

## Data on granulometric composition of calcium phosphate obtained by dispersion method

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**Abstract.** The kinetics of calcium phosphate crystallization from model solutions of saliva and liquid phase of dental plaque has been studied by the dispersion method. It was found that the composition of the saliva model system is favorable for the growth of larger crystals. The size of the particles in crystallization varies nonlinearly. As supersaturation grows, the amount of formed particles increases, however, the average rate of crystallite growth decreases.

**Keywords:** Biological fluid; Saliva; Microcrystallization; Mathematical model; Self-assembly; Nano- and micro-level analysis of variance.

### 1. Introduction

Investigation of phase formation in biological environment is one of the current issues in the field of crystallization from solution [1–3].

Crystallization of low-solubility compounds (LSC) from model solutions of biological fluids is difficult to study since they are multicomponent, and the process is multi-factorial. In addition, the formation of LSC most often occurs under non-equilibrium conditions and is controlled by kinetic factors [4]. By now, there is not much information on the nature of LSC crystallization in physiological solutions of complex composition [5, 6].

One of the new and promising optical methods to study crystallization is the dispersion analysis which allows obtaining information on particle size distribution [7]. The particle size distribution is directly related to the crystallization parameters that determine the mechanism of nucleation and crystal growth. This technique is not commonly used in the experimental study of crystallization, though it helps to accumulate different information about the origin and growth of crystals and is easy to use.

Thus, the aim is to study the kinetics of LSC (calcium phosphate) crystallization from model solutions of saliva and liquid phase of dental plaque (LPDP) by the dispersion method.

### 2. Experimental techniques

To study the process of crystallization we used the model solutions with the concentration range of basic inorganic components and saliva pH and LPDP of an average healthy adult [4].

For each series of the experiments, the solutions containing cations and anions were prepared since in these conditions, soluble compounds are not formed. In each of the solutions, pH was adjusted to the physiological value ( $6.93-7.00 \pm 0.05$ ) by adding 20% of NaOH or HCl solution (conc.). The equivalent volumes of the solutions were mixed to make a solution with a designed supersaturation ( $S$ ,



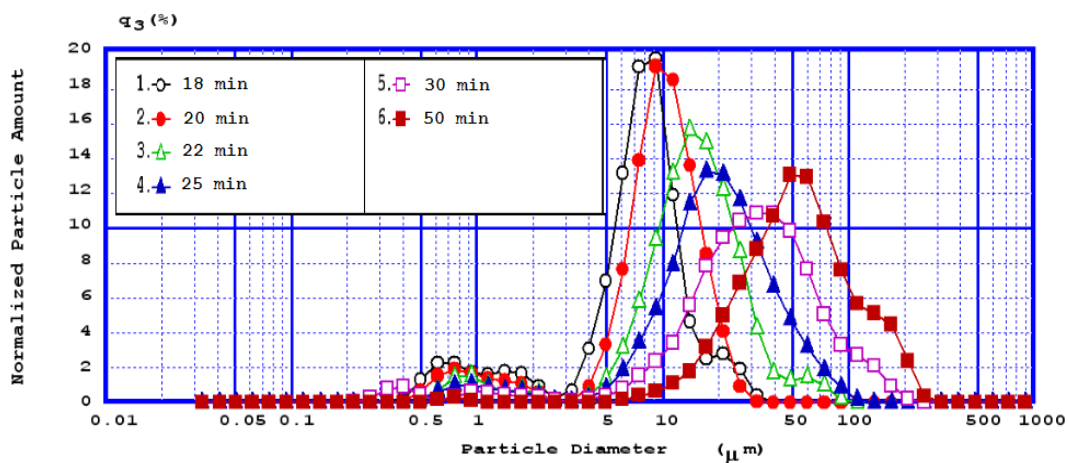
to determine the effect of supersaturation on the parameters of nucleation the following values were modeled  $S = 5, 10, 15, \dots, 50$  for both systems) and calculated concentration of the components. The prepared solution was analyzed at different time intervals with a laser diffraction analyzer. The diagrams of particle size distribution were taken, and the average size of the particles formed was calculated.

The dispersion analysis was performed with laser diffraction particle size analyzer Shimadzu SALD-2101 (Laser Diffraction Particle Size Analyzer). For more reliable results, the 4-5 fold replicated analysis of the samples was carried out. The relative standard deviation for the measurement data was  $S_r = 0.02-0.04$ .

