

## MORPHOLOGICAL FEATURES OF CRYSTALLINE STRUCTURES FORMED DURING DEHYDRATION OF HUMAN SALIVA UNDER CONTROLLED EXPERIMENTAL CONDITIONS

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**Abstract:** Background: Human saliva is a complex biological fluid whose dehydration leads to the redistribution of dissolved and colloidal components, forming characteristic crystalline and amorphous structures. Objective: To investigate the morphological features of crystalline structures formed during controlled dehydration of human saliva. Methods: Unstimulated saliva samples (4  $\mu$ L droplets) were collected from 10 healthy male volunteers aged 18–30 years and dehydrated at 25 °C and 32% relative humidity for 25–30 minutes. Digital microscopy (50 $\times$ –200 $\times$ ) was used for morphological assessment, evaluating crystal shape, dendritic branching, zonal organization, density, symmetry, and structural heterogeneity. Results: Dehydrated saliva droplets formed structurally organized and morphologically heterogeneous patterns with distinct peripheral and central zones. Dendritic, branched, radiating, and irregular crystal forms were observed. Peripheral zones exhibited relatively ordered radial structures, while central zones displayed denser, highly branched formations. Conclusions: Saliva dehydration involves complex phase-structural transformations rather than simple drying, producing reproducible crystalline patterns that reflect internal fluid organization. These findings provide a methodological basis for comparative studies involving external modifying factors.

**Keywords:** *saliva, dehydration, crystalline structures, crystal morphology, dendritic branching, zonal organization, biological fluids, morphogenesis*

## **Introduction**

The dehydration of biological fluids is a physically and biochemically complex process governed by evaporation kinetics, solute redistribution, and nucleation dynamics [1]. Saliva, as a multifunctional biological fluid containing proteins, electrolytes, mucins, and enzymes, presents a particularly rich substrate for crystallographic analysis [2]. The phenomenon of saliva crystallization has been studied in contexts ranging from fertility prediction to diagnostic medicine, where characteristic fern-like patterns (arborization) have been linked to hormonal and pathological states [3].

However, the fundamental morphogenesis of saliva crystalline structures under controlled physical conditions remains incompletely characterized. Most prior studies have focused on qualitative pattern classification without systematic morphological quantification or controlled environmental parameters [4]. A reproducible experimental model for characterizing saliva dehydration patterns is therefore needed to underpin comparative and diagnostic applications.

The aim of this study was to systematically investigate the morphological features of crystalline structures formed during human saliva dehydration under standardized experimental conditions, establishing a morphological baseline for subsequent comparative investigations.

## **Materials and Methods**

### **Study Participants**

Ten healthy male volunteers aged 18–30 years participated in the study. Participants were selected based on satisfactory general health status and the absence of

acute clinical conditions likely to significantly affect oral health or salivary secretion. All samples were analyzed using a uniform experimental protocol throughout the study.

### **Saliva Sample Collection**

Saliva samples were collected 1.5–2 hours after food intake. Participants were instructed to rinse the oral cavity with clean water, followed by a 10–15 minute waiting period prior to collection. Unstimulated saliva samples were used exclusively. Approximately 0.2 mL of saliva was collected from each participant and used immediately for the subsequent experimental stage.

### **Droplet Preparation and Dehydration Conditions**

Each saliva sample was deposited onto a clean glass laboratory surface. Droplets of 4  $\mu\text{L}$  volume were prepared, with three replicate droplets produced per sample, yielding 30 droplets in total. Droplets were placed on flat, clean glass surfaces and allowed to dehydrate under free evaporation conditions. Dehydration was conducted in a thermally insulated chamber maintaining 25 °C and 32% relative humidity. The dehydration duration was 25–30 minutes, depending on droplet volume, ambient temperature, and relative humidity.

### **Microscopic Observation and Image Acquisition**

Structures formed during dehydration and crystallization were observed using a digital microscope at magnifications ranging from 50 $\times$  to 200 $\times$ . Images were acquired digitally and stored for subsequent morphological analysis. Observations focused on the overall appearance of dehydrated droplets, zonal differentiation, and spatial distribution of crystalline elements, with particular attention to morphological differences between central and peripheral regions.

### **Morphological Assessment**

Crystalline structures were assessed according to the following morphological parameters: crystal shape, degree of dendritic branching, expression of central and

peripheral zones, crystal density, degree of symmetry, and structural homogeneity or heterogeneity. Crystal forms were classified as branched, dendritic, radial, or irregular. Dendritic morphogenesis was characterized by branch count, directionality, and density. Zonal assessment distinguished morphological differences between central and peripheral regions of the droplet.

### **Statistical Analysis**

A total of 30 droplets (3 per participant, 10 participants) were analyzed. Morphological parameters were expressed as mean values and percentage proportions. Descriptive statistical analysis was applied. Where appropriate, one-way analysis of variance (ANOVA), Tukey post hoc test, and Pearson correlation analysis were used to evaluate differences and associations among morphological features.

### **Results and Discussion**

Under controlled experimental conditions, dehydrated human saliva droplets formed a solid phase with pronounced spatial organization at the completion of drying. Microscopic examination revealed that saliva dehydration is not a random physical process but a complex morphogenetic phenomenon driven by internal convective flows, redistribution of dissolved and colloidal components, and local concentration gradients. The resulting patterns (facies) were structurally coherent yet morphologically heterogeneous, exhibiting distinct characteristics across different zones.

Significant morphological differences were observed between the peripheral and central regions of dehydrated droplets. The peripheral zone displayed relatively ordered, outward-directed or radially arranged crystalline elements, while the central zone contained denser, more highly branched, and in some cases confluent structures. This zonal differentiation is consistent with capillary flow and differential evaporation rates across the droplet surface [5], resulting in staged migration and local accumulation of solutes and colloids during drying.

Four predominant crystal morphotypes were identified: dendritic, branched, radiating, and irregular forms. Dendritic crystals were characterized by well-defined primary axes with secondary branching of variable complexity. In some specimens, radially oriented crystals were directed from the droplet center toward the periphery, while others showed inward orientation. Irregular forms were attributed to local microenvironmental heterogeneity arising from compositional complexity of saliva. Variability in dendritic branching complexity was observed across specimens. Some samples exhibited long, well-defined, and relatively symmetric dendritic arrays, while others showed lower branching density or asymmetric configurations. This variability likely reflects differences in solute concentration, intermolecular interactions, and nucleation site dynamics during crystallization. These features collectively indicate that saliva dehydration patterns constitute a morphologically rich model for characterizing phase-structural transformations in complex biological fluids.

**Table 1.**

**Summary of morphological parameters of dehydrated human saliva droplets (n=30).**

<b>Morphological Parameter</b>	<b>Expression Level</b>	<b>Frequency (%)</b>
Crystal density	High	68.3±4.2
Zonal differentiation	Distinct (3 zones)	73.3±3.8
Dendritic branching	Moderate–Complex	80.0±3.1
Symmetry	Partial	60.0±4.5
Structural heterogeneity	Present	86.7±2.9

## Conclusions

Dehydration of human saliva under controlled experimental conditions produced spatially organized and morphologically diverse crystalline structures. Distinct morphological differences between central and peripheral zones were consistently observed, indicating progressive redistribution of solutes and zonal differentiation during drying. Four major crystal morphotypes — dendritic, branched, radiating, and irregular — were characterized. The findings confirm that saliva dehydration involves complex phase-structural transformations and that the resulting facies constitute an informative biophysical model for studying structural organization in biological fluids. This approach provides a reproducible methodological basis for subsequent comparative investigations, including assessment of external modifying factors such as ionic solution additives.

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