

Formation and characterization of flow-driven and self-propelling calcium alginate-based structures

Theses of doctoral (Ph.D.) dissertation

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1 Introduction and Aims

The importance of non-equilibrium reactions in biological processes (structural transformations of proteins, self-replication) and in inorganic systems (oceanic vents, silicate tubes) is indisputably large. Their formation requires the interaction of chemical reactions and transport processes, but it is worthwhile first to explore their driving forces in simpler systems due to their complexity. This is investigated in the case of inorganic self-organizing structures by chemobrionics. Our research group has previously worked on self-organised precipitation in a three-dimensional (3D) flow-driven system, as the method allows to control the properties of the precipitation formed at both micro- and macroscales. However, the formation of organic structures (hydrogels) or hybrid (organic-inorganic) systems has not yet been understood and characterised from the reaction-transport perspective. Hydrogels are also naturally occurring (collagen, gelatine), have a high water content, and are therefore highly important in therapeutics (contact lenses, burns, hygiene products). In the first part of my work, I aimed to study in the 3D flow-controlled system the formation of calcium phosphate precipitate, which is also important in biology as it builds up bones and teeth, the calcium alginate gelation, and based on these reactions, the hybrid organic-inorganic system. During the micro- and macroscopic characterization of the resulting structures, I explored the electrochemical, diffusive, and rheological properties of the system, in addition to the construction of dynamic phase diagrams. Based on the literature, the crystallization of calcium phosphate can occur in many ways, so I thought it was important to investigate the precipitation reaction under different chemical conditions and to perform a qualitative analysis of the structures formed.

Observations of natural phenomena and the way living beings work often provide ideas for imitating them. These, so-called bio-inspired approaches can even be used to perform tasks. For example, Leonardo da Vinci designed the rudimentary construction of airplanes by studying the wings of birds. Numerous new bio-inspired solutions are still being developed today, relying also on chemistry in addition to physics. By exploiting the reactions and/or transport processes, it is even possible to create an artificially engineered autonomous movement of a cell-like "motor", which could be used as a transporter, as a switch, or as a pollutant remover in the future. Furthermore, collective patterns can be also observed in both living and non-living systems when they interact with each other (swarming birds, traffic, planetary orbits). The study of how these systems are assembled at a higher level has revealed new regularities and underlying forces. In the second part of my work, I aimed to investigate the operating principle and the dynamics of a swimming hydrogel bead made of calcium alginate, in addition to the synchronization of several hydrogels.

2 Experimental Section

The flow-driven experiments were performed in a three-dimensional plexiglas vessel into which a solution containing sodium alginate and/or sodium phosphate, or only trisodium phosphate, disodium hydrogen phosphate, or sodium dihydrogen phosphate salts were flowed through a tube (Figure 1a). I used a peristaltic pump to inject the solution through a seal at the bottom

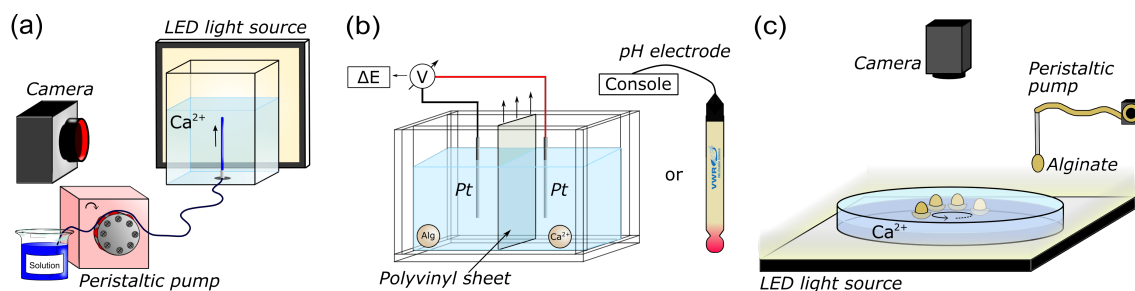


Figure 1: The a) flow-driven system, the b) electrochemical cell, and the c) Petri dish arrangement used in the experiments.