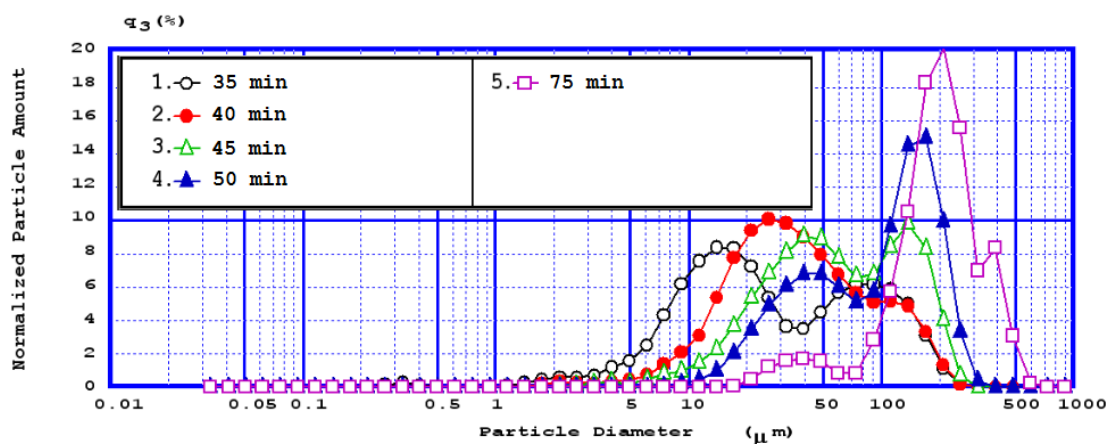
### 3. Result and discussion

In the study of crystal formation and growth in model solutions, the differential curves for particle size distribution at different intervals of crystallization (Figure 1, 2) were obtained for all supersaturations under investigation with the diffraction analyzer.

The analysis of the dependencies indicated differences in particle size distribution for the two tested systems. The LPDP system is characterized by monomodal particle distribution throughout the crystallization time, and for the system modeling the saliva composition, the distribution is polymodal.



**Figure 1.** Diagrams for particle size distribution at different time intervals in the LPDP system with  $S = 15$ .



**Figure 2.** Diagrams for particle size distribution at different time intervals in the saliva system with  $S = 25$ .

This type of particle size distribution can be explained if assume that in crystallization, calcium hydrogenphosphate (brushite) is a largely dominating compound in the LPDP, and in the model saliva solution, the dominating compounds are calcium hydrogen phosphate and octacalcium phosphate [4]. At that, in the process of solid phase crystallization, the following processes can be observed: nucleation of solids (nucleation), primary crystal growth to a critical size, aggregation of the crystals and growth of the formed nano-sized particles, aggregation of the particles and formation of crystals with a diameter of several micrometers, and their further growth. As a result, nucleation and crystal growth in the model system of saliva occurs simultaneously in several centers of different critical size. This makes the obtained distribution curves polymodal.

After appropriate mathematical treatment of the differential curves, an average size of the particles formed in crystallization was determined (table 1 and 2).

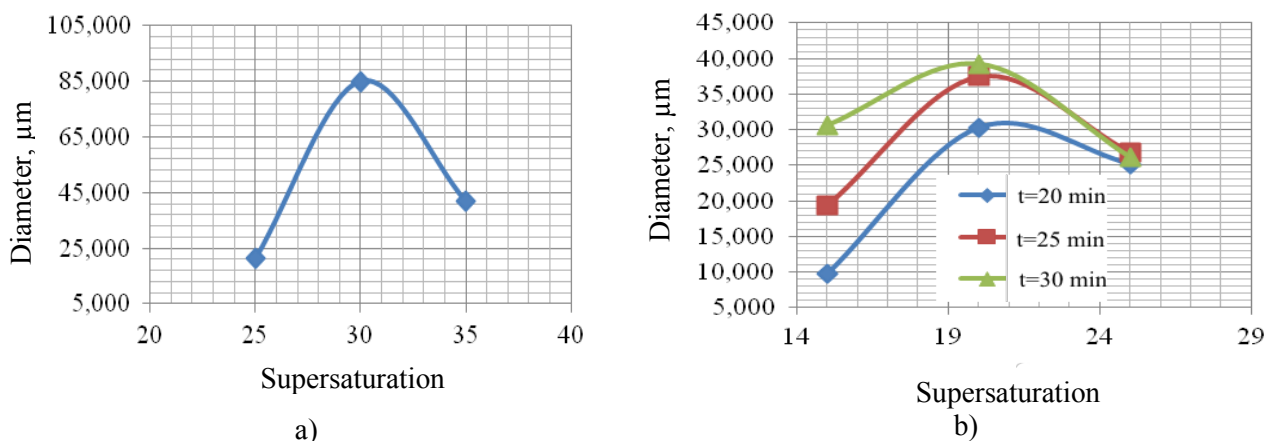
**Table 1.** Average particle size for LPDP solution in time under varied supersaturations.

