# Interface-Driven Spontaneous Differentiation-Repulsion Behavior in Isochemical Droplet Populations

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## Theoretical analysis

### Introduction and assumptions

The model described in this section was constructed to test the hypothesis that the repulsion observed in popping is a consequence of Marangoni flow maintaining an equilibrium surface concentration of the decanol present at the air-water interface, which is under constant flux due to evaporation.

We determined the velocity at which decanol moves through the monolayer by establishing a mass flux balance between Marangoni flow-mediated convection and evaporation of decanol in the surrounding air. To test the validity of this model, we compared the obtained velocity with the measured velocity of the decanol droplets that were repelled due to popping. To make such a comparison, we assumed that the velocity of the advancing decanol monolayer is equal to the velocity of the repelled droplets. We split this assumption into two parts: (i) The speed of the monolayer is roughly equal to the speed of the solvent (water) molecules; (ii) the speed of the water molecules is roughly equal to the speed of the submerged particle, i.e., decanol droplet.

For water at infinitesimally small depths, the first assumption is valid if we assume a no-slip condition.

To justify the second assumption, we note that if there would be a difference between these velocities, a drag force would be generated. Assuming Stokes' drag and using Newton's second law, we can find the acceleration that this drag force would generate:

$$F_{drag} = m_{drop}\, a = 6\,\pi\,\eta\, r_{drop}\, v_{rel}$$

Here, $v_{rel}$ is the relative velocity of the decanol droplet with respect to the surrounding water medium. Observing that $a$ is the derivative of $v_{rel}$, we can solve the differential equation to find the proportion of velocity remaining after some time *t* can be expressed as

$$\frac{v_{rel}}{v_{rel,0}} = e^{-\frac{6\,\pi\,\eta\, r_{drop}}{m_{drop}}\, t}$$

The viscosity of decanol $\eta$ is 10.9 mPa.s [28]. If we assume $r_{drop}$ = 40 µm, and use this to calculate $m_{drop}$, we can see that any relative velocity of the droplet to the surrounding medium is reduced very rapidly: for any starting value of relative velocity ($v_{rel,0}$), within 1.5 ms (5% of the frame rate), only <1% of speed remains. Therefore, the second assumption is justified.

**Deriving the model**

Based on the side-view images of the water droplets, we assume the water droplet to be shaped like a spherical cap (Fig. 1a). We can express the position of any point on the droplet surface in terms of an azimuthal angle $\psi$ and a polar angle $\varphi$. Next, we assume that a droplet pops exactly in the middle of the water droplet (the zenith position). Since the droplet is in the center, the system has circular symmetry, and therefore any velocity (or other variable) will not depend on $\psi$ (Fig. 1b).

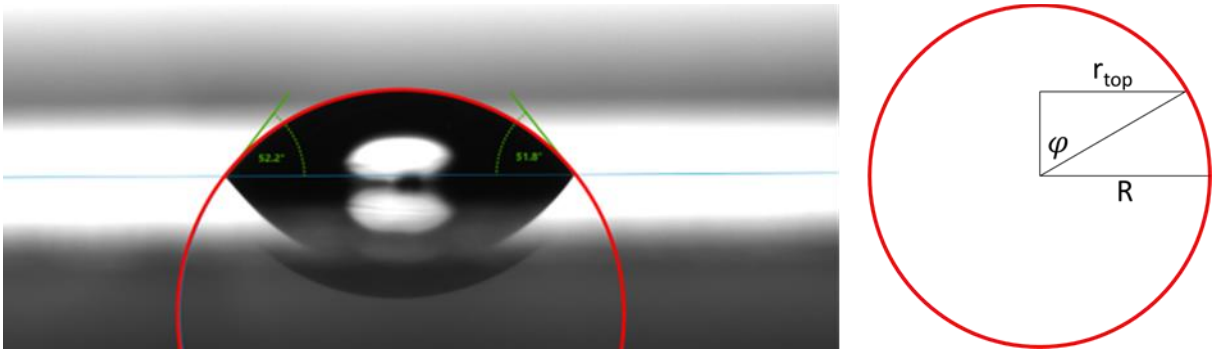


***Figure 1.*** *(a) A circle superimposed on a water droplet observed from the side. The circle fits the shape of the droplet well. (b) Schematic representation of the variables used in the derivation of a steady-state model using a spherical geometry.*

We take $\varphi_0$ to be the polar angle at the boundary of the popped droplet. For any region that is fully outside of $\varphi_0$, the steady-state assumption yields that the decanol influx should equal the decanol efflux. Next, we can draw a circle at an arbitrary angle $\varphi_1$, for which $\varphi_1 > \varphi_0$, and an angle $\varphi_2$ which marks the border of the droplet. Furthermore, we define a region 1, which is the region between $\varphi_1$ and $\varphi_2$. From

this region, the decanol enters from region 0 (the droplet) and decanol exits through evaporation and by moving to region 2 (from $\varphi_1$ to the boundary of the dish $\varphi_2$). Formally, we get the balance equation:

$$J_{0\to1} = J_{1\to2} + J_{evap,1}$$

We assume that the diffusive flux between regions is negligible, leaving only the advective flux:

$$J_{n\to n+1} = \Gamma_{\text{monolayer}} \cdot v_n \cdot C_n = \Gamma_{\text{monolayer}} \cdot v_n \cdot 2\,\pi\,R\,\sin(\varphi_n)$$

