Collective behaviour in droplet systems

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1. Introduction

Living cells are very complex objects with a lot of interesting properties. Many scientists try to create a system, which imitates behaviour of living organisms. Living cells can communicate between each other and behave collectively. Similarly non-living systems with some specific collective behaviour exist [1]. Collective behaviour can be defined as a spontaneous formation of a coherent system without external interaction. The system behaviour is very complex; the understanding of individual compounds does not explain behaviour of the whole system. The fundamental problem lies in understanding the characteristics of the population where individuals cooperate between themselves. To understand collective behaviour is important to characterize and valorise mutually cooperation among individual compounds. A remarkable example of the biological system, where collective behaviour can be observed is slime mold *Dictyostelium discoideum.* This slime occurs in favourable condition as a unicellular organism, but in case of unfavourable condition the cells start to communicate among themselves, perform a chemotactic movement and they create multicellular organism, which is able to survive unfavourable condition [2]. The aim of present work is to observe a population of decanol droplets in a sodium decaonate environment and to study life-like behaviour in this system.

1.1 Multicellular organism

During evolution significantly increasing complexity of living organisms occurred over time, for example: formation of cell life, eukaryotic cells, intelligence etc. Another big evolution jump was an origin of the multicellularity. The multicellular organisms are actually new individuals with a higher degree of the organization. There are some important properties, it should be mentioned that multicellular organisms are agglomerates of cells that live together. Cells can live also in colonies. The cells live in colonies because they can survive easier than individually. Some of the advantages of multicellularity is a mutual cooperation, the division of functions between individuals, the ability that each one specializes in a particular activity. They are also more powerful and their interconnection is better than if they were separated. The cells are connected together more or less permanently in many colonies. Usually they are connected by extracellular matrix. In other colonies (typically many bacterial colonies), free movement appears and the shape of colony is maintained by the way of communication between each other.
Ants are the illustrative example of collective cooperation individual organisms with defined function inside of the system. They have lived in very large and well-organized association. They diligently carry out their work to ensure the existing of the whole colony. The queen is responsible for the reproduction by laying eggs, ants “workers” are not capable of reproduction. They work for the

colony. The advantage of mutual cooperation is the ability to survive adverse conditions, to reduce dependence on external environment, growth form and species diversity.
Cells or multicellular organisms can move using special organelles (flagella, cilia etc.) or by amoeboid shape changes. It could be random walk or they can perform so-called chemotactic motion. A lot of laboratories around the world are interested in research of chemotactic motion in both natural and artificial systems and our laboratory is not an exception [3, 4].

1.2 Chemotaxis

Cells are able to respond to a chemical gradient, that is found outside of cells, and to move along the chemical gradient is called chemotaxis [3]. Chemoattractant indicates the chemical compounds, which cells are able to respond on. Chemotaxis play very important role in different biological systems in nature. Chemotaxis can be found in bacteria as well as in eukaryotic organism. Bacteria can respond to change of temperature or concentration of chemoatractant. The chemotaxis behaviour is very important in eukaryotic, for example: neutrophils, immunocyte and nerve tissue. *Distyostelium discoidum* is the most extensively studied model of eukaryotic organism where the chemotactic movement was found. Chemotaxis can be divided to three interrelated phenomena: chemosensing (the ability to sense a chemoattractant), polarization (the ability to rotate from/to chemoattractant) and locomotion (the ability to move).

1.3 Chemotactic movement of droplets

The chemotaxis of living cells described above was studied by biologists since the beginning of 18^{th} century, but chemotaxis of non-living objects started to be followed up in the past few decades. In our laboratory we focus on exploring the system of decanol droplets in a sodium decanoate environment, which can follow the addition of salt and hydroxides, like living cells are able to follow addition of chemoattractant [4].
Some other organic substances can mimic chemotactic behaviour of living organisms as well. For example Prakash et al. [5] show that two-component droplets of well-chosen miscible liquids such as propylene glycol and water deposited on clean glass are not subject to pinning and cause the motion of neighbouring droplets over a distance. These droplets are stabilized by evaporation-induced surface tension gradients and therefore they move in response to the vapour emitted by neighbouring droplets. Additionally, they move in response to the vapour released neighbouring droplets. Torkkeli et al. [6] described electrostatic transformation of water droplets on superhydrophobic alkylketene dimer and Teflon surfaces. The system is placed between two electrodes and by changing electrode voltage movement of droplets occurs along the electrode paths. Garcia et al. [7] introduced a movement of droplets from biological fluids in a water environment which is controlled by introducing magnetisable carbonyl iron microparticles. Martin M. Hanczyc et al. [8] introduced a system where droplets of nitrobenzene containing oleic anhydride in an oleate were moving in a pH gradient.