Supersaturation values					
S=15		S=20		S=25	
t, min	d, $\mu\text{m}$	t, min	d, $\mu\text{m}$	t, min	d, $\mu\text{m}$
18	$7.724 \pm 0.288$	5	$8.358 \pm 0.334$	2	$9.042 \pm 0.274$
20	$9.699 \pm 0.285$	7	$15.180 \pm 0.338$	3	$19.324 \pm 0.323$
22	$14.52 \pm 0.309$	10	$20.492 \pm 0.303$	5	$25.808 \pm 0.384$
25	$19.32 \pm 0.339$	15	$25.439 \pm 0.341$	8	$27.189 \pm 0.418$
30	$30.621 \pm 0.361$	20	$30.324 \pm 0.409$	10	$26.438 \pm 0.426$
35	$40.056 \pm 0.402$	25	$37.463 \pm 0.443$	15	$25.224 \pm 0.442$
40	$45.619 \pm 0.468$	27	$38.315 \pm 0.380$	20	$25.189 \pm 0.427$
45	$49.287 \pm 0.216$	30	<b><math>39.206 \pm 0.374</math></b>	22	$26.384 \pm 0.449$
50	<b><math>52.648 \pm 0.373</math></b>	-	-	25	$26.779 \pm 0.446$

**Table 2.** Average particle size for saliva solution in time under varied supersaturations.

Supersaturation values					
S=25		S=30		S=35	
t, min	d, $\mu\text{m}$	t, min	d, $\mu\text{m}$	t, min	d, $\mu\text{m}$
35	$23.388 \pm 0.489$	5	$20.216 \pm 0.423$	5	$21.696 \pm 0.454$
40	$34.775 \pm 0.374$	10	$36.124 \pm 0.420$	10	$30.682 \pm 0.353$
45	$56.738 \pm 0.370$	15	$49.138 \pm 0.333$	15	$35.737 \pm 0.392$
50	$105.759 \pm 0.334$	20	$66.761 \pm 0.316$	20	$37.050 \pm 0.398$
55	$140.910 \pm 0.360$	25	$76.418 \pm 0.281$	25	$40.577 \pm 0.433$
60	$168.842 \pm 0.549$	30	$84.962 \pm 0.417$	30	<b><math>41.824 \pm 0.438</math></b>
65	$176.731 \pm 0.316$	35	$90.112 \pm 0.312$	-	-
70	$192.877 \pm 0.293$	40	$92.540 \pm 0.288$	-	-
75	<b><math>198.350 \pm 0.256</math></b>	45	<b><math>94.831 \pm 0.349</math></b>	-	-

It is evident that during crystallization the particles increase in size, and the confidence intervals for different periods of time do not overlap. Despite the similarity of the pattern, the size of the solid phase particles in the saliva model solution and that of the particles in the LPDP solution are different. In the saliva model solution, it varies from 42 to 198  $\mu\text{m}$  in transition from  $S = 25$  to  $S = 35$ , and in the LPDP solution, it ranges from 52 to 26  $\mu\text{m}$  for supersaturations  $S = 15$  and  $S = 25$ , respectively. These results are consistent with the data obtained previously, that is, the composition of the "saliva" model system is favorable for growth of larger crystals [7]. The analysis of the table data shows that the size of the particles in crystallization changes nonlinearly with the maximum extremum and indicates similar character of the obtained patterns for the LPDP and saliva model solutions (Figure 3). However, for the "saliva" system, the crystal growth is observed before supersaturation equal to 30, while in crystallization of the LPDP, the crystals grow at supersaturation equal to 20, and these dependencies do not change over time. This behavior can be attributed to higher concentrations of the basic inorganic components of the LPDP solution if compared to the saliva model system [4]. In addition, decrease in the crystal size under increased supersaturation (saliva > 30, LPDP > 20) may be caused by transition from a heterogeneous nucleation of the crystallite to a homogeneous one or by secondary processes (aggregation, dissolution, recrystallization, etc.). The higher the value of the initial supersaturation, the larger is the aggregation. The calculated values of the particle size enabled to find the dependence of this parameter on the supersaturation in the modeled solutions (Figure 3).



