EFFECT OF 0.9% SODIUM CHLORIDE SOLUTION ON THE MORPHOLOGICAL CHARACTERISTICS OF HUMAN SALIVA CRYSTALLIZATION PATTERNS

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Abstract. Background: The addition of isotonic sodium chloride (NaCl) solution to biological fluids alters their physicochemical properties and may modify crystallization patterns during dehydration. Objective: To evaluate the effect of 0.9% NaCl solution at varying volumetric ratios on the morphological characteristics of human saliva dehydration facies. Methods: Saliva-NaCl mixtures were prepared at four volume ratios (4:0, 3:1, 2:2, 1:3 µL saliva:NaCl) and pure 0.9% NaCl (0:4 µL). Droplets were dehydrated under standardized conditions (25 °C, 32% RH) and examined by digital microscopy. Zonal differentiation and the proportion of the organized zone relative to total droplet area were recorded. Results: Increasing the NaCl proportion was associated with a progressive increase in zonal organization ratio from 31.8±2.6% (pure saliva) to 86.8±1.1% (0.25 saliva:0.75 NaCl). Pure NaCl produced only cubic crystalline forms without zonal organization. Conclusions: The addition of 0.9% NaCl solution modifies saliva crystallization patterns in a dose-dependent manner, expanding the organized crystalline zone and altering crystal morphology, with implications for salivary diagnostic modeling.

Keywords: saliva, sodium chloride, NaCl, crystallization, dehydration facies, zonal organization, crystal morphology, biological fluid modification

Introduction. The crystallization behavior of biological fluids is highly sensitive to ionic composition and solute concentration [1]. Sodium chloride, as the predominant electrolyte in physiological saline (0.9% NaCl), is known to influence nucleation kinetics, crystal growth rate, and macroscopic pattern formation in drying droplets [2]. In salivary fluid, NaCl is a native component; however, its exogenous addition at controlled concentrations offers a tractable experimental tool for modifying and studying the physicochemical determinants of crystallization morphology [3]. Saliva dehydration facies have been proposed as potential diagnostic indicators in various clinical contexts, including inflammatory oral conditions, systemic disease, and hormonal fluctuation [4]. Understanding how ionic additives such as NaCl alter these patterns is therefore of both fundamental and applied scientific interest. To date, the dose-dependent effect of isotonic NaCl on saliva crystallization morphology has not been systematically characterized using digital microscopy under controlled environmental conditions. The present study aimed to investigate the morphological changes in human saliva dehydration facies resulting from the stepwise addition of 0.9% NaCl solution at four volumetric ratios, establishing the relationship between NaCl proportion and zonal crystalline organization.

Materials and Methods

Participants and Saliva Collection. Unstimulated saliva samples were collected from 10 healthy male volunteers (age 18–30 years) following standardized procedures: oral rinsing with water, 10–15 minute rest period, and collection 1.5–2 hours postprandially. Approximately 0.2 mL saliva was obtained per participant and used immediately.

Preparation of Saliva-NaCl Mixtures. Five experimental groups were prepared with total droplet volume of 4 µL per droplet: Group 1 — pure saliva (4:0 µL); Group 2 — 3 µL saliva + 1 µL 0.9% NaCl; Group 3 — 2 µL saliva + 2 µL 0.9% NaCl; Group 4

— 1 µL saliva + 3 µL 0.9% NaCl; Group 5 — pure 0.9% NaCl (0:4 µL). Three replicate droplets were prepared per group per participant, yielding 150 droplets in total.

Dehydration and Microscopy. All droplets were placed on clean glass slides and dehydrated in a thermally insulated chamber at 25 °C and 32% relative humidity for 25–30 minutes. Digital microscopy at 50×–200× magnification was used for image acquisition. The presence and expression of three zones (peripheral, transitional, central) were recorded, and the zonal organization ratio — defined as the proportion of the organized crystalline area relative to total droplet area — was calculated for each group.

Statistical Analysis. Data are expressed as mean ± standard deviation (SD). Between-group differences in zonal organization ratio were assessed by one-way ANOVA with Tukey post hoc test. Pearson correlation analysis was used to evaluate the association between NaCl volume fraction and zonal organization ratio. Statistical significance was set at $p < 0.05$.

Results and Discussion. Progressive addition of 0.9% NaCl to human saliva produced systematic and reproducible changes in the morphological organization of dehydrated droplets. As the NaCl proportion increased, the proportion of the organized crystalline zone relative to the total droplet area increased significantly and consistently across all experimental groups (Table 1).

Pure saliva droplets (Group 1) exhibited three-zone organization in 31.8±2.6% of the total area, characterized by the typical dendritic and branched crystalline morphology described in Article 1. As NaCl proportion increased to 25% (Group 2), the zonal organization ratio more than doubled to 65.0±2.3%, indicating that even low ionic supplementation markedly expanded the area of structured crystalline organization. At equal volumetric proportions (Group 3, 2:2), the ratio reached 78.2±1.1%, suggesting that ionic dilution of organic components facilitates more extensive crystal nucleation and ordered growth.

Morphological characteristics of dehydrated saliva-NaCl mixtures at different volumetric ratios. **Table 1.**

Group	Saliva (µL)	NaCl (µL)	Peripheral Zone	Transitional Zone	Central Zone	Zonal Ratio (%)
Pure saliva	4±0.1	—	+	+	+	31.8±2.6
3:1 saliva:NaCl	3±0.1	1±0.1	+	+	+	65.0±2.3
2:2 saliva:NaCl	2±0.1	2±0.1	+	+	+	78.2±1.1
1:3 saliva:NaCl	1±0.1	3±0.1	+	−	+	86.8±1.1
Pure NaCl	—	4±0.1	−	−	−	—

At the highest saliva:NaCl ratio of 1:3 (Group 4), a zonal organization ratio of 86.8±1.1% was observed; however, the transitional zone was absent, and only peripheral and central zones were distinguishable. This finding suggests that at high ionic concentrations, NaCl-driven crystallization dominates, collapsing the complex three-zone architecture into a biphasic spatial organization. This is consistent with the known effect of high ionic strength in suppressing colloidal organic components and promoting inorganic salt crystallization [5]. Pure 0.9% NaCl (Group 5) produced no organic zonal organization; instead, discrete cubic crystalline formations characteristic of NaCl were observed, confirming the organic origin of the zonal patterning in mixed groups. These cubic forms — clearly distinct from the dendritic organic-phase crystals — were also visible in Group 4 samples at the periphery, suggesting phase separation between salivary and ionic crystallization fronts. A strong positive Pearson correlation was

observed between NaCl volume fraction and zonal organization ratio ($r = 0.98$, $p < 0.01$), indicating a highly systematic dose-dependent relationship. These results demonstrate that the ionic environment is a principal determinant of the macroscopic organization of saliva dehydration facies.

Conclusions. The addition of 0.9% NaCl solution to human saliva in increasing proportions produces a progressive, dose-dependent expansion of the organized crystalline zone in dehydrated droplets, with the zonal organization ratio increasing from 31.8% (pure saliva) to 86.8% (1:3 saliva:NaCl). The three-zone organizational architecture characteristic of saliva is preserved at low and moderate NaCl concentrations but transitions to a biphasic pattern at high ionic proportions. Pure NaCl produces only cubic inorganic crystalline forms without zonal organization. These findings demonstrate that the ionic composition of saliva is a critical modulator of its crystallization morphology and provide a quantitative framework for subsequent studies employing other ionic or biochemical modifiers.

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