1.4 Robotic platform for study collective behaviour droplets

Many research groups are studying the movement of droplets described above. However, all these works are done manually using pipette. The using of any robotic platform could make the experimental work easier and more precise.
M.M.Hanczyc et al., [9] used liquid-handling robot to explore motile droplets, monitored their dynamics as they approach equilibrium. The camera was used to track speed of nitrobenzene droplets

containing oleic acid anhydride in sodium oleate environment. Chemotactic movement is based on a chemical reaction where the anhydride is used as the "fuel" so after some time anhydride is used up and then droplets decrease its speed. In this moment, the robot is able to evaluate the line speed of droplets and if the speed of droplets decreases below a certain value, the robot adds more anhydride which indicates again the movement of droplets. The robot was able to monitor this process for several hours.

In our laboratory the experiments were performed manually, and all solutions and droplets were added to the Petri dish using a pipette. Such a procedure is very time consuming and laborious. Now the work is performed by using a robot called Evobot that was produced at IT University in Copenhagen. The robot is built using open-source robotic liquid handling platform. It has three layers. The first is the control layer - it contains a" head" which can move in the plane. In this layer there are different modules e.g.: syringe, temperature sensors, pH sensor. The syringe has two degrees of freedom. One is to move the piston up and down, the other is to move the syringe upwards and down. The second layer is called transparent experimental layer, where Petri dish, micro-wells and other vessels are placed. A final layer is a sensing layer. Here is a camera which is able to monitor the experimental layer from below.