of the vessel. The vessel was filled with a calcium chloride solution before the injection. For easier detection, I dyed the sodium alginate solution with methylene blue. Furthermore, all experiments were illuminated with an LED light source and either a black (phosphate) or a white (alginate) background was used. A camera was used to record the temporal evolution of the structures formed during the reactants' contact. In the experiments, volumetric flow rate, concentration of the solutions, and thus their density difference was adjusted. For the phosphate solutions, pH was also set by varying the ratio of the masses of the differently protonated phosphate salts. The growth of composite structures by laser illumination was investigated. Their viscoelasticity with a rheometer was determined, while the electrochemical properties of the system were measured in a cell that we constructed (Figure 1b). The quantitative distribution of ions and forming precipitates in the hydrogel was simulated in a program we wrote in Wolfram Mathematica. The composition and morphology of the different calcium phosphate precipitates were determined by powder X-ray diffraction and scanning electron microscopy.

The swimming hydrogel motor was created by dropping a drop of sodium alginate solution into the calcium chloride solution stored in a Petri dish (Figure 1c). The movement of the motor was recorded from above with a camera. The evaluation was done with home-made programs. Particle image-based velocity characterization was also performed to determine the operating principle of the self-acting hydrogel.

3 New Scientific Results

- I. *In the production of calcium phosphate–calcium alginate hybrid hydrogels, the incorporation of precipitate increases the elasticity of the hydrogel, while at higher amounts a brittle thin membrane can be obtained, resulting in a one-step decrease in the time evolution of the potential difference by preventing diffusion during gelation. [3]*

By varying the flow rate and the calcium chloride concentration, I created three different hydrogel structures in the 3D flow-controlled system: spreading, budding, and jetting tube. By varying the flow rate and the concentration of sodium phosphate, at constant concentrations of sodium alginate and calcium chloride, spreading, rupturing, and intact balloon composites were developed. By tracking the growth of the composite walls in time, it revealed that the transport process occurs mainly by diffusion. Furthermore, at the highest phosphate concentration that we used ($c(\text{Na}_3\text{PO}_4) = 263 \text{ mM}$), the precipitate in the gel prevented the diffusion so a thin membrane of constant thickness was formed. The pure alginate hydrogel was compared with the hybrid by rheological measurements. The flow point was independent of the calcium phosphate content and that the phosphate ion content reduced the viscoelasticity. Electrochemical measurements were also carried out on the system, which showed that the reaction is mainly pH-driven and that the potential difference changes in time with a one- or a two-step mechanism when decreasing the phosphate ion concentration (Figure 2).

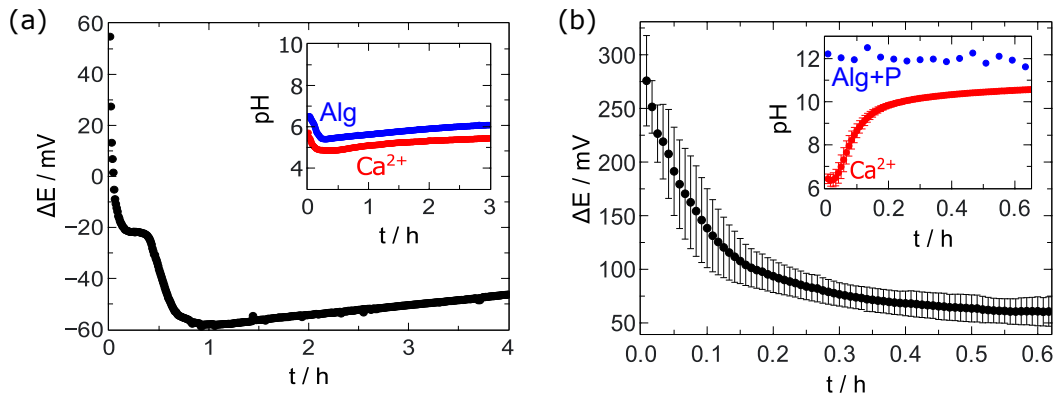


Figure 2: The temporal evolution of the potential difference with a) a two-step and b) a one-step mechanism. The small figures show the time dependence of the pH of the solutions.

