Better red than dead: On the influence of Oil Red O dye on complexity of evolution of a camphor-paraffin droplet on the water surface

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Abstract

Droplets of paraffin can show self-motion when powered by camphor. We investigated the motion of such droplets when varying concentrations of camphor and the dye, Oil Red O, as well as the droplet volume. Experiments have shown that the presence of the Oil Red O dye in the system can significantly increase the complexity of droplet evolution, if compared to the case where the dye is absent. Just by increasing the concentration of Oil Red O, the droplet achieves a complex evolution of motion before it splits into many self-propelled secondary objects expressing motion similar to the progenitor droplet. Therefore, the system replicates and the behavior persists. When many division events occur from a single droplet, the collective motion appears similar to swarming behavior of living creatures. This system provides a wet lab, artificial life model to study the temporal evolution of behavior.

Introduction

The investigation of self-propelled, soft-bodied systems plays an important role in the field of artificial life because it offers an experimental system that displays life-like behavior yet is definable from the bottom-up. The study of such systems can help to understand emergence as well as help to define life. As both animals and microbial life can follow or hunt sustenance as well as seek out partners for procreation, the ability to actively transport oneself from one spot to another is an important tool in survival and therefore crucial in the evolution of life. In recent years several simplistic artificial systems have been designed that can mimic the complex directional as well as controlled direction changing biolocomotion of microorganisms. These systems include active catalytic colloids in aqueous H_2O_2 solution (Gomez-Solano et al., 2017), oil droplet systems that can be tuned to mimic microbial chemotaxis (Hanczyc et al., 2007; Čejková et al., 2014) or swarm behavior (Tanaka et al., 2017) and systems propelled by a gradient in surface tension on a water surface using compounds such as camphor to drive itself or as a motor for a boat with a direction (Nakata et al., 2018, 2015). This article describes a novel system that combines the flexibility of a soft object with dynamic camphor systems to study motion and other dynamical behavior of embodied far from equilibrium media. Self-propelled droplets, in many cases, rely on a chemical interaction between the droplet and the surrounding media, inducing an internal convection to create movement (Hanczyc et al., 2007; Nakata et al., 2018). Camphor based systems exploit the ability of camphor to decrease the surface tension in the areas where the surface concentration is high. Such systems are characterized by a fast mass flow because camphor molecules, released from their source, simultaneously evaporate from the surface. The appearing gradients in surface tension support object motion and induce Marangoni convection in the bulk water phase (Nakata et al., 2015, 2018). Even though these systems still lack the aspect of purposeful motion and in most cases the evolution at the initial stage is governed by fluctuations, they enable us to study the principles of self-motion.

In the attempt to combine aspects of these two systems, we created a droplet system which is not driven by an internal convection but rather driven by camphor-based Marangoni flows. We discovered complex and evolving behavior in a paraffin droplet with camphor. Over time and depending on the initial conditions the system undergoes an evolution states that include processes like explosion into small droplets, locomotion, coalescence, and morphing.

In addition to varying the concentration of camphor, we discovered that the concentration of the dye, Oil Red O in the range of 0.1 to 1.5 mg/ml, also affected droplet evolution.

In typical droplet experiments a dye is used to create a contrast between the droplet and the surrounding media as a purely visual aid that makes it easier to track the behavior. We found that a high concentration of this dye had a strong effect on the camphor-paraffin droplet motion. Here we report on the evolution of paraffin droplets driven by camphor with varying concentrations of camphor and dye at two different droplet volumes.

Materials and Experimental Conditions

The droplet source material containing varying concentrations of camphor and dye were made from the following stock solutions: 150 mg/ml commercially available (1R)-

Figure 1: Structure formular of an Oil red O molecule. Illustration from (Sigma-Aldrich, 2018)

(+)-Camphor (98% purity, Sigma-Aldrich) in paraffin oil (puriss., CAS-Number: 8012-95-1, Sigma-Aldrich), 1.5 mg/ml Oil Red O (BioReagent, Sigma-Aldrich, Structure Illustrated in Figure 1) and 150 mg/ml camphor, and 1.5 mg/ml Oil Red O in paraffin. Stock solutions were prepared in bulk on a hotplate at 50°C with a magnetic stirrer. Samples made from these stock solutions were prepared in 1.5 ml Eppendorf microtubes and mixed on a vortex. All experiments were performed at 22±2°C in a 19cm diameter glass Petri Dish containing 150 ml water purified using a Millipore ELIX5 system. Amounts of oil mixture added as singe droplet were either 50 or 200 μl. The time evolution of the system was recorded for a minimum of 20 minutes from above using a mounted digital camera (NEX VG20EH, SONY) and then digitalized, edited and analyzed using on a computer using the ffmpeg and ImageJ software. The entire system, including experiment and camera, was enclosed to eliminate confounding effects from air flow in the laboratory.