**Figure 3.** Dependence of the particle size on supersaturation in the model systems: a) is Saliva and b) is LPDP.

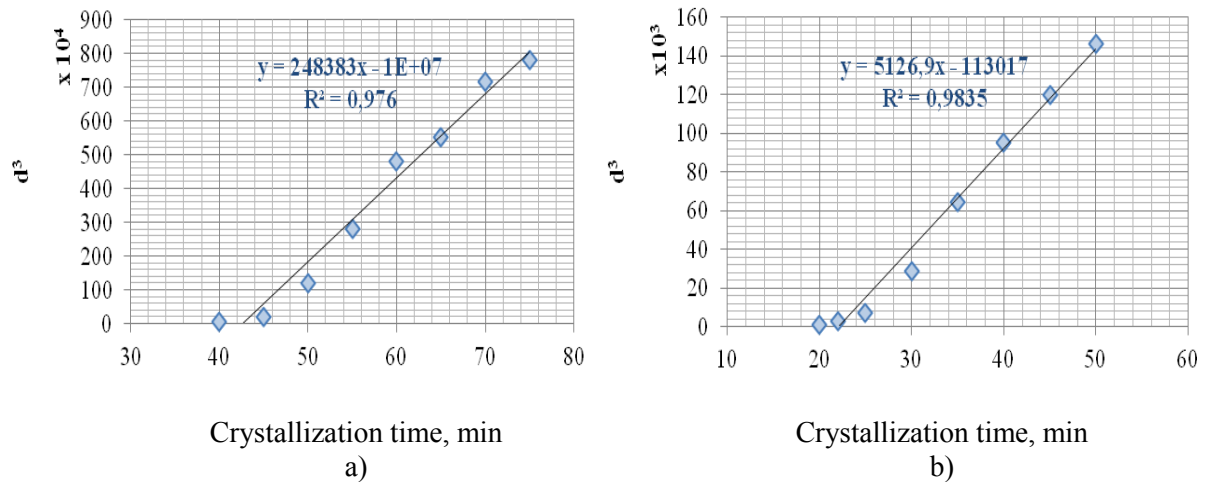
As the supersaturation increases, the number of the formed particles grows, however, the average rate of crystallite growth reduces (table 3).

**Table 3.** Average rate of the crystallite growth in the model systems.

Supersaturation (saliva)	Average growth rate $\mu\text{m}/\text{min}$	Supersaturation (LPDP)	Average growth rate $\mu\text{m}/\text{min}$
25	4.38	15	1.38
30	1.85	20	1.24
35	0.81	25	0.74

The obtained values of the particle diameter depending on the time of crystallization (except for the LPDP model system with the supersaturation of 25) are linearly correlated in the coordinates  $d^3 = f(t)$

(Figure 4). Therefore, in terms of the Lifshitz-Slyozov-Wagner theory [9], the particle growth in these systems occurs due to isothermal distillation (Ostwald ripening), that is the substance is transferred from small to large particles since the chemical potential of the latter is smaller (Kelvin effect). As a result, fine particles gradually dissolve and larger particles grow. Thus, the growth of particles is caused by the diffusion substance transport.



**Figure 4.** Dependence of the particle size on crystallization time, a) is saliva system  $S = 25$ , b) is LPDP system  $S = 15$ .

It was found that it (the correlation?) is not linear, it has the maximum extremum, and it is of the similar character for both LPDP and saliva solutions. The obtain interrelation indicates that two-dimensional nucleation is characteristic of the modeled liquids since it can be described by the equation  $\frac{dR}{dt} = k_n S^n$  (where  $n$  is the order of the reaction surface [10]), and the calculated value is equal to  $n = 2.00 \pm 0.06$ . These patterns are in good agreement with the data in [9] and with previous results [4].

The shape of the curve "size-time" for the LPDP system with the supersaturation equal to 25 is different. This may be due to a different mechanism of nucleation (transition from heterogeneous to homogeneous nucleation mechanism as the supersaturation increases) and crystal growth. It can be assumed that the particle growth in the system occurs due to coagulation, i.e. aggregation (coacervation) of the particles in a dispersed phase. Since a large number of nuclei of crystallization are formed in this system, the density of the solid-phase particles is higher, they are close to each other, and this enhances their approach and mutual aggregation.

#### 4. Conclusions

Thus, in the research by means of the dispersion analysis it was found:

1. For the liquid phase of dental plaque, the monomodal particle distribution can be observed throughout the crystallization time, however, for the system modeling saliva it is polymodal.
2. In crystallization, the particle size increases and the confidence intervals for different time periods do not overlap.
3. The composition of the saliva model system is favorable for growth of crystals larger in size.
4. The particle size in crystallization varies nonlinearly, and it has the maximum extremum. As supersaturation increases, the number of formed particles grows, however, the average rate of the crystallite growth decreases.

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