II. *By varying the pH of phosphate solutions, linear, oscillating, or stepwise growing crystalline tubes or balloon-shaped membranes can be obtained in a 3D flow-driven system in a controlled manner.* [2]

The 3D flow-driven system is suitable for the creation of calcium phosphate precipitates with different macrostructures, as illustrated in Figure 3. Using a trisodium phosphate re-

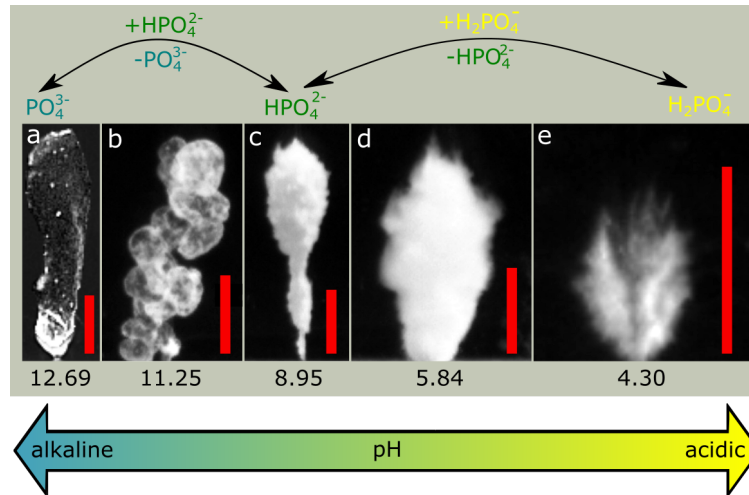


Figure 3: Effect of pH on the macrostructure of calcium phosphate precipitates. The red scale corresponds to 0.5 cm.

actant in a highly alkaline ($\text{pH} = 12.69$) solution, membrane-like structures were formed: a linearly evolving jetting tube, an oscillating popping tube, a stepwise developing irregular cylinder or a balloon. With sodium hydrogen phosphate reactant ($\text{pH} = 8.95$), crystalline precipitation tubes evolved: stuffed, hollow and branched tubes, and coral shapes. The flow created in the tubes by increased flow rate caused branching, while higher concentrations resulted in stiffer structures with less branching. In the case of dihydrogen phosphate, a crystal in the range of mm has grown. Precipitates formed at intermediate pH values carried the properties observed at higher and lower pH, and therefore different precipitate compositions by X-ray diffraction measurements were determined. In an acidic environment at $\text{pH} = 5.51$, the composition of the solid material is brushite, which was detected with more intense peaks at reflections belonging to the (020) family. The same composition can be observed at $\text{pH} = 5.84$ and $\text{pH} = 6.08$, but these reflections have lower intensity, i.e. the oriented increase is less significant. Besides brushite, chlorapatite and monetite are also formed between $\text{pH} = 6.53$ and 10.95 . The typical composition at $\text{pH} = 11.20$ is brushite and monetite. At more alkaline pH, where the membrane structure is typical, the composition could not be determined.