Results and Discussion

During initial experiments on the paraffin-camphor system the lipophilic dye, Oil Red O, was chosen in order to create a visual contrast between paraffin droplet and water phase. This is a useful tool to quantify the motion of such systems with image analysis software. We discovered that the concentration of dye played an important role. An experiment using paraffin oil containing 7.5 mg/ml camphor produced stable, moving droplets. After addition of a large amount of dye to the paraffin the behavior became surprisingly complex as the droplet rapidly expanded before breaking up into long arms forming a Turing pattern-type structure. From this extended structure, the arms coalesced into smaller droplets and the system died thereafter upon exhausting the camphor. This process was quite rapid and approached the dead state in under a minute as can be seen in Figure 2.

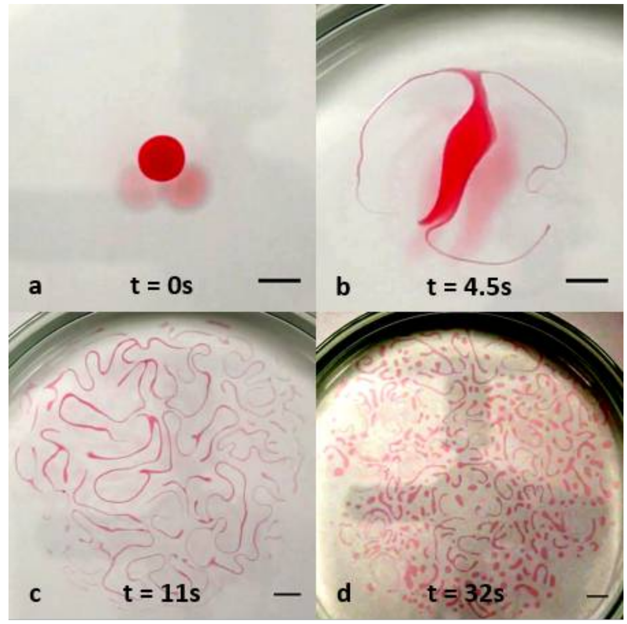


Figure 2: Droplet containing 7,5 mg/ml camphor and surplus of Oil Red O dye in paraffin placed on a water surface inside a 19 cm diameter glass Petri dish. Snapshots are taken at different times: 0s, 4.5s, 11s and 32s as shown in a, b, c and d respectively. The different snapshots show the very complex evolution of the droplets at different stages. Scale bars = 1cm. Video: (Löffler et al., 2018).

This clearly shows that the addition of the dye transformed the system from a stably moving droplet to a much more dynamic and complex system. In order to categorize the types of droplet behavior and motion seen in this system, increasingly complex stages of evolution were assigned symbols 0, A, B, C, D, E, F and G. Definitions of each category are presented in Table 1. We restrict our analysis to the evolution of initial droplet introduced to the experiment with a timescale of up to 20 minutes. In the analysis of experimental results, we use the symbol X^t to indicate that the evolution matching category, X, was maintained for t seconds. Similarly, we use the symbol $X^{<t}$ to say that the X category was maintained for less than t seconds. If no time is given, the state lasted until the end of the experiment. The symbol T describes the stage when the primary droplet becomes indistinguishable from its secondary offspring.

Table 1: Overview of defined categories of droplet evolution with example illustrations.