Where $\Gamma_{\text{monolayer}}$ is the concentration of decanol in a monolayer in mol/m$^2$, $v_n$ is the velocity of decanol at $\varphi_n$ in m/s, $C_n$ is the circumference of the circle at $\varphi_n$ and $R$ is the radius of the imaginary sphere of which the water droplet makes part. For the evaporation flux, we assume that evaporation from a surface is directly proportional to the area of that surface. Therefore:

$$J_{evap,1} = K_{evap} \cdot A_1 = K_{evap} \cdot 2\,\pi\,\mathrm{R}^2\,(1 - \cos(\varphi_n))$$

With $K_{evap}$ being the evaporation constant described in the results section. Filling in the equations for Marangoni flux and evaporation flux into the balance equation, we get:

$$v_1 = v_0 \cdot \frac{\sin(\varphi_0)}{\sin(\varphi_1)} - \frac{K_{evap}}{\Gamma_{\text{monolayer}}} \cdot R\,\frac{1 - \cos(\varphi_1)}{\sin(\varphi_1)}$$

To find $v_0$, we remember the steady-state assumption yield that total influx and total efflux from the air-water surface must be equal:

$$J_{0\to1} = J_{evap,total}$$

Filling in the term yields

$$v_0 = \frac{K_{evap}}{\Gamma_{\text{monolayer}}} \cdot R \cdot \frac{1 - \cos(\varphi_2)}{\sin(\varphi_0)}$$

Filling this into the equation for $v_1$ gives the final equation:

$$v_1 = R \cdot \frac{K_{evap}}{\Gamma_{\text{monolayer}}} \cdot \frac{\cos(\varphi_1) - \cos(\varphi_2)}{\sin(\varphi_1)}$$

**Fitting the model and generating predictions**

Finally, the model is fitted using a correction constant $k$ to show that the correct line shape is predicted by the model. Substituting the contact angle (θ) for $\varphi_2$, and removing the subscripts from $v_1$ and $\varphi_1$ for legibility outside this demonstration, the resulting equation is:

$$v(\varphi) = k \cdot R \cdot \frac{K_{evap}}{\Gamma_{\text{monolayer}}} \cdot \frac{\cos(\varphi) - \cos(\theta)}{\sin(\varphi)}$$

In our experiments, we will not be able to determine $\varphi$ directly. Instead, we need to express the answer in terms of $r_{top}$. We thus introduce the following transformation:

$$\varphi = \sin^{-1}\left(\frac{r_{top}}{R}\right)$$

To determine $R$, we use the special case of the edge of the droplet:

$$R = \frac{r_{water\ droplet}}{\sin(\theta)}$$

**Supplementary Video legends**

**Supplementary Video 1. Spontaneous differentiation-repulsion behavior in decanol droplets (example 1).** Spontaneous "popping" of a decanol droplet immediately leads to a repulsive behavior in the neighboring droplets. The repulsive droplet eventually moves to the edge of the water reservoir (top-right), and a new droplet differentiates, followed by the repulsive behavior of the surrounding droplets. Decanol droplets were stained with Nile red (0.01% w/v) and visualized using fluorescence microscopy.

**Supplementary Video 2. Spontaneous differentiation-repulsion behavior in decanol droplets (example 2).** Decanol droplets produced using microfluidic devices not treated with polyvinyl alcohol (PVA) also display similar popping behavior. This shows that the phenomenon is independent of the surface treatment and does not rely on any trace amounts of PVA that might be introduced during the microfluidic production. Decanol droplets were stained with Nile red (0.01% w/v) and visualized using fluorescence microscopy.

**Supplementary Video 3. Spontaneous differentiation-repulsion behavior in bulk-produced, unstained decanol droplets.** Unstained vortex-produced droplets show similar popping behavior confirming that neither Nile Red nor microfluidic production are necessary for the popping phenomenon to take place. The droplets were visualized using bright-field microscopy.

**Supplementary Video 4. Side-view imaging showing a decanol droplet entering the interface.** Side-view time-lapse imaging using a contact angle measurement set up shows a droplet entering the air-water

interface (at $t$ = 85 ms)**.** Surrounding droplets can be seen getting repelled in subsequent time frames. Decanol droplets were stained with Nile red (0.01% w/v) and visualized using bright-field microscopy.

**Supplementary Video 5. Popping-induced Marangoni flow.** A differentiated decanol droplet produces Marangoni flow, which is visualized using fluorescent beads. By shifting the focus, one can see that close to the surface, the flow moves radially outward from the popped droplet (20 s onwards). Approximately 150 µm below the surface, a compensatory backflow moves radially inwards towards the popped droplet (initial 20 s). The *z*-distance is measured relative to the interface and the interface is identified when the droplet is in sharp focus. Decanol droplets were stained with Nile red (0.01% w/v) and visualized using fluorescence microscopy.

**Supplementary Video 6. Popping frequency significantly increases in the presence of salt.** Bright-field images showing the increased popping frequency in presence of 50 mM salt (along with 30 µM decanoate). Within 10 s, all the droplets popped and reached a stable structure maintaining equidistance from each other.

**Supplementary Video 7. Marangoni tweezers.** In case of multiple successive popping events, the popped droplets can arrange equidistantly in the form of a polygon at the edge of the water droplet and pin the un-popped decanol droplets in the center. This happens due to the Marangoni flow-field around each popped droplet resulting in “Marangoni tweezers”, resembling the optical tweezers. Decanol droplets were stained with Nile red (0.01% w/v) and visualized using fluorescence microscopy.

**Supplementary Video 8. Population reorganization.** A dense polydisperse population of decanol droplets gets periodically disturbed and reorganized via popping, ultimately leading to an ordered, stable structure that resembles a biological tissue. This shows that popping events can not only lead to local repulsion, but also induce an impressive structural reorganization.

**Supplementary File 1. Microfluidic design used to produce decanol droplets.** A flow-focusing device was used to produce micron-sized decanol droplets. From left to right – water inlet, decanol inlet, and the outlet.