III. *Calcium phosphate balloon grows linearly with injected volume in a 3D flow-controlled system, where the volume growth rate equals to the injection rate within experimental error.* [2]

In the 3D flow-controlled system, membrane-structured calcium phosphate balloons form periodically under alkaline conditions. Symmetric structures are observed at pH = 12.46 and 11.82, while asymmetric balloons are formed at pH = 11.34. The time variation of the volume V of the symmetric balloons was calculated using Pappus' second centroid theorem

$$V = 2 \cdot \pi \cdot R_s \cdot A \quad (1)$$

where R_s is the distance between the axis of rotation and the centroid of the plane with area A . The results are illustrated in Figure 4. The volume of the balloon increases linearly with

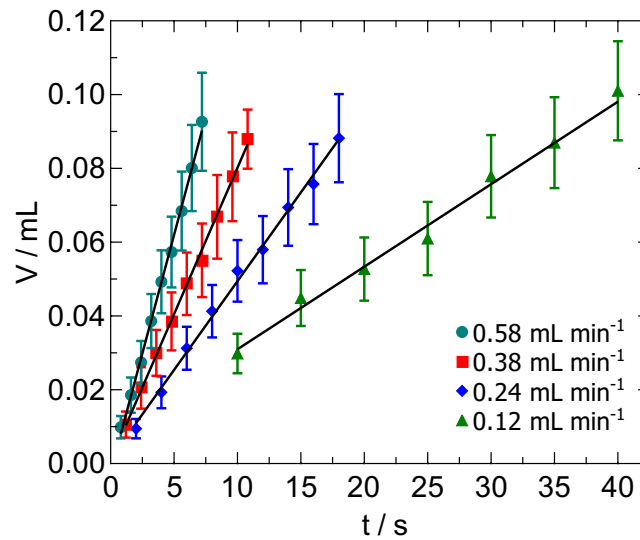


Figure 4: Increase in balloon volume over time at pH = 12.46 and different flow rates.

time and the volume growth rates calculated from the fitted slopes are equal to the flow rates within experimental error suggesting that the injected solution remained within the precipitate structure.

IV. A self-propelled swimming calcium alginate hydrogel bead can be created, where the motion induced by the Marangoni effect is due to the outflow of water by the osmotic gradient. [1]

A polymer-based, self-propelling calcium alginate structure has been created for the first time without the addition of an "extra" fuel. The hydrogel's self-propulsion principle is illustrated in Figure 5. Hydrogel is formed immediately at the interface of the calcium

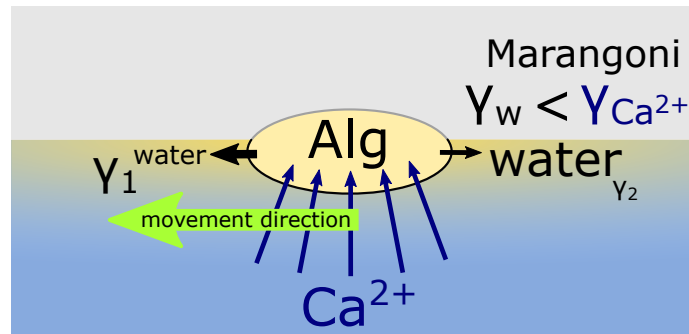


Figure 5: Illustration of how a swimming calcium alginate hydrogel motor works.

chloride solution and the sodium alginate added to it. Subsequent gelation occurs inside the droplet by diffusion of Ca^{2+} ions into the droplet. The hydrogel asymmetrically shrinks as it exerts water out due to gelation and the emergence of an osmotic gradient at the interface. I confirmed the water outflow using shadowgraphy and particle image-based velocimetry. A surface tension gradient is formed between the asymmetrically outflowing water around the hydrogel and the reactant calcium chloride solution, which causes the structure to move. The density difference required for the movement was set by selecting the initial concentrations.

V. Depending on the initial concentrations, the swimming hydrogel bead can either form a more active disc, often describing circular motion, or a less active boat, typically following a straight line, during continuous and sometimes intermittent motion. [1]

At lower sodium alginate concentrations (0.3–0.5 % w/V) a flat disc is formed, while at higher concentrations (1.0–1.5 % w/V) a boat-shaped hydrogel is evolved. The discs exhibit several types of motion, so I have distinguished four types, of which continuous is translational (I) and rotational (II) motion, besides the intermittent (III) motion observed before the quasi-equilibrium (IV). Figure 6 illustrates the velocity profile during motion and the corresponding trajectory.

