wt% fluorescent latex; nearly all particles are concentrated on the droplet top. Scale bars: A and B = 1 mm, C and D = 500 μm .

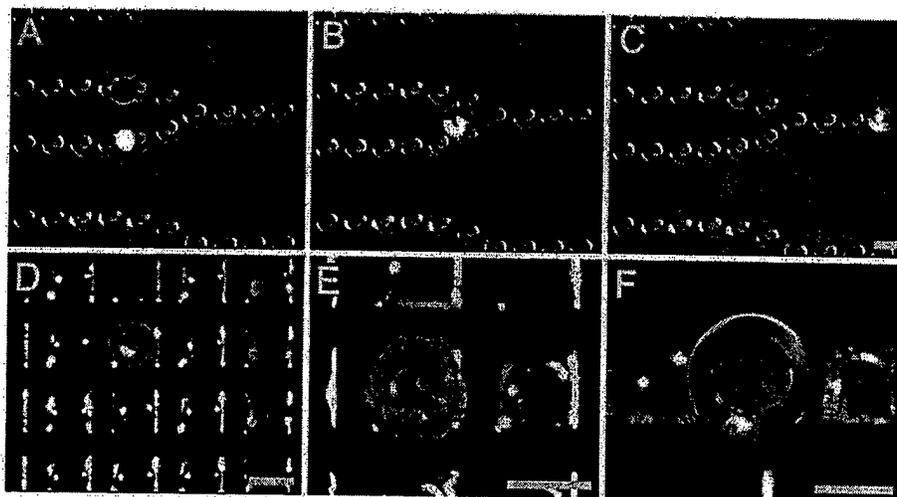


Figure 4. Mixing, precipitation and encapsulation in aqueous microdroplets suspended on a matrix fluidic chip. (A-C): Mixing of droplets containing latex microspheres (white) and gold nanoparticles (purple). (D) Two droplets containing polystyrene (white) and magnetic (brown) latex have been mixed to temporarily form anisotropic polymer aggregate. (E) A crystalline shell of calcium phosphate precipitated after mixing droplets containing solutions of Na_2HPO_4 and CaCl_2 . (F) Water droplet containing 1.0 wt% latex and 1.9 mM Na-dodecyl sulfate encapsulated inside a liquid dodecane shell. Scale bars = 1 mm.

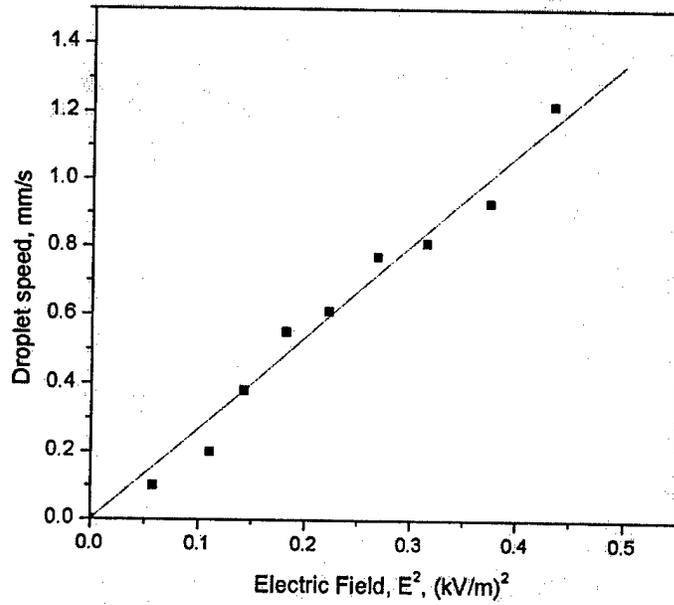


Figure 5. Droplet speed plotted as a function of the field intensity squared. The data are for 750 nL aqueous droplets submersed in a 1.15 mm deep PFMD layer. The speed was measured by the smallest time required for the droplet to traverse an automated 8-electrode sequence forwards and backwards. The field was estimated by dividing the voltage applied by the electrode pitch (1.54 mm). Frequency was 200 Hz.



Figure 6. Micrographs of particle separation in droplets caused by temperature gradient from evaporation (top) and sedimentation (bottom). The latex microspheres used are 1 μm and 4.9 μm in diameters respectively.

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