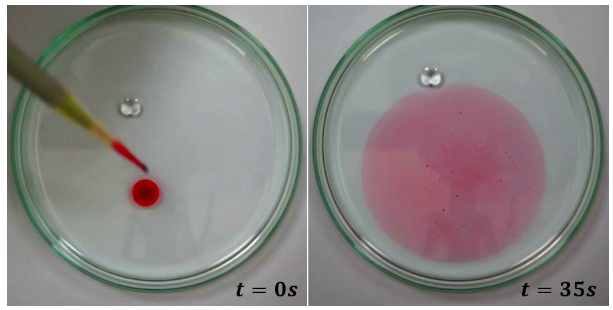
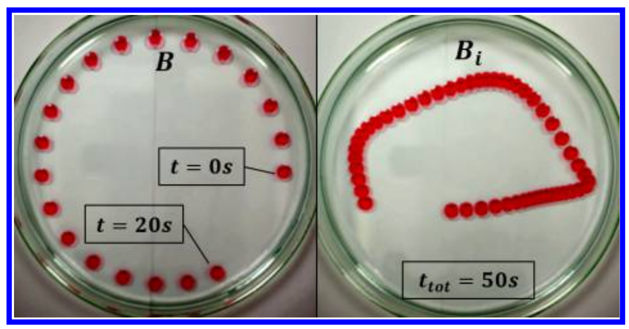
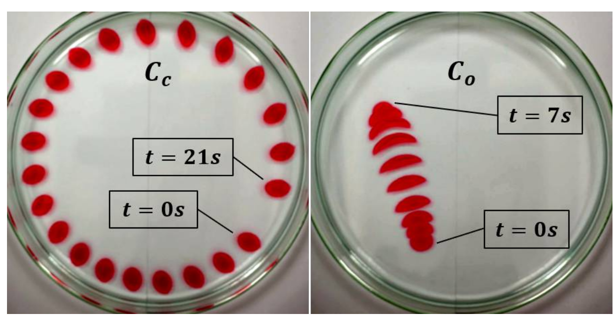
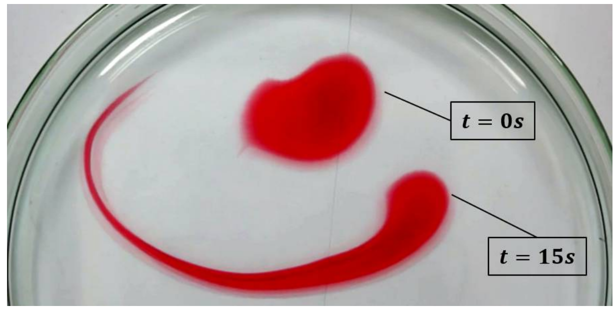
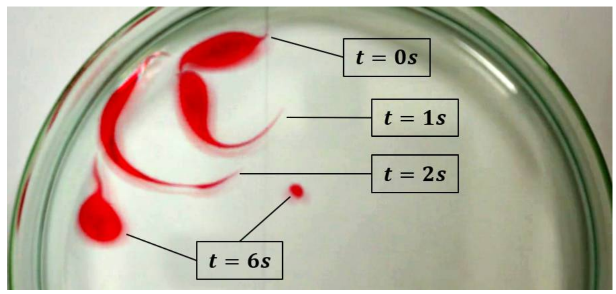
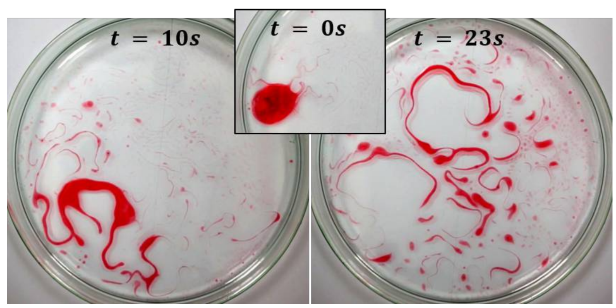
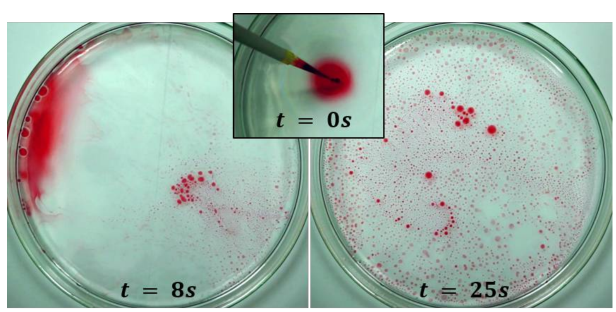
Symbol and description corresponding to motion type.		Illustration of motion type (In B and C droplet locations are superimposed at equal time steps).
0	No motion at all.	Example in the picture below where the transparent droplet does not expand and is displaced due to the expansion of the other. Video: (Löffler et al., 2018).
A	Expansion of the droplet material in a thinning layer on the water surface without displacement of the centre of mass. Videoclip: (Löffler et al., 2018).	
B	A droplet moves while maintaining circular shape. Continuous motion is denoted as (B) and non-continuous, intermittent motion as (B_i). Videoclip: (Löffler et al., 2018).	
C	Droplet elongates on the axis perpendicular to its velocity. The change can reach a continuous steady state (C_c) or oscillate (C_o). Videoclip: (Löffler et al., 2018).	

Table 1: Overview of defined categories of droplet evolution with example illustrations.

Symbol and description corresponding to motion type.		Illustration of motion type (In B and C droplet locations are superimposed at equal time steps).
D	Droplet periodically elongates, its arms extending by an order of magnitude larger than the original droplet diameter. No droplets separate from the extension. Videoclip: (Löffler et al., 2018).	
E	Droplet develops long arms from which smaller droplets occasionally separate. This occurs while the droplet is either moving in an oscillatory manner (E) or in a steady state (E_c). Videoclip: (Löffler et al., 2018).	
F	Droplet develops multiple very long arms, which arrange themselves in a maze-like structure before splitting into small droplets. Videoclip: (Löffler et al., 2018).	
G	Upon contact with the water surface, the droplet spreads out violently in a thin layer. At the edge of the layer small droplets coalesce from it. Videoclip: (Löffler et al., 2018).	