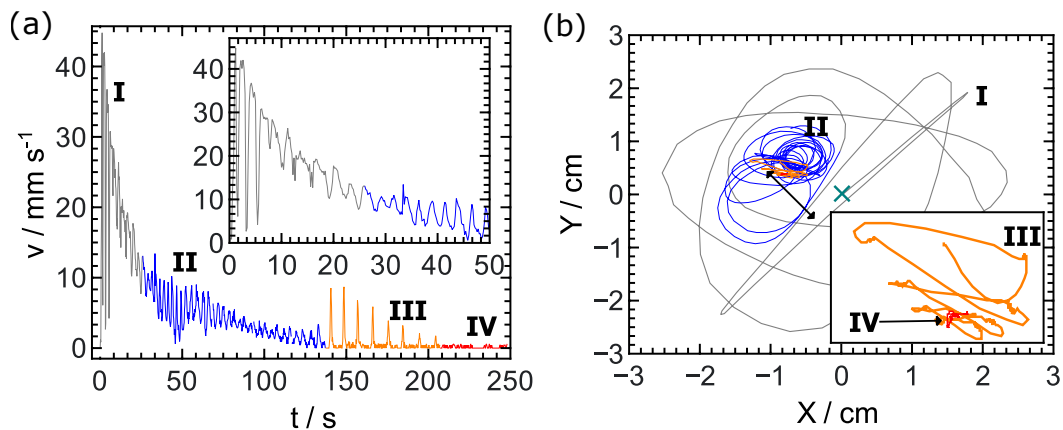


Figure 6: a) Change of the velocity (v) of a hydrogel as a function of time (t) and the trajectory b) of the experiment. The four colours represent the four distinct periods (I-IV). Reactant concentrations: 6 M calcium chloride and 0.5% w/V sodium alginate.

The capillary forces between the glass wall of the Petri dish and the hydrogel affect the direction of motion, thus the rectilinear direction is observed in smaller and smaller sections over time during the translational period. The initial concentrations also affect the translation period along with decreasing the calcium chloride concentration, which leads to trajectories close to the Petri dish wall. Nevertheless, depending on the initial concentrations, the number, and the type of periods (and thus the trajectory) can be controlled, i.e. the movement can be tuned. Discs showed more active behaviour compared to boat structures: the measurable maximum velocities and the number of periods were typically higher for discs.

VI. *Several swimming hydrogel beads can move in coordination with a time period of 0.5–2.5 s, where the smaller hydrogels of different sizes collide more often.*

Attractive (capillary forces) and repulsive (Marangoni effect) interactions occur between the swimming hydrogel beads. Since both are of comparable size in my experiments, the hydrogels periodically collide and move away from each other as they are close together. I have observed first time such a coordinated motion between two floating hydrogel beads, as illustrated in Figure 7a. Based on the time variation of the position of the hydrogels