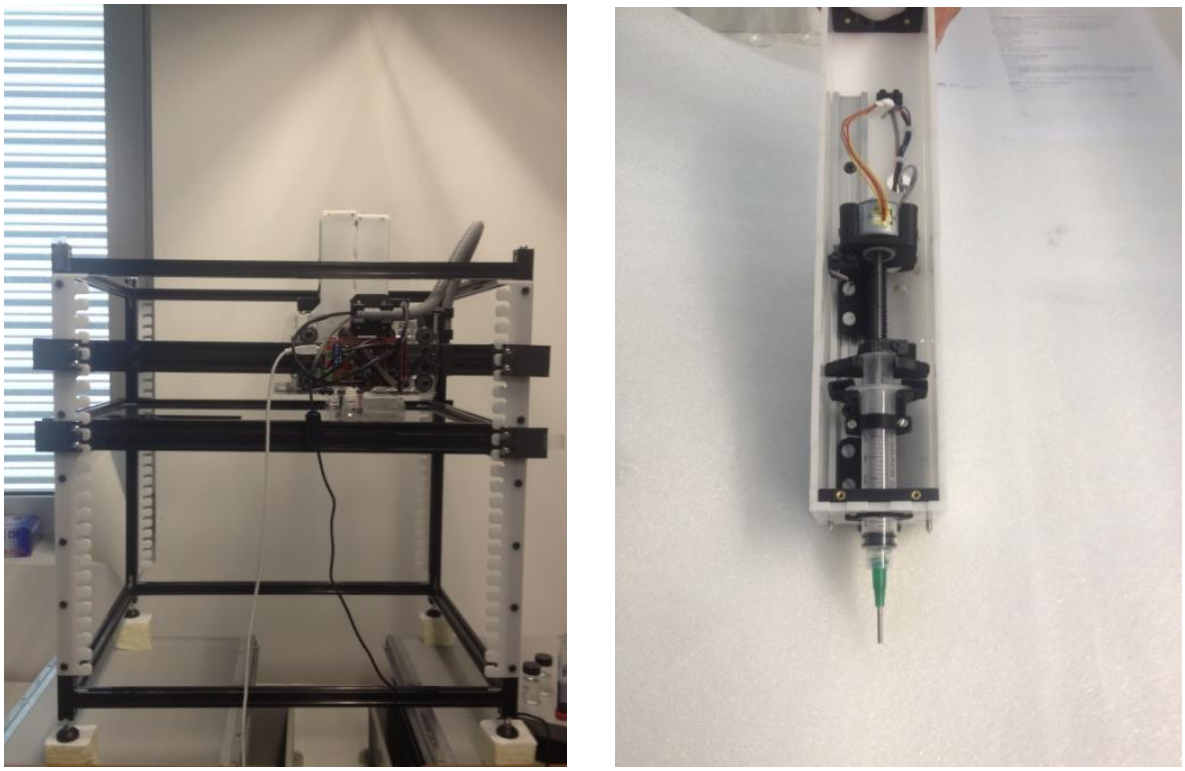

Figure 1: Evobot

2. Experimental

Experiments described in present work were performed on the Petri dishes with the size 100 x 10 mm. 10mM sodium decanoate aqueous solution was spread on the Petri dish. Into this solution 20 µl decanol droplets were added in dependence of the kind of experiment (as described in the part Results and discussion). Experiments were recorded with a camera.

Prepared solution of sodium decanoate had pH 12.4. For experiments where the effect of pH was studied, hydrochloride acid was used to reduce pH on required values (pH = 7, 8, 9, 10, 11). Petri dish was filled with 10 ml sodium decanoate and seven droplets of decanol were added.
To study the behaviour of decanol droplets in dependence on their number and different amounts of sodium decanoate was filled Petri dish with 6, 8, and 10 ml sodium decanoate and then was added different number of decanol droplets (n = 1,2,3,4,5,6,7,8) with a volume of 20 µl.

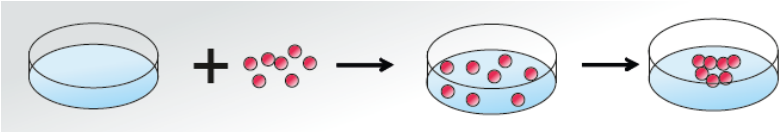

Figure 1 Scheme of the experiment.

3. Results and discussion

3.1 The behaviour of decanol droplets with different pH

In the Figure 3 we can see the behaviour of decanol droplets with volume of 20 µl in sodium decanoate solution with different pH, namely 7, 8, 9, 10, 11, 12,4 at time t = 30, 180, 270 and 540 s. The experiment was also performed with sodium decanoate pH 6. Unfortunately, decanol droplets with pH 6 start to create very quickly a clusters and fused. At time t = 45 s coalescence of decanol droplets occurred.