The resulting strings for droplet volumes 50μl and 200μl, presented in Table 2 and 3, describe the sequence of events and thereby give a measure of complexity. The results in Tables 2 and 3 show that at very high concentrations of camphor, the effect of the camphor dominates over the influence of the dye because a large amount of camphor was released from the oil in a very short amount of time, saturating the water surface with camphor and thereby approaching a steady state at which the supply rate of camphor was close to the evaporation rate. In the long term, the supply rate will decrease which lead to coalescence. The largest effect of the dye could be observed at camphor concentrations of around 7.5 mg/ml where an increase in dye drastically increased the complexity of behavior.

At low concentrations of camphor around 1.8 mg/ml the dye changed the behavior but only when a larger volume of material was added to the water surface, resulting in a larger total amount of camphor, which indicated that the ratio between the total amount of camphor and the water surface plays a role. Overall, the qualitative behavior (type of evolution and the order of changes) was found to be highly reproducible. For the simplest motion type (B/C) also the quantitative features were reproducible.

Table 2: The categories of motion observed during time evolution of 50μl droplets at different ratios of camphor to dye. Symbols 1 and 2 indicate results of separate experiments. Longer strings with "higher" letters indicate more complex behavior.

	Camphor conc. (mg/ml)			
Dye conc. (mg/ml)	150	7.5	1.8	0
1.5	$G^{<10}T$	1:$B^{20}C_o^{80}D^{40}E^{60}D^{50}E^{360}T$ 2: $C^4D^5F^{15}T$	A	A
0.75	-	1: $B^{355}C_c^{120}C_o^{120}D^{480}E$ 2: $B^{260}C_c^{150}E_c$	-	A
0.35	$G^{<10}T$	1:$B^{1140}B_i$ 2:$B^{1055}B_i^{50}C_o$	0	0
0.19	-	$B^{685}B_i$	-	0
0.1	$G^{<10}T$	B_i	0	0
0	$G^{<10}T$	B_i	0	0

Table 3: The categories of motion observed during time evolution of 200μl droplets at different ratios of camphor to dye. Longer strings with "higher" letters indicate more complex behavior.

	Camphor conc. (mg/ml)			
Dye conc. (mg/ml)	150	7.5	1.8	0
1.5	$G^{<10}T$	$A^4G^2F^{21}E^{20}T$	$A^3B_i^{200}C_o^{360}D^{480}E$	A
0.35	$G^{<10}T$	B	B_i	A
0.1	$G^{<10}T$	B	B_i	0
0	$G^{<10}T$	B	B	0

Conclusion

Within this work we have presented the diversity and evolution of paraffin droplet behavior that is dependent upon camphor concentration, dye concentration and droplet volume. We have discovered that a large concentration of Oil Red O dye significantly increases the liveness of a droplet. This work is the first step towards further studies of complex behavior of soft-bodied systems propelled by typical surface agents acting together with certain dyes. This includes the movement through increasingly complex mazes, ratio between droplet volume and water surface, swarm and cluster behavior and introduction of complex competitive chemistry inside the droplet or in the bulk. Usually a dye is used to increase contrast and is treated as a passive ingredient. As we demonstrated, the introduction of Oil Red O at high concentrations, drastically increases the complexity of evolution in a camphor-paraffin droplet which could be due to changes in the interfacial tension between oil and water (Tuck et al., 2003). Furthermore, this introduces the question whether dyes have an influence on other oil-based droplet systems. In our previous papers we used 0.2 to 2 mg/ml Oil Red O for good visualization (Čejkova et al., 2016; Caschera et al., 2013). This amount of dye is near and above the threshold where we see complex behaviors emerging . Many reports do not disclose concentrations of dyes used in the experiments, leading to some questions about the influence of dye, if any, on the results. In addition, the effect of alternative dyes on droplet systems are currently being investigated in. The diversity and evolution of behaviors in such a simple chemical system presents an opportunity for the study of emergence. In addition, the observation that divided droplets retain the behavior characteristics of the progenitor droplet will be studied in detail. For example, where is the information in the system? Is it in the droplet, in the environment or perhaps in a combination of the two? Lastly in context to the theme of the Artificial Life conference, we note that the choice of color, a primary tool for many artists and initially thought to be unrelated to experimental outcomes, becomes an active ingredient in self-producing aesthetics.

Acknowledgements

This publication is part of a project that has received funding from the European Unions Horizon 2020 research and innovation programme under the Marie Skodowska-Curie grant agreement No. 711859.
Scientific work funded from the financial resources for science in the years 2017-2021 awarded for the implementation of an international co-financed project.
The authors are grateful to Prof. H. Kitahata and Dr. Y. Koyano for their comments and suggestions.

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