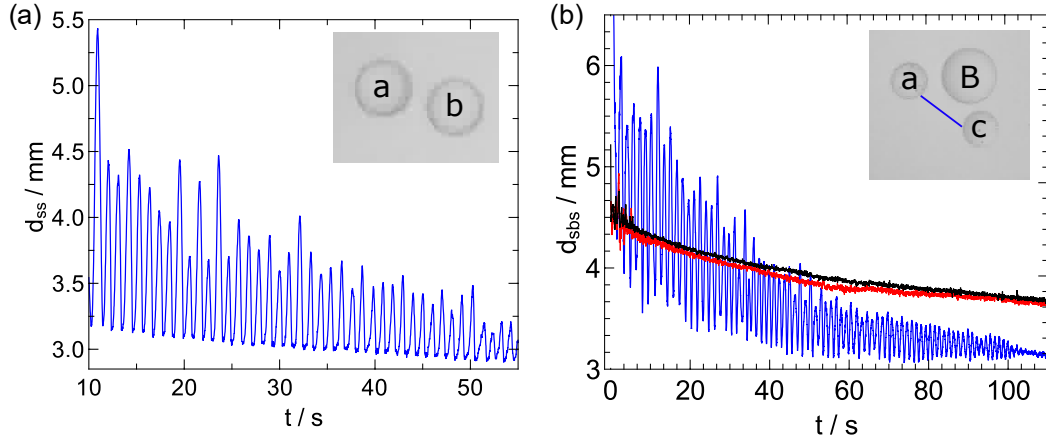


Figure 7: a) Distance between the centers of two small hydrogels (d_{a-b}), and b) of a large and two small hydrogels (d) in the function of time (t).

relative to each other, collisions were characterized by defining the period time, which is influenced by the size of the hydrogel in addition to the number of collisions, as shown in Figure 7b. Larger structures collide fewer times for shorter periods. If both small and large hydrogels are present, the small hydrogels show higher activity. For three, four and five members, synchronous motion was observed for small sizes, with period times varying between $T = 0.5\text{--}2.5$ s. For six and seven motors, an open ring was formed, while for more than eight members, holey or tightly assembled clusters appeared.

4 List of Scientific Publications

4.1 Scientific Publications Related to the Topic of the Dissertation

1. **R. Zahorán**, P. Kumar, D. Horváth, Á. Tóth
Self-propulsion of a calcium alginate surfer
Soft Matter, **19**, 8033–8039 (2023)
IF₂₀₂₃ = 2.900
2. **R. Zahorán**, P. Kumar, Á. Deák, E. Lantos, D. Horváth, Á. Tóth
From balloon to crystalline structure in the calcium phosphate flow-driven chemical garden
Langmuir, **39**, 14, 5078–5083 (2023)
IF₂₀₂₃ = 3.700
3. **R. Zahorán**, P. Kumar, Á. Juhász, D. Horváth, Á. Tóth
Flow-driven synthesis of calcium phosphate–calcium alginate hybrid chemical gardens
Soft Matter, **18**, 42, 8157–8164 (2022)
IF₂₀₂₂ = 3.400

4.2 Other Scientific Publications

1. N. P. Das, **R. Zahorán**, L. Janovák, Á. Deák, D. Horváth, Á. Tóth, G. Schusztter
Kinetic characterization of precipitation reactions: Possible link between a phenomenological equation and reaction pathway
Crystal Growth & Design, **20**, 11, 7392–7398 (2020)
IF₂₀₂₀ = 4.076
 2. **R. Zahorán**, Á. Kukovecz, Á. Tóth, D. Horváth, G. Schusztter
High-speed tracking of fast chemical precipitations
Physical Chemistry Chemical Physics, **21**, 21, 11345–11350 (2019)
IF₂₀₁₉ = 3.430
- Σ IF = 17.506

5 Oral Presentations

5.1 Oral Presentations Related to the Topic of the Dissertation

1. **Zahorán R.**, Horváth D., Tóth Á.

Áramlásvezérelt és önmeghajtó kalcium-alginát alapú szerkezetek előállítás és jellemzése

Preliminary presentation of dissertation, Reaction Kinetics and Photochemistry Working Group of HSA, October 26-27, 2023, Mátrafüred, Hungary

2. **Zahorán R.**, Kumar P., Horváth D., Tóth Á.