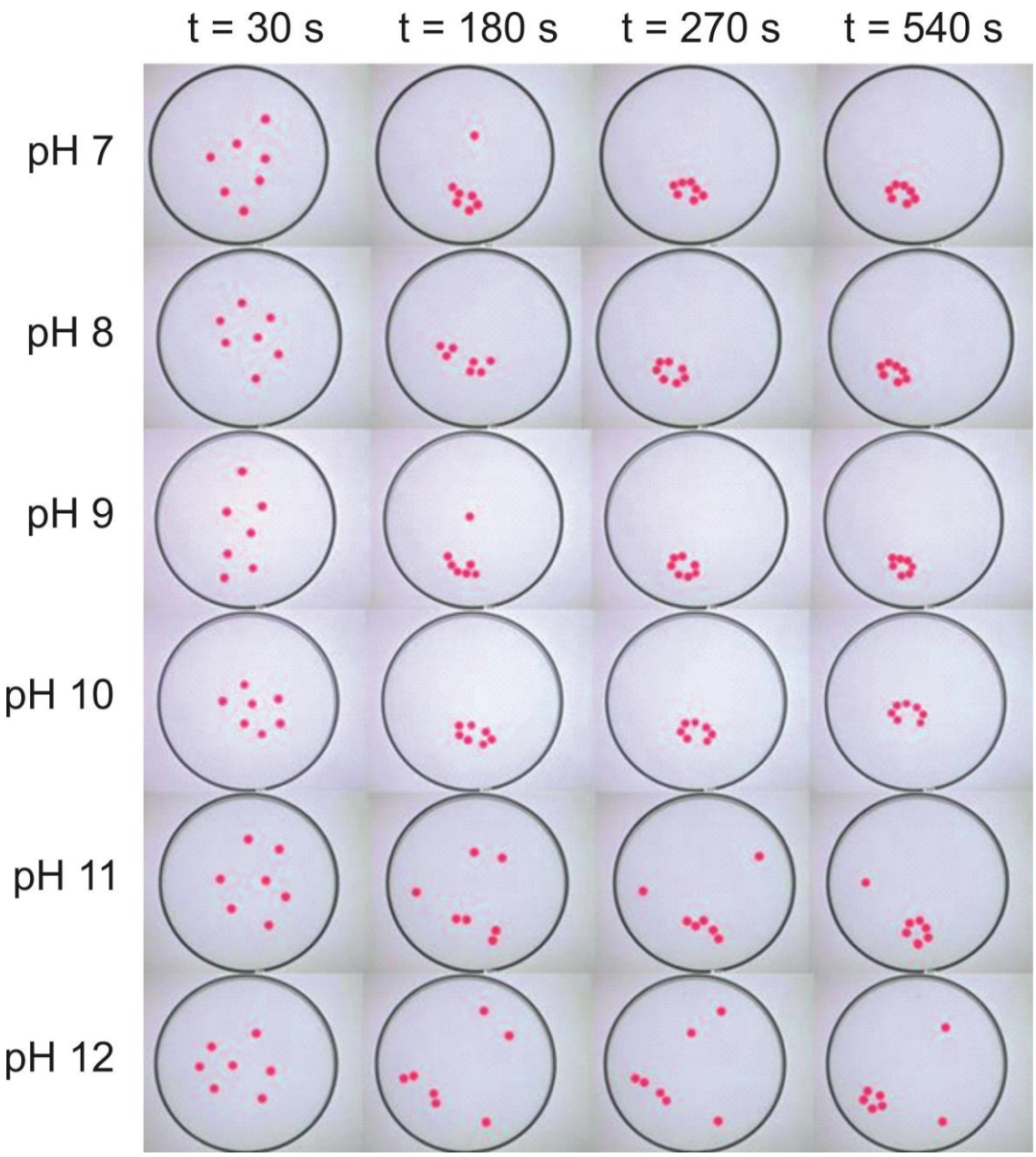

Figure 2 Behaviour of decanol droplets with volume of 20 µl in 10 ml of sodium decanoate with different pH environment. Diameter of Petri dish is 10 cm.

3.2 The behaviour of decanol droplets depending on their number

The following experiment was aimed at examining how decanol droplets with volume of 20 µl arranged according to their number. After adding of three decanol droplets clustering could be seen in the triplet, which was not stable, thus at time t = 5 min decanol droplets separated. If we increased the number of decanol droplets to 4 and more, we see clusters of decanol droplets that will last together. If we add 6-13 decanol droplets we can observe non-annular body of C shape.