Önmeghajtó kalcium-alginát gyöngyök

Reaction Kinetics and Photochemistry Working Group of HSA, 2023. június 8-9, Balatonvilágos, Hungary

3. **R. Zahorán**, P. Kumar, D. Horváth, Á. Tóth

Self-propelled calcium-alginate bead

Reaction Kinetics and Photochemistry Working Group of HSA, October 28, 2022, virtual (Mátrafüred), Hungary

4. **R. Zahorán**, P. Kumar, D. Horváth, Á. Tóth

Calcium-phosphate chemical gardens

GRS Oscillations and Dynamic Instabilities in Chemical Systems, July 16-17, 2022, Easton, USA

5. **R. Zahorán**, P. Kumar, D. Horváth, Á. Tóth

Organic-to-inorganic transition of chemical gardens

COST Action Chemobrionics Meeting, September 22-24, virtual (Ankara), Turkey

6. **R. Zahorán**, P. Kumar, D. Horváth, Á. Tóth

Organic-to-inorganic transition of chemical gardens

ALIFE 2021 Conference on Artificial Life, July 19-23, 2021, virtual (Prague), Czech Republic

5.2 Other Oral Presentations

1. **R. Zahorán**, D. Horváth, Á. Tóth, G. Schusztér

High-speed tracking of fast chemical precipitations

2nd International Conference on Reaction Kinetics, Mechanisms and Catalysis, May 20-22, 2021, Budapest, Hungary

2. **Zahorán R.**, Das N. P., Müller B., Tóth Á., Horváth D., Schusztter G.
Kémiai csapadékképződés kinetikai tanulmányozása
Reaction Kinetics and Photochemistry Working Group of HSA, May 23-24, 2019, Balatonalmádi, Hungary
3. N. P. Das, B. Müller, **R. Zahorán**, Á. Tóth, D. Horváth, G. Schusztter
Macroscale precipitation kinetics: Towards complex precipitate structure design
COST Action CA17120 on “Chemobrionics”, March 11-13, 2019, virtual (Granada), Spain
4. E. Balog, N. P. Das, B. Müller, **R. Zahorán**, Á. Tóth, D. Horváth, G. Schusztter
Towards Complex Precipitate Structure Design
M-ERA.NET “Material Synthesis in Non-Equilibrium Conditions” Workshop, January 23-26, 2019, Budapest, Hungary
5. **R. Zahorán**, G. Schusztter
Csapadékképződés indukciós periódusának vizsgálata nagy időfelbontású kamerával
XXXIV. OTDK, March 21, 2019, Budapest, Hungary
6. **R. Zahorán**, G. Schusztter
Csapadékképződés indukciós periódusának vizsgálata nagy időfelbontású kamerával
Local TDK, November 22, 2018, Szeged, Hungary
7. **R. Zahorán**, G. Schusztter
Induction period determination of fast precipitation reactions
XLI. Chemistry Lectures, October 17, 2018, Szeged, Hungary
8. **R. Zahorán**, G. Schusztter
Nagy időfelbontású kamera alkalmazása ólom-oxalát csapadék reakciókinetikai vizsgálatához
XL. Chemistry Lectures, October 18, 2017, Szeged, Hungary

6 Posters

6.1 Poster Presentations Related to the Topic of the Dissertation

1. **R. Zahorán**, P. Kumar, D. Horváth, Á. Tóth
Flow-driven fabrication of calcium phosphate/alginate composites

GRC Oscillations and Dynamic Instabilities in Chemical Systems, July 17-22, 2022, Easton, USA

2. **R. Zahorán**, P. Kumar, D. Horváth, Á Tóth

Flow-driven fabrication of calcium phosphate/alginate composites

GRS Oscillations and Dynamic Instabilities in Chemical Systems, July 16-17, 2022, Easton, USA

3. **R. Zahorán**, P. Kumar, D. Horváth, Á Tóth

Flow-driven fabrication of calcium phosphate/alginate composites

EPF European Polymer Congress, June 26 - July 1, 2022, Prague, Czech Republic

6.2 Other Poster Presentations

1. **R. Zahorán**, Á Tóth, D. Horváth, G. Schuszter

High-speed tracking of fast chemical precipitation

GRC Crystal Growth and Assembly, June 23-28, 2019, Manchester, USA

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