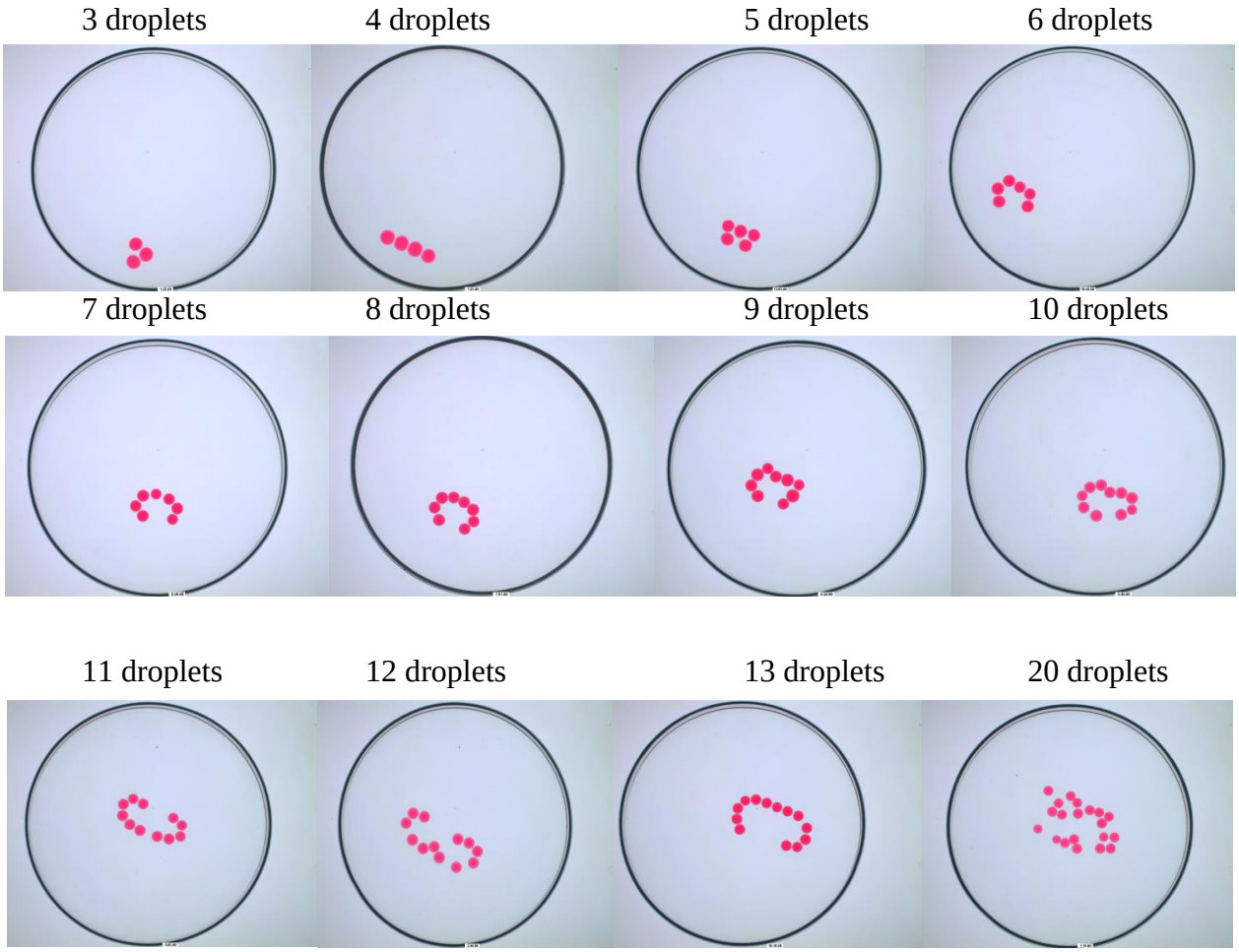

Figure 3 Behaviour of decanol droplets depending on their number. Diameter of Petri dish was 10 cm.

3.3 The behaviour of different number of decanol droplets in different volume of sodium decanoate

The aim of the experiment was to see how droplets behave in different volume of sodium decanoate with pH 12.4 and number of decanol droplets. At first Petri dish was filled with 6 ml (h = 0,076 cm) of sodium decanoate and different number of decanol droplets was added. Then the same experiment was repeated under the same circumstances except one – volume of sodium decanoate was changed to 8 ml (h = 0,102 cm). As you can see in the figures 5 and 6, if we added more decanol droplets, the droplets are going faster together.

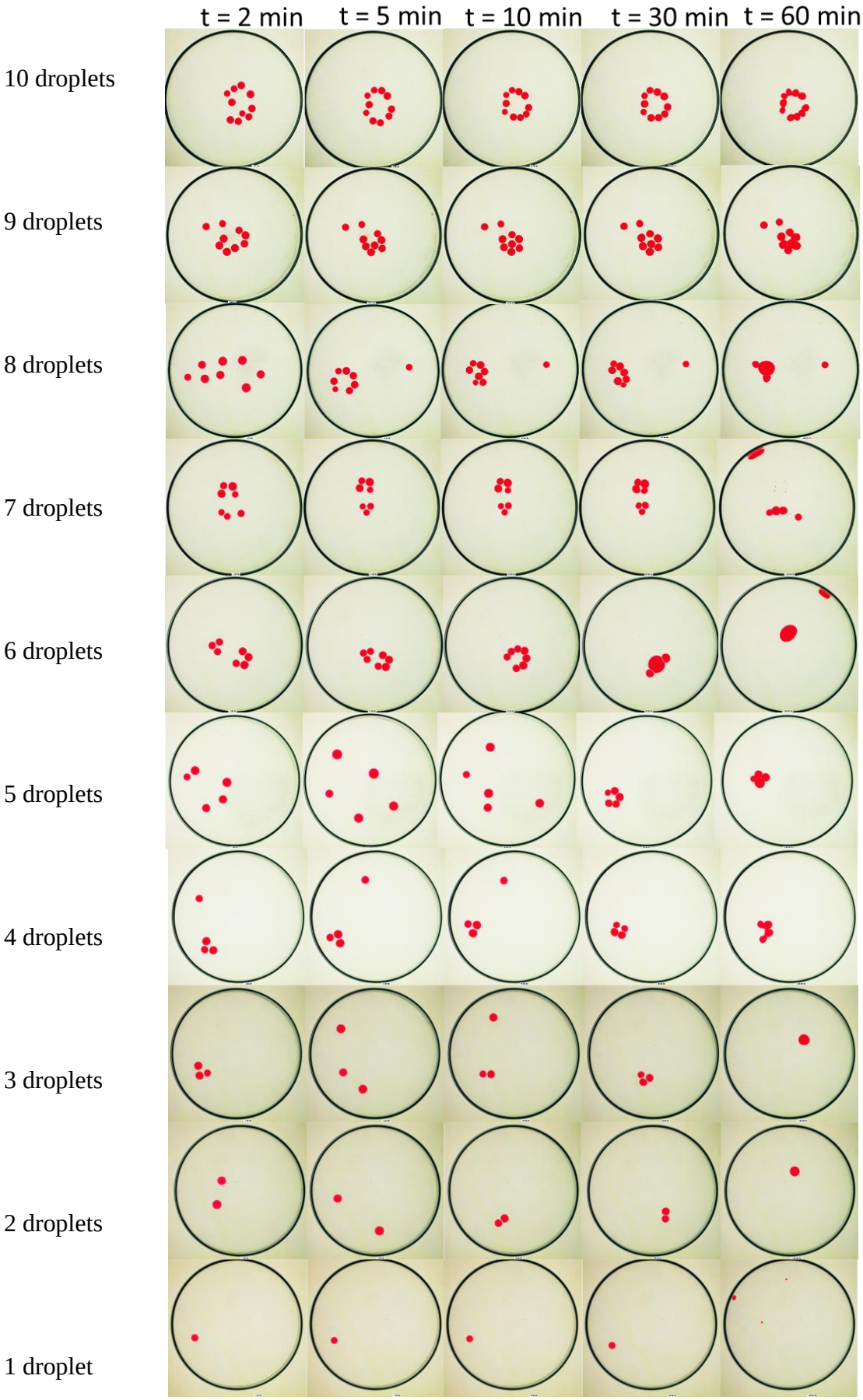

Figure 5 Behaviour of decanol droplets in dependence of their number (in 6 ml of decanoate).

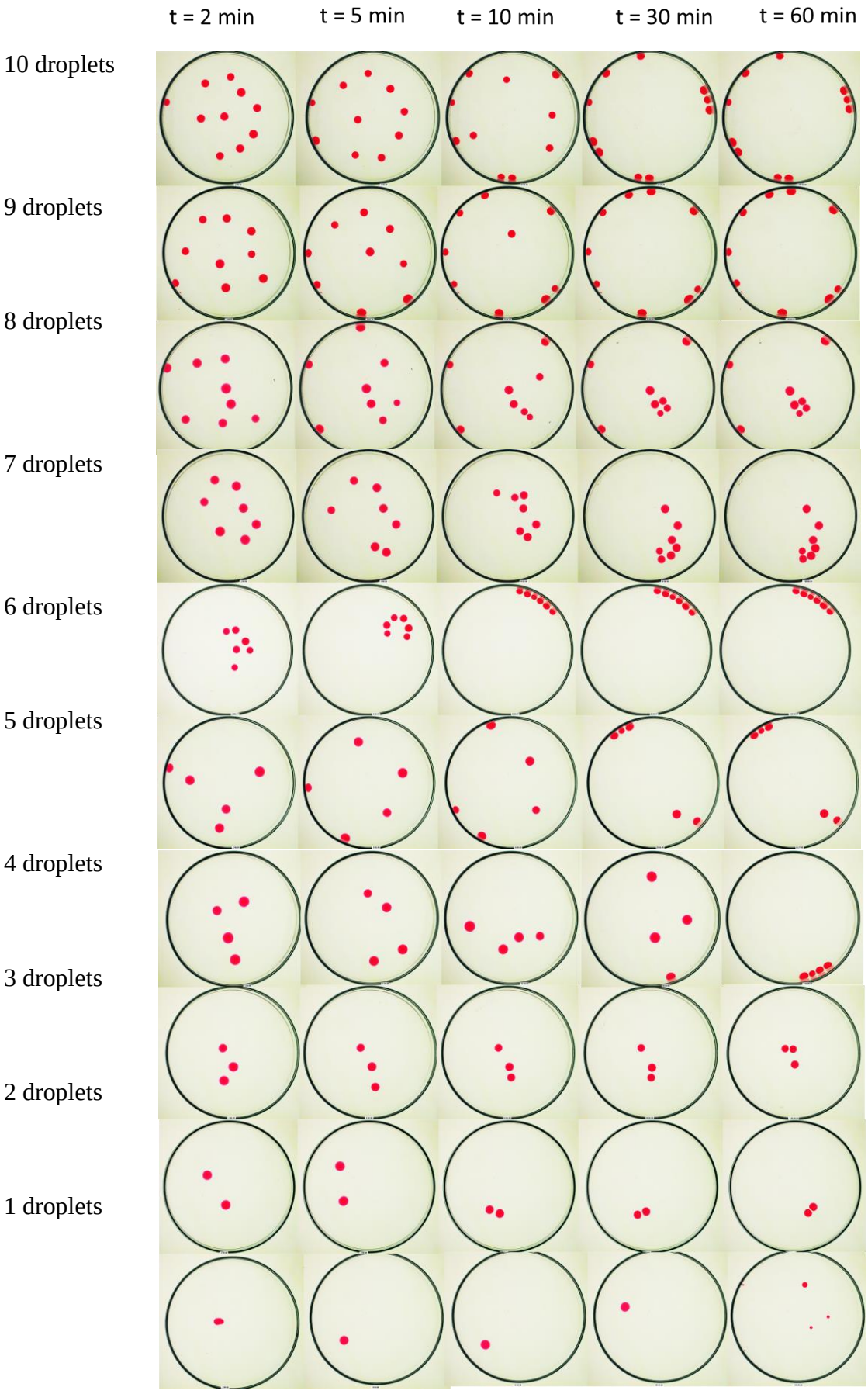

Figure 6 Behaviour of decanol droplets in dependence of their number (in 8 ml of decanoate).

4. Conclusion

In this work we have studied population of decanol droplets in sodium decanoate environment under various conditions. The behaviour of decanol droplets was tested in dependence of *(i)* different pH, *(ii)* volume of sodium decanoate (as environment) and *(iii)* the number of decanol droplets. It has been found out that the population of decanol droplets is able to behave like the population of living cells. Decanol droplets can spread in the enviroment independently as individual objects, but depending on the environmental conditions, they are able to cluster and thus mimic the formation of a multicellular organisms. It was found if we fill the Petri dish with thin layer of sodium decanoate, the decanol droplets create faster cluster, because there is the influence of bottom of the Petri dish. However, we can achieve the same effect in thicker layer if we decrease pH of sodium decanoate.

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